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THEMES:

EGF antagonizes collagen generation in human lung fibroblasts *via* induction of interleukin-13 receptor α2 expression

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IL-13 is a cytokine expressed and secreted mainly by activated Th2 lymphocytes which plays a pivotal role in fibrosis. There are two kinds of receptor of IL-13: IL-13Rα1 and IL-13Rα2. IL-13 Rα1 binds IL-13 with low affinity and transduces IL-13 signals. IL-13Rα2 serves as a critical inhibitor and a ''decoy'' receptor for IL-13. In this study, we report that EGF inhibits collagen generation through upregulation of IL-13Rα2 expression. We found that EGF induced IL-13Rα2 expression at both mRNA and protein levels. Such induction displayed a time and dose dependent manner. In addition, collagen generation in these cells reduced after treatment with EGF which was associated with increased expression of IL-13Rα2. Finally, we showed that collagen synthesis did not decrease after exposure to EGF in IL-13Rα2-knockdown fibroblasts. These results suggest that EGF antagonizes collagen generation in human lung fibroblasts *via* induction of IL-13Rα2. Grant sponsors and numbers: National natural science foundation of China 30860118, National undergraduate student innovative experiment plan of China 091040306.

The feature of lung inflammation in Clara cell depleted mice

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Clara cells are important secretive cells in respiratory conduct. Most research on Clara cells focus on their secreted protein, whereas little is known as, but poor concerns the function of intact Clara cells in host defense. This study was to evaluate the function of Clara cells in lung injury by establishing a Clara cell-depleted mice model. In brief, 100ppm naphthalene was inhaled by balb/c mice for 4 hours to establish bronchial Clara cells depleted model. Lavage total cells, protein, LDH and wet/dry ratio, MPO activities in lung tissue were detected to evaluate the lung injury in this model. 110 kinds of inflammation-associated-gene expression in lung tissues were detected using cDNA microarray method, and confirmed by real time PCR. Compared with control, there were not differences in lung wet-dry ratio, total cells and lavage LDH levels in BALF and lung tissue MPO activities, except for protein elevated in Naphthalene injured mice. But cytokine IL-4; IL-5 and IL-10 mRNA in lung tissue were up regulated and IL-1, IL-6, most chemokines were down regulated in Naphthalene injured mice. In conclusion, 100ppm Naphthalene inhaling for 4 hr by mice is a practicable way to establish Clara cell depleted model. The Clara cell depleted model had a characteristic of predominant expression of Th2 cytokines and decreased expression of chemokines, with no obvious sign of inflammation. This study provides a new way to study the Clara cell function.

Regulation of dopamine on the duodenal epithelial ion transport in rat

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Gastric epithelial cells can synthesize dopamine (DA), which was proved to have the protective effect on the gastrointestinal (GI) mucosa. However, the mechanism remains largely unknown. The present study aims to investigate the effect of DA on the duodenal epithelial ion transport in rat and the underlying mechanism. Short-circuit current (I_{SC}), pH titration, and Radioimmunoassay (RAI) were performed in the study. The result indicates that exogenous DA (10μmol/l), when added to the basolateral side of duodenal mucosa, induced a concentration-dependent I_{SC} decrease, which was partly (10μM) and completely (100μM) inhibited by D1 receptor antagonist, SCH-23390, neither by D2 receptor antagonist (sulpiride) nor β1- and β2-adrenoceptor antagonist (CGP-20712A, ICI 118,551). DA-induced I_{SC} response was also blocked by putative K^+ blockers, Ba_2^+ (5mmol/l) and TEA (tetraethylammonium) (5mmol/l). Apical pretreatment with epithelial Na^+ channel blocker, amiloride (10μmol/l), non-specific Cl^- channel transporter inhibitor, glibenclamide (1 mmol/l), and apical HCO_3^- replacement did not significantly affect the DA-induced I_{SC} response. Moreover, although apical addition of DA made no difference in I_{SC}, the pH in apical side was remarkably increased. Interestingly, the PD rats, in which the dopaminergic neurons of substantia nigra (SN) were destroyed by injection of 6-OHDA, manifested a higher DA contents in gastric mucosa, from 4.93 $\pm$ 0.82 nmol/g to 7.15 $\pm$ 0.92 nmol/g (n=5, $P<0.01$) and a stronger I_{SC} response to basolateral application of DA, from -10.00 $\pm$ 2.72μA/cm^2 to-13.93 $\pm$ 3.40μA/cm^2 (n=6, $P<0.01$), suggesting that gastric-derived DA might involved in the regulation of duodenal K^+/HCO_3^- transport. In conclusion, DA is able to promote duodenal K^+ secretion by binding with basolateral D1 receptor. Reduction of dopaminergic neurons in SN may lead to an increase of DA content in gastric mucosa, and enhanced DA-induced I_{SC} response. This study provides a new insight on the modulation and protective effect of DA on the duodenum.

Toll-like receptor 9 agonists stimulate epithelial cells to secrete immunoglobulin M

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B cells have been thought to be the only source of immunoglobulin (Ig). However, increasing evidence has revealed that some non-B cells can also produce IgG and IgA. The purpose of this study was to further reveal the existence of IgM in non-B cells. Immunohistochemistry showed that IgM was expressed predominantly in human malignant and benign epithelial tissues other than mesenchymal tissues. RT-PCR, flow cytometry, western blot, and confocal microscopy further revealed the expression of IgM in some human epithelial cancer cell lines. In addition, these cell lines could also express functional toll-like receptor 9 (TLR9). More important, CpG 2006, a TLR9 agonist, substantially increased the secretion of IgM in human epithelial cancer cells. Surprisingly, the two non-CpG controls, CpG 2078 and GpC, induced these effects at a similar level. However, the neutralizing CpG ODN208, which was reported to inhibit rather than activate TLR9, did not induce these effects. In addition, chloroquine, a TLR9 inhibitor, and siRNA of the TLR9-associated adapter, myeloid differentiation primary response gene 88 (MyD88), could abolish these effects. To further confirm these findings, we isolated murine liver epithelial cells by FACS sorting and revealed the existence of IgM. Next we treated Balb/c mice with murine CpG ODN and sacrificed them 5 days later. Immunohistochemistry using paraffined liver tissues revealed increased secretion of IgM while RT-PCR and qPCR using isolated liver epithelial cells showed enhanced IgM expression. Together, these findings indicate that TLR9 agonists could stimulate the secretion of IgM in epithelial cells and suggest that these IgM may be involved in innate immunity.

Silica-induced epithelial-mesenchymal transition (EMT) in human bronchial epithelial cells

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Epithelial-mesenchymal transition (EMT) plays an important role in fibrotic diseases. However, little is known regarding toxic silica-induced EMT in lung fibrosis. We investigated whether silica could induce EMT in human bronchial epithelial (HBE) cells *in vitro*. HBE cells were stimulated with 200 μg/mL silica for 72h. Morphologic changes were observed. The expression of mesenchymal marker a-smooth muscle actin (a-SMA) and the epithelial cell marker E-cadherin (E-cad) were detected in silica-stimulated HBE cells, using western blotting. After exposure to silica, HBE cells lost contact with their neighbor and displayed a pindle-shape, fibroblast-like morphology. In addition, incubation of HBE cells with silica induced *de novo* expression of mesenchymal marker a-SMA and loss of epithelial marker E-cad. These results suggested that silica could induce EMT in human bronchial epithelial cells.

Expression of NKCC1 and NKCC2 in gastric mucosa and their possible role in acid secretion

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NKCC1 and NKCC2 have been reported to express in mammalian stomach, but their cellular distribution and specific role in acid secretion is poorly understood. In the present study we compared the expression pattern of NKCC1 and NKCC2 in rat gastric mucosa in order to reveal their potential role in acid secretion. NKCC1 was expressed in the neck and base of the gastric glands and was abundant in parietal cells and chief cells, but had no detectable levels in the mucous cells. Compared with NKCC1, NKCC2 was expressed in the whole gastric glands and localized to the parietal cells, chief cells and mucous cells at high levels. By using H^+ selective microelectrode, the gastric acid secretion of rat and mouse was examined. The basal H^+ flux rate was inhibited by H2 receptor antagonist, cimetidine. However, when we pretreated the tissue with nonspecific NKCC inhibitor bumetanide (10μM), the H^+ flux rate was not affected by cimetidine. After stimulation with histamine *in vivo*, the distribution of NKCC1 in the parietal cells did not significantly changed, but NKCC2 transferred from the basolateral membrane to the cytoplasm. Moreover, NKCC2 diffused to the whole cytoplasm from aggregate vesicles in primary cultured parietal cells after histamine stimulation. These results suggest that the differential expression of NKCC1 and NKCC2 in gastric epithelial cells may contribute to the different gastric secretion. NKCC2 might be involved in the regulation of gastric acid secretion of rodent.

Apoptosis of lens epithelial cell induced by elemene and the cellular and molecular mechanism

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To investigate the effect of natural medicinal monomer elemene (Ele) on apoptosis of lens epithelial cell (LEC) and the cellular and molecular mechanism in vitro. The bovine LEC were cultured with Ele and were used to observe the ultrastructure changes under transmission electron microscope, and to detect DNA content and mitochondrial transmembrane potential ($\Delta\Psi m$) changes by flow cytometry. The typical morphological changes of LEC apoptosis in Ele group were observed under transmission electron microscope, such as chromatin condensation and aggregation at the nuclear periphery, and nuclear fragmentation as well. The DNA content of LEC in Ele group decreased and showed a time-dependent manner. It was significant lower than that of control group ($P<0.01$). The $\Delta\Psi m$ of LEC in Ele group decreased in early stage. The difference was significant between Ele group and control group ($P<0.01$). These results suggest that Ele can remarkably induce apoptosis of LEC *in vitro*. Decreases of DNA content in LEC nucleus are the mechanism of LEC apoptosis induced by Ele. Moreover, Collapse of mitochoondrial transmembrane potential ($\Delta\Psi m$) in cytoplasm induced by Ele results in the irreversible apoptosis process of LEC. And LEC apoptosis induced by Ele maybe mediated through two mechanisms: nuclear mechanism and cytoplasmic mechanism. Finally, the apoptosis of LEC induced by Ele may be the cellular and molecular mechanism of reducing posterior capsular opacification of lens. Therefore, Ele may become potential medicine for after cataract prevention and treatment.

Effects of Polysaccharides from *Rhizoma Atractylodis Macrocephalae* and from *Radix Astragali* on polyamine-mediated K^+ channels in the IEC-6 migration

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As the key step in the restitution of gastrointestinal mucosal injury is gastrointestinal epithelial cell migration, was designed to evaluate the effect of polysaccharides from *rhizoma atractylodis macrocephalae* and from *radix astragali* on the migration of intestinal epithelial cells (IEC-6). It was found that the polysaccharide from *rhizoma atractylodis macrocephalae* or *radix astragali* could promote the migration of IEC-6 and reverse the inhibitory effect on the migration of polyamine-depleted IEC-6 by DFMO. Our results showed that both polysaccharides from the *rhizoma* and from *the radix* could increase intracellular polyamines (spermidine) of IEC-6 after wounding and reverse DFMO-caused reduction of the intracellular spermidine. Furthermore, polysaccharides reversed the inhibitory effect of 4-Aminopyridine (4-AP, inhibitor of Kv1.1) the migration of IEC-6 and on inhibitory effect on the expression of Kv1.1mRNA and Kv1.1protein by DFMO or 4-AP. Finally, polysaccharides could promote the outflow of K^+ in the migration of IEC-6 and reverse the inhibition of K^+ outflow by DFMO. All of the results suggest that the mechanism of promoting cell migration of the polysaccharides relates to the activation of polyamine-mediated K^+ channel by the polysaccharides.

Down-regulation of integrin β4 in the airway hyperresponsiveness model by ozone stress

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It is known that ozone stress can induce AHR (airway hyperresponsiveness). The underlying cellular and molecular mechanism is not fully understood. We constructed a successive ozone stress rat model and showed that AHR caused by ozone stress presented as increased lung resistance (RL) in response to inhaled histamine, but not baseline RL. Meanwhile, structural disruption and decreased integrin β4 expression on airway epithelia were observed. Further correlation analyses revealed that increased RL to inhaled histamine (0.32 mg/ml) is negatively correlated to integrin β4 mRNA expression. Moreover, we silenced integrin β4 on human bronchial epithelial cells and observed damaged anti-oxidative ability and much more apoptosis of epithelial cells when integrin β4 was down-regulated. Overall, this study suggested that down regulation of integrin β4 was involved in the ozone-induced AHR, presumably by inducing epithelial apoptosis and anti-oxidation damage.

Cloning and function research of the novel gene BRAP

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Bombesin receptor subtype 3 (BRS-3), the orphan bombesin receptor, may play a role in the regulation of stress responses in lung and airway epithelia. BRAP is a novel gene we found in our previous study which interacts with BRS-3. This study was designed to determine the subcellular location of BRAP-expressing protein and its function in bronchial epithelial cells. The new gene ORF was amplified by RT-PCR and ligated to pEGFP-C1 vector, then the recombinant plasmid pEGFP-C/BRAP was transfected into Hela cells. The location of BRAP protein was observed by the laser confocal microscope, and the expression of transfected protein was analyzed by Western-blot. At the same time, we generate the recombinant plasmid pcDNA3.1(+)/BRAP, transiently transfected it into human bronchial epithelial cells (HBEs) and detected its impact on the cell cycle with flow cytometry. We further sort HBE cells which stably express pcDNA3.1(+)/BRAP, and observe their impacts on the wound repair ability of HBE. DNA sequencing confirmed the successful building of the recombinant pEGFP-C1/BRAP plasmid. BRAP protein was located in the membrane and cytoplasm, the expression of BRAP protein was increased significantly in the transfected recombinant cells. Flow cytometry results demonstrated that the recombinant plasmid pcDNA3.1(+)/BRAP increased the S phase + G2 phase of cell cycle by 25%. Microscopic video analysis system showed that the repair index of wounded BEC increased 20% through stably expression of BRAP. The present study demonstrated that the novel gene BRAP protein was located in the membrane and cytoplasm, suggesting that this protein may be a cytoplasmic protein and involved in cellular signal transduction; BRAP promoted the cell cycle, and increased the wound repair ability of human bronchial epithelial cells. This work was supported by the grant #30870917 from National Natural Science Foundation of China.

Expression of respiratory syncytial virus-induced gene networks in human epithelial cells by microarray analysis

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Worldwide, respiratory syncytial virus (RSV) is the most important viral pathogen of serious lower-respiratory tract illness in infants and young children. Epithelial cells are the primary and, as in the case of RSV, virtually the only cell target of viruses which enter the airway. A body of studies have been done about the complex virus-host interplay during the acute infection phase, but little is known about the response to RSV infection of airway epithelial cells during the chronic infection phase. Here, we investigated host transcriptional responses induced by RSV infection of airway epithelial cells during serial passage. Monolayers of confluent human airway epithelial cell line 16HBE cells were infected with RSV (A strain) at multiplicity of infection (MOI) of 0.0067. From sequential passages of surviving cells, chronic infect cultures of 16HBE cells were obtained, and viral persistence was verified and monitored by real-time PCR. The cells of first generation (G1) and ninth generation (G9) were subjected to RNA extraction, and gene expression was analyzed using cDNA microarrays (Illumina HumanWG-6 v3.0 Expression BeadChip). Our results show that RSV chronic infection induced significant changes in epithelial cell gene expression. Comparative analysis of infected G1 vs. control G1 revealed the differential expression of 796 genes (494 up- and 302 down-regulated), while differential expression of 71 genes (26 up- and 45 down-regulated) was found between infected G9 and control G9. Gene ontology analysis showed that downregulated genes were mainly associated with extra cellular matrix components and oxidative stress. Upregulated genes were predominantly related to interferon, chemotatic factor and cytokine. Infected epithelial cells display reductive antioxidative and cytothesis transcription and they activate antiviral and inflammatory processes. This study may pave the way for understanding epithelial cells response to RSV chronic infection. This work was supported by the National Basic Research Program of China (973 Program) (NO. 2009CB522102).

Transforming growth factor β1 induced human proximal tubular epithelial cells to produce stem cell factor

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Stem cell factor (SCF) is a multifunctional cytokine. The local production of SCF may be an important mechanism for regulating proliferation, differentiation, and migration of various cells bearing c-kit receptors, and might be susceptible to the cytokines that serve in inflammation and tissue repair. A few reports and our preliminary study have found that SCF has a high expression in renal tubular epithelial cells in pathological condition; however, its mechanism is unknown. To study the cause of it, we stimulated human proximal tubular epithelial cells (HKC), with transforming growth factor β1 (TGF-β1) respectively in the concentration of 0.5 ng/ml; 1.0 ng/ml; 5.0 g/ml and 10.0 ng/ml. RNA expression of SCF was determined by *in situ* hybridization utilizing nonradioactive oligonucleotide probes and quantitative image analysis. Our findings are as follows: in HKC, which was stimulated by different concentration TGF-β1, the SCF mRNA expression was greatly enhanced in response to TGF-β1. The flow cytometry and immunocytochemistry further confirmed that SCF protein in this cell line was significantly enhanced by TGF-β1. In brief, our results suggest that TGF-β1 can modulate SCF mRNA and protein expression in a dose dependent manner in vitro cultured human proximal renal tubular epithelial cells. These results give insights to renopathy mechanism's research.

Cellular mechanisms underlying the duel effect of formaldehyde on mouse airway epithelium

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Abnormal fluid accumulation in tissues, including respiratory edema, is a consequence of secretion and absorption disorder in epithelial cells. Previously, it has been reported that formaldehyde (FA), a common indoor air pollutant, induces respiratory edema and airway inflammation. However, the underlying mechanism for respiratory edema induced by FA is not clear. In this study, mice airway epithelium were isolated and short circuit current (Isc) were recorded. FA could produce a concentration-dependent increase of Isc in normal K-H solution, which could be inhibited in Cl^- free K-H solution or by non-specific chloride channel inhibitor DPC (diphenylamine-2-carboxylic acid) or by CFTR specific inhibitor $CFTR_{i\text{-}172}$, but not by Ca^{2+}- activated Cl^- channel inhibitor DIDS (4, 4`- diisothiocyanatostilbene- 2,2`- disulfonic acid). In addition, pretreatment of forskolin or MDL-12330A would reduce the Isc induced by FA, indicating that FA induces cAMP-dependent Cl^- secretion in airway epithelium. With the FA-inhalation for 1h, 1.5h and 2h in mice model, the wet-dry ratio, water percentage and tissue inflammation of lung tissue were significantly increased in FA-inhalation group, while the Isc of airway epithelium induced by forskolin were decreased, implying the dysfunction of CFTR and activation of the inflammation. These results suggest that inhalation of FA may instantly activate cAMP-dependent CFTR mediated Cl^- secretion to act as an innate defense response, while long-term inhalation of FA inhibits CFTR and triggers the pathway of inflammation.

Regulation of the vaginal epithelium pH by extracellular ATP *via* CFTR

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It is well known that there is high capacity ATP in male's sperm, which is essential for sperm capability. In this study, we first proved that ATP can also affect the pH environment of female reproduction duct, which might be import for the sperm capability in vagina. VK2/E6E7, a cell line from normal human vaginal epithelia immortalized by expression of human papillomavirus 16/E6E7, was used in most of our experiments. CFTR was found to be expressed in VK2/E6E cells by RT-PCR and Western Blot. Application of 400μM ATP can make VK2/E6E7 pH_i descending, while RB-2 (a potent blocker of P_2Y receptors), MDL-12, 330A (an Adenylyl Cyclase inhibitor), DPC (a non-specific inhibitor of Cl^--channel) and $CFTR_{inhibitor\text{-}172}$ (a specific CFTR inhibitor) can significantly attenuate the pH_i acidification by ATP. In short-circuit current study, we found that the ATP-induced current mainly consists of HCO_3^-. When measuring the cAMP in the cells, ATP had a positive effect on cAMP elevation which can be inhibit by MDL-12,330A. In the *in vivo* experiments when ATP was applied into female rat vagina, it induced HCO_3^- secreting and basified the Vaginal pH in a dose depended manner. The effect of ATP on vagina can be inhibited by $CFTR_{inhibitor\text{-}172}$. Taken together, these data suggest that ATP binds to the P_2Y receptor in vaginal epithelium and activates adenylyl cyclase, leading to an increase of cAMP and thus enhancing the aginal pH by secreting HCO_3^- via CFTR.

The expression of TLR_4 mRNA and CD_{14} mRNA and the effect of lipopolysaccharide in lens epithelial cells

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To investigate the expression of Toll-like receptor 4 (TLR_4) and cluster of differentiation 14 (CD_{14}) and the effect of lipopolysaccharide in lens epithelial cells (LECs). Expression of TLR_4 mRNA and CD_{14} mRNA in bovine LECs cultured *in vitro* were examined by using the one-step reverse transcription-polymerase chain reaction (RT-PCR). In addition, bovine LECs were exposed to lipopolysaccharide (LPS) in various concentrations for various time periods, after that mRNA expression of TLR_4 and CD_{14} in LECs were determined by using the RT-PCR. Two amplification bands, at 451 bp and 348 bp, were evident in the RT-PCR of LECs, and were confirmed as TLR_4 mRNA and CD_{14} mRNA respectively by genetic sequencing. The expression of TLR_4 mRNA in LECs from groups LPS 50 ng/ml, 100 ng/ml, 200 ng/ml, 500 ng/ml and 1000 ng/ml was significantly higher than that in control LECs ($p<0.01$). The expression of TLR_4 mRNA in LECs treated with 100 ng/ml LPS for 24 h, 48 h and 72h was significantly higher than that in control LECs ($p<0.01$). The expression of CD_{14} mRNA in LECs treated with 100 ng/ml LPS for 24 h was significantly higher than that in control group ($p<0.05$). These results suggest that both TLR_4 and CD_{14} are expressed in LECs and LPS enhances the expressions of TLR_4mRNA and CD_{14}mRNA in LEC, which may be related to intraocular cellular response, as well as in formation and development of after- cataract.

Dehydroepiandrosterone-induced proliferation of prostatic epithelial cell is mediated by NF-kB via PI3K/Akt signaling pathway

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Dehydroepiandrosterone (DHEA), metabolized to androgens and/or estrogens in the human prostate, is the most abundant steroids in humans. DHEA levels decline with age, and use of DHEA supplements to delay the aging process is of unproved effectiveness and safety. In the United States, DHEA is widely available as an over-the-counter dietary supplement, and is increasingly self-prescribed for its alleged anabolic and antiaging effects, with unsubstantiated claims of beneficial effects as well as uncertain long-term safety. In this study, we demonstrated that proliferation of prostatic epithelial cells and increase of PSA expression induced by DHEA were neutralized by Casodex or androgen receptor (AR) siRNA, two specific AR blockers. DHEA stimulated NF-kB DNA binding activity, with this effect being blunted by Casodex or AR siRNA. Moreover, the inhibition of the phosphatidylinositol 3-kinase (PI3K)/AKT nullified the effects of DHEA on NFKB activation. These findings suggested that DHEA stimulated normal prostatic epithelial cell proliferation, and ARis involved in DHEA-induced PSA expression in normal prostatic epithelial cells. This stimulation effect induced by DHEA is mediated by the activation of NFKB via PI3K/AKT pathway.

Na^+ reabsorption in rat vaginal epithelium *via* epithelial sodium channel

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The microenvironment formation of the vagina plays a critical role in female reproduction. The composition of the basal ion transport across the rat vaginal epithelium was studied by short-circuit current (*Isc*) technique to explore the role of epithelial sodium channel (ENaC). The basal *Isc* was 39.528 $\pm$ 12.807 $\mu A/cm^2$ in normal Krebs-Henseleit (KH) solution. Removal of extracellular Na^+ from the bathing solution completely blocked the basal *Isc*. Adding amiloride, the inhibitor of ENaC, to the apical side of vaginal epithelium significantly blocked the basal *Isc*. Furthermore, adding BaCl2, the inhibitor of K^+ channel, to the basolateral side of vaginal epithelium also inhibited the basal *Isc*. Those results revealed that, in the resting condition, the basal ion transport across the vaginal epithelium is mediated by Na^+ reabsorption *via* ENaC in the apical side. The basolateral K^+ channel may have cooperation with the apical ENaC to maintain the basal ion transport in vaginal epithelium.

Molecular mechanism of the dysregulated TERE1/UBIAD1 in bladder carcinoma

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Down-regulation of TERE1/UBIAD1 (TERE1, transitional epithelial response gene) causes multiple human cancers including bladder carcinoma. TERE1 also contributes to SCCD (Schnyder crystalline corneal dystrophy). Although the role of TERE1/UBIAD1 in lipid metabolism gradually becomes clear, its function in tumor suppression remains to be clarified. The mRNA and protein level of both TERE1/UBIAD1 and hTERT was analyzed by RT-PCR and western blotting in normal and carcinoma bladder tissues. TERE1 siRNA oligos was exploited to knock down the expression level of TERE1 in Human Urothelial Cells (HUC), hTERT expression level and the ERK phosphorylation level were measured. Our results showed that the decrease of TERE1 expression was closely related to the increase of hTERT expression and ERK phosphorylation in transitional cell carcinoma (TCC) samples. hTERT level and ERK phosphorylation in TERE1 knock down cell line increased with the subsequent cell proliferation. Adding the MEK inhibitor U0126 into the above transfected HUC inhibited the ERK phosphorylation and hTERT transcription. In conclusion, our result is the initial demonstration that down-regulation of TERE1 activated MAPK signaling pathway and induced subsequent bladder cell proliferation. TERE1 might be a new negative regulator of the MAPK signaling pathway which plays a pivotal role in the development of bladder carcinoma.

The β2-adrenoceptor agonist induced change of mRNA expression of ENaC in human alveolar epithelial cell

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Active Na^+ transport across alveolar epithelia plays an important role in alveolar fluid clearance. β2-adrenoceptor expresses mostly in alveolar area and β2-adrenoceptor agonists are clinically used in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) therapy. However, little is known about the effect of activation of β2-adrenaceptor on the activities of epithelial Na^+ channel (ENaC). This study aimed to investigate the cellular mechanisms and signaling pathways underlying Na^+ absorption in human alveolar epithelial cell (A549) under the stimulation of β2-adrenergic receptor. Our preliminary results showed that mRNA expression of β- and γ-subunt of ENaC were significantly increased after the cultured cell were exposed to β2-adrenoceptor agonist isoprenaline (10^6 M) for 24h and 48h and α-subunit of ENaC expression was not changed. mRNA expression of β- and γ-ENaC were also increased significantly with cell exposure to α-adrenoceptor antagonist phentolamine (10^6 M), indicating that inactivation of α-adrenoceptor may enhance the activities of β2-adrenoceptor and ENaC. mRNA expression of Na^+-K^+-$2Cl^-$ cotransporter (NKCC) was not detectable and NKCC expression level was not changed by isoprenaline and phentolamine treatment, indicating unchanged secretory activity. The results suggest that A549 cell appeared to be absorptive epithelial cells, and expression of ENaC may be regulated by activation of β2-adrenoceptor.

Regulation of human catenin α like-1 expression by nuclear transcriptional factor LEF-1 and AP-2 in bronchial epithelial cells

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Adhesion molecules play vital roles in airway hyperresponsiveness or airway inflammation. Our previous study indicated an alpha-catenin-related protein, catenin alpha-like 1 (CTNNAL1) was downregulated in asthma patients and animal model. We also observed that the expression of CTNNAL1 was increased with the acute ozone stress. CTNNAL1 contributes to the wound repair and proliferation of human bronchial epithelial cells (BECs). In the present study, we determined molecular mechanisms of CTNNAL1 regulation in human BECs. 8 oligonucleotide probes corresponding to various regions of the CTNNAL1 promoter were used in EMSA (electrophoretic mobilityshift assays). 5 were found to have an enhanced mobility shift with extracts from BECs. On the basis of the assay of mutated probes and antibody supershift, they were verified as LEF-1, AP-2α and CREB. Next, ChIP (chromatin immunoprecipitation) assay was used to determine the interaction between these transcription factors and CTNNAL1 promoter. Only AP-2α and LEF-1 show the binding on CTNNAL1 promoter. By site-directed mutagenesis of putative transcription-factor-binding sites within pGL3/FR/luc, we observed a reduction in human CTNNAL1 promoter activity of mutants of both AP-2α and LEF-1 sites. The time courses of AP-2α and LEF-1 activation, followed by CTNNAL1 expression were also examined. It was shown that ozone stress can activate the AP-2α and LEF-1 within one hour, ozone-inducible CTNNAL1 expression and AP-2α and LEF-1 binding activity correlated during a-16 hour time course. Our data suggest that a robust transcriptional CTNNAL1 up-regulation occurs during acute ozone-induced stress and is mediated at least in part by ozone-induced recruitment of LEF-1 and AP-2α to the human CTNNAL1 promoter. This work was supported by the grant #30800504, #30670915 from National Natural Science Foundation of China and the construct program of the key discipline 2007JF3009 in Hunan province.

The effects of different KI intake on H_2O_2 production and oxidative injury effect in FRTL cells

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To explore the effects of iodine on H_2O_2 production, elimination and oxidative injury effect of thyrocytes *in vitro*. FRTL (Fisher rat thyroid cell line) cells were incubated for 6, 12, 24, 48, 72 and 144 hours respectively with different dosage of KI ranging from 10^{-6} mol/L to 10^{-2} mol/L. We examined the number and the morphology of cells, the content of H_2O_2, the expression of specific genes to eliminate H_2O_2, and the oxidative injury effect of FRTL cells. Except for 10^{-6} mol/L KI groups, the more dosage of KI was given to FRTL cells, the fewer cells were survived, and the more H_2O_2 were detected. Furthermore, if we extended incubation period, these tendencies were more obvious. The mRNA levels of several H_2O_2-associated enzymes in thyrocytes, including glutathione peroxidase1, glutathione peroxidase4, thioredoxin peductase and peroxiredoxins, showed no difference between high iodine groups and control group. Furthermore, there were no difference in MDA content among control, 10^{-6}mol/L, and 10^{-5}mol/L KI groups. However, the MDA expression in cells and culture medium were both increased with KI ranging from 10^{-4}mol/L to 10^{-2}mol/L. In conclusion, after administration by 10^{-6}mol/L KI, there was no obvious effect on FRTL cells, while 10^{-5}mol/L KI increased H_2O_2 production. Furthermore, KI ranging from 10^{-4}mol/L to 10^{-2}mol/L induced oxidative damage on FRTL cells. However, the elevated H_2O_2 content did not have any effect on H_2O_2-associated enzymes' in thyrocytes.

Effect of iodine deficiency and iodine excess on the anti-oxidative capability of mice

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To explore the effects of iodine on the anti-oxidative capability of thyroid, liver, brain and blood *in vivo*. Babl/c mice were divided into 5 groups according to their sex and body weight, i.e., (1) low iodine (LI); (2) normal iodine (NI); (3) five-fold high iodine (5HI); (4) ten-fold high iodine (10HI) and (5) fifty-fold high iodine (50HI). After 3 and 6 months, the following parameters were measured, i.e., thyroid absolute and relative weight, thyroid hormones in serum, and thyroid morphology. We also tested the glutathione peroxidase (GPx) activity, superoxide dismutase (SOD) activity and malondialdehyde (MDA) content of liver, brain and blood by biological chemistry method. Results showed that long-term iodine deficiency induced typical diffuse goiter with follicular proliferation in Babl/c mice. On the other hand, iodine excess induced a typical colloid goiter in Babl/c mice. After given low iodine diet for 3 and 6 months, the activity of GPx and SOD in liver and brain declined, but the content of MDA of blood, liver and brain were higher than those of NI group. There was no difference in the SOD and GPx activity and MDA content among NI, 5HI and 10HI groups. However, after given 50 fold doses of KI, the activity of GPx in blood, liver and brain increased, but the activity of SOD in liver decreased, and the content of MDA of blood and brain in 50HI group were lower than those of NI group. In conclusion, low iodine intake damaged the anti-oxidative capability of thyroid, liver, blood and brain in normal Babl/c mice. Treated with high KI (50HI) would induce the change of the activity of anti-oxidative enzymes in blood, liver and brain, but no oxidative damage was found in these tissues. In a word, low iodine intake would induce oxidative damage on Babl/c mice, but the anti-oxidative system of Babl/c mice had a certain tolerance to high KI intake.

CFTR in epithelial cells plays a key role in maintaining lymphocytes survival *in vitro*

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CFTR is a cAMP-regulated chloride channel and its mutation is associated with cystic fibrosis disease, which is characterized by persistent airway inflammation and airway infection. In this study, the in vitro co-culture system was used to study the possible role of CFTR expressed on bronchia epithelial cells on lymphocytes. First, Bronchia epithelial cells with normal CFTR can maintain lymphocytes survival. Two cells lines, HBE and CFBE, were co-cultured with freshly isolated splenocytes. After 2 days culture, the lymphocytes were collected for cell survival and cell subsets analysis by flow cytometry. It was found that lymphocytes co-cultured with HBE cells survived much better than that co-cultured with CFBE cells and the percentage of $CD4^+$ T cells in CFBE group was significant higher. Second, genes associated with lymphocytes survival were detected by RT-PCR, real time PCR or immunofluorescense staining. The genes included IL-7, IL-15, TGF-beta, E-Cadherin, MAdCAM-1 and ICAM-1. In HBE cells, ICAM-1, MAdCAM-1 and E-cadherin expression were higher but IL-15 expression was much down-regulated. Third, the expression of CFTR in HBE cells was knocking down by a designed microRNA that against human CFTR coding region. Stable clones with CFTR knock down were selected to co-culture with lymphocytes. HBE cells transfected with plasmid containing no targeting sequence were used as the control cells. We also found that the CFTR expression in HBE cells was needed for better lymphocytes survival. This study indicates that CFTR expression on epithelial cells is important for better lymphocytes survival. The persistent airway inflammation and airway infection may be caused by the destruction of homeostasis of lymphocytes.

Preparation and bioactivity study of treating AHR disease from *Clerodendrum bungei*

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Clerodendrum bungei, a commonly used traditional Chinese herb, was reported to have some pharmacologic actions. The previous work of our research group suggested that Clerodendrum bungei might be a potential effective medicine aiming at airway hyper-responsiveness (AHR) diseases by its anti-inflammation effects. Among all the solvent Clerodendrum bungei extracts, the chloroform extract had more activity. The aim of this study is to further separate the active components of Clerodendrum bungei. The present study divided chloroform extract in to six active ingredients by Silica gel chromatography. Mouse AHR model was established by ozone stress methods and then the mice were treated by each of the active ingredients for one week. We measured the value of pulmonary resistance (R_L) by BUXCO system under different excitation conditions, counted the WBC number of blood and BALF, and observed the injury of lungs by lung biopsy. The results showed that: Active components A, B and C were useful to attenuate the ozone-increased R_L value; Active components B and C decreased number of white blood cells in BALF, compared with control group; Active components B, C and D had less WBC in blood; Active components B, C and D had minor pathological changes in lung. These results suggest that active components B and C that divided from chloroform extract might contain the most effective monomer which can reduce airway inflammation and treat the disease of airway hyper responsiveness. This work was supported by the grant # 09JJ3056 from Natural Science Foundation of Hunan.

Involvement of cystic fibrosis transmembrane conductance regulator (CFTR) in epithelial-mesenchymal transition during cancer development

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Epithelial-Mesenchymal Transition (EMT) is an intricate process by which epithelial cells lose their epithelial characteristics, like loss of tight junctions, and acquire a mesenchymal-like phenotype. It is essential for numerous developmental processes. Cancer cells have also been described to undergo EMT acquiring a more invasive and metastatic phenotype. The reverse process, known as mesenchymal-to-epithelial transition (MET), is also involved in the formation of a secondary metastatic tumor. Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated Cl^- channel, which is expressed in epithelial cells of various organs. To investigate the role of CFTR in EMT during cancer metastasis, we employed a highly differentiated colorectal cancer cell line LIM1863. Using this cell model, we report that the expression of CFTR was down regulated in cells undergoing EMT and the down regulation was reversed during MET. In addition, a specific CFTR inhibitor CFTRinh-172 could induce EMT in a dose-dependent manner in LIM 1863. On the contrary, MET was blocked by CFTRinh-172 at similar concentrations. These results suggest that functional CFTR is required for cells to keep epithelial phenotype. Further studies demonstrated expression of actived urokinase-type plasminogen activator (uPA) and uPA-receptor were increased in LIM1863 with CFTRinh-172 treatment, which revealed that CFTR may regulate the processes of EMT through activating uPA/uPAR system.

Human mammary epithelial cells are sensitized to ionizing radiation by knockdown of caveolin-1

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At present, cell response to ionizing radiation is attracting growing interests all over the world. Ionizing radiation could interfere with cell division of cancer and normal cells by introducing oxidative stress and injury to DNA. Caveolin-1 (Cav-1) is an integral transmembrane protein and a critical component in interactions of integrin receptors with cytoskeleton-associated and signaling molecules. Numerous cellular biology functions of Cav-1 have been investigated, including vesicular transport, cholesterol homeostasis, cell proliferation and differentiation, and signal transduction. Previous research suggested that ablation of Cav-1 gene expression induced an abnormal amplification of crypt stem cells, resulting in increased susceptibility to radiation. Cav-1 knockdown enhanced the radio-sensitivity of 3D human pancreatic cancer cell cultures. On the basis of these findings, we evaluated radiation-dependent expression of Cav-1 as well as the role of Cav-1 in the radiation survival and DNA damage response in a number of human mammary epithelial cell lines. Through si-RNA technology, we have obtained a cell line, named MCF10A-ST1-7SD8, which efficiently mediating silencing of Cav-1 expression compared with parental cell line MCF 10A. The colony formation assay showed that Cav-1 knowdown significantly enhanced the radiosensitivity of MCF10A-ST1-7SD8. Western blot technique was applied for detection of DNA-PKcs, ATM and Ku80. The results showed that DNA-PKcs expression was increased with the passage of repair time in the two cell lines. And there were no significant differences in ATM and Ku80 expressions. These data illustrated Cav-1 knowdown strongly affected the cellular radiosensitivity of human mammary epithelial cells not by DNA repair pathway, but by regulation of cell cycle.

Expression of vasohibin in placentae from pregnancies complicated by severe pre-eclampsia

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The aim of this study was to compare the expression of vasohibinin in placentae from pregnancies complicated by severe pre-eclampsia with that from normal pregnancies. Placenta tissues were obtained following delivery by caesarean section in 30 cases of pregnancy with severe pre-eclampsia and in 30 matched women with uncomplicated pregnancies. Protein expression of vasohibinin placenta tissue was quantified by Western blot analysis. Immuno-histochemistry was used to determine the localization of the protein. The mean value of vasohibinin in normal pregnant women was 0.91 $\pm$0.12, while 0.58 $\pm$ 0.09 in women with severe pre-eclampsia. These values were statistically different between the two groups ($P<0.05$). Vasohibinin is specifically expressed in the vascular endothelial cells in normal term villous placenta, and no positive staining of other cell types was found such as syncytiotro phoblast cells, cytotrophoblast cells, and chorionic villi interstitial cells. These results suggest that reduced vasohibin may be responsible, at least in part, for the impaired vascular development which occurs in the pre-eclampsia.

Involvement of TRPV2 in sexual excitation transduction in rat penile peripheral nerve fibers

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During sexual behavior, excitation is generated by intromission stimulation and then conducted to central nervous system. Previously, researches about sexual excitation focus on central nervous system and little is known about excitation in distal glans penis. Transient receptor potential vanilloid 2 (TRPV2) has been proposed to be activated by diversiform stimulus including mechanical stimuli. However, whether TRPV2 playing a role in transduction of sexual excitation is unknown. Here we show that TRPV2 participates in conduction of sexual excitation. In this study, we used RT-PCR, western blot and Immunohistochemistry to confirm that TPRV2 expressed in the peripheral nerve fibers of glans penis which originated from sacral vertebrae 2-4(S2-S4). We also demonstrated that the nonselective TRPV channel blocker, ruthenium red (RuR) could affect the sexual behavior in vivo experiments: increasing intromission frequency (IF); prolonging ejaculatory latency and decreasing intromission frequency per min (IF/min). Moreover, RuR and tranilast, an inhibitor of TRPV2 could significantly inhibit the neural potential of penile dorsal nerve (DNP) initiated by electrical stimulus (ES) of the glans penis. Simultaneously, 2-aminoethoxydiphenyl borate (2-APB) could enhance the neural potential initiated by ES. Together, these results suggest not only that TRPV2 may be involved, directly or indirectly, in transducing mechanical stimuli into electrical signals and mediating sexual excitation transduction, but also that TRPV2 may be a new curing target for sexual dysfunction.

Co-localization of CD147 and matrix metalloproteinases and its implication for spermatogenesis during mouse testis development

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Matrix metalloroteinases (MMPs) are the critical proteinase in the restructuring events during the testis development. CD147, as extracellular matrix metalloproteinase inducer (EMMPRIN), is known to stimulate the production of MMPs in tumor metastasis and its knockout mice an infertile, suggesting its possible involvement in spermatogenesis. We here showed that CD147, both detected by RT-PCR and western blot, was present at all stages of testicular development from day 7 to day 56. Interestedly, CD147 was increased dramatically after 21 days compared to 7 days and 14 days. Of eight MMPs studied, MMP-2, MMP-7, MMP-9 and MMP-23 were detected changes in expression, especially MMP-2 with dynamic change during testicular development. MMP-2, observed by immunofluorescence, was present around the spermatogonia cell and elongated spermtids. Furthermore, CD147 and MMP-2 were co-localized on the surface of spermatogonia. These data suggest that CD147 might regulate germ cell migration via induction of MMP production during spermatogenesis.

Inhibition of human sperm fertilizing capacity by adjudin

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Previous studies have shown that adjudin is a derivative of lonidamine and a potential male contraceptive. Adjudin exerts its effect by disrupting Steroli-germ cell adhesive and detaching spermatids from the seminiferous epithelial cells in the rat testis, thereby inducing their premature release into the tubular lumen leading to rat infertility. However, the exact function of adjudin on human sperm fertilizing ability has not been evaluated. The objective of the present study was to assess the effect of adjudin on human sperm fertilizing capacity and its possible mode of action. Semen samples were obtained from 25 fertile men at the Sperm Bank of Zhejiang Province according to WHO (1999) standard andrology criteria. The results showed that sperm hyperactivated motility and capacitation were promoted in the HTF medium for incubation up to 5 h. Conversely, adjudin significantly inhibited sperm hyperactivated motility, but not affect sperm motility. Adjudin inhibited capacitation is reversible. Adjudin prevented the acrosome reaction stimulated by rhuZP3β or progesterone in a dose-dependent manner (10 nm-10 μM). Also, adjudin blocked sperm penetration of zona-free hamster egg and intracellular cAMP production. The forskolin activates adenylyl cyclase (AC) activity in spermatozoa and increases intracellular cAMP production that was inhibited significantly by adjudin. Adjudin exerts its inhibition to sperm capacitation in the complete Cl^-- and Cl^--deficiency HTF media through blocks the conductance of Cl^- which may play a role. Taken together these results provide evidence that adjudin is a potent blocker of CFTR, mediated by disrupting Cl^- function leading to failure of sperm capacitation and male fertility.

Study of the best equilibrium time of vitrification in large pieces of ovarian cortex of sheep

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The purpose of the cryopreservation and transplantation of ovarian tissue is not only to improve the technology to obtain more survival follicles, but also to regain the function and restore the long-term maintenance transplanted ovary. Previous studies shown ovarian cryopreservation using small piece of cortex cannot maintain its long-term function. Therefore this study intended to improve the conditions of vitrification in large pieces of ovarian cortex, exploring the simplest, economic and effective ovarian tissue cryopreservation. The ovarian cortex from 2 to 6 month old sheep were divided into three different groups, small size group: 5mm $\times$ 4mm $\times$ 2mm (40mm^3); medium size group: 10mm $\times$ 4mm $\times$ 2mm (80mm^3) and large size group: 10mm $\times$ 8mm $\times$ 2mm (160 mm^3). The ovarian tissue were immersed in 1.5mol/L EG for 15 minutes first, then in vitrication solution for different time at room temperature. Finally, the ovarian tissue was plunge into liquid nitrogen immediately for storage. The effects of cryopreservation were assessed by microscopy and apoptosis-related immunohistochemical examination. Results showed that the three size of ovarian cortex were vitrificated after appropriate equilibrated. (1) Small size (40mm^3) group: The percentage of morphologically normal follicles at 7min group were similar to fresh control group (86.61 $\pm$ 5.37) / 88.30 $\pm$ 4.71), and higher than that of 5min; 6min and 8min group, $P<0.05$; Medium size (80mm^3) group: the percentage of normal follicles at 11min group were similar to that of in fresh control group (88.33$\pm$3.95/ 88.76$\pm$5.05); Large size (160mm^3) group: the percentage of normal follicles at 19 min group were similar to that of in fresh control group (88.33 $\pm$ 3.95 and 87.24$\pm$3.40). (2) The results of TUNEL annlysis: the rate of apoptosis of vitrification groups were increased, compared with the fresh control group, $P<0.05$. In conclusion, optimal equilibrium times have preserved larger pieces ovary tissue of sheep by vitrification.

CFTR is aberrantly expressed in the glandular epithelium of endometroisis

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Endometriosis is an estrogen-dependent inflammatory disease. The main clinical features are chronic pelvic pain and infertility. However, the mechanisms of infertility in endometriosis are still unclear. Recent studies have suggested that the dysfunction of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) contributes to various reproductive disorders or infertility. Here we investigated expression pattern of the CFTR in normal endometrium (n=3) and ectopic endometriotic (n=7) tissues of the patients who had never received any hormonal treatment before surgery in order to determine the potential correlations between CFTR and endometriosis. H&E staining revealed that the glands (destructive and non-destructive type) in the foci of endometrosis exhibited different size and shape with three distinctive differentiation state: well differentiated, poorly differentiated and fragmented glands. The consecutive and regular distribution of CFTR were detected both in the apical membrane of glandular epithelial cells of normal endometrium and well differentiated endometriotic glands by immunoflurescence. However, CFTR expression was irregular and fragmented in the poorly differentiated and fragmented glands of endometriotic tissue. The semiquantitative analysis of CFTR demonstrated that the average intensity of CFTR expression in the glandular epithelial cells of endometriotic tissue (Mean$\pm$SEM, 18.39$\pm$0.94) was significantly higher than that of the normal endometrium tissue (Mean$\pm$SEM, 14.48$\pm$0.80). These results suggest that CFTR might be involved in the pathogenesis of endometriosis. More extensive studies are required to understand the biological function of CFTR in this disease.

The possible role of ENaC in pathogenesis of preeclampsia and the underlying mechanism

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The pathology of preeclampsia includes the inhibition of trophoblast invasion, differentiation and reduced vascular remodeling. ENaC is a sodium channel widely expressed in a variety of epithelial cells and plays crucial roles in different physiological processes, including regulation of blood pressure. Recent studies suggest that ENaC is involved in tumor metastasis and invasion process. Since trophoblast layer invasion is similar to the biological behavior of tumor, and can be activated or reduced by protease. We hypothsized that ENaC is also involved in the process of trophoblast invasion and regulate angiogenesis; therefore, abnormal expressin of ENaC may be involved in the pathogenesis of preeclampsia. We detected the expression of ENaC in placenta of preeclampsia patients (n=12) and normal pregnancy control (n=12) by reverse transcription polymerase chain reaction. Immunohistochemical method was applied to study the localization of ENaC in the placenta. The expression of ENaC mRNA was significantly lower in women with preeclampsia than in normal pregnancy group (0.3158 $\pm$ 0.1136 vs 1.3151 $\pm$ 0.1218) ($p<0.01$). Immunohistochemical analysis revealed that ENaC was selectively present in trophoblastic cell in human placenta and was down-regulated in preeclampsia patients. We conclude that ENaC may play an important role in the pathogenesis of the preeclampsia.

Activated p38 MAPK in chronic nonbacterial prostatitis

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Chronic nonbacterial prostatitis is a poorly understanding syndrome of autoimmune etiology. To further clarify its pathogenesis, our study investigated the role of p38 MAPK, a crucial protein kinase, in chronic nonbacterial prostatitis. Eugamic masculine rats were castrated and subcutaneously injected with estradiol benzoate for 4 weeks to induce chronic nonbacterial prostatitis. Histomorphometric changes of the prostate tissue showed that the induced chronic nonbacterial prostatitis of rats was very similar to clinical cases in pathological characteristic. The expression of p38 MAPK was extremely increased in prostatitis. Meanwhile, elevated expression of TNF-α, MMP-9 and COX-2, which are post-transcriptionally regulated by p38 MAPK, was assessed with RT-PCR and Western-blot in prostate tissues. If intraperitoneal injection of phosphorylated SB203580, a specific inhibitor of p38 MAPK, was executed while chronic nonbacterial prostatitis was induced, the protein expression of TNF-α, MMP-9 and COX-2 was down-regulated. The expression of phosphorylated p38 was also reduced. After SB203580 treatment, mitigatory inflammation response in prostate was identified according to histological phenotype. In conclusion, p38 MAPK was involved in chronic nonbacterial prostatitis via regulating the expression of TNF-α, MMP-9 and COX-2. Obstruction of p38 MAPK maybe a potential therapeutic method of chronic nonbacterial prostatitis.

Heat shock protein 70 increases tyrosine phosphorylation by the purified CaM in the medium during mouse sperm capacitation

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Capacitation is an important physiological pre-requisite that enables sperm to undergo acrosome reaction and to fertilize the egg. Researchers have found that the protein phosphorylation especially at tyrosine residues is one of the most important events that occur during capacitation.In order to investigate the effect of CaM on protein tyrosine phosphorylation, cauda epididymal spermatozoa of mouse were incubated in EKRB medium supplemented with 3 mg/ml BSA in the absence or presence of 10 μM CaM. After 90 minutes at 37°C under 5% CO2 in air, sperm cells were pelleted, washed and extracted. Protein tyrosine phosphorylation was detected by Western blotting after SDS-PAGE and two dimension gel electrophoresis. After analysed by ImageMaster, MALDI-TOF MS was used to identify the 82 kDa proteins which increased tyrosine phosphorylation by the purified CaM in the capacitation medium. The results showed that inclusion of purified CaM in the capacitation medium significantly increased tyrosine phosphorylation of 82 kDa and 95 kDa components. Two PMF of proteins tyrosine phosphorylation were obtained by MS analysis. One of two proteins identified by MS is unknown. Heat shock protein 70 is one of tyrosine phosphorylation proteins which responds to the CaM during mouse sperm capacitation.

Expression and effect of proto-oncogene c-erbB2 during the initiation of primordial follicle growth

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In this study, the expression and effect of c-erbB2 were investigated during the initiation of primordial follicle growth in rats. Ovaries from 2-day-old Sprague-Dawley rats were isolated and randomly divided into five groups (n=20, ovaries/group): ovaries of the control (ovaries weren't cultured), cultured for 4 d, cultured for 8 d, cultured for 4 d supplemented with 50ng/ml epidermal growth factor (EGF) and cultured for 8 d supplemented with 50ng/ml EGF, respectively. In-situ hybridization, immunohistochemistry, RT-PCR and western blot were performed to assess the expressions of c-erbB2 mRNA and protein, proliferating cell nuclear antigen (PCNA) and p-extracellular signal-regulated kinase1/2 (p-ERK1/2) during the initiation of primordial follicle growth. The results showed that c-erbB2 mRNA expressed in oocyte endochylema and ErbB2 protein expressed in oocyte membrane. In addition, the expressions of c-erbB2 mRNA were significantly increased in 8 d culture group ($P<0.05$) as well as groups supplemented with EGF for 4 d ($P<0.05$) or 8 d ($P<0.01$) compared to the control group respectively, and the expressions of c-erbB2 mRNA in groups cultured with EGF were significantly higher than those in groups cultured without EGF, respectively ($P<0.05$). Western blot analysis showed that culture for 4 and 8 d or supplement with 50ng/ml EGF induced a significant increase of PCNA and p-ERK1/2 protein levels compared to the control group, respectively ($P<0.01$), and the protein levels of PCNA and p-ERK1/2 in the groups cultured with EGF were significantly higher than those in the groups cultured without EGF, respectively ($P<0.05$). Our results suggest that proto-oncogene c-erbB2 could promote the initiation of primordial follicle growth through effect of EGF involving enhancement of ERK-MAPK signal transduction. This study was supported by National Natural Science Foundation of China (No.30660053).

Apoptosis during the development of ovarian follicle

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Apoptosis, also named programmed cell death, is a necessary process in a living organism, involving in maintaining proper development and eliminating damaged or excess cells. This physiological process is apparent during proper development for tissues to eliminate unwanted cells. Folliculogenesis is a complex process involving dramatic morphological and functional changes in granulosa and theca cells. This process is sequential and dictated by specifically, tightly regulated response to endocrine hormones and intra-ovarian regulators. In mammalian ovaries, only a few number of presented follicles in a fetal ovary can reach ovulatory status during follicular development, more than 99% of follicles undergo a degenerative process known as ''atresia'' induced by cell apotosis. It is characterized by distinct biochemical and morphological changes such as DNA fragmentation, plasma membrane blebbing and cell volume shrinkage. Apoptotic process follows a particular pattern during different phases: in fetal ovaries, most of apoptotic activity was detected in germ cells; while in adult quiescent cortical follicles apoptosis was occurred originating from both oocyte and granulosa cells. It has been demonstrated that it is the oocyte which initiates the apoptotic process and induces the follicular atresia. The process always begins from the oocyte and then extends to the surrounding follicular cells leading to the growing follicle atresia. Thus, it seems that the apoptotic signals can communicate from a single cell to all over the other cell types inside the follicle. Finally, the whole follicular structure has become atretic, while the surrounding stromal cells remain viable. Apoptosis in ovary is regulated by a number of endocrine, locally produced intracellular mediators in a stage-specific and time-dependent manner. New knowledge of hormones and cell factors which regulate granulosa cell or oocyte apoptosis and their possible signaling pathways underlying intracellular events has made important contributions in advancing our understanding mechanism of follicular atresia.

