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TALKS BY INVITED SPEAKERS

A BIOPHYSICAL DISSECTION OF NEUROTRANSMITTER RELEASE AT A GLUTAMATERGIC SYNAPSE

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The unique capabilities of our brain as an information processor are critically dependent on the correct function of some 10 billions of neurons, each of which is connected to about 10 000 other neurons by way of synapses. Unlike in electronic computers these connections are not rigid but adapt their coupling strengths in response to the information flow in the system – a phenomenon called synaptic plasticity. A dissection of the process of synaptic transmission as well as of the mechanisms underlying plasticity is essential for understanding some of the major neurological diseases. It has been known since the early fifties, that synaptic transmission is initiated by the release of a signalling substance, the neurotransmitter, from the presynaptic neuron. This, in turn, is triggered by an influx of Calcium ions (Ca^{++}) into the nerve terminal. The neurotransmitter, once liberated, induces an increase in the conductance of the postsynaptic membrane. When synaptic strength changes during 'plasticity' this can be a consequence of changes in any of the steps of this complicated process. Unfortunately, most nerve terminals are very small and not readily accessible to detailed investigation, such that usually it is very difficult to assign a given change to one of these molecular mechansims. Quite recently, however, it was discovered that a specialized synapse in the auditory pathway, the 'Calyx of Held', has presynaptic terminals, which are large enough that quantitative biophysical techniques can be applied. Particularly, the postsynaptic current can be measured precisely, while the presynaptic calcium concentration ($[Ca^{++}]$) can be increased or decreased – either by opening and closing of Ca^{++} channels or by releasing Ca^{++} from a chemically caged form by photolysis. Furthermore, $[Ca^{++}]$ can be measured by introducing fluorescent Ca^{++} indicators into the terminal. Using these experimental possibilities, we have studied the role of Ca^{++} and other second messengers in short-term changes of synaptic strength. We found that there are two steps, which are strongly modulated: i) action potential waveform and Ca^{++} influx is modulated in multiple ways by second messengers ii) during ongoing activity new synaptic vesicles have to be recruited, to replace those that have undergone exocytosis. This step of recruitment is also modulated strongly by $[Ca^{++}]$, cAMP and other second messengers. The release process itself – although steeply dependent on $[Ca^{++}]$ – is relatively immune to other forms of modulation.

IMMEDIATE-EARLY GENES AS MASTER SWITCHES IN DISEASE

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Immediate early genes are genes that share the characteristic of having their expression rapidly and transiently induced upon stimulation without dependence on de novo protein synthesis. Our studies over recent years have demonstrated that restenosis, angiogenesis and inflammation can be suppressed using novel ''anti-gene therapeutic'' strategies targeting certain immediate-early genes. We targeted the basic-region leucine zipper protein, c-Jun with a catalytic DNA molecule, Dz13, a 34-bp oligonucleotide capable of cleaving both murine and human c-Jun transcripts at position G967 or G1311 respectively. Since both angiogenesis and inflammation depend on vascular permeability, we investigated whether the DNAzyme, Dz13, could suppress retinal neovascularisation in a murine model of proliferative retinopathy (ROP). We demonstrated that knockdown of c-Jun by Dz13, inhibited retinal neovascularisation. The control DNAzyme, Dz13scr, a size-matched molecule retaining the catalytic core, but with scrambled hybridizing arms was unable to influence neovascularisation. This led us to investigate whether Dz13 could influence a number of inflammatory processes. Dz13 suppressed vascular permeability and transendothelial emigration of leukocytes in murine models of vascular permeability, acute inflammation and collagen antibody induced arthritis (CAIA), whereas its scrambled counterpart, Dz13scr had no influence over these processes. Treatment with Dz13 reduced vascular permeability due to cutaneous anaphylactic challenge or VEGF administration in mice. Dz13 also abrogated monocyte endothelial cell adhesion in vitro and abolished leukocyte rolling, adhesion and extravasation in a rat model of inflammation. Dz13 attenuated neutrophil infiltration in the lungs of mice challenged with endotoxin, a model of acute inflammation. Dz13 also reduced joint swelling, inflammatory cell infiltration and bone erosion in a mouse model of rheumatoid arthritis (CAIA). FITC-conjugated DNAzyme localised within the tissue and was still catalytically-active 1 hr following intradermal injection. We demonstrated a reduction in c-Jun immunoreactivity in Dz13-treated joint (CAIA), lung (sepsis) and retina (ROP). Further, we showed that Dz13 blocks cytokine-inducible endothelial c-Jun, E-selectin, ICAM-1, VCAM-1 and VE-cadherin expression but has no effect on JAM-1, PECAM-1, p-JNK-1 or c-Fos. Previous studies by our group demonstrated that Dz13-mediated inhibition of c-Jun leads to suppression of SMC proliferation and wound repair *in vitro*, the reduction of neointima formation following a rat carotid artery injury model, and markedly reduced intimal hyperplasia and increased lumen size in balloon-injured segments in rabbits. Our recent findings thus implicate c-Jun as a useful target for anti-inflammatory, anti-angiogenic and anti-restenotic therapies.

NEGATIVE REGULATION OF SIGNAL REGULATORY PROTEIN ON CANCER SIGNALING

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SIRPα1 is a member of the signal regulatory protein (SIRP) family that undergoes tyrosine phosphorylation and binds SHP-2 tyrosine phosphatase in response to various mitogens. The expression levels of SIRPα1 were decreased in human HCC tissues, as compared with the matched normal tissues. Exogenous expression of wild-type SIRPα1, but not of a mutant SIRPα1 lacking the tyrosine phosphorylation sites, in SIRPα1 negative Huh7 human HCC cells resulted in suppression of tumor cell growth both in vitro and in vivo. Treatment of Huh7 transfectants with EGF or HGF induced tyrosine phosphorylation of SIRPα1 and its association with SHP-2, which were accompanied by reduced ERK1 activation. Expression of SIRPα1 significantly suppressed activation of NF-κB and also sensitized Huh7 cells to TNFα or cisplatin-induced cell death. In addition, SIRPα1-transfected Huh7 cells displayed reduced cell migration and cell spreading in a fashion that was dependent on SIRPα1/SHP-2 complex formation. In conclusion, these results suggest a negative regulatory effect of SIRPα1 on hepatocarcinogenesis through, at least in part, inhibition of ERK and NF-κB pathway. The heightened sensitivity of cells restoring SIRPα1 function could be exploited in the development of therapeutic regimens which may potentiate the antineoplastic effect of conventional cytokines or chemotherapeutic agents.

TALKS ON SPECIAL TOPICS

EPIGENETIC REGULATION OF GENE EXPRESSION BY LINKER HISTONE H1

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H1 linker histones play a key role in the folding of chromatin into higher order structures. Mice contain at least eight H1 subtypes that differ in expression during development. Our previous studies showed that mice develop normally when any one of six different H1 genes is inactivated homozygously, whereas mice lacking three H1 subtypes, H1c, H1d and H1e, generated by three rounds of gene inactivation in ES cells, die by mid-gestation with a broad range of defects. To further understand the role of H1 in chromatin structure and

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gene expression, mouse embryonic stem cells null for three H1 genes were derived and were found to have 50% of the normal level of H1. H1 depletion caused dramatic chromatin changes, but surprisingly, gene-profiling analysis revealed that expression of only a small number of genes is affected. Interestingly, a significant proportion of the affected genes are normally regulated by DNA methylation, such as imprinted genes and X-linked genes. Analysis of DNA methylation status showed significant changes in the specific CpGs within the regulatory regions of several H1 regulated genes. These results indicate that H1 can participate in epigenetic regulation of gene expression by contributing to the maintenance or establishment of specific DNA methylation pattern. The mechanisms of H1 mediated epigenetic gene regulation will be discussed. This study was supported, in part, by Georgia Cancer Coalition.

INCREASED CARDIOGENIC DIFFERENTIATION EFFICIENCY AND SIGNIFICANT MYOCARDIAO REGENERATION

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Despite significant therapeutic advances, replacement of infarcted heart tissue with regenerating functional myocardium remains a therapeutic ideal due to the negligible ability of adult cardiomyocytes to repopulate. In this study, we hypothesized that three prerequisites are critical for significant repair of myocardial infarction (MI), namely (i) increased survival potential of the cardiac progenitors; (ii) early reconstitution of damaged coronary vasculature in ischemic region; and (iii) enhanced cardiogenic differentiation efficiency of the progenitor cells. To satisfy these three prerequisites, we have found and isolated a novel angiogenic factor (angiogenin) and a cardiogenic factor (Cardiogenin) from *Dagencao*. Angiogenin was demonstrated to specifically activate angiogenesis specific pathways and promote revascularization in ischemic hearts. Cardiogenin appeared to induce gene expressions associated with cell survival and cardiogenic differentiation specific pathways in mesenchymal stem cells (MSCs) and myocardial regeneration in infarcted hearts. Therefore, angiogenin and cardiogenin were used, which appeared to satisfy the 3 postulated prerequisites, for the treatment of MI and chronic coronary heart disease, demonstrating clear evidences of newly regenerated cardiomyocytes and reconstitution of damaged coronary vasculature as well as significantly improved functional performance. Transplantation of MSCs with the specifically activated signaling pathways induced by pretreatment with cardiogenin into a MI animal model also resulted in significant regeneration of functional myocardia. The presently demonstrated therapeutic effects of cardiogenin and angiogenin for significant repair of ischemic hearts and the associated signalling pathways identified for effective cardiogenic differentiation of MSCs and for effective growth of new vessels in ischemic region of the hearts may lead to immediate development of new treatment strategies for ischemic heart diseases.

MAINTENANCE OF TELOMERES AND CELL PROLIFERATION: SIGNALING FROM HORMONES AND GROWTH FACTORS TO TELOMERES

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Telomeres are the chromosomal ends that are a vital for a variety of functions of chromosomes. Essential for all eukaryotic cells, telomeres regulate cell proliferative potentials; maintenance of telomere lengths underlies cancer cell immortality. However, little is known of how telomere length is programmed by extracellular cues under physiological and pathological conditions. This presentation will show that steroid hormone estrogen regulates the sizes of telomeres *in vivo*. Mice with estrogen deficiency showed compromised mechanisms of telomere maintenance in association with aberrant cell proliferation in the ovarian tissues and adrenal gland. In addition, a cytokine bone morphogenetic protein (BMP) negatively regulated telomere length in cultured cells and in xenograft tumors of mice. BMP induced tumor cell senescence and apoptosis through a mechanism of telomere shortening. Thus, hormones and cytokines are effective programmers of telomere homeostasis, from acting on cell surface, so as to regulate the fate of cell development under physiological conditions.

INTEGRATIVE CANCER EPIGENETICS IDENTIFIES NOVEL TUMOR SUPPRESSOR GENES FOR COMMON ASIAN TUMORS

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Carcinogenesis is a multi-step process, involving multiple genetic and epigenetic alterations including the epigenetic disruption of tumor suppressor genes (TSGs) through promoter CpG methylation. The frequent presence of epigenetic abnormalities in tumors provides us with not only potential epigenetic tumor markers for molecular diagnosis, but also a novel way of identifying new TSGs. Frequent epigenetic inactivation of a gene specifically in tumors but not in normal tissues indicates that it is likely a candidate TSG. Using DNA methyltransferase inhibitors as demethylation agents, epigenetic inactivation of TSGs can also be reversed and exploited as a cancer therapeutic strategy. Using nasopharyngeal (NPC) and esophageal carcinomas (ESCC) as tumor models, both prevalent in southern China, I attempted to identify novel putative TSGs, epigenetically/and genetically inactivated, by employing various integrative epigenetic approaches such as methylation subtraction, array-CGH, high-throughput expression profiling coupled with methylation analysis, and functional studies. A few novel candidate TSGs have been identified, including cell signaling-related and transcriptional regulatory genes. These genes are frequently methylated and downregulated in NPC and ESCC, as well as other common carcinomas. Functional analyses showed that these candidate genes could induce apoptosis of tumor cells and suppress tumor cell growth, are thus functional TSGs. The epigenetic silencing of these TSGs would disrupt normal cell signaling control and contribute to tumor pathogenesis.

A SCHIZOPHRENIA-SUSCEPTIBLE GENE AFFECTS QUANTA SIZE OF TRANSMITTER RELEASE

Xiao Wei Chen* [1], Ya Qin Feng* [2], Chan Juan Hao [2], Xiao Li Guo [2], Xin He [2], Zhi Yong Zhou [2], Ning Guo [1], Hong Ping Huang [1], Wei Xiong [1], Hui Zheng [1], Pan Li Zuo [1], Claire Xi Zhang [1], Wei Li [2], Zhuan Zhou [1]
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Schizophrenia is one of the most debilitating neuropsychiatric disorders, which affects approximately 0.5–1% of the population worldwide. Schizophrenia is a disease of the synapse, but its synaptic pathology or abnormal synaptic transmission remains far from clear. *DTNBP1* gene, which encodes a coiled-coil protein dysbindin, is a major susceptible gene of schizophrenia. Our previous results have demonstrated that sandy (*sdy*) mouse harbors a spontaneously occurring deletion in the *DTNBP1* gene and expresses no dysbindin protein. Here, by using amperometry, whole-cell patch clamp, and electron microscopy in *sdy* mice, we provide first characterization of the specific defects of neurosecretion and vesicular morphology in neuroendocrine cells and brain hippocampal synapses at the single vesicle level. These defects include larger vesicle size, slower quantal release, and less release probability. The current results reveal that dysbindin plays a crucial role in regulating exocytosis and biogenesis of vesicles in endocrine cells and neurons, suggesting its possible involvement in the pathogenesis of schizophrenia at synaptic levels.

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GENERAL SUBMISSIONS

EPIGENETICS AND REGULATION OF GENE EXPRESSION

THE KRAB DOMAIN-CONTAINING ZINC FINGER PROTEIN ZNF382 IS A POTENT TUMOR SUPPRESSOR WITH FREQUENT EPIGENETIC INACTIVATION IN NASOPHARYNGEAL, ESOPHAGEAL AND OTHER CARCINOMAS

Ying Duan Cheng [1], Yan Bai [2], Ada H.Y. Wong [1], Pei Liang [2], Ji Sheng Li [1], Jian Ming Ying [1], Ya Cao [3], Anthony T.C. Chan [1], Xiu Shan Wu [2], Qian Tao [1]
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Zinc finger protein (ZFP) is the largest transcriptional factor family, with some members involved in tumorigenesis. We identified a KRAB domain-containing Zinc finger protein (KRAB-ZFP), ZNF382, downregulated in nasopharyngeal carcinoma (NPC) and esophageal squamous carcinoma cell (ESCC), which are major tumors in southern China and Southeast Asia. ZNF382 is broadly expressed in normal tissues, but silenced or downregulated in 7/7 nasopharyngeal, 15/17 (88%) esophageal, 7/10 (70%) gastric, 6/12 (50%) breast, 2/5 lung, 3/4 cervical, 1/3 prostate, and 4/4 colon carcinomas. We further found that the *ZNF382* promoter is a CpG island, and frequently methylated in primary tumors of NPC and ESCC, but seldom in paired surgical marginal tissues and not in any normal epithelial tissue. The transcriptional silencing of *ZNF382* could be reversed by pharmacologic demethylation with 5-aza-2′-deoxycytidine or genetic demethylation with double knockout of DNMT1 and DNMT3B, indicating a direct epigenetic mechanism mediated by DNMT1 and DNMT3B. Ectopic expression of ZNF382 in NPC, ESCC, lung and colon carcinoma cells inhibited their colony formation. Thus, ZNF382 is a new tumor suppressor for multiple carcinomas. Its frequent methylation may thus be an epigenetic biomarker for patients with these cancers.

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN GROUND AND SPACE CELL LINES BY SUPPRESSION SUBTRACTIVE HYBRIDIZATION

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The space environment consisting of microgravity and radiation poses significant health risks for human cells. The 48A9 Caski cells were exposed to space environment by way of being carried on ‘‘Shen Zhou’’ spaceship. We have already observed the phenotypic change of cell in ground (Caski) and space cell lines (48A9 Caski). To understand genetic differences and similarities between ground and space cells, suppression subtractive hybridization (SSH), based on suppression PCR and a combination of normalization and subtraction in a single procedure, was applied. We set up a subtractive cDNA library using cDNA from the 48A9 cell line as ‘‘tester’’ and the other from the ground Caski cell line as ‘‘driver’’. The library was screened and identified by reverse Northern dot blot technique. Positive clones were sequenced, compared with known sequences in the public databases of GenBank/EMBL/DDBJ using NCBI BLAST for homology analysis, and then detected by RT-PCR. Among 480 clones, 35 were identified as differentially expressed genes of which the expression of 31 genes were up while 4 genes were down. Those genes are associated with cell cycle, apoptosis, differentiation, skeleton and transcription. These results suggest that space environment exposure can make significant biological impact on uterine cervix cancer cell by differential expression of some genes, which cause heritage phenotypic change of the cell.

STUDY ON RELATIONSHIP BETWEEN POLYMORPHISM OF CYP2A6 AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN INNERMONGOLIA HANS

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To investigate the relationship of Cytochrome P450 2A6 (CYP2A6) gene polymorphism with Chronic Obstructive Pulmonary Disease (COPD) heredity susceptibility in Innermongolia Hans, we demonstrated that a case-control study which detected CYP2A6 alleles of 60 patients with COPD and 60 control by polymerase chain reaction-restriction fragment length polymorphism, which included CYP2A6*1 (CYP2A6wt or wt), CYP2A6*2, CYP2A6*3 and cyp2A6*4 (CYP2A6del or del). The frequencies of CYP2A6wt allele were 96.7% and 81.7% in COPD group and control group, and those of CYP2A6del allele were 3.3% and 18.3% in the two groups respectively. A highly significant difference was found between the two groups ($P < 0.05$). The results of Binary Logistic Regression showed that people who carried CYP2A6wt allele had a 5.04 fold higher risk of COPD than those who carried CYP2A6del allele (95% CI = 1.03 - 25.11), but no relationship was found between CYP2A6del and COPD, and between CYP2A6*2 and CYP2A6*3. Meanwhile, we found smokers who carried CYP2A6del allele had a 31.09 fold higher risk of COPD than those who carried CYP2A6del allele. Smokers who carried CYP2A6wt allele had a 6.56 fold risk of COPD than nonsmokers who carried CYP2A6wt allele. These results suggest that gene polymorphism of CYP2A6 may be related to the occurrence of COPD in Innermongolia Hans. CYP2A6wt may be a risk factor of COPD, and remarkably increase COPD susceptibility of the smokers. In addition, a close interaction exists between CYP2A6wt and smoking in the occurrence of COPD.

THE STUDY OF PROMOTER METHYLATION OF MULTIPLE TUMOR-RELATED GENES IN SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

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Aberrant methylation of CpG islands in promoter regions can permanently inactivate tumor-suppressor genes and therefore plays an important role in the occurrence and development of cervical cancer. In this study, we investigated the methylation status of the promoter region of five genes (APC, P16, E-cadherin, ASC and FHIT) in 20 normal cervical squamous epithelial tissues and 31 cervical cancer tissues. The objective of our study was to reveal the relationship between methylation status of multiple tumor-related genes and their frequencies in the occurrence of cervical cancer. 20 normal control samples were obtained from the normal cervical squamous epithelial tissues of patient with benign lesions and 31 cancer samples were acquired after the surgery resection (all samples are squamous cell carcinoma of the uterine cervix with pathological diagnosis). Genomic DNA was extracted from the samples and methylation specific PCR method (MSP) was employed to detect the methylation profiles of the promoter CpG islands of the following five genes: P16, APC, E-cadherin, ASC and FHIT. Methylation was not detected in any normal cervical squamous epithelial tissues. In cervical cancer tissues the methylation rates of P16, APC, E-cadherin, ASC and FHIT were 66% ($P < 0.05$), 61% ($P < 0.05$), 55% ($P < 0.05$), 26% ($P > 0.05$) and 19% ($P > 0.05$) respectively. Methylation of two or more tumor-related genes was detected in 14 (45%, $P < 0.05$) tumor samples. We also found that the higher the clinical stage and histological grade is, the higher the positive rate of methylation of P16, APC and E-cadherin genes. Our results show that the hypermethylation frequency of single genes and the accumulation of epigenetic alterations in individual samples of cervical cancer patients may vary considerably. Combined detection of methylation status of multiple

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genes may be a more reliable method for the early diagnosis of cervical carcinoma.

EFFECT OF DNA METHYLATION ON E-cadherin/Connexin32 EXPRESSION IN HT-29 COLON CANCER CELLS

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Previous researches showed E-cadherin (E-cad) / Connexin32 (Cx32) expression was downregulated in colon cancer tissues, and was associated with an increase of invasive and metastatic potential. This study was to study the mechanisms responsible for inactivation of E-cad/Cx32 gene in colon carcinoma. We investigated the methylation status around the 5′ promoter region of E-cad/Cx32 gene of HT-29 colon cancer cell line by methylation-specific polymerase chain reaction, the E-cad/Cx32 protein expression by immunocytochemistry, and compared the E-cad/Cx32 methylation status with E-cad/Cx32 protein expression. We found that hypermethylation of E-cad/Cx32 was involved in HT-29 cell line; while E-cad/Cx32 protein expression was almost lost in it. Therefore, methylation of the E-cad/Cx32 promoter region was correlated with the loss of E-cad/Cx32 protein expression in HT-29 colon cancer cell line. Treatment of E-cad/Cx32-negative carcinoma cells with the demethylating agent, 5-aza-2′-deoxycytidine, induced re-expression of this gene. Our findings indicate that 5′ CpG island methylation is common in colon carcinoma and may play an important role in the inaction of E-cad/Cx32. Our results also suggest that demethylation of the E-cad/Cx32 gene may be a potential therapeutic strategy for colon carcinoma.