Cdh1, the anaphase promoting complex or cyclosome (APC/C) cofactor, inhibits trophoblast cell proliferation and invasion

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Well controlled trophoblast invasion into uterine decidua is a pivotal event during normal pregnancy, which is tightly regulated by various factors produced in the maternal-fetal interface. Cdh1 (Fzr1 genes coding) is involved in tumor suppression and genomic stability of trophoblast cell. However, little is known about the expression and the role of Cdh1 on the maternal–fetal relationship. In this study, we found that Cdh1 level of placental villi was significantly higher in the first trimester compared to in third trimester. Immunostaining revealed that Cdh1 proteins were strongly expressed in decidualized stromal cells and moderately in villous cytotrophoblasts and syncytiotrophoblasts, but absent in the trophoblast cell lines (JEG-3 and HTR-8/SVneo cell). Functional assays with Fzr1 stable transfected HTR-8/SVneo cells revealed that Fzr1 expression significantly inhibited the proliferation by MTS assays, and restricted invasion of HTR-8/SVneo cells by transwell assays compared to the control. Together, our data suggest that Cdh1 protein was temporally and spatially expressed at the human maternal-fetal interface, and may regulate embryo implantation by inhibiting trophoblast cell proliferation and invasion.

Lectin histochemical studies on distribution and maturation of mouse uterine natural killer cell during periimplantation

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Uterine natural killer (uNK) cells have been shown to play a vital role in reconstructing uterine spiral arteriole and subsequent placental development. In this study, we aimed to investigate the distribution and regulation of uterine NK cells in mouse uterus during periimplantation. Using a unique uNK cell marker, Dolichos biflorus agglutinin (DBA) lectin, the expression and distribution of uNK cell were detected in uterus of normal pregnancy, delayed implantation and artificially induced decidualization. The results showed that DBA staining was mainly distributed in some vascular endothelial cells of mouse uterine stroma and myometrium during embryo preimplantation. In the pregnancy of D5, uNK cells were localized in the stroma of uterine mesometrial pole, with round, immature and small lymphocyte-like cells. During D7 and D8 of pregnancy, the immunostaining results showed that uNK cells were increasingly accumulated in the mesometrial pole of implantation sites, with their morphology changing from small round cells with scattered cytoplasm to large and mature cells with heavy cytoplasmic granules. Little DBA staining signal was exhibited in the uterine stroma in delayed implantation. After delayed implantation was terminated by estrogen treatment and embryo implantation was initiated, DBA staining signal was strongly detected in the stroma of uterine mesometrial pole. However, in the artificially induced decidualization model, the distribution and maturity of NK cells are similar to those of the normal pregnancy in the mouse uteri. In conclusion, our data suggest that the distribution and maturation of uterine NK cells are dependent on uterine decidualization in mouse early pregnancy. Supported by the National Natural Science Foundation of China (No 30760071, 30960118).

Anti-cyclin D1 intrabody inhibits the growth and proliferation of cervical cancer cells

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Cancer of the cervix is one of the most frequent malignancies and is the second most prevalent cancer in women worldwide. The expression of CyclinD1 increases distinctly during the progression from the normal epithelium to preneoplastic lesion and to invasive cervical cancer. So cyclin D1 may become a potential target for cervical cancer therapy. This study is designed to block and inhibit cyclin D1 by using intrabody technology in order to inhibit the growth and proliferation of cervical cancer cells. Using the anti-cyclinD1 scFv as template, nuclear localization signal (NLS) peptides and E-tag was introduced into anti-cyclinD1 intrabody gene (AD5N) by PCR. Then the intrabody gene (AD5N) was subcloned into pcDNA3.1 to construct the recombinant expression vector pAD5N. The control plasmids (pcDNA3.1) and recombinant plasmids with intrabody (pAD5N) were stably transfected into HeLa cells. Then G418 selection was performed to obtain the positive cell clones HeLa/pcDNA3.1 or Hela/pAD5N. Western blot, Immunoprecipitation, MTT assay and fluorescence activated cell sorting was conducted to investigate the expression and anti-tumor effect of intrabody in HeLa cells. The results showed that AD5N was expressed efficiently and bind to Cyclin D1 of HeLa cells. AD5N inhibited significantly the proliferation, arrested distinctly the cell cycle, and induced the apoptosis of HeLa cells compared with the control ($P<0.01$). The ratio of G0-G1 phase cells increased by 10.35%, S phase cells decreased by 15.77%, and the apoptosis index increased by 6.38%. The above results showed that the anti-cyclinD1 intrabody (AD5N) significantly inhibited the growth and proliferation and distinctly induced apoptosis of HeLa cells. This study suggested that anti-cyclinD1 intrabody might be further used in cervical cancer gene therapy. This study is supported by the National Natural Science Foundation of China (30200256, 30972806) and the S&T Development Planning Program of Jilin Province (20090727).

Lentivirus-mediated RNA interference reveals that a testis-specific gene, *LM23,* is essential for spermatogenesis in *Rattus norvegicus*

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LM23 is a gene with testis-specific expression in *Rattus norvegicus* previously reported by our laboratory. To reveal the function of *LM23* in the testis, we used lentivirus-mediated RNA interference (RNAi) to knock down *LM23* expression in a tissue-specific manner *in vivo*. In our approach, a lentiviral vector expressing a short hairpin RNA targeting *LM23* was microinjected into the efferent ducts of *R. norvegicus* testes. The expression of *LM23* in the treated testes was significantly knocked down compared with controls. These LM23-shRNA testes contained germ cells arrested at the spermatocyte stage, and suffered from increased apoptosis and dyregulation of some meiotic genes. The results demonstrate the validity of the RNAi approach and reveal that *LM23* expression in the testis is crucial for meiosis during spermatogenesis in *R. norvegicus*. This work was supported by the National Natural Science Foundation of China (No. 30670784).

CD1d-independent NKT cells and feto-maternal immune tolerance

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To study the effects of NKT cell on the Th1/Th2 of immune balance in the endometrium during the period of embryo implanting maternal uterus. The experimental groups were determined according to mouse on the 4d of pregnancy for intraperitoneal injection of the antigen α-GalCer with the concentration of 2.4 nmol, 4.8 nmol and 9.6 nmol in saline. The ficoll was used to separate the peripheral mononuclear cells (PMBC) and then the expression of IL-4, γ-IFN in PMBC and uterine decidua was measured by RT-PCR. Results: the levels of γ-IFN mRNA in PMBC and uterine endometrium of the 2.4 nmol, 4.8 nmol and 9.6 nmol groups were decreased significantly ($P<0.05$); the levels of IL-4 mRNA in PMBC of the 2.4nmol, 4.8nmol, 9.6nmol groups were decreased significantly ($P<0.05$), but the levels of IL-4mRNA in uterine endometrium of the 4.8nmol group were increased significantly ($P<0.05$); in PMBC the relative optical density of γ-IFN/IL-4 mRNA of the 4.8nmol group was increased significantly ($P<0.05$); On the contrary the relative optical density of γ-IFN/IL-4 mRNA of the 4.8nmol group in uterine endometrium was decreased significantly ($P<0.05$). Conclusions: In the window of implantation the active NKT cells produced high level of IL-4 and promoted the humoral immune response in uterine mucosa. In PMBC the active NKT cells regulated the Th1/Th2 of immune balance towards the Th1 immune response, but in the uterine endometrium they adjusted the Th1/Th2 of immune balance towards the Th2 immune response and maintained the formation of feto-maternal immune tolerance in uterine endometrium. This work was supported by the Educational Department of Jiangxi Province (No. GJJ09111 and No. GJJ09444).

Germ cell apoptosis and proliferation during male mice temporary infertility after heat shock treatment

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The apoptosis of differentiated germ cell after heat shock treatment led to short-term infertility in male mice, while the survival of spermatogonial stem cells does not seem to be affected. The spermatogonial stem cells after 1-2 spermatogenic cycle of self-renewing and differentiation generate a large number of sperms which makes the resumption of fertility. In order to further clarify the destiny of testicular germ cells remained in heat shok-treated mice during short-term infertility; this experiment systematically studied the germ cell apoptosis and proliferation after heat shock treatment. The results show that: The apoptotic round spermatids and spermatocytes accumulated 2 hours after heat shock treatment. Eight hours after heat shock treatment, the apoptosis round germ cells reached the greatest degree. Eighteen days after heat shock treatment, germ cell apoptosis in heat shock treated-group returned to normal levels as same as that in the control group. The survival of spermatogonial stem cells does not seem to be conspicuously affected. After heat shock treatment, the proliferation of spermatogonia has been inhibited. The expression levels of stem cell proliferation promoting regulatory factor GDNF significantly ($P<0.05$) down-regulated from 4 h to 15 days after shock treatment; Stem cell differentiation regulatory factor SCF expression levels was also significantly ($P<0.05$) down-regulated from 4 h to 10 days after heat shock treatment. In short, at the stage in which mouse short-term infertility appeared after heat shock treatment, round spermatids and spermatocytes produced apoptosis; Spermatogonia proliferation was inhibited, this may contributed to the down-regulated expression of stem cell self-renewing factor GDNF and differentiation factor SCF.

CFTR protein expression in healthy men spermatozoa is not correlated with fertilization rate of ova

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Our previous studies have shown that the cystic fibrosis transmembrane conductance regulator (CFTR) is important for capacitation and male fertility in mouse, guinea pig and human spermatozoa. However, it is unclear whether sperm CFTR expression rate of healthy men is correlated with the ova fertilization rate. To explore this question, CFTR protein expression rate in healthy men spermatozoa related to fertilization rate of ova was investigated. 93 infertility couples using IVF were investigated. Immunofluorescence staining was used to assess sperm CFTR expression rate of male partners. According to the Ministry of Health Decreed Law in 2003 on the human assisted reproductive technology and specifications, oocyte fertilization rate was calculated. All the patients were divided into three groups based on the fertilization rate of ova in vitro. A group of 30 cases, fertilization rate $>60\%$; B group of 32 cases, fertilization rate between 30% and 60%, C Group of 30 cases, fertilization rate $<30\%$. It was performed by measure the expression rate of CFTR in each group and explored the relevance between the expression rate of sperm CFTR and the fertilization rate of ova. The CFTR expression rates of spermatozoa from healthy men in three groups are from $30.7\pm20\%$ to $36.2\pm25\%$ ($P>0.05$), and the relevance between spermatozoa CFTR expression rates and the fertilization rates of ova in 93 patients are no statistical significance ($P>0.05$). Although the fertilization rate of ova from 30% to 76% is significantly different, the rates of CFTR protein expression in healthy men spermatozoa are no significant different. It is probably correlated with ova quality which further needs research. These results further suggest that the fertilization rate may be correlated with ova quality whereas the expression rate of the CFTR in healthy men spermatozoa is not correlated with the fertilization rate of ova.

Study on the optimum condition of three freezing methods in preserving fetal ovary tissue

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This study in tends to explore the most simple and effective ovary tissue cryopreservation through comparing the three methods: program freezing method (PFG), rapid freezing method (RFG) and vitrification (VG). Xenografting under the capsule of the kidney was performed and the ratio of oestrum recovery, the beginning days of recovery of oestrous cycle, the level of serum FSH as well as the morphologic structure of survival ovaries were examined to assess the growth of follicles in survival ovaries, so as to compare the effect of different freezing methods. In terms of estrous cyclicity recovery rate as well as the days of estrous cyclicity opening, there was no significant difference between each freezing group and the fresh transplanting group (FTG)($P>0.05$). 14 weeks after xenografted, value of serum FSH in each experimental group was pretty much the same with that in normal control group. There was also no difference among each experimental group in terms of serum FSH value which was found lower than that in alter control group. No significant difference was indicated among each transplanted group in grafts' survival rate ($P>0.05$). RFG showed significant difference with either VG or FTG with its number of follicles in grafts lesser than that of the latter, but it showed no significant difference with PFG in the same regard. 18 weeks after being xenografted, antrofollicular development was observed in each experimental group except in FTG. The follicles at developmental phases in VG were much more than those in either FTG or RFG ($P<0.05$). The three methods can all effectively preserve fetal ovary tissue and vitrification is the most effective ovary tissue cryopreservation. The combination of freezing method and transplanting method can effectively improve ovary cortex' survival and development potential when being cryopreserved and grafted.

Aerolysin as a probe for GPI-anchored proteins on spermatozoon

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We introduced aerolysin, a bacteriotoxin which can recognize GPI-anchored proteins (GPI-APs) specially, as a bioprobe to identify the GPI-APs on spermatozoa. High-purity aerolysin and high specific anti-aerolysin polyclonal antibody were prepared by our Lab. The lipid raft where GPI-APs are enriched on spermatozoa was extracted by TX-114. Sandwich western-blotting showed that there were 12 protein bands recognized by aerolysin which were ranged between 15~130 kD. Fluorescence-immunocytochemistry of spermatozoa with FLAER, Alex488 labeled aerolysin, showed that the proteins recognized by FlAER distributed around the head and tail of spermatozoa. Primary Results suggest that aerolysin recognizes GPI-APs on spermatozoa effectively and there are several GPI-APs on the surface of spermatozoa. Further identification of GPI-APs on spermatozoa is worth investigating.

Differential expression library of human endometrium during the window of implantation

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The uterine endometrium is receptive to the embryo for only a short period during the menstrual cycle. Using a suppression subtractive hybridization (SSH) approach, we studied the profile of specific genes expressed during the implantation window in the human endometrium. Forward and reverse subtractive cDNA libraries were constructed and 192 and 162 positive clones were identified, respectively. The differentially expressed genes were assigned to several groups based on their functions. Most of the known genes function in transcription, cytoskeleton, and signal transduction. Some genes were expressed or up-regulated and some were suppressed or down-regulated. *In situ* hybridization experiments showed that NID-1 expression was restricted to stromal cells during the mid-secretory phase. This present study provides the basis for finding new potential genes that are involved in the window of implantation.

Effect of follicle stimulating hormone on fetal ovarian tissue vitrifiation

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Vitrified fetal ovary tissue can be a suitable source for ovary xenogenic transplantation. In this study, we explored whether the using of follicle stimulating hormone (FSH) during vitrifiation could protect primordial follicles or initiate primordial follicle development during cultivation. The fresh ovarian cortex of a 20 week fetus was cutted into fragments of two different size: 5mm × 4mm × 2mm and 10mm × 4mm × 2mm, and cryopreserved in the cryopreservation solution with 10 μg/ml FSH. After thawing, the ovary fragments cultured in the medium with 10 μg/ml FSH. We detected the follicle diameter and follicle constituent ratio of thawed cultured ovary fragments and compared with the fresh ovary fragments. The result showed that follicle constituent ratio and follicle diameter of fresh ovary fragments was similar with vitrified ovary fragment, but differed from cultured ovary fragment for 4.5 hours after thawing. After 4.5 hours culture with 10 μg/ml FSH, primary follicle start development. These results suggest that FSH addition did not make primordial follicles well loss during vitrifiation, and could start primordial follicle development during following *in vitro* culture.

The effects of mouse hemi-ovaries transplantation pretreated with HMG

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The cryopreservation and transplantation of ovarian tissue is a way of conservation and recovery of the fertility of the young adults with cancer. However, the ischemic hypoxia and ischemic-reperfusion injury after the operation often causes great loss of primordial follicles, which results in low survival rate and worse function and shorter life span of the transplanted ovary. The purpose of this study is to explore the effect of the revascularization and followed follicular development after ovarian tissues transplantation in mice pretreated with HMG. The hemi-ovaries were autotransplantated under kidney capsule after cultured for 3h in the medium without HMG (control group) or with 0.15 IU/ml HMG (treated group). The conditions of the vascular anastomosis of the ovarian cortex were checked by Chinese ink perfusion after transplanted for 24h and 36h. The VEGF expression in ovarian tissue was observed by immunohistochemistry after cultured for 12h, 24h and 48h. The total number of survival follicles was counted after transplanted for 1 month in each group. The blood supply can be seen between the grafts and recipients by the way of ink-perfusion after transplanted for 36h in treated group, but not in the control group, it was observed only after transplantated for 48h. The VEGF positive cells were more than control group in the treated group. The total number of survival follicles was evidently higher in treated group compared with control group after transplanted for 1 month, $P<0.05$. The higher expression of VEGF could be induced by HMG in vitro and during transplantation, which might promote the revascularization and the permeability to macromolecules which were nutrients for the grafts. The results showed that pretreated with HMG *in vitro* is beneficial for a larger pieces of ovarian tissue transplantation.

GABA-A receptor δ subunit is expressed in sperm and important for progesterone-induced AR reaction

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GABA is a major inhibitory neurotransmitter in central nervous system (CNS) and it functions mainly through GABA receptor. There are two major classes of GABA receptor: $GABA_A$ and $GABA_B$. Previous studies have shown that GABA-AR may functional not only in CNS, but also in peripheral nonneuronal tissues, including reproductive tissues. However, the exact mechanism is still not clear. Using RT-PCR, quantitative real time PCR, Western blot, immunostaining and functional study including CTC staining, calcium imaging, we investigated the expression, localization and the function of GABA-A receptor subunits in sperm and their possible interaction with GABARAP, a protein interacted with $\gamma 2$ subunit containing GABA-A receptor and required for trafficking of GABA-A receptor. Using GABA subunit specific peptide and calcium imaging, our study indicated that δ subunit is involved in calcium increase in progesterone induced sperm activation. While $\gamma 2$ subunit seems not essential for sperm activation in our experimental conditions. Sperm form δ-subunit KO mice has attenuated calcium response to progesterone, further suggesting that δsubunit containing GABA-A receptor maybe important for progesterone induced calcium increase and AR reaction in physiological condition.

Cyclooxygenase: an important factor playing role in shedding and bleeding of endometrium in mice menstrual-like model

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Endometrium undergoes shedding and bleeding under the precise control of hormone. Vascular changes and inflammation are the key characters in the process. Prostaglandins (PGs) are considered to be proinflammatory, vascular permeability and play important roles during the female reproduction. Cyclooxygenase (COX) is the rate-limiting enzyme that produces PGs from arachidonic acid. In mice menstrual-like model, we used real time-PCR, Western, immunohischemistry, vaginal smears and cyclooxygenase inhibitor to verify the function of cyclooxygenase in breakdown and bleeding of endometrium. The mRNA expression of COX-1 and COX-2 were reduced and increased respectively from 0 h to 24 h which is the phage of breakdown and bleeding of endometrium in mice and the protein expression of them showed sharp increase. Location of their protein was shifted from close to luminal epithelium to outer sub-decidual zone where the endometrium will shed. Thus, COX maybe involved in the broken and bleeding of endometrium. Then, we used cyclooxygenase inhibitor indomethasin to inhibit the activity of cyclooxygenase in the mice model, bleeding was reduced and breakdown was partially prevented comparing to control treatment. Meanwhile, vascular permeability was decreased and the number of leucocyte sharply reduced. Our results show cyclooxygenase plays an important role in vascular permeability and inflammatory in shedding and bleeding of endometrium in mice menstrual-like model.

Effect of the alpha-lipoic acid on apoptosis in HIT-T15 cells induced by high glucose

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High glucose plays an important role in the pathogenesis of diabetes. Alpha-lipoic acid (LA) has been used to prevent and treat diabetes, mainly through increasing insulin sensitivity in many tissues. In this study, we assessed whether LA could inhibit high glucose-induced apoptosis in islet beta cells. HIT-T15 was treated with high glucose (35mmol/L) in the presence or absence of 0.5mmol/L alpha-lipoic acid from 1day to 8 day. LA significantly reduced the amount of apoptotic HIT-T15 cells as well as inhibited cell growth induced by high glucose. LA can also efficiently reverse the elevated gene expression of Bax induced by high glucose and increase Bcl2 expression. It protects against high glucose-induced apoptosis in HIT-T15 cells involved in two mechanisms. LA can not only slightly attenuate ROS production and augment mitochondrial membrane potential ($\Delta\Psi_m$) by its antioxidant activity. Most importantly, LA can significantly promote the expression of Pdx1 (a master regulator of beta cells cytoprotection) and phosphoralated Akt (one of critical mediators of survival). Additionally, LA cannot improve the insulin secretion on HIT-T15 cells with high glucose treatment. Our data suggest that alpha-Lipoic acid can effectively attenuate high glucose-induced islet beta cell apoptosis not only by its antioxidant activity but also by activating cell survival factors. These findings provide new interpretation on the role of alpha-lipoic acid in the treatment of diabetes.

Ultrastructure of a special tail stump sperm defect in male infertile offspring of Chinese consanguineous marriage.

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The effect of consanguinity on semen parameters were observed in 37 male first-offspring of consanguineous marriages in the infertility clinic of the University Hospital. Routine analysis of semen samples from 37 infertile male of consanguineous marriage was conducted. Transmission electronic microscope (TEM) was used to detect the sperm malformation characteristics with a special short-tailed form of sperm at the ultrastructural level. Most patients present severely poor semen quality and some special phenotypes in spermatogenesis and development were observed. In semen analysis, 89.2% presented poor semen quality and were much higher than those happened in the general subfertile population. Oligozoospermia constituted about 51.4%, while only one patient showed azoospermia, and 62.2% of the patients were severe asthenozoospermia. 29.7% of patients with asthenozoospermia presented immobile sperm (i.e. 0% motility) which was characterized by predominant short-tail sperm in 54.5% of the semen samples. Under TEM observation on the short tail sperm, no structural abnormalities in the sperm head were found, while a variety of structural defects in the tail was obvious, which include: thinner diameter of middle piece than that of principal piece without mitochondrial helix, or limited scattered mitochondria, or mitochondria overlap arrangement; peripheral fiber defects or scattering fibers in the principal-piece region of the tail, a variety of microtube absence among individuals, such as ''9+1'' or ''8+1''. Our results suggest that poor semen quality and those sperm characteristics in a high degree of consanguineous offspring are most likely due to a genetic origin and one or more gene defects may cause the abnormal development of the sperm tail structure.

Protective and proliferating effects of ginsenosides on cultured mouse spermatogonia through antioxidant action

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Development of spermatogonia is influenced by many internal and external factors. Using a germ-somatic cell co-culture model, we evaluated the effects of ginsenosides (GS) on proliferation and oxidative damage of type A spermatogonia. Type A spermatogonia were isolated from 7-day-old mice and characterized by c-kit immunocytochemical staining. Spermatogonia were challenged with GS alone or in combination with a reactive oxygen substance (ROS)-producing system. Results showed that ROS inhibited spermatogonial proliferation and induced an elevation in thiobarbituric acid reactive substances but a decrease in superoxide dismutase activity and glutathione content. However, the oxidative damage induced by ROS was attenuated by simultaneous supplementation with GS. In addition, GS significantly stimulated proliferation of spermatogonia, but the proliferating effect was suppressed by combined treatment of PKC inhibitor H7. These results indicate that GS may restore the intracellular antioxidant system to attenuate the oxidative damage in mouse spermatogonia and promote spermatogonial proliferation *via* antioxidant action involving the PKC signal pathway.

Functional characterization of a novel testis-specific Rho GTPase in spermatogenesis

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Mammalian spermatogenesis is a tightly regulated developmental process. Exploring the testis-specific genes has expanded our knowledge of the mechanism of spermatogenesis. We identified a new testis-specific gene and characterized its coding product, RSA-14-44 as a novel member of Rho GTPases subfamily in terms of its structural and enzymatic features. Distinct from the canonical Rho GTPases, RSA-14-44 associates with PSMB5, a catalytic subunit of proteasome in various developmental stages of spermatogenesis. Our results demonstrate that instead of the mature PSMB5 finally assembled in proteasomes, RSA-14-44 associates with the precursor form of PSMB5, and accordingly, we found less significant variation in the cellular proteasome activity with the overexpression of RSA-14-44. However, overexpressed RSA-14-44 down-regulates the stability of the PSMB5 precursor, which requires maintaining the association between these two proteins. These results provide the first line of evidence for the functional links between Rho GTPases and the core subunits of proteasome, thus proposing some new clues for deciphering the secrets of mammalian spermtaognesis.

***c-erbB*$_2$ and *c-myb* induce oocyte maturation *via* activation of maturation promoting factor (MPF)**

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In this experiment, we investigated the effects of protooncogenes *c-erbB*$_2$ and *c-myb* on oocyte maturation and the upstream or downstream relationship with MPF by using RT-PCR, western blot analysis and recombinant proto-oncogene protein microinjection. Our results showed both *c-erbB*$_2$ and *c-myb* antisense oligodeoxynucleotide (*c-erbB*$_2$ASODN and *c-myb* ASODN) inhibited mouse oocytes germinal vesicle breakdown (GVBD) and the first polar (PB1) extrusion in a dose-dependent manner, and delayed maturation process. When recombinant *c-erbB*$_2$ or *c-myb* protein was microinjected into cytoplasm of GV stage oocytes, the GVBD rate after cultured for 6hrs was increased by 23.1% ($P<0.05$) and 32.2% ($P<0.05$) respectively, and the PB1 extrusion rate after cultured for 12 hrs was increased by 17.3% ($P<0.05$) and 23.5% ($P<0.05$) respectively. RT-PCR result showed the expression of *c-erbB*$_2$ and *c-myb* were detected in oocytes, *c-erbB*$_2$ASODN inhibited *c-erbB*$_2$mRNA and *c-myb*mRNA expression; *c-myb*ASODN inhibited *c-myb*mRNA expression but did not show effect on *c-erbB*$_2$mRNA expression. Roscovitine did not change the expression level of *c-erbB*$_2$mRNA and *c-myb*mRNA though it inhibited oocyte maturation significantly, but blocked the effects of recombinant *c-erbB*$_2$ and *c-myb* protein-induced oocyte maturation. Furthermore, cyclinB1 expression was inhibited remarkably when oocytes were treated with *c-erbB*$_2$ASODN, *c-myb*ASODN and Roscovitine. Nonsense *tat* ODN had no significant effect on the expression of *c-erbB*$_2$mRNA, *c-myb*mRNA and cyclinB1. These results suggest that *c-erbB*$_2$ and *c-myb* play an important role in oocyte maturation, the effects of *c-erbB*$_2$ and *c-myb* might depend upon the action of MPF, it activiations are the event that occurs downstream of *c-erbB*$_2$ and *c-myb* in maturation signal pathway.
This study was supported by the National Natural Science Foundation of China (No. 30260035).

Khat affects apoptosis and related gene XIAP and Smac expression following transient focal ischemia in rats

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Khat chewing can cause stroke but the underlying mechanism is not fully understood. The aim of this study is to explore the regulation of Khat treatment on the expression and its cellular localization of Smac/Diablo (second mitochondrial activator of caspase) and XIAP (X chromosome-linked inhibitor of apoptosis proteins) in the model of ischemia/reperfusion injury of the brain. Adult male Sprague-Dawley rats were given Khat for one month, then the ratswere operated by right middle cerebral artery occlusion (MCAo) for 2 h and reperfusied for 1, 3, 6, 12 and 24 h. Their behaviour was estimated with neurological score. Apoptosis was assessed by terminal deoxynucleotidyltransferase- mediated dUTP-biotin nick end labeling (TUNEL). The expression of Smac/DIABLO and XIAP in the left cerebral ischemia of rats were examined by methods of immunohistochemistry and Western blot. The mRNA levels for Smac/DIABLO and XIAP were evaluated by reverse transcriptase PCR (RT-PCR). The results showed that Khat significantly decreased the neurological outcome compared with control group ($p<0.05$). In addition, Khat-treatment significantly increased the number of TUNEL-positive cells ($p<0.01$), reduced expression level of XIAP immunoreactivity and decreased mRNA level of XIAP within the peri-infarct area. However, the expression level of mRNA and protein of Smac/DIABLO increased significantly at 1~3 h after reperfusion, peaked at 12h, then declined markedly at 24 h but they all remained at higher levels in the Khat treatment group than the controls. Our findings suggest that Khat treatment can induce apoptosis through depressing the expression of the XIAP but not the Smac genes after transient focal ischemia. Therefore, Khat chewing should be avoided in persons who have any cerebrovascular problems.

The function and related mechanism of leptin in neuropathic pain

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Leptin, the product of the obese (ob) gene, is known to function as an immunomodulatory regulator. ATP receptor ($P2X_{2/3}$) was expressed in dorsal root ganglion (DRG). The role and related mechanism of leptin in neuropathic pain were investigated in this study. Chronic constriction injury (CCI) rats were used as the animal model of neuropathic pain. Sprague-Dawley male rats were randomly divided into 5 groups: sham group, CCI+PBS group, CCI+leptin group (10μg/kg, 50μg/kg and 200μg/kg). On day 7 post-operation, leptin was administrated by intrathecal injection in CCI+ leptin group once daily for 7 days. Mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL) were measured at pro-operation, pro-injection and 1h, 2h, 12h, 24h post-injection on day 7 post-operation. The expressions of $P2X_{2/3}$ and TNFα protein and mRNA on L4/L5 DRG neurons were detected by immunohistochemistry and RT-PCR. MWT in CCI+ leptin (200μg/kg) group was obviously increased compared with that in sham group at 1h, 2h,12h, 24h post-injection ($P<0.01$) while the data in CCI+PBS group was obvously decreased. TWL in CCI+ leptin (50μg/kg) group and CCI+leptin (200μg/kg) group were obviously increased compared with those in sham group at 1h, 2h and 12h post-injection ($P<0.01$). On the day 14 after operation, the expressions of $P2X_{2/3}$ and TNFα protein and mRNA on L4/L5 DRG neurons in CCI +PBS group were sharply increased compared with those in sham group. The expressions in leptin-treated groups were lower than those in CCI +PBS group ($P<0.01$) but still higher than in sham group ($P<0.05$). Leptin reduced the hyperalgesia in CCI rats. And the expressions of $P2X_{2/3}$ and TNFα receptors on L4/L5 DRG of CCI rats were decreased by leptin. Leptin may reduce the nocicetive transmission of neuropathic pain mediated by $P2X_{2/3}$ and TNFα receptors resulting in an effective action in the managemeant of neuropathic pain. This work was supported by the grant (№s 30800424, 30860086, 30860333 and 30660048) from National Natural Science Foundation of China.

The involvement of spinal P2X7 receptor in the mirror-image pain induced by tetanic stimulation of the sciatic nerve

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Mirror-image pain refers to the pain sensation from healthy body region contralateral to the actual site of trauma or inflammation. Previous work reported that glia and proinflammatory cytokines were key mediators in the creation of mirror-image pain. Given that P2X7 receptor (P2X7R) was expressed predominantly in glia and played a key role in the maturation and release of cytokines, it may be an important signal molecule contributing to the induction of mirror-image pain. The present study investigated the role of P2X7R in the mirror-image pain induced by tetanic stimulation of the sciatic nerve (TSS). TSS produced contralateral mechanical allodynia, so-called mirror-image pain. Intrathecal administration of a P2X7R antagonist oxidized ATP (oxATP) partially alleviated contralateral nociceptive allodynia, indicating the implication of P2X7R in mirror-image pain. Immunofluorescence staining revealed that TSS induced the activation of ipsilateral microglia from day 3 and bilateral astrocytes from day 7 after tetanic stimulation in the spinal dorsal horn. The activation was suppressed by pre-injection of oxATP 30 min before tetanic stimulation. Double immunofluorescence showed colocalization of P2X7R with microglial marker OX-42, but not with astrocytic marker glial fibrillary acidic protein (GFAP) or neuronal marker neuronal nuclei (NeuN). These results suggest that P2X7R in microglia plays a pivotal role in mirror-image pain induced by TSS and the process may be achieved by mediating the activation of ipsilateral microglia and the subsequent bilateral astrocytes in the spinal dorsal horn.

Role of $P2X_{2/3}$ receptors on nociceptive transmission of trigeminal neuralgia

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Extracellular ATP can play a key role on nociceptive transmission by activating $P2X_{2/3}$ receptors. This study intends to investigate the role of $P2X_{2/3}$ receptor in nociceptive transmission of trigeminal neuralgia *via* trigeminal ganglion (TG). Twenty SD rats were divided into two groups randomly, 10 rats in each group. One was trigeminal neuralgia (TN) group, the other was sham group. In the TN group, right unilateral chronic constriction injury (CCI) of rat was produced by placing loose chromic gut ligature around the infraorbital nerve (ION). In the sham-operative group, the ION was only exposed using the same procedure, but not ligated. Mechanical response thresholds were observed before operation and on day 3, 5, 7, 9, 11, 13, 15 after operation. TGs of rats in both groups were taken to measure the expression of $P2X_{2/3}$ receptor by immunohistochemisty, *in situ* hybridization and RT-PCR. After operation, allodynia in response to mechanical stimulation of the territory of the ligated nerve on post-operative day 9-15 in the TN group was higher than that in the sham group ($p<0.05$). The results of immunohistochemisty, in situ hybridization and RT-PCR all showed that the $P2X_{2/3}$ receptor expressions of right TG in the TN group were significantly higher than those in the sham group ($p<0.05$). Those results suggest that $P2X_{2/3}$ receptors in trigeminal ganglion may play important role in nociceptive transmission of trigeminal neuralgia. This work was supported by the grant (№s 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№s 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province.

Study of targeted blocking STAT3 by the decoy oligodeoxynucleotide inhibiting the proliferation of glioma cells

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STAT3, a signal transducer and activator of transcription 3, has been suggested to be involved in tumor genesis of glioma. Our previous work ovserved that STAT3 were over activated in human malignant glioma cell lines. In order to inhibit proliferation of glioma cells, a 15-mer double-stranded decoy oligodeoxynucleotide (decoy ODN), which mimicking the STAT3 specifics-elements, was designed, optimized and synthesized, then transfected into glioma cells as mediated by Lipofectamine^{TM}2000. Then, MTT assay was used to analyze the proliferation in glioma cells. [^{3}H]-thymidine incorporation assay was performed to detect the inhibiting consequence of two cell lines DNA synthesizing. Additionally, the changes of mRNA expression of cell cycle relative genes CylinD1 and c-myc were checked by RT-PCR. The results showed that treatment with STAT3 decoy ODN significantly suppressed proliferation in glioma cell U251 and A172 by, 6.81% and 6.16%, respectively. Furthermore, STAT3 decoy ODN dramatically inhibited DNA synthesis of glioma cells ($P<0.05$). After treated with STAT3 decoy ODN, the transcription levels of CylinD1 and c-myc were significantly decreased both in U251 and A172. These results suggest that targeted inhibition of STAT3 with a decoy ODN can inhibit the proliferation of malignant glioma cells, so it may potentially be used as an effective anti-glioma therapeutic approach.

Caffeine effect on the calcium response evoked by NMDA and GABA on primary cultured hypothalamus neurons.

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Caffeine is widely used as a psychoactive drug. Although it has been reported that caffeine could result in fetus development retardation, the mechanism involved in this is unclear. In the present study, we aimed to investigate the effect of caffeine on neural development of fetus and explore the underlying mechanism. Briefly, the pregnant SD rats were fed with water ad libtum containing 1 g/l caffeine during pregnancy. We found that caffeine consuming rat gave birth to offspring with lower birth weight than that of control ones. Moreover, the offspring of caffeine exposed animals maintained their low body weight for a long period. Given that aberrant hypothalamus function is linked to reduced fetal growth, we used microarray to compare the gene expression profile of hypothalamus between caffeine exposure rats and control ones. Our results demonstrated that the exposure to caffeine down-regulated expression of various neurotransmitter receptors, such as glutamate receptor and GABAA receptor of hypothalamus neuron. Glutamate mediated pathway plays a pivotal role in regulating the release of growth hormone in hypothalamus. During early stage of neuron development, GABAA receptor exhibits an excitatory role, which promotes the growth and the development of neuron. In addition, by using an in vitro culture of primary hypothalamus neuron, we measured intracellular calcium mobilization in response to various stimuli. Our results showed that caffeine treatment decreased the calcium response evoked by NMDA in primary cultured hypothalamus neuron. Besides, caffeine treatment could also decrease the calcium response evoked by GABA in immature neurons. Based on our observation, we propose that fetal caffeine exposure impairs neural development through dysregulation of neuro-transmitter receptors-mediated Ca^{++} responses.

A novel variant of ER-α, ER-α36 effects on apoptosis induced by MPP$^+$ in PC12 cells

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Many reports show that estrogen receptors, ERα and ERβ, widely expressed in central nervous system and might play a very important role in normal brain functions and neurodegenerative diseases. ERα-36, a newly discovered estrogen receptor subtype, was identified and cloned by Wang in 2005. Compared with traditional ERα (ERα-66), it lacks both transcriptional activation domains (AF-1 and AF-2) but retains the DNA-binding domain, partial dimerization and ligand-binding domains. Recently, some groups found that ERα-36 has multiple roles in many cancer cells, which was different from ERα-66. ERα-36 is predominantly expressed on the plasma membrane and could transduce both estrogen-and antiestrogen-dependent activation of the MAPK, Akt signaling pathway and stimulate cell proliferation. However, in central nervous system, the expression and function of ERα-36 is unknown. Previously, study in our group indicated that ERα-36 mediated PI3K/Akt cell signaling when Caveolin-1 gene was silenced in MCF10A^{CE} cells. Here, we report that the expression of ERα-36 and contribution of PI3K/Akt mediated by ERα-36 to the MPP$^+$ in PC12 cells by using Western blot. We established the ERα-36 downregulated PC12 cell model (PC12-36L1) by transfected ERα-36 shRNA plasmid. And then, MTT method was used for apoptosis assay. Lastly, we examined p-Akt signal proteins related to cell apoptosis after treated with MPP$^+$(48h). The results showed that ERα-36 was expressed in PC12 cells. Both PC12 cell and PC12-36L1 cell showed apoptosis after treatment with MPP$^+$ (48h). The survival rate of PC12-36L1 cell was higher than that of PC12 cell, and the expression of p-Akt in PC12-L1 cell that related to cell apoptosis was also higher than that of PC12 cells ($p<0.05$). It is suggested that the novel estrogen receptor subtype-ERα-36 might be involved in regulation of apoptosis signaling pathways. This work was supported by the National Natural Science Foundation of China (No.30970353).

Alteration of retinoic acid receptors expression and cellular translocation during the postnatal development of the rat hippocampus

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Retinoic acid (RA) is a key modulator of hippocampus development, and neural plasticity can be regulated via changes in the transcription of RA receptors. The temporal expression of RA receptors in the rat hippocampus during postnatal development has not been studied in detail. To address this, we studied the mRNA expression patterns of RA receptors (RARs and RXRs) in the rat hippocampus during postnatal, 3, 7, 10, 14, 21 and 30 days. We found that mRNA expression levels of RARα, RARβ, RARγ and RXRβ followed the manner of a bimodal distribution. Their expression levels were increased at the postnatal 1d, down-regulated to the lowest levels from postnatal 3d to 10d, and sharply up-regulated to the highest peak at postnatal 14d which was about 5 fold than that of postnatal 1d, and then down-regulated slightly again to remain a plateau during the period of postnatal 21 to 30d. However, mRNA levels of RXRα and RXRγ were only up-regulated at postnatal 1d, and then remained at lower levels after postnatal 1d to 30d. In addition, immunohistochemistry staining of the rat brain revealed that the RARα protein translocated from the nucleus to the cytoplasm after postnatal 1d and mainly remained in the cytoplasm with the hippocampus postnatal development. More interestingly, the cellular expression pattern of RARα protein shifted from the pyramidal to the molecular layers of the hippocampus following postnatal development of nervous system. These results support that the RA receptor plays an important role in the hippocampus during postnatal development, and that changes in its expression pattern are important for its synaptic plasticity functional role in the developing hippocampus. This work was supported by the key project of National Natural Science Foundation of China (No. 30830106).

Continuous VA deficiency during postnatal development decreases rat learning and memory function via excitatory Ca^{2+} neuron

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Vitamin A (VA) is an important nutrient during postnatal brain development, many studies have shown that VA deficiency (VAD) can cause learning and spatial memory ability damage in rats, however, the specific injury mechanism is not clear now. To address this, We controlled rats VA intake by using elemental dietary and achieved that the peripheral blood VA levels of VAD and VA normal (VAN) group rats are 0.5-0.8 μM and 1.0-1.3 μM, respectively. Using Morris water maze and shuttle box tests, we found that the spatial memory and active learning function of the VAD group was significantly lower than that in the VAN group during their adolescence (postnatal 30-35 days) ($P \leq 0.01$) ($P \leq 0.05$). The Ca^{2+} activity detection of hippocampal slices in both groups during the postnatal different period (1, 3, 7, 10, 14, 21 and 30 days) revealed that the responsiveness of neurons to calcium shock by NMDA challenge in the VAD group was significantly weaker than that in the VAN group ($P \leq 0.05$). The expression levels of RARα in the postnatal 1-3d period of the VAD group were significantly up-regulated than that of the VAN group ($P \leq 0.05$), but during the postnatal 10-30d, down-regulated than that of the VAN group ($P \leq 0.01$). Moreover, the expression levels of NMDA-NR1 (N-methyl-D-aspartic acid receptor NR1) in the VAD group were significantly decreased than that in the VAN group in the postnatal 1-30d period ($P \leq 0.05$). These results demonstrate that continuous VA deficiency during the postnatal development may repress the expression of NMDA-NR1 by decreasing RARα expression to inhibit neuronal calcium excitability, further lead to spatial memory and active learning function injury in adolescent. This work was supported by the key project of National Natural Science Foundation of China (No. 30830106).

Research on nerve regeneration and recovery in xenogeneic acellular nerve scaffold graft through co-application of NGF and GM_1

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To explore the effect of co-application of the NGF and GM_1 on nerve regeneration and function recovery after xenogeneic acellular nerve scaffold transplantation. SD rats were randomly divided into four groups: NS control, NGF-treated, GM_1-treated and NGF + GM_1-treated group. The rabbit tibial nerves were sectioned to take chemical extraction for making the scaffold. The sciatic nerve defects were bridge-connected with 10 mm long xenogeneic acellular nerve scaffold. Before transplantation, the xenogeneic acellular nerve scaffolds were dipped into NS, NGF, GM_1, NGF and GM_1, respectively. After operation, the rats were injected with NS, NGF, GM_1, NGF and GM_1, respectively, into muscles of the lower limb on the surgical side. A series of examinations were carried out, such as SFI, recovery rate of the motor nerve conduction velocities (MNCV%), triceps surae recovery rate of the complex muscular action potential (CMAP%), HRP retrograde trace, the recovery rate of wet weight of triceps surae muscle. Such activities were also carried out in this period as grafting part and its proximal and distal parts as well as stoma nerve fibers of the proximal part and distal part were taken histomorphologic observation and immunohistochemical staining for the presence of nerve fine NF and S-100.The results showed that in the same time span, the jointly treated group was superior to the two solely treated groups in the following measurements ($P<0.05$), SFI, MNCV%, CMAP%, L_3-L_5 the dorsal root ganglion and the labeled cells HRP in L_3-L_5 the spinal cord segment motoneurons, the recovery rate of wet weight of triceps, and myelinated nerve fibers in grafting part and in its distal part. Meanwhile, the jointly treated group and the two solely-treated groups outshone the physiological saline-treated group ($P<0.05$) in those regards. The present results suggest that co-application of NGF and GM_1 can obviously enhance the peripheral nerve regeneration and function recovery in xenogeneic acellular nerve scaffold graft.

Effects of matrine on the expression of $P2X_3$ receptor in stellate ganglion of rats after myocardial ischemia

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The stellate ganglion (SG) neurons in cardiac afferent nerve pathway are involved in the nociceptive transmission of myocardial ischemic injury. Most of the nociceptive response to peripheral ATP is mediated by $P2X_3$ receptor, so the $P2X_3$ receptor play a crucial role in facilitating pain transmission. This study was aimed to investigate the effects of matrine on the expression of $P2X_3$ receptor in stellate ganglia of rats after myocardial ischemia. Spsaruge-Dawley (SD) rats were treated on back with a multiple subcutaneous injection of isoprenaline to induce rat myocardial ischemic model, which was done once a day for 14 days. The changes of ECG was detected as the indication of myocardial ischemia. The abnormal Q wave or ST-segment displacement in myocardial ischemic group was obviously appeared compared with that in the control rats. The results showed that the expression of the $P2X_3$ protein and $P2X_3$ mRNA in SG of the myocardial ischemic rats were enhanced compared with those in the control ($p<0.05$). The expressions of the $P2X_3$ protein and $P2X_3$ mRNA in myocardial ischemic rats treated with matrine group were lower than those in myocardial ischemic rats. Matrine may inhibit the signal transmission of myocardial ischemia by reducing the expression of $P2X_3$ receptor in the rat SG. This work was supported by the grant (№s 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№s 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province.

The effect of lead exposure on DCX positive cells in layer II of adult guinea pigs neocortex

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Lead, as a kind of neurotoxic heavy metal, has been widely explored as for its influence on neural development in the children, however, it is still unveiled that effect of lead exposure on neurogenesis in adult rodent. Our previous data showed that double-cortin (DCX) positive cells (immature neuron) in the layer II of adult guinea pig neocortex, maintained in the whole adulthood, even in the aged guinea pig. In the present study, we tried to investigate the effect of lead exposure on DCX positive cells in layer II of adult guinea pig. The normal adult guinea pigs were given the drinking water which contained lead acetate (0.2 g/L) daily, the exposured animals were sacrificed in 2, 4 and 6 months, the same aged guinea pigs served as control. The neocortex slices were stained by DCX immunohistochemisty and DCX positive cells were counted. We found that density of DCX positive cells in frontal cortex, temporal cortex, parietal cortex and occipital cortex was decreased ($P<0.05$) as lead exposure period extended. These results show that lead exposure results in loss of immature neurons in adult neocortex, at least, suggest lead exposure could affect neurogenesis in the adulthood negatively.

Neurochemical phenotype and function of endomorphin-2-containing neurons in the myenteric plexus of the rat colon

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The previous studies have shown that μ-opioid receptors (MOR) are distributed in gastrointestinal tract, but the endogenous ligands with highest affinity and specificity, endomorphins, are not. So we investigated the neurochemical phenotype of endomorphin-2 (EM2) - containing neurons in the myenteric plexus of the rat colon, and evaluated the effects of EM2 on colonic motility by immunofluorescent histochemistry on whole-mount sections and neuronal blocking agents with electrical field stimulation (EFS) on intact mid colonic segments. In the myenteric plexus of the mid colon between the right and left flexures, 53%, 37%, 26%, and 49% of EM2-immunoreactive (-IR) neurons were also positive for choline acetyl transferase (ChAT), 5-hydroxytryptamine (5-HT), vasoactive intestinal peptide (VIP)- and nitric oxide synthetase (NOS), respectively, but EM2 did not colocalize with calcitonin gene-related peptide (CGRP). In vitro EM2 did not directly affect the spontaneous activity of intact colonic segments or carbachol-prestimulated colonic segments. However, EM2 inhibited the electrically- induced colon contractile response up to 62% $\pm$ 7.1%. These inhibitory responses were blocked by the opioid receptor antagonist, naloxone (10^{-5} M), and the MOR antagonist β- FNA (10^{-6} M). In colons blocked by atropine (10^{-6} M) or ICS (10^{-6} M), EM2 (10^{-6} M) still inhibited electrically-induced colonic contractions. L-NAME (10^{-4}M) blocked the inhibitory effect of EM2. These result suggested that EM2-containing neurons, acting as the interneurons of the myenteric plexus, may mediate motility of the rat colon through inhibitory neuronal pathways.

Thrombin induced TGF-β1 pathway: a cause of communicating hydrocephalus post subarachnoid hemorrhage

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Subarachnoid hemorrhage (SAH) frequently results in complications including intracranial hypertension, rebleeding and vasospasm. The extravasated blood is responsible for a cascade of reactions involving release of various vasoactive and pro-inflammatory factors from blood and vascular components in the subarachnoid space. The communicating hydrocephalus following SAH is one of the complex and multifactorial neurological disorders, which arises from fibrosis in the subarachnoid space. Spinal arachnoiditis and periradicular ''inflammation'' of the arachnoid membrane and adjacent peridural structures lead to fibrosis within and around the lumbar dural sac and the spinal nerve roots. Fibrosis causes cavitas subarachnoidalis stenosis, which in turn blocks the cerebrospinal fluid circulation. The mechanism of communicating hydrocephalus after subarachnoid hemorrhage (SAH) is unclear. Revealing a signaling cascade may offer us significant insights into the molecular etiology of an accumulation of the cerebrospinal fluid in cerebral compartments during SAH. To establish the mechanism of the communicating hydrocephalus following SAH, we infused cerebrospinal fluid (CSF) with thrombin (TH), resulted proinflammatory and proliferative responses in rat meninges of SAH. The effect of TH could be completely blocked by a transforming growth factor β1 (TGF-β1) inhibitor, SB-431542, suggesting that TH stimulated proliferation of meninges is through TGF-β1 signaling pathway. The cascade of TGFβ1-Smad3-CTGF was significantly up-regulated by TH, which in turn, stimulated the proliferation of subarachnoid meninges. TH induced over-expression of TGF-β1 and its downstream factors activating might be one of mechanism of communicating hydrocephalus after SAH.

Effects of matrin on the transmission of primary sensory signalling mediated by $P2X_{2/3}$ receptor in pathological pain

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ATP is closely related to neuropathic pain. $P2X_{2/3}$ receptor is mainly expressed on the middle and small neurons of dorsal root ganglia (DRG). Matrine, a traditional Chinese medicine, has analgesic action. Chronic constriction injury (CCI) rats were adopted as the neuropathic pain model. Mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL) in CCI group were obviously reduced compared with that in sham group on days 7 - 14 after operation. The data in CCI + matrine group were significantly increased compared with that in CCI group. On the day 14 after operation, $P2X_2$ and $P2X_3$ immunoreactivity on L4-L6 DRG in CCI group was sharply increased compared with that in sham group; $P2X_2$ and $P2X_3$ expression in matrine-treated group was lower than that in CCI group, while it was still higher than that in sham group. ATP-activated currents in CCI group were enhanced compared with those in sham group. ATP-activated currents in matrine-treated group was lower than those in CCI group. Matrine reduced the hyperalgesia in CCI rats. The expression of $P2X_{2/3}$ receptor on L4/L5 dorsal root ganglia of CCI rats was decreased by matrin. Matrin may inhibit the nocicetive transmission of primary sensory neurons mediated by $P2X_{2/3}$ receptor and thus produce an effective action in the treatment of neuropathic pain. This work was supported by the grant (№s 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№s 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province, the grant (YBP08A01) from Jiangxi Province Excellent Ph.D. Students Foundation, and the grant (YC08B009) from the Innovation Foundation of Graduate School of Nanchang University.

Protective effects of naloxone on oligodendrocyte injury caused by LPS-activated microglial cells

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During a few neurodegenerative diseases, microglia cells will be activated and can release a lot of cytokines which can cause other cell death including neurons and oligodendrocytes. To explore the possible protective effects of naloxone on oligodendrocytes injury caused by LPS activated microglia cells, primary isolated and purificated rat microglia cells were cocultured with oligodendrocytes, and microglia cells were activated by LPS (1 μg/ml),then the cells were treated with 0.1 μM naloxone for 18 h, the protective effects of naloxone were measured by IHC staining. After microglia cells were activated by LPS, the injury of cocultured oligodendrocytes could be obviously observed. However, treatment of LPS activated microglia cells with 0.1 μM naloxone significantly prevented the oligodendrocytes injury caused by microglia activation. So Naloxone can protect oligodendrocytes injury from activated microglia cells, and it could be a good candidate for treating some neurodegenerative diseases. This work is supported by ''Special Talent Project'' No.XT200910 of Ningxia Medical University, Ningxia Natural Science Foundation No. NZ09103 and National Natural Science Foundation of China No.30960108.

Effects of xenotransplantation of microencapsulated Schwann cells on the nerve regeneration after spinal cord injury in rats

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Transplantation of Schwann cells can enhance neuronal survival, axonal regeneration and functional recovery after spinal cord injury. Barium-alginate microcapsules, which develop an effective immunoisolation system, make it possible to overcome the immune rejection in xenotransplantation. Here the effects of microencapsulated Schwann cells xenotransplantation on the nerve regeneration after spinal cord injury in rats were determined. Predegenerated rabbit sciatic nerves were cultured by modified repeated explanation of Schwann cells. Adult rats were performed a lateral hemisection of spinal cord at left T10 level, then gelatin sponge with the Schwann cells microencapsulated in alginate- Barium were implanted into the injured spinal cord area. Using histological and immunohisto-chemical staining, we found the number of the Nissl stained neurons rose remarkably and their volume increased after xenotransplantation. The expression of Growth-associated protein-43 increased. The expression of Neurofilament-200 showed an increasing tendency after transplantation. The locomotor function of the hindlimbs also recovered. These results indicate that xenotransplantation of micro-encapsulated Schwann cells can promote the spinal cord nerve regeneration and improve the functional recovery.

The effects of sodium ferulate on the expression of P2X3 receptor in cervical dorsal root ganglia of rats after myocardial ischemic injury

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Previous evidence indicated that dorsal root ganglion (DRG) neurons, the primary sensory neurons expressed multiple P2X receptors, including P2x1-6. Among them, P2X3 receptor was present predominantly in a subpopulation of small-diameter sensory neurons related with nociceptive reception. P2X receptors belong to a superfamily of ligand-gated, non-selective cation channels. Spsaruge-Dawley (SD) rats were randomly divided into the normal control group, myocardial ischemia injury group and myocardial ischemic rats treated with sodium ferulate (SF) group. Myocardial ischemic rat model was established as following: SD rats were treated on back with a multiple subcutaneous injection with isoprenaline. Intraperitoneal injection of sodium ferulate (SF), which was done once a day for 14 days. Compared with the normal control group, the abnormal Q wave or ST-segment displacement appeared obviously in myocardial ischemic group. The results showed that the expression of the P2X3 protein and P2X3 mRNA in cervical dorsal root ganglia of the myocardial ischemic injury group was higher than that in control group ($p<0.05$). Compared with the myocardial ischemic group, the results of SF treatment group showed that the expression of the P2X3 protein and P2X3 mRNA in cervical dorsal root ganglia were significantly reduced ($p<0.05$). The myocardial ischemia is often painful. Pain arises from activation of nociceptive primary afferents innervating visceral organs. SF may inhibit the signal transmission of myocardial ischemic pain by reducing the expression of P2X3 receptor in cervical dorsal root ganglia of the myocardial ischemic rat. This work was supported by the grant (№s 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№s 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province.