POTENTIAL ROLES OF TRANSCRIPTION FACTOR Elf-1 DURING FOLLICULAR DEVELOPMENT, CORPUS LUTEUM FORMATION, MAINTENANCE AND DEGENERATION IN RAT OVARY

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Elf-1 is a member of the Ets transcription factor family. It is associated with cell proliferation and differentiation, and can also regulate vascular-specific gene expression during the blood vessel development. Cell proliferation and angiogenesis play important roles during follicular development, ovulation, corpus luteum (CL) formation and degeneration. However, the relationship between Elf-1 and ovarian follicular development, luteal formation, maintenance and degeneration has not been reported. In the present study, we investigated the expression of Elf-1 mRNA and protein in rat ovary during the estrous cycle and pregnancy by in situ hybridization, immunohistochemistry, RT-PCR and Western blot. Elf-1 mRNA was primarily localized in oocytes, granulosa cells and luteal cells, and the result of RT-PCR showed that the expression of Elf-1 mRNA was higher at proestrus and diestrus, compared with those at estrus and metestrus. The expression of Elf-1 mRNA increased during the early pregnancy, and reduced at the late pregnancy. In addition, our results of immunohistochemistry and Western blot indicated that the expression pattern of Elf-1 protein was similar to those of Elf-1 mRNA. Our results suggest that Elf-1 may be involved in the development of rat ovarian follicle and may play critical roles in the regulation of CL formation, maintenance and degeneration in rat ovary.

EPITOPE ANALYSIS OF A NOVEL Homo SPAINS SYNAPSE ASSOCIATED PROTEIN, PREPARATION OF ITS ANTIBODY AND PROTEIN EXPRESSION STUDY

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A family of synapse-associated protein (SAPs) has recently emerged as a central player in the molecular organization of synapses. This work is to analyze the epitope of a novel Homo sapiens synapse associated-protein FRG4, synthesize polyclonal antibody, and then study FRG4 protein expression in smooth muscle cells and endothelial cells. FRG4 full-length sequence was obtained by PCR from human fetal liver library. Its epitope, motifs and the second structure of amino acids encoded were detected by bioinformatics technique. FRG4 peptides synthesized by solid-phase peptide synthesis method were immunized to rabbits. Expression of FRG4 in vascular smooth muscle cells and endothelial cells was detected by immunohistochemistry. The 13-peptides PKLVKEEVFWRNY was selected by bioinformatic analysis to synthesize rabbit anti-human FRG4 polyclonal antibody. Antiboby purity was 82.79% and antibody dilution was 1:16,000 detected by ELISA. The antibody had a good reaction and specificity in Western blot. Expression of FRG4 was detected mainly in the cytoplasm of smooth muscle cells and endothelial cells by immunohistochemistry. Our results showed that a novel Homo sapiens synapse associated protein (FRG4) antibody was synthesized successfully.

UNIQUE MICRORNA MOLECULAR PROFILES IN LRRC4 RE-EXPRESSED GLIOBLASTOMA CELLS

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MicroRNAs (miRNAs) are short non-coding RNA molecules playing regulatory roles in animals and plants by repressing translation or cleaving RNA transcripts. Although miRNA differential expression between normal human brain tissue and glioblastoma has been identified, the precise mechanisms regulating miRNA expression are unknown. However, several mechanisms, including genetic and epigenetic alteration, might affect miRNA expression in cancers. Leucine-rich repeats containing 4 (LRRC4) is a potential glioma suppressive gene. The conspicuous absence of LRRC4 in high-grade gliomas directly contributes to increasing tumor grade. Re-expression of LRRC4 can significantly suppress glioblastomas U251 cells tumorigenesis in vivo, and cell proliferation and invasion in vitro. In this study, we examined the effect of re-expressed LRRC4 on miRNA expression profile in glioblastoma U251 cells by 743 microRNAs microarry. The analysis of both U251/vector and U251/LRRC4 cell lines allowed us to identify a group of microRNAs whose expression was significantly altered in re-expressed LRRC4 cells. A set of miRNAs (hsa-miR-155, hsa-miR-143, hsa-miR-455, hsa-miR-9, hsa-miR-612 and has-miR-381) was up-regulated in LRRC4 re-expressed U251 cells, while another set (hsa-miR-15b, hsa-miR-185, hsa-miR-590, hsa-miR-182 and hsa-miR-487b) was down-regulated in LRRC4 re-expressed U251 cells. Among these microRNAs, hsa-miR-185 and hsa-miR-182 have been identified by real-time PCR. The most interesting result was that has-miR-381 is predicted to be the target microRNA of LRRC4 by three softwares (TARGETSCAN, PICTAR and Sanger). This work was supported by National Key Project of Scientific Research Program (2006CB910502, 2006CB910504); National Natural Sciences Foundation of China (30770825, 30600224); China Postdoctoral Science Foundation (20060400265); the Special Funds of Science & Technology Departments of Hunan Province, China (05SK1001_1), Hunan Province Natural Sciences Foundations of China (No. 06JJ20080).

DIFFERENTIAL GENE EXPRESSION PROFILE OF ASTROCYTES

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Experimental studies showed that astrocytes have two morphologically and functionally distinctive cell lineages. Type 1 astrocytes are derived from progenitor cells that lack the A2B5 antigen and are responsive to injury in the central nervous system to generate scar tissues, while type 2 astrocytes arise from O-2A progenitor cells that bear A2B5 antigen and are associated with neurons to maintain the functional integrity of axons. The present study is aimed to

observe and analyze the difference in gene expression profile of purified type 1 and type 2 astrocytes in order to gain more information on their biological characteristics. Type 1 astrocytes and type 2 astrocytes were isolated with a standard shaking method and then purified with the differential adhesion method. The cDNA microarray chips comprising 4096 clones were employed to identify the gene expression profile of type 1 astrocytes and type 2 astrocytes. The differentially expressed genes were compared and analyzed. Results from four experiments revealed that 138 points in 4096 measured points were differentially expressed in type 1 astrocytes and type 2 astrocytes, 60 of which had higher expression in type 1 astrocytes than that in type 2 astrocytes. Among them there were 99 genes with known functions up to now. There were 42 genes highly expressed in type 1 astrocytes and 57 genes highly expressed in type 2 astrocytes among the 99 known ones. In conclusion, by studying the gene expression profile of cultured type 1 and type 2 astrocytes, we identified 99 genes with known functions that were differentially expressed between these two types of astrocytes.

INVOLVEMENT OF Smad PATHWAY IN CLEFT PALATE INDUCED BY ALL-TRANS RETINOIC ACID

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An excess of all-trans retinoic acid (atRA) leads to the formation of cleft palate. atRA regulates the expression of TGF-β in a variety of systems, including embryonic cells and tissues. The effect of TGF-β is mediated by Smad signaling pathways. We harvested the mouse palatal shelves from embryonic day 13 mice and cultured in media with different concentration of atRA *in vitro*. After 24 hours, we observed Smad2 and Smad3 were endogenously activated and expression of Smad7 was inhibited during the fusion process in normal control group. atRA treatment abrogated phosphorylation of Smad2 and Smad3 and stimulated expression of Smad7 in medial edge epithelial (MEE). After 72 hours, the palate of vehicle control group fused completely, but the opposing shelves were not intact in atRA treated groups. The fusing extent was negatively correlated with the concentration of atRA. Migration and apoptosis of MEE cells and degradation of basal lamina within midline epithelial seam (MES) markedly characterized in vehicle control palatal shelves in culture, while atRA treated group induced apoptosis in mesenchyme and inhibited apoptosis of MEE cells and degradation of basal lamina within MES. At the same time, we observed the development of palate shelves of pregnant females gavaged with atRA at different time points delayed while the control group fused completely. The palatal shelf of the control mice was remodeled to a horizontal position above the tongue. p-Smad2 protein was spread in the palatal epithelium, and the palate shelf of atRA-induced mice were still vertical along the lateral sides of the tongue. No p-Smad2 was detected in the palate shelf. Our results suggest that atRA treatment inhibits apoptosis of MEE cells and basal lamina degradation, which results in cleft palate, by inhibiting Smad signaling pathways.

GENE THERAPY OF MICE PERITONEAL FIBROSIS BY NANO-CARRIER PAMAM MEDIATED pCTGF-shRNA

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We constructed plasmid containing the CTGF-shRNA (pCTGF-shRNA) and transfect mouse peritoneal mesothelial cells in vitro and mouse peritoneal fibrosis model in vivo through nano-carrier PAMAM. We then investigated the effects of pCTGF-shRNA on the expression of CTGF, TGF-β1 and FN in mouse peritoneal mesothelial cells or mouse peritoneum. Transfection of several pCTGF-shRNA to primary mouse peritoneal mesothelial cells (induced by 4.25% high glucose) was performed using PAMAM G9. The pCTGF-shRNA that has the most depression effect was selected. C57BL/6 mice were randomly divided into four groups: CTGF RNA interference-treated group, negative control group, peritoneal fibrosis group and normal control group. After corresponding treatments for 28 days, the thickness of parietal peritoneum was measured by Masson′ dye staining. The expression of CTGF, TGF-β1 and FN mRNA were determined by semi-quantification reverse transcription PCR and Western blot. Introduction of pCTGF2-shRNA resulted in significant reduction of CTGF expression in mouse peritoneal mesothelial cells treated by 4.25% glucose. CTGF RNA interference-treated group had significantly thinner peritoneum compared to negative control group and peritoneal fibrosis group ($P < 0.01$). In addition, the mRNA and protein levels of CTGF, TGF-β1 and FN in CTGF RNA interference-treated group were markedly decreased compared to negative control group and peritoneal fibrosis group ($P < 0.05$), but still significantly increased compared to the normal control group ($P < 0.05$). Nano-carrier PAMAM could efficiently transfect pCTGF2-shRNA into primary mouse peritoneal mesothelial cells or mouse peritoneal fibrosis model and result in inhibition on peritoneum thickening or on increased expression of CTGF, TGF-β1 and FN level in mouse peritoneum. All these results indicate that CTGF-RNAi through PAMAM can be used as a very efficient strategy in gene therapy of peritoneal fibrosis.

INFLUENCE ON METOPROLOL ANTIHYPERTENSIVE EFFECT OF β1-ADRENORECEPTOR GENE POLYMORPHISM AND METHYLATED MODIFICATION

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To investigate whether polymorphism and methylation of β1-adrenoceptor (β1-AR) gene have impact on antihypertensive efficacy in patients with hyprtension. Methods: Three hundred hypertensive patients enrolled began taking metoprolol by the same dose. We also tested the genotype of their β1-AR gene by PCR-RFLP to compare the antihypertensive efficacy among patients with different genotype. Through methylation specific PCR (MSP), we detected the methylation state in β1-AR gene from peripheral blood of patients with the same genotype controlled by the subjects with normal blood pressure, and investigated the relationship between the modification and the antihypertensive response to metoprolol. Patients with Arg389 Arg genotype had significantly greater decrease in diastolic blood pressure as compared to those with Gly389 Arg and Gly389 Gly (8 ± 1.3 vs. 4 ± 1.5, 3 ± 1.1, $P < 0.05$). In addition, β1-AR gene from peripheral blood of all subjects was methylated. Polymorphism at Gly389Arg of β1-AR gene was associated with antihypertensive response to metoprolol. Methylation modification on β1-AR gene from human peripheral blood found not related to the response.

INHIBITORS OF DNA METHYLTRANSFERASE AND HISTONE DEACETYLASE REGULATE THE EXPRESSION OF β1-ADRENOCEPTOR GENE IN MYOCARDIAL CELLS

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Some recent researches have demonstrated individual differences exist in the population with the same β1-adrenoceptor (β1-AR) gene polymorphism. Whether the epigenetic modification of β1-AR gene, including DNA methylation and histone acetylation, could effect on its expression is still unknown. The aim of this study was to investigate whether 5-aza-2′-deoxycytidine (5-AZA-CdR) and trichostatin A (TSA), either administered alone or in combination, play a role in the control of expression of β1-AR gene in H9c2 myocardial cells. H9c2 myocardial cells were treated with 5-AZA-CdR and TSA. Bisulfite genomic sequencing was used to explore the demethylation status of β1-AR gene promoter. Quantitative real-time PCR analysis and Western blot were performed to determine the effects of the treatment on the expression of β1-AR

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gene. Methylation-sensitive PCR (MSP) analysis was used to confirm the reduction in DNA methylation and β1-AR gene promoter methylation following drug treatment. Although both 5-AZA-CdR and TSA alone did not activate the methylated β1-AR gene promoter in H9c2 myocardial cells and upregulate the expression of β1-AR gene, the combination of the two drugs not only appeared to produce a greater activation, but also seemed to be synergistic with respect to cell kill. β1-AR gene promoter activity was robust following demethylation of only a few CpG dinucleotides by brief exposure to 5-AZA-CdR and persisted even after prolonged treatment. In contrast, TSA did not facilitate demethylation of the β1-AR gene promoter. These findings may offer some new evidence to explain why the individuals with the same β1-AR gene polymorphism still have different antihypertensive response to metoprolol.

AGING AND DEGENERATIVE DISEASES

EFFECTS OF DENDRANTHEMA MORIFOLIUM ON LEARNING, MEMORY AND MOVING ABILITIES IN AGING MICE INDUCED BY D-galactose

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Dendranthema morifolium (ramat.) tzvel. (DM) is a chinese herb which has been used as a sudorific, antipyretic and antidote medicine. This study is to investigate the effects of DM on learning, memory and moving abilities in aging mice induced by D-galactose. Sixty mice were divided into control group, aging group, and DM group. The aging mice were induced by D-galactose. Learning and memory abilities were tested with Morris water maze and step down tests. Moving ability was evaluated with exhaustive swimming and anti-static fatigue tests. The activities of cerebral superoxide dismutase (SOD), glutathione peroxidase (GSH-px) and acetylcholinesterases (AChE), and the contents of cerebral malodialdehyde (MDA) and lipofuscin (LF) were assayed. Our results showed that DM significantly ameliorated the impairments of learning, memory and moving abilities. DM decreased the activities of SOD and GSH-px, and increased AChE, MDA and LF concentrations in aging mice induced by D-galactose. These findings suggest that DM can effectively prevent the aging-induced decrease in learning, memory and moving abilities. The mechanisms might be related to the enhancement of the activity of anti-oxidative enzyme, elimination of the free radical, and decrease in the activity of AChE.

PROFECTIVE EFFECTS OF SALIDROSIDE ON GLUTAMATE INDUCED NEUROTOXICITY IN CULTURED HIPPOCAMPAL NEURONS

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Excitotoxicity is a cell death caused by excessive exposure to glutamate (Glu), due to neuronal degeneration in many acute and chronic central neuron system diseases. In this study, we explored the effects of salidroside, a phenylpropanoid glycoside isolated from Rhodiolarosea L, on cultured hippocampal neurons exposed to excitotoxic doses of Glu. Pretreatment with salidroside for 24 hours markedly attenuated Glu-induced cell viability loss and increased LDH contents in a dose-dependent manner. Fluorescent microscope detection of Hoechst 33342 staining cells and Flow cytometric analysis of annexin-V and propidium iodide labeling cells at the cellular level showed that apoptosis was significantly reduced in the cultured hippocampal neurons pretreated with salidroside. Moreover, confocal microscope assessment using fluorescent dyes (Flu-4, AM) revealed that pretreatment with salidroside significantly reduced intracellular Ca^{2+} overload of hippocampal neurons induced by Glu. These results suggest that salidroside has protective effects against Glu-induced cell damage in hippocampal neurons, providing experimental data for the treatment of acute brain injury and neurodegenerative diseases.

EFFECT OF ANTHOCYANIN-RICH EXTRACT FROM BLACK RICE ON THE SURVIVAL OF MOUSE BRAIN NEURON

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The aim of this study is to observe the effect of anthocyanins on the survival of mouse brain neuron. The primary cultured mouse brain cells were exposed to the extract from black rice. The anthocyanins concentration of extract was examined by spectrophotometer. After cultured for 5 days, the primary neuron were exposed to the extract with different concentrations, and then the viability of the cells was detected by microscope after 3 days. The study showed that the extract (5; 10; 20 and 40 microg/mL) significantly increased cell viability up to 17.3 - 25.4% compared with that of the untreated cells. But when the concentration of extract was in the range of 80−400 microg/mL, the cell viability started to decrease. When the concentration exceeded 400 microg/mL, the cell viability was lower than that of control group. These results suggest that the anthocyanins extract could protect neuron against death at low concentration.

PROTECTION OF HEPATOCYTE GROWTH FACTOR ON ASTROCYTES EXPOSED TO SIMULATED ISCHEMIA/REPERFUSION *IN VITRO*

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The present study was conducted to investigate whether hepatocyte growth factor (HGF) had a direct protection on cerebral cortical astrocytes subjected to oxygen-glucose deprivation/reperfusion (OGD/R) and explored the intracellular signaling pathway mediating these effects. Primary cultured cerebral cortical astrocytes were prepared from Sprague-Dawley rats. Cell injury was evaluated by lactate dehydrogenase (LDH) release rate, and cell viability was assessed by 3-(4,5-dimethylthazol-2-yl)-2,5-diphenyltetrazolinum bromide (MTT) assay. Percentage of apoptotic cells was analyzed by flow cytometry and Hoechst 33258 staining. To investigate the effects of HGF on astrocytes under oxygen-glucose deprivation 12 h/reperfusion 12h (OGD_{12}/R_{12}) condition, the cultures were treated with HGF at different final concentrations (20-120 ng/ml). Our results showed that the increase in LDH release rate, the decrease in cell viability, and the increase in number of apoptotic cells resulting from OGD_{12}/R_{12} were significantly prevented by HGF treatment. Application of 80 ng/ml HGF exhibited the maximum effect. As detected by semi-quantitative RT-PCR and Western blotting analysis, c-Met mRNA and protein were expressed in primary cultured cerebral cortical astrocytes, and c-Met receptor in astrocytes exposed to OGD_{12}/R_{12} was remarkably upregulated at both the protein and mRNA levels. Application of HGF (80 ng/ml) did not affect the amount of c-Met mRNA and protein in astrocytes subjected to OGD_{12}/R_{12}. Treatment with c-Met inhibitor SU11274 (5 μM) significantly blocked the HGF mediated protection in cortical astrocytes exposed to OGD_{12}/R_{12}. As measured by Western blotting analysis, HGF stimulated both Akt and ERK1/2 activities in cortical astrocytes. Inhibition of Akt activation with the phosphatidylinositol-3 (PI-3) kinase inhibitor abolished the HGF mediated protection. In addition, prevention of ERK activation with the MEK inhibitor reduced the protective effects of HGF. These results suggest that HGF can directly protect cortical astrocytes against oxygen-glucose deprivation/reperfusion induced cell injury in a dose-dependent manner, and the protective effect is mediated by PI3-K /Akt and MEK/ERK signaling pathways. This work was supported by the Key Project of Chinese Ministry of Education (No. 02147).

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COMPARISON OF THE INHIBITORY ACTIVITIES OF THE β-SHEET BREAKERS ON β-AMYLOID PROTEIN

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Alzheimer's disease (AD) is a major progressive neurodegenerative disorder occurred in center nervous system. It has been shown that the aggregation of Aβ causes the neurotoxic change of the peptide. Therefore, inhibition of this process seems to be an effective therapeutic strategy for AD. Based on the stereochemistry structure and aggregation of $A\beta_{1\text{-}42}$, our group designed six β-sheet breakers. To compare the effect of these breakers on β-amyloid protein (Aβ) fibril formation and cytotoxicity to SH-SY5Y cells, the inhibitory effects of β-sheet breakers on $A\beta_{1\text{-}42}$ fibril formation were determined by using fluorescence analysis with Thioflavin T (ThT) and electron microscopic image, and the protective effects against cytotoxicity induced by $A\beta_{1\text{-}42}$ in SH-SY5Y cells were evaluated by MTT reduction assay. With the background intensity of fluorescence from aged group as 100 percent, we estimated the inhibitory rate of each group for aggregation and fibril formation of Aβ and found that H102 exhibited strongest inhibitory effect. Aggregation and fibril formation of $A\beta_{1\text{-}42}$ by TEM were observed and H102 exhibited strongest inhibitory again. Then we checked the protection of these breakers on SH-SY5Y cells form $A\beta_{1\text{-}42}$ − induced cytotoxicity. H102 was better than the other five. These results suggest that H102 was much more efficient than the other five β-sheet breakers in inhibiting $A\beta_{1\text{-}42}$ aggregation and in protecting SH-SY5Y cells from $A\beta_{1\text{-}42}$ − induced cytotoxicity.

COMPARISON OF AMINO ACIDERGIC NEUROTRANSMITTERS IN HIPPOCAMPUS AND BEHAVIOR BETWEEN DIFFERENT AGED h-APP TRANSGENIC MICE WITH ALZHEIMER'S DISEASE

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The amyloid precursor protein (app695 751) transgenic mouse is a model for amyloid deposition, and like AD, the mice develop memory deficits as amyloid deposition during the period of cognitive decline and formation of amyloid plaques and neurofibrillary tangles. Our aim was to determine changes in amino acidergic neurotransmitters concentration in hippocampus and cognitive behavioral alterations in hAPP transgenic mouse in different aging period using high performance liquid chromatography (HPLC) with fluorometric detection and step down test, water mazz test respectively. The data showed that hAPP transgenic positive App (+) mice had a loss in learning and memory ability with aging. In water maze test, the latent period of seeking for platform of 9 months old App (+) mice (32.8 ± 11.9s) was longer than that of 6 months old App (+) mice (23.2 ± 8.1s). In step down test, numbers of errors were more in 9 months old App (+) mice (2.38 ± 1.26) than in 6 months old App (+) mice and the latent period were shorter in 9 months old App (+) mice (208.7 ± 85.4 s) than in 6 months old App (+) mice (244.1 ± 96.2 s). The results of the amino acidergic neurotransmitters assay were more variable. The concentrations of glutamine and asparate were higher in 9 months old App (+) mice (0.58 ± 0.24 and 0.53 ± 0.23μg/g tissue wet weight) than that in 6 months old App (+) mice (0.34 ± 0.18 and 0.28 ± 0.17). Inhibitory amino acid taurine and GABA concentrations were 0.45 ± 0.18 and 0.49 ± 0.22 (μg/g tissue wet weight) respectively. There was no significant difference comparing with 6 months old App (+) mice. These results suggest that the change of amino acidergic neurotransmilters in hippocampus may play a role in the development of Alzheimer's disease.

EFFECT OF CHRONIC STRESS ON THE BEHAVIOR AND THE SPATIAL LEARNING AND MEMORY OF MICE IN MORRIS WATER MAZE TASKS

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To study the effects of the chronic stress on the exploratory behavior and the spatial learning and memory of mice in Morris water maze tasks, Kunming mice were randomly divided into control group and stress group. The chronic stress model in 7 days with multiple stressors (cold water, bondage, bondage + heat, unexpectedly given to mice) was applied. The exploratory behavior and spatial learning-memory ability in mice were tested by using open field and Morris water maze tasks. The changes of synaptosomal free calcium concentrations ($[Ca^{2+}]_i$) of the hippocampus in mice brain were measured by fluorescent spectrophotometry, and Fura 2-AM was used as an indicator for $[Ca^{2+}]i$. The results showed that chronic stress induced a remarkable decrease in the spontaneous behaviors in the tested mice. The escape latency of the treated mice by chronic stress was significantly increased, and the time spent in quadrant for the platform was significantly decreased in Morris maze tasks compared with the control group mice. The $[Ca^{2+}]_i$ of synaptosomes was significantly increased in the chronic stress mice. The results indicate that chronic stress induced a significant decline in spatial learning-memory ability in mice, which may be related to the changes of free calcium concentration of synaptosomes in mice brain. This work was supported by the 11th Five-year Plan Provincial Key Construction Project and the Scientific Research Foundation of Qufu Normal University.

FUNCTIONAL EXPRESSION OF P2X3 ION CHANNELS MEDIATES FORMALIN-INDUCED PAIN

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ATP-gated P2X receptors in nociceptive sensory neurons participate in transmission of pain signals from the periphery to the spinal cord. In the present study, the expression of P2X3 receptors in dorsal root ganglia (DRG) was characterized using immunohistochemistry, image analysis and patch clamp methods. SD rats were distributed into normal control and experimental groups, while experimental groups were divided randomly into 30 min, 1 h, 3 h, 6 h, 12 h, 24 h and 48 h groups which were treated with 5% formalin 100 μl injected into the rats hind paw. The expression and distribution of P2X3 in the lumber spinal cord and $L_{5\sim6}$ dorsal root ganglion were detected. Western blots showed P2X3 antibody recognized a major monomer of ~64KDa (SDS page) in DRG. Immuno-positive product of P2X3 was detected predominantly in endochylema and ecphyma of small and medium-larger DRG neurons. The expression of P2X3 in DRG small neurons had no change in 30 min and 1 hour group, but was up-regulated at 3 h group. The 24 h group had an obvious difference compared with other groups. P2X3 expression inmedium-larger DRG neurons was up-regulated at 6 hour group and the 48 h group had an obvious difference compared with other groups. There were no distinct changes of immuno-positive product of P2X3 in lamina II of spinal cord in 30 min and 1 h groups after formalin test compared with normal control group, while the expression of P2X3 of 3 h group was increased. In isolated small and middle-size DRG neurons, 10 mM alpha, beta-me ATP evoked an inward current, which was inhibited completely by 1 mM A-317491, a potent and selective P2X3 receptor antagonist. The data suggest that the early stage up-regulation of P2X3 by formalin test in the spinal cord and DRG may be one of the mechanisms that give rise to nociceptive effect.

EFFECT OF CHRYSANTHEMUM ON ANTI-OXIDATIVE ABILITY AND TELOMERASE ACTIVITY IN D-GALACTOSE-INDUCED SENILE MICE

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Chrysanthemum, a traditional Chinese herb, has been reported to have an anti-oxidative ability. The present study is to investigate the effect and mechanism of chrysanthemum on D-galactose-induced aging. To induce aging, D-galactose (60 mg/kg) was injected subcutaneously everyday for 7 weeks. In treated group, chrysanthemum (150 mg/kg/day) was i.g. administrated for 6 weeks from the second week. The content of malondialdehyde (MDA) and activities of telomerase, superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) were measured. The result showed chrysanthemum significantly increased

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the activity of SOD and GSH-PX, and decreased the content of MDA in heart, liver and brain treated with D-galactose. Chrysanthemum also enhanced telomerase activity of heart in aging mice. So chrysanthemum had the anti-aging ability. The mechanism might be via regulation of free radical metabolism, enhancement of antioxidative enzyme and telomerase activities, and alleviation of lipid peroxide level.

POLYPEPTIDES EXTRACT OF *ACHYRANTHES Bidentata Blume* PROTECTS AGAINST NMDA-INDUCED APOPTOSIS IN RAT CULTURED HIPPOCAMPAL NEURONS

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Glutamate is a major excitatory neurotransmitter in the mammalian central nervous system (CNS), and produces a marked effect by interacting with its receptors. The N-methyl-D-aspartate (NMDA) subtype of glutamate receptors is known to play a pivotal role in triggering excitotoxicity. Overstimulation of NMDA receptors results in a significant increase of the intracellular Ca^{2+} level followed by neuronal cell death, especially apoptosis. In contrast, NMDA receptor antagonists are known to rescue neuronal cell death. Therefore seeking a natural bioactive substance, which can block NMDA receptors, becomes a hotspot of Chinese crude drug's research. Achyranthes Bidentata Blume is a traditional Chinese medicine, which has been collected in Pharmacopoeia of the PRC. Polypeptides were extracted from the water-solution of Achyranthes Bidentata Blume. At the same time NMDA excitatory neurotoxicity model was developed in rat cultured hippocampal neurons. Using MTT assay, Hoechst/PI double staining and DNA ladder detection, our results indicated that the polypeptides had protective effect on neuronal cell death, especially apoptosis. Moreover, our data demonstrated that the protection was probably related to NMDA receptors by Ca^{2+} image and whole-cell NMDA-evoked currents.

POTASSIUM CHANNELS WERE INVOLVED IN ZINC-INDUCED APOPTOSIS IN MES23.5 CELLS

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Previous researches have demonstrated that zinc may be involved in the pathogenesis of Parkinson's disease by apoptotic pathway. However, the mechanisms underlying zinc-induced apoptosis are unknown. Previous studies showed that 6-hydroxxydopamine (6-OHDA)-enhanced potassium channels were involved in the apoptosis of dopaminergic neurons. Our study is to illustrate whether zinc-induced apoptosis was also mediated by potassium channels. First we demonstrated cell apoptosis with zinc treatment by hoechst staining assay. The results showed that $13.4 \pm 0.6\%$ of MES23.5 cells were apoptotic after 24 h incubating with 60 μM zinc sulfate. Then we observed that the tyrosine hydroxylase (TH) mRNA expression and the dopamine content were decreased detected by semi-quantitative RT-PCR and high-performance liquid chromatography-electrochemical detection (HPLC-ECD). Further study was conducted to elucidate the mechanism of cell apoptosis using whole-cell patch clamp recording. The data demonstrated that MES23.5 cells exhibited a tetraethylammonium (TEA)-sensitive outward K^+ current with delayed rectifier characteristics. Increases of K^+ current density were recorded following the treatment by 60 μM zinc for 2 - 8 hours. After incubation with 20 mM TEA, the zinc-induced enhancement of K^+ currents were fully blocked. Furthermore, incubation with TEA also could block zinc-mediated caspase-3 activation and cell apoptosis. These data suggest that zinc-induced apoptosis on MES23.5 dopaminergic cells may be due to the enhancement of TEA-sensitive K^+ channel activity. This work was supported by the grants from the National Program of Basic Research sponsored by the Ministry of Science and Technology of China (2007CB516701, 2006CB500704) and the National Foundation of Natural Science of China (No. 30570649).

IRON REGULATORY PROTEINS WERE INVOLVED IN THE 6-HYDROXYDOPAMINE (6-OHDA) INDUCED FERROPORTIN1 DOWN-REGULATION

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Based on our previous study, we proposed that ferroportin1 (FP1) might account for the nigral iron accumulation in 6-hydroxydopamine (6-OHDA) lesioned Parkinson's disease models. In the present study we observed the effect of 6-OHDA on iron efflux, as well as FP1 expression, in ventral mesencephalic neurons, cultured astrocytes and MES23.5 cells. The findings showed that 6-OHDA (10 μmol/L) decreased the iron efflux in these cells with decreased mRNA and protein expressions of FP1. To further clarify that the down-regulation of FP1 was not due to the increased intracellular iron, these cells were overloaded with ferric ammonium citrate. Under iron overload conditions, FP1 showed a dose-dependent up-regulation. 6-OHDA treatment could increase both iron regulatory protein 1 (IRP1) and IRP2 mRNA expression. Silencing of IRPs by small interfering RNA dramatically abolished 6-OHDA-induced FP1 down-regulation. These results suggest that decreased expression of FP1 was responsible for the decreased iron efflux with 6-OHDA treatment in ventral mesencephalic neurons, cultured astrocytes and MES23.5 cells. Down-regulation of FP1 by 6-OHDA was in an IRE/IRP-dependent manner. This work was supported by the grants from the National Program of Basic Research sponsored by the Ministry of Science and Technology of China (2007CB516701, 2006CB500704) and the National Foundation of Natural Science of China (No. 30600190 and 30570649).

TRICHOSTATIN A INDUCES APOPTOSIS AND PROMOTES NEUROTOXIC EFFECTS OF NEUROTOXINS ON DOPAMINERGIC NEURONAL CELLS

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Parkinson's disease (PD) is a progressive neurodegenerative disease related to age, and primarily resulted from the death of dopaminergic neurons in the substantia nigra. Increasing evidences have shown that epigenetic factors play important roles in the aging and disorder of human being and animals. Trichostatin A (TSA) as a histone acetylation inhibitor was usually used to induce status alteration of histone acetylation in cells. In our studies, SH-SY5Y cells (human), N27 cells (rat) and MN9D (mouse) were used as cell models of dopaminergic neurons treated with TSA. Our results showed that treatment with TSA led to decreased cell viability and apoptosis in dopaminergic neuronal cells. Combination of TSA and typical neurotoxins in PD, i.e. MPP+, 6-OHDA or rotenone, resulted in stronger damages in dopaminergic neuronal cells than either of them. These results suggest that epigenetic regulation might play roles in pathogenesis of Parkinson's disease through association with cell death of dopaminergic neurons.

β-SECRETASE-1 (BACE1) EXPRESSION IN CEREBRAL NEOCORTEX SHOWS A MODULAR DISTRIBUTION PATTERN: INVERSE CORRELATION WITH ENDOGENOUS NEURONAL ACTIVITY

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Reduced brain or neuronal activity and metabolism may play an important role in Alzheimer's disease (AD) etiology, as suggested by epidemiological and imaging studies in humans. Stimulatory experimental paradigms, e.g., environmental enrichment, voluntary exercise and learning, reduce the levels and accumulation of amyloid peptides (Aβ) in the cerebral cortex of transgenic mouse models of AD. Neuronal hypoactivity in the rat olfactory bulb, induced by naris-occlusion, elevates protein levels and activity of β-secretase-1 (BACE1), a prerequisite for Aβ genesis. We determined BACE1 expression relative to endogenous neuronal activity, measured by cytochrome c oxidase (CO) reactivity, in the adult rhesus monkey (*Macaca mulatta*) striate cortex (V1) and guinea pig barrel cortex (SI) following whisker trimming. BACE1 expression in V1 occurred mainly in the neuropil over layer II-IV, appearing as alternating patches of high and low immunoreactivities. The periodic patches of BACE-1 immunoreactivity correlated spatially with CO blob and inter-blob areas, but the labeling intensities of the two markers were complementary to each other within a given area. In SI, BACE-1 expression in the barrels increased following unilateral trimming of vibrissae, which was associated with a decreased CO reactivity in the same barrels. As with the olfactory system, our present findings again revealed an inverse relationship between BACE1 expression and endogenous neuronal activity. Importantly, deprivation-induced BACE1 upregulation can occur trans-synaptically in the cerebral neocortex. Our results emphasize a biological role of BACE1 in neuronal and synaptic plasticity, but also implicate a potential mechanism whereby neuronal hypoactivity may lead to Aβ overproduction and accumulation in the neocortex.

OVEREXPRESSION OF DIVALENT METAL TRANSPORTER 1 ENHANCED IRON INDUCED CELL DAMAGE

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Elevated iron accumulation has been reported in brain regions in some neurodegenerative disorders. However, the mechanism for this is largely unknown. Divalent metal transporter 1 (DMT1) is an important divalent cation transporter. Our previous studies have demonstrated increased DMT1 expression and elevated iron levels in the SNpc of PD mouse model, indicating the important role of increased DMT1 expression in iron accumulation. In the present study, we generated the stable MES23.5 dopaminergic cell lines with overexpression of DMT1 to investigate the role of increased DMT1 expression in cellular oxidative stress and cell damage caused by iron accumulation. Results showed that the expression of DMT1 increased significantly in stable transfected cells compared to control. The upregulation of DMT1 enhanced the iron influx inducing the subsequent increase of reactive oxygen species and reduced mitochondrial membrane potential. This led to the activation of caspase-3. These results suggest that increased DMT1 expression in MES23.5 cells caused the increased intracellular iron accumulation, which resulted in the increased oxidative stress leading to ultimate cell damage. This work was supported by the grants from the National Program of Basic Research sponsored by the Ministry of Science and Technology of China (2007CB516701, 2006CB500704) and the National Foundation of Natural Science of China (No. 30400139 and 30570649).

HISTAMINE ALTERATION IN h-APP TRANSGENIC MICE WITH ALZHEIMER'S DISEASE

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Neurotransmitter histamine (HA) has been implicated in the regulation of numerous and important activities of the central nervous system as arousal, cognition, circadial rhythms and neuroendocrine regulation. The role of histamine in central nervous system disorders is not clearly demonstrated and contradictory results have been reported. Our study is to investigate the role of histaminergic system in central nervous system by assaying the content of histamine in hippocampus in the hApp transgenic mouse model of neurodegeneration using high performance liquid chromatography (HPLC) with fluorometric detection. Simultaneously hippocampus tissues were taken for dehydration and embedding, congo red staining and immunohistochemical staining. Our experimental data indicated the participation of histamine in AD progress. The contents of histamine in hippocampus in 3 and 6 months old App (+) mice were 12.28 ± 1.36 and 20.65 ± 3.45 (μg/g wet tissue weight) respectively, while in 3 and 6 months old App (-) mice HA were 9.87 ± 1.64 and 13.45 ± 2.11 (μg/g wet tissue weight). The data showed that concentration of HA in hippocampus increased with mice growth and HA in 6 months old App (+) mice was higher than that in 6 months old App (-) mice. Immunohistochemistry revealed plaques in hippocampus mice and Congo red staining showed amyloid deposition in intercellular space in 6 months old App (+). Our work indicates that histamine levels increase as AD progresses.

PROTECTIVE EFFECT OF HYDROGEN SULFIDE ON MPP^+ INDUCED DAMAGE IN PC12 CELLS

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Hydrogen sulfide (H_2S) is the third gaseous mediator apart from nitric oxide (NO) and carbon monoxide (CO). Eto K *et al*. show that the level of H_2S in the brains of AD patients is severely decreased. However, it remains incompletely defined whether the low level hydrogen sulfide is responsible for the oxidative stress-induced neurodegeneration in Parkinons disease. To investigate the protective effect of hydrogen sulfide against MPP^+ damage in PC12 cells and explore the involved underlying molecular mechanism, we used MPP^+ induced PC12 cells damage as the model of Parkinons disease and pretreated with NaHS (used as H_2S donor) to explore the potential protective effect. The cell shape and nuclear morphology were observed by nuclear staining with hoechst33258; the proliferation of PC12 cells was observed by 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. The apoptosis of PC12 cells was detected by flow cytometry (FCM) with propidium iodide (PI) stain. The mitochondrial membrane potential ($\Delta\Psi m$) and the level of reactive oxygen species (ROS) in PC12 cells were analyzed by rhodamine123 and dihydrohodamine123 stain FCM respectively. RT-PCR and western blot were used to detect the level of Bax mRNA and protein expression of Bcl-2 and Caspase3 in the PC12 cells respectively. We found that H_2S decreased the inhibition ratio, the apoptosis, and the ROS accumulation, increased the loss MMP, down-regulated Bcl-2, up-regulated Bax and Caspase-3 induced by MPP^+ in PC12 cells. The results explained that H_2S has neuroprotective effect against MPP^+-induced damage in PC12 cells, the mechanism of which might be related to attenuate the MPP^+-induced intracellular ROS generation, preserve $\Delta\Psi m$, prevent the MPP^+-induced down-regulation of Bcl-2 and up-regulation of Bax and Caspase-3.

PERIPHERAL ZINC (ZN^{2+}) INDUCED DOPAMINERGIC NEURONS APOPTOSIS IN THE NIGROSTRIATAL SYSTEM OF RATS

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Clinical studies have demonstrated an excess of zinc in the substantia nigra (SN) of Parkinsonian patients, suggesting the involvement of zinc in Parkinson's disease (PD). To demonstrate the relationship between peripheral zinc overload and dopaminergic neuron loss in rat SN and to investigate the possible mechanism, in the present study we used Inductively Coupled Plasma (ICP-2) detector, fast cyclic voltammetry, tyrosine hydroxylase (TH) immunohistochemistry, Nissl staining and high-performance liquid chromatography-electrochemical detection to study the degeneration of dopaminergic neurons and increased zinc content in the SN of rats. Our study showed that peripheral zinc overload increased the zinc content and induced damage of dopaminergic neurons in the SN. We also observed that lipid peroxidation elevated and SOD activity decreased in the midbrain of rats. The results of Western blots showed

cytochrome c released into cytosol from mitochondria after a higher dose of zinc injection (50 mg/kg). Both Hoechst 33258 staining and caspase-3 activity assay indicated the involvement of apoptosis in the SN of rats treated with zinc. These results suggest that peripheral zinc could increase the zinc amount in the SN, where excessive zinc caused the apoptosis of dopaminergic neurons. Our findings provide evidences that zinc might be involved in the etiology of PD. This work was supported by the grants from the National Program of Basic Research sponsored by the Ministry of Science and Technology of China (2007CB516701, 2006CB500704) and the National Foundation of Natural Science of China (No. 30570649).

UP-REGULATION OF DIVALENT METAL TRANSPORTER 1 IS INVOLVED IN 1-METHYL-4-PHENYLPYRIDINIUM (MPP^+)-INDUCED APOPTOSIS IN MES23.5 CELLS

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Apoptosis has been identified as one of the important mechanisms involved in the degeneration of dopaminergic neurons in Parkinson's disease (PD). Our previous study showed increased iron levels in the substantia nigra as well as loss of dopaminergic neurons in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced PD mouse models. 1-Methyl-4-phenylpyridinium (MPP^+) is commonly used to establish a cellular model of PD. Although intracellular iron plays a crucial role in MPP^+-induced apoptosis, the molecular mechanism linking increased iron and MPP^+-induced neurodegeneration is largely unknown. In the present study, we investigated the involvement of divalent metal transporter 1 (DMT1) that accounts for the ferrous iron transport in MPP^+-treated MES23.5 cells. In the treated cells, a significant influx of ferrous iron was observed. This resulted in a decreased mitochondrial membrane potential. Additionally, an elevated level of ROS production and activation of caspase-3 were also detected, as well as the subsequent cell apoptosis. These effects could be fully abolished by using iron chelator desferal (DFO). Increased DMT1 (-IRE) expression but not DMT1 (+IRE) accounted for the increased iron influx. However, there were no changes for iron regulatory protein 1 (IRP1), despite decreased expression of IRP2. Iron itself had no effect on IRP1 and IRP2 expression. Our data suggest that although DMT1 mRNA contains an iron responsive element, its expression is not totally controlled by this. MPP^+ could up-regulate the expression of DMT1 (-IRE) in an IRE/IRP-independent manner. Our findings also show that MPP^+-induced apoptosis in MES23.5 cells involves DMT1-dependent iron influx and mitochondria dysfunction. This work was supported by the grants from the National Program of Basic Research sponsored by the Ministry of Science and Technology of China (2007CB516701, 2006CB500704) and the National Foundation of Natural Science of China (No. 30400139 and 30570649).