Study of puerarin on sympathoexcitatory reflex induced by myocardial ischemic nociceptive signaling *via* $P2X_3$ receptor in rat superior cervical ganglion

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Sympathoexcitatory reflex induced by myocardial ischemia can increase blood pressure and sympathetic nerve activity. Puerarin is an active component extracted from Chinese herbal medicine Ge Gen. The present study investigated the effect of puerarin on sympathoexcitatory reflex induced by myocardial ischemic nociceptive signaling *via* $P2X_3$ receptor in rat superior cervical ganglion (SCG). The findings showed that the systolic blood pressure, heart rate and respiration in the myocardial ischemic rats were higher than those in control rats. After the treatment with puerarin in the myocardial ischemic rats, systolic blood pressure, heart rate and respiration were lower than those in the myocardial ischemic rats ($p<0.05$). TH and $P2X_3$ receptor were co-expressed in SCG neurons by immunofluorescence. The co-expression of TH and $P2X_3$ receptor in myocardial ischemic injury group exhibited more intense staining than that in control group ($p<0.05$). Puerarin can decrease the expression of TH and $P2X_3$ immunoreactivity in myocardial ischemic rats. $P2X_3$ protein and mRNA expression in SCG neurons of myocardial ischemic injury rats were increased compared with those in control rats ($p<0.05$). Puerarin decreased the $P2X_3$ protein and mRNA expression in SCG neurons of myocardial ischemic injury rats. In conclusion, puerarin could decrease the expression of $P2X_3$ protein in SCG neurons in myocardial ischemia rats, and then reduce systolic blood pressure, slow down heart rate and respiration in myocardial ischemia injury rats. These results suggest that puerarin may depress sympathoexcitatory reflex induced by myocardial ischemic nociceptive signaling *via* $P2X_3$ receptor in rat superior cervical ganglion. This work was supported by the grant (№s 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№s 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province.

Effects of nimodipine on brain AQP4 and ZO-1 expressions in rat's brain after serious systemic thermal injury

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Mortality after serious systemic thermal injury may be linked to significant increases in cerebral vascular permeability, but its mechanism is not yet fully understood. In this research, SD rats were randomly divided into control, thermal injury and nimodipine groups, of which the later two groups were divided into five post-scald groups: 1h; 3h; 6h; 12h and 24h. The 30% TBSA scald rats were made, and nimodipine was injected into the scald rat's peritoneal cavity immediately after scald in the thermal injury group. Changes of the BBB permeability were determined by detection of Evans blue (EB) content in rat brains with chemic-quantitative analysis. The expression levels of zonula occludens-one (ZO-1) were analyzed by real time PCR. The changes of brain AQP4 expression after severe scald were determined by immunohistochemical method. Compared with control group, a significant increase of EB content was found in brain of severe scald rat of which the peak was emerge at 6h after. However, the EB extravasations were significant restricted in rats that were treated with nimodipine. The expression of ZO-1 mRNA in rats brain with severe scald decreased quickly compared with that of the normal control, of which the result was most marked at 3h after scald. However, the expression of ZO-1 mRNA of the severe scald rat's brain treated with nimodipine was increase ($P<0.01$) comparing with that of scald rats model, peaks of which emerged at 3 and 6h after scald. The expression of brain AQP4 began to increase 2h after severe scald, then reached peak after 6h. After nimodipine treatment, permeability of BBB, and expression of brain AQP4 were decreased. These results suggest that nimodipine can protect the function of BBB by lowering the AQP4 expression and enhancing ZO-1 mRNA expression after severe scald.

Cognitive function and expression of α4 nAChR in the insular kindling model of epilepsy

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The findings that the seizures originating in the insular cortex persisted after temporal cortectomy is likely to explain some of the failures of the surgical procedure in temporal lobe epilepsy (TLE). Patients with TLE surgery may be at risk for a more rapid decline in selective verbal memory skills. The α4 nicotinic acetylcholine receptors (α4nAChR) were reported to modulate aspects of memory and attention. So the purposes of this study were to investigate memory function and emotional deficits in the insular kindling model of epilepsy and the expression of the α4 nAChR in hippocampus. The male SD rats were randomly divided into the control group and the insular kindling group. The insular kindling group was subdivided into 1 and 2 week groups (K_1 and K_2 groups).The kindling group that have been evoked for 5 consecutive stage V seizures were established by the electrical stimulation fully-kindled insular seizures. Emotional deficits were tested by open field test, learning and memory abilities were tested by Morris water maze and the expression level of α4nAChR in hippocampus was determined by immunohistochemical staining and western blotting. In the open-field exploratory maze, the numbers of the feces of K_1 and K_2 groups were both obviously increased compared with the control group. There was no significant difference between learning and memory abilities of K_1 and K_2 group and the control group. However, the expression of α4nAChR of K_1 and K_2 groups increased significantly. Kindling differentially might affect cognition disorders and emotional deficits and α4nAChR may play a role in it.

Antidepressant-like effect of flavonoids from artificial cultivation Chinese traditional herb *Glycyrrhiza uralensis* fisch.

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Combining with the extracellular unitary recordings of dorsal raphe nucleus(DRN) serotonergic neurons and the assessment of brain-derived neurotrophic Factor (BDNF) level in hippocampus, the ethological approaches, such as forced swimming test (FST), tail suspension test (TST) and the chronic unpredictable mild stress (CUMS), have been proposed as potential methods for screening the new anti-depressant drug in mammals. We demonstrate that the flavonoids from artificial cultivation Chinese traditional herb *Glycyrrhiza uralensis* Fisch can antagonize the depressant-like behavior in mice, and potentiate the BDNF level (ELISA) in hippocampus. Compared with fluoxetine, the flavonoids can not impact the dynamic change of the DRN neuron firing rate in 3ds and 21ds ig treatment. These results suggest that the flavonoids from artificial cultivation Chinese traditional herb *Glycyrrhiza uralensis* Fisch can produce the anti-depressant effect by modifying the hippocampus BDNF level in animal models. The mechanism of this effect on enhancing CNS serotonergic neuron system needs more investigation.

Effect of astragalus injection on ultrastructure of neurons that surrounding intracerebral hemorrhage in rats

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To study the protective effect of astragalus injection on neurons that surrounding intracerebral hemorrhage (ICH) in rats. ICH model was established by injecting collagenase VII into the brain of rats. 25 male rats were randomly divided into the astragalus remedial group, control group with ICH and the sham group. Then the astragalus remedial groups were divided into 3 groups which were 0 h, 6 h and 24 h remedial group. All rats involved in the experiment were killed at 72 h after ICH. Then the influence of astragalus injection on the ultrastructure of the apoptotic neurons after ICH at different time points was observed with an electron microscope. Result showed that in normal control group, ultrastructure of neurons was nearly normal. But in ICH group, most neurons around haematoma exhibited varied characteristics of apoptosis: the cell body shrinked, nuclear membrane collapsed, karyopycnosis nucleus pycnosised, chromatin moved to nuclear membrane, nucleolus was rare; some mitochondria magnitude was different, presenting dense-type changes, most mitochondria swelled, volume increased, mitochondrial crista collapsed, cavity-like degeneration; rough endoplasmic reticulum broaden. However, the degree of injury of neuronal chondriosome, nucleolus, nuclear membrane, rough endoplasmic reticulum and so on in astragalus injection remedial group were much less severe than those of ICH control group. In remedial group, neuron ultrastructure in 0 h and 6 h after ICH was better than that in 24 h group. In conclusion, astragalus could protect neurons located near the hemorrhage brain area, alleviated the apoptosis of neurons after ICH, and this effect was more effective at an early stage following hemorrhage.

The effect of matrine on $P2X_3$ receptor expression of rat cervical dorsal root ganglion after myocardial ischaemia

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Myocardial ischemia causes the production of a variety of chemical substances, which acts on the cardiac afferent nerve to cause pain. $P2X_3$ receptors play a crucial role in facilitating pain transmission. The cervical dorsal root ganglion (DRG) in rat express the $P2X_3$ receptor. Matrine is a kind of alkaloid which is extracted from sophora root. Matrine has many pharmacology functions, such as improving arrhythmia and reducing pain. The present research explored the effect of matrine on the expression of $P2X_3$ receptor of DRG in myocardial ischemic rats. Sprague-Dawley rats were randomly divided into the saline control group, the myocardial ischemic group and the myocardial ischemic rats treated with matrine group. Two weeks later, the electrocardiogram of the myocardial ischemia group appears deep Q wave and ST-segment displacement compared with that of the saline control group, it shows the myocardial ischemic model has been established. The expression of $P2X_3$ protein and $P2X_3$ mRNA of DRG were respectively measured by immunohistochemistry and RT-PCR. The results show the expression of $P2X_3$ protein and $P2X_3$ mRNA of DRG in myocardial ischemic rats are obviously higher than those in the control rat ($p<0.05$), but it is decreased after the myocardial ischemic rats treated with matrine ($p<0.05$). There are no significant different between the saline control group and the myocardial ischemic rats treated with matrine group ($p>0.05$). According to these results, matrine may decrease the expression of $P2X_3$ receptor of rat DRG after myocardial ischemia. This work was supported by the grant (№s 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№s 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province.

Roles of HSP60 in oligodendrocyte injury and the mechanism

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Heat shock protein (Hsp) 60 is thought to function as an endogenous danger signal to the immune system. We observed that Hsp60 level is significantly increased in perventricular leukomalacia (PVL), which is the principal form of brain injury in the premature infant and accounts for most of the cerebral palsy. Lethal injury to premyelinating oligodendrocytes progenitors (preOLs) in the immature cerebral white matter has been postulated to be a key feature of PVL. The function of Hsp60 in PVL is not well studied. So our hypothesis is that Hsp60 may play an important role in preOLs injury. Our *in vitro* study shows that Hsp60 level increased in both microglia and oligodendrocytes when cells were treated with LPS or IFN-r, and lots of Hsp60 was released into the culture media from microglia, but very few from oligodendrocytes. The binding study shows that Alex647 labeled Hsp60 only binds to oligodendrocytes, but not to microglia. The possible explanation for these findings is that upon activation, microglia overexpress Hsp60 and then part of Hsp60 is released to the extracellular space. These released Hsp60 then causes immune stimulation of oligodendrocytes. However, the clarification of this hypothesis requires further studies. Additionally, to address the mechanism of Hsp60 in oligodendrocytes inflammation, we here tested whether Hsp60 expression is regulated by poly (ADP-ribose) polymerase-1 (PARP-1), which was reported to trigger the protection against heat shock and inflammation in PARP-1 deficient mice. We found that Hsp60 level was increased when oligodendrocytes were treated with DPQ, a potent PARP-1 inhibitor, but decreased with either the treatment of etoposide, an activator of PARP-1, alone or together with DPQ. The data indicate that PARP-1 may inhibit Hsp60 expression in oligodendrocytes. However, the detailed mechanism of the regulation of HSP60 by PARP-1 needs to be further studied. This work is supported by ''Special Talent Project'' No.XT200910 of Ningxia Medical University, Ningxia Natural Science Foundation No. NZ09103 and National Natural Science Foundation of China No.30960108.

Scavenging of blood glutamate for enhancing brain-to-blood glutamate efflux

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The glutamate levels in cerebrospinal fluid (CSF) can be regulated by decreasing blood glutamate levels and accordingly accelerating the brain-to-blood glutamate efflux. This process can be accomplished by activating the blood resident enzymes in the presence of the respective glutamate co-substrates. A few glutamate co-substrates and co-factors were studied with the attempt to find the optimal condition to reduce blood glutamate levels. The administration of the mixture of 1mM pyruvate and oxaloacetate (Pyr/Oxa) for 1 h was found to decrease blood glutamate up to 50%. However, adding lipoamide to this mixture could further reduce blood glutamate levels to more than 80%. In vivo experiments also showed that lipoamide together with Pyr/Oxa could reduce more blood glutamate than with Pyr/Oxa alone, accordingly to enhance glutamate efflux from brain to blood. The results may outline a more effective strategy for the removal of excess glutamate in various neurodegenerative disorders. This work is supported by ''Special Talent Project'' No.XT200910 of Ningxia Medical University, Ningxia Natural Science Foundation No. NZ09103 and National Natural Science Foundation of China No. 30960108.

Modulation of rat visceral pain by brain-derived neurotrophic factors (BDNF)

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Brain derived neurotrophic factor (BDNF) plays an important role in the development of nerve system, the survival of neurons and the synapse-plasticity. Recent researches have shown that BDNF is a modulator of pain in the inflammatory, neuropathic and incision-induced pain hypersensitivity. The present study was designed to observe the behavior index and the expression of c-Fos in anterior cingulate cortex (ACC) of the rat with visceral pain injected of anti-BDNF peritoneally. To explore the role of BDNF in visceral pain hypersensitivity. The result indicated an obvious abnormal behavior after the injection of acetic acid with a great increase in the number of abdominal contraction. Correspondingly, the result of immunohistochemistry revealed that after the injection of acetic acid, the expression of c-Fos in ACC was up-regulated. However, for the male experimental group rats that received anti-BDNF antibody before acetic acid, the behavior index increased along with an enhancement of the expression of c-Fos in ACC. But for the female, the behavior index decreased and the expression of c-Fos weakened. This study well proved that BDNF plays a critical role in the modulation of visceral hypersensitivity, which is sex-dependent. BDNF might contribute to the visceral hypersensitivity in female while perform an opposite effect on male.

The effects of emodin on the expression of $P2X_3$ receptor in cervical dorsal root ganglia of rats after myocardial ischemic injury

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Myocardial ischaemia (MI) is a disease characterized by ischaemia (reduced blood supply) to the heart muscle, usually due to coronary artery disease (atherosclerosis of the coronary arteries). The emodin is the effective component of the traditional Chinese medicine Rhubarb. This study explores the effects of emodin on the expression of P2X3 receptor in cervical dorsal root ganglia (CDRG) of rats after myocardial ischemic injury. The ST-segment changes were recorded by the electrocardiogram (ECG) and the expression changes of P2X3 receptor in CDRG were detected by immunohistochemistry, RT-PCR and western blotting after 14–day treatment. The obvious changes of ST-segment were found in myocardial ischemic rats and the expressions of P2X3 immunoreactivity and mRNA in the CDRG neurons of myocardial ischemic injury rats were significantly increased compared with those in the normal rats ($p<0.05$). The results of MI+emodin group showed that the expression of the P2X3 protein and P2X3 mRNA in cervical dorsal root ganglia were significantly reduced compared with those in the myocardial ischemic group ($p<0.05$). There are no difference among normal control group, emodin control group, MI + emodin group ($p>0.05$). In conclusion, emodin could improve the aberrant changes of T wave and the ST section of the abnormal ECG, reduce the expression of P2X3 protein and mRNA in CDRG neurons in myocardial ischemic rats. These results may indicate that emodin may reduce myocardial ischemic nociceptive signaling via P2X3 receptor in rat cervical dorsal root ganglia. This work was supported by the grant (№s 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№s 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province.

Role of sodium ferulate on myocardial ischemic nociceptive signaling mediated by $P2X_3$ receptor in rat stellate sympathetic ganglion

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Coronary heart disease is one of the most common vascular diseases and its basic pathological process is myocardial ischemia. $P2X_3$ receptor is the most abundant P2X-receptor subtype in heart, which is expressed in primary sensory neurons that mediate nociception. And the stellate sympathetic ganglia (SG) in cardiac afferent nerve pathway is involved in the nociceptive transmission of myocardial ischemic injury. It also has been reported that sodium ferulate (SF) is an active component from Chinese herbal medicine and has anti-inflammatory activities. The present research investigated the role of sodium ferulate on signalling of myocardial ischemic rats mediated by $P2X_3$ receptor in stellate sympathetic ganglia. Spsaruge-Dawley (SD) rats were treated on back with a multiple subcutaneous injection of with isoprenaline, which was done once a day for 14 days. The expression of $P2X_3$ mRNA in SG neurons was tested by RT-PCR. And the expression of $P2X_3$ immunoreactivity was tested by immunohistochemistry. ST-segment changes were recorded by electrocardiogram (ECG). There were obvious changes of Q wave or ST-segment in myocardial ischemic rats compared with that in the normal rats ($p<0.05$). The $P2X_3$ immunoreactivity and mRNA expression in the SG neurons of myocardial ischemic injury rats were significantly increased compared with those in the control group ($p<0.05$). However, the expressions of $P2X_3$ immunoreactivity and mRNA in the myocardial ischemic injury rats treated with sodium ferulate were reduced compared with those in myocardial ischemic rats ($p<0.05$). According to these results, sodium ferulate may reduce myocardial ischemic nociceptive signaling mediated by $P2X_3$ receptor in rat stellate sympathetic ganglion. This work was supported by the grant (nos 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (no 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (nos 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (nos 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province.

Effects of puerarin on acute nociception mediated by $P2X_3$ receptor of dorsal root ganglion neurons

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The $P2X_3$ receptors play a crucial role in facilitating pain transmission. Puerarin extracted from the traditional Chinese medicine Ge-gen is widely used in the treatment of cardiovascular disease in China. The effects of puerarin on acute nociception mediated by $P2X_3$ receptor of rat dorsal root ganglion neurons was investigated at the present research. Frequency of lift foot, flinching and licking of rat hindpaw (times/5 minutes) were measured and the expressions of $P2X_3$ protein and mRNA in L_4-L_6 dorsal root ganglion were detected by immunohistochemistry and RT-PCR. Puerarin could obviously inhibit the acute nociception induced by intraplantar injection of formalin in rat. Co-administration of intrathecal puerarin with ATP into the rat, produced a significant reduction in nociceptive paw flinching, licking and guarding behavior increased by intrathecal injection of ATP in rat ($p<0.05$). Co-administration of intrathecal puerarin with α,β-meATP into the rat, produced a significant ($p<0.01$) reduction in nociceptive paw flinching, licking and guarding behavior increased by intrathecal injection of α,β-meATP in rat. Intraplantar injection of puerarin could decrease the upregulation of $P2X_3$ receptor expression induced by intraplantar injection of formalin in DRG. These results suggest that the anti-nociceptive effects of puerarin may mainly be associated with inhibiting the transmission of nociceptive signaling mediated by $P2X_3$ receptor activation in primary sensory neurons. This work was supported by the grant (№s 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№ 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province, the grant (YBP08A01) from Jiangxi Province Excellent Ph.D. Students Foundation, the grant (YC08B009) from the Innovation Foundation of Graduate School of Nanchang University and the grant (2007A117) from the Health Department of Jiangxi Province.

Expression of Src suppressed C kinase substrate in rat neural tissues during inflammation

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Src-suppressed C kinase substrate (SSeCKS), an *in vivo* and *in vitro* protein kinase C substrate, is a major lipopolysaccharide (LPS) response protein which markedly upregulated in several organs, including brain, lung, heart, kidney et al, indicating a possible role of SSeCKS in inflammatory process. However, the expression of SSeCKS during inflammation and its biological function remains to be elucidated. Here, we established an inflammatory rat model with lipopolysaccharide to investigate the gene expression patterns of SSeCKS in neural tissues using TaqMan quantitative real-time PCR and immunohistochemistry. Real time PCR showed that SSeCKS mRNA expressed in a dose- and time- dependent manner in sciatic nerves, spinal cords and dorsal root ganglions. Immunohistochemistry showed that SSeCKS colocalized with nerve fibers in sciatic nerve after LPS administration, but no localization with Schwann cells, and neurons in dorsal root ganglions and spinal cords. These findings indicated that SSeCKS might play important roles in nerve fibers and neurons during the inflammation.

Sleep architecture alteration involve in up-regulation of A_{2A} receptor expression in rat hypothalamus following cocaine withdrawal

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To further study the characteristics of sleep disturbance resulting from cocaine withdrawal and whether it is related to the changes of adenosine receptor expressions in rats. Adult rats were instrumented with sleep-wake recording electrodes. Following post-surgical recovery, cocaine (20 mg/kg) was administered subcutaneously to rats once per day for 7 days. Polygraphic signs of undisturbed sleep-wake activities were recorded for 24 h during withdrawal 1 day (acute withdrawal), 8 days (subacute withdrawal), and 14 days (subchronic withdrawal) from repeated cocaine treatment and before cocaine administration. Western blots were performed to examine the expression of the adenosine receptor in hypothalamus. Non rapid eye movement (NREM) sleep was increased during dark and light periods in cocaine withdrawal 8 days rats; and the increase of NREM sleep was observed during dark and light periods in withdrawal 14 days rats, whereas both of daytime and night time REM sleep were significantly reduced following 8 and 14 days abstinence from cocaine treatment. However, in cocaine withdrawal day 1, wakefulness was significantly increased, total sleep was decreased, NREM sleep was markedly reduced, and REM sleep enhanced. Sleep/wake cycle were significantly increased just in cocaine withdrawal 1 day; during NREM and REM sleep as well as wakefulness, there were no marked differences in percentage of δ, θ and α power density among control and withdrawal 1 day, 8 and 14 days rats in both light and dark periods. A_{2A} receptor expression was significantly enhanced during 8 and 14 days cocaine abstinence, whereas A1 receptor level reduced only in withdrawal 14 days rats and A_{2B} subunit expression remained unchanged during the entire experimental periods. Our preliminary findings suggest that sleep architecture changes caused by subacute and subchronic cocaine withdrawal may be at least partially mediated by A_{2A} not A_1 or A_{2B} subunits in rat hypothalamus.

Quercetin attenuates cell apoptosis in the rat brain after focal cerebral ischemia

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This study was to investigate the effects and mechanisms of quercetin anti-apoptosis by in the rat brain after stroke. Quercetin was intragastrically administered to rats in doses of 10 and 20 mg/kg/day, starting 3 h after the onset of middle cerebral artery occlusion (MCAO). The behavioral test was performed by using the modified neurological severity score (mNSS). We assessed the effects of quercetin on cell apoptosis by TUNEL and immunohistochemistry staining and western blot. Quercetin significantly improved neurological function, decreased the number of TUNEL and Bax positive cells and increased the number of Bcl-2 positive cells in the cortex as compared with model groups. Furthermore, quercetin significantly increased Bcl-2 protein level and decreased Bax protein level as compared with model groups. We further investigated the mechanisms involved in the effects of quercetin decreasing cell apoptosis. Results suggest that quercetin significantly increased brain derived neurophic factor (BDNF) and its receptor TrkB protein level. Due to cell survival is associated with the activation of PI-3K /Akt signaling pathway. We also examined the expression of p-Akt by western blot. We found that quercetin dramatically elevated the p-Akt expression as compared with control groups. These results suggest that quercetin can decrease cell apoptosis in the rat brain after stroke and the mechanism may be related to the activation of PI-3K/Akt signaling pathway.

Effect of morphine addiction and withdrawal in parents prior to mating on foraging behavior in adult offspring rats

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In the present study, the effect of morphine addiction and withdrawal in parents prior to mating on foraging behavior were investigated in adult offspring rats. Adult rats were intraperitoneally injected with morphine for consecutive 10 days, control animals were injected with saline. After 21 days of morphine withdrawal, male and female rats were mated and classified: Group 1: morphine males mated with morphine females, Group 2: morphine males mated with saline females, Group 3: saline males mated with morphine females, Group 4: saline males mated with saline females. Half of offspring rats in each group at 15-d old were exposed to enriched environment (EE) for 2 weeks. All offspring rats were detected of their foraging behavior at 8 weeks of age. After that, the anterior cingulate cortex (ACC) was excised for Golgi impregnation and the dendritic morphology were observed. Results showed that the amount of foraged food of offspring rats in group 1, 2, 3 were reduced compared with that of the same gender offspring rats in Group 4. In group 1, 2, 3 the amount of foraged food of offspring rats which exposed to EE during juvenile were significant increased compared with those have no experience of EE. Golgi staining revealed that the dendritic length, spine density, and numbers of branch points in the apical dendrites of the neurons in ACC were significantly reduced in the group 1, 2, 3 when compared with the group 4, while the EE stimulation to offspring can reverse those changes. These results suggest that the experience of morphine addiction and withdrawal of parental rats prior to mating can transgenerational affect the foraging food ability of offspring, EE stimulation to offspring during juvenile can promote the recovery of foraging behavior, the mechanism may be related to the morphological integrity of the ACC neurons in offspring rats.

Research on central pharmacological effect and mechanism of oxysophoride

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Oxysophoride is extracted from one of the local herbs in Ningxia Hui Autonomous Region .Research has showed that oxysophoride is of multiple pharmacological effects including anti-inflamation, anti-neoplastic and dis-infection. This study aimed to detect the central pharmacological effect of oxysophoride and its basic mechanism. Behavior pharmacological method was adopted to observe and monitor the effect of oxysophoride on mice's general behavior and autonomic activities, on the action of hypnotic of Pentobarbital with threshold and sub-threshold dosage and on mice's passive movement as well as the effect on Pentylenetrazol induced convulsions in mice. Monopole leads were employed to record the bioelectricity of cerebral frontal cortex, amygdale, hippocampus, and reticular formation relatively. FFT technique was adopted to analyze the cerebral electrical waves for revealing the effect of oxysophoride on cerebral bioelectricity of different parts of brain in rats. SABC was introduced to measure the effect of oxysophoride on glutamate (Glu) and gamma amino butyric acid (GABA) immunoreaction positive cells of frontal cortex and hippocampus in CNS (central nerves system). The results showed that oxysophoride prolonged the sleeping time by and enhanced the hypnotic effect of Pentobarbital at subthreshold dosage. Our study shows no effect on passive moment and convulsion caused by Penlylenetrazol by ip administrating Oxysophoride 1000 mg/kg. Oxysophoride administrated icv to groups (10 mg/rat, 5 mg/rat and 2.5 mg/rat) made most EEG appear in low frequency and slow wave with shuttle shaped sleeping wave. δ waves take high proportion of total power in cortex while α and β waves take high proportion of total power in subcortex (amy, hip and rt). In addition to the electrophysiological changes, oxysophoride increased the number of GABA immunoreactions positive nerve cells and significantly decreased the number of GLU immunoreactions positive cells in rat's cerebral cortex and hippocampus administrated icv (10 mg/rat). The results showed that Oxysophoride possesses the action of central inhibition. Its mechanism may relate to the expression imbalance of GABA and Glu in CNS.

AMPK mediates neuronal activity-dependent mitochondrial transcriptional regulation and neuroprotection in rat visual cortical neurons

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Neuronal activity and energy metabolism are tightly coupled. However, the signaling mechanism is poorly understood. Peroxisome proliferator-activated receptor γ coactivator-1-α (PGC-1α) coactivates a number of transcription factors critical for mitochondrial biogenesis, including nuclear respiratory factor 1 (NRF-1). The present study aimed at testing the regulatory mechanism and physiological significance of neuronal activity-dependent mitochondrial transcriptional regulation in visual cortical neurons in vitro and in vivo. Cultured primary visual cortical neurons were subjected to 25 mmol/L KCl depolarizing treatment for varying times. PGC-1α, NRF-1 and mitochondrial transcription factor A (mtTFA) expression was analyzed by RT-PCR and immunoblots. Neuronal activity significantly increases PGC-1α, NRF-1 and mtTFA level. Following the withdrawal of KCl, both PGC-1α and NRF-1 expression were significantly reduced. AMPK inhibitor completely repressed the neuronal activity induced up-regulation of PGC-1α, NRF-1 and mtTFA mRNA and AMPK phosphorylation as well as cellular ATP content. AICAR and resveratrol treatment markedly increased AMPK phosphorylation leading to up-regulate the PGC-1α and NRF-1mRNA levels in visual cortical neurons. Visual deprivation significantly decreases AMPK activity, PGC-1α and NRF-1levels in visual cortical neurons as compared with non-deprived ones. Resveratrol treatment caused markedly upregulation of AMPK, PGC-1α, NRF-1 levels and mitochondria amount in the deprived visual cortices. These results strongly indicate that neuronal activity-induced up-regulation of PGC-1α, NRF-1 and mitochondrial biogenesis in visual cortical neurons is mediated by AMPK activation. Our results also point to the therapeutic possibility of AMPK for the treatment of neuronal energy dysregulation disease.

Activation of ERK in ACC contributes to the pain hypersensitivity and anxiety-like behaviour following surgical incision in rats

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Our previous studies showed that surgical incision could induce anxiety-like behavior in rat, but the mechanism is poorly understood. The present study we explored the role of anterior cingulated cortex (ACC) extracellular signal-regulated kinase (ERK) activation in surgical incision induced pain-related negative effect in rat. Longitudinal incision in one plantar hind-paw produced a two-phase ERK activation in ACC bilaterally, the first phase is 15 min to 30 min after incision and the second phase is 6 h to 3 d after incision. We found that blockade of ERK activation in the first phases in the ACC with U0126 eased incision-evoked mechanical hypersensitivity and anxiety-like behavior, while blockade of ERK activation in the second phase in the ACC prevented the expression of incision-evoked anxiety-like behavior, but this blockade in the second phase did not affect incision-evoked mechanical hypersensitivity. These findings suggest that surgical incision induced a two-phase ERK activation in the ACC, the first phase may involve in the incision-induced pain hypersensitivity and anxiety-like behavior, and the second phase may contribute to the maintenance of incision-induced anxiety-like behavior.

Puerarin has an analgesic effect on procedural pain in burn dressing changes by acting on $P2X_3$ receptor

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The procedural pain evoked by burn dressing changes is common and most painful in patients. Puerarin can inhibit inflammation and oxidative damage. This study was aimed to evaluate the effects of puerarin on procedural pain in burn dressing changes and the possible involvement of $P2X_3$ receptor. The visual analog scale (VAS) score was assessed. Blood pressure, pulse at pre., mid. and post-dressing changes after administration of puerarin was monitored. Blood glucose, insulin and cortisol in peripheral blood were measured. Healthy volunteers were observed as control. The effect of puerarin on the expression of $P2X_3$ receptor in PMCs of peripheral blood was observed by immunohistochemistry and RT-PCR. Blood pressure and pulse at pre., mid. and post-dressing changes after administration of puerarin showed no difference ($p>0.05$). Blood glucose, insulin and cortisol in peripheral blood in puerarin treated group were lower than those in 0.9% sodium chloride treated group. The expression of $P2X_3$ receptor protein and mRNA in puerarin-treated group at post-dressing changes significantly reduced ($p<0.01$) compared with pre-dressing changes. The expression of $P2X_3$ receptor protein and mRNA in 0.9% sodium chloride treated group at post-dressing changes had no obvious change ($p>0.05$). The expression of $P2X_3$ receptor protein and mRNA in puerarin-treated group and 0.9% sodium chloride treated group was higher than that in healthy volunteers ($p<0.01$) . Results showed that puerarin had analgesic effect on procedural pain in dressing changes of the burn wound by acting on $P2X_3$ receptor. This work was supported by the grant (№s 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№s 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province.

Maternal caffeine exposure impairs contexture learning and memory of rats

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Caffeine is a widely used substance consumed by over 80% of the populations in western countries. The risk of daily caffeine intake in pregnancy women on fetal growth and development, and the long-term outcomes on the offspring, is still unclear and controversial. In this study, we aimed to investigate if maternal caffeine exposure could have any effect on the learning and memory ability of the offspring. Pregnant female SD rats were given by drinking water containing caffeine (1g/L) in the whole gestation period. Contexture fear conditioning (CFC) test was performed when the male offspring were 3 month old. On CFC test day 1, rats were put into a chamber with steel grid for 3 min and then followed with 10s tone stimuli; at the end of tone stimuli an electrical foot shock (0.5A, 0.5s) was given. On test day 2, rats were put into the same chamber for 5 min and the 'freezing' time was recorded. On test day 3, rats were put into a new chamber for 3 min followed with another 3 min conditional tone stimuli. The freezing time was also recorded. The results showed that the freezing time of maternal caffeine exposure rats on test day 2 was significantly shorter than the control rats (105 ± 14s vs 173 ± 21s, $p<0.05$), indicating impaired hippocampus dependent contexture learning and memory in caffeine exposure rats. On test day 3, caffeine exposure rats showed similar freezing time compared with the controls (31 ± 15s vs 47 ± 40s, $P>0.05$) in the novel environment, indicating unchanged anxiety and locomotor activity levels in caffeine exposure rats. During tone stimuli period, caffeine exposure rats showed reduced freezing time compared with the controls (112 ± 33s vs 167 ± 4s, $p<0.05$), indicating that the hippocampus independent memory was also impaired in caffeine exposure rats. In summary, our results showed that maternal caffeine exposure has significant impact on the learning and memory of the offspring.

Relationship between cord vitamin A level and neurodevelopment at two years of age in a suburb of Chongqing, China

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The present study was conducted to measure vitamin A (VA) concentrations in pair-matched maternal and cord serum samples and to investigate the relationship between cord VA level and intelligence development at two years of age. A total of 157 maternal-neonatal pairs in Tongliang, a suburb of Chongqing, China, were recruited and demographic information was investigated. Blood from cord and mothers were collected after delivery to measure serum retinol by high-performance liquid chromatography. Intelligence development at two years was measured by the Gesell Development Schedules (GDS) which was expressed as development quotients (DQs). The concentration of cord blood retinol level was (0.68 ± 0.19) μmol/L. After adjusting for the confounding factors such as gestational age, sex, maternal education level, environmental tobacco exposure and birth head circumference, the vitamin A placental transport ratio (cord VA level / maternal VA level) presented significantly positive correlations with motor DQs and average DQs ($p<0.01$), and cord vitamin A level showed significantly positive correlations with language DQs and social DQs ($p<0.05$). The adaptive and average DQs in high cord blood VA group were significantly higher than those of low cord blood VA group ($p<0.05$). Our data suggested that perinatal vitamin A status may have long-term impact on intellectual development. Close correlation possibly exists among VA level in cord blood as well as VA placental transfer ratio and intellectual development at two years of age.

Effects of marginal vitamin A deficiency beginning from pregnancy on hippocampus synaptic plasticity related genes expression in offspring rats

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The present study was conducted to investigate the effects of marginal vitamin A deficiency (MVAD) beginning from pregnancy on hippocampus synaptic plasticity related gene expression in offspring rats. Sixteen female rats were randomly divided into control and MVAD groups. The dams and pups were fed with normal diet (VA 6500 IU/kg) and MVAD diet (VA 400 IU/kg) in control and MVAD group, respectively. Eight female pups were respectively killed at postnatal day 1 (P1d), P2w, P4w and P8w in two groups. Serum vitamin A (VA) concentration was monitored by high-performance liquid chromatography. The mRNA expressions of NMDA receptor (NR) subunits NR1, NR2A, NR2B, CAMK?α, Arc and CBP in hippocampus were detected by real time PCR. The serum VA concentration of MVAD group was significantly lower than that of control group at P8w ($P<0.05$). Real time-PCR results showed that mRNA levels of NR1 and NR2B in control group were significantly higher in most time points than those in MVAD group. The expression of Arc in control group was significantly higher than that in MVAD group at P4w and P8w ($p<0.05$), and CAMKIIα level in control group was higher than that in MVAD group at P2w and P4w ($p<0.05$). There was no difference of CBP level between the two groups. The data showed that MVAD beginning from pregnancy have influences on postnatal mRNA expressions of important genes related to synaptic plasticity pathway, such as NR1, NR2B, Arc and CAMKIIα.

Changes of RARα and synaptic plasticity signaling pathway related gene expressions in hippocampus of young rats after morris water maze

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The study investigated the changes of retinoic acid receptors (RAR) and synaptic plasticity pathway related gene expressions in hippocampus of young rats after Morris Water Maze (MWM) test. Sixteen 7 weeks female rats provided with normal diet (VA 6500IU/kg) were randomly divided into MWM group and non-MWM group. A ten days MWM test was conducted in the two groups. Eight female rats with MWM or non-MWM test were killed at 8 weeks old, respectively. Serum vitamin A (VA) concentration was monitored by high-performance liquid chromatography. The mRNA and protein expressions of RARα, RARβ, NMDA receptor subunits NR1, NR2A, NR2B, CAMK II α, Arc and CBP in hippocampus were detected by real time PCR and immunofluorescence respectively in the two groups. There was no difference of serum VA level between MWM and non-MWM groups, while the mRNA expressions of RARα, NR1, NR2A, Arc, CAMK II α and CBP in hippocampus were higher in MWM group than those in non-MWM group ($p<0.05$), and the mRNA levels of RARβ and NR2B remained unchanged after MWM test ($p>0.05$). The protein expressions of RARα, and NR1 increased after MWM test as well. There were positively correlation between the mRNA levels of RARα and NR1, NR2A, NR2B, Arc and CBP. There are increases of some synaptic plasticity signaling pathway related genes after MWM test, and RARα may have close relationship with their changes in the formation and maintain of learning and memory. The results indicate some relationship between retinoic acid signaling pathway and synaptic plasticity pathway.

Effects of methionine and choline on SV2C in lead exposed cells

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The lead can affect neurotranmitters and impair learning and memory in the nervous system. The release of the neurotranmitters depends on the proteins of synaptic vescial transport. It is still not clear whether the lead can damage the proteins of synaptic vescial transport. We aimed to explore the changes of synaptic vesicle protein 2C (SV2C) in lead exposed cell and study the combination effect of methionine and choline on SV2C level. Neuro2a (N2a) cells were divided into normal group, lead exposed group (lead acetate 10^{-3} mol/L), methionine and choline treatment group (methionine and choline 20 mmol/L, lead acetate 10^{-3} mol/L). We used reverse transcription polymerase chain reaction (RT-PCR) and Western blotting techniques to detect the expression of SV2C mRNA and SV2C protein respectively in the 24, 48 hours in different groups. The expression of SV2C was markedly decreased in lead exposed cells comparison with that in the normal group, and there were significant differences ($P<0.05$) between the lead exposed group and the normal group. The level of SV2C was obviously increased in methionine and choline treatment group comparison with that in lead exposed cells. There were significant differences ($p<0.05$). These results suggest that lead can damage the protein of synaptic vescial transport and affect the release of neurotransmitters and lead to damage of nervous system. Methionine and choline can improve the level of SV2C in lead exposed cells. Methionine and choline may play an important role in the prevention and treatment of lead poisoning. This work was supported by the grant (№ 30760211) from National Natural Science Foundation of China.

NMDA receptor activation injures pancreatic islets of C57BL/6 mice *in vivo*

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N-methyl-D-aspartate receptor (NMDAR) is an important subtype of ionotropic glutamate receptor, which is well known in the central nervous system, yet it's not clear enough that the function of NMDAR in peripheral non-neural tissues. NMDAR has been detected in the pancreas of adult rats. Our earlier study showed that over-activation of NMDAR played important roles in hypoxia induced impairment of rat fetal pancreas development. The aim of this study was to investigate the effects of prolonged exposure to N-methyl-D-aspartate (NMDA) on pancreatic islets of C57BL/6 adult female mice in vivo. C57BL/6 mice were divided into control group and NMDA group (NMDA, 8mg/kg). Each mouse received intraperitoneal injection for 7 consecutive days. Blood samples were collected from tail vein at the seventh day and the pancreatic tissues were stained with hematoxylin and eosim (H&E). Out result show that the blood glucose of the NMDA group is significant higher than the control group (10.0 ± 1.1 vs. 6.5 ± 0.7 mmol/L, $P<0.01$). HE stain showed that both the number of the islets in unit area and the ratio of the islet area to the pancreas area of NMDA group is lower than control group ($P<0.05$). These results suggest that over-activation of NMDA receptors can cause pancreatic islet damage.

Effect of histone acetylation on transcriptional regulation of amyloid precursor protein activity

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The histones acetylation (HA) is thought to be involved in the destabilization and restructuring nucleosomes, which is probably a crucial event in the control of the accessibility of DNA templates to transcriptional factors (TF). A current working hypothesis is that recruitment of co-activators with HAT activity by promoter-bound TF results in the acetylation of histone residues of nearby nucleosomes, which increase the accessibility of DNA to the transcription machinery. The histone deacetylase are critically involved in cell cycle regulation, cell proliferation and the development of human cancer. To better understand the link between chromatin modification and amyloid precursor protein (APP) expression, we have investigated the level of HA at APP promoter. HDAC inhibitor, TSA and butyrate, activated the transcriptional activity of APP promoter in U87mg cells, Pur α knock-out cells as well as HeLa cells. It seems there were more transcriptional activities on HeLa cells than on U87mg cells. It means the degree of HA is different in these cell lines. Pur α can suppress the HA inhibitor Butyrate and TSA induced APP transcriptional activity. Egr-1 expression level was dramatically increased in HeLa cells and U87mg cells. The sequence analysis demonstrated that there is an Egr-1 binding site in the APP promoter and located in the +65 to +79. Our experiments on ChIP's assay already showed that Egr-1 binds to the App promoter, GST-Pull-down assay and co-IP results showed the interaction between Pur α and Egr-1 in vitro and in vivo. Together, our findings indicate that APP promoter can be activated by HA. Purαcould completely suppress the activation induced by histone deacetylase inhibitor. All these might be closely related to the increased level of Egr-1 since Egr-1 and Pur α has associated interaction *in vitro* and *in vivo*.

Interplay between Pur α and Egr-1 in the transcriptional regulation of amyloid precursor protein gene expression

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The Alzheimer's disease was first described in 1906 and was identified as an ''unusual disease of the cerebral cortex'', which caused memory loss, disorientation, hallucinations. The most important hallmark in Alzheimer's disease study was the establishment of amyloid hypothesis (Amyloidβ). The Aβ aggregates on the CNS to form the senile plaque which disrupts the brain cells, clogs the points of cell-to-cell communications, activates immune cells that trigger the inflammation and devour the disable cells and ultimately, kills cells. In our previous study, we found that the Pur α protein can down-regulate the amyloid precursor protein (APP) promoter activity. In order to better understand the mechanism, we checked the effects of Egr-1 on the transcriptional regulation of APP promoter. The experimental results proved that Egr-1 was a positive regulator for APP promoter. Our data of luciferase assay showed that when Egr-1 binding sites were deleted, there is no function to up-regulate the APP promoter activity. The Pur α also can suppress the function of Egr-1. Using the $Pur^{-/-}$ cells for transactivation check, the suppression was removed. The further data of gel shift, ChIP's assay as well as western blotting suggested that Pur α suppress both the endogenous expression and the exogenous stimuli induced Egr-1 expression. The ChIP's assay and gel shift also showed that the Egr-1 and Pur α can competitively bind to the same site in APP promoter. The immunohistological results also showed the colocolization of these two proteins. From the above, we believe that there might be a displacement mechanism of Pur α for Egr-1 in the transcriptional regulation and the interplay between Pur α and Egr-1 can control APP expression.

Protective effect of chitooligosaccharides on hydrogen peroxide-induced PC-12 cells death by inhibition of oxidative damage

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Oxidative stress which manifested by protein oxidation and lipid peroxidation has long been linked to cell death in many neurodegenerative diseases, especially in Alzheimer's disease (AD). Currently, although no reliable drug or remedy could be used for treating or preventing the symptoms of these diseases, treatment with antioxidant is a promising approach to slow the process. Chitooligosaccharides (COSs) are biodegradation products of chitosan with the properties of free radical scavenging and anti-tumor, etc. In this paper, we assessed the effect of COSs (M.W. 1500, DD. 90%) on hydrogen peroxide (H_2O_2)-induced cytotoxicity in PC-12 cells. The results showed that pretreatment of PC12 cells with COSs reduced H_2O_2-induced toxicity, intracellular reactive oxygen species (ROS) generation and lipid peroxidation in a dose-dependent manner. H_2O_2-induced apoptosis represented as the DNA fragmentation, the concentration of intracellular Ca^{2+} and caspase-3 activity were also attenuated by COSs. These results suggest that COSs could protect the PC-12 cells from H_2O_2-induced injury through the inhibition of oxidative damage, intracellular calcium influx, DNA fragmentation and eventually prevention of cell apoptosis. Taken together, these data suggest that COSs may be a promising approach for the treatment of AD and other oxidative stress related neurodegenerative diseases.

Effects of chronic stress on ability of spatial learning-memory and expression of brain-derived neurotrophic factor in mice

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Many studies have shown that chronic stress is harmful to health, even led to diseases, meanwhile it impacts the brain cognitive functions and leads to learning-memory impairment. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family. In the present study, we evaluated the effects of chronic stress on the ability of spatial learning-memory, and the expression of brain derived neurotrophic factor (BDNF) in the hippocampus and prefrontal cortex (PFC) of mice. The ability of spatial learning and memory of mice was measured using Morris water maze, and the expressional changes of BDNF in mice were detected by immune-histochemical method. The results showed that compared with control group mice, the ability of spatial learning and memory of mice were significantly decreased; and the BDNF expression in the CA1, CA3 region and DG of hippocampus and PFC were significantly reduced in stress group mice. On the seventh day after stress period, the BDNF expression of the hippocampus and PFC in stress group mice showed a remarkable decrease, compared with control group mice. The results indicate that chronic stress causes the damage of mice's spatial learning and memory function which may be closely related to the down-regulation of BDNF expression. This work was supported by Shandong Province Natural Sciences Fund ZR2009DL009 and the 11th Five-year Plan Provincial Key Construction Project.

Study on the Cognitive Functions and the Regulation of α7 Nicotinic Acetylcholine Receptors by Fructus Lycii in aging mice

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Nicotinic acetylcholine receptors (nAChRs) containing α7 subunits are thought to assemble as homomers. α7-nAChR has been implicated in modulating learning and memory, and alterations of α7-nAChR have been found in patients with Alzheimer's disease (AD). Fructus lycii (the fruit of Lycium barbarum) has long been used in oriental medicine as an anti-aging agent. As herbal medicine has received increasing attention for the treatment of AD, the purposes of our study were to investigate the cognitive functions and the regulation of α7 nicotinic acetylcholine receptors **by** fructus lycii in aging ICR mice. The aging ICR mice were operated different dosages of fructus lycii by intragastric administration, then the cognitive functions were measured by Morris water maze, the expression levels of α7nAChR in cortex and hippocampus were detected by immunohistochemistry and western blotting. Compared with the aging ICR mice, the abilities of learning and memory, the expression levels of α7nAChR in cortex and hippocampus were all increased in two groups of receiving fructus lycii. The fructus lycii can remarkably improve the cognitive functions of aging mice and the mechanism may be due to the upregulation of cerebral α7nAChR.

Effects of prenatal administration of PCP on hippocampal neurogenesis in neonatal rats

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To evaluate whether chronic prenatal exposure to Phencyclidine (PCP) could affect the development of hippocampal neurogenesis of the offspring, the pregnant female SD rats received repeated s.c. injections of PCP during the last two weeks of gestation. Behavioral assessments were performed on dams to confirm expression of behavioral sensitization. Locomotor activity was recorded by a Supermex analyzing system. All animals received s.c. BrdU At 24 h after the last injection of PCP, then rats were perfused and the brains were removed. For BrdU immunostaining, sections were incubated with rat anti-BrdU monoclonal antibody and with Cy3-conjugated donkey anti-rat IgG. The results showed that female rats received repeated PCP administrations showed a marked increase of locomotor activities on days 1, 5 and 10 and a development of behavioral sensitization. At 21 days after birth, the numbers of BrdU-positive cells in the granule cell layer in the male or female of PCP-treated group were significantly increased by approximately 77%, the locomotor activities in the PCP-treated group were significantly decreased by approximately 30% than those in the control group. Concerning a differentiation of BrdU cells, there was no difference in the ratio of neuron to glia between the PCP-treated and the control group. These results suggest that increase of cell proliferation in the hippocampus may possibly affects behavioral abnormalities during infants in the offspring.

Effects of triptolide on neuronal apoptosis of hippocampus in AD cellar model

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A recognized and effective method for Alzheimer disease (AD) has not been found up to now. The rise of the immunoinflammatory theory of AD prompts people to look for anti-inflammatory drugs or immunomodulators to treat AD. Our previous studies have shown that anti-inflammatory traditional Chinese medicine triptolide (TP) can improve learning and memory abilities of AD rat model. The study was intended to explore effects of TP on neuronal apoptosis of hippocampus in AD cellar model. The AD cellar model was established by action of microglial conditioned medium induced by aggregated Aβ1-40 (20μg/ml) on rat cultured hippocampal neurons. MTT assay and TUNEL staining were used to observe the effects of TP at different dosages (5μg/ml and 25μg/ml respectively) on neuronal apoptosis of hippocampus in AD cellar model in different phases (2 hours, 24hours respectively). After 2 hours of cultivation, the apoptotic number of hippocampal neurons in model group was more than normal control group ($P<0.05$), and the apoptotic number of hippocampal neurons in other groups have no significant difference. After 24 hours of cultivation, the apoptotic number of hippocampal neurons in model group was obviously increased as compared with normal control group ($P<0.01$), and the apoptotic number of hippocampal neurons in low-dose TP group and high-dose TP group was obviously decreased as compared with model group ($P<0.01$), and the apoptotic number of hippocampal neurons in high-dose TP group was obviously decreased as compared with low-dose TP group ($P<0.01$). It is concluded that TP can inhibit neuronal apoptosis of hippocampus in AD cellar model.

Insulin-like effects of visfatin on human osteoblasts

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To investigate the effects of visfatin on human osteoblasts (HOBs) and the involved possible mechanisms. HOBs were treated with visfatin or insulin. Alizarin Red S staining was used for assessment of human osteoblasts matrix mineralization. The expression of visfatin in HOBs was determined by reverse transcription-polymerase chain reaction (RT-PCR); HOBs proliferation was assessed by measuring [^{3}H]thymidine (2μCi/ml) incorporation into trichloroacetic acid (TCA)-insoluble material and by cell count. Expression of Alkaline phosphatase (ALP), procollagen I C-terminal propeptide (PICP) and osteocalcin (OC) mRNA was determined by Real-time quantitative RT-PCR. ALP activity in the cell monolayer and PICP in the cell-conditioned medium were measured using ELISA kit, osteocalcin (OC) protein secretion was measured by RIA, the expression and tyrosine phosphorylation of insulin receptor (IR), insulin receptor substrate-1 (IRS-1) and IRS-2 were detected by immunoprecipitation and Western blot. IR tyrosine phosphorylation inhibitor HNMPA-$(AM)_3$ was used for the signaling pathway study. Visfatin mRNA was not expressed in human osteoblasts. Visfatin induces the tyrosine phosphorlation of IR, IR-1 and IR-2. Visfatin promotes glucose transport, proliferation, type I collagen production and mineralized nodule formation, and HNMPA-$(AM)_3$ suppresses these effects of visfatin. Furthermore, high concentration of visfatin treatment down-regulated production of osteocalcin, and visfatin has no effect on expression and activity of ALP. visfatin can stimulate the tyrosine phosphorylation of IR, IRS-1, and IRS-2 in human osteoblasts and can promote osteoblast glucose transport, proliferation, type I collagen production, and matrix mineralization through the IR transduction pathway. This work was supported by the National Natural Science Foundation of China (30872708), the National Natural Science Foundation of Hunan Province (05C0163) and the Post-doctorate Scientific Research Special-purpose Project of Hunan Province (2008RS4012).

The role of asymmetric dimethylarginine in myocardial aging of aged rats and its mechanisms

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Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS), plays an important role in the development of cardiovascular diseases, and accumulated evidence indicates that oxidative stress, change in aging-related gene expression and mitochondrial dysfunction are closely related to aging. This study was to determine the role of ADMA in myocardial aging of aged rats, subsequently to investigate the direct effect of ADMA on senencence of primary cardiocytes and finaly to explore the potencial mechanisms for ADMA-induced myocardial aging. Furthermore, the effects of calorie restriction (CR), antioxidant N-acetylcysteine (NAC) and resveratrol on ADMA-induced myocardial aging were also investigated. Results showed that endothelium-dependent relaxation of aortas was impaired in 24-month-old rats fed ad lib (AL) compared to aged CR rats or young rats. This impairment was accompanied with increased senescence-associated β-galactosidase staining, upregulation of aging genes P53 & P21 transcriptions and downregulation of longevity gene Sirt1 transcription in myocardium of aged AL fed rats. Simultaneously, the concentration of endogenous ADMA was significant elevated, whereas transcription & activity of dimethylarginine dimethylaminohydrolase (DDAH, the major metabolic enzyme of ADMA), nitrite/nitrate concentration and mitochondrial ATP production were distinctly decreased in myocardium of aged AL fed rats compared to aged CR rats or young rats. All of above effects of aged rats could be significantly improved by treatment with CR. Interestingly, similar senecent effects observed in aged rats could be induced by treatment of primary cardiocytes with ADMA, and these senescent effects induced by ADMA could be improved by treatment of primary cardiocytes with NAC and resveratrol. These results indicate that the elevated ADMA plays an important role in myocardial aging of aged rats, and the mechanism may be related to the increases of oxidative stress & aging-related gene transcription and downregulation of longevity gene transcription & mitochondrial ATP generation.

Involvement of longevity gene sirtuin-1 in switch of white/brown adipocytes

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Mechanisms responsible for age-related obesity are not well known. Recent study reported that brown adipose tissue, which has antiobesity effects, was detectable in adults and negatively associated with age. Longevity sirtuin-1 (SIRT-1) gene is of great importance in preventing aging and in energy metabolism, and is also decreased with age. In the present study, we examined whether SIRT-1 participats in brown adipose formation by modulating expression of PR domain-containing 16 (PRDM16), a newly reported determinative gene controlling brown adipose formation, which can initiate switch of white preadipocytes to brown adipocytes. We found that in visceral white adipose of obesity patients (30∼39 years old male, BMI⩾28, exclude other metabolic disorders, n=13), positive immunoreactivities of both SIRT-1 and PRDM16 were decreased compared with age and sex matched lean control (BMI<23, without any metabolic disorder, n=13) by immunohistochemical staining. In cultured 3T3-L1 white preadipocytes, treatment of SIRT-1 gene agonists including resveratrol (10 μM) and (E)-3, 5, 4'-trimethoxystilbene (BTM-0512, 0.3 μM) increased the expressions of SIRT-1 and PRDM16 mRNA, while SIRT-1 antagonist nicotinamide (NAM, 5mM) significantly reversed the effects of BTM-0512 on PRDM16 expression. These results indicated that SIRT-1 gene might be involved in the switch of white preadipocytes to brown adipose by promoting PRDM16 express. It is reasonable to postulate that age-related obesity may be at least partly due to the decrease of SIRT-1 expression and subsequently reduction of brown adipose formation.