EFFECT OF ELECTROACUNPUNCTURE ON BEHAVIOR AND LTP HIPPOCAMPAL Ca1 IN MEMORY IMPAIRED RATS

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In the present study, we investigated the effects of D-galactose induced learning and memory impairment and LTP in hippocampal CA1 region of rats, and explored the mechanism of electroacupuncture (EA) on learning and memory. Morris water maze test showed the escape latency of Model was significantly longer than that of Control. The distance percentage was shorter, however, the escape latency of EA was shorter than of Model, and the distance percentage was longer. LTP in Hippocampus Ca1 in Model was significantly inhibited, but inhibition of LTP in Hippocampus Cal electroacupunctrer was recovered to some extent, and the synaptic plasticity was improved. These results suggest that electroacupuncture can improve the lowered learning and memory of aging rats induced by D-galactose, and the mechanism of action is probably related to the increase of LTP in Hippocampus Ca1.

INFLUENCE OF H102 ON THE EXPRESSION OF AMYLOID PROTEIN AND AMYLOID PRECURSOR PROTEIN IN THE HIPPOCAMPUS OF APP 695 TRANSGENIC MICE

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Alzheimer's disease (AD) is a major progressive neurodegenerative disorder occurred in center nervous system. It has been shown that the aggregation of β-amyloid protein (Aβ) causes the neurotoxic change of the peptide. Therefore, inhibition of this process seems to be an effective therapeutic strategy for AD. Based on the stereochemistry structure and aggregation of $A\beta_{1\text{-}42}$, our group designed six β-sheet breakers. We have already checked that H102 was more efficient than the other five β-sheet breakers in inhibiting $A\beta_{1\text{-}42}$ aggregation and in protecting SH-SY5Y cells from $A\beta_{1\text{-}42}$ − induced cytotoxicity through Thioflavin T (ThT), electron microscopic and MTT. In this experiment we wanted to observe the influence of H102 on the expression of amyloid protein and amyloid precursor protein in the hippocampus of APP 695 transgenic mice. The 9-month-old APP 695 transgenic mice were randomly divided into the model group and the H102 group; C57BL/6 J mice were adopted as normal control group. The H102 group was injected with H102 in a dose of 3 μl per mouse in lateral ventricle, once a day; while the model group and the control group were injected with saline. 10 days latter, the brain sections from transgenic mice and wild type female mice were subjected to immunohistochemistry and Congo red histological staining, and observed under microscope. Aβ and APP immunohistochemistry showed density of positive cell in the CA1 region of hippocampus of control group were less than those of model group. H102 peptide reduced the area, and density of positive cells. Congo red staining showed there were lots of amyloid plagues in the brains of model mice but not in the brains of normal control. H102 significantly decreased the amyloid plagues. These results suggest that H102 can decrease the level of APP, Aβ in APP transgenic mice.

CARDIOVASCULAR DISEASES AND TREATMENT

INHIBITORY EFFECTS OF ADRENOMEDULLIN ON THE EXPRESSION OF VASCULAR CELL ADHESION MOLECULE-1 IN MYOCARDIAL ISCHEMIA-REPERFUSION INJURY

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It has been reported that CGRP can reduce the damage to heart after myocardial ischemia and reperfusion. Adrenomedullin (Adm), a novel and potent hypotensive peptide, shows slight structural homology to the calcitonin gene-related peptide (CGRP) family. There is yet no research in cardioprotective effects of Adm on myocardial ischemia-reperfusion injury. In this study, we investigated the influence of adrenomedullin1−50 (Adm1−50) on vascular cell adhesion molecule-1 (VCAM-1) expression in isolated rat heart. Twenty-four rats were randomly divided into A; B; C and D group ($n = 6$ for each group). The isolated rat hearts were perfused in a Langendorff mode for 20 min and followed by 60 min of global ischemia. Then, all groups were reperfusion with Kerbs-Henseleit bicarbonate for 60 min, but group B, C and D were perfused with buffer in the presence of 1×10^{-9}; 1×10^{-8} and 1×10^{-7} mol/L Adm1−50, respectively, for 15 min after the onset of reperfusion. The post-ischemic change of creatine kinase-isoenzyme (CK-MB) in coronary effluent and the expression of cardiac VCAM-1 mRNA were measured. After reperfusion, Adm1−50 dose-dependently decreased the expression of VCAM-1 and the CK-MB activity. The ratio of VCAM-1/GAPDH mRNA were 1.2 ± 0.52. 1.1 ± 0.45, 0.6 ± 0.31 and 0.5 ± 0.36 for group A, B, C and D respectively ($p < 0.01$, group C and D vs group A). Adm1−50 may protect the myocardium from ischemia-reperfusion injury by inhibiting the expression of VCAM-1.

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PRETREATMENT WITH BCG ATTENUATE CARDIAC REMODELING VIA ACTIVATION OF TLR4

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Hypertension-induced cardiovascular hypertrophy and fibrosis are critical in the development of heart failure. We wondered if Bacillus Calmette Guerin (BCG), a safe vaccine which has strong immune modulating effect, could prevent cardiac hypertrophy and fibrosis in mice by regulation of immune microenviroment through Toll like receptor 4 (TLR4) and DC-SIGN. Animals received BCG intraperitoneally with or without TLR4 antagonist (msbB) or DC-SIGN inhibitor (mannan) every 3 days for a week before abdominal aortic constriction (AAC). Myocardial hypertrophy and fibrosis were evaluated by echocardiography and pathohistology. Biochemical and immunological changes were investigated by RT-PCR, immunohistochemistry and confocal. Pretreatment of BCG attenuated myocardial hypertrophy and fibrosis, which up-regulated expression of IFN-γ and decreased TGF-β, IL-10 in the myocardium. We also found BCG reversed AAC-induced increase in the number of myocardium-infiltrate in M2-macrophages. Moreover, inhibition of TLR4 significantly attenuated the protective effects of BCG, while mannan did not. Our results suggest that BCG can prevent cardiac remodeling induced by pressure overload by modulating immune microenvironment via activation of TLR4.

DAMAGE EFFECTS OF SLEEP DEPRIVATION ON MYOCARDIUM AND ITS ANTIOXYGEN INDEX IN RATS

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Sleep deprivation is a very common phenomenon in our society now and can cause damaging effects to our body. Using the ''flower pot technique'' sleep deprivation model, we demonstrated that sleep deprivation could cause damaging effects on myocardium. After sleep deprivation, heart rate increased and S-T segments of ECG rose or mixed with T waves showing ischemia of myocardium, P-R interval and Q-T interval prolonged. In addition, cadiocytes lysis or necrosis, subcellular organelles were impaired: the chromosome dissolved gently, the endoplasmic reticulum expanded accompanied with evidence of Ca^{2+} over loaded. Following morphological changes were observed: structure of the mitochondria blurred; the intercalated disk dissolved; thrombocytes accumulated in microvessels; protein deposited in endotheliocytes interstitium; edema, bleeding and monocytes invasion in the lumen; and lipid peroxidation reaction effects spread widely. In addition to morphological changes, myocardium mitochondrial showed increased malondialdehyde level and increased superoxide dismutase activity followed by a decreased trend as sleep deprivation prolonged. These results suggest that sleep deprivation can induce damage on myocardium and the stress especially oxygen stress caused by sleep deprivation may be the possible mechanism, indicating potential use of antioxidants to treat sleep deprivation.

DISTRIBUTION OF − 159 C/T GENE POLYMORPHISM IN PROMOTER REGION OF CD14 AND ITS SIGNIFICANCE FOR PLASMA LIPIDS LEVELS

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To investigate the distribution of −159 C/T gene polymorphisms in the promoter region of CD14 and its relation to plasma lipid levels in normal Chinese Han population in Hunan, genotypes of CD14 were typed in 118 normal men by PCR - RFLP and their plasma lipid levels were assessed. The - 159C/T gene polymorphism was present in normal Han population of Hunan, and the frequencies of CC, CT and TT were 19.5 %, 51.7 %, and 28.8 %, respectively. The TT genotype carriers had lower plasma low - density lipoprotein cholesterol (LDL - C) level than that of the CT and TT genotype carriers ($P < 0.001$, $P < 0.05$). No association was found between the genotypes and plasma levels of total cholesterol, triglyceride, high -density lipoprotien, apolipoprotein A1, apolipoprotein B100 and body mass index. TT genotype significantly influenced the plasma levels of LDL - C in the normal Chinese Han population.

RECONSTITUTION OF CORONARY VASCULATURE IN ISCHEMIC HEARTS

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In the remodeling process of post myocardial infarction, limited neoangiogenesis to the infarct-bed capillary is insufficient to support the greater demands of the hypertrophied but viable myocardium, which results in further ischemic injury to the viable cardiomyocytes at risk. Here we reported the rapid angiogenic effect induced by the extracts from Dagencao (angio-T) to form functional vasculature and the promoted survival potential of the viable cardiomyocytes at risk after myocardial infarction. Our results demonstrated dual effects of angio-T on up-regulating expression of angiogenic factors and protection of the cardiomyocytes against further ischemic injury. Echocardiograph studies demonstrated significant functional improvement of the infracted hearts by 2 days after infarction and angio-T treatment. These therapeutic properties of angio-T to induce early reconstitution of a blood supply network, and improve heart function post infarction appear entirely novel and may provide a new dimension for therapeutic angiogenesis medicine for the treatment of ischemic heart diseases.

COATING PREPARATION AND DRUG DELAYED RELEASE *IN VITRO* AND *IN VIVO* OF DEXAMETHASONE-ELUTING INTRAVASCULAR STENTS

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To observe the effect of drug delayed release *in vitro* and *in vivo* in dexamethasone-eluting intravascular stent, we prepared stents by dip coating, and then high performance liquid chromatography (HPLC) was used to detect the drug loading. The weight of bare stents and drug eluting stents were determined. NETZSCH thermal analyser was used to analyze dexamethasone. The drug slow-release rate in vitro was detected with parallel plate flow chamber perfusion system, and then the dexamethasone-eluting intravascular stents were implanted into rabbit abdominal aorta to detect drug slow-release character in vivo. The blood flow velocity of rabbit abdominal aorta was detected by ultrasonic inspection. The results showed the drug loading of stents prepared by dip coating for four days was 93.15 ± 7.83μg. The weight of drug load was about 13.70 ± 0.84 % in the coating. Differential scanning calorimetry (DSC) indicated that the melting point was 259.3°C, which was coincided with 254–264°C in the pharmacopoeia of the People's Republic of China. The thermogravimetry (TG) curve indicated the rate of loss in weight of dexamethasone was 76.67% from 250°C to 400°C. DSC and TG curve hinted the course of evaporation and sublimation of dexamethasone on the temperature. Drugs were released slowly and the release rate reached 84 % at 15d. The maximum blood flow velocity of abdominal aorta was 27.03 ± 3.25 cm/s, while the minimum velocity was 12.13 ± 2.20 cm/s. The drug concentration detected after implantation 2 hours was 4.59μg/g in vessel wall and 1.91 μg/g in liver, respectively. The drug concentration detected after implantation 10 days was

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0.22 μg/g in vessel wall and 0.73 μg/g in liver, respectively. These results suggest that the prepared dexamethasone-eluting intravascular stents have evident effect of delayed release. This study was supported by grants from the MOST of China (2004DFA06400), the Scientific Research Foundation of Third Military Medical University (06XG030), and Chongqing Municipality of China (CSTC2006AA5014-3, DRC2005-1006).

IN VITRO DRUG SLOW-RELEASE BEHAVIOR OF LIGUSTRAZINE ELUTING INTRAVASCULAR STENTS

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In the present study, we assessed in vitro adsorption and elution characteristics of ligustrazine eluting intravascular stents by high-performance liquid chromatography (HPLC). Ligustrazine eluting stents have two layers: the inner layer is L-polylactide (L-PLA) coating, and the outer is polymethyl methacrylate. Ligustrazine hydrochloride was absorbed onto L-PLA coating, and covered by polymethyl methacrylate coating. L-PLA coated stents were immersed into ligustrazine hydrochloride solutions diluted to 5, 10, 20 mg/ml at 37 °C for 1 to 5 days, and then eluted in 20 ml PBS buffer solution for two weeks. Adsorption quantity was measured by drug eluting concentration using HPLC. Stents for slow-release were immersed in 20 mg/mL ligustrazine hydrochloride solutions for 72 hours as described above, and then covered by outer layer at 37 °C after drying placed into parallel plate flow chamber perfusion system which contains 150 ml pH 7.4 PBS buffer solution at the flow rate of 10 ml/min for 16 days. Samples were obtained at specific time points after eluting. The quantity of ligustrazine hydrochloride adsorbed onto the L-PLA coated stents was dependent on the concentration and duration of immersion in the solution. More than 95 % of the final amount bound at each concentration was adsorbed within 72 hours. The eluting curve was biphasic with initial rapid elution for the first 24 hours followed by a gradual slow elution. After two weeks, we still could detect drug release. Our results indicate that Ligustrazine hydrochloride could be passively adsorbed onto L-PLA coated stents, influenced by the concentration and duration of immersion, and eluted in a predictable manner. These studies suggest that a potent anti-platelet aggregation drug may be used on intravascular stents. This study was supported by grants from the MOST of China (2004DFA06400) and Chongqing Municipality of China (CSTC2006AA5014-3, DRC2005-1006).

MESENCHYMAL STEM CELLS FOR REGENERATING MYOCARIDUM

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Myocardial infarction (MI) due to coronary artery diseases remains the most prevalent cause of premature death. Replacement of infarcted heart tissue with regenerating myocardium from native progenitor pools or exogenously introduced stem cells remains a therapeutic ideal but not practical mostly due to their low efficiency in cardiogenic differentiation. In this study, we report the activation of cardiogenic specific signaling pathways in mesenchymal stem cells (MSCs), which increases the cardiogenic differentiation efficiency of MSCs, significantly repaired infarcted hearts in humans and in MI animal models, with clear evidence of newly regenerated myocardium from MSCs of endogenous origin throughout the infarct zone with striated characteristics and ultrastructural integration as well as significantly improved functional performance. Depletion of cardiomyogenic differentiation specific signaling pathways resulted in decreased cardiogenic differentiation efficiency of MSCs *in vitro* and defected repair of MI *in vivo*, indicating the necessity of the specific signaling pathways for efficient and significant repair of MI. Our results demonstrated that the increased cardiogenic differentiation efficiency in progenitor cells plays a critical role in significant regeneration of functional myocardium for repair of infarcted hearts.

K_{ATP} CHANNEL, ADENOSINE AND NEURONAL PATHWAYS EQUALLY CONTRIBUTE TO REMOTE PRECONDITIONING-MEDIATED CARDIAC PROTECTION FROM I/R INJURY

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Numerous studies have shown that K_{ATP} channel, adenosine and nervous pathways are involved in the remote ischemia preconditioning (RIPC) mechanism, a powerful strategy to protect heart from ischemia-reperfusion injury (IRI). However, it remains elusive whether they contribute to RIPC independently or coordinately. The present study compared the effects of K_{ATP} channel, adenosine and neuronal pathways on RIPC. Four groups were randomly assigned in New Zealand rabbits. Group 1: RIPC was set up as the control group; Group 2: control plus 8- (*p*-sulfophenyl) theophylline (8-SPT); Group 3: control plus hexamethonium (Hex); Group 4: control plus Glibenclamide (Gli) 0.3 mg/kg. Enzymatic assay and triphenyltetrazolium (TTC) staining were performed to assess the heart injury. MAO15 dramatically reduced the infarct size from 55.2 % ± 1.4 % to 21.3 % ± 0.7 % and greatly inhibited the rise of creatine kinase (CK), CK-MB and lactate dehydrogenase (LDH). Pretreatment of either 8-SPT, Hex or Gli greatly blocked the protection of IPC from 21.3 % ± 0.7 % to 34.2 ± 1.1, 32.8 ± 0.9 and 34.7 ± 1.2 respectively. The levels of CK, CK-MB and LDH were similar in the groups of 8-SPT, Hex or Gli. In conclusion, blocking neuronal pathway, adenosine and K_{ATP} channel could similarly inhibit the RIPC-mediated cardioprotection suggesting that these three signaling act on RIPC coordinately. The nerve afferents by RIPC may be the upstream signaling to release the adenosine, which in turn may activate K_{ATP} channel to protect the heart from IRI.

PROTECTIVE EFFECT OF CURCUMIN AGAINST HIGH GLUCOSE-INDUCED ENDOPLASMIC RETICULUM STRESS BY DOWNREGULATION OF COX-2 EXPRESSION IN HUVECS

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The aim of this study is to investigate whether curcumin could protect against high glucose-induced apoptosis by suppressing endoplasmic reticulum (ER) stress pathway in human umbilical vein endothelial cells (HUVECs). HUVECs were exposed to low glucose (5.5 mmol/L) or high glucose (30 mmol/L) for 12~48 h. The expression of ER stress-related markers (glucose-regulated protein 78 (GRP78/Bip) and CAAT/enhancer binding protein homologous protein (CHOP)) and cyclooxygenase-2 (COX-2) were also evaluated by western blotting analysis. Annexin V-FITC/PI flow cytometry analysis revealed a time-dependent increase of apoptotic cells in HUVECs treated with high glucose. Curcumin (10–100 μmol/L) inhibited the high glucose-induced apoptosis in a dose-dependent manner. High glucose induced a time-dependent increase in the levels of COX-2, GRP78 and CHOP proteins expression in HUVECs. Curcumin and nimesulide (a COX-2 inhibitor) downregulated high glucose-induced overexpression of COX-2 and CHOP expression in HUVECs. In conclusion, these data suggest that curcumin can protect the HUVECs against high glucose-induced apoptosis by downregulation of COX-2 and inhibition of endoplasmic reticulum stress. This work was supported by the National Natural Science Foundation of China (No. 30400094).

IMPROVEMENT OF ENDOTHELIAL DYSFUNCTION IN HYPERCHOLESTEROLE-MIC RABBIT AORTAS BY *EX VIVO* GENE TRANSFER OF DIMETHYLARGININE DIMETHYLAMINOHYDROLASE -2

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Endothelial dysfunction plays an important role in the development of atherosclerosis. Asymmetric dimethylarginine (ADMA) is an independent risk factor for endothelial dysfunction. Dimethylarginine dimethylaminohydrolase (DDAH) is the major metabolic enzyme of ADMA, and DDAH2 is the predominant isoform in cardiovascular system. Both elevated endogenous ADMA and reduced DDAH activity are involved in the mechanisms of endothelial dysfunction in atherosclerosis. This study was designed to investigate whether *ex vivo* gene transfer of DDAH to atherosclerotic vessels could improve endothelial dysfunction induced by hypercholesterolemia. The recombinant adenovirus encoding the human DDAH2 gene driven by a cytomegalovirus (CMV) promoter was constructed, and human DDAH2 gene was transferred to thoracic aortic rings from hypercholesterolemic rabbits by infection with recombinant adenoviral Ad5CMVhDDAH2. Vascular DDAH2 transcription and activity, serum ADMA concentration and endothelium-dependent relaxation of aortic rings were determined in high fat diet fed rabbits. Results showed that the endothelium-dependent relaxation response to acetycholine was significantly impaired while serum ADMA levels were markedly elevated in atherosclerotic rabbits compared to control rabbits. Both DDAH2 transcription and DDAH activity were distinctly suppressed in aortas of atherosclerotic rabbits. *Ex vivo* gene transfer of hDDAH2 to atherosclerotic rabbit aortas not only increased the functional expression of hDDAH2 gene resulting in the enhancement of vascular DDAH activity but also markedly improved the impaired endothelium-dependent relaxation in atherosclerotic arteries. These results suggest that suppression of DDAH2 gene expression and vascular DDAH activity contribute to the endothelial dysfunction of atherosclerotic rabbit. *Ex vivo* gene transferring of DDAH2 can normalize the endothelial dysfunction and inhibition of DDAH activity, indicating that targeted DDAH2 gene transferring to blood vessel may be a novel approach for the treatment of endothelial dysfunction in atherosclerosis. This study was supported by grant from the Natural Science Research Foundation of China (30271507).

EFFECTS OF (-)-EPIGALLOCATECHIN-3-GALLATE ON THE DEVELOPMENT OF ATHEROSCLEROSIS AND TOLL-LIKE RECEPTOR 4-NUCLEAR FACTOR KAPPA B SIGNALING PATHWAYS IN apoE-/- MICE

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(-)-Epigallocatechin-3-gallate (EGCG), a flavonoid found in green tea, is known to inhibit NF-kB activation induced by many pro-inflammatory stimuli. Previous studies showed that Toll-like receptor 4- nuclear factor kappa B (TLR4/NF-κB) was involved in atherogenesis. Although EGCG has anti-atherosclerosis (AS) effects in apoE-/- mice, relatively a little is known about the precise mechanisms of the inhibitory effects of EGCG on AS. In the present study, we attempted to identify whether EGCG can prevent the development of atherosclerosis in apoE-/- mice by inhibiting TLR4/NF-κB signaling pathways. Sixty male apoE-/- mice (5 wk old) were randomly divided into four groups: control group, EGCG group, high-fat diet group and high-fat diet+ EGCG group. EGCG (10 mg/kg) was injected intraperitoneally every day until death (24th W). Areas of aorta atherosclerosis were measured by oil red O staining. Western blotting and immunohistochemistry were used to detect the change of expression of TLR4 and NF-κB proteins in mouse aorta. Serum concentrations of IL-1β, TNF-α, sICAM-1and MCP-1 were determined by enzyme-linked immunoabstorbent assay (ELISA). Results showed that in EGCG group and high-fat diet + EGCG group, EGCG reduced areas of aorta atherosclerosis by 78 % and 63 %, respectively, compared with high-fat diet group ($P < 0.01$), and expressions of TLR4 and NF-κB (p65) were obviously higher in aorta atherosclerosis lesions than that of other groups ($P < 0.01$). Compared with high-diet group, the serum concentrations of IL-1β, TNF-α, sICAM-1 and MCP-1 were markedly decreased in groups treated with EGCG. These results suggest that EGCG could inhibit the development of atherosclerosis in apoE-/- mice effectively, which possibly is correlated with the down-regulated expressions of TLR4, NF-κB, IL-1β, TNF-α, sICAM-1 and MCP-1 proteins.

PAMAM DENDRIMERS DELIVERED shRNA STRONGLY INHIBITS CTGF EXPRESSION OF H9C2 RAT EMBRYONIC CARDIOMYOCYTES

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Connective tissue growth factor (CTGF) is involved in many cellular processes underlying fibrosis such as cell proliferation, adhesion, and migration. It is regarded as a potent profibrotic factor. It is known that CTGF is upregulated by Ang II in rat cardiac myocyte, however, a recent report has revealed that some hypertrophic agonists upregulated CTGF in neonatal rat cardiac myocytes but had no effect in cardiac non-myocytes. In this study, the short hairpin RNA (shRNA) was used to investigate whether CTGF is necessary for the hypertrophy of cardiac myocyte. CTGF specific shRNA expression vectors were constructed and introduced by polyamidoamine (PAMAM) dendrimer G9 to H9C2 rat embryonic cardiomyocytes stimulated with 10-6 mol/ L Ang II. The proliferation of cardiac myocytes was assessed by MTT assay. The mRNA and protein expression of CTGF and fibronectin were detected by real time PCR and Western blot, respectively. The expression of CTGF and fibronectin were upregulated significantly in H9C2 stimulated with 10-6 mol/ L Ang II. The MTT value, CTGF and fibronectin expression in PAMAM-mediated CTGF shRNA groups were obviously downregulated compared to the Ang II group and missense shRNA group ($P < 0.05$). Our results suggest that PAMAM-mediated CTGF shRNA could efficiently decrease CTGF and fibronectin upregulation caused by Ang II, which may be a novel method for the treatment of cardiac fibrosis.

CARDIOPROTECTION BY POSTCONDITIONING IS MEDIATED BY CALCITONIN GENE-RELATED PEPTIDE IN ISOLATED RAT HEARTS

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Previous studies have demonstrated that endogenous calcitonin gene-related peptide (CGRP) plays an important role in mediation of ischemic preconditioning. Postconditioning, a novel cardioprotective strategy, was afforded by transient and repeat insult and reperfusion in post-ischemia phrase. Isolated male rat hearts were subject to 60 min of left coronary artery occlusion and followed by 60 min of reperfusion. Postconditioning was induced by three cycles of 1-min ischemia and 1-min reperfusion after ischemia-reperfusion. The cardiac function was recorded constantly during the test. Creatine kinase (CK) and CGRP release from coronary aorta and infarct area were determined. Ischemia-reperfusion caused a significant decrease in cardiac function and a significant increase in CK release and infarct size. Postconditioning produced a marked improvement of cardiac function and decreased CK release and infarct size, concomitantly with an increase in the release of CGRP release in coronary effluent, and the cardioprotection afforded by postconditioning was abolished by CGRP 8-37 (10^{-7} M), a selective CGRP receptor antagonist, or pretreatment with capsaicin (50 mg/kg, s.c.), which depletes transmitters in sensory nerves. Exogenous CGRP (5×10^{-9} M) administration of CGRP reappeared postconditioning like cardioprotection in the rats pretreated with capsaicin. In summary, these results suggest that the protective effects of ischemic postconditioning are related to stimulation of endogenous CGRP release in rat hearts.

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CHANGES OF ENDOTHELIN-1 EXPRESSION IN CEREBRAL BASILAR ARTERY OF SCALD RATS

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The purpose of the present study was to investigate the influences of scald to endothelin-1 (ET-1) expression on cerebral vessels in rats and to further elucidate the relationship between changes of ET-1 expression and scald-induced cerebral vascular remodeling resulting cerebral vasospasm. The ET-1 levels were detected in cerebral basilar artery by the method of radioimmunoassay. Expression of ET-1 and its mRNA were determined by western blotting and reverse transcription polymerase chain reaction(RT-PCR), respectively. Changes of cerebral basilar artery histological structures were observed and its diameter was measured by angiography. The results showed that the ET-1 level at scald 3 hours was enhanced, higher than that of the control group, being 32.55 ± 10.56 and 13.51 ± 5.65, respectively, and reached a peak at 12 hours (72.05 ± 8.71). The expression of ET-1 at 6 and 12 hours were 1.68 ± 0.42 and 2.67 ± 0.38 times that of the control group, respectively. The expression of ET-1 mRNA was also enhanced. At scald 6 hours group, the level reached a peak (1.204 ± 0.106), being about 5 times that of the control group (0.252 ± 0.12) and there were significant differences ($P < 0.05$) between at scald 6 hours group and control group. Coincidently, Vascular remodeling was observed in cerebral basilar artery of rat after scald, and the diameter of cerebral basilar artery was decreased at scald 6 h group compared with control group rats, especially at scald 12 h group obviously. We speculate scald may cause an increase of ET-1 in cerebral basilar artery, and elevated ET-1 may promote the vascular remodeling of basilar artery in scald rats. It suggested that ET-1 might play an important role in the pathogenesis of scald-induced cerebral vasospasm and cerebral circulation disorder.

PERIPHERAL THERMAL INJURY CAUSES BLOOD–BRAIN BARRIER DYSFUNCTION AND ZONULA OCCLUDENS-1 EXPRESSION CHANGES IN RAT

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Mortality after serious systemic thermal injury may be linked to significant increases in cerebral vascular permeability and edema due to blood-brain barrier (BBB) disruption. Zonula occludens-1 (ZO-1), a protein of the tight junctions (TJ), lines the cytoplasmic face of intact TJ. The current study investigated whether disruption of BBB in a rat thermal injury model was associated with ZO-1 expression and activity. The experimental Sprague-Dawley rat models with severe scald (30 % TBSAIII degree) were established. In this research, healthy Sprague-Dawley rats were separated into two groups: normal control and scald group which were divided into five post -scald groups: 3 h, 6 h, 12 h, 24 h and 48 h. Changes in BBB permeability were determined by detection of Evans blue (EB) content in rats' brain with chemical method. Brain edema was detected by calculating water content. Furthermore, in order to explore the molecular mechanism of BBB disruption in rats' brain after severe scalds, the expression levels of gene and protein of ZO-1were analyzed by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and western-blot analysis, respectively. Expression of brain ZO-1 mRNA was significantly decreased 3 h through 48 h after burn. Expression of brain ZO-1 protein was significantly decreased at 24 h and 48 h post thermal injury, however ZO-1 expression changes were not marked at 3 h, 6 h and 12 h post thermal injury. Water content and Evan's blue dye of brain were significantly increased beginning at 3 h and reached significant levels between 6 h and 24 h after thermal injury. On the other hand, ZO-1 protein levels continued to decrease significantly through 48 h. The decreases in ZO-1mRNA expression and protein were associated with increased BBB permeability following thermal injury, indicating ZO-1 may contribute to observed cerebral edema in peripheral thermal injury.

PUERARIN INHIBITS HIGH GLUCOSE-INDUCED INJURY THROUGH UPREGULATION OF HO-1 EXPRESSION IN BLOOD VESSELS

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To investigate the effect and mechanism of puerarin on high glucose induced injury in blood vessels. The thoracic aortic rings of male Sprague-Dawley rats were mounted on a bath system. Isometric contractions of aortic rings were measured. Human umbilical vein endothelial cells (HUVECs) were exposed to puerarin for 0~12 h. The expression of HO-1 was evaluated by western blotting analysis. After incubated with high glucose (44mmol/L) for 2 h, the relaxation responses to ACh decreased in aortic rings of rat. Incubation with high glucose for 4 h also induced decrease in contraction responses to phenylephrine (PE). Puerarin (10^{-9} - 10^{-8} mol/L) prevented the high glucose induced decrease in contraction responses to PE and relaxation responses to ACh. Pretreatment of heme oxygenase-1 (HO-1) inhibitor ZnPPIX (1 μmol/L) abolished the protection of puerarin in aortic rings. HO-1 activity of aorta increased after the treatment of puerarin. MTT analysis revealed a time-dependent decrease of cell viability in HUVECs treated with high glucose (30 mmol/L). Puerarin inhibited the high glucose-induced cell death which was abolished by ZnPPIX. Puerarin also induced overexpression of HO-1 expression in HUVECs. These data suggest that puerarin can protect blood vessels against high glucose-induced dysfunction and cell death likely through upregulation of HO-1 protein expression. This work was supported by the National Natural Science Foundation of China (No. 30400094)

EFFECTS OF PROBUCOL ON ATHEROSCLEROTIC LESIONS AND SCAVENGER RECEPTOR CLASS B TYPE I EXPRESSION IN MICE

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Probucol or its metabolites can increase the selective uptake of cholesterol ester which may depend on the scavenger receptor class B type I (SR-BI). In this study, twenty male C57 mice and twenty apo E knockout ($apoE^{-/-}$) mice were randomly divided into two groups ($n = 10$, each group), respectively. All mice were fed normal diet enriched with 15 % lard and 0.25 % cholesterol for 12 weeks, and 0.5 % probucol to diet was added for the intervention groups. We investigated the changes of blood lipid level, atherosclerotic lesions and expression of SR-BI and peroxisome proliferator-activated receptor gama (PPARγ) on $apoE^{-/-}$ mice and C57BL/6 J mice fed with high fat and high cholesterol by probucol. We found that the levels of serum lipids were markedly lowered in mice fed with probucol ($P < 0.05$), and this lowering was more significant in C57 mice than in $apoE^{-/-}$ mice ($P < 0.05$). The group fed with probucol had less aorta lesion area than controls in $apoE^{-/-}$ mice ($P < 0.05$). Probucol decreased the lipid content and upregulated the expressions of SR-BI and PPARγ in the mouse livers of both C57 and $apoE^{-/-}$ mice ($P < 0.05$). The results suggest that probucol could decrease the levels of serum lipids and reduce aorta lesion in mice fed with HFHC diet. The lipid lowering and antiatherosclerotic effections may be associated with upregulation of SR-BI and PPARγ expression in livers.

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INVOLVEMENT OF ENDOTHELIAL DDAH/ADMA PATHWAY IN NITROGLYCERIN TOLERANCE: ROLE OF ALDH-2

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Nitroglycerin (GTN) tolerance is closely related to oxidative stress induced decrease in activity of mitochondrial isoform of aldehyde dehydrogenase (ALDH-2), and prolonged GTN treatment causes endothelial dysfunction. Asymmetric dimethylarginine (ADMA), a major endogenous NO synthase (NOS) inhibitor, could inhibit NO production and induce oxidative stress in endothelial cells. ADMA and its major hydrolase dimethylarginine dimethylaminohydrolase (DDAH) have recently been thought of as a novel regulatory system of endothelium function. The aim of the present study was to determine whether the DDAH/ADMA pathway is involved in the development of GTN tolerance in endothelial cells. Tolerance reflected by the decrease in cyclic GMP (cGMP) production was induced by exposure of the human umbical vein endothelial cells (HUVECs) to GTN (10 μM) for 16 h. While the treatment increased reactive oxygen species (ROS) production/malondialdehyde (MDA) concentration and decreased ALDH-2 activity as well as cGMP production, it markedly increased the level of ADMA in cultured medium and decreased DDAH activity in endothelial cells. Exogenous ADMA significantly enhanced the ROS production/MDA concentration and inhibited ALDH-2 activity, and overexpression of DDAH2 could significantly suppress GTN-induced oxidative stress and inhibit ALDH-2 activity, which was also attenuated by L-arginine. Therefore, our present results for the first time suggest that the endothelial DDAH/ADMA pathway may play an important role for the development/maintenance of GTN tolerance.

TOLL-LIKE RECEPTOR 4 MEDIATES PRO-INFLAMMATORY EFFECTS OF VISFATIN ON ENDOTHELIAL CELLS

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It is well-known that toll-like receptor 4 (TLR4) plays a key role in vascular inflammation, which contributes to the pathogenesis of cardiovascular diseases. Visfatin, a novel adipocytokine, was recently reported to induce inflammatory reactions and damage endothelial function. The aim of the present study was to investigate whether TLR4 mediates the pro-inflammatory effects of visfatin on vascular endothelial cells. In cultured human umbilical vein endothelial cells (HUVECs), incubation with visfatin (0.1 − 10 nM) for 24 h significantly up-regulated the mRNA expression of tumor necrosis factor-α (TNF-α), a key inflammatory factor, and increased its level in the cultured medium in a concentration-dependent manner. As shown by the results of electrophoretic mobility shift assay, visfatin concentration-dependently activated nuclear factor-κB (NF-κB), and NF-κB inhibitor PDTC could markedly attenuate TNF-α production induced by visfatin in HUVECs. In addition, visfatin markedly upregulated the expressions of both mRNA and protein of TLR-4 in endothelial cells as determined by real time PCR and western blot, respectively. Co-incubation with anti-TLR-4 mAb could completely block visfatin-induced activation of NF-κB and the elevation of TNF-α production. In summary, the present results suggest that visfatin could induce inflammatory cytokine TNF-α production in endothelial cells via TLR4-mediated and NF-κB-dependent signaling pathway. Our data provide a novel mechanism of pro-inflammatory effect of visfatin.

FACTOR ANALYSIS OF EFFECTS ON ENDOTHELIAL CELLS ABSORBING LDL EXPOSED TO SHEAR STRESS

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In atherogenesis, low-density lipoprotein (LDL) plays a very important role in its happening and promoting. With the aim of finding out the factors effects on endothelial cells absorbing LDL, we studied that the factors that may affect the absorption of LDL changed. We used 2D-flat plate flow chamber in the study. Synchronization cultural HUVECs that passage to third generation were preferred. Purifying LDL from fresh human plasma using routine methods, we marked LDL as done by Z.F. Stephan. Time of perfusion was 2 hours, and flow rate, pressure and DiI-LDL concentration (which we called Co) were the parameters of perfusion. We kept the two factors of them invariably, observing the effects on the absorption of ^{125}I-LDL in endothelial cells caused by the other factor. When we kept the DiI-LDL concentration as 10 μg/ml (C_0) and pressure as normal, absorption of ^{125}I-LDL in endothelial cells had decreased 35 %, 63 % and 78 % as shear stresses were 15, 30 and 45 dynes/cm^2 correspondingly contrast to static condition. When we kept the DiI-LDL concentration as 10 μg/ml (C_0) and flow rate invariably, absorption of ^{125}I-LDL in endothelial cells had increased 85.4 % and 120 % as pressures were 100 and 200 mmHg correspondingly contrast to zero pressure. When we kept the pressure and flow rate invariably, absorption of ^{125}I-LDL in endothelial cells had increased obviously 43.8 % and 114 % as concentrations were 100 and 150 μg/ml correspondingly contrast to concentration of 50 μg/ml. The difference was extremely significant ($p < 0.01$). Normal absorption of LDL in endothelial cells was in inverse proportion to shear stress, and was proportional to the pressure and LDL concentration. These findings contribute to analyze the dominant factor in deposition of atheromatous lipids in the three factors.
This study was supported by grants from the MOE of China (No.104158, No. B06023), the MOST of China (2004DFA06400), as well as CSTC (2006AA5014-3).

ANALYSIS OF NEOINTIMAL HYPERPLASIA AFTER IMPLANTATION OF A MONOCLONAL ANTIBODY ELUTING STENT

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We set out to study the neointimal hyperplasia induced by the stents and the impact of monoclonal antibody to the platelet glycoprotein (GP) IIIa receptor on the migration and hyperplasia of vascular smooth muscle cells (VSMC) by implanting this mAb eluting stents in rabbit iliac artery. Stent deployment conditions were less than 1.2:1 stent: artery ratio by quantitative coronary angiography (QCA). The stents mounted on a balloon catheter were positioned into the iliac artery from the femoral artery through a 5F sheath introducer under fluoroscopy using a standard micro-guide wire. The balloon was inflated at a pressure of 10 atm for 30s, deflated and then slowly withdrawn, leaving the stent in place. Animals received no anticoagulant or antiplatelet therapy were killed 4 weeks after operation. The segments of stented vessel were polymethyl methacrylate (PMMA) embedded before further sectioning and stained with hematoxylin-eosin. A computerized morphometry system consisting of a microscope fitted with a digital develop image workstation was used to

measure the images. All the data were obtained from three replications for each arterial section. Four animals were examined for each group. Neointimal formation was observed in all stented vessel segments. Neointimal areas were 0.70 ± 0.12 mm^2 for arteries with mAb eluting stents and 1.92 ± 0.21 mm^2 for vessels with control stents ($P < 0.01$). The restenosis rates of these two groups were 9.35 ± 1.08 and 28.37 ± 1.86, respectively ($P < 0.01$). These promising results indicate that the monoclonal platelet GP IIIa receptor antibody significantly reduced neointimal hyperplasia and improved arterial patency rates in a rabbit iliac artery model of stent thrombosis. This study was supported by grants from the MOST of China (2004DFA06400), Chongqing Municipality of China (CSTC2006AA5014-3, DRC2005-1006) as well as the MOE of China (B06023).

LIVER X RECEPTOR AGONISTS UP-REGULATE NIEMANN-PICK TYPE C1 AND NIEMANN-PICK TYPE C2 EXPRESSION IN apoE$^{-/-}$ MICE

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In the present study, we investigated the effect of T0901317, a kind of LXR agonists, on Niemann-Pick Type C1 (NPC1) and Niemann-Pick Type C2 (NPC2) expression in apoE$^{-/-}$ mice, and discussed the roles of NPC1 and NPC2 in the process of atherosclerotic lesion. 52 male apoE$^{-/-}$ mice were randomly divided into four groups: a. base group ($n = 10$); b. control group ($n = 14$); c. LXR agonist treatment group ($n = 14$); d. LXR agonist prevention group ($n = 14$). All mice were fed a high-fat/high-cholesterol diet; at the same time, the base group was treated with vehicle for 8 weeks; the control group was treated with vehicle for 14 weeks; the LXR agonist treatment group was treated with vehicle in the former 8 weeks and treated with T0901317 in the latter 6 weeks; the LXR agonist prevention group was treated with LXR agonist for 14 weeks. NPC1 and NPC2 level were determined by real-time RT-PCR, western blot and immunohistochemistry. Plasma lipid contents were determined by full-automatic biochemical analyser. Atherosclerotic lesion in the aorta was detected by oil red O staining method and sudan IVstaining method. Lipid accumulation in the liver was detected by oil red O staining method. Plasma total cholesterol (TC), triglyceride (TG), Low density lipoprotein-cholesterol (LDL-C), High density lipoprotein-cholesterol (HDL-C) and Apolipoprotein AI (apoAI) concentrations were markedly increased in LXR agonist treatment and prevention groups compared with control group ($p < 0.05$); atherosclerotic plaques and lipid stripes in aorta of LXR agonist treatment and prevention groups were markedly less than control group ($p < 0.05$); NPC1 and NPC2 expression of LXR agonist treatment and prevention groups in liver, aorta and small intestine were up-regulated when compared with control group ($p < 0.05$). Our results suggest that LXR agonist could reduce the development of atherosclerosis and up-regulate the expression of NPC1 and NPC2 in liver, aorta and small intestine in apoE$^{-/-}$ mice fed with high-fat/high-cholesterol diet. The authors gratefully acknowledge the financial support from the National Natural Sciences Foundation of China (30470720) and Hunan Provincial Natural Sciences Foundation of China (06jj5058).

P2X2/3 RECEPTOR OF NODOSE GANGLIA NEURON INVOLVED IN MYOCARDIAL ISCHEMIA INJURY

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Extracellular ATP acts on purinergic receptors as a potent agonist for a variety of different cell types, including cardiomyocytes and nodose ganglia. ATP can be released from endothelial cells, ischemic myocardium or sympathetic nerve endings. ATP released from different cell types is implicated in the initiation of pain by acting on purinoceptors on sensory terminals. ATP is known to depolarize DRG neurons via P2X3 and P2X2/3 receptor activation. Therefore, P2X2/3 receptor is involved in nociceptive transmission. This study was aimed to explore the role of P2X2/3 receptor in the myocardial ischemic injury and nociceptive transmission via nodose ganglia. The content of ATP was measured by HPLC. The expression of P2X2 and P2X3 protein were analyzed by western blotting. Myocardial ischemia enhanced the ATP content in rat heart and nodose ganglia. ATP content was decreased in the myocardial ischemic rats treated with P2X2/3 receptor antagonist A-317491. The myocardial ischemic injury increased the expression of P2X2 and P2X3 protein in nodose ganglia. In rats treated with A-317491, the expression of P2X2 and P2X3 protein in nodose ganglia was reduced. According to these results, we conclude that P2X2/3 receptor is involved in nociceptive transmission of myocardial ischemic injury. This work was supported by grant (No. 30460040 and No. 30660048) from National Natural Science Foundation of China and grant (No. 2007-60) from the Educational Department of Jiangxi Province.