Aβ4-8 is one of the key sides of amyloid precursor protein (APP) interactions

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Amyloid precursor protein (APP) has been believed to play an important role in the development of Alzheimer's disease (AD). It has been proposed that APP molecules could interact with each other and these interactions might promote an adhesion of cells with APP on their plasma membrane. In order to explore the pathogenic mechanism of AD and to reveal the effects of the interactions between APP on occurrence and development of AD, the key sites and phenomena for the APP interactions were investigated. Immunofluorescence, co-IP, Cell Wound Healing Assay, Transwell Assay, cell aggregation Assay and MTT Assay were used in this study. In this study, we verified these effects of APP in COS-7 cells and demonstrated that the APP interactions could inhibit the migration or promote the adhesion of target cells with APP on their plasma membrane. However, these effects mediated by APP interactions could be inhibited by Aβ42 or the antibodies induced by Aβ28. On the other hand, further studies revealed that APP could mediate the adhesion of target cells on the different matrixes including Aβ4-8, Aβ1-11, Aβ1-28, Aβ1-35, Aβ1-42 and C99, but could not mediate the adhesion on the matrixes including Aβ28-42 and rat Aβ1-42, rat C99. Similarly, these APP-mediated effects on the adhesion of target cells on different matrixes were also inhibited by Aβ42 or the antibodies induced by Aβ28. Results suggested that the extracellular region of Aβ42, especially Aβ4-8, might play an important role in the interactions between APP molecules. It was suggested that the adhesion between neurons and extracellular Aβ42 aggregates might be mediated by APP interactions on their plasma membrane and extracellular Aβ42 aggregates, eventually leading to neuron dysfunction.

Packaging of rAAV-VEGF165 and testing the biological activity

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To construct recombinant adeno-associated virus (AAV) simultaneously carrying the human vascular endothelial growth factor 165 ($VEGF_{165}$) gene, and assess its specifically expression in human umbilical vascular endothelial cell (HUVEC) and biological activity in vitro. Plasmid pAAV-$VEGF_{165}$ was constructed by $VEGF_{165}$ segments amplified from pET-32a(+)-$VEGF_{165}$ carrying $VEGF_{165}$ gene. The recombinant expression plasmid pAAV-$VEGF_{165}$ and pAAV-GFP were co-transfected into HEK293 cells with pAAV-Helper and pAAV-RC for recombinant AAV replication and package through homologous recombination, respectively. After identifying the virus titer by real-time PCR method, cultured HUVEC were infected with this recombinant virus to detect overexpression and the effect of proliferation. The $VEGF_{165}$ gene was successfully amplified and recombinant pAAV-$VEGF_{165}$ was verified by double digestion and DNA sequencing. The system provided a high packing ratio of more than 90% and the purified recombinant virus had a high titer of 6.0×10^{10} pfu/mL. After HUVEC were infected with this recombinant virus, the expression of $VEGF_{165}$ were specifically confirmed by western blot analysis. Infection with rAAV mediated $VEGF_{165}$ gene dramatically increased the effect on proliferation of HUVEC relative to that observed in non-infected or rAAV-GFP infected groups. rAAV-$VEGF_{165}$ was packaged successfully with the ability of infecting HUVEC to highly express $VEGF_{165}$ and biological effect of proliferation, thus $VEGF_{165}$ targeted gene therapy of human diseases is possible.

Puerarin protects against high glucose-induced apoptosis by inhibiting calpain activation in HUVECs

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The aim of this study was to investigate whether puerarin could protect against high glucose-induced apoptosis by suppressing calpain activation in human umbilical vein endothelial cells (HUVECs). METHODS: HUVECs were exposed to normal glucose (5.5mmol/L) or high glucose (33mmol/L) for 48 h. Then cell apoptosis and caspase-3 activity were determined. The expression of heme oxygenase-1 (HO-1) mRNA was evaluated by RT-PCR analysis. The activation of calpain and HO activity were also detected. RESULTS: Compared with the normal glucose group, exposure of HUVECs with high glucose for 48 h resulted in the significant increases in calpain and caspase-3 activity, and apoptosis, which were prevented by co-incubation with puerarin (10^{-6}, 10^{-5}, or 10^{-4} mol/L) in a concentration-dependent manner. HO-1 mRNA expression and HO activity were decreased in HUVECs treated with high glucose for 48 h. Compared with high glucose group, co-incubation HUVECs with puerarin and high glucose induced the increases in HO-1 mRNA expression and HO activity. HO-1 inhibitor protoporphyrin IX zinc (II) abolished the inhibitive effect of puerarin on high glucose-induced calpain and caspase-3 activation, and apoptosis. Conclusion: The data show that puerarin protects against high glucose-induced endothelial cells apoptosis by a mechanism involving upregulation of HO-1 expression and inhibition of calpain activity. This work was supported by Scientific Research Fund of Zhejiang Provincial Education Department (Y200908945).

The primary research of $cPLA_2$ in vascular remolding of big arteriole in hypertension

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Hypertension can result in Vascular remolding of big arteriole. The main reason is due to the imbalance between proliferation and apoptosis of Vascular Smooth Muscle Cells (VSMC). We used immunohistochemical technique, configuration measure and western blot analyis to determine the expression of Phospho-cytosolic phospholipase A_2 ($cPLA_2$) and Phospho-extracellular Signal-regulated kinase kinase (MEK) of the aorta' VAMC. We demonstrated that the blood pressure was significantly increased in 8-week-old SHR, 16-week-old SHR, 24-week-old SHR, it was statistically correlated to blood pressure of the same old WKY rats ($P<0.05$). Each SHR group's ratio of heart weight to body weight was significantly higher than that of the same age in the control WKY group with increased age. The positive rate of $cPLA_2$ was lower in every SHR group compared with the same age control WKY group and was statistically correlated between the different rats ($P<0.00$), and between the different age SHRs ($P<0.00$). The rule of expression of MEK was different from that of $cPLA_2$, with increased week age while increased the expression of p-MEK positive immunostaining rate, and it was significantly higher in every SHR group than in the same age control WKY group and was statistically correlated between the different rats ($P<0.00$), and was statistically correlated between the different week age rats ($P<0.05$). Western blot had the same result as immunohistochemical techniques. The conclusion is $cPLA_2$ can inhibit apoptosis and increase proliferation in the the spontaneous hypertension rats aorta' VSMC, and it can also promote generation and assembly of the collagen fibers, which result in the remolded vascular. The possible mechanism is through MEK/ERK1/2 signal passage, subsequently leads to downstream phosphorylation of $cPLA_2$.

Suppression of 11beta-hydroxysteroid dehydrogenase type 1 activity diminish insulin resistance and hypertriglyceridemia in metabolic syndrome

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Metabolic syndrome, a constellation of the interrelated risk factors that appear to directly promote the development of cardiovascular disease including hypertension, dyslipidemia, insulin resistance and obesity. Changes in the secretion or metabolism of glucocorticoids, which have important functions in the adipose, liver, kidney and vasculature, have been reported to be associated with Metabolic Syndrome and hypertension. The intracellular conversion of cortisone to cortisol by 11beta-hydroxysteroid dehydrogenase type 1 (11β-HSD1) controls the tissue concentrations of the active glucocorticoid cortisol. Because the various cardiovascular and metabolic activities depend on the level of glucocorticoids, the hypothesis that 11β-HSD1 plays an important role in the hypertension, dyslipidemia, and insulin resistance of metabolic syndrome has come to light and it is necessary to be experimentally tested. The obese and lean SHR/NDmcr-cp (SHR-cp) rats which were implanted with radiotelemetry were administrated with vehicle or compound 11 (10 mg/kg/d, gavage) for 3 weeks. Cardiovascular, metabolic, and renal function were measured before and during these 3 weeks of administration. 11β-HSD1 activity in adipose tissue and liver of SHR-cp was significantly decreased after the chronic administration of compound 11. The mean arterial pressure, glucose intolerance, insulin resistance, and hypertriglyceridemia with no effect on heart rate, body weight gain, or microalbuminuria in obese SHR-cp group were significantly decreased by administration of compound 11. In association with the changes in cardiovascular and metabolic function, plasma renin activity was decreased in obese SHR-cp treated with compound 11. From all above, these results indicate that an important mechanism in the elevated blood pressure, plasma triglycerides and insulin resistance associated with metabolic syndrome is related to 11β-HSD1 activity in liver and adipose tissues.

Regulation of renin expression and its signal pathway

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The renin angiotensin aldosterone system (RAAS) plays a major role in the regulation of systemic blood pressures, fluid and electrolytes homeostasis, and in kidney development. Renin release is tightly controlled by several mechanisms including a beta receptor mediated mechanism, a macula densa control, and a baroreceptor-mediated mechanism. Recent work suggested a crucial involvement of the cyclic AMP (cAMP) pathway in activation of the renin gene. In many cell types, the intracellular increase of cAMP induces the phosphorylation of numerous transcription factors, including the cAMP Responsive Element Binding protein (CREB). Phospho CREB is then able to interact in a complex with CBP (CREB Binding Protein) and p300 and occupy the cAMP Responsive Element (CRE) of the promoter. We hypothesized that the stimulation of renin expression by the cAMP pathway involved the recruitment of CREB at the CRE of the renin gene promoter, its association with CBP and p300, and their acetyltransferase activities. To address this hypothesis, Chromatin Immunoprecipitation was used to identify modifications of histone, which usually associated with gene activation or repression in in vivo tissue or cultured cells, with forskolin stimulation or gene knock down with either one or combination of specific siRNA for CREB, CBP and p300. In rennin expressing cells, knock down of CREB, CBP or p300 is associated with a lower expression of the renin gene and the combination of the three siRNA induced a drastic loss in renin expression associated with the loss of activation markers around the CRE of the renin promoter. Therefore these results show that the complex [CREB/CBP/p300] is involved in the activation of renin gene expression *in vitro* and *in vivo*, *via* post-translational modifications of histones 3 and 4 by CBP and p300.

The CYP2D6 polymorphism in relation to the metabolism of TMG and Sparteineis in the Uighur population

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TMG and Sparteineis are first line treatment for hypertension and arrhythmia separately, a common cause of cardiovascular disease. Response to TMG and Sparteineis are variable and unpredictable. CYP2D6 are major contributors to the metabolism of TMG and Sparteineis, and their genetic polymorphism influences plasma concentration of TMG and Sparteineis used for hypertensive and antiarrhythmic treatment. Our objectives were to identify CYP2D6 genetic polymorphisms in Uighur population in China, to compare the allele frequencies with those of Han groups, and to evaluate variant-induced functional variations in TMG and Sparteineis metabolism. The mutations of CYP2D6 gene were detected by PCR-based DNA sequencing in 100 unrelated Uighur subjects. Then, HRM assays were used to genotype CYP2D6 variant alleles in 500 unrelated Uygur subjects. On this basis, Weston-blotting and HPLC were used to detect the enzyme activity of variants. The results showed that the frequency of CYP2D6*2 is 30% in Chinese Uygur population, has a difference compared with the previously reported 15% in Han groups. The frequency of CYP2D6*35 is 70% in Chinese Uygur population, has a significantly difference compared with the previously reported 10% in Han groups. Both CYP2D6*2 and CYP2D6*35 are extensive metabolizers by detecting their enzyme activities, which were consistent with the previously reported. These findings indicate that the dose of TMG and Sparteineis should be increased used for antihypertensive and antiarrhythmic therapy in Uygur population.

An experimental study in reducing blood pressure in spontaneously hypertensive rats through extracellular signal-regulated kinase inhibition

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This study was planned to investigate the effect of the extracellular signal-regulated kinase 1/2 (ERK1/2) inhibitor PD98059 on the blood pressure. This study used immunohistochemistry and in situ hybridization methods to investigate the effect of ERK1/2 inhibitor PD98059 on the SHR rat blood pressure, as well as comparative study of the relationship with ERK1/2 signal transduction pathway. SHR group of 8-week-old of age began to show an elevated blood pressure. There was significant difference in blood pressure in 16-week-old group and 24-week-old group comparing with the control group. There was no significant arterial blood pressure difference between PD98059 intervention group and the same age of SHR group. In PD98059 intervention group of 24-week-old, the ratio of inner/outer diameter of the renal small artery is higher than the SHR control group. However, there is no significant difference between PD98059 group and the SHR control group ($P>0.05$). In SHR 16 and SHR24 groups, the positive staining of ERK1/2 phosphorylation on the renal small artery endothelial cells and smooth muscle cells was significantly higher than the Wistar control group. In PD98059 group, the phosphorylation of ERK1/2 positive rate on the renal small artery VSMC, was significantly lower than SHR control group ($P<0.05$). The results suggested that even though PD98059 can inhibit phosphorylation of ERK1/2, it is not able to significantly lower arterial blood pressure. This work was supported by Natural Science Foundation of China (30860095) and supported by Natural Science Foundation of Ningxia (NZ0651).

Cardio-protective effects of endothelin-1 mRNA antisense oligodeoxynucleotide on streptozotocin-induced myocardial injury in diabetic rats

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Cardiomyopathy is one of the most severe complications in diabetic patients. Endothelin has potent effect of vasoconstriction .This study used the endothelin-1 mRNA antisense oligodeoxynucleotide (ET-1AS-ODN) on diabetic rats to examine the protective effects of ET-1AS-ODN on diabetic cardiomyopathy (DC) and discuss the pathogenesis and mechanism of DC in a rat model. The result showed the cardiac function of the animals in the ET-1 AS-ODN treatment groups improved significantly, and the survival rate was raised significantly (66.67%, 90%, 70% and 100% for ET-1 AS-ODN 12; 6; 3 and 1.5 OD/kg groups, $P<0.05$, $P<0.01$). Serum MDA, LDH, ET and ET/ ON decreased. SOD and GSH-PX activities were increased in the ET-1 AS-ODN groups (12; 6 and 1.5 OD/kg). The result shows that Endothelin-1 mRNA antisense oligodeoxynucleotide can prevent the myocardium from injury in diabetic rats and has a protective effect on the myocardium. The mechanism of the protective effect of Endothelin-1 mRNA antisense oligodeoxynucleotide involves inhibition of endogenous ET-1 production, enhanced capacity of clearing oxygen free radicals and resistance to oxidative injury. It indicates that ET-1 does play an important role in the pathogenesis and mechanism of cardiomyopathy in diabetes.

In vitro study of Api6 expression in lipid loading and inflammatory macrophages

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Apoptosis inhibitor 6 (Api6), also known as AIM (Apoptosis Inhibitor expressed by Macrophage) or SPα (Soluable Protein alpha), is a newly defined member of the group B scavenger receptor cysteine-rich (SRCR) superfamily. Previous studies have suggested its important roles in immune system regulation, but the involvement of Api6 as a scavenger recepteor in lipid metabolism is rarely studied. The aim of this study was to define the expression pattern of Api6 in lipid loading and inflammatory macrophages. The human monocyte/macrophage lineage THP- 1 cells and murine Raw264.7 macrophages were incubated in the presence of 100μg/ml oxidized low-density lipoprotein (oxLDL) alone, oxLDL plus 200ng/ml lipopolysaccharide (LPS) and LPS alone. Foam cell formation after treatment was evidenced by red oil O staining. Real-time PCR results showed that after LPS or oxLDL treatment, the mRNA levels of Scavenger receptor SRA were increased 6-fold, 2.5-fold and 6-fold, 44-fold in Raw264.7 cells and THP-1 cellls respectively, but the expression of Api6 in Raw264.7 had no change before and after treatment. The mRNA levels of Api6 increased 2.7-fold after LPS and oxLDL treatment in THP-1 cells. The expression levels of Api6 in these macrophages were highly related to the presence of nuclear receptor liver X receptor (LXR) isoform LXRα. This study reveal that due to the lack of LXRα isoform, the basic expression level of Api6 is low in Raw264.7 macrophages, which makes it an ideal cell line for further study related to physiopathologic function of Api6 in lipid metabolism.

Effect of emodin on nociceptive signal transmission of myocardial ischemia mediated by $P2X_{2/3}$ receptor on stellate ganglion neurons.

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The stellate ganglia (SG) in cardiac afferent nerve pathway are involved in the nociceptive transmission of myocardial ischemic injury. $P2X_3$ and $P2X_{2/3}$ receptors in DRG neurons contribute to nociception. The present research is to investigate the effects of emodin on the nociceptive signal transmission in myocardial ischemia mediated by $P2X_{2/3}$ receptor in SG. Thirty SD rats were divided into three groups randomly, 10 rats in each group. A: Control group, made subcutaneous injection of normal saline; B: myocardial ischemic injury rat model was established by subcutaneous injection of isoproterenol 5mg/kg; C: myocardial ischemic injury rats treated with emodin (ip.) in final concentratioin of 50mg/kg and then made subcutaneous injection of isoproterenol 5mg/kg. The electrocardiogram (ECG) of rats at pre-injury and post-injury 7 and 14 days were tested. The expressions of $P2X_2$ and $P2X_3$ receptors in SG neurons were measured by immunohistochemisty and RT-PCR. ECG shows that, compared with Group B, emodin could against T wave and J point changes effectively ($p<0.05$). $P2X_{2/3}$ receptor expression of SG in Group B were significantly higher than those in the other ones ($p<0.05$). The results suggest that emodin may inhibit the nociceptive signal transmission of myocardial ischemia mediated by $P2X_{2/3}$ receptor in SG to alleviate myocardial ischemia. This work was supported by the grant (№s 30860086, 30860333 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№s 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province.

CyclophilinA inhibits cholesterol accumulation *via* the caveolin-1 bonding-dependent pathway in vascular smooth muscle cells

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Epidemiological studies have shown cholesterol- decreasing could reverse angiosclerosis in foam cells. CycolphilinA and caveolin-1 are key proteins participating in the cholesterol transport of foam cells. However, the functions and expressions of these proteins in vascular smooth muscle cells (VSMCs) are still unclear. We undertook the research to examine the expressions of cyclophilinA and caveolin-1 in lipid-loaded VSMCs induced by oxidized low density lipoprotein (Ox-LDL) and their effects on cellular cholesterol accumulation. When VSMCs were treated with 75mg/L Ox-LDL for different times (0h, 24h, 48h and 72h), the expression of cyclophilinA and caveolin-1 were significantly decreased in a time-dependent manner by Western-blot and Immunofluorescence analysis. Oil Red O dye showed that lots of lipid droplets accumulated in VSMCs with Ox-LDL treatment after 48 hours. HPLC analysis revealed that content of cellular total cholesterol and cholesterol ester increased greatly. Moreover, the content of cellular total cholesteorl was increased in VSMCs treated with cyclosporinA as an inhibitor of cyclopilin A. IP showed that cyclophilinA was combined with caveolin-1 and the combination was weakened in VSMCs treated with cyclosporinA. Yet the expressions of caveolin-1 and cyclopilinA didn't change significantly in cyclosporinA groups. Our results have shown that cyclophilinA can inhibit the intracellular cholesterol accumulation through combining with the caveolin-1 in VSMCs. This work was supported by the National Natural Science Foundation of China (30770868, 30600249 and 30971170) and the construct program of the key disciplines in human province.

PUMA is critical for cardiomyocyte apoptosis induced by ischemia reperfusion injury

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Cardiomyocyte loss induced by apoptosis is one of the characteristic pathological phenomena during ischemia reperfusion injury (IRI). As a member of BH3-only proapoptotic Bcl-2 family, PUMA (p53 upregulated modulator of apoptosis) is an essential mediator of p53-dependent and -independent apoptotic pathways. Cardiomyocyte hypoxia/reoxygenation (H/R) on neonatal rats were modeled as cardiac ischemia/reperfusion injury *in vivo*, and PUMA targeted siRNA was transfected into cultured primary cardiomyocytes *in vitro*. LDH activity was determined by Automatic Biochemical Analyzer. Cell apoptosis was determined by Annexin V apoptosis assay and PI staining combined Flow Cytometry. Cell viability was determined by MTT assay. RT-PCR and Western blot was used to detect the mRNA and protein of PUMA, Bcl-2 and Bax. Spectrophotometer was used to test caspase-3 activity. It was shown that very few PUMA protein was expressed in normal cardiomyocytes. PUMA expression was significantly up-regulated upon H/R *in vivo*, and positively correlated with cell apoptosis rate, Caspase-3 activity and Bax expression, while, negatively correlated with Bcl-2 expression. Moreover, it was found that PUMA targeted siRNA could significantly improve the tolerance of cardiomyocyte to H/R injury and reduce cardiomyocyte apoptosis rate by down-regulating Bax expression, Caspase-3 activity and up-regulating Bcl-2 expression. PUMA, therefore, appears to be a novel therapeutic target in cardiac IRI.

Calcitonin gene-related peptide-mediated protective effect of rutaecarpine against endothelial cell injury induced by lysopbosphatidylcholine

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It has been shown that the cadioprotection of rutaecarpine is mediated by calcitonin gene-related peptide (CGRP) *via* activation of vanilloid receptor subtype 1 (VR1). Vascular endothelial cells can also synthesize CGRP and CGRP could protect against endothelial dysfunction induced by lysopbosphatidylcholine (LPC). In the present study, we explored whether the endothelial cell-derived CGRP is involved in the protective effect of rutaecarpine against endothelial cell damages. Human umbilical vein endothelial cells (HUVECs) were treated with rutaecarpine (10^{-7}, 3×10^{-7} or 10^{-6} M) for 24 h. Endothelial cell injury was induced by LPC (5 mg/l), and evaluated by cell viability and lactate dehydrogenase activity. The level of CGRP mRNA was detected by RT-PCR, and protein level was measured by radioimmunoassay. Treatment with rutaecarpine increased the production of endothelium-derived CGRP in a concentration-dependent manner, which was abolished by capsazepine, a competitive antagonist of VR1. Rutaecarpine significantly attenuated the endothelial cell damage induced by LPC, which was prevented and aggravated by capsazepine or CGRP (8-37), antagonist of CGRP receptor. The present results suggest that rutaecarpine prevent LPC-induced endothelial injury, which is related to upregulation the expression of CGRP via activation of VR1 in endothelial cells.

Study on the protective effects of ebselen on myocardial ischemical reperfusion injury in rats

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To study the protective effects of Ebselen (Ebs) on ischemical reperfusion myocardial cell apoptosis in rats. The improvement experimental model of MIR injury was established by ligation of left anterior descending coronary artery for 30 min and reperfusion for 60 min in rats. Myocardial infarct size (TTC staining) was measured, and histopathological changes of myocardiurn were observed by light microscope. The apoptosis of cardiomyocytes were detected by *in situ* end labeling method. The activity of caspase-3 were detected by immunohistochemical, the protein expression of caspase-3 were determined by Western blot. The results showed that compared with the model of MIR, the changes of myocardial pathological damages were improved by Ebs (1 mg/kg; 2 mg/kg and 4 mg/kg). In Ebs group, it was not an obvious swelling of cells and infiltration of inflammatory cells, the myocardial infarction size were reduced ($P<0.05$, $P<0.01$). The rate of myocardial apoptosis were significantly decreased ($P<0.05$, $P<0.01$), the expression of caspase-3 positive cells were significantly decreased ($P<0.05$), and a dose-dependent, the protein expression of caspase-3 were also significantly decreased. In conclusion, Ebs preconditioning has the protective effects on myocardial ischemia-reperfusion injury in rats such as reducing myocardial apoptosis, to reduce myocardial injury in pathology, Ebs can relieve the myocardial infarction size. The mechanism of which may be related to the increasing of antioxidase activity, and the decreased myocardial apoptosis.

Aldehyde Dehydrogenase 2 (ALDH2) rescues myocardial ischemia/reperfusion injury

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Mitochondrial isoform of aldehyde dehydrogenase (ALDH2) may be beneficial to ischemic heart diseases although the underlying mechanism is unknown. This study was designed to investigat the mechanism involved in ALDH2-induced cardioprotection against ischemia/reperfusion (I/R) with a special focus on autophagy, which is known to be triggered by I/R. Wild-type (WT) and ALDH2 overexpression mice were subjected with ischemia and reperfusion and cardiac function was assessed using the Langendroff and cardiomyocyte edge-detection systems. Compared to WT mice, ALDH2 overexpression significantly reduced the infarct size, facilitated the recovery of post-ischemic LV function following I/R and reversed cardiomyocyte contractile dysfunction in response to hypoxia/reoxygenation. Autophagy was induced in the ischemia phase with a further increase during reperfusion in WT mice. ALDH2 transgene significantly upregulated autophagy during ischemia, the effect of which was accompanied with activation of AMPK and inhibition of mTOR. On the contrary, ALDH2 transgene overtly inhibited autophagy during reperfusion which was associated with activation of Akt and mTOR. Additionally, levels of cardiac 4-hydroxy-2-nonenal (4-HNE), endogenous toxic aldehyde, were increased by ischemia and maintained during reperfusion. Elevated levels of HNE resulted in the formation of HNE adducts that inactivated AMPK and Akt, induced cardiomyocyte contractile dysfunction, the effects of which were alleviated by ALDH2. Furthermore, ALDH2 transgene prevents HNE modification of the LKB1 and PTEN signaling axis. ALDH2 offers a beneficial myocardial protective effect against I/R injury involved in the detoxification of toxic aldehtde. The ALDH2-elicited beneficial effect was associated with an increase of autophagy during ischemia and a decrease of autophagy during reperfusion, via AMPK- and Akt-dependent mechanisms, respectively.

The novel steroidal glycoside from the dried bulb of Allium Macrostemon Bunge inhibits platelet activation

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The dried bulbs of Allium macrostemon Bunge is the main sources of traditional Chinese medicine ''xiebai'', which is used for the treatment on myocardial ischemia, but the mechanism is still far from clear. Recently, a new furostanol glycoside, were isolated from the dried bulbs of Allium macrostemon Bunge. The structure of the new compound was elucidated by the spectral data elucidation as: (25*R*)-26-*O*-β-D-glucopyranosyl-5α-furostane-3β, 12β, 22, 26-tetraol-3-*O*-β-D-glucopyranosyl(1→2)[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside. This study was designed to investigate inhibitory effects of the new compound on the expression of activation-dependent platelet membrane surface glycoproteins and phosphorylated AKT. We performed flow cytometric analysis using monoclonal antibodies, anti-CD62P (P-selectin), and anti-CD61. *In vitro* ADP stimulation of platelets taken from healthy volunteers produced significant increases in the mean channel fluorescence intensities (MFI) for CD62P (126% increase) and CD61 (60% increase). The enhanced MFI for CD62P and CD61 was suppressed to the control level by pretreatment with 80 μM (53% suppression) and 320 μM (78% suppression) of the compound. Moreover, pretreated human platelets with different concentrations (5; 20; 80 and 320 μM) of the compound, the expression of phosphorylated AKT was significantly decreased in a concentration dependent manner. The results suggested that the novel steroidal glycoside inhibits the ADP induced platelet activation by decreased CD61, CD62P expressions and the AKT signal pathway maybe contributes to the antiplatelet effect.

Observation on antiatherosclerosis ability of total flavonoids from Jumi

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Jumi is medicinal plant and its main producing areas situate in Suichang of Zhejiang Province. In this study, we observed antiatherosclerosis ability of food-roune hyperlipidemia by total falconoid from Jumi in SD rats. We established hyperlipidemia model rats, and intervened by total falconoid from Jumi. Rats were divided into normal control groups, hyperlipidemia model groups, higher dose groups and lower dose groups. We determined the level of total cholesterol (TC), trilaurate glycerin (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and atherogenic index (AI), and histopathology of liver. Total falconoid from Jumi significantly reduced the serum levels of TC, TG, LDL and AI, and increased HDL/TC. It had significant curative effects on hepatic adipose infiltration in rats. The results suggest that total flavonoids from Jumi could regulate disturbance of lipid metabolism, hepatic adipose infiltration and arteriosclerosis.

The Protective Effect of Recombinant Adenovirus-HSP20 on CHF rats

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This study was aimed to explore the protective effect of recombinant adenovirus-HSP20 on CHF rats. The HSP20 gene was amplified by reverse transcription polymerase chain reaction using human heart DNA as template. The product of RT-PCR were ligated with shuttle plasmids Ad-Track and then Ad-HSP20 plasmid were obtained from homologous recombination in E coli-BJ5183, into which the AdeasyTM plasmid of adenovirus framework. The recombined Ad-Track plasmid or Ad-hsp20 plasmid were transfected into 293 package cell line. The CHF rats were prepared by abdominal aorta constriction and were infected by recombined adenovirus via intravenous injection after twelve weeks. To observe the distribution and the expression of HSP20 gene in CHF rats, 36 rats were randomly divided into 4 groups: Ad-HSP20 group; Ad-Track group; NS group and sham-operated as control group. The cardiac function was evaluated by echocardiography and hemodynamics, the serum levels of hsp20 was detected by Elisa, the expression of HSP20 by RT-PCR and immunohistochemistry were detected in different groups. Apoptosis of cardiomyocytes was determined by Tunel staining. Ad-HSP20 were constructed successfully and the titer of purified adenovirus was 1.25×1011 vp/ml. Maximal rate of pressure increase (+dp/dt(max)) and decrease (-dp/dt(min)) values, and Left ventricular end systolic pressure were increased significantly, while left ventricular end diastolic pressure was decreased significantly in Ad-HSP20 group. The ratio of Tunel-positive cardiomyocytes to total number of cardiomyocytes in the Ad-HSP20 group was significantly reduced as compared with the vector and Ad-Track group. These data indicates the cardioprotective effects of HSP20 in CHF rats.

Contrast of hemodynamic parameters and aortic tension induced by CLP & LPS septic shock in rats

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To observe the differences of mean arterial blood pressure (MABP), heart rate (HR) and thoracic aorta tension induced by two septic shock models in male Sprague-Dawley rats. We used cecal ligation and puncture (CLP, the cecum was ligated and punctured twice with an 18-gauge needle) 20 hours and lipopolysaccharide (LPS, the rat was injected with 10 mg/kg lipopolysaccharide intraperitoneally) 6 hours to establish septic shock in rats. The carotid artery was cannulated and connected to a pressure transducer to determine MABP and HR. Isolated thoracic rings were mounted on an organ bath and the tension of the vessel was recorded. Our results shown that, the mortality was 65.2% (30/46) in CLP shock rats, but no rats dead in LPS shock rats (0/24). The two models showed significant decrease in MABP and HR, and CLP model decreased more ($P<0.01$) (CLP decrease 55.7% and 71.5%, LPS decrease 41.5% and 58.3%). And constriction by high K^+ (60 mmol/L) or 10^{-6} mol/L phenylephrine (PE) in endothelium-intact aortic rings were all decreased, and LPS model decreased more ($P<0.01$) (CLP decrease 22.8% and 26.4%, LPS decrease 70.1% and 72.9%). Constriction by high K^+ or PE in endothelium-denuded aortic rings had the similar decrease. In conclusion, MABP and vasoconstriction responsiveness of aorta were all decreased in two septic shock models in rats. CLP model decreased more in the MABP, though LPS model decreased more in vasoconstriction responsiveness of aorta.

Identification and Functional Characterization of CYP2D6*10 and CYP2D6*15 in the Uighur population

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CYP2D6 is an enzyme of potential importance for the metabolism of clinically used anti-arrhythmic and antihypertensive drugs, include Metoprolol and TMG. And it exhibits genetic polymorphism with inter-individual differences in metabolic activity. The aim of this study was to investigate the functional characterization of CYP2D6 variants identified in Chinese Uygur population. HRM assays were used to genotype CYP2D6*10 and CYP2D6*15 variant alleles in 500 unrelated Uygur subjects. The results showed that Chinese Uygur population of CYP2D6*10 and CYP2D6*15 in the distribution of frequencies were 15% and 35%. This result is significantly different from previous report, which indicated the frequency of CYP2D6*10 was 51.3% in the Chinese population while the frequency of CYP2D6*15 was less than 1% in the Eastern population. However, there is no report on frequency of CYP2D6*15 in Chinese Uygur population. On this basis, Weston-blotting and HPLC were used to detect the enzyme activity of variants. We found the CYP2D6*10 and CYP2D6*15 are both PM phenotype, the findings indicate that the dose of metoprolol should be increased for anti-arrhythmic therapy in Uygur population and the extensive monitoring for adverse drug reaction should be taken when using TMG for antihypertensive therapy in Uygur population.

From global to individual: the discovery of a novel C-type lectin protein from five-pace snake venom

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Viper venom was known to possess complex proteins and multiple biological activities which attracts drug researchers' attentions and efforts. In our study, coupled chromatographic technique was built up for five-pace snake venom proteome assay and protein mining. The results indicated that the venom was characteristically hemorrhagic and anticoagulative. Thus a novel protein that can affect platelet aggregation was discovered, and then proved to be a new member of C-type lectin family due to their homologous N terminal sequences when Blast with protein database. The protein was a 26.59 kD dimer that consists of two subunits with Mw of 14.14 kD and 13.03 kD and the two chains share 45% identity with each other. The protein was finally nominated as agacutegrin. Similar with other members, it could mainly inhibit human platelet aggregation induced by ADP and collagen, with IC_{50} value of 4.07 and 5.70 μg/mL, respectively. It acted as an analog of glycoprotein Ib, binding with von Willebrand Factor (vWF) competitively. What's more, agacutegrin had an inhibitory effect on proliferation in carcinoma cells *in vitro* with ID_{50} among 18-35 μg/mL, as well as the adherence of carcinoma cells to extracellular matrix, e.g. fibronectin, laminin and collagen. Moreover,agacutegrin had no thrombin like enzyme, phospholipase A_2, arginyl esterase activities and hemorrhagic toxin. These results suggested that agacutegrin had the potential to be a promising drug to treat arterial thrombus, atherosclerosis, even the metastasis of tumor cells.

Interaction of SK2 channel with ryanodine receptor type2 in heart

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Small conductance calcium-activated potassium (SK) channels are the calcium-dependent potassium channels which link intracellular calcium to membrane potential changes. However, little is known about modulation of SK channel activity. Ryanodine receptors (RyRs) induce calcium-triggered calcium release and modulate cardiac calcium handling. This study explored the possibility of SK2 (SK channel subfamily N, member 2) -RyR2 protein interaction using a yeast two hybrid system, coimmunoprecipitation and immunohistochemistry. Anti-SK2 antibody which was bound to protein A-agarose precipitated RyR2 protein from mice atrial and ventricular tissues and RyR2 protein could be detected on immunoblots using anti-RyR2 antibody. The reverse experiments were tested using anti-RyR2 antibody bound to protein A-agarose to precipitate the SK2 from mice mice atrial and ventricular tissues, and SK2 channel protein could be detected on immunoblots using anti-SK2 antibody. We next assessed the regional localization of SK2 channel and RyR2 in isolated mouse atrial cells using immunohistochemistry. Confocal images showed that double immunostaining of SK2 and RyR2 had distinct patterns around and in the Z-line in atrial myocytes. Our study first presents that SK2 protein interacts with RyR2 in native cardiac tissues and colocalizes in part with RyR2 in isolated single mouse cardiomyocytes.

Inhibitory effects of garlic polysaccharide pretreatment on cultured cardiocytes in anoxia/reoxygenation injury

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To investigate the inhibitory effects of garlic polysaccharide pretreatment on cultured cardiocytes in anoxia/reoxygenation (A/R) injury and its mechanisms. The model of anoxia/reoxygenation injury was developed by $Na_2S_2O_4$ (5 mmol·L^{-1}) in cultured neonatal rat cardiocytes. The cultured cardiocytes were pretreated with different concentrations of garlic polysaccharide (10 mg·L^{-1}; 30 mg·L^{-1} and 100 mg·L^{-1}) before anoxia/reoxygenation. Cell viability was determined by MTT, the activity of LDH, CK, AST, SOD, iNOS, and the contents of MDA and NO were determined by colorimetry respectively. The apoptosis ratio of cardiocytes was determined by flow cytometry. Compared with A/R model group, garlic polysaccharide could significantly improve myocardial cell viability in anoxia/reoxygenation injury, increase the activity of SOD, inhibit the release of LDH; CK and AST in supernatant, decrease the contents of MDA, NO and apoptosis rate of cardiocytes in a concentration dependent manner. Garlic polysaccharide has protective effects on cardiocytes in anoxia/reoxygenation injury and the mechanism may be related with its oxygen free radical scavenging activity.

Protective effect of Humulus flavonoids against high glucose-induced vascular endothelial dysfunction in rat thoracic aorta

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To investigate the protective effect of Humulus flavonoids on vascular dysfunction induced by high glucose. We used aortic rings with endothelium from male Sprague-Dawley rats, and mounted in an organ bath. Isometric contraction of the rings was recorded. Results showed that after incubation with high glucose (44 mmol/L), the relaxation evoked by acetylcholine was significantly decreased in an endothelium-dependent manner. When coincubated with Humulus flavonoids and high glucose, the vasodilator dysfunction induced by high glucose was reversed in a dose-dependent manner. N-nitro-L-arginine methyl ester, an inhibitor of NOS, offset the protective effect of Humulus flavonoids. The non-selective guanylate cyclase (GC) inhibitor methylene blue also completely abolished the protective effect of Humulus flavonoids. In conclusion, Humulus flavonoids alleviate the endothelium-dependent vasodilator dysfunction induced by high glucose in rat aortic rings. Increased NOS activity and stimulation of GC may be involved in the protective effects of Humulus flavonoids.

MSCs improve restoration of H_2O_2 injury PC12 cells by anti-apoptosis and cytokines release

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It has become interestingly clear that transplantation of mesenchymal stem cells (MSCs) can effectively restore injury tissue's biological function *in vivo*. However, few MSCs are induced to the biological functional cells under the injury local microenvironment. Our previous studies have found that MSCs transplantation into the hypoxic-ischemic brain damage (HIBD) of new-born rats enhances learning and memory function, and modulates expressions of some inflammatory cytokines, especially IL-6 in damaged area. Using co-culture system of H_2O_2 treated PC12 cells with MSCs *in vitro*, this study determined how MSCs modulated local immune responses in the injury microenvironment. MSCs increased proliferation ability and lactated dehydrogenase (LDH) activity of H_2O_2 treatment PC12 cells after 24h co-culture, and the number of apoptotic cells reduced in the co-culture group of PC12 cells and MSCs by using Annexin-V apoptosis assay. The bcl-2 mRNA was up-regulated and caspase-3 mRNA was downregulated in the co-culture group compared with the H_2O_2 treated PC12 alone group. Studies using ELISA revealed that the expression of IL-10 was gradually induced in the PC12 treatment alone group, and reduced in the co-culture group with MSCs following increase of H_2O_2 concentration. Compared with H_2O_2 treated PC12, increases release of IL-6 was found in the co-culture group of MSCs and H_2O_2 challenged PC12, however, lower than that of the MSCs alone group. Taken together, the current results indicate that MSCs repress apoptosis of PC12 cells following H_2O_2 treatment and modulate some cytokines of injury microenvironment in the condition of the co-culture system, which is advantaged to recover biological function of hypoxic-ischemic injury PC12 cells. This study was supported by the National Natural Science Foundation of China (No.30872670), and the key project of Chongqing Municipal Health Bureau (No.2009-1-41).

Phototherapy as a novel method to treat abnormal scar

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Hypertrophic scar and keloid are abnormal scarring tissues resulting from the imbalance of synthesis and degradation of collagen. The lesions are usually unsightly and associated with pruritus and pain. We tested whether phototherapy can be used to treat abnormal scar. Light emitting diode (LED) light source was used to treat fibroblasts *in vitro* and hypertrophic scar on animal. Type I collagen and MMP I and *in vitro* wounding assay were examined after exposing cells to different light doses. Hypertrophic scar on rabbit ear was created with a whole layer skin punch wounding. Low dose phototherapy inhibited fibroblast migration, collagen I production; increased collagenase secretion and its activity. Irradiation with 150 J/cm2 for 15 times reduced scar formation on rabbit ear. All are in a dose dependent manner. The results suggest phototherapy may be a potential noninvasive therapy for treating abnormal scar.

Directed differentiation of mouse-induced pluripotent stem cells generates dopaminergic nerve

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Induced pluripotent stem cells (iPSC) provide an invaluable resource for regenerative medicine as they allow the generation of patient-specific progenitors with potential value for cell therapy. We generated mouse induced pluripotent stem (iPS) cells from neural stem cells (NSCs) by using nonintegrating adenoviruses transiently expressing Oct4, Sox2, Klf4, and c-Myc. These adenoviral iPS (adeno-iPS) cells show DNA demethylation characteristic of reprogrammed cells, express endogenous pluripotency genes and form teratomas. The method of phase induction was used to culture iPS cells. After cultured in the serum-free medium containing bFGF and LIF for 10 d, 70.2 ± 7.8 percent of iPS cell masses were nestin-immune positive. After changing the medium with the serum-free medium containing B27 and IL-1 for 7 d, 22.7 ± 2.5 percent of cells were NSE-immune positive and 30.5 ± 3.4 percent GFAP-immune positive, which showed typical neuron-like morphology, with a round cell body and long processes. The positive rate of tyrosine hydroxylase (TH) was 10.1 ± 2.1 percent. The TH-immune cells were clustered, neuron-like shaped, but with less processed. Our results showed that iPSCs derived from NSCs can be induced and differentiate into dopaminergic neurons, which cast new insight into the mechanism research in the field of neuron regeneration.

Comparison of DNA methylation in Tau Promoter region between BMSC and BMSC-derived neurons

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Bone marrow mesenchymal stem cells (MSCs) have the capacity to be induced to differentiate in vitro into neuron-like cells and express neuron-specific biomarkers *in vitro*. We demonstrated that the expression of microtubule associated protein Tau, which is neuron-specific, increased when MSCs differentiated into neurons by the combination induction of DMSO and BHA. And we also found the *Tau* promotor was hypermethylated in MSCs, significantly higher than that in MSC-derived neurons. In order to prove the affair of demethylation of *Tau* promotor is a trigger to Tau expression, MSCs were induced following three different directions: neurons, cardiac muscle cells and hepatic cells. The activity of DNA methyltransferase (DNMT) decreased in all three cell types origin of MSCs, while the high level of DNA hypermethylation of *Tau* promotor just maintained in MSC-derived cardiac muscle cells and hepatic cells, in which Tau protein was not expressed. It is like that only when MSCs differentiated into neurons, the decrease of DNMT activity can make *Tau* promotor demethylated, the mechanisms of which is still unknown. These results suggested that the demethylation of *Tau* promotor is the key mechanism to trigger Tau expression during MSCs differentiation into neurons and the demethylation is related to the decrease of DNMT activity.

STK31 acts as a cell fate determinant in spermatogonial stem cell

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Spermatogenesis is an essential process for mammalian reproduction. The process starts with the mitosis of male germline stem cells (GSC) or spermatogonial stem cell (SSC). However, genes that are involved in regulating the self-renewal and differentiation of GSC are largely unknown due to the lack of *in vitro* model. Recent advances in GSC culture provide a valuable tool for functional genomics studies in GSC. *STK31* has been previously identified in spermatogonia, which exhibits a testis-specific expression pattern. In the present study, we aimed to determine the function of *STK31* in GSC. Using the GFP tagging method, we first demonstrated that *STK31* was localized to the cytoplasm and formed granular structure that divide asymmetrically during mitosis. Co-immunofluorescent staining with E-cadherin in mouse testis suggested that *Stk31* was expressed in the transition state between undifferentiated and differentiated spermatogonia. Using the GSC culture, we showed that the expression of *Stk31* was induced in retinoic acid (RA)-induced differentiation. Interestingly, *Stk31* proteins demonstrated mitotic asymmetry during GSC division after RA induction. Ectopic overexpression of *Stk31* using retroviral transduction induced clump dissociation in GSC colonies, which is a phenotypical change of GSC differentiation. These data indicate the involvement of *Stk31* in mouse spermatogonia cell fate determination. Further studies of *Stk31* in spermatogenesis *in vivo* are required for the identification of the asymmetry machinery of GSC and the signaling mechanism underlying cell fate determination.

All-*trans* retinoic acid pre-induction facilitates neuronal differentiation of mesenchymal stem cells

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All-*trans* retinoic acid (ATRA) plays an important role in nervous system development. However, the effect and mechanism of ATRA on neuronal differentiation of MSCs is still poorly realized. Here we reported that ATRA pre-induction could improve neuronal differentiation of rat MSCs. When MSCs were exposed to 1 μmol/L ATRA for 24h before induced by modified neuronal induction medium (MNM), induced neural-like cells expressed higher levers of neurons marker (Nestin, NSE, and MAP-2), but lower levers of neuroglia cells markers (CD68, GFAP, and GDNF), as well as exhibited higher resting membrane potential and intracellular calcium concentration than those of cells induced by MNM alone. MSCs expressed weakly retinoid X receptors (RXRs) including RXRα, RXRβ, RXRγ. The expressions of RARα and RARγ in MSCs were distinctly detectable, whereas RARβ almost could not be detected. However, the RARβ expression of MSCs after 24h ATRA pre-induction, not RARα, RARγ or RXRs, increased dramatically. Surprisingly, the redistribution of RARβ near the nuclear membrane in the differentiated neurons indicated that RARβ may be involved in neuronal differentiation of MSCs. We further detected transcription factors (TFs) activity of MSCs induced with or without ATRA using TranSignal^{TM} Protein/DNA Arrays. Our data showed that ATRA might cross talk with glucocorticoid signaling pathway, TP53, MAX, SMAD3/4, GATA and STAT family members to regulate cell development, cell death, cell-mediated immune response, cellular function and maintenance. ATRA treatment suppressed a set of TFs which involved in neurological disease (CREB1, THRA, THRB and STAT1) and developmental disorders (CREB1, FOXM1, MYOD1, NFATC4, NR2F2, PPARA, SRF, THRB, XBP1). In conclusion, our results demonstrate that ATRA per-induction facilitates neuronal differentiation of MSCs through pre-activating retinoid signaling pathway and regulating some transcription factors activity. This work was supported by the National Natural Science Foundation of China (No. 30830106).

The mRNA of four subtypes of muscarinic receptors are expressed in the gallbladder smooth muscle of Guinea pigs

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Muscarinic acetylcholine receptors are known to be in the regulation of smooth muscle constractions in various organs, but the muscarinic receptor subtypes in the gallbladder smooth muscle of guinea pigs are not clear. We detected the mRNAs of four muscarinic receptor subtypes in gall- bladder smooth muscle of guinea pigs. The tissue of the gallbladder was collected from six 10-week- old pigmented guinea pigs to determine mRNA expressions of muscarinic receptors with RT-PCR. The rank order of expression extent was M_3, M_2, M_4 and M_1 receptor. The result suggests that four subtypes of muscarinic receptors are present in the gallbladder smooth muscle of guinea pigs (M_1 to M_4), indicating the involvement of the four muscarinic receptors in the gallbladder contractions aroused by vagal neurotransmitter acetylcholine.

Growth of cultured neural stem/progenitor cells in chitosan-alginate- hyaluronate-heparan scaffolds

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Neural stem/progenitor cells (NS/PCs) therapy is a promising strategy for brain disorders, traumatic brain injuries and neurodegenerative disorders. However, a major problem is to build a compatible culture model for NS/PCs proliferation and differentiation. In this study, a new porous three-dimensional natural polymer scaffold comprised of chitosan, alginate, hyaluronate and heparan sulfate (C-A-Ha-He) was fabricated, and several structural determinants of its biological activity including the mean pore size, the porosity and degradation rate were identified. By evaluating the cellular proliferation, adhesion rate and alkaline phosphatase activity, we assessed the status of NS/PCs in the C-A-Ha-He scaffolds. Compared with chitosan-alginate scaffolds, C-A-Ha-He scaffolds provided a more suitable environment for NS/PCs proliferation and differentiation after 7 days. Moreover, immunostaining and observation by electron microscope indicated that NS/PCs in the C-A-Ha-He scaffolds might form an artificial neural network where neurons and glia establish connections and exhibit synaptic activities. These findings provide a biological basis for future application in screening medicine or transplantation of this artificial construct in neural repair. The study was supported by the National Science Foundation of China (30800288) and Doctoral Program Foundation of Institutions of Higher Education of China (20070141050) is greatly acknowledged.

Mechanical stimulus accelerate mesenchymal stem cells differentiating toward cardiomyocytes

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The adipose tissue-derived mesenchymal stem cells were induced to differentiate with 5-azacytidine, then stimulated by 8%, 1 Hz mechanical stretch for 10 days continuously. α-sacromeric actin and GATA4 were expressed little in the cells stimulated only by mechanical stretch, connexin43 (Cx43), trial natriuretic peptide (ANP), α-myosin heavy chain (α-MHC) and Nkx2.5 were not expressed, and the cell's ATPase activity was not increased. Except α-MHC, α-sacromeric actin, GATA4, ANP and Nkx2.5 were all expressed in the cells induced only with 5-azacytidine. After the cells induced with 5-azacytidine, the expressions of α-sacromeric actin, Cx43, GATA4, ANP, α-MHC and Nkx2.5 in the cells stimulated by mechanical stretch were all more than in those not stimulated by mechanical stretch, the cell's ATPase activity was also higher. Sole mechanical stimulus had little effect on adipose tissue-derived mesenchymal stem stem cells differentiating toward cardiomyocytes by itself, but mechanical stretch could accelerate the cells treated with 5-azacytidine differentiating toward cardiomyocyte. This work was supported by grants from the National Naturnal Science Fountation of China (30700156), Key Project of Tian Jin Naturnal Science Fountation (06YFJZJC02000) and Medical science Foundation (06MA344).

Pax6 maintains the EGF-responsive neural stem cell pool in the SVZ

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At late embryonic days of the cortex development, the main pool of neural stem cells, the subventricular zone (SVZ), formed in the cerebral cortex, along with an important event that neural stem cells (or intermediate progenitors) transit from FGF-responsive to EGF-responsive. Stem cells in the SVZ are both FGF and EGF-responsive. They maintain a steady pool in the SVZ; and keep a fine balance between proliferation and differentiation, as well as their population. In this study we provide evidences that Pax6 plays a crucial role in the regulation of the EGF-responsive stem cell pool in the SVZ. In the Pax6 homozygously mutant mice, we found that the neurospheres formed from mutant cortex are less than that from wild type mice cortex (E18.5d), which means less stem cell in the SVZ pool. Furthermore we found that the expression of EGFR in these neurospheres is lower than that of the wild type (by western blot). The EGF-activable cells was also decreased from 16.8% (wild) to 4.5% (mutant), in the mutant dorsal cortical SVZ (E18.5d) by flow-cytometry. These results suggest that Pax6 controls the balance of the SVZ EGF-responsive stem cell pool. By immunostaining of the cortex (E18.5d), we further confirmed the down-regulation of EGFR expression in the dorsal SVZ of mutant cortex. And then we detected the decreasing of a special EGFR+/Sox2+ population stem cells in the SVZ of mutant cortex, while not of the EGFR-/Sox2+ population. This suggests that Pax6 controls only one population of SVZ stem cells. In a further effort to study the possible mechanisms, although we found a DNA binding site of Pax6 in the EGFR promoter region by CHIP assay, but by luciferase assay, we failed to detect the direct activation of EGFR transcription by Pax6. So Pax6 regulate the EGF-responsive population not through the EGFR expression.

Establishment and characterization of a liver stem cell line with stable expression of functional ALB promoter and luciferase reporter gene

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Albumin (ALB) has been widely used as a liver-specific marker for the detection of hepatocyte differentiation. In this report, we constructed a liver stem cell line with stable expression of functional ALB promoter and luciferase reporter gene. ALB promoter was amplified by PCR, then ligated with pBGLuc plasmid containing luciferase reporter gene to get a pBGLuc-ALB plasmid. Relative ALB-GLuc activity of pBGLuc-ALB transfected HEK293, HP14.5 (Hepatic progenitor cells derived from E14.5 mouse fetal liver), LC14 (Live Cells derived from 14-day old mouse liver) and Hepa1-6 cells was coincident with ALB expression level in each cell lines. Afterward, HP14.5 cells were infected by ALB-GLuc retrovirus to get stable cell line, which could survive in medium containing high concentration of Blasticidin. Stable cell line were cultured with dexamethasone and HGF induction medium *in vitro*, relatively ALB-Gluc activity gradually increased from 3 to 12 days after induction and had a uniform increasing tendency compared with ALB expression detected by immunofluorescence. Therefore, we successfully constructed a liver stem cell line with stable expression of ALB promoter and luciferase reporter gene for further study of hepatocyte differentiation.

Sox2 regulate Erbb2 expression in cancer stem cells

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The cancer stem cell hypothesis proposes that cancers arise in stem/progenitor cells through disregulation of self-renewal pathways generating tumors which are driven by a component of ''tumor initiating cells'' retaining stem cells properties. Cancer stem cells are defined by a capacity for sustained self-renewal, persistent proliferation and tumor initiation or propagation. Sox2 is a core transcription factor in normal stem cells, maintaining the ''stemness'' characteristics and plays a crucial role in sustaining growth and self-renewal. Overexpression of Sox2 in glioma could increase its tumorigenicity. But the detailed mechanisms and the main target molecules of Sox2 in cancer stem cells are still not well-known. In this study we provide evidence that Sox2 could directly regulate the expression of Erbb2 (or Her2) in human breast cancer cell line MCF-7. There are several predicted Sox2 binding sites in the promoter region of the Erbb2 gene. Four different deletion constructs of Erbb2 promoter region were tested by luciferase assay. Transfection of Sox2 could upregulate the Erbb2 promoter mediated luciferase activity, in a dose dependent manner. Overexpression of Sox2 could also upregulate the expression of Erbb2 in MCF-7 cells, by realtime RT-PCR method. Another marker of cancer stem cells Cd133 was slightly elevated. All these results suggest that there is a Sox2/Erbb2 pathway in regulating the behavior of cancer stem cells.

RXRs antagonist RARs signal's effect of inducing apoptosis for OGD injury in PC12 cells

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Retinoid acid (RA) plays a dual role in cell apoptosis. How does RA effect apoptosis in oxygen- and glucose-deprivation (OGD) injury PC12 cells by RA receptors pathway? We studied apoptosis of OGD injury PC12 cells with different concentrations of ATRA and 9-Cis-RA challenge using flow cytometry, and the injury PC12 membrane damage by the LDH enzyme release detection. We found that apoptosis rates of the injury PC12 cells exposed ATRA from 5μM to 20μM concentration were significant increased ($P \leq 0.05$), however, no different between ATRA 1μM and control group (0μM). Surprisingly, the apoptosis rates of the PC12 cell exposed in the 10μM and 20μM 9-Cis-RA were significantly lower than that in the same concentration of ATRA ($P \leq 0.05$). However, the apoptosis rate in the 20μM 9-Cis-RA challenge was lower than that of the control group. The level of LDH release of ATRA stimulation was significantly increased from the beginning of the 5μM to 20μM concentration ($P \leq 0.01$), and no significant difference was found among the 9-Cis-RA stimulation groups compared with the control group ($P \geqslant 0.05$). As known, ATRA specificly activates RARs and 9-Cis-RA activates RARs and RXRs. These results suggest that activation of RARs by RA may play an enhancing apoptosis, while this effect could be to inhibit by the activation of RXRs in OGD injury PC12 cells. This work was supported by the key project of National Natural Science Foundation of China (No. 30830106).

The mechanism of bone marrow mesenchymal stem cell differentiation into islet-like cells in diabetic pancreas microenvironment *in vivo*

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Mesenchymal stem cells (MSCs) transplantation to overcome deficient β-cell population is an attractive proposal. Nevertheless, the efficiency of adult stem cells to differentiate into islet-like cells *in vitro* is low at present. The present study aimed to explore whether diabetic microenvironment could induce MSCs to transdifferentiate to islet cells and compensate insufficient β-cell. To investigate the mechanism of MSCs differentiated into insulin-producing cells. We transplanted male EGFP transduct bone marrow-derived MSCs to experimental diabetic female SD rats by multipoint injecting into subcapsular pancreas. MSCs not only act as ''seed'' cells, but also may exert a clinically useful immune modulatory effect, because of their immune privilege. 18 days later, glucose level in serum began to decrease gradually, and was approximate to normal value. Insulin and C peptide level in serum began increasing from 14 days. Reversal of diabetes was evidenced by normal insulin level in serum and normal intra-peritoneal glucose tolerance test. Pancreatic neo-genesis islets were verified by histology and morphometry. EGFP and insulin protein coexpression indicated that some of neo-genesis islet cells derived from transplanted MSCs and had normal function. Coexpression of Y-chromosome SRY gene and PDX-1 mRNA future clarified the results. RT-PCR and Quantitative Real-Time PCR revealed MSCs in pancreas microenvironment expressed transcription factor PDX1, Ngn3, Nestin, Nkx2.2 and PDX-4 at 1 weeks after transplation, these genes up-regulated and reached to peak at 4 weeks after transplation, then followed a down-regulating. Ngn3, Nestin, PDX-4 didn't detected 12 weeks after transplation. The PDX-1, Insulin and Glucagon up-regulated to peak. There are no polyploid and aneuploid cells in EGFP-labeled MSCs in pancreas. This study shows that bone marrow derived MSCs could contribute functional islets and expressed mark transcription factors/genes correlated β-cells' expression time series of developing in pancreatic microenvironment, there were no cell fusion of MSCs with pancreatic tissue cells. Which provide a theoretical and clinical applications of pancreas regeneration.

Extension of proliferative lifespan by introduction of telomerase reverse transcriptase gene into human epidermal stem cells *in vitro*

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Telomerase extends the proliferative lifespan and prevent replicative senescence in most somatic cells. Whether it has similar function in human epidermal stem cells remains to be determined. In this study, the expression features of human telomerase reverse transcriptase (hTERT) gene, telomerase activity and the gene expression spectra in the human epidermal stem cells from different developed stages were observed and compared by using cDNA microarray chip, TRAP-ELISA, western blot, immunocytochemistry and RT-PCR techniques and the method of cultured epidermal stem cell *in vitro*, respectively. Recombinant eukaryotic expression plasmid containing both hTERT and enhanced green fluorescent protein (EGFP) genes were provided by Professor Shiming Yang and transfected into the epidermal stem cells with the liposome mediated gene transfection method. The proliferation of the transduced cells was examined. The results showed that the telomerase activity and the cytalytic subunit of telomerase were positive and in low level in cultured epidermal stem cells derived from different developed stages but no expression in the keratinocyte cells. The gene expression spectra were obviously different in the cultured epidermal stem cells derived from different developed stages which were mainly associated with protein translation, energy synthesis and DNA replication. They are closely related to the development process of human epidermal stem cells. Introduction of hTERT into the epidermal stem cells not only yielded the expression of the hTERT gene and protein but also exhibited significantly elevated telomerase activity and proliferation. All these works establish the base of further gene modification, monoclone bolting, cellular special surface markers and cell differentiation mechanism. The ability to maintain the biological characteristics and proliferative lifespan of epidermal stem cells could have important applications in wound healing and skin tissue engineering.

Repair of deep skin burn defects with cultured epidermal stem cells and acellular amniotic membranes

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Tissue engineered skin constructed by cultured seed cells provides a new way to accelerate skin wound healing and improve the quality of tissue repair. Conventional models with dermal scaffold material based on collagen hydrogels, however, have poor stability and unsatisfactory longevity. Here, we describe an improved acellular amniotic membrane as a scafford with epidermal stem cells for tissue engineering skins and observe their clinical effect on deep burn wounds. The human epidermal stem cells was isolated from the skin samples by trypsin digesting method and purified by collagen adhering method, and then seeded on the acellular amniotic membrane to form the engineered skin. The biological attachment and growth of cultured epidermal stem cells were observed. Twelve cases of deep burn wounds were applied with the engineered skin. The results showed that the epidermal stem cells adhered to the surface of acellular amniotic membrane quickly after being seeded and exhibited a high colony formation efficiency. The wounds treated with engineered skin healed rapidly with good clinical ''take''. The average time of wound closure in engineered skin group was shortened than that of control group. There were no obvious evidence of immunological rejection and inflammatory reaction during the observation. This indicated that the acellular amniotic membrane with good histocompatibility may be a suitable scaffold with epidermal stem cells in the construction of tissue engineering skin to repair deep burn wounds.

Mesenchymal stem cells enhances the viability of primary culture of rat hippocampal neurons under oxidative stress

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Mesenchymal stem cells (MSCs) are known to have pluripotency to differentiate into cells of various multi-lineages and their therapeutic potential in treatment of neuronal lesions and degenerative diseases has been proposed. Excessive oxidative stress is thought to be a key factor inducing brain damages and neuronal death, especially in the hippocampus. In this study, we co-cultured rat bone marrow MSCs with primary hippocampal neurons under H_2O_2-induced oxidative stress and the effect of MSCs on rescuing neuron death was evaluated. The primary hippocampal neurons of neonatal rat were cultured for seven days before H_2O_2 stress. Two concentrations of H_2O_2 were used (100μM and 250μM) and the treatment lasted for 2 hours. After oxidative stress the hippocampal neurons were co-cultured with MSCs (1:1) for 24 hours and the viable cells were then measured by MTS assay. 24 hours after 100μM and 250μM H_2O_2 treatment, the viable hippocampal neurons were significantly reduced to 48% and 34%, compare with the blank control group ($p<0.05$ and $p<0.01$, respectively). Co-culture with MSCs after the H2O2 treatments significantly increased the viable cells to 98% ($p>0.05$, *vs* co-culture control group) and 91% ($p<0.05$, *vs* co-culture control group). These data indicated that in either mild or severe oxidative stress conditions, MSCs could enhance the viability of hippocampal neurons in vitro. The underlying mechanisms, including the differentiation of MSCs into neurons and the release of anti-apoptotic factors from MSCs, are currently under investigation.

Valproic acid eliminates quiescent cancer stem-like cells in pediatric GBMS by driving them into cell cycle and promoting radiation induced-DNA damages: an *in vivo* study in orthotopic xenograft models

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Clinical outcomes in patients with glioblastoma multiforme (GBM) remain dismal. Failure to eliminate cancer stem cells, particularly those in the quiescent G_0 phase, is believed to be a primary cause of tumor recurrence. This is because quiescent cancer stem cells are inherently resistant to chemo- and radiation therapies that are designed to kill rapidly proliferating cells. Here, we examined our hypothesis that valproic acid, a histone deacetylase inhibitor, can activate cell cycle progression of quiescent GBM cells and sensitize them to radiation induced cell killing in a panel of primary tumor-based orthotopic xenograft mouse models of pediatric GBMs. Our results showed that systemic treatment of GBM xenografts with VPA (600 mg/ml) through a subcutaneous osmotic pump at 1 ul/hr for 7 days activated the cell cycle progression of the quiescent GBM stem cells *in vivo*, leading to depletion of G0 phase $CD133^+$ GBM stem cells with concomitant increase of $CD133^+$ populations as detected with flow cytometric analysis of Hoechst 33342/Pyronin Y stained cells. In the activated cycling cells, VPA significantly elevated the intracellular levels of reactive oxygen species, the key mediators of radiation induced cell killing. As expected, VPA radiosensitized a panel of glioma stem-like cells and inhibited DNA repair, as evidenced by enhanced expression of DNA strand break marker histone r-H2AX, leading to significantly suppressed neurosphere formation *in vitro*. More importantly, VPA combined with radiation could prolong survive time of IC-1406GBM orthotopic xenograft model *in vivo*. In conclusion, our study identified a novel activity of valproic acid on quiescent GBM stem-like cells. Since VPA is an established drug that can pass through blood-brain-barrier, our findings have a great potential of expedited application in clinical applications.

Involvement of BMP/Smad and Smurf mediated ubiquitin-proteasome pathway in mechanical strain induced osteoblastic differentiation

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Osteoblast and its precuror are sensitive to mechanical strain, mechanical loading play an important role in osteoblast differentiation and bone formation, but the molecular event involved in mechanical signals transduction are poorly understood. In this study, osteoblastic cells MC3T3-E1 were exposed to cyclic uniaxial tenlile strain at 2000 μstrain via a four-point bending system. Mechanical strain had no significant effect on the cell proliferation by MTT analysis, but the differentiation marker gene expression and alkaline phosphatase (ALP) activity were enhanced. Induction of osteoblast differentiation by tensile strain was associated with increased bone morphogenetic protein-2 (BMP-2) production and the activation of Smad1/5 (p-Smad1/5). Addition of purified BMP-2 further increased the up-regulation of differentiation marker gene expression, ALP activity and p-Smad1/5 induced by mechanical strain, whereas these were blocked by BMP-2 antagonist Noggin. In addition, the mRNA level of Smad1and Smad 5 was not in accordance with their protein level, tensile strain had no effect on the the mRNA expression of Smad1 and Smad5, but Smad1/5 protein increased after loading. The degradation of Smad1/5 was inhibited by tensile strain, because Smuf1, a Smad ubiquitin regulatory factor that mediates Smad1and Smad5 degradation in a proteasome depended manner, decreased after tensile strain. Application of a proteasome inhibitor MG132 enhanced osteoblat differentiation gene expression induced by mechanical strain, this indicated that Smurf1 mediated ubiquitin- proteasome pathway was also involved in the mechanical regulation of osteoblast differentiation. Collectively, mechanical strain can induce osteoblast differentiation through activising BMP-2-Smad1/5 pathway and suppressing Smurf1 mediated ubiquitin-proteasome pathway.

Induction and culture of neural stem cells derived from human induced pluripotent stem cells

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Induced pluripotent stem cell (iPS cell) is a kind of stem cell which can be generated from adult cells. Neural stem cells (NSCs) derived from iPS cells which can be generated from patients are optimal seed cells for NSC-based therapy because it can avoid immunological rejection. Here, NSCs derived from iPS cells were obtained through three steps and a long term culture system was established. Briefly, iPS clones were cultured in embryoid bodies (EBs) medium for 4 days to form EBs. EBs were induced by retinoic acid (RA) for 4 days and then screened in serum free medium. After 7 days screening, EBs were collected and named iPS cell derived NSCs. For terminal differentiation, iPS cell derived NSCs were cultured in DMEM/F12 contained 10% fetal boving serum. For clonal analysis, iPS cell derived neurospheres were dissociated and diluted in 96-well plates. After one day's screening, EBs sticked to the bottom of flask and rosette constructions appeared on the 4th day. Then most EBs detached from the bottom and form uniform neurosphere-like clones. The immunostaining results showed nestin positive cells were significantly higher in induced EBs than control EBs ($p<0.05$). When cultured in serum containing medium, these iPS cell derived NSCs clones differentiated to neurons and astrocytes which expressed β-tubulin III and GFAP respectively. Additionally, real-time PCR indicated that the expression level of nestin gene in induced EBs were also significantly higher than control EBs ($p<0.05$). Moreover, there were a few single iPS cell derived NSCs survived and formed neurospheres in 96-well plates. Furthermore, iPS cell derived NSCs can be cryopreserved, and proliferated well when thawed. Our results suggest this inducing method is an effective mode for the differentiation of iPS cells to NSCs. Serum free medium used in our experiment is suitable for the long-term propagation of NSCs derived from iPS cells.

bFGF gene transfected heterotopic transplantation of autologous bone marrow mononuclear cells promote wound healing of skin burn

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Promoting wound healing, reducing scar formation and seeking more donor skin are dominating methods for treating skin burns especially over deep second degree burns. Stem cell technology has been widely used in the treatment of skin burn. Autologous bone marrow mononuclear cells are believed to function as stem cells which show a high capacity for differentiation and play a critical role in a variety of disease therapies, including skin wound healing. In this study, we report that basic fibroblast growth factor (bFGF) gene transfected autologous bone marrow mononuclear cells promote wound healing and reduce scar synthesis of skin burn in experimental mice. In addition, we found that autologous bone marrow mononuclear cell transplantation show an increased wound healing rate and decreased repair time after the bFGF gene is transfexted in deep second degree burns of the mice. Finally, these results suggest that bFGF gene transfected heterotopic transplantation of autologous bone marrow mononuclear cells represents an effective therapeutic procedure in wound healing and may contribute to the repair of skin burn. This study was supported by the Scientific and Technological Program Granted by Jiangxi Provincial Health Department (20093158).

Wnt signaling pathway regulates the differentiation of hepatic progenitor cells

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Wnt signaling plays an important role in embryonic liver development, morphogenesis, and organogenesis. Hepatocytes differentiate from the endoderm during embryonic development. Hepatic progenitor cells (HPCs) have been isolated from fetal liver and extrahepatic tissues. Here, we sought to determine the role of Wnt signaling pathway in regulating hepatic differentiation of fetal liver-derived HPCs. Using mouse liver tissues from E12.5 to postnatal day 28, we found that 13 of the 19 Wnt genes and almost all of Wnt receptors/co-receptors expressed in most stages. However, the expression of Wnt antagonists SFRP2, SFRP3/FrzB, and DKK2 was only detected in the early stages (i.e., before E16.5). We next established reversible stable HPCs derived from the E14.5 mouse fetal liver (aka, HP14.5). The stable HPCs were shown to express high levels of early liver stem cell markers (such as Oct4, DLK, Thy1, and N-CAM) but low levels of late liver markers (such as albumin and UGT1A), and can be induced to mature hepatocytes by dexmethasone. Furthermore, dexmethasone-induced ALB-GLuc activity, glycogen synthesis and ICG-uptake positive of HP14.5 cells were significantly inhibited by SFRP3/FrzB. Therefore, our results suggest that a tight regulation of Wnt signaling activity may be critical to mouse liver development and hepatic differentiation. This work was supported by the National Natural Science Foundation of China (No. 30771925).

Gender difference in FSHR positive cells derived from adult bone marrow stem cells indirect co-cultured with sertoli cells

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The experiment is aimed to investigate whether bone marrow stem cells (BMSCs) indirectly co-cultured with Sertoli cells (SCs) are able to differentiate into follicle- stimulating hormone receptor (FSHR) positive cells and to compare the sex difference. SCs were isolated from 12 day old SD rat testes and BMSCs were separated SD rat bone marrow. An indirect co-culture system was established using 0.4μm pore Transwell membranes. BMSCs and SCs were co-cultured for 7 days. Two days after the BMSCs were co-cultured with SCs, some of the male BMSCs expressed FSHR, which is a SCs specific marker in the testicular cells, by immunocytochemistry and RT-PCR. The cells were medium sized and had elongated spindle appearance. Meanwhile some of the female BMSCs were also FSHR positive, which is a granulosa cell (GCs) specific marker in the ovary cells. The cells were small or medium sized, most of them presented spindle and a few cells were round in shape. Most of these FSHR positive cells derived from male and female BMSCs were found in small clusters. Following longer in culture (from 5 ~ 7 days), a good few male FSH positive cells changed their shape from spindle to polygonal or triangular shape, the most of female FSH positive cells became round and were larger. In control group, the BMSC still kept undifferentiated relatively elongated or spindle-shaped cells that they were FSHR negative. These results suggest that the soluble factors secreted by SCs could induce Male and female BMSCs to differentiate into FSHR positive SC-like and GC-like cells respectively *in vitro*. That adult BMSCs could differentiate into SC-like and GC-like cells is dependent on the BMSCs sex on our experimental condition. This research was supported by National Natural Science Foundation of China Grant 30960409.

ATRA pre-induction improves neuronal differentiation of rat mesenchymal stem cells and maintains induced neurons survival by inhibiting apoptosis

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Increasing evidence indicates that MSCs have a potential to differentiate into neuron cells under an appropriate cellular condition. Retinoid signaling pathway has been shown to regulate cell proliferation, differentiation, and apoptosis. However, the effect and mechanism of *all-trans*-retinoic acid (ATRA) on regulating neuronal differentiation process of MSCs are still poorly understood. Here, we reported that ATRA pre-induction improved neuronal differentiation of rat MSCs and regulated apoptosis of induced neuron cells. MSCs harvested from SD rat were exposed in different concentration of ATRA (0.01 -100μM) for 24hr, then cultured with modified neuronal induction medium (MNM). We found that, 1μM ATRA pre-induction significantly improved neuronal differentiation efficiency and neural-cells survival. Compared with MNM alone induced neural-like cells, ATRA/MNM induced cells expressed higher levels of NESTIN, NSE, and MAP-2, but lower levels of CD68, GFAP, and GDNF, supporting that ATRA pre-induction promotes neurons but not neuroglia cells differentiation from MSCs. Interestingly, ATRA alone only up-regulated NESTIN expression. Trypan blue and hoechst staining results showed that low concentration (0.01-1.0μM) of ATRA improved induced neuron survival and inhibited it's apoptosis with dose-dependent manner, whereas at higher concentrations of ATRA (e.g., 10μM and 100μM), the cell death and apoptosis rate of induced neurons were significantly higher than that of the controls. Therefore, our results indicate that exposure to 1μM ATRA before neuronal induction can improve neuronal differentiation of MSCs and maintain cell survival through inhibiting apoptosis. This work was supported by the National Natural Science Foundation of China (No. 30830106).

Study on the expression and significance of Notch-1 in non-keratin nasopharyngeal carcinoma

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To investigate the expression of Notch-1 in non-kertain nasopharyngeal carcinoma (NK-NPC). Sixty biopsy specimens of clear pathological diagnosis with NK-NPC were prepared using the immunohistochemical analysis. The expression of Notch-1 protein in tissues was examined. Our results shown that, the positive expression rate of Notch-1 in NK-NPC was 85%, which was significantly higher than 20% in chronic nasopharyngitis ($P<0.05$). And the Notch-1 expression in NK-NPC was positively correlated with differentiated degree ($P<0.05$), while no significant differences were observed in sex and age ($P>0.05$). Positive staining could be seen in the cancer cells and lymphocyte of differentiated NK-NPC and undifferentiated NK-NPC. The Notch-1 expression in cancer nest of undifferentiated NK-NPC was lower, only focused on the lymphocyte. No positive staining was found in vesicular-nucleus cells. But we could see a lot of positive expression in cancer cells and lymphocyte in differentiated NK-NPC. In conclusion, Notch signaling participated in the occurrence of non-keratin nasopharyngeal carcinoma and, the Notch-1 expression was positively correlated with the differentiated degree of NK-NPC.

Study on the transplantation of microencapsulated hepatic-like cells derived from umbilical cord blood cells for the treatment of acute hepatic failure in rats

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To investigate the therapeutic effect of intra-peritoneal transplantation of microencapsulated hepatic-like cells derived from umbilical cord blood cells on experimental rats with toxic hepatic failure. CD34+ cells were isolated by magnetic cell sorting (MACS). Purified CD34+ cells were amplified and induced to hepatic-like cells by a combination of FGF4 and HGF. The mRNA and protein lever of hepatocyte lineage expressions were evaluated by RT-PCR, immunohistochemical analysis and immune-fluoresce. Then cells were encapsulated by the alginate and transplanted into abdominal cavity of rats 48 hr after the induction of acute hepatic failure (AHF) with D-galactosamine. The survival rate, hepatic pathologic changes and biochemical test were determined. The morphology and structure of the microenpsulate in the greater omentum was examined. The result shown that, in comparisons with free hepatic-like cells transplantation group, the survival rate of rats in microencapsulated hepatic-like cell group was significantly higher ($P<0.05$). And the value of ALT, AST and TB is significant different compared with PBS control group ($P<0.01$). Histological staining also supported this result. After 1-2w post-transplantation, survival cells in the microenpsulate were found in the greater omentum of transplanted microencapsulated hepatic cells group by HE staining, but we also found some fibrous tissue around microencapsulate appeared. In conclusion, transplantation of microen-capsulated hepatic-like cells derived from umbilical cord blood cells preliminarily alleviates the symptoms of AHF rats.

Regulatory effect of dimethylarginine dimethylaminohydrolase on endothelial progenitor cells differentiation and function

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Endothelial progenitor cells (EPCs), which can differentiate into mature endothelial cells, promote neovascularization, participate in the preparation of vessels and angiogenesis, and are used for the treatment of ischemic diseases. Vascular endothelial growth factor (VEGF) is a potent factor regulating EPCs functions, including reendothelization and angiogenesis. The effects of VEGF have been shown to be mediated by type 2 VEGF receptor (KDR). It is know that dimethylarginine dimethylaminohydrolase (DDAH) metabolizes asymmetric dimethylarginine (ADMA), which is endo-genous inhibitor of nitric oxide synthase (NOS) leading to inhibition of nitric oxide (NO) production. It has been demonstrated that the DDAH/ADMA pathway is involved in senescence of endothelial cell and angiogenesis. SIRT1, human silent information regulator 2 (Sir2), is a potent NAD+-dependent protein deacetylase and plays an important role in the maintenance of gene silencing. SIRT1 inhibits senescence of endothelial cells through the DDAH2/ADMA pathway. It has also been shown that SIRT1 is involved in the senescence of EPCs induced by high glucose. Based on the regulatory effect of DDAH/ADMA and SIRT1 on angiogenesis and cell senescence, and SIRT1-mediated the effect of DDAH/ADMA, therefore, in the present study we tested the effect of DDAH/ADMA and SIRT1 on senescence and function of EPCs. In this study, we demonstrated that peripheral blood-derived EPCs predominantly expressed DDAH2 which was increased with EPCs differentiation. Interrupting DDAH2 expression induced EPCs senescence and dysfunction including impairment of angiogenesis and adhesion, and the mRNA expression of VEGF and KDR was also down-regulated. The expression of SIRT1 was increased with EPCs differentiation. Interrupting SIRT1 inhibited the expressions of DDAH2, VEGF and KDR, but had no effect on the level of ADMA. In conclusion, DDAH2 participated in the differentiation of EPCs and regulated the senescence and function of EPCs through the VEGF/KDR pathway by the activation of SIRT1.

Electrically guiding migration of human induced pluripotent stem cells

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Poor homing and integration of transplanted stem cells with the targeted host tissues is one of the road blockers for stem cell therapy. We tested the feasibility of using electric fields (EFs) to guide migration of human iPS (induced pluripotent stem) cells, because iPS cells are an excellent alternative to embryonic stem (ES) cells. Applied EFs guided and stimulated migration of iPS cells toward the anode in both 2D and 3D cultures with a threshold less than 30mV/mm. EF-exposure did not alter expression of iPS markers - SSEA-4 and Oct-4. We compared electric field-guided migration of hiPS cells and hES cells. hES cells migrated in an opposite direction, toward the cathode. hiPS cells are more sensitive than ES cells in showing earlier electrotaxis. Rock inhibition, a method to aid expansion and survival of stem cells, significantly increased the motility, but reduced directionality of migration of iPS cells in an EF by 70~80%. We conclude that EFs can be an effect method to guide migration of human iPS cells, through a Rho kinase dependent manner.

Gender difference in FSHR positive cells derived from adult bone marrow stem cells induced by retinoic acid

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The aim is to investigate whether adult male and female bone marrow stem cells (BMSCs) are able to differentiate into follicle-stimulating hormone receptor (FSHR) positive Sertoli cells (SCs)-like and granulosa cells (GCs)-like cells respectively in vitro induced by all-trans retinoic acid (RA) and to compare the sex difference. Male and female BMSCs from adult male or female SD rat bone marrow were cultured respectively in DMEM supplemented with 10% fetal bovine serum. The BMSCs were cultured above medium with 10^{-6} M RA or without RA (control group) for 7 days. Two days after induction culture, some of the male BMSCs began to expresse FSHR, which is a SCs specific marker in the testicular cells, by immunocytochemistry staining and RT-PCR. The cells were middle in size and had elongated spindle appearance. Meanwhile some of the female BMSCs were also FSHR positive, which is a GCs specific marker in the ovary cells. The cells were small or medium sized, most of them presented spindle and a few cells were round in shape. Most of these FSHR positive cells derived from male and female BMSCs were found in small clusters. Following longer in culture (from day 5 to day 7), a good few male FSH positive cells gradually changed their shape from spindle to polygonal or triangular shape, the most of female FSH positive cells gradually became round and were larger. In control group, the BMSCs still kept undifferentiated relatively elongated or spindle-shaped cells that they were FSHR negative. These results suggest that RA could induce Male and female BMSCs to differentiate into FSHR positive SC-like and GC-like cells respectively *in vitro*. It is dependent on the BMSCs sex on our experimental condition. This research was supported by National Natural Science Foundation of China Grant 30960409.

Induce adult female bone marrow stem cells to differentiate into Scp3 positive cells by retinoic acid

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The Scp3 (synaptonemal complex protein 3) is a component of the synaptonemal complex, a meiosis-specific protein structure essential for synapsis of homologous chromosomes during meiotic prophase I and is a marker that stem cells differentiate into germ cells. The aim is to investigate whether adult female bone marrow stem cells (FBMSCs) are able to express the meiosis–specific gene Scp3 *in vitro*. FBMSCs were separated from adult female SD rat bone marrow and were cultured in DMEM supplemented with 15% fetal bovine serum with 10-5M all-trans retinoic acid(ATRA group) or without ATRA(control group). The FBMSCs of ATRA group expressed the Scp3 mRNA by RT-PCR from 8 to 15 days. Some of the FBMSCs in ATRA group were Scp3 positive on 10, 15 and 29 day after culture with ATRA by immunocytochemistry. The cells were middle and round and were found in small clusters. Following longer in culture (from day 10 to day 29), the number of Scp3 positive cells gradually increased and the cells became larger. In control group, a few Scp3 positive cells began appearance on 15 day and were able to detecte the expression of Scp3 protein from 15 day by immunocytochemistry. The FBMSCs in control group were not able to detect the expression of Scp3 mRNA by RT-PCR. It is possible that the number of the survival cells in control group was too small to obtain the enough total mRNA needed by RT-PCR. In conclusion, some of the adult FBMSCs are able to develop and differentiate into meiotic prophase I oocyte-like cells. ATRA can accelerate the expression of Scp3 protein in the FBMSCs. This research was supported by National Natural Science Foundation of China Grant 30960409.

Preliminary research of the aldosterone synthesized by bone morrow stem cells of adult male rats *in vitro*

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Aldosterone plays an important role in salt and water homeostasis and blood pressure control. Recently systemic or local aldosterone has emerged as a multifunctional hormone exhibiting profibrotic and proinflammatory actions. The renin-angiotensin-aldosterone system (RAAS) is central to the pathogenesis of hypertension, cardiovascular disease, and kidney disease. The variability in effects of RAAS on cardiovascular progenitor cells might reflect differences between the various models with respect to circulating and local tissue RAAS activation. The aim was to investigate whether the bone marrow stem cells (BMSCs) are involved in the local tissue RAAS activation. The BMSCs were isolated from male rat bone mallow and cultured in the DMEM containing 10% fetal bovine serum (FBS). The primary BMSCs from the adherent cell fraction were cultured for 21 days. The whole medium was replaced 2 times every week and was then collected. The levels of aldosterone in the media were measured by radioimmunoassay (RIA). The concentration of aldosterone in the culture medium on day 0 was low ($M \pm SD$, 16.472 ± 11.594 pg/ml). The aldosterone content in the culture medium on day 7 was 52.718 ± 37.162 pg/ml ($P > 0.05$). The higher levels of aldosterone in the culture media were detected by RIA on days 11 (125.46 ± 17.439 pg/ml, $P < 0.01$); 14 (115.012 ± 24.200 pg/ml, $P < 0.01$); 18 (133.028 ± 11.141 pg/ml, $P < 0.01$) and 21 (99.982 ± 34.423 pg/ml, $P < 0.01$). In conclusion, the BMSCs of adult male rats can synthesize aldosterone *in vitro*. The results suggest that the BMSCs might be involved in the local RAAS and its role need to be explored in a further step of the research work. The research was supported by National Natural Science Foundation of China Grant 30960409.

Involvement of CFTR in photodynamic therapy-mediated apoptosis

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Photodynamic therapy (PDT) is a revolutionary medical technology that uses lasers to activate light-sensitive pharmaceuticals to treat cancer and other diseases in a non-surgical, minimally invasive way. Photodynamic therapy (PDT) induces cell death, mostly through apoptosis, by producing reactive oxygen species (ROS) in many cancer cells *in vitro* or *in vivo*. Transmembrane Conductance Regulator (CFTR) has been implicated in the regulation of apoptosis possibly through the control of intracellular ROS balance. In this study, we aimed to investigate the role and the underlying mechanisms of CFTR in PDT-mediated apoptosis. To achieve this, we compared the PDT response in both control and CFTR-knockdown 16HBE4o- human lung epithelial cells. We pretreated the cells with same concentration of 300uM photosensitizer (ALA) for 4hrs and subsequently exposed them to red light, Cell survival and apoptosis were determined by MTS assay and TUNEL assay. Our results showed that PDT significantly induced cell death and apoptosis in both control and CFTR knockdown cells as evaluated by MTS assay and western detection of caspase 3 and poly(A)DP ribose polymerase (PARP) cleavage. However, there were much less cells survived after PDT treatment in CFTR knockdown group compared with 50% of the control group. In addition, we found that the decreased cell number was due to enhanced apoptosis in CFTR knockdown cells as determined by TUNEL assay, our results indicate that CFTR is involved in the regulation of PDT-induced cell apoptosis. Further studies are needed to understand the biological function of CFTR in controlling the intracellular ROS and modulation of mitochondrial function.

Roles of Kras^{G12D} in immunoglobulin expression and secretion in tumor cells

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Immunoglobulins (Igs) are found to be produced in human carcinomas and may have important implications in cell growth regulation. To investigate the growth promoting functions of Igs in cancer cells, we established cell proliferation model in Panc-1, A549, HT-29 and HeLa MR cell lines by transfection with *Kras^{G12D}* plasmid, and observed the expression and secretion of Igs by RT-PCR, Western Blot and flow cytometry. Sensitivity to proliferation by *Kras^{G12D}* was determined by MTT assay. The results indicated that the expressions of IgG in the tumor cell proliferation models were increased on mRNA level and decreased on intracellular protein level; meanwhile the secretions of IgG were increased, especially in 48 hours. We further established *Kras^{G12D}* stable expressing HT-29 cell strain, and identified IgG expression and secretion in the HT-29- *Kras^{G12D}* stable transfectant. We demonstrated that the expression and secretion of IgG increased in HT-29- *Kras^{G12D}*. Taken together, HT-29- *Kras^{G12D}* stable transfectant is a rapid proliferation cell line, associated with IgG high expression and secretion, which may constitute a new approach in the study of Igs functions in human carcinomas.

Baicalin induces caspase-dependent apoptosis in human colon cancer HT-29 cells through Bax- and Bcl-2-triggered mitochondrial pathway

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Baicalin (BA) is a flavonoid compound purified from *Scutellaria baicalensis* Georgi and has been shown to possess potent anticancer activities. Human colon cancer cell line HT-29 cells were incubated with different concentrations of baicalin then cell morphological changes, cell viability decreased and apoptosis dose- and time- dependently were observed by [^{3}H] thymidine incorporation, clone formation assay and flow cytometry. More importantly, administration of baicalin also suppressed the growth of HT-29 cells in immunodeficient nude mice. Furthermore, we investigated the role of ROS, Ca^{2+}, Bcl-2, Bax, and caspases proteins and mitochondria membrane potential in baicalin-induced apoptosis in HT-29 cells. Western blot analysis was used to determine the levels of Bcl-2, Bax and apoptosis associated proteins, and confocal laser microscope for examining the translocation of associated proteins. The results indicated that baicalin promoted the levels of ROS, Ca^{2+} and decreased mitochondrial membrane potential in HT-29 cells leading to the release of cytochrome c, causing the activation of capase-9 and -3, then cell apoptosis through Bax- and Bcl-2-triggered mitochondrial pathway. As shown by treatment of HT-29 cells with an inhibitor of caspase-3 (z-VAD-fmk), we demonstrated that HT-29 cell apoptosis was associated with the activation of caspase-3. Collectively, baicalin induces caspase-dependent apoptosis in human colon cancer HT-29 cells through Bax- and Bcl-2 triggered mitochondrial pathway.

Anthocyanin-rich extract from black rice inhibits colon cancer formation in mice

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The present study is to investigate the effect of anthocyanins on the tumor formation in C3/H/HCN mice. Mice were fed with the azoxymethane (AOM) diet (control) or AOM diet supplemented with anthocyanins-rich extract from black rice for 8 weeks. The incidence of tumor and the hyperplasia of colon epithelial cells were measured. The results showed that the extract from black rice significantly reduced the rate of tumor formation about 12.7% when compared with the control group. The hyperplasias of colon epithelial cells were inhibited about 20.5% to 43.5% depend on the concentration of anthocyanins-rich extract. The results suggest that the black rice extract of anthocyanins can inhibit colon cancer formation in mice.

MicroRNA-122 is down-regulated in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3

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MicroRNAs (miRNAs) are a class of short non-coding RNAs with posttranscriptional regulatory functions and participate in diverse biological pathways. MiR-122, a liver-specific miRNA, is frequently down-regulated in hepatocellular carcinoma (HCC) and HCC-derived cell lines. In an effort to identify novel miR-122 targets, we performed an in silico analysis and detected a putative binding site in the 3'-untranslated region (3'-UTR) of NDRG3, a member of N-myc down-regulated gene (NDRG) family. Our previous DNA microarray data had shown that NDRG3 up-regulated in hepatitis B virus (HBV)-related HCC. The present study showed that miR-122 modulates NDRG3 expression by directly targeting the binding site within the 3'-UTR. The cellular mRNA and protein levels of NDRG3 were repressed by transfection of miR-122 expression vector, which subsequently reversed the malignant phenotype of HepG2.2.15 cell line. This study demonstrated that miR-122 plays an important role in HBV-related hepatocarcinogenesis by inhibiting NDRG3. Thus miR-122 represents a key diagnostic marker and potential therapeutic molecule for HBV-related HCC. This study was supported by NSFC Grants (No. 30772031 and 30801036) and Doctoral Fund of Ministry of Education of China for new teachers (No. 200804221065).

Properties of the human ezrin gene enhancer in HeLa cells: position, orientation and promoter dependence

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We previously demonstrated that the region -87/+134 of human ezrin gene exhibits promoter activity in HeLa cells, and a further upstream region containing enhancer sequences positively regulates transcription. Here, using reporter gene expression assays in transiently transfected HeLa cells, we identified the enhancer properties of the region -1541/-706 of human ezrin gene. We found that when the ezrin -1541/-706 segment located upstream luciferase reporter gene without promoter in forward or reverse orientation, its transcriptional activation was about 5-16% relative to that of ezrin promoter, which was set to 100%. Within plamids containing reporter gene controled by ezrin promoter, when the ezrin -1541/-706 segment located upstream promoter in forward orientation, expression levels of reporter gene increased about 113% relative to that of ezrin promoter; when this segment located upstream promoter in reverse orientation, downstream reporter gene in forward or reverse orientation, expression levels of reporter gene respectively decreased 32%, 39% and 75%, relative to that of ezrin promoter. In addition, within plamids containing reporter gene controled by SV40 promoter, when the ezrin -1541/-706 segment located upstream promoter in forward orientation, expression levels of reporter gene increased about 21% relative to that of SV40 promoter; when this segment located upstream promoter in reverse orientation, downstream reporter gene in forward or reverse orientation, expression levels of reporter gene respectively decreased 71%, 43% and 92%, relative to that of SV40 promoter. These data suggest that the region -1541/-706 of human ezrin gene possesses enhancer properties in HeLa cells, and that enhances transcription in a relatively position, orientation, and promoter-dependent manner.

Continuous fall in hemoglobin level is a poor prognostic factor in patients with nasopharyngeal carcinoma treated with radiotherapy

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Anemia can not only reduce the quality of life of patients with cancer, but also affect their survival. This study was to investigate the prognostic value of hemoglobin (Hb) level in patients with nasopharyngeal carcinoma (NPC) treated with radiotherapy. Clinical data of 520 NPC patients received definitive radiotherapy between 2000 and 2002 at Sun Yat-sen University Cancer Center were analyzed. Patients were stratified into normal Hb level and anemia groups according to their Hb levels before, during, and after radiation. Anemia was defined according to World Health Organization criteria as Hb $<$130 g/L in men and $<$120 g/L in women. Hb continuous decrease group and non-decrease group were defined according to Hb changes in the patients during radiotherapy. Loco-regional recurrence-free (LRFS) and overall survival (OS) rates were estimated using the Kaplan–Meier method. Before radiation ,the 5-year LRFS rates were 60.9% in anemia group and 63.9% in normal Hb level group (P=0.337); the 5-year OS rates were 65.2% and 71.0%, respectively (P=0.299). During radiation, the 5-year LRFS rates were 56.7% in anemia group and 67.9% in normal Hb level group (P=0.013);The 5-year OS rates were 61.0% and 75.9%, respectively (P=0.001) .After radiation ,the 5-year LRFS rates were 59.6% in anemia group and 64.9% in normal Hb level group (P=0.169); the 5-year OS rates were 65.0% and 71.9%, respectively (P=0.090).The 5-year LRFS and OS rates were significantly lower in Hb continuously decrease group than in Hb non-decrease group (59.1% vs 69.3%, P=0.032; 66.2% vs 76.4%, P=0.011). Multivariate analysis showed that the continuous decrease of Hb was an independent prognostic factor for OS. These results suggest that the change in Hb level during radiotherapy is an important prognostic factor affecting the OS of NPC patients.

HAb18G/CD147 enhances cell proliferation by promoting G0/G1 to S transition by ERK1/2 dependent cyclin D1 synthesis in human hepatoma cells

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Previous studies have indicated that overexpression of HAb18G/CD147 enhances proliferation potentials of human hepatoma cells, however, mechanism of HAb18G/CD147 underlying the proliferation process of human hepatoma cells has not been determined. In our present study, up-regulation of HAb18G/CD147 resulted in significant growth enhancement, G0/G1 phase proportion reduction, and upregulated expression of cyclinD1. In addition, Silenced expression of HAb18G/CD147 decreases store-operated Ca2+ entry, thus significantly reduced phosphorylation of ERK1/2. Moreover, blockade of ERK1/2 function by U0126 inhibited HAb18G/CD147-dependent cyclinD1 expression. EGTA, a specific chelating agent of Ca^{2+}, suppressed HAb18G/CD147-induced ERK1/2 activity and cyclinD1 expression. Both EGTA and U0126 reversed HAb18G/CD147-mediated growth enhancement and G0/G1 to S transition promotion. Taken together, these results suggested that HAb18G/CD147 enhances cell proliferation by promoting G0/G1 to S transition by ERK1/2 dependent cyclin D1 synthesis in human hepatoma cells.

Cloning, expression, purification and characterization of HSP105

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In this paper we described the cloning, expression, purification, and identification study of HSP105 gene from human. The hsp105 expression vector pPICZα/hsp105 was constructed via PCR using specific primers with Xho I and ClaI target sites and a part of α-mating factor signal peptide. The recombinant vector was identified by endonuclease digestation assay and sequencing, then linearized and transformed into Pichia pastoris X-33 *via* electroporation. 10 of zeocin resistant positive clones of Pichia pastoris were screened for high level-expressing strains by PCR, SDS-PAGE of the fermental supernatants and western blot. These results indicated that the recombinant hsp105 was identical with the native HSP105. The HSP105 protein was purified by centrifugation, cation-exchange chromatography and gradient elution. The purity coefficient of HSP105 could be more than 85% and the concentration of HSP105 in the broth can reached to 150 mg•L^{-1}.We further explored the zymotechnique of 80 L of HSP105. The study was focused mainly on pH value, culture medium, dissolved oxygen, methanol feeding speed, initial biomass. The results indicated that in the FM21 medium with 0.5% peptone, the best pH was 4.8, DO between 25% to 30% and the supply speed of methanol is 11 mL•h^{-1}•L^{-1} multiplied by initial fermentation volume. And the supernatant fermentation was purified by SP Sepharose XL on pH3.8 and by stepwise elution using 0.4 mol•L^{-1} NaCl and 0.8 mol•L^{-1} NaCl. The purity coefficient of HSP105 could be more than 80% and the concentration of HSP105 in the broth can reach to 250 mg•L^{-1}.

Suppression of RBP-WH inhibits cell cycle progression and induces apoptosis in glioma cells

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Human RNA binding proteins (RBPs) have been reported to be involved in human malignancies and several RBPs have been found as promising biomarkers of lung, head and neck, colon, breast, and pancreatic cancers. The present study investigated the biological significance of RBP-WH in human gliomas. Human RBP-WH was significantly up-regulated in glioma tissues and cell lines compared to normal brain tissues as determined by Real-time PCR and Western Blot. Transient transfection siRNA of RBP-WH in human glioma cell lines resulted in inhibition of cell cycle progression and induction of apoptosis of cells. Compared to negative controls, cells with knockdown of RBP-WH expression showed a G1/S arrest by flow cytometry and an increase in the number of apoptotic cells by TUNEL in situ cell death detection kit-POD assay. Furthermore, employing the RIP-Chip assay we consistently identified a group of 35 mRNAs in three independent analyses of RBP-WH-RNP complexes biotin-purified from a human glioma cell line (T98G). In our RIP-Chip analysis we found that 25% of the RBP-WH associated mRNAs (9 genes) were involved in regulation of cell cycle and apoptosis. These results suggest that RBP-WH affects a network of genes and could function as a master regulator during glioma development and formation.

Expression of LOX and MMP-2 in gastric cancer and effects on tumor invasion and metastasis

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Lysyl oxidase (LOX) is an extracellular matrix enzyme that maintains the integrity of the extracellular matrix and basement membrane of blood vessels by catalyzing lysine-derived cross-links in fibrillar collagens and elastin. Recent results have shown that LOX is expressed in invasive tumor cells and promoted cell migration. But the relation of LOX and MMP-2 in cancer metastasis is still unknown. To compare the expressions of lysyl oxidase (LOX) and matrix metalloproteinases-2 (MMP-2) in gastric cancer tissues and peficancerous tissues, in gastric cancer tissues with and without lymph node metastasis, and to analyze the effects of LOX and MMP-2 on tumor invasion and metastasis, we collected gastric cancer tissues and peficancerous tissues from 46 patients that underwent surgery. Levels of LOX and MMP-2 mRNA were detected by RT-PCR. Protein abundances of LOX and MMP-2 were examed using Western blot. The enzyme activities of LOX and MMP-2 were evaluated through Amplex Red and zymography respectively. Results showed that the expressions of LOX and MMP-2 mRNA, protein and enzyme activity in 46 gastric cancer tissues were significantly higher than that in 46 peficancerous tissues. In gastric cancer tissues with lymph node metastasis, the levels of LOX and MMP-2 mRNA, protein and enzyme activity were higher than those in gastric cancer tissues without lymph node metastasis. The activity of LOX in cancer tissues of III-IV stage was significantly stronger than that I-II stage. Compared with I-II stage, the levels of MMP-2 mRNA, protein and enzyme activity were higher in III-IV stage. In the groups of gastric cancer tissues and gastric cancer tissues with lymph node metastasis, expressions of LOX were positively correlated with MMP-2 ($P<0.01$). These results suggested that LOX and MMP-2 may promote the growth and metastasis of gastric cancer collectively.

Transcriptional repression of breast cancer resistance protein (BCRP) by wild type p53 through a decrease in NF-κB activity in MCF-7 cells

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The breast cancer resistance protein (BCRP) is an efflux drug transporter that confers resistance against various clinically relevant compounds, such as mitoxantrone, topotecan, SN38. In the present study, we demonstrated that overexpression of wild type (wt) p53 inhibited BCRP and NF-κB subunit p50 expression and enhanced the chemosensitivity to mitoxantrone, while knockdown of p53 by RNA interference (RNAi) in MCF-7 cells, resulted in BCRP and p50 expression increases with decreases chemosensitivity to mitoxantrone was decreased. Cotransfection assay also showed that wt p53 repressed BCRP and NF-κB promoter activity. After inhibition of NF-κB by a dominant-negative IκB-α mutant, the inhibitory effect of BCRP promoter activity by wt p53 was lower, and the expression levels of BCRP was down-regulated in MCF-7 and JAR cells. Furthermore, we found that NF-κB can efficiently bind to the NF-κB (p50) binding site within the BCRP promoter using EMSA assay. These data suggest that NF-κB is an activator of BCRP expression through direct DNA binding, and wt p53 downregulates BCRP at mRNA and protein levels through a decrease in NF-κB activity in MCF-7 cells. Given that BCRP accounts for the atypical multidrug resistance and p53 plays important role in tumor development, our data should contribute to a better understanding of not only regulation of BCRP, but also cancer multidrug resistance (MDR) especially the relationship between MDR and the development of tumor.

Upregulation of BCRP is essential for HER2-mediated breast cancer resistance *via* nuclear factor-κB activation

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HER2 (erbB2, neu) is a member of the ErbB receptor tyrosine kinase family, which is associated with breast carcinogenesis, metastasis and therapeutic resistance. However, the underlying mechanisms of resistance to chemotherapy has also far from being completely understood. Breast cancer resistance protein (BCRP, ABCG2), is a drug efflux pump responsible for multidrug resistance in a variety of cancer cells, including breast carcinomas. Here, we show that HER2 enhances the expression of BCRP, which is involved in HER2-mediated multidrug resistance (MDR). We set up HER2-overexpressing breast cancer cell lines (MCF7/HER2) to examine the BCRP expression by RT-PCR, Western blot and Luciferase Reporter Assay. The results showed that HER2 upregulate the expression of BCRP both at mRNA and protein levels. Western blots reveal that HER2 overxpression dramatically increase the levels of p-PI3K, p-Akt and p- IκB, and induce p65 nuclear translocation to regulate BCRP expression, but not in vector control cells. Consistently, BCRP upregulation in MCF7-HER2 cells was prevented when silence of HER2 and inhibition of HER2-driven signalling by tyrosine kinase inhibitor AG825, PI3K/ AKT inhibitor LY294002 and NF-κB inhibitor-dexamethasome. We then demonstrated that BCRP contributes to HER2-associated Taxel, MMC, VP16, 5-Fu and Mit resistance. Furthermore, the MCF7-HER2 cells, when treated with dexamethasome or knock-down of BCRP expression, sensitizes the MCF7-HER2 cells to these chemotherapeutic agents. Collectively, the results suggest that HER2 overexpression upregulates BCRP via NF-κB activation and which may play critcal roles in HER2-mediated MDR.

Monitoring the expression profiles of tongue cancer chemotherapy resistance associated protein1 (TCRP1) silence cells by drug resistance microarray

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Tongue cancer chemotherapy resistance-associated protein1 (*TCRP1*) was a novel multidrug resistance (MDR) candidate gene cloned from tongue cancer multi-drug resistance cell line (Tca8113/PYM) lately established by ourselves. We found in primary study that the *TCRP1* gene especially mediated resistance of anti-cancer drug PYM or cDDP. To identify genes involved in the various mechanisms of TCRP1 associated MDR, The Oligo Toxiology & Drug Resistance Microarray was exploited to analyze the multidrug-resistance cell line (Tca8113/PYM) and stable TCRP1 silence expression cell lines (Tca8113/PYM-i). The microarray characterizes expression of a panel of 263 key genes related to four metabolic processes, including drug resistance; *et al*. A standard >1.5 or <0.67-fold cutoff value was used to determine differentially expressed genes. The results show that 21 genes were up-regulated and 9 genes were down-regulated in Tca8113/PYM cells. Which including MTIX (3.89-fold), Akt (2.14-fold), NF-κB (1.87-fold), EGFR (1.7-fold), TP53 (0.62-fold), HIF1 (0.63-fold), *et al*. These results were corroborated by quantitative real-time reverse transcription-PCR and western blotting and should contribute useful information for revealing the action mechanisms of chemotherapy resistance in malignant tumors including tongue cancer. This study was supported by the National Natural Science Foundation of China (30873088).

Study of the mechanism responsible for multidrug resistance in breast cancer MCF-7 cell mediated by 14-3-3σ

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Multidrug resistance (MDR) is a major obstacle to successful cancer treatment. To understand the mechanism of MDR, many drug resistant breast cancer cell lines have been established and various mechanisms of resistance, involving ATP-binding cassette (ABC) transporters dependent or independent, have been discovered. But it is still far from understood. 5-fluorouracil (5-Fu) is used as fistline anticancer drug in breast cancer chemotherapy and there are only a few reports on its resistance mechanism. In our previous study, while characterizing a multidrug resistant breast cancer cell line MCF-7/5-Fu, established in our lab via 5-Fu selection, we found that BCRP is overexpressed, but 14-3-3σ is downregulated. In the present study, we showed that ectopic overexpression of 14-3-3σ by cDNA stable transfection indeed increased the sensitivity of MCF-7/5-Fu to 5-Fu, Mitoxantrone and Cisplatin, accompanied with p53 upregulation and Akt phosphorylation (p-Akt), NF-κB-p50, survivin, Bcl-2 and BCRP downregulation, and that reducing the 14-3-3σ expression by RNAi in MCF-7 cells caused opposite results. The molecular change can be explained by the observation that Akt activation can be diminished by enhanced 14-3-3σ expression via physical binding mediating Akt phosphorylation inhibition. In addition, 5-Fu treatment can induce the up-regulation of 14-3-3σ and p53 synergistically in MCF-7 cells, and downregulation of p-Akt, NF-κB-p50, Survivin and Bcl-2, time-dependently. Our studies suggested that the deregulation of 5-Fu induced 14-3-3σ/p53/Akt pathway and the final overexpression of BCRP after long-term selection most likely to be responsible for the MDR acquirement induced by 5-Fu in MCF-7 cell and that the 14-3-3σ intervention maybe play positive role in MDR reversion.

Clinicopathologic features of gallbladder carcinosarcoma - adenocarcinoma and angiosarcoma

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We found a rare case of gallbladder carcinosarcoma composed of adenocarcinoma and angiosarcoma. A 67-year-old man was treated in hospital, we studied this disease with its clinical data, histopathology and immunohistochemistry, and relevant existing literature, in order to characterize of this disease. The size of gallbladder is 10.0cm × 5.0cm × 4.5cm, and the tumor had infiltrated into serosa of gallbladder, but non-existent in the liver. Histopathology and immunohistochemistry showed infrequent adenocarcinoma and angiosarcoma simultaneously coexisted, and no transitional areas were detected between carcinoma and sarcoma. Gallbladder carcinosarcoma with coexistence of adenocarcinoma and angiosarcoma is a rare tumor with high malignant neoplasm, which needs to be discriminated from several other diseases. The stage of the tumor is an important prognostic factor in carcinosarcoma of gallbladder and it may be used for prognosis.

Studies on relativity of p73 gene methylation and non-Hodgkin lymphoma

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To investigate the effects of methylation of p73 on pathogenesis of non-Hodgkin lymphoma, the methylation status of p73 gene promoter and the expression of the p73 gene mRNA were examined in 24 NHLs by methylation-specific PCR (MSP) and reverse transcription polymerase chain reaction (RT-PCR) respectively. The level of p73 protein was detected by Western blotting. Furthermore, the p73 gene mRNA level in several NHLs treated by 5-Aza-2'-deoxycytidine was analyzed. MSP revealed that the promoter of p73 was methylated in 21 (87.5%) of 24 NHLs and not methylated in all of 12 reactive hyperplasia and 22 health adult peripheral blood. p73 mRNA was not expressed in 20 (83.33%) of 24 NHLs and expressed in all of 12 reactive hyperplasia and 22 health adult peripheral blood, p73 protein was not expressed in 22 (91.67%) of 24 NHL and expressed in 12 reactive hyperplasia. p73 mRNA was expressed in several NHLs treated by 5-Aza-2'-deoxycytidine. We concluded that p73 inactivation may be involved in the pathogenesis of NHLs and that methylation was the predominant mechanism of inactivation of p73 gene in NHL.

Liver-specific expression of an exogenous gene controlled by human apolipoprotein A-I promoter

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Liver-specific gene therapy is advantageous to minimize the possible adverse effects caused by non-target gene expression. The CMV promoter of the enhanced green fluorescent protein (EGFP) expressing plasmid CMV-EGFP was replaced with the liver-specific promoter apolipoprotein A-I (ApoAI) generating ApoAI-EGFP plasmid. In vitro expression experiments performed in various cell lines including HepG2, SMMC-7721, MCF-7, ACC-2 and Lo2 indicated that pCMV-EGFP treatment caused gene expression in all the cell lines, whereas pApoAI-EGFP treatment only induced EGFP expression in cells of liver origin including the liver cancer cells HepG2 and SMMC-7721 and the normal liver cells Lo2. Either pCMV-EGFP or pApoAI-EGFP was formulated as pegylated immuno-lipopolyplexes (PILP), a novel and efficient gene delivery system. Following intravenous administration of the PILP in H22 tumor-bearing mice, there was significant EGFP expression in liver, tumor, spleen, brain and lung in the pCMV-EGFP treated mice, whereas in the pApoAI-EGFP treated mice there was only gene expression in liver and tumor and the non-liver organ gene expression was eliminated. This study suggests that the use of the PILP technology and liver-specific promoter enables efficient and liver-specific expression of an exogenous gene.

Combined gene therapy against hEGFR and hTERT for experimental human liver cancer

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The present study was designed to establish the feasibility and efficacy of combination nonviral RNA interference gene therapy targeting hEGFR and hTERT for experimental human liver cancer with the pegylated immuno-lipopolyplexes (PILP). Firstly, Two expression plasmids encoding a short hairpin RNA directed at the human EGFR (hEGFR) mRNA and directed at the human TERT (hTERT) mRNA (anti-hEGFR pDNA and anti-hTERT pDNA) were formulated as PILP with receptor-specific monoclonal antibodie (MAb). Human hepatoblastoma SMMC-7721 cells were transfected with these PILP. Cell cycle distribution and apoptotic were evaluated on 2, 4, 6 day after transfection. Results indicated that the silencing effect was processed in a time-dependent manner in cultured hepatoblastoma cells. In hepatoblastoma cells treated with anti-hEGFR pDNA and con-treated with anti-hEGFR pDNA and anti-TERT pDNA, these plasmid all formulated as PILP with 8314 MAb, we displayed that the percentage of cells in G0/G1 phase were increased by 17.62% and 45.62% respectively compared with controls; the total apoptotic rate were 12.47% and 29.59% respectively on 4 day after transfection. Finally, SMMC-7721 cells were implanted subcutaneouly into scid mice, and every 5 day i.v. gene therapy was started at day 10 after implantation of 2000,000 cells. In two groups i.v. anti-hEGFR pDNA and con-i.v. anti-hEGFR pDNA and anti-TERT pDNA, these plasmid all formulated as PILP with 8314 MAb, every five days i.v. RNAi gene therapy resulted in 46.13% and 74.04% of tumor gowth inhibition rates, 76.47% and 94.12% increase in survival time of mice with liver cancer. Combined gene therapy against hEGFR and hTERT could cause more significant suppression human hepatoblastoma cells in vivo and vitro compared with one gene therapy. Combination of the two gene therapy may be additive in antitumor effect.