A COMPARATIVE STUDY OF THE EFFECT OF EPCs AND MSCs DERIVED FROM THE RAT BONE MARROW ON ATHEROSCLEROSIS

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Atherosclerosis (AS) is a disorder with endothelial dysfunction. Some researches showed that both endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs) derived from the bone marrow could differentiate into endothelial cells (ECs) to replace dysfunctional endothelium and may potentially limit atherosclerotic lesion formation. We studied and compared the effect of EPCs and MSCs derived from the rat bone marrow on AS. When AS model of rats was made successfully by high fat diet feeding for three months, EPCs and MSCs were transduced by recombinant adeno-associated virus (rAAV)-mediated green fluorescent protein (GFP) gene (rAAV-GFP) in order to display the location of the cells. The transduced cells were injected via the tail vein to two groups of recipient rats respectively. And the model control group received normal saline. Two months after the injection, the level of serum lipid was tested. The change of aorta histology was observed by HE staining and GFP-labeled cells by frozen section. The endothelial nitric oxide synthase (eNOS) and intercellular adhesion molecule 1 (ICAM-1) gene expression in artery vessel were detected by reverse transcription polymerase chain reaction (RT-PCR). The results showed that the labeled cells could be observed in the section of artery vessel in two cell- treated groups. Compared with the model control group, two cell- treated groups had an obvious downward tendency in the levels of serum lipid and the lipid deposit in aortic endothelium was less. The ICAM-1 expression levels of the two cell-treated groups were obviously lower, while eNOS expression levels were markedly higher than the model control group. Our results suggest no significant difference in the therapy of AS between EPC-treated group and MSC-treated group. These studies provided the further support for potential applications of EPCs and MSCs derived from the bone marrow in the therapy of AS.

CXCR4 EXPRESSION IN ATHEROSCLEROTIC LESIONS INDUCED BY LOW SHEAR STRESS

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The stromal cell-derived factor-1 receptor CXCR4 has been proven to involve in several problematic diseases, including AIDS, cancer cell metastasis, leukemia cell progression and rheumatoid arthritis (RA), but there are few reports on atherosclerotic induced by low shear stress. To test the role of CXCR4 in atherosclerosis, we set up an animal model with local low shear stress which was modeled by a ring attached to the wall of common carotid artery. Rabbits were fed with normal diet for 4 weeks. Numerical simulation was performed to

obtain the distribution of flow field and wall shear stress. Rabbits were sacrificed and relative arteries were obtained at 4 weeks. Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were determined by commercially enzymatic methods. The pathological slides were prepared and stained for optical microscopic observation. The content of cholesterol in arteries wall was assayed with High Performance Liquid Chromatography (HPLC). Immunohistochemistry staining was used to detect the expression in atherosclerotic lesions. Results showed that there was a low shear stress region (0–0.3 Pa) at distal of ring. At the end of 4 weeks, plasma total cholesterol, HDL cholesterol and triglyceride had no obviously changed in rabbits. HE staining showed that there were obvious atherosclerosis lesions in low shear stress field. There was obvious lipid deposition in atherosclerosis lesions. CXCR4 was highly expressed in atherosclerotic plaque without expression in control. These data suggest that CXCR4 may play important roles in atherosclerosis formation and development induced by low shear stress. The authors gratefully acknowledge the financial support from the National Natural Sciences Foundation of China (30700325) and Hunan Provincial Natural Sciences Foundation of China (06jj50051).

DIFFERENTIAL PROTEOMIC STUDY OF INFLUENCE ON ANGIOTENSIN II IN MONOCYTES

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Accumulating clinical and laboratory data have demonstrated that renin-angiotensin system (RAS), the main regulator of cardiovascular and renal function, is closely relevant to cardiovascular and renal diseases. Historically, AngII was only seen as a regulatory hormone that governs fluid and electrolyte balance and arterial pressure. With the discovery of RAS within various tissues, it has been gradually accepted that local tissue AngII could activate the cells regulating the expression of many substances, including growth factors, cytokines, chemokines, and adhesion molecules, which are involved in cell growth/apoptosis, fibrosis, inflammation, coagulation and fibrinolysis. Although tremendous progresses have been made in studies of RAS, it is clear that RAS is far more complex than that we can imagine and we are just beginning to understand its role in disease processes at tissue level. The proteomics approach provided us a new tool to study RAS. Here proteomic tools were used to analyze the influence of AngII in monocytes. The stcomparative two-dimensional gel electrophoresis (2-DE) technology was performed to separate the total protein of U937 cells. Then, PDQuest software was used to analyze 2-DE images, and the 23 differential expression proteins were identified by peptide mass fingerprint (PMF) based on Matrix-assisted laser desorption/ionization time of flight mass spectrometry. The differentially expressed proteins could be divided into seven main groups based on their functions: chaperones, glucose metabolic enzymes, protein metabolic enzymes, proteins relative to signal transduction, proteins related to cellular structure, proteins involved in detoxification and proteins related to DNA replication.

ANIMAL MODEL FOR ESTABLISHMENT OF THE CONTROLLABLE LDL CONCENTRATION POLARIZATION IN RABBITS

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Because of penetration flow, lipid molecules such as LDL will accumulate at blood vessel wall interface and generate a phenomenon called concentration polarization in the area of engineering. Furthermore, LDL concentration at blood vessel wall interface will decrease with shear stress increases. LDL concentration heightens locally at those regions where shear stress is low. It is a very important factor for inducing atherosclerosis localization and the generation of arterial stenosis. We had confirmed that LDL concentration polarization existed in the three different stenosis degrees (30 %, 40 %, and 50 %) through numerical simulation and in vitro model system in our former study. This study was aimed at establishing a controllable LDL concentration polarization animal model to investigate the different effects of local hemodynamic change and LDL concentration polarization on atherogenesis *in vivo*, and for the further analysis on the mechanism of it. Sixty New Zealand white rabbits (weight 1.8–2.2 kg) were randomly divided into surgical group and control group. We selected the same different stenosis degree (30 %, 40 %, and 50 %), which caused by gel-silica pipe loop ligature operation on left common carotid artery in rabbits. Then three types of forages which contain different concentration of cholesterol (1 %, 2 % and 3 %) were used to feed surgical group for 8 weeks, which could form controllable LDL concentration in the blood vessel. And then we detected hemodynamics parameters of the bilateralis common carotid artery with color Doppler flow imaging (CDFI), and analyzed data with statistics software. An animal experiment model with controllable LDL concentration polarization degree was established. This study was supported by grants from the Chinese MOE (No.104158, No. B06023) and the MOST of China (2004DFA06400).

ROLE OF LDL CONCENTRATION POLARIZATION IN THE ATHEROGENESIS BY NUMERICAL SIMULATION AND ANIMAL EXPERIMENT

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To confirm the hypothesis that concentration polarization of atherogenic lipids, which may occur in the arterial system and play an important role in the localization of atherosclerosis, we tested the role of LDL concentration polarization in the atherogenesis by numerical simulation and animal experiment. We simulated and measured the distribution of flow fluid and shear stress at the axifugal part of local stenosis vascular *in vitro.* To establish a 3D-stenosis vascular with 30 %, 40 %, 50 % stenosis, angioaccess was 0.15 cm and length was 5 cm. We gave two different flow with RE of 250 and 500 correspondingly to each stenosis. Numerical solution was implemented by FLUENT 6.2. The distribution of flow at axifugal part was determined by PIV system, and results were analyzed by image manipulation software. Then we obtained lucidification vascular as routine and got three different vascular stenosis, 30%, 40%, 50%, to compare the concentration of LDL by laser scan confocal technique. We found that the wall concentration of LDL was largest in the round tube with 40% narrow at the same velocity in numerical stimulation. To this end, we used the rabbit carotid artery stenosis model to carry out animal experiment to test the role of LDL concentration polarization on the atherogenesis. A cylinder with a round lumen was placed around the carotid artery, inducing a region of low shear stress and oscillatory shear stress down-stream of the device. Treatment group was fed with high fat/cholesterol diet for 4 weeks, and then was sacrificed and carotid artery was obtained through dissection. Results were analyzed with several routine staining methods. The animal experiment revealed that atherogenic plaque was detected at the distal of stenosis artery where concentration polarization occurred in a thin layer close to the luminal surface of the artery. This study was supported by grants from the MOE of China (No.104158, No. B06023) and the MOST of China (2004DFA06400).

EFFECTS OF PROBUCOL ON PARAOXONASE 1 EXPRESSION AND OXIDATIVE STRESS IN HYPERLIPIDEMIC MICE

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Probucol is a hypolipidemic drug that can regulate the blood lipid levels, improve the function of endothelial cell, and prevent the formation of foam cells

and lipid oxidation. In the present study, we aimed to investigate the effects of probucol on high fat-high cholesterol (HFHC) diet-induced hyperlipidemia and atherosclerotic lesions, and the effects of oxidative stress in mice. Thirty male C57BL/6 J mice and thirty male apo E knockout ($apoE^{-/-}$) mice were randomly divided into three groups (control group, HFHC group and HFHC-probucol group; n = 10, each group). The animals in control group were fed normal diet. HFHC mice were fed a high fat/high cholesterol diet (15% lard and 0.25% cholesterol). HFHC-probucol group was supplemented with 0.5% probucol in HFHC diet. We examined the correlative index at the end of 12 weeks. The levels of serum lipid were increased in HFHC groups compared with the control group ($P < 0.05$), and were significantly lowered in probucol treatment group compared with respective HFHC group ($P < 0.05$). Serious lesions of the aorta in C57BL/6 J mice were not observed. HFHC and probucol treatment resulted in a significant reduction of aorta lesion area in $apoE^{-/-}$ mice compared with HFHC group. Probucol contributed to a significant decrease of lipid content of the liver tissue in both C57BL/6 J and $apoE^{-/-}$ mice as showed by hematoxylin-eosin staining. Furthermore, compared with respective control groups, the serum (MDA) contents, oxidized low density lipioprotein (ox-LDL) contents, C-reactive protein (CRP) activity, and glutathione S-transferase (GST) activity were increased, and the levels of total antioxidative capacity (T-AOC), total superoxide dismutase (T-SOD) activity, and paraoxonase 1 (PON1) activity were decreased in HFHC-treatment mice ($P < 0.05$), and these changes were attenuated or reversed by probucol treatment ($P < 0.05$). Probucol improved PON1 activities and upregulated PON1, CRP mRNA expression in liver ($P < 0.05$). These results show that probucol contributed significantly to prevent the oxidative stress, upregulate the PON1 expressions in the livers and increase the serum PON1 activity in the high fat-high cholesterol diet-induced hyperlipidemic mice, suggesting the potential therapeutic effects of probucol in atherosclerosis.

EFFECTS OF POLYMORPHISM OF β1-RECEPTOR AND CYP2D6 ON THERAPEUTIC EFFECTS OF METOPROLOL

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Hypertension is one of the most common cardiovascular diseases in the world, and the major risk factor of heart disease, stroke and end-stage renal diseases. Metoprolol is a lipophilic β1-selective adrenergic receptor antagonist for the treatment of hypertension. It is primarily metabolized by cytochromosome P450 (CYP) 2D6 (CYP2D6) gene. The β1-adrenoreceptor is known to mediate many of the regulatory effects of endogenous catecholamines on the key physiological events in heart, kidney and adipocytes. However, there is still little study about association between β1-adenoreceptor, CYP2D6 polymorphism and blood pressure. Here we reported a prospective, observational clinical study on the combinational influences of the CYP2D6 and β1-adenoreceptor polymorphisms on therapeutic effects of metoprolol in 403 outpatients with essential hypertension. It was observed that the same dosages of metoprolol could reach different therapeutic effects in patients with different CYP2D6 and β1-adenoreceptor polymorphism. Meanwhile, different dosages of metoprolol could achieve the same therapeutic effects in patients with different CYP2D6 and β1-adenoreceptor polymorphism. It was demonstrated by our study that combination of polymorphisms of CYP2D6 and β1-adenoreceptor might be used as guidance for treatment for Chinese Han hypertensive patients by β1-adrenoreceptor antagonists.

THE INHIBITORY EFFECT OF REINIOSIDE C ON TNF-α PRODUCTION INDUCED BY ASYMMETRIC DIMETHYLARGININE VIA ROS/NF-κB DEPENDENT PATHWAY IN HUMAN MONOCYTIC CELLS

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Many studies have demonstrated that development of atherosclerosis is associated with inflammation. Asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase (NOS) inhibitor, has been implicated in vascular inflammation through induction of reactive oxygen species (ROS) and pro-inflammatory genes in endothelial cells. However, relatively few attentions have been paid to the effect of ADMA on monocytes, one of the important cells throughout all stages of atherosclerosis. The present study was to explore the mechanism of tumor necrosis factor-α (TNF-α) elevation induced by ADMA and studied the inhibitory effect of reinioside C, which is the main component extracted from *Polygala fallax* Hemsl. In ADMA-treated monocytes, the TNF-α levels, ROS production, and the activity NF-κB were detected. We found that ADMA (3−30 μM) time- and concentration-dependently increased TNF-α production, and that ADMA (30 μM) time-dependently increased intracellular ROS production and activated NF-κB activity, and these effects were inhibited by L-arg (NOS substrate) and PDTC (inhibitor of NF-κB). In the present study, we also found that reinioside C (1−10 μM) dose-dependently inhibited TNF-α production induced by ADMA (30 μM) in monocytes. Furthermore, reinioside C (1−10 μM) attenuated ADMA-induced generation of reactive oxygen species and activation of nuclear factor-κB (NF-κB) activity in monocytes in a dose-dependent manner, and these effects were also inhibited by L-arg (0.5 mM) and PDTC (100 μM). These data suggest that reinioside C could attenuate the increase of TNF-α induced by exogenous ADMA through inhibition ROS/NF-κB pathway in monocytes.

CHANGING IN ATRIOVENTRICULAR CONDUCTION IN MICE OVER-EXPRESSING Ca^{2+}-ACTIVITED K^+ CHANNELS

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Small conductance Ca^{2+}-activated K^+ (SK2) channels exist in most neurons and mediate the afterhyperpolarizations following action potential (AP). Evidences indicated that the presence of SK2 channel, a subtype of SK channels in cardiac myocytes, played a crucial role in cardiac AP profile and was involved in many physiological processes. Here, we further examined the effects of over-expression of SK2 channel in the atrioventricular node (AVN) in a transgenic mouse model using *in vivo* and *in vitro* electrophysiological studies. Electrocardiographic (EEG) recording showed that PR and RR intervals were shortening in the over-expression of SK2 channel mice. The spontaneous APs recorded from an isolated AVN exhibited a significant increase in the beating rate and a shortening of AP duration in the over-expression of SK2 mice compared with WT mice. Results from whole-cell patch-clamp techniques showed a significant increase in Ca^{2+}-activated K^+ current in AVN myocytes isolated from the transgenic mice compared with WT mice. Data from immunofluorescence confocal further showed that the small conductance Ca^{2+}-activiated K^+ channel was not only expressed in the working myocytes, and also in the AVN conduction system. Our data confirmed the predominant roles of SK2 channels in mice AVN.

OPENING OF MITOCHONDRIAL K_{ATP} CHANNEL IMPROVING LONG-TERM HYPOTHERMIC PRESERVATION HEART BY UPREGULATION OF HSP 70 PROTEIN

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The aim of this study was to investigate the mechanism of cardioprotective effects of diazoxide, a selective opener of mitochondrial ATP-sensitive potassium channel (mitoK_{ATP}) in preserved heart. The isolated rat heart Langendorff model was used. The hearts were stored in 4°C Celsior solution in the presence or absence of diazoxide for 3, 6 or 9 h followed by 60 min of reperfusion. Apoptotic cardiomyocytes were detected by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) method. The expression of heat shock protein 70 (HSP 70) was also evaluated by western blotting analysis. The results showed that the percentage of apoptotic cells increased in a time-dependent manner after hypothermic preservation. When compared with the 9 hours control group, diazoxide (15, 30 or 45 μmol/L) reduced the percentage of apoptotic cells and increased the expression of HSP 70 protein in rat hearts suffered from 9 hours of hypothermic preservation in a dose-dependent manner. These results indicate that diazoxide could alleviate rat myocardial injury induced by ischemia- reperfusion through upregulation of HSP 70 protein expression. (This work was supported by the National Natural Science Foundation of China (No.30470635)).

ELEVATED ASYMMETRIC DIMETHYLARGININE CONTRIBUTES TO THE HEPATIC MITOCHONDRIAL DYSFUNCTION OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Mitochondrial dysfunction is one of the characteristics in diabetes mellitus. Accumulating evidence showed that endogenous nitric oxide synthase (NOS) inhibitor asymmetric dimethylarginine (ADMA) played important roles in insulin resistance, diabetes and diabetic vascular complications. This study was to explore the roles of ADMA in the development of hepatic mitochondrial dysfunction in diabetic rat and its potential mechanisms. Diabetic model was induced by a single injection of streptozotocin (60 mg/kg) to male Sprague-Dawley rats. Serum ADMA levels were analysed by high performance liquid chromatography. Mitochondrial transmembrane potential, ATP content, succinate dehydrogenase and cytochrome C oxidase activities in diabetic rat liver and ADMA-treated hepatocytes were measured to evaluate mitochondrial function. Copy numbers of mitochondrial gene COX I and nuclear gene β-actin were used to reflect mitochondrial biogenesis. UCP2 and PGC-1α transcriptions, NOS and SOD activities, nitrite/nitrate and malondialdehyde contents were detected to explore the mechanisms of hepatic mitochondrial dysfunction induced by ADMA. Results showed that serum endogenous ADMA levels were elevated in accompany with impaired hepatic mitochondrial function as shown by the reductions of mitochondrial membrane potential, ATP production, succinate dehydrogenase and cytochrome C oxidase activities, and mitochondrial biogenesis in diabetic rats. Similar mitochondrial dysfunction was observed in cultured hepatocytes (H4IIE) after exposure to exogenous ADMA. Treatment with antioxidant PDTC not only improved mitochondrial function but also increased mitochondrial biogenesis. Further studies revealed that hepatic mitochondrial dysfunction induced by ADMA was associated with enhancements of UCP2 transcription and oxidative stress. These results suggest that the elevation of endogenous ADMA contributed to hepatic mitochondrial dysfunction in diabetic rats through increasing oxidative stress and upregulating UCP2 transcription. This study was supported by grant from the Natural Science Research Foundation of China (30271507).

MMP-2, 9 EXPRESSION ON ATHEROSCLEROTIC PLAQUE IN apoE OR LDLR KNOCKOUT MICE WITH AND WITHOUT CHOLESTEROL DIET

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Matrix metalloproteinases (MMPs) are an ever-expanding family of endopeptidases with common functional domains and mechanisms of action discovered because of their ability to degrade ECM components. MMP activity is thought to be regulated at multiple levels: the growth, destabilization, and eventual rupture of atherosclerotic lesions. Apolipoprotein E knockout ($ApoE^{-/-}$) and low-density lipoprotein receptor knockout (LDLR-/-) mice exhibit hypercholesterolemia and develop complex atherosclerotic lesions similar as human. Using these $ApoE^{-/-}$ and $LDLR^{-/-}$ mice model with and without cholesterol diet, C57BL/6 mice served as controls, we evaluated the role of MMP-2, 9 on atherosclerotic lesion. Histological and histochemical observations of atherosclerosis revealed significant correlation of MMP-2, 9 express with macrophage and smooth muscle cell. $ApoE^{-/-}$ and $LDLR^{-/-}$ mice with cholesterol diet exhibited cellular compositional changes indicative of an unstable plaque phenotype and had the high MMP-2, 9 express. Compared with these mice, $ApoE^{-/-}$ and $LDLR^{-/-}$ mice without cholesterol diet had reduced lesion size and increased smooth muscle cell and decreased macrophage content in the plaque, indicative of a stable plaque phenotype. These data demonstrate high cholesterol diet effect MMP-2, 9 express in macrophages and degrade vascular matrix directly involved in atherosclerotic plaque destabilization.

EFFECT OF NITRIC OXIDE SYNTHASE INHIBITORS ON HEMODYNAMIC PARAMETERS AND THORACIC AORTA TENSION IN SEPTIC SHOCK RATS

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The present study was to investigate the effect of nitric oxide synthase inhibitors on hemodynamic parameters and thoracic aorta tension in septic shock rats. We used cecal ligation and puncture (CLP) method to establish septic shock in rats, and nitric oxide synthase inhibitors (L-NAME, AMG or 7-NI) were injected after CLP. The carotid artery was cannulated and connected to a pressure transducer to determine mean arterial blood pressure (MABP). Ventricular dynamic parameters, including heart rate (HR), left ventricular developed pressure (LVDP), maximal rise/fall velocity of ventricular pressure (+/-dP/dtmax), were determined following intraventricular cannulation via the carotid artery. Isolated thoracic rings were mounted on the organ bath and the tension of the vessel was recorded. We found that (1) after treatment with L-NAME, AMG or 7-NI, the mortality decreased to 50.0 %, 37.5 %, and 42.1 %, respectively (65.2 % in septic shock rats); (2) the MABP in septic shock rats partly recovered after using the NOS inhibitors, and all ventricular dynamic parameters partly recovered after using the inhibitors; (3) the hyporeactivity of endothelium-denuded aortic rings to vasoconstrictors induced by septic shock was partly recovered by pretreatment with the inhibitors. However, only L-NAME or 7-NI could inhibit the decrease of vasoconstriction induced by septic shock in endothelium-intact aortic rings. The results indicate that the three nitric oxide synthase inhibitors had different improved effects on hemodynamic parameters and thoracic aorta tension in septic shock rats, which may be related to the specificity of the inhibitors.