Observation of Caffeic Acid Ge's inhibitory effect on U14 tumor cell proliferation by culture *in vitro*

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This study aims to investigate the inhibitory effect of Caffeic Acid Ge on U14 tumor cell proliferation *in vitro*. The MTT assay was used to detect the growth inhibition rates of U14 tumor cells at 24, 48 h which cultured with Caffeic Acid Ge in different concentrations. Trypan blue stain method was used to calculate cell number and cell death. The cell cycle distribution and apoptosis were measured by flow cytometry. As shown by MTT assay, Caffeic Acid Ge at the concentration of 0.01 to 10 $\mu g \cdot mL^{-1}$ could inhibit the proliferation of U14 cells, which showed obvious concentration-effect relationship, and the maximum inhibition rate was 45.58% at 1 $\mu g \cdot mL^{-1}$. After being stained by Trypan blue, the mortality rates of all drug groups increased significantly, compared with the control group ($P<0.01$). Flow cytometry results indicated that most of the U14 cells treated with caffeic acid Ge were arrested at the sub-G0-G1 phase and the U14 cells were blocked in S phase. These results suggest that Caffeic Acid Ge has inhibitory effect on U14 tumor cells *in vitro*, which can induce apoptosis.

Vaccination with G22-I50 bispecific anti-idiotype antibody induces a protective immune response against nasopharyngeal carcinoma in a Hu-PBL-SCID mouse model

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We have reported that the G22-I50 bispecific anti-idiotype antibody vaccine was able to induce more powerful humoral and cell-mediated immune responses in Balb/c mouse model. In the present study, we further evaluated the protective efficacy of G22-I50 using human nasopharyngeal carcinoma cell line(HNE2) and a hu-PBL- SCID mouse model (severe combined immunodeficient mice reconstituted with $40x10^6$ human peripheral blood lymphocytes). We demonstrated that immunization with G22-I50 vaccine caused significant inhibition of tumor growth with the delay time to tumor detection (tests *vs* controls, 14 d *vs* 8 d, $p<0.05$) and much smaller tumor size ($p<0.05$) *in vivo*. Furthermore, the activated lymphocytes ($CD8^+$) were capable of infiltrating into the tumor site, and much more apoptotic cells along with activation of caspase-3 were observed in the tumors from vaccinated-mice. Also, Spleen cells from G22-I50-immunized mice gave a significant proliferative response and higher expression level of IFN-γ, IL-2. These results suggest that G22-I50 immunization could effectively inhibit nasopharyngeal carcinoma growth in hu-PBL-SCID mice. Therefore, G22-I50 may be a promising candidate as a nasopharyngeal carcinoma vaccine. This study was supported by Innovation Fund of Central South University (2009bsxt059).

Stanniocalcin 1 enhanced sensitization of cervical cancer CaSki cells to anti-tumor drugs

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The drug resistance is very common and formidable problem for treating tumor. It is necessary to explore the mechanism of drug resistance. Stanniocalcin 1 (STC1) is associated with tumorigenesis and development. When human cervical cancer CaSki cells were treated with cisplatin, thapsigargin, and rapamycin separately, the proliferation of CaSki cells was inhibited, and the cell cycle was arrested in G1 phase. To investigate the function of STC1 in sensitization to drugs, the eukaryotic expression vector of STC1 (pcDNA3.1(+)-stc1) was constructed. The pcDNA3.1 (+)-stc1 and empty pcDNA3.1(+) were separately transfected into CaSki cells, and the stably transfected CaSki cells (CaSki/STC1 and CaSki/control) were selected. RT-PCR and western blot showed that the expression of STC1 in stably transfected CaSki cells (CaSki/STC1) was upregulated. When drugs (cisplatin, thapsigargin, and rapamycin) were added into CaSki/STC1 and CaSki/control cells, the growth of CaSki/STC1 was evidently suppressed compared with CaSki/control cells, and cell-cycle G1 was strongly arrested compared with CaSki/control cells. These demonstrated that STC1 sensitized cervical cancer cell to drugs, which represents a potential cancer treatment strategy. This study was supported by National Natural Science Foundation of China, No. 30672352.

Human cervical cancer CaSki cells adapted to stimuli by regulating the expression of genes

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Until now, cancer is still difficult to treat, while the difficulty of curing tumor is associated with the response of tumor cell to stimulus. To understand the molecular mechanism of tumor cell responding to stimulus, human cervical carcinoma Caski cells were flown on ''Shen Zhou IV''space shuttle mission. The electron microscope was used to observe the morphological character in ground and space cervical cancer CaSki cells. Reverse Northern blot and RT-PCR were applied to exam differentially expressed genes in cervical on ground and in space cancer CaSki cells using the previous constructed cDNA library. RT-PCR was used to check the expression of the genes while the CaSki cells were treated with drugs, heat, and rays. We found that the ultrastructure of space cervical cancer CaSki cells changed compared with ground group, including large mitochondrion, high density of cytoplasm, enhanced ribosome, and wide microtubule. We obtained 35 differentially expressed genes which were associated with cytoskeleton, apoptosis, cell cycle, transcription, and immune. The genes accordingly altered when CaSki cells were treated with drugs, heat, and rays. In conclusion, the survival CaSki cells adapted to the change of environments by regulating the expression of genes and then changing cell morphous, when the outside conditions changed. Thus, these genes formed a protective network to react quickly in the face of external stimuli. This study was support by National Natural Science Foundation of China, No. 30672352 and Innovation Fund of Central South University (2340-74334000006).

The proliferation of breast cancer cells are inhibited by the human intrabody targeting cyclinD1

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Cyclin D1 belongs to the core cell cycle machinery, and it is overexpressed in the majority of human breast cancers. Mice lacking cyclin D1 are resistant to mammary carcinomas triggered by the ErbB-2 oncogene. The induction of cyclin D1 degradation and inactivation may offer a useful avenue for therapeutic intervention. This study is designed to inhibit the growth and proliferation of breast cancer cells through blocking and inhibiting the biological activity of cyclin D1 overexpressed in cancer cells by using intrabody technology. An anti-cyclinD1 intrabody (NLS-AD) was generated by introducing nuclear localization signal sequence and E-tag sequence to the anti-cyclin D1 scFv and then was cloned into the pcDNA3.1 to construct the expression vector pNLS-AD coding anti-cyclinD1 intrabody. Then pNLS-AD was transfected into the breast cancer MCF-7 cells. The RT-PCR and western blotting showed the anti-cyclinD1 intrabody was expressed efficiently. Immunofluorescence assay showed that anti-cyclinD1 intrabody was localized in the nuclear area. Immunoprecipitation results suggested that anti-cyclin D1 intrabody could recognize and combine its target protein cyclinD1 of MCF-7 cells. MTT assay showed that the anti-cyclin D1 intrabody significantly inhibited the growth of MCF-7 cells ($P<0.01$). The results of FACS showed that anti-cyclin D1 intrabody arrested most of MCF-7 cells at G1 phase and distinctly induced apoptosis of MCF-7 cells ($P<0.01$). This study suggested that anti-cyclin D1 intrabody might be further used in breast cancer gene therapy. This study was supported by the National Natural Science Foundation of China (30200256, 30972806) and the S&T Development Planning Program of Jilin Province (20090727).

EMBJ08, a novel gene promoting cell proliferation

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In the present study, we concern on the functional study of a novel gene EMBJ08, which may be involved in promoting cell proliferation. Base on the Renilla luciferase (pRL) activity of Dual Luciferase Reporter Gene Assay, the effect of the novel gene EMBJ08 was detected in large scale screening of predicted secretory proteins. And the cell growth curve and flow cytometry were used to determine its effect on proliferation. Finally, RT-PCR was used to survey its expression profile in 17 different cell lines. Our results showed that the activity of pRL was up-regulated obviously by EMBJ08 in the large scale screening of three reporter genes. The bioinfromatic analysis shown its full length was 4131 bp, with 9 exons and located in 8q12.1; the electronic expression profile in the oncomine database demonstrated EMBJ08 ranked in the top 6 of these genes with different expression levels between the normal bone marrow and the T-cell acute lymphoblastic leumkemia. Moreover, the result of RT-PCR also showed that EMBJ08 was highly expressed in Jurkat and Raji cells, which consists with the data of oncomine database. The cell growth curve showed overexpressing EMBJ08 could promote cell proliferation ($P<0.05$), and the cell cycle analysis indicated EMBJ08 could increase the percentage of S phase significantly compared with the vector, that was 7.88% at 24h and 14.63% at 48h, respectively. In conclusion, these results indicate that the novel gene EMBJ08 can promote cell proliferation and is highly expressed in immunological related tumors. Further work should be done to explore functions and application of EMBJ08.

PDCD5 regulates cell autophagy

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Programmed Cell Death 5 (PDCD5) is a protein that accelerates apoptosis in different cell types in response to various stimuli, and has been shown to be down-regulated in many cancer tissues. Further investigation reveals that PDCD5 interacts with Tip60 and function as a co-activator to promote apoptosis *via* the Tip60-p53 signaling pathway. In the present study, we demonstrate a novel feature in the action of PDCD5 on programmed cell death. We found that the clonogenic formation was suppressed in both HEK293 and HeLa cells when PDCD5 expression was stably inhibited by RNA interference. Simultaneously, typical morphological characteristics of autophagy were observed by transmission electron microscopy, including extensive cytoplasmic vacuolization and enclosure of cell organelles by double-membraned structures. Knockdown of PDCD5 expression of enhanced starvation-induced cell autophagy in HeLa. This was indicated by an increase in MDC dot staining and proportion of punctate pattern of LC3 localization, enhancement of Beclin 1 protein expression and decrease of beta-catenin level. Overexpression of PDCD5 partially reversed starvation-induced autophagy. Our preliminary data suggest that PDCD5 may regulate autophagy activation through Wnt signal pathway and function in making trade-offs between apoptosis and autophagy.

Identification of binding site in integrin β1 subunit in complex with HAb18G/CD147

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There are several lines of evidence suggesting that HAb18G/CD147 interacts with integrin $\alpha3\beta1$ and $\alpha6\beta1$. However, the mechanism of HAb18G/CD147 interacting with integrin and the binding site in integrin $\beta1$ subunit for HAb18G/CD147 are still unknown. In our present study, results of mammalian protein interaction trap (MAPPIT) indicated a binding site in integrin $\beta1$ subunit for CD147. We found that both the extracellular segment (I like domain) of HAb18G/CD147 and peptides GRGDS containing the Arg-Gly-Asp sequence bind at MIDAS pocket of βA domain in integrin $\beta1$ subunit, and peptide GRGDS could prevent HAb18G/CD147 colocalizing with integrin $\beta1$. In human 7721 hepatoma cells, migration, invasion capacities and matrix metalloproteinases (MMPs) secretion potential were decreased by inhibiting HAb18G/CD147 expression or incubation of GRGDS ($p<0.01$). However, migration and invasion capacities as well as MMPs secretion potential weren't further decreased by incubation of GRGDS after deletion of HAb18G/CD147. Levels of integrin downstream molecules including focal adhesion kinase (FAK) and phospho-FAK (p-FAK) were decreased in human hepatoma cells with HAb18G/CD147 deleted or incubated with GRGDS, and cytoskeleton in human hepatoma cells were rearranged after treatment. Taken together, our findings suggest that I like domain of HAb18G/CD147 binds at the MIDAS pocket of βA domain in integrin $\beta1$ subunit. Moreover, peptide GRGDS could prevent downstream activation of HAb18G/CD147/integrin $\alpha3\beta1$/FAK signaling pathway by competitively inhibiting the interaction of HAb18G/CD147 and integrin $\beta1$, subsequently attenuating invasive and metastatic potential of human hepatoma cells.

Effect of Chrysanthemum flavonoids on growth and cell cycle regulators of gastric cancer cell

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The present study is to investigate the effect of Chrysanthemum flavonoids containing serum on growth and cell cycle regulators of gastric cancer cell. Chrysanthemum flavonoids containing drug serum was prepared by gastric perfusion of Chrysanthemum flavonoids in different do sages to SD rats. Gastric cancer cells were cultured in medium contained the drug serum with different concentrations of Chrysanthemum flavonoids. The change of cell cycle was detected by flow cytometry, and the mRNA and protein expressions of CyclinD2, CyclinE, Cyclin-dependant kinase2 (CdK2), Cdk4, Cdk6 and p16INK4a, p27KIP1 were detected with immunohistochemistric (IHC) SABC method and RT-PCR. Results showed that after Chrysanthemum flavonoids intervention, the cancer cells were constrained in stage G0/G1, unable or-retardatory to enter stage G (namely, the DNA synthesis stage). IHC examination showed the grey scale values of CyclinD2, CyclinE, Cdk2, Cdk4 and Cdk6 were higher, and that of p27KIP1 and p16INK4a were lower in cells after moderate/high dosage Chrysanthemum flavonoids intervention than those in the blank control ($P<0.01$). RT-PCR showed the OPTD values of CyclinD2, CylinE, Cdk2, Cdk4 and Cdk6 were lower, and that of p27KIP1 and p16INK4a were higher in cells after moderate/high dosage Chrysanthemum flavonoids intervention than those in the blank control ($P<0.01$). In conclusion, Chrysanthemum flavonoids can inhibit the expressions of cell cycle promoting related factors of gastric cancer cell, including CyclinD2, CyclinE, Cdk2, Cdk4 and Cdk6, and enhance the expressions of cell cycle inhibiting factors of gastric cancer cell, asp27KIP1 and p16INK4a. These underlie the action of the remedy in preventing the growth of gastric cancer cells.

Proteomics analysis reveals dynamic expression patterns of proteins in different stages of colorectal cancer

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Colorectal Cancer (CRC) is a well-known malignant tumor. Although there are many studies about the differential proteins expression between the normal and tumor or the tumor with metastasis, the dynamic proteins expression model during the whole process of CRC carcinogenesis is still unknown. In this study, twenty-five specimen were recruited, which included 20 specimen in TNM I-IV clinical stages of CRC (each 5 samples) and 5 their matched normal mucosae as normal control. After preparation, proteins were separated by two-dimensional fluorescence difference gel electrophoresis (2-D DIGE) in 13 parallel gels with internal standard. By using the DeCyder 6.5 software analysis system, we got 199 differentially expressed proteins (ANOVA analysis; $p \leq 0.05$) among 4463 matched protein spots. Forty proteins were unambiguously identified by MALDI-TOF-MS/MS, which mainly involved in disease mutation, energy metabolism, folding of proteins, signaling pathway, reversible hydration of carbon dioxide and so on. Pattern analysis shows that the proteins expression changed dynamically during the progression of CRC progression. In addition, the stage I was the most important stage, because most molecules changed from the initiation of CRC carcinogenesis. It demonstrated that the molecular events occurred before histological changes. In summary, we profiled proteome alterations in different stages of CRC, and found dynamic expression patterns of proteins. These results may provide useful insights for understanding the mechanism involved in the process of CRC carcinogenesis.

Expression of the $p21^{WAF1/CIP1}$ protein and P53-gene mutation in papillary thyroid carcinoma

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In this study, we discussed the relationship of p53-gene mutation and quantitative expression of the $p21^{WAF1/CIP1}$ protein in papillary thyroid carcinoma (PTC). 38 PTC samples were stored by liquid nitrogen and 141 PTC samples were stored by wax block and the normal thyroid tissue in each specimen retained as control group. P53-gene purified with polymerase chain reaction single strand conformational polymorphism (PCR-SSCP) method and $p21^{WAF1/CIP1}$ expression was quantitatively by mean optical density method. The results display there were no statistically significant differences between PTC cases without p53-gene mutation and with p53-gene mutation in primary tumor (T), lymph nodes metastasis (N), and distant metastasis (M) ($P > 0.05$). The optical density levels between two groups were significantly different ($P < 0.01$). There is no direct relationship between the progression of PTC and the p53-gene mutation. The expression of $p21^{WAF1/CIP1}$ in PTC was induced by wild p53-protein, which serves as a regulative route during carcinogenesis and development of PTC.

Resvereatrol induced apoptosis in breast cancer cells involves upregulation of ASPP1 through E2F-1

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Resveratrol is a natural polyphenolic compound which is found in grape skin, wine and peanuts. Many studies indicated resveratrol triggering intracellular events involved in p53 and Rb/E2F pathway. ASPP1 is a new family member of ASPP (apoptosis stimulation protein of p53) which play a important role in regulating the apoptotic. To examine resveratrol's effect as an anticancer nutrient in breast cancer cells, we treated MCF-7 and MDA-MB231 cells in different concentration of resveratrol. We found that resveratrol increases ASPP1 expression in MCF-7 and MB231 cells. We also found that resveratrol enhanced cancer cell apoptosis in MCF-7/ASPP1 cells, and with company of higher expression of bax, p21 as compared to control. Furthermore, when ASPP1 were knocked down by siRNA in MB231 cells, apoptosis will not induced obviously as well as control. On the other hand, in order to understand the mechanisms in which ASPP1 enhance the apoptosis induce by resveratrol, we detected the expression of ASPP1 with the link E2F change in MCF-7 and MB231 cells. We found higher expression of ASPP1 is associated with increased E2F by infecting E2F1/, meanwhile, down-regulation of ASPP1 expression with E2F1 koncked down by siRNA. In conclusion, our results demonstrate that over expression of ASPP1 rendered MCF-7 and MDA-MB231 cells more sensitive to resveratrol. ASPP1 enhanced resveratrol-mediated apoptosis via E2F pathway. This study suggested that ASPP1 over-expression may represent a novel therapeutic approach for resveratrol in human breast cancer and that this may involve the E2F pathway.

HAb18G/CD147 inhibits hepatoma cells apoptosis *via* FAK-Src pathway mediated TFII-I activation and Bip up-regulation

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The role of HAb18G/CD147 underlying endoplasmic reticulum stress (ERS) condition of human hepatoma cells has not been determined. In the present study, we found that the expression of HAb18G/CD147 was increased as time-dependent manner in tunicamycin (Tm) or Thapsigargin (Tg) incubated 7721 cells. The expression levels of ERS markers-CHOP and Bip were also increased as time-dependent manner. Upregulation of HAb18G/CD147 in HCC cells markedly increased the Ca^{2+} level in ERS condition. In ERS condition, the expression of Bip was in positive correlation with HAb18G/CD147. No significant expression and phosphorylation modifications of IRE1, PERK and ATF6 were found in HAb18G/CD147 upregulated HCC cells and HAb18G/CD147 down-regulated HCC cells. It is known that TFII-I and YY1 can form trimer with ATF6 to regulate Bip expression. Upregulation of HAb18G/CD147 in HCC cells markedly increased TFII-I phosphorylation level ($p < 0.01$), and accumulation of p-TFII-I in the nucleus. c-Src and FAK phosphorylation levels were enhanced in HAb18G/CD147 upregulated HCC cells ($p < 0.01$). There were no significant expression modifications of c-Src and FAK in HAb18G/CD147 upregulated HCC cells and HAb18G/CD147 downregulated HCC cells ($p > 0.05$). The addition of FAK inhibitor significantly decreased the phosphorylation levels of FAK, Src, TFII-I and expression level of Bip in HAb18G/CD147 upregulated HCC cells ($p < 0.01$). Upregulation of HAb18G/CD147 in HCC cells obviously inhibited apoptosis and derceased caspase4 activity ($p < 0.01$). Taken together, these results suggest that in ERS condition, by FAK-Src signal pathways, HAb18G/CD147 may phosphorylate TFII-I and promote the accumulation of p-TFII-I in the nucleus, thus promoting Bip expression. By promoting Bip expression and decreasing the activity of caspase4, HAb18G/CD147 may inhibit HCC apoptosis.

Overexpression of human papillomavirus type 16 E6 oncoprotein increased hypoxia-inducible factor (HIF)-1 protein accumulation and vascular endothelial growth factor (VEGF) expression in lung cancer cells A549

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Lung cancer is the most common malignancy worldwide. Cigarette smoking is the major cause of lung cancer, but many non-smokers can suffer from lung cancer. Recently, more and more epidemiological evidences have showed that high–risk HPV infection may be related to lung cancer. However, the effect of HPV-16 E6 oncoprotein on angiogenesis remains unclear. Accumulating evidence has demonstrated that hypoxia-inducible factor (HIF)-1 and vascular endothelial growth factor (VEGF) play a pivotal role in angiogenesis. In this study, non-small cell lung cancer (NSCLC) cells (A549) were transiently transfected with hemagglutinin (HA)-pSG5-HPV-16 E6 construct, and transfection with HA-pSG5-HPV-16 E6 mutant or empty vector HA-pSG5 served as negative controls, and cells exposed to transfection reagent (Lipofectamine 2000) served as mock transfection controls. The expression of HPV-16 E6 oncoprotein, HPV-16 E6 mutant, empty vector pSG5 in the transfected-cells was confirmed using specific monoclonal antibody against HA. HIF-1a protein expression in the transfected-cells was analyzed by Western blot, and VEGF protein concentration in the conditioned media was determined by ELISA. HIF-1a and VEGF mRNA levels were determined by real-time RT-PCR. Our results showed that HPV-16 E6 oncoprotein enhanced HIF-1a protein accumulation but had no obvious effect on HIF-1a mRNA expression. In addition, HPV-16 E6 oncoprotein significantly increased VEGF protein secretion and mRNA expression. Taken together, these results suggest that HPV-16 E6 oncoprotein may play an important role in lung cancer angiogenesis. This work was supported by the grants from National Natural Science Foundation of China (No. 30872944), Department of Science and Technology of Guangdong Province (No. 2009B030801330), Guangdong Administration of Traditional Chinese Medicine (No. 2008166), and Department of Science and Technology of Dongguan (No. 2008108101029).

The establishment of radiotherapy-related models of nasopharyngeal carcinoma using serum comparative proteomics

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The present study determined specific serum peptide profile by comparing the serum differences between four radiotherapy-ralated types of nasopharyngeal carcinoma patients (NPC) and normal control group. The study collected four radiotherapy-ralated types of NPC and normal control serum samples. Four radiotherapy-ralated types were radiosenstive and not liable to metastasize type (type I), radio-resistant and not liable to metastasize type (type II), radiosenstive and liable to metastasize type (type III) and radio-resisitant and liable to metastasize type (type IV). The above sesum were followed by automated MALDI-TOF MS analysis after peptides extracted on magnetic beads coated with WCX phase. Mass spectrographic data were analyzed with ClinproTool TM software. The specific serum peptide model of four types was established by using genetic algorithms. The sensitivity and specificity of model were tested by blind testing. The differential expression of the peptide peaks of type I, type II, type III and type IV compare to nomal group are 55 (n=25); 36 (n=23); 42 (n=25) and 15 (n=25), respectively. The study selected 809.01Da; 3954.95Da and 5965.52 Da peptide peaks to establish model I for type I, 3954.67Da; 5336.91Da and 5634.35 Da peptide peaks to establish model II for type II, 808.95Da; 8142.39Da and 7765.96 Da peptide peaks to establish model III for type III and 808.92Da and 7766 Da peptide peaks to establish model IV for type IV, which were statistically significant differences between the two groups. The recognition rate of model I, model II, model III and model IV are 96%; 91.48%; 94% and 90%, respectively. The predictive powers are 95.04%; 86.11%; 91.39% and 93.28%, respectively. The sensitivities are 0%; 20%; 80% and 20%, respectively. The specificities are 80%; 95%; 65% and 75%, respectively. The present study established the four radiotherapy-ralated models of nasopharyngeal carcinoma, which has provided the basis for directing individual treatment and developing a specific tumor marker of nasopharyngeal carcinoma.

MicroRNA-370 is down-regulated in hepatitis B virus-related hepatocellular carcinoma and targets insulin-like growth factor binding protein 4

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MicroRNAs (miRNAs), a class of short non-coding RNAs, participate has diverse biological pathways and function as gene regulators. Our previous study shown that miR-370 is down-regulated in hepatitis B virus (HBV) related hepatocellular carcinoma (HCC). The target genes of miR-370 were predicted using the algorithms including TargetScan, Pictar, and miRanda. Insulin-like growth factor binding protein (IGFBP) 4, which was commonly predicted by the three algorithms and also screened out from previous DNA microarray data, was selected for further investigation. The expression vectors of miR-370 and IGFBP4 were constructed and transfected into HepG2 and HepG2.2.15 cell lines. Both restriction analysis and sequencing result proved that recombinant plasmids were correctly constructed. The expression of mRNA and protein of IGFBP4 was detected by RT-PCR and Western blot respectively. The cellular mRNA and protein levels of IGFBP4 were repressed by elevated miR-370 after transfection, which subsequently led to inhibition of the cell viability. In addition, a direct interaction of miR-370 with the target site of the 3' UTR of IGFBP4 was demonstrated. Our study clarified that miR-370 plays a pivotal role in blocking HBV-related hepatocarcinogenesis by inhibiting IGFBP4. Thus miR-370 represents an early diagnostic marker and potential therapeutic factor for HBV-related HCC. This study was supported by NSFC Grants (No. 30772031 and 30801036) and Doctoral Fund of Ministry of Education of China for new teachers (No. 200804221065).

Role of epidermal growth factor-like domain 7 in metastasis of human gastric carcinoma

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Epidermal growth factor-like domain 7(Egfl7) is a recently identified secreted protein that is believed to be primarily expressed in endothelial cells (ECs). Although its expression was reported elevated during tumorigenesis, whether and how Egfl7 contributes to human gastric carcinoma remains unknown. In this study, overexpression of Egfl7 was found in gastric carcinoma cells in tissues and positively correlated with its invasion and metastasis. To further confirm this phenomenon, BGC823 cell, a gastric carcinoma cell line with a high level expression of Egfl7, was selected from a series of gastric carcinoma cell lines. We depleted its expression of Egfl7 by using small interfering RNA. Interestingly, reduction of Egfl7 expression resulted in significant inhibition of invasion and migration of BGC823 cells in vitro. In conclusion, our study suggests Egfl7 might serve as a novel prognostic marker for invasion and metastasis of gastric carcinoma.

Human SBK1 is dysregulated in multiple cancers and promotes survival of ovary cancer SK-OV-3 cells

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Protein kinases are involved in comprehensive cellular processes and also implicated in many human diseases. SH3-binding domain kinase 1 (Sbk1) was first cloned and characterized in rat and the human cDNA was cloned in our lab in 2006, but the expression and function of endogenous protein have not been well studied in human. In this follow up study, we screened a panel of cell lines and tissues, as well as a tumor tissue array for SBK1 expression at both RNA and protein levels. To detect the protein, we generated the first rabbit polyclonal antibody against human SBK1. We show that the SBK1 is expressed in most of the cells and tissues examined, and the protein is highly up-regulated in ovarian serous adenocarcinoma while down-regulated in esophagus squamous cell carcinoma and stomach adenocarcinoma. When over-expressed in an ovarian cancer cells SK-OV-3 by adenovirus infection, SBK1 protected the cells from apoptosis induced by the viral infection, therefore promoting cancer cell survival. Given that a missense mutation K92E in human SBK1 was identified recently from ovarian mucinous carcinoma, together, these results suggest that the wide-spread expression pattern of human SBK1 may predict a broad cellular function, and its dysregualated in certain cancers suggests an involvement of the protein in the pathogenesis of human cancers.

Metastasis promoted by caveolin-1 gene silence in human mammary epithelial cell MCF10A

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Caveolin is an essential structural molecule of Caveolae and could inhibit the anchorage-independent cell growth in breast cancer. To study the relationship between Caveolin-1 and metastasis in breast cancer, we developed MCF10A-ST1 and MCF10A^{CE} cell lines which relatively express 30% and 15% of Caveolin-1 compared with mammary epithelial cells (MCF10A) by means of gene trapping and RNAi technology. Then we examined the expression of ERα and associated proteins. The results indicated that the expression of ERα66 and ERα36 was up-regulated, and the phosphorylation of ERK1/2 was increased. Then, MCF10A, MCF10A-ST1, MCF10A^{CE} and MCF-7 as well as estrogen treated group were cultured in monolayer by wounded healing assay. Western blot, RT-PCR, immunocytochemistry and gelatin zymography detection were used. The results showed that migrant distance was different in the four cell lines, 50% in MCF10A^{CE} and 30% in MCF10A-ST1 compared with MCF-7, while little in MCF10A. In MCF10A-ST1 and MCF10A^{CE} cell lines, the expression and activity of MMP-2 in wounded healing group showed an increasing tendency, while the expression of E-cadherin decreased. RT-PCR showed that tomor metastasis related gene, VEGF, β-catenin, was sigificantly high in MCF10A^{CE} cells, while E-cadherin was low. These results indicate that Caveolin-1 plays an important role in mammary tumorigensis and may affect cell metastasis *in vitro*. Such evidences will greatly advance the progress in prevention and treatment of human breast cancer. This work was supported by the National Natural Science Foundation of China (No.30570225).

Anticolorectal carcinoma activities of Zuojin Pill and Fanzuojin Pill *in vitro* and *in vivo*

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We cultured HT29 cells and investigated the intervention effect of Zuojin Pill's major constituents against colorectal carcinoma *in vitro*, and then established transplanted nude mice and colorectal carcinoma model in rats to investigate the intervention of Zuojin Pill and Fanzuojin Pill on the progressive colorectal carcinoma *in vivo*. Cultured HT29 cells in the logarithmic growth phase were treated with berberine (26.25; 52.5; 105; 210 and 420 μmol/L) and evodiamine (5; 10; 15; 20; 25 and 30 μmol/L) for 24; 48; 72 and 96 hours. The results suggested that evodiamine and berberine dose-dependently inhibited the proliferation of HT29 cells in vitro. The suppression ratios achieved using evodiamine (7.5, 15 and 30 μmol/L) were 39.3 $\pm$ 2.13%, 52.8 $\pm$ 5.34% and 64.1 $\pm$ 7.19%, while those achieved using berberine (52.5; 105 and 210 μmol/L) were 44.1 $\pm$ 3.97%, 55.9 $\pm$ 4.12% and 65.3 $\pm$ 6.94%. Moreover, berberine and evodiamine could effectively inhibit the telomerase activity of HT29 cells. The single cell suspension of HT29 cells was subcutaneously injected into the left abdominal region of nude mice at a concentration of 5×10^5 cells/0.2 mL to produce tumor bearing nude mice. Rat model of colorectal carcinoma was induced with 1, 2-dimethyl hydrazine (DMH, i.h. 25 mg/kg; once a week for 12 weeks). The rats were then randomly divided into 3 groups: Zuojin Pill, Fanzuojin Pill and control. Rats in each group were killed at weeks 11; 21 and 34 after treatment. The intervention effects were evaluated by pathological examination. The results suggested that Zuojin Pill and AZT had no significant influence on tumor size but had weak effect on telomerase activity in tumor- bearing nude mice. In DMH-induced rat colon cancer model, Zuojin pill and Fanzuojin pill can repress the invasion of the early rat carcinoma obviously, while in late cancer, this action is not obvious. This study was supported by National Natural Science Foundation of China, No. 30400602, No. 30672687.

Inhibitory effects of myocardial cells culture medium on growth of human nasopharyngeal carcinoma and its mechanism in nude mice

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This study aims to investigate the antitumor effects and its mechanism of myocardial cells culture medium (CMCM) in nude mice with human nasopharyngeal carcinoma xenograft, and to investigate its sensitizing effect for chemotherapy. CNE-2 cells were implanted subcutaneously into nude mice and divided into 6 groups: saline group (25ml/kg), DDP group (2mg/kg), low, middle, high dose CMCM groups (7.5mg/kg, 15mg/kg and 30mg/kg, respectively), CMCM+DDP group (CMCM, 15mg/kg; DDP, 2mg/kg). CMCM and saline were administered intraperitoneally for 14 consecutive days, and DDP was administered intraperitoneally once every other day. Nude mice were killed at 24 hours after the final treatment, and the tumors were isolated and weighed. TUNEL and CD34 immunohistochemistry were used to analyze cell apoptosis and intratumor microvessel, respectively. The growth of the implanted tumor in mice was significantly inhibited following treatment with CMCM, DDP, and CMCM+DDP, respectively. The inhibition rate of low, middle, and high dose CMCM groups were 42.45%, 51.42% and 54.31%, respectively, all showing significant difference versus saline group ($P<0.05$). There was no significant difference among three CMCM groups ($P>0.05$), although there was a dose dependent trend among them. The inhibition rate of CMCM, DDP and CMCM+DDP groups were 51.42%; 54.21% and 73.41%, respectively, all showing significant difference versus saline group ($P<0.05$). Both CMCM and DDP showed significant difference versus CMCM + DDP group ($P<0.05$). However, there was no significant difference between CMCM and DDP group ($P>0.05$). TUNEL showed that compared with the saline group, other groups presented more apoptosis ($P<0.05$). Moreover, CMCM+DDP group presented more apoptosis than individual DDP group ($P<0.05$). CD34 Immunohistochemistry showed there was no significant difference for intratumor microvessel density in all groups ($P>0.05$). In conclusion, CMCM has inhibitory effects on growth of human nasopharyngeal carcinoma in mice due to cell apoptosis. CMCM could be developed as a promising chemotherapy sensitizer.

LRRC4 inhibits in glioblastoma cells invasion by blocking proMMP-2 activation

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The previous research indicated that LRRC4 is involved in nervous system development and neurite outgrowth, and being a glioma suppressive gene, inhibits glioblastoma cells tumorigenesis *in vivo* and cell proliferation *in vitro*. Glioblastoma multiforme is the most common malignant tumor of the adult central nervous system. The highly lethal nature of this tumor results from the acquisition of an invasive phenotype that allows the tumor cells to infiltrate surrounding brain tissue. In this work, we studied the effect of the reexpression of LRRC4 on glioblastoma U251 cells chemotaxis, invasion and secretion of MMP-2 and-9 proteinases activity using gelatin zymography. LRRC4 could inhibit chemotaxis and invasion in cell lines and transplanted tumor of left reni-theca of Kunming mice. SDF-1α stimulation induced the activation of pro MMP-2, and this activated effect was inhibited by LRRC4. However, activated form of MMP-9 (82KD) was not tested in all cell lines. Thus, LRRC4 inhibit in glioblastoma cells invasion by blocking proMMP-2 activation, and the research represents a new target for development of new therapeutic strategies in glioma. This study was supported by National Key Project of Scientific Research Program (2006CB910502, 2006CB910504); National Natural Sciences Foundation of China (30770825); Program for New Century Excellent Talents in University (NCET-08-0562).

TMEM161A, a transmembrane protein, regulates cells apoptosis

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Apoptosis is one of the most important processes in programmed cell death. We describe the biological activities of TMEM161A (transmembrane protein 161A, also known as AROS-29, FLJ20422 and FLJ39645), which mainly locates to plasma membrane and endoplasmic reticulum and contains eight putative TM domains as well as a potential signal peptide, TMEM161A protein is conserved in evolution across different species. Overexpression of TMEM161A markedly inhibited cell growth and colony formation in 293T cells and HeLa cells respectively. In our study, TMEM161A-transfected HeLa and 293T cells succumbed to cell death with hallmarks of apoptosis including phosphatidylserine externalization, loss of mitochondrial transmembrane potential, caspase activation and chromatin condensation. Importantly, TMEM161A overexpression also strongly activated AP-1 and NF-κB signaling pathways. So far, our data show that TMEM161A may act as a transcriptional activator in AP-1 and NF-κB signaling pathway for the first time and regulate cell apoptotic pathway.

High throughput screen on biomarkers and corresponding aptamers of breast cancer

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The present study aimed at high throughput screening on biomarkers of breast cancer cells and their aptamers, which would contribute to the diagnosis and treatment of breast cancer. Hs578Bst and Hs578T were taken as the healthy and cancerous breast cells in the study. After separated by SDS-PAGE and transferred to the nitrocellulose, proteins in Hs578T were used to screen ssDNA aptamers against cancer targets, while proteins in Hs578Bst were used as substractive objects. ssDNA aptamers were developed by an in vitro selection process, Systematic Evolution of Ligands by Exponential Enrichment (SELEX). By western blot, the differential proteins between Hs578T and Hs578Bst and active aptamers with high-affinity for these proteins were all found. Then as high-affinity and specific capture agents, the active aptamers were used to capture the biomarkers only expressed in Hs578T. The biomarkers of Hs578T were identified by matrix-assisted laser desorption ionization time-of-flight time-of-flight mass spectrometry and database searching. In the results, The western blot, in which aptamers were used instead of antibodies, showed six luminescence strips special in breast cancer cells, three of them were identified. They were vimentin, TUBA1C and mutant β actin, which stood for the potential biomarkers of breast cancer cells. And the individual aptamers of differential proteins were obtained. In conclusion, these findings show for the first time the possible role of vimentin, TUBA1C and mutant β actin in breast cancer. The integrated approach of protein electrophoresis, immunoblotting and SELEX could make it possible that finding and analyzing differential protein, selecting aptamers and evaluating aptamer activity were all achieved synchronously, while the high-affinity and specific aptamers are suitable to be used for the investigation of differential protein function. Since the sensitivity of luminescence is better than gel dyeing's, the method seemed to be highly sensitive to the low concentration targets. Also, the approach is applicable to the study of protein modification because of the aptamer advantage in distinguished molecular group.

Reduced expression of TMEM166 is associated with esophageal carcinoma and gastric cancer

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TMEM166 (transmembrane protein 166) is a novel autophagy-related protein. Some evidence shows TMEM166 is a novel regulator involved in both autophagy and apoptosis. However, the expression level of TMEM166 in various human tumors has not been characterized. In this study, we investigated the expression of TMEM166 and its correlation with clinicopathologic characteristics of esophageal carcinoma and gastric cancer. Our data revealed that the positive rate of TMEM166 expression in the esophageal and gastric tumor tissues was significantly lower than that of the normal tissues ($P<0.05$). Furthermore, we studied the correlation of the expression level of TMEM166 with the clinicopathologic characteristics of patients with esophageal carcinoma or gastric cancer. The decreased expression of TMEM166 in esophageal squamous cell carcinoma was correlated with tumor infiltration ($P<0.05$), but not with age, differentiation or lymph node metastasis. Furthermore, overexpression of Ad5-TMEM166 in KYSE150 and BGC823 cell lines resulted in dose-dependent and time-dependent cell death. In conclusion, our preliminary data indicate that reduced expression of TMEM166 may contribute to the pathogenesis of human esophageal and gastric tumors, and that Ad5-TMEM166 may be a novel candidate for gene therapy for the treatment of esophageal and gastric tumors.

NYD-SP8 expression in nasopharyngeal carcinoma and its clinical significance

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NYD-SP8, a novel uPAR-like protein, has been shown to be involved in metastasis of various kinds of cancers, but its biological activity and clinical significance in nasopharyngeal carcinoma (NPC) are still unclear. This study was to determine the expression of NYD-SP8 in NPC and to analyze its correlations to growth and infiltrative metastasis of tumor. Western blot was applied to detect NYD-SP8 expression in different NPC cell lines including radiation-resistant CNE1, radiation-sensitive CNE2, high-metastatic 5-8F and low-metastatic 6-10B. Additionally, the expression of NYD-SP8 in 61 specimens of NPC was determined by immunohistochemistry; the content of NYD-SP8 in the 103 specimens was detected by ELISA. Data were subjected to statistical analysis with respect to clinicopathologic variables. NYD-SP8 protein levels in CNE2 and 6-10B were higher than that in CNE1 and 5-8F, respectively. There was NYD-SP8 protein expression in NPC tissues, but not detected in their normal tissue counterparts. In summary, NYD-SP8 expression was higher in TNM ~ stage of cancerous tissues than that in I~ II stages ($P<0.01$). The serum NYD-SP8 level in NPC patients was (62.2 ± 4.1ng/ml), which was significantly higher than that of normal subjects (13.3 ± 3.8ng/ml) ($P<0.01$). NYD-SP8 expression is different in NPC cell lines with various biological behaviors, and the detection of NYD-SP8 either in NPC tissue or in serum could be regarded as a valuable indicator for predicting progress of NPC.

Effective anti-tumor responses on nude mice with breast cancer after chemotherapy with vaccinating tumor-lysate pulsed dendritic cells

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The breast cancer nude mice model had been successfully established by inoculating 10^6 MCF-7 breast tumor cells subcutaneously (s.c) in situ to 94 balb/c female nude mice in this experiment. After cyclophosphamide treatment, we compared the therapeutic effects of tumor-lysate pulsed dendritic cells(DCs) along with cord blood mononuclear cells (DCs+CBMNCs), dendritic cells (DCs) only, cord blood mononuclear cells (CBMNCs) only and PBS only on breast cancer nude mice. Immunotherapy maintained 174 days, tumor size was assessed by measuring the long (a) and short diameters (b) using calipers every three days and the tumor volume was calculated by $1/6\Pi ab^2$. We compared the mean tumor volumes and survival conditions among these groups for 15 days. A higher survival rate was shown in group DC+CBMNC compared with other three groups throughout the whole treatment, but it merely was statistically significant on day 93 ($p=0.017$), day 107 ($p=0.026$) and day 134 ($p=0.024$) when compared to control groups. The mean tumor volume of group DC+CBMNC was the smallest throughout the whole treatment as well. One-way ANOVA about the tumor volumes among these groups showed statistic significance on day 75 ($p=0.018$), day 93 (0.000), day 107 (0.000), day 121 (0.001) and day 134 ($p=0.018$). In conclusion, our work show that after chemotherapy, vaccinating tumor-lysate pulsed DCs and CBMNCs can induce anti-tumor responses on breast cancer nude mice, especially in 3-4 months time.

Expression of nm23, BRCA-1, ki-67 and their clinical significance in breast cancer

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To investigate clinicopathological significance of nm23, BRCA-1 and ki-67 expression in breast carcinoma. We use immunohistochemistry SP applied to detect the expression of nm23, BRCA-1 and ki-67 in 54 cases of breast carcinoma specimens. Results shown that high-staining of nm23 accounted for 25 in 33 cases without lymph node metastasis of breast carcinoma and 9 in 21 cases with lymph node metastasis cases, respectively, which had statistical significance. Expression deletion of BRCA-1 was positively correlated with the malignant grade, which was observed in dysplasia, carcinoma *in situ* and invasive carcinoma. The positive staining of ki-67 was positive significantly associated with the extent of breast tissue hyperplasia and malignant degree. The expression levels of nm23, BRCA-1and ki-67 were independent of tumor diameter, age and ER. In conclusion, nm23, BRCA-1 and ki-67 together with the traditional markers (ER, PR, etc.) can determine the prognosis and metastasis with more confidence.

Inhibitory effect of garlic polysaccharide on proliferation of BEL-7402 human liver cancer cell *in vitro*

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Garlic polysaccharide is an active ingredient which extracted from garlic bulbs. Many studies have demonstrated that garlic polysaccharide has an antioxidation, scavenging oxygen free radical effects, can protect the heart against myocardial fibrosis. However, it remains incompletely defined whether it can induce tumor cell apoptosis. To investigate the effects of garlic Polysaccharide on the human hepatocellular carcinoma BEL-7402 cells in terms of inhibition of proliferation and induction of apoptosis. We used BEL-7402 Cell in cultural *in vitro*, given with different concentration of garlic polysaccharide (10 $mg \cdot L^{-1}$, 30 $mg \cdot L^{-1}$, 100 $mg \cdot L^{-1}$, 300 $mg \cdot L^{-1}$) at different times such as 24 h; 48 h and 72 h. Cell morphological changes were observed in inverted microscope. The inhibitory rate of cells was observed by MTT assay respectively, cell apoptotic rate was detected by flow Cytometry (FCM) with Annexin V/PI staining. The results indicated that garlic Polysaccharide inhibited the proliferation of BEL-7402 cells significantly. The suppression was both in a time-dependent and dose-dependent manner. Morphological examination of garlic Polysaccharide-treated samples showed cell with chromatin condensation. We also found that garlic Polysaccharide increased the cell apoptotic rate. The results show that Garlic Polysaccharide can inhibit the growth of BEL-7402 human liver cancer cell in vitro by inducing programmed cell death.

Effect of Dureping Injection on Activity of Nuclear Factor Kappa B

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Viral infection cannot be controlled effectively, especially the influenza virus. Many Chinese medicines have antiviral effect. To investigate the influence of Dureping Injection anti-influenza virus to Ana-1 on activity of nuclear kappa B (NF-κB), Ana-1 cell line was infected by influenza virus FM1 strain then treated with the Dureping Injection, 12 h and 24 h later, collected the cells, extracted mRNA and measured the expression of TLR7, MyD88 and NF-κB p65 respectively by RT-PCR; extracted the nuclear protein and measured the expression of NF-κB p65 by Western-blot. Results shown that Dureping Injection down-regulated the expression of TLR7; MyD88; NF-κB p65 mRNA and NF-κB p65 protein in Ana-1 cell line infected by influenza virus, and shows dose dependent relationship significantly. These results suggest that Dureping Injection has an obvious effect against influenza virus FM1 strain by regulating the MyD88-dependent signal pathway, which depressed the activity of NF-κB.

HAb18G/CD147 regulates interconversion between amoeboid movement and mesenchymal movement by regulating the activation of RhoA and Rac1 signaling pathways in HCC cells

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Tumor cells can move as individual cells in two different modes: mesenchymal mode and amoeboid mode. The two modes of movement are interconvertible, in which the cytoskeleton rearrangement plays an important role. Our previous studies indicated that HAb18G/CD147 and annexin II are interaction protein, and they are involved in the cytoskeleton rearrangement. Therefore, the present study focused on the mechanisms of HAb18G/CD147 and annexin II in HCC cell cytoskeleton rearrangement and cell movement. The results showed that downregulation the expression of HAb18G/CD147 and annexin II caused morphological changes of HCC cells, but the morphological changes caused by the two molecules were diametrically different. When the expression of HAb18G/CD147 was downregulated, HCC cells became rounded morphology. When the expression of annexin II was downregulated, HCC cells became elongated morphology. The former is similar with the amoeboid mode of movement and the later is similar with the mesenchymal mode of movement. Next, we detected the interaction site of annexin II and HAb18G/CD147 using MAPPIT system. We found that the extracellular portion of HAb18G/CD147 can interact with a phosphorylation inactive mutant (A II-Y23F) prey, and inhibit phosphorylation of annexin II. We also found that HAb18G/CD147 inhibited Rho signaling pathways and ameboid movement in HCC cells by inhibiting annexin II phosphorylation. HAb18G/CD147 can also promote the membrane localization of WAVE2 and Rac1 activation in HCC cell by integrin-FAK-PI3K/PIP3 signaling pathway, and then, promote the formation of lamellipodia and mesenchymal movement. These results suggest that interaction between HAb18G/CD147 and annexin II is involved in the interconversion between mesenchymal and amoeboid movement of HCC cell.

Anti-inflammatory effect of TANREQING combined with cefoperazone in established murine model of AECOPD

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Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) is a common and growing clinical problem that is responsible for a major cause of hospital admission and substantial worldwide health burden. AECOPD is characterized by a fixed obstruction of the airway, accelerated decline in lung function, poorer health-related quality of life and increased mortality. To observe the anti-inflammatory effect of TANREQING combined with cefoperazone in rats with acute stage of chronic bronchitis, we established the murine AECOPD model by smoking and dropping Klebsiella pneumoniae with stimulation of wind. The successfully established model rats were randomly divided into two groups, the control group was treated with conventional therapy of cefoperazone by intragastric administration, and the TANREQING group was added 2 ml TANREQING injection. The pulmonary function and the blood gas analysis were observed before treating and the 28 day after treating. The combination with TANREQING significantly improved the VT (Tidal volume) from 2.94 ± 0.60 ml/min to 4.07 ± 0.43 ml/min, and MV (minute ventilation) from 625.24 ± 112.85 ml to 755.69 ± 142.32 ml, as well as in the control group from 2.97 ± 0.24 ml/min to 3.58 ± 0.65 (VT) and from 614.75 ± 114.01 ml to 717.45 ± 132.39 ml (MV), and there were statistical differences between the two groups ($P<0.01$). After 28 days' treatment, the total numbers of WBC in the bronchoalveolar lavage fluid (BALF) both decreased in the two groups, and the TANREQING group ($3.43 \pm 0.67 \times 10^8$/L) was significantly lower than the control group ($3.92 \pm 0.52 \times 10^8$/L) ($P<0.01$). The contents of Interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α), important factors implicated in the pathogenesis of inflammation, in BALF of TANREQING group significantly decreased to 0.85 ± 0.14 ng/mL and 33.47 ± 6.28 ng/mL respectively, and 0.92 ± 0.26 ng/mL and 35.31 ± 5.31 ng/mL in control group, the TANREQING group were less than the control but there was no significant difference between them ($P>0.05$). Thus, TANREQING may reduce the airway inflammation and improving lung function by decreasing the level of cytokine IL-8 and TNF-α in the AECOPD.

AY25 induces activation of interferon regulatory factor 7

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The type-I interferon response is critical to immunity against viruses and can be triggered in a high level expression by Toll-like receptor 9 (TLR9) subfamily, and IRF7 play an important role in the response. Interferon regulatory factor 7 is one of the interferon regulatory factors family (IRFs). Activation of interferon regulatory factor 7 is essential for the induction of Type I interferons (IFN-α/β) and innate antiviral responses. In our study, we established a high-throughput, cell-based screening platform based on automated fluorescence microscopy system, which can fast acquire and quantitatively analyze images of IRF7-GFP location in cotransfected cells. From the screening of 3,000 novel human genes that we cloned in our lab, we identified AY25 could induce the translocation of IFR7 obviously. AY25 is located on chromosome 17q12-q21 and encodes 432 aa. Bioinformation analysis shows that AY25 is conserved in Homo sapiens, Pan troglodytes, Canis familiaris, Mus musculus, and Rattus norvegicus. Location assay showed that AY25 located to mitochondria. Western blot analysis validated that AY25 could enhance the ability of translocation to nuclear of activated IRF7. Moreover, overexpressing AY25 could up-regulate IFN-β expression level based on reporter gene assay. To further determine the role of AY25 in activation of IFR7, we also need to detect the expression of their downstream molecular by RT-PCR and analyze IFN-β level by ELISA.

The expression of TGF-β 1, TβR and Smad2/3 in liver infected with Echinococcus multilocularis

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The aims of the study to observe systemically and dynamically the expression of TGF-β1, TβRI/II and Smad2/3 in the liver of Balb/c mice infected by Echinococcus multilocularis (Em), we demonstrated that the expression of TGF-β1 mRNA increased gradually at 8w post infection with Em. Smad3 mRNA was lower than infect group beside 2days and 36w. At 8w post infection, some alveclar sphereuesicae could be observed obviously and become bigger at 24w infection. The level of protein TGF-β1 increased gradually at 8w, peak at 12w. The level of protein Smad2/3 was lower in infected mice than the normal group, but its expression increased gradually after 8d, peak at 4w, then decreased gradually. TGF-β1, TβRI/II, Smad2/3 only observed in hepatic sinusoid in the normal group, while in hepatic sinusoid, hyalomitome, cell membrane, fiber organization, focus germinal layer surrounding in infected group. TGF-β1 reached peak at 12 weeks but TβRII peak at 24w. Smad2/3 and TβRI was all reached peak at 8 weeks. In the different Em infected time, the inflammation and fibrosis were observed in the liver of infected group. These results suggest that the pathway of TGF-β/Smads signal protein activated in Em infection and was useful the liver fibrosis and hepatic injury possibly. This study was supported by the grants from NSFC (No.30860263, 30960358and 30560146).

Human microRNA miR-151 in monocytes was identified to be related with the etiology of rheumatoid arthritis

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MicroRNAs (miRNAs) are short noncoding RNA molecules that regulate gene expression by targeting mRNAs. Recently, miRNAs have been implicated important in the etiology of various diseases. However, little is known about their roles in the development of rheumatism. Rheumatism is an autoimmune disease and cellular immunity is more important in its pathways. It is characterized by cytokine included T cell and monocytes. Circulating monocytes produce various factors important for rheumatoid arthritis (RA). We first explored differential expression of miRNAs in human circulating monocytes between 20 RA patients and 10 normal. Expression level of each miRNA was normalized with RNU48. Differential miRNAs were selected by t-test and miR-151 was up-regulated ($P=0.015$) in two groups, furthermore, it was confirmed in individual assays with qRT-PCR. Moreover, we investigated mRNA profilings in human circulating monocytes isolated with the subjects used in miRNA array analysis. Pearson correlation analysis between the expression level of miR-151 and the mRNA array expression data was performed. We found significant correlation of miR-151 with TNFSR11 ($r= -0.87$, $P=0.000176$), LRCH1 ($r=0.73$, $P=0.0087$) and FZD5 ($r=0.72$, $P=0.02143$) genes. LRCH1 and FZD5 genes are also predicted as the targets of miR-151 (http://www.targetscan.org). LRCH1 and FZD5 were also down-regulated expressed in RA patients compared with normal person. Genetic epidemiologic studies have shown the association of LRCH1 gene with human osteoarthritis. Expression profiling studies also found the relationship between FZD5 gene and human rheumatoid arthritis. Hence, miR-151 may affect the differentiation of monocytes by regulating the expression of LRCH1 and FZD5 genes. This is the first *in vivo* miRNA profiling analysis and miRNA and mRNA correlation analysis in human circulating monocytes in the development of rheumatoid arthritis. Our study suggested that human miR-151 may be involved in monocyte and thus the etiology of rheumatoid arthritis.

Comparisons between gargling glucosyltransferase activity test and resazurin disc test for evaluating deciduous caries susceptibility

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Dental caries is a prevalent infectious disease in China, so it is of much constructive significance to distinguish the susceptible in the prevention of dental caries. The Resazurin disc (RD) test is one of the common methods. Its use was limited by qualitative rather than quantitative. In addition, operation of the test requires timely measurement after sampling impacting. The purpose of our experiment is that to compare sensitivity between glucosyltransferase activity (GGA) test and RD test to find a new convenient index for evaluating caries susceptibility. A kindergarten in changsha was selected randomly as subject investigated. Based on dmft which distinguished by oral health examination, children between three to five years old were divided into group CF (caries free, dmft=0, n=22), CE (caries exist, dmft<=4, n=22) and CS (caries susceptible, dmft>4, n=10). Sampling time and results were compared respectively between GGA test and RD test. GGA test sampling takes 67.6 ± 8.1 seconds while RD test takes 42.0 ± 5.4 seconds ($p<0.05$). The GGA test is a kind of quantitative text. The GTF activity of group CS is significantly higher than group CF and CE ($p<0.05$), but no statistical difference between the group CF and CE ($p>0.05$). By RD test is a qualitative test. It states that group CS and CE are higher than group CF ($p<0.05$) but no statistical difference is found between the CE and the CS group ($p>0.05$). The Results showed that the GGA test can distinguish caries susceptible effectively and be sensitive only in serious dental caries population. GGA test is in quantitative. Sampling and testing can be done by batch make it more suitable for general survey.

The study on the response of Th1/Th2 cytokines to autoimmune acute liver injury in mice

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Liver disease is one of the most serious threats to human health. In our study, concanavalin A (ConA) was used to make an autoimmune acute liver injury model. The adult and healthy Balb/c mice were divided into two groups: normal control group and liver injury group. Normal control group was received single injection of 0.3 ml saline and liver injury group was received 12.5mg/Kg ConA by caudal vein injection. The serum, liver tissues were collected at 8h, 24h and 72h after ConA injection. The morphologic of liver tissues and the concentration and variation of Th1/Th2 cytokines were observed by tissue section and FlowCytomix technique. The edema of hepatocytes, scattered liver necrosis,the infiltration of lymphocyte and sinusoids congestive could be found at 8h after ConA injection. At 24h, there were more edema liver cells and enlarged necrotic foci which surrounded by a large number of lymphocyte infiltration, but the congestion of sinusoids was reduced. At 72h, the edema of hepatocytes and congestion of sinusoids were significantly reduced, and the necrotic foci began to be absorbed. There were significant differences in the concentration of Th1 (IL-1, IL-2, IFN-γ and TNF-α) and Th2 (IL-4, IL-6 and IL-10) in the liver between liver injury Group and normal control group at 8h ($P<0.05$), At 24h, the concentration of Th1 and Th2 were decreased to normal level or below the normal level, with a downward trend. The serum concentrations of Th1 (IL-2 and IFN-γ) and Th2 type cytokines (IL-6) in liver injury group were higher at 8h, compared with the normal control group ($P<0.05$) and decreased gradually after 24h. The results showed that the cytokines secreted by Th1 lymphocytes, macrophage and Th2 lymphocytes were involved in acute immunological liver injury.

The role of hepatocyte cyclooxygenase-2 in the acute fulminant hepatic failure

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Cyclooxygenase-2 (COX-2) -derived PGs participate in a number of pathophysiological responses such as inflammation, carcinogenesis, and modulation of cell growth and survival. This study was designed to examine the role of COX-2 in Fas-induced and LPS-induced liver injury *in vivo*. C57/BL6 wild type, COX-2 transgenic (Tg) mice with targeted expression of COX-2 in the liver were treated with Jo2 (0.5 μg/g body weight) or LPS in combination with D-galactosamine (GalN) for 4 h to induce acute fulminant hepatic failure. Following 4 h after Jo2 challenge, the COX-2 Tg mice showed resistance to Fas-induced liver injury when compared to the wild type mice, as reflected by the lower ALT and AST levels, less liver damage and less hepatocyte apoptosis ($P<0.01$). The liver tissues from the COX-2 Tg mice express higher levels of EGFR and several key molecules of the downstream Akt signaling pathway when compared to the wild type mice. Pretreatment with the COX-2 inhibitor (NS-398) or the EGFR inhibitor (AG1478) exacerbated Jo2-mediated liver injury and hepatocyte apoptosis. These results demonstrate that hepatocyte COX-2 protects against Fas-induced liver injury by activation of EGFR/Akt pathway. However, following 4 h after LPS/D-GalN challenge, the COX-2 transgenic mice exhibited higher serum ALT and AST levels and more prominent liver tissue damage than wild type mice. Western blot analysis of the liver tissues showed that LPS/D-GalN treatment for 4 hours induced the cleavage of PARP, caspase-3 and caspase-9 in COX-2 transgenic mice but not in wild type mice. Increased hepatic expression and activation of JNK2 in COX-2 transgenic mice suggests that upregulation of JNK2 may represent a potential mechanism for COX-2-mediated exacerbation of liver injury. Our experimental findings suggest that COX-2 is not a general cytoprotective mediator in the liver.

Study on the correlations between Glucosyltransferase activity in saliva and deciduous caries susceptibility of preschool children

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Dental caries is a kind of infectious disease, which is found prevalent among 70% children in China. Deciduous caries is subject to susceptibility. And some children have high dmft (decay miss and fill total) while some have low or none, so it is beneficial to distinguish the susceptible in the prevention of dental caries. S.mutans (Streptococcus mutans) and S.sobrinus (Streptococcus sobrinus) are primary pathogenic bacterium. The Glucosyltransferase (GTF) from these pathogen, redound to the formation of plaque. Therefore it is of much significance to find a convenient and cheap testing method to assess deciduous caries susceptibility for preventing childhood dental caries. The test purpose is determine the GTF activity in saliva, and analyzes the correlation with susceptibility of deciduous caries. The probability which is use the GTF activity as an index to screen the susceptible population is investigated. A kindergarten in Changsha was selected randomly as subject investigated. Based on dmft which distinguished by oral health examination, children between three to five years old were divided into groups CF (caries free, dmft=0, n=22), CE (caries exist, dmft<=4, n=22) and CS (caries susceptible, dmft>4,n=10). Collect the saliva of the sampled children by normal saline gargling posterior to cleansing their mouth for 48 hours. Test the GTF activity based on UV spectrophotometric method. The results showed that the GTF activity of group CS (0.182 ± 0.338 IU/L) is significantly higher than group CE (0.018 ± 0.212 IU/L) and group CF (-0.066 ± 0.116 IU/L) ($p<0.05$). No statistical difference is found between the group CE and CF ($p>0.05$). The correlations test illustrates that there is a significant correlation between GTF activity and dmft, with correlation coefficient (r) of 0.348 ($p<0.05$). These results suggest that the GTF activity in saliva relates closely to the severity of dental caries. It is an effective index to screen the deciduous caries susceptible high-risk population.

Difference of immunological response against *Schistosoma japonicum* induced by primary cells and cell fractions from schistosomulum in mice

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Our previous studies had shown that primary juvenile worm cells (pJCs) elicited the significant protection against challenge infection of *Schistosoma japonicum*. The differences in immunological responses of primary juvenile worm cells (pJCs) and fractions of juvenile worm cells (JCFs) from 12 day-old *schistosomulum* were evaluated in this study. Firstly, duration of existence of pJCs and JCFs in the vaccination spot tissues of mice immunized subcutaneously with both immunogens was observed. Then the difference of humoral and cellullar immune responses induced by pJCs and JCFs was analyzed. Subsequently, protection efficacy of pJCs given intravenously (i.v.) and subcutaneously (s.c.) respectively was evaluated. The results illustrated that *schistosomulum* cell protein had existed in the vaccination tissues of pJCs group on the whole tested time, while only detected at 1 and 3 days after immunization in JCFs group. Compared to control group, both mice immunized with pJCs and JCFs showed high level of antibody, but there were no significant differences between pJCs and JCFs groups. The pJCs administration significantly enhanced spleen lymphocyte proliferation such as $CD4^+$ and $CD8^+$ T cell, and a decline in $CD4^+/CD8^+$ ratio and the percentages of $CD4^+$ $CD25^+$ regulatory T cells in $CD4^+$ T cells in comparison with JCFs was also observed. Mice immunized with pJCs given i.v. or s.c. exhibited high level of specific antibody and protection effects, but given i.v. was significantly higher than given s.c. ($P<0.05$) and showed significant reduction in worm burden, liver eggs per gram (LEPG) load worm average weight. These results indicated that pJCs could induce a stronger cell immune and stay longer at the immunization site when compared to JCFs.

Association between two SNPs in the monocyte chemoattractant protein-1 (MCP-1) gene and tuberculosis in Ningxia Chinese

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Monocyte chemoattractant protein-1 (MCP-1; gene name CCL2), has been suggested to play an important role in the initiation and development of tuberculosis (TB) by recruiting monocytes to sites of infection. Recently, single nucleotide polymorphisms (SNPs) in the MCP-1 regulatory region have been identified. Controversial results regarding the association of SNPs of the MCP-1 gene with TB have been reported. In the present study, we examined a possible association between the -2518G/A, -2076A/T polymorphisms of the MCP-1 gene and TB in the population of Ningxia area. A total of 459 Ningxia patients with TB and 654 healthy controls were included in the study. Two SNPs in the promoter (-2518A/G, -2076A/T) of the MCP-1 gene were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-PFLP) or direct sequencing. Results shown that patients with TB had significantly higher frequency of the MCP-1(-2518) AG+GG genotypes compared to controls [83.6% vs 75.7%; OR (95%CI), 2.019 (1.466-2.781); P=0.000], The TB patient group showed a significant higher frequency of the G allele compared to the controls [60.5% vs 52.1%; OR (95%CI), 1.408 (1.186-1.670) P=0.000]. Significant differences of MCP-1-2076 (A/T) variants were also found between patients and controls. AT genotype of TB cases was significantly different from that of controls [16.1% vs 10.2%; OR (95%CI), 1.624 (1.115-2.236), P=0.004]. Strong linkage disequilibrium was found among 2 SNPs (-2518A/G, -2076A/T), resulting in 4 major estimated haplotypes. A significant difference was found in G-T haplotype frequencies between patients with TB and controls [6.1% vs 1.6%; OR (95%CI), 4.483(2.668-7.533), P =0.000]. In conclusion, the present study showed a significant but not independent association between the -2518G/A, -2076A/T polymorphisms of the MCP-1 gene (presence of -2518 G allele and -2076 T allele) and TB in the Ningxia population. MCP-1 -2518(GA+GG) genotype and MCP-1 -2076(AT) genotype may be a risk factor for TB among Ningxia population.

Immune activation of T lymphocytes induced by bronchial epithelial cells chronically infected with RSV

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Respiratory syncytial virus (RSV), an important pathogen, preferentially infects airway epithelial cells and causes bronchiolitis. It has been reported that RSV infection may be responsible for susceptibility of asthma, but the underlying mechanism is not clear. We hypothesize RSV infection can disturb the homeostasis of immune system through changing the function of antigen presentation cells, such as bronchial epithelial cells. To observe the immune activation of T lymphocytes *in vitro* stimulated by RSV chronically infected human bronchial epithelial cells (HBECs). We established the chronically infected HBECs model by RSV (virulence is $1.4x10^6$ pfu/mL) with MOI 0.0001. Enlarged fusion cells were seen in later period, edema of mitochondrias, expansion of endoplasmic reticulum, fissure around nucleus, generous lysosomes in cytoplasm and intracellular virus particles were all observed under electron microscope. The infection rates were between 30% and 50% tested by immunofluorescence. Lymphocytes were isolated from peripheral blood and divided into four groups. They were Lymphocytes (Group A); lymphocytes interfered by RSV (Group B); co-culture of lymphocytes and normal HBECs (Group C) and co-culture of lymphocytes and infected HBECs (Group D). After 24 hours, lymphocytes were collected and examined for apoptosis rate, cell cycle and CD3+ cells number by flow cytometry analysis. Supernates were tested by ELISA for IL-4, IFN-r and IL-17. The results showed that apoptosis rate of Group D was significantly higher than other three groups ($P<0.05$). The cell number of Group D is most in S Period and least in G1 Period during four groups ($P<0.05$). And cell number in S Period of Group C is more than other groups. The number of CD3+ cells is most in Group D ($P<0.05$), and Group C is more than A and B. The level of IL-4, IFN-r and IL-17 in supernate is highest in Group D ($P<0.05$). In conclusion, RSV chronically infected HBECs can induce T lymphocytes proliferating, apoptosis in response to immune activation and releasing IL-4, IFN-r and IL-17.

Effect of TANREQING on airway inflammation in rats with chronic bronchitis

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Chronic bronchitis (CB) is a common disorder with high morbidity and mortality that is characterized by the prolonged airway inflammation, and it represents a substantial burden on health care resources. TANREQING liquid has been showed to treat c**hronic bronchitis** successfully, while the anti-inflammatory action mechanism of TANREQING in the treatment of CB is still unclear. In this study, we established the rat chronic bronchitis by passive cigarette smoking plus intratracheal instillation of lipopolysacchride (LPS). The successfully established model rats were then randomly divided into two groups, the control group was treated with normal saline by intragastric administration, and the treated group was added 2 ml TANREQING liquid. After four weeks' therapy, the characteristic structural changes in airway, including infiltration of inflammatory cells, smooth-muscle cell layer thickening and goblet cell hyperplasia, were dramatically alleviated in the treated group compared to the control group shown by HE staining. The total numbers of WBC in the bronchoalveolar lavage fluid (BALF) of the treated group ($1.34\pm0.79\times10^8$/L) was significantly decreased against the control group ($2.95\pm1.37\times10^8$/L), and there were statistical differences between the two groups ($P<0.01$). Interleukin-8 (IL-8) was subsequently identified as an important factor implicated in the pathogenesis of inflammation, and it is believed that tumor necrosis factor-α (TNF-α) are capable of augmenting IL-8 production. We found that the expression levels of serum IL-8 was significantly lower than that of rats in control group (53.27 ± 18.98 ng/L versus 67.86 ± 12.29 ng/L), as well as TNF-α (37.05 ± 2.74 ng/L versus 58.42 ± 6.15 ng/L), and there was positive relationship between the expression of IL-8 and TNF-α (both $P<0.05$). Our results reveal that TANREQING liquid may reduce the airway inflammation of the chronic bronchitis rats by reflecting the infiltration of WBC and depressing the synthesis of IL-8 and TNF-α, and TANREQING injection appears safe and effective in treating CB disease.

The research of cellular immune function of Human Papillomavirus Type 16 E7 protein

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Human Papillomavirus Type 16 (HPV16) infection is a main risk factor for HPV16-related diseases, including tumors such as cervical cancer. To develop the therapeutic vaccines based on HPV E7 gene or protein, we immunized BALB/c mouses with purified HPV16 E7-TRX fusion protein and tested their cellular immunity by MTT and 51Cr release assay. MTT results showed that A570 values of lymphocytes from the mice immunized by HPV16 E7-TRX fusion protein, pET32a(+) expressing protein were 1.0308 0.0896 and 1.0292 0.0468 respectively, they were significantly higher than the ConA positive control group (0.9656 0.1036) and the 1640 medium negative control (0.9075 0.0454). 51Cr release assay showed that the 51Cr release rates were 36%, 25% and 22% respectively, when we used the lymphocytes from the HPV16 E7-TRX fusion protein immunized mouse as the effective cells and NIH3T3 cells which were stably transfected by the verified pcDNA3.1(+)/E7 as the target cells, and keep the ratios of the effective cells versus target cells were 100:1; 50:1 and 25:1 respectively. On the other hand, the rates from the unimmunized control mouses were 14%; 9% and 5% respectively. There is statistical significance between the rates from immunized and unimmunized mouse at all these three levels. These primiry results suggested that HPV16 E7 protein can effectively stimulate BALB/c mouse producing specific cellular immune reaction.

Construction of Recombinant Adenovirus Fusion Expression VP1-2A of Foot and Mouth Disease Virus and Porcine IL-2

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Foot-and-mouth disease (FMD) is caused by FMDV, which is a highly contagious, acute vesicular disease in cloven-hoofed animals. In order to prevent and control this disease, several novel approaches to vaccine development are emerging, and these may enjoy significant advantages over conventional vaccines. However, as yet they remain too costly for general veterinary use, and their immunogenicity is often limited. One way to remove this shortcoming is to improve the adjuvant used in the formulation of the vaccine. In this research, we choose porcine Interleukin-2 as the adjuvant to Construct Recombinant Adenovirus Fusion Expression VP1-2A of Foot and Mouth Disease Virus and Porcine IL-2.VP1-2A gene of FMDV was and porcine Interleukin-2 gene were amplified and cloned into pAdeno Vator-CMV5-IRES-GFP, named pA5VP1-2A-PoIL-2. Recombinant vectors were linearized by *Pme*?, and then linearized recombinant vectors and pAdeno VatorΔE1/E3 co-transformed into *E.coli* BJ5183 competent cells by electrophoration. The homologous recombinant plasmids were selected and linearized by *Pac*? to expose the encapsidation signal. Linearized virus plasmids were transfected into 293A cells by Lipofectamine^{TM}2000, and recombinant viruses were named rAd5-VP1-2A-PoIL-2. Western-blot indicated that rAd5-VP1-2A-PoIL-2 had specific antigenicity of FDMV and VP1-2A-PoIL-2 was cleaved by 2A with VP1-2A and PoIL-2. The lymphocyte proliferation test indicated that rPoIL-2 had biological activity, thus providing valuable support for further development of FMD genetic engineering vaccines.

Vasoactive intestinal peptide regulates the dynamic balance of TREM-1/TREM-2 expression

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Acute lung injury (ALI) is characterized with pulmonary capillary endothelial and alveolar epithelial damage and increased vascular permeability due to inflammatory reaction. Triggering receptors expressed on myeloid cells-1 (TREM-1) and TREM-2 are newly discovered inflammatory molecules. TREM-1 can combine with TOLL-like receptors (TLRs) to stimulate the according immune signal, resulting in the excessive inflammatory response. TREM-2 can inhibit the reaction between macrophage and TLR, and alleviate the inflammatory effects of TREM-1. Vasoactive intestinal peptide (VIP) is an abundant neuropeptides in lung, and has strong inhibitory effect on inflammation. The biological effects of unbalanced of TREM-1/TREM-2 in ALI and the regulatory role of VIP have not been reported. RT-PCR analysis and FCM showed that the TREM-1 expression in mouse lung of ALI was significantly increased, whereas the TREM-2 expression decreased. The results suggest that changes of TREM-1 and TREM-2 expression in physiological and stress state are opposite, and there may be a dynamic equilibrium of TREM-1/TREM-2 ratio, and the loss of steady state of TREM-1/TREM-2 plays an important role in the development of ALI. We observed that TREM-1 only expresses on macrophages, while TREM-2 exists in a variety of lung cells, such as bronchial epithelial cells, fibroblasts, lung adenocarcinoma cells and macrophages. The distribution and biological effects of TREM-2 in lungs may be more extensive than TREM-1, and TREM-1/TREM-2 ratio remained low in physiological conditions which is related to the micro-environment stability of normal lung. Further study shows that VIP could down-regulate TREM-1 expression and up-regulate TREM-2 expression of LPS-induced macrophages in a time- and dose-dependent manner, and its signaling pathway was related to PKC and PKA. It suggests that VIP is involved in regulation of TREM-1 and TREM-2 expression while in ALI, and is an endogenous substance which could re-balance the TREM-1/TREM-2 ratio. This study was supported by the National Natural Science Foundation of China (No.30870915).

The infection and viral DNA replication of *Bocavirus* minute virus of canine in permissive cells and non-permissive cells

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Minute virus of canine (MVC) is a member of genus *bocavirus* in *parvovirdae* family. We demonstrate that MVC only infected WRD cells(permissive cells) since specific MVC mRNAs were only detected in infected WRD cells among tested cell lines using RNase protection assays, after we infected various cell types (324k, A549, A293, WRD, CrFK, EBTr, COS-7 and A9 cells) with MVC. Furthermore, we transfected infectious clone of MVC to different cell lines. The results showed that, with the exception of 293 cells, non-human cells (CrFK, EBTr and COS-7) supported a significant replication following transfection. In addition, two-rounds of reinfection on WRD cells (blind passage) of the transfected cell lysates in CrFK, EBTr and COS-7cells tranfected with infectious clone of MVC was confirmed by detection of specific viral mRNAs. The result suggests that MVC can produce infectious virions in some types of non-permissive cells. Although, 293 cells supported very poor DNA replication of MVC following transfection, we did not detect viral infection by the RNase protection assay after four- rounds of reinfection in WRD cells. However, using western blot and immunofluorescence, we found that the NS1 protein of MVC was expressed in both 293, CrFK, EBTr and COS-7cells after transfected with infectious clone of MVC, and the NS1expression in 293 cells was high than that in other cells, which suggests that maybe other steps of virus production (e.g., single strand DNA synthesis or capsid assembly) were blocked in 293 cells and DNA replication of MVC required specific cellular factors that were only present in certain types of cells. Together, these results indicate, unlike other parvoviruses, Bocavirus infection had a critical limiting step of viral DNA replication and infection in permissive cells and non-permissive cells.

Effect of vasoactive intestinal peptide on IL-17 expression in lung and macrophage

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Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) still represent a substantial problem, with a mortality rate around about 50%. ALI progressed to pulmonary fibrosis in the late stage, which will seriously affect the quality of life of patients. Interleukin-17 (IL-17) is a newly described proinflammatory cytokine produced by a subtype of T helper lymphocytes (Th17). And studies suggest that IL-17 could promote the fibrosis in lever and lung. The relevance between IL-17 and ALI is unknown. Our study demonstrates that the IL-17 expression in broncho-alveolar lavage fluid (BALF) and lung homogenate after lipopolysaccharid (LPS)-stimulating significantly increased comparing with normal group mice. Alveolar macrophages (AMs) play a vital role in the procession of ALI through secreting a variety of proinflammatory cytokine. To study whether the macrophage secretes IL-17, RT-PCR and ELISA were employed to determine the IL-17A mRNA and IL-17 of macrophage respectively. Results showed that both IL-17A mRNA and IL-17 expression of LPS-induced macrophage increased significantly. As one of the most important neuroimmune peptides in lung, vasoactive intestinal peptide (VIP) exerts protective-property through multi-ways. We tested that VIP could reduce the IL-17 mRNA and IL-17A in a time- and does-dependent manner, which could be partially reversed by PKC inhibitor and PKA inhibitor. These results suggest that macrophage can secrete IL-17 during ALI, which might exert an alternative effect on the procession of ALI, and VIP may decrease the IL-17 expression of LPS-induced macrophage, whose signal transduction pathways are associated with PKC and PKA. This study shows a novel insight into the pathophysiological mechanisms in ALI/ARDS, and provides us with new property of VIP in lung injury/repair. This study was supported by the National Natural Science Foundation of China (No.30870915).

The protective effects of desipramine on lipopolysaccharide-induced acute lung injury in mice

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Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are common diseases with high mortality. The aim of present study is to investigate the protective effects of desipramine (DP), a selective norepinephrine reuptake inhibitor, on lipoplysaccharide (LPS)-induced ALI. The mice model of ALI was induced by LPS (10 mg/kg ip). DP (20 mg/kg) was injected at 10 min before LPS. The results showed that LPS significantly increased the total protein concentration and WBC number in bronchoalveolar lavage fluid (BALF) ($P<0.01$). The lung wet-to-dry weight ratio (W/D) and myeloperoxidase (MPO) activity were both increased by LPS ($P<0.01$). Pretreatment with DP attenuated the LPS-induced increase in the total protein concentration and WBC number in BALF and the lung W/D ratio and MPO activity in lungs ($P<0.05$). In DP pretreatment group, the pathological changes of lung tissue were less severe than that in LPS only group. In control group, few NF-Bp65 positive cells were detected in lung tissue by immunohistochemistry. Pretreatment with DP attenuated the LPS-induced increase in NF-κB P65 protein expression ($P<0.05$). These results suggested that pretreatment with DP protected lung from LPS induced lung injury in mice, through, at least in part, inhibiting NF-κB signalling pathways. The work was supported by the National Natural Science Foundation of China (No. 30370531, 30471835).

Roles of TIPE2 in hepatitis B virus-induced chronic hepatic inflammation

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Hepatitis B virus (HBV)-induced hepatic inflammation afflicts hundreds of millions of people worldwide and is a leading cause of hepatic cancer. While the deleterious effect of the chronic hepatitis is well recognized, the molecular mechanisms underlying the pathogenesis of HBV-induced hepatic inflammation are not well understood. We report here that the tumor necrosis factor-α-induced protein-8 like-2 (TIPE2 or TNFAIP8L2), a newly identified regulator of immune receptor signaling, plays a critical role in controlling HBV-induced hepatitis. Patients with chronic hepatitis B had significantly reduced levels of TIPE2 expression in their peripheral blood mononuclear cells (PBMC) as compared to healthy individuals. The TIPE2 expression negatively correlated with the blood levels of alanine aminotranferease (ALT), aspartate aminotransferase (AST), and total bilirubin (Tbil) as well as the HBV load of the patients. These results indicate that TIPE2 plays a critical role in taming HBV-induced hepatic inflammation and that strategies targeting TIPE2 may be effective for preventing the hepatic disease.

Transduction of *Schistosoma japonicum* juvenile worms by pantropic retroviral vectors pseudotyped with the glycoprotein of vesicular stomatitis virus (VSVG)

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Although draft genome sequences of *Schistosoma japonicum* (*S. japonicum*) and *Schistosoma mansoni* (*S. mansoni*) are available, the structures and characteristics of most genes and influence of exogenous genes on metabolism of schistosomes remain uncharacterized. Furthermore, which functional genomics approaches will be tractable for schistosomes are not yet apparent. Here, the retroviral vector pBABE-puro based on Moloney murine leukemia virus was modified to incorporate the human telomerase reverse transcriptase gene (*hTERT*) as a reporter gene, under the control of retroviral long terminal repeat. The construct and pVSV-G plasmid encoding vesicular stomatitis virus envolope glycoprotein (VSVG) were employed to co-transfect GP2-293 cells and the VSVG-pseudotyped replication incompetent retrovirus particles were produced. Subsequently, these retrovirus virions were utilized to infect *S. japonicum* juvenile worms for investigation of integration, transcription and expression of *hTERT* transgene in these parasites. Juvenile somules were cultured for 6 days after exposure to the virions after which genomic DNAs from these parasites were extracted. PCR and Southern blot hybridization confirmed the integration of *hTERT* transgene in genomic DNAs extracted from virus exposed schistosomes. RT-PCR, immunoblot analysis and immunohistochemistry staining revealed transcription and expression of *hTERT* in the transduced worms. These findings indicated that *S. japonicum* could be effectively transduced by VSVG-pseudotyped retroviral virion, carrying the *hTERT* gene, and suggest that the pantropic retroviral vectors offer a potential tool to develop large-scale genetic analysis through insertional mutagenesis and to establish transgenic schistosome line.

The protective effect of oxymatrine on the cardiac muscle in rats with septic shock

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To explore the protective effect of oxymatrine (omt) on the cardiac muscle in rats with septic shock. Using a septic shock model produced by cecal ligation and puncture (CLP), fifty-six male SD rats were randomly divided into 7 groups: sham operation group, OMT control group, model group, CLP+OMT (52, 26, 13mg•kg^{-1}) group, positive control (CLP+Dexamethasone) group. To observe the changes of organization in the cardiac muscle tissue and the effect of oxymatrine on heart function. Changes in plasma lactic dehydrogenase (LDH), succinic dehydrogenase (SDH) and calcium content in the myocardium were determined by colorimetric method. In model group, The ultrastructure showed that severe cardiac muscle derangement, inflammatory cell infiltrate wide-ranging and the mitochondrion intumesced, intimal and adventitial integrity were demolished obviously, some crista confused and amount were reduced, the other were vanished or vacuolization. Compared with the normal control, HR increased 15%, LVEDP increased 47%, MAP decreased 33%, LVSP decreased 24%, LVdp/dt$_{max}$ decreased 38%, - LVdp/dt$_{max}$ negative value decreased 32%. LDH activity increased 35%, SDH activity decreased 28%, calcium contents increased 34% ($P<0.01$). iv. OMT 52, 26, 13mg·kg^{-1}, myocardial damage was ameliorated with different degree, but still have slight inflammatory cell infiltrate and exudation. From the electron microscope, cardiac muscle fiber was aligned normal, nucleus and mitochondria damages were relieved with different degree. Meanwhile, in the CLP+OMT (52, 26 mg·kg^{-1}) group, HR and LVEDP were decreased, MAP, LVSP and LVdp/dt$_{max}$ were increased, -LVdp/dt$_{max}$'s negative value increased, LDH activity decreased, SDH activity increased, calcium contents decreased ($P<0.01$). In the CLP+OMT (13mg·kg^{-1}) group, LVEDP, $\pm$LVdp/dt$_{max}$ and calcium contents were not improved obviously ($P>0.05$). Above-mentioned parameters all have improved in the OMT+DEX group ($P<0.01$). These results suggest that oxymatrine can produce protective effects on cardiac muscle in rats with septic shock. That protective effects in the CLP+OMT (52, 26 mg·kg^{-1}) group as well as in the OMT+DEX group.

Association between RANTES functional polymorphisms and tuberculosis in Ningxia Chinese Hui people

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Chemokines play a major role in leukocyte recruitment during the formation of tuberculous granulomas. One chemokine, regulated on activation normal T cell expressed and secreted (RANTES), has been suggested to play an important role in the initiation and development of tuberculosis (TB). In the present studied, we examined a possible association between genetic polymorphisms (RENTES-403G/A, -28C/G and In1.1T/C) and TB of the Hui people in the Ningxia Haiyuan area. A total of 188 Haiyuan Hui patients with TB and 200 healthy cotrols were included in the study. REANTS-403 and -28 promoter and intronic In1.1T/C polymorphisms were analyzed by polymerase chain reaction-restriction fragement length polymorphism (PCR-RFLP) or direct sequencing. Results shown that patients with TB had significantly higher frequency of the REANTS (-28) CG+GG genotypes compared to controls [12.7% vs. 27.1%; OR (95%CI), 2.541 (1.472-4.414); $P=0.001$], The TB patient group showed a significant higher frequency of the G allele compared to the controls [6.6% vs. 14.0%; OR (95%CI), 2.298 (1.378-3.833) $P=0.001$]. The distribution of REANTS-403G/A and In1.1T/C was not found to be different between patients with TB and healthy control subjects of the hui population in the Haiyuan area. Strong linkage disequilibrium was found among 3 SNPs (RENTES-403G/A,-28C/G and In1.1T/C), resulting in 4 major estimated haplotypes. One risk haplotypes of RANTES, C-A-G, at positions IN1.1, –403 and –28, respectively, were identified. In conclusion, the present study showed a significant but not independent association between the (-28) C/G polymorphisms of the REANTS (presence of -28G allele) and TB of the Hui people in the Ningxia Hai yuan area. Our findings support the association Between RANTES functional polymorphisms and TB.

SARS-CoV S protein induces strong mucosal immune responses and provides long-term protection against SARS-CoV infection

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We have previously reported that a subunit protein vaccine based on receptor binding domain (RBD) of SARS-CoV S protein and a recombinant adeno-associated virus (rAAV)-based RBD (RBD-rAAV) vaccine could induce highly potent neutralizing antibody (NA) responses in immunized animals. In this study, systemic, mucosal and cellular immune responses and long-term protective immunity induced by RBD-rAAV were further characterized in a BALB/c mouse model, with comparison of the intramuscular (i.m.) and intranasal (i.n.) routes of administration. Our results demonstrated that: (1) the i.n. vaccination induced systemic humoral immune response of comparable strength and shorter duration than the i.m. vaccination, but local humoral immune response was much stronger; (2) the i.n. vaccination elicited stronger systemic and local specific cytotoxic T cell responses than the i.m. vaccination, as evidenced by higher prevalence of IL-2 and/or IFN-γ producing CD3+/CD8+ T cells in both lungs and spleen; (3) the i.n. vaccination induced a similar protection as the i.m. vaccination against SARS-CoV challenge in mice; (4) higher titers of mucosal IgA and serum NA were associated with lower viral load and less pulmonary pathological damage, while no antibody-mediated disease enhancement effect was observed; and (5) the vaccination could provide long-term protection against SARS-CoV infection. Taken together, our findings suggest that RBD-rAAV can be further developed into a vaccine candidate for prevention of SARS and that i.n. vaccination may be the preferred route of administration due to its ability to induce SARS-CoV-specific systemic and mucosal immune responses and its better safety profile.

Artificial ribozyme, M1GS, intracellularly silences expression of the human cytomegalovirus gene UL54.

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The extracellular cleavage activity of an artificial ribozyme, RNase P-based M1RNA and guide sequence (M1GS), on its target gene unique long 54 gene (UL54) has been confirmed by in vitro experiment. UL54 is an mRNA segment of deoxyribonucleic acid polymerase gene in human cytomegalovirus. The present study is to explore its intracellular inhibitive effect on this target gene expression. A specific ribozyme, T6-M1GS, was constructed. To test the intracellular activity of T6-M1GS, Hela cells were co-transfected with ribozyme plasmid T6-M1GS-pLXSN and the target recombinant plasmid UL54-D-GFP. After 48 h of transfection, the expression of green fluorescent protein was detected by fluorescence microscope to determine UL54-D expression. Meanwhile, UL54-D expression was also evaluated by northern blot analysis. The results showed that T6-M1GS expressed in Hela cells can specifically silence the expression of UL54-D gene. The results suggested that T6-M1GS might be a specific inhibitor of UL54 mRNA, and it might be developed into a promising anti-virus agent.

Effects of lipoxin A4 on lipopolysaccharide-induced oxidative damage in macrophages and the possible mechanisms

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To explore the effects of lipoxin A4 (LXA4) on lipopolysaccharide (LPS)-induced oxidative damage of macrophages, the RAW264.7 macrophages cells were exposure to LPS in the absence or presence of LXA4. Then, the cell activity was analyzed by [3H]-TdR incorporation assay; reactive oxygen species (ROS) were quantified through flow cytometry (FCM) and laser confocal scanning microscopy; IκBα degradation and NF-κB translocation were determined via Western blot; NF-κB transcriptional activity was tested by transfections and luciferase activities assay; the levels of nitric oxide (NO) and malondialdehyde (MDA), besides the activities of SOD, GSH-Px, CAT, and iNOS were all detected using assay kits. In this study, the data indicated LXA4 was able to increase the survival activity of LPS-treated macrophages; decrease the levels of ROS, NO and MDA; inhibit the activity of iNOS; promote the activity of SOD, GSH-Px, and CAT; restrain LPS-induced IκBα degradation, NF-κB translocation and NF-κB transcriptional activity. It suggested LXA4 could antagonize LPS-induced oxidative damage through decrease the production of ROS and NO also increase the activity of antioxidative enzymes, and the mechanism involving in inhibition of NF-κB signaling pathway.

Vasoactive intestinal peptide reduces IL-17R expression in lipopolysaccharide-induced lung fibroblast

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Pulmonary fibrosis is a destructive inflammatory disease with limited therapeutic options, and it is a common complication of acute lung injury (ALI). The exact inflammatory response preceding and concurring with collagen deposition remains unclear. Some studies have identified the critical role for Interleukin-17A (IL-17A) in fibrosis. And we have verified IL-17A expression increased significantly in lung during ALI. IL-17A can recognize IL-17 receptor (IL-17R) A and C, which are cell surface receptors. This receptor, especially anchored on lung fibroblast synthesizing and secreting collagen, plays a vital role in the procession of fibrosis. We tested the IL-17RA/C mRNA expression in lung after intraperitoneal injection with lipopolysaccharide (LPS), and the results showed that IL-17RC mRNA not IL-17RA mRNA increased significantly. Further research found the increased IL-17RC mRNA and IL-17R expression in LPS-induced lung fibroblast, illustrating that the change of IL-17R expression may be relevant with fibrosis. Vasoactive intestinal peptide (VIP), an abundant neuroimmune peptide in lung, shows its protective-property for ALI from our previous studies. To examine its anti-fibrosis activity, we found that with VIP could reduce IL-17RC mRNA and IL-17R expression in LPS-induced lung fibroblast in time- and does-dependent manners. And this effect could be partially reversed by PKC inhibitor and PKA inhibitor. These results suggest that VIP can reduce IL-17R expression in LPS-induced lung fibroblast and its signal transduction pathways are related to PKC and PKA. Together, this study provides a logical relationship between IL-17R and ALI, and shows us a novel therapeutic property of VIP in anti-fibrosis. This study was supported by the National Natural Science Foundation of China (No.30870915).

Orientation and directed migration of cultured trophoblast cells in small electric fields

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The invasion of maternal deciduas and uterine spiral arteries by a trophoblast subpopulation called extra villous trophoblast (EVT) is essential for the establishment of a normal placenta and an adequate blood flow toward the fetus. Electric field as a potential guidance cue might be direct to a variety of cell behaviors, including migration and invasion of cells. The mechanism by which direct electric fields (dc EFs) direct cell movement, however, is not yet understood, and the effects on trophoblast cells are entirely unknown. Single HTR8 cells, representing a first trimester trophoblast cell line with invasive properties, cultured in media containing 20% calf serum showed significant galvanotropism, including directed migration and orientation in the electric field with a field strength of 200 mV/mm. Cultured in the mediumsingle cells showed obvious cathodal migration at 200 mV/mm. The mRNA level of matrix metallo proteinase (MMP)-9, one of the key molecules involved in migration andinvasion of cells was elevated when the cells were cultured in the electric field. These data suggest that the trophoblast cells were responsive to small dcEF stimulation. This could have important implications for the invasive process of extravilloustrophoblast and has clinical implications. Galvanotaxis could thus play a significant role in both cellular physiology and pathophysiology.

Effects of vitamin A and vitamin A plus zinc on serum erythropoietin concentrations in preschool children in Chongqing, China

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Erythropoietin (EPO) is a key agent in haematogenesis. Previous studies suggest that vitamin A (VA), zinc and some other micronutrients may relate to anaemia. However the relationships between VA, zinc and some other micronutrients with EPO are not clear. The study was to determine whether supplementation of VA, VA+zinc or VA combining with multiple-micronutrient improved EPO concentration in preschool children. In a suburb of Chongqing China, 290 preschoolers aged between 36-72 months old were assigned randomly to 3 treatment groups: VA group that supplemented 25000 IU VA two times per months; VA+zinc group that supplemented 10 mg elemental zinc five times per week, and supplemented 25000 IU VA two times per months; the VA combined multiple-micronutrient group that supplemented 5000 IU VA, and calcium, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin C, folic acid, niacinamide, as well as pantothenic acid five times per two weeks. The supplementation lasted for 6 months. At baseline, serum retinol, serum zinc, hemoglobin, and EPO concentrations were no differient among groups (control for ages). The EPO concentrations were significant increased in all treatment groups compared with the baseline values ($P<0.05$). The EPO concentrations gain in VA and VA+zinc group was significant higher than the multiple-micronutrient group ($P<0.05$). The EPO concentrations increase in VA+zinc group was higher than VA group ($P>0.05$). These results suggest that VA and zinc supplementation increases serum EPO concentrations in preschool children living in less developed community.

Phosphorylation of spinal signaling-regulated kinases by acute colorectal distension in rat

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Spinal extracellular signaling-regulated kinase 1 and 2 (ERK 1/2) have been found to contribute to nociceptive processing, but little is known about the role of spinal ERK 1/2 in visceral pain related to colorectal irritation. The aim of this study was to investigate ERK activation (phosphorylation) in spinal dorsal horn neurons after acute colorectal distension. Methods: Under intraperitoneal anesthesia using chloral hydrate 300 mg/kg, male Sprague-Dawley rats were exposed to a 20-s colorectal distension of 2, 4, 6, 8, 10 and 12 kPa or no distension (sham). The electromyographic response in the rectus abdominis muscle and mean arterial blood pressure and heart rate changes to colorectal distension were determined. The numbers of phosphorylated-ERK 1/2-immunoreactive (pERK 1/2-IR) dorsal horn neurons in cervical (C5-8), thoracic (T5-8), thoracolumbar (T12-L2) and lumbosacral (L(6)-S(1)) segments were counted using immunohistochemistry. Results showed that compared with the non-distended sham rats, colorectal distension resulted in a stimulus-dependent increase in electromyographic activity and the number of pERK-IR neurons that selectively located to the thoracolumbar segment, mostly in the deep dorsal and the central canal regions. The time course study demonstrated that spinal ERK activation peaked at 90 min with a slow decline for 150 min after uterine cervical distension stimulation. In conclusion, this study suggests that activation of spinal ERK might be involved in acute visceral pain arising from the colorectal.

Selection of human stem cell factor mimetic peptides from phage-displayed random peptide library

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The hematopoietic growth factors (HGFs) control the proliferation and differentiation of blood stem cells and progenitor cells and play a role in modulating the immune system to some degree. Mimetic peptides of human EPO and human TPO had been generated respectively in 1990s. However, mimetic peptides of human stem factor cell factor (hSCF) have not been reported. Here, we described that screening phage clones with high hSCF receptor (rc-kit/Ig1-3) binding activity from phage-display random hepta/dodeca peptide library by using phage ELISA assay, abstracting and sequencing phage single DNA was carried out. 11 ph.D-C7C clones and 8 ph.D-12 phage clones with high binding activity with hSCF receptor were selected. Sequence analysis showed that there were no consensus sequence between hSCF and these screened mimetic peptides, except one consensus sequence DPSPHTH found in heptapeptid library. Four kinds of peptides (CE3, CE16, LE4 and LE20) with higher c-kit/Ig1-3 binding activity were chemically synthesized and characterized by using cell proliferation assay with MTT in UT-7 cells. These four kinds of synthesized peptides could stimulate UT-7 cell proliferation shown by MTT assay, especially CE16 and LE20. These results illustrated that four kinds of hSCF mimetic peptides were successfully isolated from phage-displayed random peptide library, laying a foundation for probing the function of hSCF mimetic peptides and clinical application.

Ghrelin and Obestatin Modulate GHRH and Somatostatin Release from Mouse Hypothalamus

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Ghrelin, a 28-amino acid peptide primarily synthesized in the stomach, has been identified as an endogenous ligand for the growth hormone secretagogue receptor, to stimulate GH secretion. Obestatin, a 23 amino acid peptide derived from the same propeptide as ghrelin, is able to antagonize ghrelin-induced increase of GH secretion *in vivo* but not *in vitro*. Thus, the blockade of ghrelin-induced GH release by obestatin could be mediated at the hypothalamic level by the growth horman releasing hormone (GHRH) and somatostatin (somatotropin releasing inhibitory factor, SRIF) which controls pituitary GH secretion. In this study, we investigated the effect of ghrelin and obestatin on GHRH and Somatostatin release from hypothalamic explants. The basal release of GHRH was not modified in the presence of ghrelin or obestatin (1μM). Exposure to 14 or 28mM potassium (K^+) induced a similar increase in GHRH release while 56mM K^+-induced GHRH release reached a maximum. In the presence of 14 or 28mM K^+, ghrelin further increased GHRH release by 25% without reaching the maximal effect induced by 56 mM K^+. The ghrelin-induced GHRH release was totally blocked by obestatin. Obestatin alone did not significantly modify K^+14/28-induced GHRH release. The basal release of SRIF was not modified in the presence of ghrelin or obestatin (1μM). Exposure to 14 or 28mM K^+ induced a similar increase in SRIF release while 56mM K^+ stimulation of SRIF release reached a maximal response. In the presence of 14 or 28mM K^+, ghrelin significantly decreased SRIF release by 24%. Adding obestatin did not interfere with the effect of ghrelin nor with the effect of K^+14/28. In summary, ghrelin increased GHRH and decreased somatostatin release from hypothalamic explants while obestatin only reduced ghrelin-induced increase of GHRH release, thus indicating that the effect of ghrelin and obestatin is targeted to GHRH neurons.

Observation of movement behavior of mouse primordial germ cells *in vitro*

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Mouse primordial germ cells (PGCs) are known to reside originally in the extra-embryonic epiblast of the gastrulating embryo. The germ cell precursors migrate back into the embryo, through the yolk sac, hindgut and dorsal mesentery, and arrive in the genital ridge finally. The mechanism of their migration behavior is going to be discovered. Data from the literature suggested that the PGCs migrate by extension of their simple amoeboid movement over the underlying cell surfaces and the extra cellular matrix. Our work on seminiferous tubule transplantation of mouse PGCs unexpectedly revealed a peculiar morphology and migration behavior of PGCs. PGCs isolated from 15 day mouse embryo testis, stained with either Neutral Red or PKH26, were observed by fluorescence microscopy. Initially, the PGCs appeared as big round cells. Shortly thereafter, they were noted to extend several crescent filopodia, one of which then became elongated into a drumstick-like process which quickly swung the swollen end side to side, seemingly in search of an attractant. These processes were rapidly, subsequently becoming long cytoplasmic processes. The addition of a drop of crude extract from 15 day embryonic testis onto one side of the cover glass induced most of the PGCs to direct their processes toward the extract. Thirty minutes later, the processes of the PGCs formed very long fine fibers, some of which measured several hundred micrometers long, resembling the axons of nerve cells. The drumstick-like end of these thin axon-like processes attached to the surface of other PGCs to form a meshwork structure. This is the first description of the behavior and morphology of live PGCs *in vitro* under fluorescence microscope, which can provide a useful model for studying PGC migration behavior and its mechanism.

Scatchard analysis of AF-1 binding to living cells by quantitative flow cytometric analysis

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Uteroglobin (UG) is an evolutionarily conserved protein that has multiple physiological functions. Of interest, out of UG protein more than 400 amino acid long, small peptides drawn for the C-terminal region, including antiflammin-1 (AF-1), retain biological activity. In the present study, quantitative analyses of labeled AF-1 binding to NIH 3T3 were done using flow cytometric analysis on NIH 3T3 cells. For assay of total binding of the [Cy5]-AF-1, cell suspensions were diluted to 3×10^6 cells/ml in PBS and kept in 37°C for 30min. The cells were then incubated with graded concentrations of Cy5-AF-1 (0.5～100nM), and cell-associated Cy5 was measured by flow cytometry, as the mean fluorescence intensity in each sample determined by flow analysis of 10,000 cells. Nonspecific binding of [Cy5]-AF-1 was determined in a parallel set of samples that were preincubated with 5μM of AF-1 to occupy the binding sites. The specific binding of Cy5-AF-1 was calculated as the difference between total and nonspecific binding. The data showed that, with increasing concentration of unlabeled peptide AF-1, the decrease in fluorescences indicates a progressive occupation of Cy5-AF-1 binding sites on the cell surface. Excess unlabeled peptide AF-1 displaced labeled AF-1 binding with the following values: 60%, 80%, 85%, and 91% for the concentrations of 1μM, 5μM, 10μM, and 20 μM, respectively. The binding data were then subjected to mass law analysis by the method of Scatchard to yield the K_d and B_{max} values. The results showed that $K_d = 37.75 \pm 4.003$ nmol/L, $B_{max} = 93.29 \pm 4.267$ RFI. Collectively, these data suggest that AF-1 bound to UG-binding protein in a ligand–receptor-like manner. Whereas AF-1 was considered to be a PLA_2 inhibitor, in light of its ability of interacting with UG-binding protein, it appears that the mechanism(s) involved in AF-1 are complex and multimodal. This study was supported by Grants 30670770 and 30870916 from National Natural Science Foundation of China.

The effect of neferine on blood glucose, serum lipids and insulin concentrations of type 2 diabetic rat model

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Diabetes mellitus is a common but serious metabolic disorder associated with many functional and structural complications. Glucose metabolism is disturbed due to an absolute or relative insulin deficiency. Neferine is a bisbenzylisoquinline alkaloid isolated from a Chinese medicinal herb (Nel umbo nucif era Gaertn). Pharmacological activities of neferine have been studied including antihypertensive, antiarrhythmic, antiagglutinating, antithrombotic, antioxidant, protecting against organphosphorus esters toxicity, inhibiting the proliferation of hypertrophic scar fibrohlasts and chemosensitive effects. The experiment was carried out to determine the effect of neferine on blood glucose, serum lipids and insulin concentrations in streptozotocin (STZ)- induced diabetic rats. Spsaruge-Dawley (SD) male rats were treated by a single intraperitoneal (i.p.) injection of STZ (30 mg/kg) after fed high glucose and fat diet for 4 weeks to induce rat model of type 2 diabetes. The level of fasting blood glucose (FBG) was judged as the indication of diabetic rat model. Total cholesterol and triglyceride were detected by the method of enzyme chromometry. Fasting insulin (FINS) was measured by radioimmunoassay. Index of insulin secretion was equal to ln [20 × FINS/(FBG-3.5)]. The STZ-treated rats appeared to decrease in insulin secretion and an increase in blood glucose, total cholesterol and triglyceride concentration. The sharp decrease in blood glucose, moderate decrease in the raised serum total cholesterol and triglyceride concentration, slight increase in the lowered serum insulin concentrations and increased index of insulin secretion were caused by neferine treatment. It is concluded that type 2 diabetic rats treated with neferine facilitate the control of the blood glucose and blood lipids and improve function of islet cell. This work was supported by the grant (№s 30860086, 30860333, 30660059 and 30660048) from National Natural Science Foundation of China, the grant (№ 20070403007) from Doctoral Fund of Ministry of Education of China and the grant (№s 0640042 and 2008GZY0029) from Natural Science Foundation of Jiangxi Province, the grant (№s 2007-60 and GJJ08049) from the Educational Department of Jiangxi Province.

TRPC expressions in the lung tissue of chronic asthmatic mice

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Asthma is a complex inflammatory disease of the lungs characterized by reversible airway obstruction, chronic airway inflammation and airway hyperresponsiveness. Chronic inflammation of the airways in asthma is characterized by increased inflammatory cells in the airway mucosa and lumen, especially eosinophils and $CD4^+$ T-lymphocytes. Non-selective cation influx through cononical transient receptor potential channels (TRPCs) is thought to be an important event leading to airway inflammation. This study was to investigate the expression of TRPC in the chronic asthmatic mice lung tissues. Mice model of chronic asthmatic were established by ovalbumin (OVA) peritoneal injection and inhalation. The airway responsiveness, bronchoalveolar lavage cytology and histological change was assessed. The TRPC expressions in lung tissue of the mice were detected with RT-PCR. Results demonstrated that the airway responsiveness of chronic asthmatic group was significantly increased compared to control group when mice were stimulated with MCh(6.25; 25 and 50mg/ml) ($P<0.05$). The numbers of total white blood cells and the EOS in chronic asthmatic group were $118.14\pm6.16\times10^4$/ml and $42.57\pm2.99\%$, respectively, while those in control group were $25.64\pm1.64\times10^4$/ml and $0.86\pm0.18\%$, respectively ($P<0.01$). Mass infiltration of inflammatory cells in airway wall or its vicinity in asthma group, and airway wall was thicker in asthma group than that in control group. The expression of TRPC1, TRPC2, TRPC3 and TRPC6 mRNA were detected in the normal mice lung tissues. Compared with those of control group, the expression of TRPC1, TRPC2 and TRPC6 mRNA in lung of chronic asthmatic group were increased ($P<0.05$). These data demonstrated TRPC1 and TRPC6 may contribute to asthma and provide an important novel target for the treatment of airway disease.

Antiflammin-1 inhibits the adhesion of neutrophils to endothelial cells induced by LPS

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The adhension between neutrophils and pulmonary vascular endothelial cells is the primary step in the inflammatory response. Uteroglobin (UG) is a potent anti-inflammatory protein from clara cells. Antiflammin-1 (MQMKKVLDS, AF-1) is equivalent to the 9 C-terminal amino acids of α-helix 3 of uteroglobin and have been showed have potent anti-inflammatory effects. But the anti-inflammatory mechanism of AF-1 is still unclear. The present study was to investigate the effects of AF-1 on adhesion between neutrophils and human umbilical vein endothelial cells (HUVECs) induced by LPS. Results showed that AF-1 (1-100μmol/L) could inhibit neutrophils adhesion to endothelial cells induced by LPS in a dose-dependent manner. With flow cytometry (FCM) assay we found that AF-1 could inhibit the expression of adhesion molecule CD11b on neutrophils and CD54 on endothelial cells induced by LPS, but the AF-1 itself does not affect the expression of adhesion molecule on the resting neutrophils and resting endothelial cells. Both neutrophils and endothelial cells could express uteroglobin-binding protein (UGBP), a AF-1 receptor protein on cell membrane, detected by FCM assay and RT-PCR. Anti-UGBP antibody pretreatment could attenuate the inhibition of adhesion rate and the expression of adhesion molecules induced by AF-1. Western-Blot assay indicated that AF-1 could reduce LPS-induced the phosphorylation level of P38/MAPK in endothelial cells significantly, and anti-UGBP antibody could inhibit the down-regulated effect of AF-1. In conclusions, AF-1 can inhibit the adhesion of neutrophils to endothelial cells and the adhesion molecules expression on them induced by LPS, which mechanism is related to decreasing the phosphorylation level of P38/MAPK through binding to the UGBP. The work was supported by the National Natural Science Foundation of China (No. 30670770, 30870916).

Serum vitamin A status of preschool children closely correlated with iron metabolic index but not body total iron contents

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To investigate the correlation between vitamin A nutritional status of preschool children and iron metabolic homeostasis and the total body iron content by cross-sectional descriptive study. Preschool children from were randomly chosen from the 20 kindergartens. Children's demographic data, socio-economic status and eating habits, etc. were investigated by questionnaires. The concentration of serum vitamin A was measured by HPLC, serum ferritin (SF) by ELISA, serum transferring receptor (sTfR) by microparticle-enhanced immunoassay, C-reactive protein (CRP) by particle-enhanced immunoturbidimetry and hemoglobin (HB) by hemiglobincyanide. The sTfR-SF index (TFR-F index) and total body iron content (TBIC) were computed, respectively. A total of 471 preschool children, 236 for boys and 235 for girls, were included in the study with ageed (4.0 ± 0.85) yrs. The concentrations of HB, SF, sTfR, serum vitamin A, TFR-F index and TBIC were (115.8 ± 9.2)g/L, (24.75 ± 14.71)μg/L, (1.28 ± 0.33)mg/L, (1.21 ± 0.36)μmol/L, 0.9595(0.7257, 1.2226) [media (P25, P75)] and 8.868 (6.986, 10.470) mg/kg, respectively. After adjustment for the covariate factors,the partial correlation coefficient (radjust) between vitamin A and HB, log SF, sTfR, TFR-F index and TBIC were 0.16 ($p<0.001$), -0.13 ($p=0.037$), 0.17 ($p=0.0011$), -0.013 ($p=0.7935$) and -0.05 ($p=0.3652$), respectively. With a multiple logistic regression model, VAD was an independent risk factor for prevalence of deficient iron storage [(odds ratio, OR) (95% CI): 1.88(1.01, 2.97)] defined by SF but not a risk factor [OR (95% CI): 1.365(0.286, 6.513)] when defined by TFR-F index. Serum vitamin A was closely correlated with iron biochemical index reflecting iron reserves and mobilization, while not iron absorption and body total iron contents.