STEM CELL RESEARCH AND INJURY REPAIR

A NOVEL ISOLATED SUBPOPULATION OF BONE MARROW STEM CELL

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Stem cell therapy sheds light on the cure of ischemic heart infarct. One of the key issues is to establish an adult stem cell line, which has high potent proliferation and cardiac differentiation. We have screened a novel clonogenic cells (AMCSCs) from adult rat bone marrow, which is negative for CD4, CD10, CD13, CD31, CD34, CD45, CD85, CD102, ICAM, VCAM, c-kit and Flk-1. The results suggest that AMCSC was not heamotopoietic stem cells, mesenchymal stem cells or endothelial progenitors, but a novel clone of adult stem cell. AMCSCs possessed strong potent to proliferation and could grow consecutively for 220 passages. After being treated with differentiation medium, cardiac differentiation and smooth muscle differentiation could be induced in AMCSCs.. The β-galactosidase staining for AMCSCs from the 22nd, 52nd, 104th, 129th, and 184th passages indicated that AMCSCs were negative for the aging marker β-galactosidase. In addition, after being cultured under low-serum condition, the proliferation capacity of AMCSCs declined. Furthermore, AMCSCs did not develop into tumors after being injected into nude mice. Our results suggest that AMCSCs could be a novel clone of adult stem cells, which had high potent in proliferation and was able to differentiate into cardiomyocyte and smooth muscle. We may be able to apply AMCSCs to conduct adult stem cell therapy for the ischemic heart infarct.

ROLE OF PROTO-ONCOGENE *C-SRC* IN THE PROLIFERATION OF RAT SPERMATOGONIAL STEM CELLS *IN VITRO*

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We aimed to study the role of proto-oncogene c-src in the proliferation of rat spermatogonial stem cells. The percoll discontinue density gradient centrifugation, followed by removal of contaminating somatic cells through adhesion to plastic dishes, was used to purify the spermatogonial stem cells. The special antibody of telomerase-reverse transcriptase (TERT) was used to identify the spermatogonial stem cells. The MTT was used to observe the viability of the spermatogonial stem cells *in vitro*. RT-PCR was utilized to observe the expression of c-src mRNA and western blot was used to observe the c-Src, p-STAT3 and p-ERK1/2 contents. The percentage of cells expressing TERT was 90.5 % after isolation of the rat spermatogonial stem cells. The proliferation inhibition rate of spermatogonial stem cells was 12.6% after being treated with 10 μmol/L antisense-src ODNs for 12 h ($P < 0.05$). The expression of c-src mRNA decreased significantly ($P < 0.05$). Compared with the control group, c-Src, p-STAT3 and p-ERK1/2 contents decreased 33.8% ($P < 0.01$), 45.3 % ($P < 0.01$) and 38.4 % ($P < 0.01$) respectively after transfected with antisense c-src ODNs. Our results show that the percoll discontinue density gradient centrifugation, followed by removal of contaminating somatic cells through adhesion to plastic dishes, was an efficiency method for isolation of rat spermatogonial stem cells. The immunocytochemistry of TERT could be used to identify spermatogonial stem cells. Proto-oncogene c-src could proliferate the spermatogonia stem cells and the effect of c-Src on spermatogonial stem cells may correlate with the p-STAT3 and p-ERK1/2 protein. This work was supported by the National Natural Science Foundation of China (No.30360032).

INDIVIDUAL FUNCTION DIFFERENCE OF Runx2 ISOFORMS IN BONE FORMATION BY RNA INFERENCE

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Runx2 is a key transcription factor in bone formation. There are two major isoforms of Runx2 in human, type I and type II, with different structures and spatiotemporal expressions. Human bone marrow mesenchymal stem cells (hBMSCs) are capable of differentiating into osteoblasts, so we aimed to determine the individual function of type I and type II in osteogenic differentiation by RNA inference in hBMSCs cultures. We set up an immortalized hBMSC line that stably expressing osteocalcin-luciferase (OC-Luc) gene. Then using retroviral gene transfer of transcription cassettes for anti-type I / type II / full Runx2 shRNAs, we established three individual hBMSC lines that stably silencing of type I or type II or full Runx2 expressions and observed time-course changes (4, 8, 12 and 16 days) of OC-Luc activity, ALP activity, calcium deposition, Runx2 isoforms mRNA and total protein levels. Interestingly we found that osteocalcin expressions were greatly increased in type I or full Runx2 knockdown groups, but was decreased in type II knockdown group. In the differentiation, full Runx2 knockdown group displayed the lowest ALP activity and calcium depositions, while type II knockdown group displayed the modest and type I knockdown group exhibited the highest. We also found that type II mRNA levels time-dependently increased in the differentiation and reached the maximal level at day 16, whereas type I mRNA levels displayed higher expressions at early stage. Additionally type I mRNAs was greatly enhanced when type II was knockdown, especially at early stage. Therefore we demonstrated that retrovirus vector-mediated expression of RNAi could achieve effective, stable gene silencing in hBMSCs, and found that type I and type II played different roles in osteoblastic differentiation, during which type I major functioned at early stage and was important for cell proliferation while type II predominantly functioned at late stage and was necessary for cell maturation, thus providing more information of bone forming mechanism and giving new hints to the clinical therapy of bone-related diseases.

THE EFFECT OF NEURAL STEM CELLS TRANSPLANTATION ON THE PROTECTION OF RETINAL GANGLION CELLS OF SPRAGUE-DAWLY RATS WITH OPTIC NERVE INJURY

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Optic nerve injury (ONI) is a common complication of craniocerebral trauma in neurosurgery. It has been confirmed that the neural stem cells (NSCs) can integrate with retinal and differentiate to neuron or glial cell after transplanted into retinal of ONI rats. Then we investigated the protection of NSCs on retinal ganglion cells (RGCs). Forty-Eight adult Sprague-Dawley (S-D) rats were randomly divided into two groups (Group N and Group C). Calibrated optic nerve crush injury model was induced in the right eyes with the left eyes served as control. NSCs were taken from embryonic rat hippocampus and cultured *in vitro* and grafted *in vivo*. Then NCSs were injected into the right eyes of Group N rats and PBS into Group C rats. And according to the time interval between optic nerve crush and the sacrifice, both Group N and C were further divided into four subgroups (7, 14, 21 and 28 days), leading to 6 rats each subgroup. Three days before sacrifice, 3 % fast blue was injected into superior colliculi bilaterally. The eyes were enucleated after the rat was sacrificed, and flat mounts of the retina from both eyes were prepared on a slide and observed under a fluorescence microscope. The labeled RGCs were counted by a computerized image analyzer and rate was statistical analyzed. Our results show that the labeled RGCs rate of Group N was significantly higher than that of Group C at every time period ($p < 0.01$). The labeled RGCs rate of both Group N and C continued to fall as time went, but the trend of falling

of Group N was evidently slower than that of Group C. These results suggest that NSCs could increase the survival rate of the RGCs and could rescue and/or restore the injured RGCs.

BEHAVIORAL IMPROVEMENT INDUCED BY BONE MARROW MESENCHYMAL STEM CELLS IN NEONATAL RATS AFTER HYPOXIC-ISCHEMIC BRAIN DAMAGE

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Neonatal hypoxic-ischemic brain damage (HIBD) harms the lives and health of newborn infants and children severely. Studies have shown bone marrow mesenchymal stem cells (rMSCs) have therapeutic potential in nervous system disease. Here we demonstrate behavioral improvement of different rMSCs treatment in neonatal HIBD rats by shuttle box test. HIBD models were successfully established in 7-day-postnatal SD rats. At 5 days after hypoxia-ischemia, the rats were randomly divided into 4 groups and respectively transplanted with PBS, CM-DiI marked rMSCs ($1-2\times10^5$), RA-induced rMSCs ($1-2\times10^5$) or dedifferentiated rMSCs ($1-2\times10^5$) into their lateral cerebral ventricle. At 2 months after MSCs administration, shuttle box test was performed to evaluate the condition of learning and memory in 6 continuous sessions for 6 days. And the 7th session was followed one month later. The learning curves of three rMSCs treatment groups were respectively steeper than PBS control group. Rats with transplantation of rMSCs exhibited higher number of active avoidance and lower number of passive escape, showing significant improvement on shuttle box test ($P<0.05$). The numbers of change in explore time and inter-trial-interval were also higher in every rMSCs treatment group than control group ($P<0.05$). But there was no noticeable difference between rMSCs treatment groups in the 6 preceding sessions. Dedifferentiated rMSCs group showed highest number of active avoidance in the 7th sessions. These results suggest that rMSCs treatment in neonatal rats after HIBD can bring out behavioral improvement, indicating rMSCs can enhance ability of learning and memory and dedifferentiated rMSCs may especially have important influence on the longer-term functional recovery.

PCREB IS INVOLVED IN NEURAL INDUCTION OF MOUSE EMBRYONIC STEM CELLS BY RETINOID ACID

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Mouse embryonic stem (ES) cells can be induced by various inductors to differentiate into a variety of cell types *in vitro*. Retinoid acid (RA), one of the most important inductors, was found to induce ES cells differentiating into neural progenitor cells (NPCs) at a concentration of 5μM in our study. During embryoid bodis (EBs) differentiation, the active CREB was relatively high when 5μM RA treatment was unremittingly performed. Inhibition the CREB activity committed EBs into other germ layers whereas increased expression of CREB enhanced the NPCs differentiation. Moreover, RA could increase the expression of active CREB by enhancing the activity of JNK. Our results suggest that CREB might play a role in RA induced NPCs differentiation by high expression of active JNK.

GENERATION OF GABAERGIC NEURONS FROM MOUSE EMBRYONIC STEM CELLS BY COMBINATION OF HIGH-RETINOID ACID AND EXTRACELLULAR MATRIX

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Neural cells derived from embryonic stem (ES) cells are promising candidates for therapeutic application to dysfunctional or aging neural tissues. During embryonic bodies (EBs) formation, exposure to high-concentration all-trans retinoic acid (RA) strongly drives neural induction. Our results demonstrate that 5 μM RA and extracellular matrix (ECM) interactions were essential for the formation of neural precursor cells and the lineage selection of neurons. In particular, we showed ES cells derived neurons were mainly GABAergic neurons by immunofluorescence and RT-PCR analysis. Moreover, ES-derived GABAergic neurons had the same electrophysiological characteristics as adult neurons. Our study thus provides a suitable model for producing specific neuron from ES cells. In addition, we found high-concentration RA promoted the differentiation of neural progenitor and delayed the progress of mesoderm, and the signaling pathway of MAPKs and PKCα were involved in this process. This work provides valuable insights into the molecular mechanism of ES cells differentiation induced by RA.

THE EFFECT OF β-AMYLOID PEPTIDE (Aβ) AND GINSENOSIDES (GS) ON THE PHOSPHORYLATION LEVEL OF TAU DURING THE DIFFERENTIATION OF RAT NEURAL STEM CELLS

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It is believed that the expression of tau is closely related to the differentiation level of neural stem cells and the phosphorylation level of Tau influences its function. We observed the expression of the Tau protein and phosphorylation of tau at Serine 262, Serine 396, and GSK-3β [pT279, 216] in the differentiation of rat neural stem cells. We found the expression of Tau [pS396], Tau [pS262], and GSK-3β [pT279, 216] in rat hippocampi neural stem cells with three groups (the normal control group, the ginsenosides Rb1 and $A\beta_{25\text{-}35}$ treated group and the $A\beta_{25\text{-}35}$ treated group). The expression of Tau [pS396] and Tau [pS262] in the ginsenosides Rb1 and $A\beta_{25\text{-}35}$treated group was higher than the normal control group, and the expression was highest in the $A\beta_{25\text{-}35}$ treated group. In addition, the expression of GSK-3β [pT279, 216] in these groups was positively correlated with Tau [pS396] and Tau [pS262]. These results suggest that in the differentiation of rat neural stem cells $A\beta_{25\text{-}35}$ could make tau over-phosphorylated and the ginsenosides Rb1 could restrain this effect of $A\beta_{25\text{-}35}$. It shows the regulation of phosphorylation level of Tau in the differentiation of rat neural stem cells was possible. $A\beta_{25\text{-}35}$ and ginsenosides Rb1 may regulate phosphorylation of Tau through enhancing or restraining the activity of GSK-3β.

STUDY ON THE EFFECT OF CRYOPRESERVATION OF CORD BLOOD HEMATOPOETIC STEM CELLS USING LOW CONCENTRATION OF VITRIFICATION LIQUOR BY VITRIFICATION

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We aimed to explore the method of cryopreservation of hematopoetic stem cells. Good results have been obtained when vitrification cryopreserved method was used to cryopreserve hematopoetic stem cells, but high concentration of vitrification liquor was toxic to cells. On the other hand, although demethyl sulfoxide (DMSO) is toxic to cells, it has been used as a cryoprotectant in all the research about the cryopreservation of hematopoetic stem cells. Ethylene glycol (EG) and propylene glycol (PG) have been widely used to cryopreserving embryo and oocyte. However, whether they could be used for cryopreservation of hematopoetic stem cells is still unclear. The effects of 1.5 M EG, 1.5 M PG and 1.5 M DMSO were compared in vitrification

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cryopreservation of cord blood hematopoetic stem cells. The recovered cells' viability was tested by typan blue staining, and the effect of EG group was best. The same result get from the recovery rate tested by MNC counted and receiver rate of CFU-GM. The result of the test of CFU-S showed that the effect of EG was better than DMSO ($P < 0.05$) and the diameter of CFU-S in EG group was the highest ($P < 0.05$), either about the WBC count in the peripheral blood of mouse ($P < 0.05$). These result suggest that the effects of protection were best when EG was composed of 1.5 M EG+ 0.25 M Suc + 1% Dex-40 in the vitrification cryopreservation of hematopoetic stem cells.

EFFECT OF EPO-ACTIVATED ASTROCYTE CONDITIONED MEDIUM ON DIFFERENTIATION OF NEURAL STEM CELL AND ITS PROTECTIVE EFFECT ON DIFFERENTIATED NEURAL STEM CELLS AFTER INJURY *IN VITRO*

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Erythropoietin (EPO), a hematopoietic cytokine, has been shown to have developmental functions not only in erythrocyte but also in the nervous system. We previously found that EPO promoted differentiation of astrocytes, and astrocytes could promote neurogenesis. Thus, it is highly possible that neurotropic factors derived from EPO-treated astrocytes could influence neural stem cells (NSCs) differentiation into neuron. Therefore, in the present study we investigated whether EPO-activated astrocytes could secrete some factors to either promote the differentiation of NSCs or protect differentiated NSCs under injury condition *in vitro*. NSCs and astrocytes were separated from newborn rat cortex, and media were collected from astrocytes (ACM) and EPO activated astrocytes (EACM). The cells included three groups: the experiment group also called the EACM group (NSCs with EACM), the ACM group (NSCs with ACM) and the control group (NSCs with DMEM/F12 + 10% FBS). The expression of NF-200 in the NSCs showed strong positivity whereas the NSCs in control group scarcely exhibited morphological changes, and NF-200 immunocytochemical staining was nearly negative in corresponding period. The percentage of neuron positive in the EACM group was 59.18 ± 2.77, while those in the ACM group and the control group were 23.58 ± 1.21 and 16.98 ± 0.91. As for EACM protective effects on differentiated NSCs after hydroxyl free-induced injuries generated by addition of $FeSO_4$ and H_2O_2, the OD value was 0.381 ± 0.027, while that in control group was only 0.270 ± 0.013 ($P < 0.05$). The percentage of survived cell in experimental group ($61.0 \pm 2.48\%$) was higher than control group (31.54 ± 1.35 %) ($P < 0.01$). These results suggest that EPO activated astrocytes could secrete some factors to either promote the differentiation of neural stem cells or protect differentiated neural stem cells under injury condition *in vitro*.

THE EFFECT OF Notch1 SIGNAL TO THE EXPRESSION AND PHOSPHORYLATION OF TAU DURING THE DIFFERENTIATION OF EMBRYONIC STEM CELLS INTO NEURONS

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The Notch signaling pathway has been implicated in the regulation of cell-fate decisions such as differentiation of embryonic stem cells into neurons. In addition, The Notch signaling pathway is closely related to microtubule-associated protein Tau and its phosphorylation. We cultured mouse embryonic stem cells (ESCs) in vitro and transfected two plasmids, pSINsi-U6-Notch1 and PEGF-C1-Notch1, which respectively increased and decreased the expression of Notch1. Then we induced ESCs to differentiate into neural cells by Retinoic acid (RA). Respectively on days 1, 3, 5, 7 and 9 after induction, we examined the Tau protein and phosphorylation of tau at Serine 262 and Serine 396. Phosphokinase GSK-3β and phosphorylase PP2A were detected by immunocytochemistry. Western blotting was utilized to observe the expression of tau, GSK-3β, andPP2A, and the phosphorylation level of tau at Serine262 and Serine 396 on day 5. We found that in ESCs transfected with pSINsi-U6-Notch1 neuron-like cells differentiated from it increased regularly and formed a network structure. GSK-3β, PP2A and tau ser262 and tau ser396 were upregulated simultaneously from around day 3and on day 5, the expressions of tau, GSK-3β, PP2A, Serine262 and Serine 396 were more than the control group. In ESCs transfected with PEGF-C1- Notch1, neuron-like cells differentiated from it were not significantly changed on day 5, and the expressions of tau, GSK-3β PP2A; Serine262 and Serine 396 were lower than the control group. These findings suggest that the Notch signaling pathway promoted the differentiation of ESCs into neurons and at the same time enhanced the expression of tau but inhibiting its phosphorylation.

THE EXPRESSION AND EFFECTS OF PROTO-ONCOGENE *C-RAF* IN SPERMATOGONIAL STEM CELLS *IN VITRO*

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Spermatogonial stem cells were separated by discontinuous Percoll gradient centrifugation from 9-day-old SD rat testes and purified by the different cells adhering speeds to dish. Spermatogonial stem cells were identified by immunohistochemistry of c-Kit protein expression. We then identified the homology between the amplified spermatogonial stem cells' DNA and *c-raf* by sequencing and SeqMan. MTT assay was used to detect survival and proliferation of spermatogonial stem cells. TUNEL was used to assay the apoptosis index of spermatogonial stem cells and RT-PCR was utilized to observe the expressions of *c-raf* mRNA and *caspase*-3 mRNA in spermatogonial stem cells. The survival rates of cells separated by discontinuous Percoll gradient centrifugation and purified by the different cells adhering speeds to dish were 92.3 % and 91.5 % respectively. The percentage of spermatogonial stem cells expressing c-Kit was 90.1 %. Spermatogonial stem cells proliferation increased significantly in the DMEM containing 10 % NBS and decreased continuously in the DMEM without NBS. The highest proliferation rate of spermatogonial stem cells was seen in 120 h culture. The homology between the amplified spermatogonial stem cells' DNA and the c-*raf* was 98 %. The survival rate of spermatogonial stem cells decreased significantly at culture 12 hours in 15 μmol/L *c-raf* AON group ($P < 0.05$). The expression of *c-raf* mRNA decreased and the expression of *caspase*-3 mRNA increased in *c-raf* AON group ($P < 0.05$). The apoptotic index of c-*raf* AON group (40.5 %) was much higher than those of the *c-raf* MON group (29.4 %) and control group (30.2 %) ($P < 0.05$). Our results show that discontinuous Percoll gradient centrifugation combined with the different cells adhering speeds to dish was an effective method for isolating and purifying spermatognial stem cells. C-kit could be used as a molecular marker in identifying the spermatogonial stem cells. The spermatogonial stem cells proliferated significantly at culture 120 hours in DMEM containing 10% NBS *in vitro* and the survival rate of spermatogonial stem cells decreased continuously in the DMEM without NBS. c-*raf* was expressed in the rat's spermatogonial stem cells. The survival of spermatogonial stem cells could be prolonged by *c-raf*. *C-raf* might inhibit the apoptosis of spermatogonial stem cells by decreasing the expression of the capspase-3.

EXPERIMENTAL RESERCH ON THE CHEMOTHERAPEUTIC SENSITIVITY OF BRAIN TUMOR STEM CELLS

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In order to explore the chemotherapeutic sensitivity of brain tumor stem cells (BTSCs), we obtained fresh specimens of astrocytoma and then cultivated them primarily into BTSCs and brain tumor cells (BTCs). By drug

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sensitivity testing in vitro (MTT assay), the sensitivities of Vumon-26 (Vm-26), bischloronitrosourea (BCNU) and diamminedichloroplatinum (DDP) in BTSCs and BTCs were examined. We found the sensitivity rates of Vm-26, BCNU and DDP in BTSCs were 39.3 %, 64.2 % and 16.7 % respectively, and those of Vm-26, BCNU and DDP in BTCs were 71.4 %, 89.3 % and 53.6 % respectively. The sensitivity rates in BTSCs were lower than those in BTCs. These results suggest that BTSC was more drug-resistant than BTC. The obvious drug-resistance could be one of the characteristics of BTSC and Colorimetric MTT assay could be used to screen for chemotherapeutic agents.

INVESTIGATION OF C-MET$^+$ CELLS IN ADULT SKELETAL MUSCLE

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Stem cell therapy offers the promising effect for injured and aged skeletal muscles. Recent studies reveal that skeletal muscle stem cell-satellite cell (C-met$^+$) might include different subpopulations, which might play different roles in regeneration of injured muscle. In the present study, the subpopulations of C-met$^+$ cells which were isolated from adult fast-twitch and slow-twitch muscles respectively were investigated. It was found that the morphology of C-met$^+$ cells in fast-twitch and slow-twitch muscles was different. The morphology of C-met$^+$ cells from fast-twitch muscle was mainly flat spindle shape, while that of slow-twitch muscle was mainly long spindle shape. C-met$^+$ cells included in fast-twitch muscle were TnIfast positive, while the C-met$^+$ cells included in slow-twitch muscle was positive for both TnIslow and TnIfast. In addition, the growth rate of C-met$^+$ cells from slow-twitch muscle was faster than that of fast-twitch muscle. The results suggest that fast- and slow-twitch muscles might have their unique subpopulations which are specific for its myofiber type.