Compared with vitamin A plus iron and multiple micronutrient supplementations, solely vitamin A fortified seasoning powder affect iron metabolic index without changing body total iron contents in children

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The present study to evaluate the effect of vitamin A on iron metabolic homeostasis and total body iron content. A total of 226 2-7 years old preschool children were recruited and randomly assigned into three diet groups for 6 months. Group I was fortified with vitamin A; Group II and III were fortified with vitamin A plus iron and vitamin A plus iron, thiamine, riboflavin, folic acid, niacinamide, zinc and calcium. The concentration of serum vitamin A, serum ferritin (SF), serum transferring receptor (sTfR), C-reactive protein (CRP) and hemoglobin (HB) were measured and TFR-F index and total body iron content (TBIC) were computed before and after intervention. The levels of HB significantly increased after intervention in all groups ($p< 0.05$) but no marked difference was observed between groups ($p>0.05$). Levels of SF and sTfR significantly decreased after intervention in all groups ($p< 0.05$) especially in group II and group III for SF ($p< 0.05$) and group I for sTfR ($p< 0.05$). No marked change of TFR-F index and TBIC was observed in group I ($p>0.05$), while TFR-F index decreased and TBIC increased in group II and group III ($p< 0.05$) after intervention. Compared with vitamin A intervention, the other two supplementations were significant protective factors for deficient iron storage defined by TFR-F index [(relative risk, RR) (95% CI): 0.410(0.218, 0.992)]. Vitamin A intervention has significant effect on iron storage and mobilization, but has no significant effect on TFR-F index and TBIC which prompted the possibility of seldom effect of vitamin A on iron absorption in small intestine.

miRNA-21 improves insulin resistace in 3T3-L1 adipocyte

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MicroRNAs (miRNAs) are a new class of highly conserved, noncoding small RNAs that regulate gene expression on the posttranscriptional level. There is increasing evidence that miRNAs are involved in multiple biological processes such as energy homeostasis, sugar and lipid metabolism, and cell differentiation, these researches suggested the role of miRNAs in insulin resistance (IR). In this study, we investigated the differentially expressed miRNAs between normal insulin-sensitive 3T3-L1 adipocytes and 3T3-L1 adipocytes rendered insulin resistance following treatment with high glucose (25mmol/L) and high insulin (1μmol/L), and showed microRNA-21 (miR-21) expression was significantly down-regulated. Over-expression of miR-21 increased insulin induced glucose consumption and glucose uptake rate by 14.9% and 46.2% respectively in IR- adipocytes. We also found PI3K p85a, p-Akt protein expression and the translocation of GLUT4 significantly increased in miR-21 treated IR-adipocytes. Overall, these studies for the first time provide evidence that miR-21 improve insulin resistace in 3T3-L1 adipocyte, and miR-21 may be potential used in treatment of insulin resistance. This work was supported by grants from the National Natural Science Foundation of China (30 770 868, 30971170).

Antiflammin-1 inhibits TGFβ-induced fibrosis in lung explants culture

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Antiflammin-1 (AF-1, MQMKKVLDS) is a synthetic nonapeptide with a similar sequence to the conserved sequence of CCSP secreted by lung Clara cells. Our previous study has manifested AF-1 could significantly inhibit the collagen deposition and fibroblast proliferation in bleomycin-induced pulmonary fibrosis. Now, we investigated whether the AF-1 had a protective role against fibrosis in mouse lung explant culture. The lung explant was treated with TGF-β(5ng/ml)under sterile conditions to establish the lung tissue fibrosis model. To investigate the anti-fibrosis function of AF-1, we treated the lung explant with AF-1($5x10^{-5}$mol/L) in the presence of TGF-β. The lung explant viability was assessed through lactate dehydrogenase (LDH) leakage and MTT assay after incubation of l; 3; 5 and 7 days. The collagen deposition was detected by hydroxyproline content and the expression of precollagen I mRNA was measured by real-time PCR at 7th day. The results showed that LDH raised up firstly and lowered down rapidly with time. The MTT level maintained steadily with a little fluctuation; AF-1 could reduce the hydroxyproline content and down regulate the expression of precollagen I mRNA. In conclusion, Antiflammin-1 has a protective effect on TGF-β induced lung explant fibrosis and it might have potent clinical application value. The work was supported by the National Natural Science Foundation of China (No. 30670770, 30870916) and Hunan Province Natural Science Foundation (No.05C0163).

The infiltration of mast cells and expression of stem cell factor in the renal tissue of patients with lupus nephritis

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The role of mast cell in kidney organization infiltration and the relations with the nephrosis have aroused the interest of kidney experts in recent years. Some research has shown that mast cells and their growth factor (stem cell factor, SCF) have implications in the pathogenesis of certain kidney diseases. A research of the mast cells and SCF distribution in lupus nephritis (LN) kidney may reveal new insights into its implications in LN and its association with disease states. This study examined the intensity of mast cell infiltration and the expression patterns of SCF in renal biopsies from 47 patients with LN and 5 nomal kidneys acting as control. They are tested, by toluidine blue and immunohistochemical staining, using specific antibodies (anti-human mast cell tryptase antibody, anti-SCF antibody). The mRNA expression of SCF was investigated by in situ hybridization. Findings of the study are as follows. The expression of SCF and mast cells were scarce or absent in normal kidneys; however they have increased significantly in LN kidneys, particularly in type IV LN. Mast cells were present in cortical tubulointerstitium, especially in fibrosis area, but were not seen in the glomeruli. SCF staining was detectable in the tubular cells, fibroblasts, peritubular capillaries, and the renal interstitium. Correlation analysis showed that the number of mast cells in renal interstitium was positively correlated with the intensity of interstitial SCF immunostain, the fibrosis of renal interstitium, and renal pathological chronic indexes. The expression of SCF was positively correlated with renal pathological active index, chronic index, albuminuria, and the injury of renal interstitium. These results suggest that mast cell may play a role in the development of LN chronic process, especially tubulointerstitium fibrosis. Furthermore, SCF is not only an important factor in mast cells accumulation but also might be actively participating in the whole process of initiation and progression of renal injuries in human lupus nephritis.

The activation of mGluRI in endothelial cells promotes the adhesion of neutrophils to endothelial cells

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L-Glutamate (Glu) is an excitatory neurotransmitter in the mammalian central nervous system. Recently, much attention has been paid to functional expression of Glu signaling molecules in peripheral tissues. The present study was aimed to investigate the expression of groupI metabotropic glutamate receptor (mGluRI) in endothelial cells, and the effect of mGluRI activation on the adherence of neutrophils to endothelial cells. Neutrophils were obtained from healthy donors. The adhesion of neutrophils to human normal umbilical vein endothelial cells (HUVE-12) was tested by colorimetric method. The results confirmed the expression of mGluR1 and mGluR5 in HUVE-12 cells using immunocytochemical methods and real-time quantitative RT-PCR. The pretreatment with the mGluRI specific agonist S-3,5-dihydroxy-phenylglycine (DHPG) (1×10^{-8}-1×10^{-6} mol/L) for 1 h on HUVE-12 cells increased the rate of neutrophils adhesion to endothelial cells in a dose-dependent manner, with a maximum effect at 10^{-6} mol/L ($P<0.01$). Following time extension (0.5-5 h), the treatment with DHPG (1×10^{-6} mol/L) increased the rate of neutrophils adhesion to HUVE-12 cells with a maximum effect at 1 h ($P<0.05$). The pretreatment with DHPG (1×10^{-6} mol/L) for 1 h increased the expression of adhesion molecule ICAM-1 in HUVE-12 cells determined by flow cytometry. ($P<0.01$). Application of selected mGluRI antagonist, (*RS*)-alpha-methyl-4- carboxyphenylglycine (MCPG, 0.5 mmol/L), abolished the effect of DHPG (1×10^{-6} mol/L) on neutrophils adhesion to HUVE-12 ($P<0.01$), and on the ICAM-1 expression of endothelial cells ($P<0.01$). These results suggest that the activation of mGluRI in endothelial cells can up-regulate the adhesion molecule ICAM-1 expression, and promote the adherence of neutrophils to endothelial cells. The work was supported by the National Natural Science Foundation of China (No. 30370531, 30471835).

Expression of PDCD5, Mdm2, Tip60 and p53 in esophageal carcinoma in Xinjiang Kazakh and Han

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This study aims to examine the relationship of the expressions of PDCD5, Mdm2, Tip60 and p53 in esophageal carcinoma in Xinjiang Kazakh and Han. The positive incidence of PDCD5, Mdm2 and Tip60 mRNA expressions in 40 cases of the esophageal carcinoma tissues were 80.0%, 65.0% and 97.5%. Expressions of PDCD5, Mdm2 and p53 protein have statistical significance in esophageal carcinoma and normal esophagus mucosa. The positive expression rate of PDCD5 protein in esophageal carcinoma was significantly correlated with tumor differentiation. The expression of Tip60 was positively correlated with p53 protein. There was a negative correlation between PDCD5 mRNA and Mdm2 mRNA. These results suggest that the expressions of PDCD5, Mdm2, Tip60 and p53 among Kazakh and Han in Xinjiang have common way. Expression of PDCD5 in esophageal carcinoma was significantly lower than in normal esophagus mucosa. Expressions of Mdm2, Tip60 and p53 in esophageal carcinoma were significantly higher than in normal esophagus mucosa. It was speculated that there may be a common transcription mechanism which constructed multiple pathways in esophageal carcinoma.

Fank1 antigonizes AP-1-mediated apoptosis by interacting with Jab1

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The identification of testis highly expressed genes involved in apoptosis is valuable to delineate the mechanism of spermatogenesis. In the present study, we reported that Fank1, a novel gene highly expressed in testis, functioned as an anti-apoptotic protein. We provide evidence for an interaction between Fank1 and Jun activation domain-binding protein 1 (Jab1) *in vivo*. In addition, we investigate the biological effect of this interaction on the modulation of the anti-apoptotic effect of AP-1. Our results indicated that by interacting with Jab1, Fank1 could suppress cell apoptosis by activating the AP-1-induced anti-apoptotic pathway. These results will allow us to gain further insight into the biological and molecular functions of Fank1 in spermatogenesis.

Selection of the functional domains of Daxx and its effect on cholesterol contents in HepG2 cells

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Fas death domain-associated protein (Daxx) as a highly conserved nuclear protein contains four different structural domains, and plays a significant role in regulation of transcription. AR (androgen receptor), which could promote the activation of SREBPs and SCAP and also the synthesis of cholesterol by effecting transcription. However, the domain of Daxx which can bind with AR and effect cholesterol contents remains undefined. Here, we want to find the activated domain of Daxx and investigate it's function. Four functional domain of Daxx were respectively amplified by PCR and subcloned to a prokaryotic expression vector (pGEX-6P-1), which included pGEX-6P-1/Daxx/DM1-240; pGEX-6P-1/Daxx/DM241-501; pGEX-6P-1/Daxx/ DM502-625 and pGEX-6P-1/Daxx/DM626-740 by gene recombination. SDS-PAGE assay showed that the size of inserted fragment in the recombinant gene corresponded to functional domain of Daxx. The molecular weight of expressing protein induced by IPTG is matched with our expect. The results from sequencing experiment indicated that there is no reading frame shifts and mutations in recombinant. The protein expressing from pGEX-6P-1/Daxx/DM626-740 can interact with AR by using GST pull-down in vitro. The cholesterol contents decreases in HepG2 cells transfected with pCDNA3.1 (+)/ Daxx/DM626-740 by oil red O stain and HPLC. At the same time, the expression of SREBPs and SCAP decreased respectively by western blot. These results have shown that Daxx could bind to AR by functional domain, which is important to reducing the cholesterol contents in HepG2 cells. This work was supported by the National Natural Science Foundation of China (30770868, 30600249 and 30971267), the Doctor Research Starting Foundation of the University of South China (2007XQD24) and the construct program of the key disciplines in human province.

Mammalian rhomboid family member RHBDD1 cleaves TSAP6 to regulate exosomal secretion and apoptosis

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Rhomboid proteins represent a widely conserved family of intramembrane serine proteases found from bacteria to humans. In our previous work, a testis highly-expressed rhomboid family member RHBDD1 was obtained from human testis Cytotrap cDNA library and it was found that RHBDD1 could mediate the cleavage of proapoptotic Bcl-2 family member Bik. Herein, by using yeast two-hybrid screening we found a novel proapoptotic protein, TSAP6, interacts with RHBDD1. TSAP6 is a p53-inducible protein with six transmembrane domains. Our work found that TSAP6 and RHBDD1 could be co-immunoprecipitated with each other and they co-localized. Further, RHBDD1 cleaves TSAP6 in a dose-dependent manner and the serine144 residue at the active center of RHBDD1 is essential for the cleavage. In addition, we found only the posttranslationally glycosylated form of TSAP6 could be cleaved in physiological state. Then, AAV-mediated knock-in of the RHBDD1 mutation was applied to genetically inactive the endogenous RHBDD1 in colonic epithelial cell line HCT116. TSAP6-mediated exosomal secretion and the apoptotic rate are significantly elevated in RHBDD1 mutated cell, indicating that RHBDD1 is participated in regulation of exosomal trafficking and apoptosis by cleaving TSAP6. These findings suggest that TSAP6 would be the first physiological multiple transmembranal protein cleaved by Rhomboid protease and expand our knowledge on the function of Rhomboid proteases.

Antiflammin-1 inhibits the proliferation of lung fibroblasts via mediation of UGBP *in vitro*

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Uteroglobin is an anti-inflammatory protein secreted from Clara cells. Our previous studies showed that UG active fragment, Antiflammin-1, could affect the level of ERK phosphorylation in mice lung fibroblasts (NIH3T3) through uteroglobin binding protein (UGBP). However, the effect of AF-1 on lung fibroblasts proliferation was investigated in vitro. Our results showed that AF-1 could inhibit the proliferation of NIH3T3 induced by TGF-β1 in a dose-dependent manner *in vitro*, and the inhibitory effect could be blocked by anti-UGBP antibody ($P<0.01$). Using flow cytometry to detect the difference of cell cycle, we also found that AF-1(10^{-5}mol/L) pretreatment could inhibit increased proliferation index (PrI) of NIH3T3 induced by TGF-β1 and the inhibitory effect could be blocked by anti-UGBP antibody ($P<0.01$). In conclusion, AF-1 can inhibit NIH3T3 proliferation induced by TGF-β1 via mediation of UGBP *in vitro*. The work was supported by the National Natural Science Foundation of China (No. 30670770, 30870916).

Effect of amikacin on the expression of phosphorylated JNK in cochlea of guinea pig

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The activation of c-Jun N-terminal kinase (JNK) by phosphorylation has been shown to mediate cell death. In this study, we investigated the effect of amikacin (AMK) on the expression of phosphorylated JNK (p-JNK) in cochlea of guinea pig and explored the role of JNK pathway in AMK-induced hair cell death. Guinea pigs were randomly assigned to control group, AMK 3 days group, 7 days group and 11 days group. Animals from AMK groups were received intramuscular injection of AMK (400 mg/kg) for 3 days, 7 days and 11 days, while control animals were received an equivalent volume of saline. Auditory brainstem response (ABR) test was used to observe the changes of auditory function before and after the drug treatment. Immunohistochemistry and Western blotting were used to detect the expression of p-JNK in cochlea of guinea pig. We found that ABR threshold shifts in AMK 7d and 11d group were significantly increased as compared with control and AMK 3d group ($P<0.01$). Immunohistochemical staining showed that there was no p-JNK expression in guinea pig cochlea in control group, while after the injection of AMK, p-JNK expression was observed in OHCs, spiral ganglion and stria vascularis, and the expression of p-JNK was greater remarkably with prolonged administration of AMK ($P<0.01$). Western blotting analysis showed that there were no expression of p-JNK1 and p-JNK2 in control group, while the expression of p-JNK1 and p-JNK2 was greater remarkably with prolonged administration of AMK ($P<0.01$), and the expression of p-JNK and ABR threshold shift were significantly correlated in each AMK group, indicating that the greater the expression of p-JNK, the greater the ABR threshold shifts. These results suggest that AMK can affect the expression of p-JNK in hair cells of guinea pig cochlea, which shows that JNK signal pathway may participate in AMK-induced hair cell death.

Protein analysis of pediatric parapneumonic pleural effusion after intrapleural urokinase treatment

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Although there have been many clinical reports about the utility of intrapleural fibrinolytic agents to improve the outcome in both adults and children, uncertainties about fibrinolytic treatment remain. This study investigated the proteomic profiling data of pediatric complicated parapneumonic pleural effusion (CPE) before and after intrapleural fibrinolytic therapy obtained by two-dimensional gel electrophoresis (2D-GE) and protein identification using electrospray ionization tandem mass spectrometry (ESI-MS/MS). Pleural effusion samples were collected from 30 pediatric patients with CPE. All patients received intrapleural urokinase treatment (urokinase 5000 IU/Kg/dose). Samples collected before urokinase treatment were classified as the control group, while those after urokinase treatment as the comparison group. We compared the 2D-GE images differences with computer software. Significant image spots were detected and identified by ESI-MS/MS. Product ion scan data obtained from MS/MS experiments were further analyzed by the protein database software. A total of 640 pairs of silver-stained protein spots was observed and 76 differences (13 increased, 63 decreased) were detected. Altogether, 37 significant changes to gel spots were selected for protein identification by in-gel digestion, liquid chromatography–tandem mass spectrometry and sequence database search. Among these proteins, those that significantly increased after fibrinolytic therapy reflected the effects of fibrinolytics, such as the fibrinogen gamma chain and fragments of fibrinogen. In those significantly decreased proteins, haptoglobin and related proteins, and alpha-1 antitrypsin and related proteins were observed. We concluded that intrapleural urokinase therapy could break the fibropurulent bonds of complicated parapneumonic effusion. The significance of the use of proteomics analysis of intrapleural fibrinolytic therapy in parapneumonic effusion could gain deep insights into therapeutic mechanism and further prognostic factors.

Renal protective effect of extract from peanut shell on acute renal failure rats: role of NOS-IR in locus coeruleus.

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This study is to investigate the protective effect of extract from peanut shell (EPS) on acute renal failure (ARF) rats, and role of NOS-immunoreactivity (NOS-IR) in locus coeruleus (LC). Male SD rats were randomly divided into four groups: control group, ARF group, EPS group and ARF+EPS group. Glycerol-induced acute renal failure in rats was employed. Blood urea nitrogen (BUN) and creatinine (Cre), and nitric oxide (NO) in renal cortex homogenate were measured by commercial kits. Meanwhile renal histopathological changes were detected by HE stain and the changes of NOS-IR in LC were evaluated by immunohistochemistry. ARF rats administrated orally with 2mL of NS for 48h showed a significant increase in BUN and Cre ($P<0.05$), when compared with that in control group. After treatment of ARF rats with EPS for 48h, BUN and Cre were significantly decreased ($P<0.05$), and the severity of tubular necrosis was alleviated when compared with that in ARF group. Meanwhile, compared with that in ARF group, the level of NO in renal cortex homogenate was significantly increased in ARF+EPS group ($P<0.05$). Immunohistochemistry showed an obvious increase of NOS-IR in LC in ARF group ($P<0.05$) but NOS-IR was further enhanced in ARF+EPS group ($P<0.05$). The results indicated that EPS could protect against the renal dysfunction in glycerol-induced ARF rats. The modeling mechanism may involve in the increase in NOS-IR in LC.

Collagen secretion mediated by NMDR activation in human fetal lung fibroblasts

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Pulmonary fibrosis is associated with a number of disorders that affect the lung. Although there are several cellular types that are involved in the pathogenesis pulmonary fibrosis, the resident lung fibroblast has been viewed traditionally as the primary cell involved in promoting the deposition of ECM that culminates in pulmonary fibrosis. N-methyl-D-aspartate (NMDA) receptors were reported in other fibroblasts, however, the existence and function of NMDA receptors in Lung Fibroblasts have not been confirmed. The purpose of this study was to examine the expression of NMDA receptors and their role in Human Fetal Lung Fibroblasts. Expression of NMDAR1 and four NR2 subunit (NR2A, NR2B, NR2C and N2D) mRNA were examined by real-time PCR. Detection of HYP in cell supernatant at different concentrations of NMDA. I, III collagen secretion in cell supernatant were measured by ELISA. Result shown that real time PCR detected the expression of NR1 and four NR2D subtypes(A,B,C and D) mRNA, the strongest two subtypes are NR2A and NR2D, followed by N2C, N2B and NAR1. NMDA induced secretion increase in HYP and I / III collagen, MK-801 could significantly inhibit the NMDA-induced increase of HYP and I / III collagen. In conclusion, NR1 and four NR2D subtypes (A, B, C and D) all express in human fetal lung fibroblasts. NMDA can promote the secretion of collagen in human fetal lung fibroblasts. These results suggest that NMDA receptors may play an important role in the collagen deposition of Pulmonary fibrosis.

Influence of mycophenolate mofetil on BTLA and ICOS signaling in renal transplant rejection

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Mycophenolate mofetil (MMF) is an immune suppressor approved for the prevention of acute rejection following renal transplantation. Inducible CO-stimulator molecule (ICOS) and B and T lymphocyte attenuator (BTLA) play an important role in the acute organ transplantation, chronic rejection and immune tolerance. To study the influence of MMF on ICOS and BTLA expression, sera and tissue of pereutaneous renal puncture were collected for patients given MMF or not. The expressions of ICOS and BTLA were detected by RT-PCR and flow cytometry for mRNA and protein level respectively. And the secretion of IFN-gamma, IL-2, IL-4 and IL-10 were measured by ELISA procedure. The expressions of mRNA and protein of ICOS and BTLA in MMF therapy group were both lower than those in control groups ($P < 0.05$). ICOS and BTLA gene silencing reduced the levels of IFN-gamma, IL-2, IL-4 and IL-10. The results were consistent in both clinical observation and cell study. In conclusion, MMF can suppress ICOS and BTLA expression in human T-lymphocytes significantly and further study along this line may be helpful for renal transplantation immune tolerance.

Molecular cloning and characterization of human novel JNK2 (MAPK9) transcript variants that show different activities on AP-1

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The JNK pathway represents one sub-group of MAP kinases that are activated primarily by cytokines and exposure to environmental stress, and participates in many physiological functions. As a continued work to identify human novel transcript variants according to the 3'-end exon sites (Wang, et al, 2009(10), 518, BMC Genomics), herein we reported cloning and characterization of five novel JNK2 transcript variants, which were designated as *JNK2*α*3*, *JNK2*α*4*, *JNK2*β*3*, *JNK2*γ*1* and *JNK2*γ2, respectively, for four JNK2 isoforms (JNK2α1, JNK2β1, JNK2α2 and JNK2β2) have been well known. Among them, *JNK2*α*4* and *JNK2*γ*2* are possibly non-coding RNAs because of pre-mature stop codons. Both JNK2α3 and JNK2β3 contain an intact kinase domain, and both encode a protein product of 46 kDa with the same size to JNK2α1 and JNK2β1, whereas JNK2γ1 contain a disrupted kinase domain, and thereby show a disable function. Over-expression of these JNK2 isoforms in 293T cells revealed that JNK2α3 show greater activity on AP-1, including cis- or trans- AP-1 stimulation activity, than that of JNK2β3 and JNK2γ1. Furthermore, JNK2α3 and JNK2β3 show different activities on phosphorylation of substrates, though both JNK2α3 and JNK2β3 could promote the proliferation of 293T cells. Our results enriched repertoire of spliced JNK isoforms and further revealed different isoforms preferentially targeted various substrates, and show difference on AP-1 stimulation activities.

Investigation analysis of metabolic diseases-associated indexes in Chongqing children

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With increasing levels of life standard, more and more metabolic abnormality diseases threaten to human health. Abnormal glucose metabolism may lead to diabetes, and lipid metabolism disorders can cause hyperlipidemia. The prevalence of chronic conditions such as type 2 diabetes, hypertension and nonalcoholic fatty liver disease (NAFLD) increases with increasing weight. Adult chronic diseases may be largely resulted from obesity in childhood period. The present study is aimed to explore the metabolic status of obese children in Chongqing by analysizing the associated indexes of metabolic diseases. 1941 children aged 7-11 were recruited with multi-stage sampling and the physical examination and biochemical test by automatic biochemical instrument including serum GLU, lipids were measured. We found that higher proportion of overweight and obese children was in Chongqing. Using BMI analysis, there were 196 overweight children and 135 children obesity, which were 10.01% and 6.96% of the investigated children, respectively. The levels of TG; TC and LDL-C were significantly higher in overweight and obesity groups than those in control group ($P<0.05$), however, the level of HDL-C was significantly lower than that in control group ($P<0.05$), and there was no significant difference of GLU among the three groups ($P>0.05$). These findings indicate that we should take effective measures to prevent and control further increase in childhood obesity. At the same time, abnormal metabolism can appear in the obese children, and these dangerous factors potentially formed during the childhood can increase the risk of adult chronic disease such as blood vessel disease, type-2 diabetes mellitus and so on. Therefore, we positively recommend that overweight and obese children be subjected to lipids detection and early intervention. This work was supported by the Key Projects in the National Science & Technology Pillar Program during the Eleventh Five-Year Plan Period of China (No. 30830106) and the key project of Chongqing Municipal Health Bureau (No.2009-1-39).

Effect of angiotensin(1-7) on the expression of E-selectin induced by angiotensinII and its mechanism in vascular endothelial cell

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This study aims to examine the effect of angiotensin-(1-7) [Ang-(1-7)] on the expression of E-selectin (Es) induced by angiotensinII (AngII) in HUVECs and its mechanism on the expression of Es induced by AngII. HUVECs were cultured in DMEM. The cell activity was determined by MTT assay. Es antigen was measured by ELISA. Es mRNA was examined by RT-PCR. We found a gradual increase in Es antigen and Es mRNA were observed in HUVECs stimulated with increasing concentration of AngII (10^{-10}-10^{-6} mol/L) ($r=0.965$, $P<0.05$), and peaked at 10^{-7}mol/L. Ang-(1-7) (10^{-9}-10^{-6} mol/L) alone could not affect the expression of Es in HUVECs ($P>0.05$). When pretreated with Ang-(1-7) (10^{-9}-10^{-6}mol/L), Ang-(1-7) could inhibited the expression of Es antigen and mRNA induced by AngII in dose-dependent manner ($r=-0.943$, $P<0.05$), and 10^{-6} mol/L was the strongest concentration. Ang-(1-7) at 10^{-6} mol/L decreased Es antigen in HUVECs in a time dependent manner, reaching a maximum level after 4h. *L*-NAME alone, which is the inhibitor of NOS, had no marked effects on Es antigen and Es mRNA in HUVECs, but *L*-NAME significantly inhibited the effects of Ang-(1-7) on Es expression induced by AngII ($P<0.05$). These results suggest that Ang-(1-7) can inhibit the expression of Es induced by AngII at mRNA level in HUVECs and it is associated with NO pathway.

The densities of spleens and the extent of pathologic changes

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In this study, we designed a new device to measure the density of internal organs and correlated with their pathologic changes. The densities of spleens from 132 autopsy cases were measured with different extent of pathologic changes by an integrated volume-density meter. All samples were divided into 2 groups: intact and pathologic groups. The extent of pathologic changes was classified into 3 stages: slight edema, severe edema, fibrosis according to the histological features. Results show that there were significant differences between each subgroup and intact group and the density was closely correlated with pathologic changes. The density of severe edema is 0.8357 ± 0.1311 g/cm^3, the density of slight hydropic spleen is 1.0546 ± 0.0265 g/cm^3, the density of normal spleen is 1.185 ± 0.0791 g/cm^3, and the density of fibrotic spleen is 1.6282 ± 0.1645 g/cm^3. Therefore, the external parameter of density might offer some clues to its pathologic changes, such as edema and fibrosis.

Involvement of asymmetric dimethylarginine in hepatic endoplasmic reticulum stress of type 2 diabetic rats

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The endoplasmic reticulum (ER) stress is provoked under diabetic conditions and involved in the development of insulin resistance and β-cell dysfunction. Accumulation of asymmetric dimethylarginine (ADMA) is also prevalent in diabetes mellitus. This study was to evaluate whether there was a connection between ADMA and ER stress in the liver of type 2 diabetic rats and further determine their causal relation and the mechanism underlying ADMA induced ER stress in rat hepatoma carcinoma cells (H4IIE). High performance liquid chromatography was applied to analyze serum ADMA concentration, and reversed transcription-polymerase chain reaction (RT-PCR) was used to detect mRNA levels of immunoglobulin binding protein (Bip), X box-binding protein-1 (XBP-1), C/EBP homologus protein (CHOP) and dimethylarginine dimethylaminohydrolase (DDAH), the key enzyme response for the metabolism of ADMA. Furthermore, the activity of NOS and concentration of nitrite/nitrate, the stable metabolites of nitric oxide (NO), were measured to reflect the production of NO. The content of malondialdehyde, derived from lipid peroxidation, and activity of antioxidant enzyme superoxide dismutase (SOD) were detected to evaluate oxidative stress. The results showed that ER stress as expressed by increases of immunoglobulin binding protein (Bip) transcription, splicing of X box-binding protein-1 (XBP-1) mRNA and C/EBP homologues protein (CHOP) transcription was provoked in the liver of type 2 diabetic rats in parallel with the increase of serum ADMA concentration. Exposure of H4IIE cells to exogenous ADMA also induced ER stress which was associated with the inhibition of NO production and oxidative stress. Treatment of H4IIE hepatocytes with antioxidant pyrrolidine dithiocarbamate (PDTC) not only reduced ADMA-induced ER stress but also decreased oxidative stress and increased NO production. These results indicate that elevated endogenous ADMA contributes to hepatic ER stress in type 2 diabetic rats, and the mechanism underlying ADMA-induced ER stress may relate to oxidative stress via facilitating NO synthase uncoupling.

Gadolinium Chloride Attenuates Hypoxic Pulmonary Injury in the Adult Rat

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This study used gadolinium chloride to inhibit the activities of alveolar macrophages under hypoxia in order to explore the role of alveolar macrophages in hypoxic lung injury. Wistar rats exposed to 9% O2 ventilatory room 7 hours every day for 7 days. Rats were divided into control group, hypoxic group and gadolinium chloride treatment group. Gadolinium chloride treatment group intravenously injected gadolinium chloride (10mg/kg body weight) before hypoxia, the control group injected with saline. Results shown that the rats were observed for 7 days no significant changes in body weight, showed that under hypoxia did not significantly affect rats metabolism. Control group and the hypoxic group lung wet / dry weight ratios were 4.05 ± 0.36 and 5.04 ± 0.57 respectively, hypoxia group was significantly higher than control group($P<0.05$). The lung wet / dry weight ratio of gadolinium chloride treatment group was 4.33 ± 0.16, significantly lower than hypoxic group ($P<0.05$). Control group, hypoxic group and gadolinium chloride treatment of right ventricular weight were 0.12 ± 0.0092 and 0.14 ± 0.019, 0.12 ± 0.12 respectively, each group showed no significant difference ($P>0.05$), each group left ventricular wall and septum was also no significant change ($P>0.05$). Lung pathological analysis revealed the infiltration of inflammatory cells, cell gap widened, alveolar red blood cell leakage in hypoxia group, gadolinium chloride treatment of hypoxic rat lung slices showed no significant infiltration of inflammatory cells. In conclusion, Gadolinium chloride can reduce the hypoxic lung injury in adult rat and, alveolar macrophages play an important role in hypoxic lung injury.

IRS-2, but not IRS-1, can sustain proliferation and rescue UBF stabilization in InR or InR defective signaling of 32D myeloid cells

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32D myeloid cells are murine myeloid precursor cells that behave almost in the same way as in the bone marrow. It has been hypothesized that in hemopoietic cells, like 32D myeloid cells, cell proliferation requires not only stimulation of cell growth, but also extinction of the differentiation program. We demonstrated the important evidence here that in 32D myeloid cells expressing the insulin receptor (InR) or selected mutants of the InR, IRS-2, but not IRS-1, is required for promoting the proliferation and inhibiting the granulocytic differentiation, thus restore ERKs phosphorylation and UBF1 stabilization. We showed that the certain mutants of the InR failed to stabilize UBF1, even in the presence of IRS-1, that resulted in induction of cell differentiation and the cease of cell proliferation. However, the combination InR/IRS-2 can rescue the disability of IRS-1, almost as effective as the combination mutant InR/IRS-2 in ERKs phosphorylation and UBF1 stabilization. In addition, the sequences of IRS-2 outside the PHPTB domains were necessary and sufficient for InR/IRS-2 regulation of cell proliferation in myeloid cells. Recruitment of IRS-2 to the rDNA and Cyclin D1 Promoter may lead to the activation of cell cycle progression and proliferation genes and compensate the insulin defective signaling. Our results indicate a predominant role of IRS-2 in InR signaling of 32D myeloid cells.

Clinicopathologic and epidemiological features of cor biloculare

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In this study, four cases of congenital cor biloculare (two boys, one girl and one epicene infant) were treated in our hospital. We studied the clinicopathological and epidemiological feature of congenital cor biloculare and to investigate its clinical diagnosis by clinical data, histopathology, and relative literature were reviewed, in order to reveal the characteristics of this disease. The infants with two-chamber heart were died soon after birth, the shortest survival time was 4 days, the longest survival was 50 days, and all cases accompanied with congenital malformations of other organs. One baby boy's left lung associated with hypoplasia, the other baby boy had no spermatic cord and seminal vesicle, baby girl occur lung abnormalities are two leaves in right lung, the epicene infant had male external genital and pelvic deformity of a closed womb. One mother had a history of smoking during pregnancy; the other one mother engage electrical components involved in the demolition waste recycling work, the last two mothers in the paint industry for the six months before pregnancy last 2 years. Congenital cor biloculare is the most primitive types of congenital heart disease, and one of the rarest, and are associated with other organ abnormalities, infant deaths. Many pregnant women may have close contact with the teratogenic factors at one time.

Human PMNs are phagocytosed by dendritic cells and interfere with DCs maturation: a new link between innate and acquired immunity

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Polymorphonuclear neutrophils (PMNs) and dendritic cells (DCs) are key component of the human immune system. In this study, we demonstrate that the alive PMNs can be phagocytosed by DCs and this results in modulating the maturation and function of DCs. Intriguingly, exposing immature monocyte-derived DCs (iMDDCs) and peripheral blood DCs (PBDCs) to freshly isolated PMNs for 3 hours, about 50-70% iMMDCs and 80-90% $CD11c^+$ PBDCs engulfed the alive PMNs. The phagocytose process was clearly observed by microscopes and flow cytometry, and can be partially inhibited by class A scavenger receptor (SR-A), calreticulin (CRT) Abs and serine protease inhibitors, but not DC-SIGN, LRP, Sirp-α and CD47 Abs. CD11b (Mac-1) and CD15 Abs can promote the uptake process by 15-25% percent. When co-cultured the two kinds of cells, PMNs strongly cluster with iMDDCs and induce maturation of MDDCs and $CD11c^+$ PBDCs that enable these DCs to trigger allogeneic T cell proliferation. Furthermore, I found that SR-A and CRT and serine protease involved in the phagocytosis ability of live PMNs by DCs. Taken together, these results suggest that alive PMNs can be phagocytosed DCs and the process modify the maturation and function of DCs. There may represent an as yet unidentified host-cell interfere DCs maturation, thereby possibly providing a novel cellular link between innate and adaptive immunity.

Cytotoxicity and internalization of single-wall carbon nanotubes in human umbilical vein endothelial cells

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The application of carbon nanotubes (CNTs) as biomaterial has greatly developed in recent years. The issue of interfacing between CNTs and mammalian cells in vitro need to be addressed before application of CNTs in vivo. To investigate the interacting effect of single-wall carbon nanotubes (SWCNTs) in cells, we utilized one commercial product of SWCNTs which have length from 1 to 3μm and diameter from 1 to 2 nm to inspect the cytotoxicity and internalization in human umbilical vein endothelial cells (HUVECs). SWCNTs were sonicated extensively in the aqueous solution of polyethylene glycol (PEG) for 1.5 h. Then the compounds were centrifuged at 1, 2000 r/min for 40 minutes twice. The suspension after the second centrifugation was collected to measure the concentration with ultraviolet-visible spectroscopy. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and transmission electron microscopy (TEM) were utilized for investigating the cytotoxicity and internalization of SWCNT respectively. MTT assay indicated that SWCNTs did not demonstrate any cytotoxicity at concentration of 0.0005 mg/ml, 0.001 mg/ml, 0.0015 mg/ml, 0.002 mg/ml, and 0.0025 mg/ml compared to the PBS control group. TEM images of HUVECs cultured with SWCNTs (0.0005 mg/ml) for 24 h showed several vacuoles containing SWCNTs in the cells. It was concluded that SWCNTs with length from 1 to 3μm can phagocytized by HUVECs and have no toxic effect on the function of mitochondrion. Further investigation is needed to be done for cytotoxicity of SWCNT all round.

Effect of adipocyte-derived dimethylarginine dimethylaminohydrolase on insulin sensitivity

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Dimethylarginine dimethylaminohydrolase(DDAH) is an enzyme responsible for hydrolyzation of asymmetric dimethylargine (ADMA), an endogenous inhibitor of nitric oxide(NO). Recent study reported that DDAH/ADMA system was detectable in normal adipose tissue. In the present study, we revealed that in adipose tissue of high fat-fed diabetic rats as well as in high glucose and insulin-treated 3T3-L1 adipocytes, the expression of insulin receptor substance-1 (IRS-1) and glucose transporter-4 (GLUT-4), two important genes in insulin-stimulated glucose uptake, was significantly down-regulated, accompanying with decreases in cellular DDAH-2 expression and DDAH activity, and increase in ADMA contents. Over-expression of human DDAH-2 reversed the impairment of IRS-1 and GLUT-4 mRNA expression in high glucose and insulin-treated adipocytes, which may attribute to the reduction of cellular ADMA contents by amelioration of DDAH activity. These findings suggested that adipocyte-derived DDAH can improve insulin sensitivity by regulating expressions of IRS-1 and GLUT-4.

Mechanism of apoptosis in ototoxicity induced by kanamycin in mice

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Inner hair cells including spiral ganglion cells and stria vascularis cells play a major role in transporting hearing signals and regulating inner ear homeostasis. However their functions are often abnormal induced by aminoglycoside ototoxicity. The injury of these inner ear cells from aminoglycoside treatment is considered apoptosis, and caspase is an important participant in the apoptosis pathway in many organs. It has been reported that calpain, a calcium-dependent protease, is essential for mediation and promotion of cell death. The purpose of the present study was to investigate details of effects of calpain during ototoxicity induced by kanamycin, one kind of aminoglycoside, and relations between expressions of calpain protein and cells apoptosis during kanamycin ototoxicity in mice inner ear. We demonstrated that kanamycin treatment induced cochlear cells including stria vascularis cells and spiral ganglion cells death by apoptotic pathway in time-dependent. Furthermore, our data showed that inner ear cells died in apoptotic pathway mediated by calpain, PAPI, Bcl-2, caspase-3 and even by microRNA34a, microRNA34c. These results suggest that calpain, miRNA34a, miRNA34c are involved in mediating the apoptosis in time-dependent in cochlear cells induced by kanamycin.

Blockade of NMDA receptors improves impaired development of rat fetal pancreas induced by intrauterine hypoxia

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The N-methyl-D-aspartate receptor (NMDAR) has been detected in the pancreas of adult rats, and regulates the insulin secretion from pancreatic β cells. Intrauterine hypoxia is an unchallenged cause resulting in intrauterine fetal growth retardation and impaired development of fetal pancreas. However, the expression of NMDARs in rat fetal pancreas and the role of NMDARs in the hypoxia-induced impaired development of pancreas are not clear. The mRNA expression of NMDAR in rat fetal pancreas was determined by Real-time PCR. The results revealed the mRNAs expression of NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C, NMDAR2D in fetal pancreas of rats, with the highest the expression of NMDAR2D. To test the role of NMDAR activation in the impaired development of fetal pancreas induced Intrauterine hypoxia. The pregnant SD rats were randomly divided into four groups: control; MK-801 treated; intrauterine hypoxia; and MK-801(a NMDAR antagonist) treatment groups. Intrauterine hypoxia significantly impaired the development of fetal pancreas (decreased the fetal pancreas weight and the ratio of fetal pancreas weight to birth weight, reduced the population of islets in unit area of fetal pancreas, decreased the fraction of β cells in the fetal pancreatic islets). But pretreatment with MK-801 dramatically improved the hypoxia-induced impairment of fetal pancreas development in rat. In summury, our results suggested that overactivation of NMDAR played critical roles in hypoxia-induced impairment of rat fetal pancreas development.

The role of NMDA receptor activation in the impairment of rat fetal lungs development induced by intrauterine hypoxia

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The NMDA receptor (NMDAR) is the important subtype of Glutamate receptor. The NMDAR is composed by the NMDAR1 and NMDAR2 subunits (NMDAR2A, N-MDAR2B, NMDAR2C and NMDAR2D). Our previous report showed that endogenous glutamate mediated newborn rat lung damage induced by hyperoxia through NMDARs Intrauterine hypoxia is an important cause resulting in intrauterine fetal growth retardation. However, the expression of NMDARs in rat fetal lungs and the role of NMDARs in the hypoxia-induced impaired development of lungs are not clear. We assume that the Glutamate and NMDAR over-activation plays an important role in retardation of the fetal lung development under intrauterine hypoxia. The results revealed the mRNAs expression of NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D in fetal lungs of rats, with the highest the expression of NMDAR2D. In order to test the role of NMDAR activation in the impaired development of fetal lungs induced by intrauterine hypoxia, the pregnant SD rats were given MK-801(a NMDAR antagonist). Intrauterine hypoxia significantly impaired the development of fetal lungs (decreased the fetal lungs wet weight, reduced radial alveolar count and abnormal alveolar structure). But pretreatment with MK-801 dramatically improved the hypoxia induced impairment of fetal lungs development in rat. In summary, our results suggested that over-activation of NMDAR played critical roles in intrauterine hypoxia induced impairment of rat fetal lungs development. The work was supported by the National Natural Science Foundation of China (No. 30471835 and 30370531).

Antiflammin-1 enhanced IL-10 gene expression and secretion in activated macrophages challenged by LPS

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Antiflammin-1 (MQMKKVLDS, AF-1) is a potent anti-inflammatory nonpeptide, with equivalent sequence to the 9 C-terminal amino acid recidue of α-helix 3 of uteroglobin. But the anti-inflammatory mechanism of AF-1 is still unclear. Interleukin-10 is a potent anti-inflammatory and immune regulatory cytokine, Alveolar macrophages are important host defence cells and one of the important sources of IL-10 in the lung. This research investigated the effect of AF-1 on LPS induced IL-10 production and gene expression in RAW264.7 cells in order to elucidate its possible anti-inflammatory mechanism. Results showed that IL-10 expression and secretion in RAW264.7 cells challenged by LPS (1 μg/ml) was increased in time dependent way, with ultimate peak at 24h ($p<0.01$). AF-1 (100μmol/L) alone had no effect on IL-10 mRNA expression and secretion in RAW264.7 cells ($p>0.05$). However, AF-1 (1-100 μmol/L) increased IL-10 mRNA expression and secretion in activated RAW264.7 cells challenged by LPS in a dose- and time-dependent way ($p<0.05$ and $p<0.01$). Pretreatment with anti-uteroglobin-binding protein (UGBP) antibody could attenuate the enhanced effect of AF-1 on LPS induced IL-10 gene expression in macrophages. Conclusion: AF-1 could significantly increased IL-10 mRNA expressions and secretion in RAW264.7 cells induced by LPS, the increased effect of AF-1 on LPS induced IL-10 gene expression and secretion in macrophages was mediated by UGBP. The work was supported by the National Natural Science Foundation of China (No. 30670770, 30870916).

ATRA-induced increase in intracellular Ca^{2+} concentration in primary hippocampal neurons

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The aim was to investigate the effects and possible mechanisms of ATRA on the intracellular Ca^{2+} level of primary cultured hippocampal neurons. Hippocampal cells were isolated from newborn rats within 24 hours and cultured for 1−15 days prior to experiments. Immunofluorescence was performed to identify cultured cells. The cultured primary hippocampal neurons were labeled with Fura-2AM, and fluorescence images were monitored using a CCD camera, and subsequently analyzed using imaging analyzing software. Ca^{2+} activities were presented as Ratio 340/380, the ratio of fluorescence intensities excited by alternating illumination of 340 nm and 380 nm beams. We show that ATRA causes an increase in the intracellular calcium concentration in primary hippocampal neurons in a dosage- and age-dependent manner. Furthermore, the increase in calcium level caused by ATRA was mainly through influx of extracellular calcium. Finally, we found that Ro41-5253 (Ro) and (+)-5methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK801) both reduced the increased Ratio340/380 of hippocampal neurons caused by ATRA. This study suggests that ATRA can modulate calcium influx in hippocampal neurons in a dose- and age-dependent manner. ATRA plays a role in modulating calcium levels in hippocampal neurons possibly by binding to RARa. NMDA receptors might be the target of the ATRA activating signaling pathway. Finally, the role of ATRA in regulating calcium concentration might be related to its effect on long-term potentiation (LTP), learning, and memory.

A novel human protein AC3-33 inhibits AP-1-mediated transcriptional activity

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Transcription factor AP-1 is a dimer formed by DNA-binding proteins of the Jun, Fos, and ATF families. AP-1 mediates cell response to growth factors, cytokines, neurotransmitters, and other intercellular signaling molecules. We report a novel human gene, AC3-33 (GenBank: c3orf33, FLJ31139), which encodes a secretory protein that can inhibit AP-1 transcriptional activity via ERK1/2 pathway. Over-expression of AC3-33 significantly inhibits AP-1 activity and DNA-binding ability. Further study indicated that over-expression of AC3-33 significantly inhibit transcriptional activity of Elk1 and c-jun, but not c-fos. As for the upstream of signaling pathway of Elk-1, our study demonstrated that over-expression of AC3-33 significantly down-regulates phosphorylation of ERK1/2, but not JNK/SAPK or p38 MAPK. These results indicate that AC3-33 is a novel member of the secretory family and inhibits Elk1 transcriptional activity via ERK1/2 MAPK. The work was supported by the National Natural Science Foundation of China (No. 30671092).

Molecular cloning of a novel splicing variant of the rat LM23 gene

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LM23 is a gene with testis-specific expression and crucial for meiosis during spermatogenesis in Rattus norvegicus. In this study, we have isolated a novel splicing variant of LM23 that we called svLM23 from rat testis, which is 858 bp, encoding a 286-amino acid polypeptide that lackes exon 6 and 7, 5'end extension in 3'-end of exon 5. The LM23 and svLM23 are both located on the rat chromosome 6q13, and the svLM23 protein is located in the nucleus. RT-PCR analysis in our work showed that svLM23 was detected in rat testis. The work was supported by the National Natural Science Foundation of China (No. 30671092) and Basic Research Foundation of National Commonweal Research Institute (No. 2009GJSSJKB03).

Effect of marginal vitamin A deficiency during pregnancy on retinoic acid receptors and NMDA receptor expression in the offspring of rats

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This study examined whether pregnancy-related marginal vitamin A deficiency (MVAD) influences postnatal development of retinoic acid receptors (RAR) and N-methyl-D-aspartate receptor subunit 1 (NR1) in hippocampus of rat pups. Sixteen female rats were randomized equally into control and MVAD groups. Dams and pups were fed with a normal control diet or one deficient in vitamin A. Eight female pups in each group were killed at 1 day postnatally, 2 weeks, 4 weeks and 8 weeks. Serum retinol levels were monitored. The expression and subcellular localization of RARα, RARβ and NR1 in postnatal hippocampus were detected by real time polymerase chain reaction, immunofluorescence and western blotting. At 1 day, 2 weeks and 8 weeks postnatally, serum retinol levels in the MVAD group were significantly lower than the control group. Eight pups in each group were randomly selected for Morris water maze test at 7 weeks of age. Results showed that spatial learning and memory in the MVAD group were affected. Vitamin A deficiency resulted in decreased mRNA levels of RARα, RARβ and NR1 ($p<0.05$). The level of protein expression of RARα and NR1 in the MVAD group were lower than the control group ($p<0.05$). There was no significant difference in RARβ between the groups ($p>0.05$). A mass of RARα and NR1 colocalized in hippocampal cell cytoplasm on postnatal day 1. Vitamin A deficiency in pregnancy may affect the postnatal expression of RARα and NR1, affecting learning and memory function in the hippocampus and synaptic plasticity of the calcium signaling pathway.

The role of NMDA receptor activation in the dystrophy of the lung of fetal rats induced by intrauterine hypoxia

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Clinically, maternal diseases during pregnancy may affect blood supply to the placenta, causing fetal hypoxia, metabolic disorders in fetal growth or retarded growth. We get the model of intrauterine hypoxia rats by decreasing the concentration of inspired oxygen of pregnant rats to $10.5 \pm 1.0\%$, hypoxia lasted eight hours a day. As the hypoxic time lasting, the survival rate of animals is obviously decreased. Fetal rat weights, humid weight of fetal lung were significantly lower. Right ventricle / left ventricle + septum (RV / LV + S) were significantly higher. Fetal lung /fetal rat weight of hypoxic 2-day group is decreased significantly lower than the air control group, as the hypoxic time lasting to increase the fetal lung /fetal rat weight. Alveolar structure is not ruley, arrangement is not orderly and distribution of alveolar septal is not uniformity, significant exudative in alveolar space. The Radial alveolar count (RAC) is decreased significantly lower, pulmonary vascular morphometry changes: pulmonary small artery is wall thickening\area increasing. However hypoxia has no obvious effect on the structure of the placenta. The Glutamate (Glu) is an important excitatory neurotransmitter, the NMDA receptor is the important subtype of Glu receptor .The NMDAR is composed by the NMDAR1, NMDAR2 subunits el (NMDAR2A, N-MDAR2B, NMDAR2C, NMDAR2D). We assume that the Glu and NMDAR may also participate in the process of fetal lung development, NMDAR over-activation plays an important role in dystrophy of the lung development under intrauterine hypoxia. We observe these is NMDAR expression in fetal lung tissue, mainly expressed NMDAR2D; as the hypoxia time lasting, the expression of the NMDAR's expression in fetal lung tissue increased, mainly expressed NMDAR2D too. The expression is peak in hypoxic six-day group, however In ten-day group the expression is no difference between the air control group. Into the NMDA receptor antagonist MK-801, In hypoxic group the survival rate of fetal rat is notable increased, the trend of Fetal rat weights. RV/LV+S. lung humid weight is decreased, the alveolar structure, RAC, pulmonary vascular morphometry are ameliorated. Alone MK-801 has certain toxic effects on fetal rat development. It indicates the GLU and the NMDAR involve in the dystrophy of the lung of fetal rats induced by intrauterine hypoxia, the mechanism needs further study.

Development of a simple method for the determination of single nucleotide polymorphisms

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Single nucleotide polymorphisms (SNPs) are the most common type of polymorphisms and occur at a frequency of approximately 1 in 1000 base pairs throughout the genome. They are associated with diversity in the population, individuality, susceptibility to diseases, and individual response to medicine. There is a need to develop a precise and simple method for SNPs typing. During SNPs typing by denaturing high performance liquid chromatography (DHPLC) and sequencing, we used PAGE (polyacrylamide gel electrophoresis) to detect PCR products. Some PCR products demonstrated that there were two discrete bands in PAGE, which yielded at least two peaks on chromatogram of DHPLC. Corresponding to this, most of samples demonstrated with one single band by PAGE exhibited one single peak when analyzed by DHPLC. These findings indicated that PAGE may be used to genotype SNPs. Therefore, we optimized gel polymerizing coefficient, gel concentration, buffer, and electrophoresis conditions, and established a PAGE method for SNPs genotyping. We found that: 8% gel with polymerizing coefficient of 49:1 can satisfactorily detect SNPs in most cases. This system has been verified to be a rapid, precise, simple and low-cost method for SNPs genotyping through genotyping of 4 SNPs. Furthermore, this system is sensitive enough to detect sequence variation even when the component percentage of the minor homozygote in the mixed homozygotes solution is scaled down to 5%.

Preliminary Research of the aldosterone synthesized by Bone Morrow Stem Cells of adult male rats *in vitro*

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Aldosterone plays an important role in salt and water homeostasis and blood pressure control. Recently systemic or local aldosterone has emerged as a multifunctional hormone exhibiting profibrotic and proinflammatory actions. The renin-angiotensin-aldosterone system (RAAS) is central to the pathogenesis of hypertension, cardiovascular disease, and kidney disease. The variability in effects of RAAS on cardiovascular progenitor cells might reflect differences between the various models with respect to circulating and local tissue RAAS activation. The aim was to investigate whethere the bone marrow stem cells (BMSCs) are involved in the local tissue RAAS activation. Methods: The BMSCs were isolated from male rat bone mallow and cultured in the DMEM containing 10% fetal bovine serum (FBS). The primary BMSCs from the adherent cell fraction were cultured for 21 days. The whole medium was replaced 2 times every week and was then collected. The levels of aldosterone in the media were measured by radioimmunoassay (RIA). Results: The concentration of aldosterone in the culture medium on day 0 was low ($M \pm SD$, 16.472 ± 11.594 pg/ml). The aldosterone content in the culture medium on day 7 was 52.718 ± 37.162 pg/ml ($P>0.05$). The higher levels of aldosterone in the culture media were detected by RIA on days 11 (125.46 ± 17.439 pg/ml, $P<0.01$); 14 (115.012 ± 24.200 pg/ml, $P<0.01$); 18 (133.028 ± 11.141 pg/ml, $P<0.01$) and 21 (99.982 ± 34.423 pg/ml, $P<0.01$). In conclusion, the BMSCs of adult male rats can synthesize aldosterone *in vitro*. The results suggest that the BMSCs might be involved in the local RAAS and its role need to be explored in a further step of the research work. The research was supported by National Natural Science Foundation of China Grant 30960409.