THERAPEUTIC EFFECT OF HUMAN UMBILICAL CORD BLOOD CELLS ON DIEBETIC MICE

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Human adult stem cell research has been paid more attention because of the compelling ethical and legal issues surrounding human embryonic stem cells. Mononuclear cells from human umbilical cord blood (MNCs) have been shown to have plasticity. In our study, human umbilical cord blood MNCs could survive in diabetic mice without using immuno-suppressive drugs and could decrease serum glucose levels at definite time period. To observe the therapeutic effect on diabetic mice of mononuclear cell from human umbilical cord blood, experimental diabetes animal model was induced in 8w kun-ming mice by a single intraperitioneal injection of streptozotocin freshly dissolved in 0.1 M of citrate buffer, pH 4.5. Serum glucose level was observed to monitor the effect on diabetic model. Freshly isolated human umbilical cord blood cells were labeled using PKH26, and then transplanted at $2x10^6$ /ml via tail vein into the model animal. Glucose and insulin levels in serum were determined at different time points. After transplantation, the distribution of transplanted cells in vivo was also examined by flow cytometer and fluorescence microscope. After 4 days of cell transplantation, serum glucose level significantly decreased and the serum insulin level increased in comparison with pre-transplantation. After 14 days, serum glucose level was recovered to the pre-transplantation level but the serum insulin level decreased in comparison with 4d and no significant difference was observed when compared with no-transplantation STZ treated group. PKH26 positive cells were found in panaceas, spleen, liver and bone marrow of transplanted group mice at 4d, but not in above-mentioned positions at 14d by flow cytometer. Results of fluorescence microscope on tissue section also supported this consequence.

FSHR AND PREMEIOTIC MARKER *STRA8* EXPRESSION IN MALE BONE MARROW STEM CELLS INDIRECT COCULTURED WITH SERTOLI CELLS

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Adult bone marrow stem cells (BMSCs) have the ability to differentiate into multiple mesodermal cells and are able to generate cells of all three germ layers under appropriate experimental conditions. Male BMSCs also could differentiate into Sertoli cells (SCs) and spermatogonia after they were injected into testis. It is unclear which factors induced BMSC to differentiate into gonadal cells. In this study, we investigated whether adult male BMSCs from SD rat were able to differentiate into SCs and spermatogonia in vitro by two different treatments: all-trans retinoic acid (RA) and indirect cocultured with SCs. In both treatments, some of the BMSCs expressed FSHR (follicle-stimulating hormone receptor), which is a SCs specific marker in the testicular cells, shown by immunohistochemistry and RT-PCR, and the cells had elongate appearance. In addition, BMSCs also expressed stra8 (stimulated by retinoic acid gene 8), which is a specific expression gene in mammalian germ cell's transition from mitosis to meiosis and is observed only in male spermatogonia after birth, detected by RT-PCR. The effects of the two treatments were similar. In control group, the BMSC still kept undifferentiated flattened cells and relatively elongated or spindle-shaped cells and they were FSHR negative and stra8 negative. These results suggest that RA and the soluble factors secreted by SCs could all induce BMSC to differentiate into SCs and spermatogonia *in vitro*. One of the soluble factors might be RA. The research was supported by grant from National Natural Science Foundations of China (No.30360032).

CARCINOGENESIS, CANCER DIAGNOSIS AND TREATMENT

NATURAL ANTI-TUMOR EFFECT OF PREGNANT MICE AND NEWLY BORN MICE AND THE EFFECT OF MICE EMBRYO EXTRACTIVE ON PROLIFERATION, DIFFERENTIATION, APPOPTOSIS OF H22 CELLS

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In the present study, we investigated the natural anti-tumor effect of pregnant mice and newly born mice and the effect of mice embryo extractive on proliferation, differentiation, and apoptosis of H22 cells. In experiment 1, 15 pregnant Kunming mice and 15 non-pregnant female Kunming mice were used. About $1x10^5$ H22 cells were injected into the peritoneal cavity of each mouse, and then, the tumor growth conditions and mice's survival period were observed. In experiment 2, 10 newly born Kunming mice and 10 adult female Kunming mice were used. About 750 H22 cells were injected into the peritoneal cavity of each newly born mouse and each adult female Kunming mouse. Then, the tumor growth conditions and the mice's survival period were observed. 5 mice of pregnant group were cured absolutely and there were no tumor growth in 60 days. The mean survival period of another 10 pregnant mice was 36.3 ± 1.95 days, while the mean survival period of control group was 20.67 ± 2.35 days ($P < 0.001$). In experiment of anti-tumor effect of newly born mice, the mean survival period of newly born mice was 34.9 ± 1.45 days, while the mean survival period of adult mice was 24.8 ± 2.30 days ($P < 0.001$). Mice hepatocarcinoma (H22) cells were cultured *in vitro* and treated with E (extractive, E). The effects of E on proliferation, differentiation, and apoptosis of H22 cells were then investigated. Results of MTT experiment showed that E could effectively inhibit the proliferation of H22 cells in a dose-

dependent and time-dependent manner. The activity of γ-GT decreased ($P < 0.05$) and the activity of ALP increased ($P < 0.05$) in the cells treated with 2.5 % E after 120 hours compared with control group. FCM analysis showed that apoptosis rate of E group was higher than control group ($P < 0.01$). In conclusion, there was a natural antitumor (H22 cell) effect in the body of pregnant mice and newly born mice. 2.5 % E induced significant growth inhibition, differentiation and apoptosis of H22 cells *in vitro*.

THE EXPRESSION AND STUDY OF MIDKINE, CD105 AND D2-40 IN ORAL MUCOSA CARCINOMA

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This work was to study and compare the expressions of Midkine, microvessel density (MVD) and lymphatic vessel density (LVD) marked by CD105 and D2-40 respectively at various degrees of differentiation and invasive extent in the cancer tissues, and evaluate the correlation of Midkine, MVD and LVD. The surgical specimens of 27 patients with oral mucosa cancer (without pre-operative radiation, chemical and immunological therapy) at the Department of Oral and Maxillofacial Surgery, Stomatology College of Harbin Medical University, were collected. Using immunohistochemical Streptavidin-Perosidase (SP) method, the positive expression rates of Midkine, CD105 and D2-40 were tested and the count of microvessels and lymphatic vessels was conducted quantitatively. The expression of Midkine, MVD and LVD showed respectively significantly positive correlation with cellula differentiation and invasive extent in the tissues of oral mucosa cancer. With the decrease of tumor differentiation, the number of microvessel at the invasion margin increased significantly, and Midkine protein was highly expressed in this region. Statistical analyses showed that ratio of positive expression of Midkine protein was closely correlated with MVD and LVD. P values were $p < 0.01$ and $p < 0.05$ respectively. The results show that, as one of promoting angionenesis growth factors, MK may function to stimulate vascular endothelial cell proliferation in tumor and facilitate the growth of tumor blood and lymphatic vessel. The study demonstrates the possibility of blocking the production of MK and counteracting its function could inhibit the production of microvessel and lymphatic vessel of oral mucosa cancer and limit the tumor growth and invasion.

THE C-TERMINAL COMBINING PROTEIN OF TACE REGULATES STNF-A ECTODOMAIN SHEDDING

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Like most ADAMs, tumor necrosis factor-a converting enzyme (TACE) is a multi-domain, type I transmembrane protein, which is responsible for ectodomain release of numerous membrane proteins. TACE is a member of the 'A Disintegrin And Metalloprotease' family, and has notable metalloprotease hydrolyzationactivity. TACE contains several noncatalytic domains whose roles in ectodomain shedding have yet to be fully resolved. We sought to identify the cause of ectodomain shedding. We explored the function of the c-terminal domain of TACE by constructing an expression vector of the c-terminal domain of human tumor necrosis factor-a converting enzyme (TACE) and expressing its protein in *E. Coil*. There was a protein anchorage of TACE to the lipid bilayer through a TM required for efficient cleavage of a broad spectrum of substrates, and that the constitute of TACE TM may play a role in regulatory specificity among TACE substrates. The c-terminal domain assembly (694−824) in the cytoplasm contains a latent phosphorylation position to select KKLDKQYESL and unifies the Src homology 3 areas with (Src homology 3 domain, SH3) the position selects PAPQTPGR. Our observations suggest that it is very possible for TACE cytoplasm domain to have the function in signal transmission and another vital role to the cell localization. Therefore, it is important for us to study the TACE cytoplasm domain structure and combining protein.

ISOLATION AND IDENTIFICATION OF BRAIN TUMOR STEM CELLS WITHIN TUMORS OF HUMAN NEUROEPITHELIAL TISSUE *IN VITRO*

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The aims of this study were to establish a simplified culture system for the isolation of brain tumor stem cells (BTSCs) in tumors of human neuroepithelial tissue, to observe the growth and differentiation pattern of BTSCs, and to investigate their expression of specific markers. 26 cases of tumor specimens from patients undergoing tumor resections were acutely dissociated and triturated into single cells in sterile DMEM-F12 medium and then filtered. The tumor cells were seeded at a concentration of 200,000 viable cells per mL into serum-free DMEM-F12 medium simply supplemented with B27, human basic fibroblast growth factor (20 μg/L), human epidermal growth factor (20 μg /L), insulin (4 u/L), L-Glutamine, penicillin and streptomycin. After the primary brain tumor spheres (BTS) generated, they were triturated again and passaged in fresh medium. We performed limiting dilution assay to observe the monoclones' formation and induced BTSC differentiation in mitogen-free DMEM-F12 medium supplemented with 10 % fetal bovine serum. At last we performed immunocytochemistry of BTSs for CD133 and Nestin expression and immunohistochemistry of tumor specimen sections for $CD133^+$ cells. The results show that a minority subset of cells in tumors of neuroepithelial tissue had the capacity to self-renew, proliferate and generate free-floating neurosphere-like BTSs in simplified serum-free medium *in vitro*. They could attach to poly-L-lysine coated coverslips in serum-supplemented medium and differentiate. The BTSCs were $CD133^+Neatin^+$ cells in tumor tissue. The $CD133^+$ cells in the tumor specimens ranged from 21 ± 6.2 to 38 ± 7.0 %. These results suggest that tumors of human neuroepithelial tissue contained $CD133^+$ $Neatin^+$ tumor stem cells which could be isolated, proliferate and differentiate *in vitro* and give rise to brain tumor spheres. This tumorigenic subset may provide both a platform for brain tumor research and a target for clinical treatment.

CAVEOLIN-1 GENE SILENCING STIMULATES EXPRESSION OF ERα AND THE METASTASIS IN HUMAN MAMMARY EPITHELIA MCF10A

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Breast cancer is a common malignant tumor, threatening women's health seriously. Metastasis of malignant tumor is the main cause of tumor related death, which is a complicated biologic process. Constitutive activation of estrogen receptor alpha (ER-α) expression is an early event in breast cancer tumorigenesis. Recent evidence supports the existence of a plasma membrane ER. Caveolins proteins constitute important structural components of caveolae, and participate in signal transduction. Caveolin-1 could inhibit the anchorage-independent cell growth. To study the relationship between Caveolin-1 and ER in breast cancer, we transcripted the siRNA into normal epithelial cells-MCF 10A to silence the expression of Caveolin-1. Then we examined the expression of Caveolin-1, ERα and associated proteins by Western Blot. The results indicate that Caveolin-1 was silenced by 85 % at 96hr, whereas we found that the expressions of protein ERα66 and ERα36 were up-regulated. With the Caveolin-1 down-regulation, the phosphorylation of ERK1/2 was increased. Theses data show that the down regulation of Cavoin-1 could activate MAP kinase, including the phosphorylation of ERK1/2, which played critical roles in the control of cell proliferation, differentiation, homeostasis, and survival via the expression of ERα which is residing in the caveolae. Then, monolayer culture of MCF-10A, MCF10A-ST1, MCF10A-7SD8 and MCF-7 were scrape wounded and allowed to grow for 24 hours, the distance traveled was measured as the distance between the wound edge and migrating front. And we also obtained the evidence that migrant distance was different in four

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cell lines: 50 % in MCF10A-7SD8 and 30 % in MCF10A-ST1 comparing to MCF-7, and little in MCF-10A by wounded healing. These results indicate that Caveolin-1 could significantly mediate membrane-initiated estrogen-signaling pathway, and may play an important role in mammary tumorigensis and may have effect on cell metastasis *in vitro*. Such evidence will greatly advance the progress in prevention and treatment of human breast cancer. This work was supported by a grant from the National Natural Science Foundation of China (No. 30570225).

STUDY ON mRNA EXPRESSION, ALLELIC LOSS AND DNA METHYLATION OF ZNF403 GENE IN LARYNGEAL CARCINOMA

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ZNF403 gene is localized at chromosome 17q12, which is one of the loss of heterozygosity (LOH) regions in laryngeal carcinoma (LC). In this study, mRNA expression, allelic loss and DNA methylation of *ZNF403* gene were investigated in LC. We first detected mRNA expression of *ZNF403* gene in 30 primary LCs and the corresponding noncancerous tissues by RT-PCR. The results demonstrated that the expression of *ZNF403* gene was absent or down-regulated in 60% of primary LCs (18/30), compared with the corresponding non-cancerous tissues. We also identified that -1370 bp to -607 bp in the upstream of *ZNF403* gene was its functional promoter region through bioinformatic analysis and luciferase reporter assay. DNA methylation of the *ZNF403* promoter region was detected in 83% (25/30) of primary LCs and 46.7% (14/30) of the corresponding non-cancerous tissues with methylation specific PCR (MSP). Statistical analysis indicated that there was a significant correlation between the promoter methylation of *ZNF403* gene and its mRNA expression ($p < 0.001$). The treatment of Hep2 laryngeal carcinoma cells with 5-aza-2'-deoxycytidine activated the expression of *ZNF403* gene along with its promoter demethylation. Finally, we performed allelic loss analysis in 29 of 30 primary LCs using three microsatellite marks (WI22201, WI16428 and RH78009) surrounding the *ZNF403* gene. We found the frequencies of allelic loss for WI22201, WI16428 and RH78009 were 0 (0/29), 10.3% (3/29) and 6.9% (2/29), respectively. Therefore, mRNA expression of *ZNF403* gene was absent or down-regulated in LC, and the promoter hypermethylation may be a mechanism leading to the down-regulation of *ZNF403* gene in LC.

TRANSCRIPTIONAL REGULATION OF SURVIVIN BY P53 MEDIATED BY EBV-LMP

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The Epstein-Barr virus (EBV) encoded latent membrane protein 1 (LMP1), an oncogenic protein, plays an important role in the carcinogenesis of nasopharyngeal carcinoma (NPC). Survivin, an inhibitor of apoptosis protein (IAP), is a dual mediator of apoptosis resistance and cell cycle progression, and is highly expressed in cancer. We have previously shown that LMP1 can upregulate survivin by NF-κB and AP-1, and in addition LMP1 can upregulate p53 protein and its transcriptional activity by phosphorylation. However, the molecular mechanisms underlying LMP1's regulation of survivin by p53 have not been elucidated. Here, we found that survivin protein level was decreased in LMP1 positive cells that transfected p53siRNA. This indicated that LMP1 could upregulate survivin by p53. Since there is a p53-binding site in survivin promoter, to confirm p53 could transactive the survivin promoter, the assay of reporter gene was utilized. To confirm p53 could physically associate with the survivin promoter, the assay of EMSA was utilized. Our results suggest that the function of p53 in NPC may not be a tumor suppressor gene. Instead, it may act as a transcription factor. The oncogenic protein LMP1 upregulated survivin by p53, which contributes to its dual effect of increasing cell proliferation and inhibiting cell apoptosis.

ACTIVATION OF THE MAPK SIGNALING PATHWAY IN HCV NS3 TRANSFORMED HEPATOCYTE

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HCV nonstructural protein 3 (NS3) is a multi-functional viral protein that plays a key role in the life cycle of virus and interacts with host cellular protein. In our previous study, we have shown that HCV NS3 has transforming and oncogenic potential. In order to further investigate the carcinogenesis of HCV NS3, human hepatocyte line QSG7701 stably expressing HCV NS3 protein was constructed, which was named pRcHCNS3/QSG. Two-dimensional electrophoresis profiles with high resolution and reproducibility were set up to separate the total protein of pRcHCNS3/QSG cells and blank plasmids transfected cells (pRcCMV/QSG), respectively. Among the differentially expressed protein spots, we identified the kinase Ras and P38 were increased in pRcHCNS3/QSG cells. Western blot analysis showed that pRcHCNS3/QSG cells resulted in a higher activity of phosphorylated P44/42, P38 and JNK in comparison with pRcCMv/QSG cells and QSG7701 cells while the basal expression of P44/42, P-38 and JNK showed no difference. Immunoprecipitation analysis displayed higher level of tyrosine phosphorylation in pRcHCNS3/QSG cells. We showed here that HCV NS3 might affect cell signaling pathway through activating tyrosine kinase and following with several cytoplasmic signaling molecules such as MAPKs, and result in hepatocyte transformation and tumor development. Further study on the signal transductions and their relationship would not only be helpful to explore the mechanism of HCV related HCC, but also provide a new idea for molecular treatment of HCC.

PHOSPHOPROTEOMICS ANALYSIS OF TRANSFORMATION POTENTIAL OF THE EPSTEIN-BARR VIRUS-ENCODED LATENT MEMBRANE PROTEIN 1 IN NASOPHARYNGEAL EPITHELIAL CELLS NP69

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Epstein-Barr virus is often associated with a variety of human malignancies including Burkitt's lymphoma, nasopharyngeal carcinoma (NPC), et al. EBV-encoded latent membrane protein 1 (LMP1) is a EBV oncoprotein involving activation of a number of signaling pathways such as NF-κB, AP-1, P38/MAPK and JAK3/STAT, several of which are controlled by tyrosine phosphorylation events. However, many signaling molecules and downstream target proteins triggered by LMP1 have not been identified. We have found in previous studies that wild type LMP1 (wtLMP1) can, while mutant LMP1 defective in TRADD site cannot, induce primary MEF and Rat-1 cells to behave a malignant transformed phenotype. In this study, we used concentrated retrovirus (RV-pLNSX, RV- $LMP1^{WT}$, RV-$LMP1^{TRADD}$) to infect the nasopharyngeal epithelial cells(NP69) and discovered a similar phenotype changes, combing two-dimensional electrophoresis, antiphosphotyrosine imumuoblotting and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS), identified 12 differentially tyrosine-

phosphorylated proteins between NP69-PLNSX and NP69-LMP1^{WT} cell lines and 11 differentially tyrosine-phosphorylated proteins between NP69-LMP1^{WT} and NP69-LMP1^{TRADD} cell lines. Some of which had previously been implicated in LMP1 signal pathway. The other proteins, including Heat shock protein 70, Cytoskeletal 7, Phosphotidylethanolamin binding protein 1, Tubulin, et al were novel signaling molecules and targets with no previously known function in LMP1 signal transduction. These data will be helpful to elucidate the molecular mechanism of LMP1 in EBV-associated nasopharyngeal carcinogenesis and guide the discovery of new drug targets and the rational utilization of pathway-specific chemotherapies. This study was supported by grants from National Science Foundation of China (30470668).

THE EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 PARTICIPATES IN MEDIATING THE JANUS KINASE 3 SIGNALING PASSAGEWAY IN THE NASOPHARYNGEAL CARCINOMA CELL

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The Epstein-Barr virus (EBV) latent membrane protein 1 (LMP1) is a critical oncogenic protein in EBV-related tumorigenesis and usurps cellular signaling pathways resulting in the induction of NFkB and AP1 via two C-terminal activating regions (CTAR1 and CTAR2), To make sure whether there exists a third activating region (CTAR3) in its C-terminal and whether there is a relation between CTAR3 and JAK3/STAT1 pathway, a plasmid encoding a mutant LMP1 defective in binding sequence (aa 232−351) for Janus kinase3 (JAK3) protein and a reporter plasmid containing JAK3 promoter and luciferase gene fused sequence were constructed. This LMP1$^{\Delta 232\text{-}351}$ gene was almost completely defective in JAK3 activation in comparison with wild type LMP1 (wt LMP1) and was almost consistent with wtLMP1 in NFkB and AP1 activation. When wtLMP1 and LMP1$^{\Delta 232\text{-}351}$ were introduced into nasopharyngeal cancer cell line CNE-2 through retrovirus, respectively, the CNE-2-wt LMP1 cells showed an increased expression of non-phoshated and phosphated JAK3 than CNE-2 control cells; while CNE-2- LMP1$^{\Delta 232\text{-}351}$ cells showed a lower expression of two kinds of JAK3 than CNE-2-wtLMP1 cells. When CNE-2-wt-LMP1 cells were treated with WHIP-131, an inhibitor of JAK3, no change in non-phosphated JAK3 expression and decreasing expression in phosphated JAK3 was observed. Further assays showed wt-LMP1 had a STAT1-binding and anti-apoptosis activity, while LMP1$^{\Delta 232\text{-}351}$ was missing the activity in CNE-2 cells. These results substantiate that LMP1 could activate the JAK3/STAT1 signaling pathway and inhibit cellular apoptosis of CNE-2 cells depending on CTAR3 and the action may be one of the mechanisms of EBV-LMP1 participating in nasophayngeal carcinogenesis. This study was supported by grants from National Science Foundation of China (30470668).

THE EFFECT OF SURVIVIN ON MULTIDRUG RESISTANCE BY EPIDERMAL GROWTH FACTOR RECEPTOR 2 VIA NF-κB ACTIVATING IN BREAST CANCER CELLS

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Survivin is a structurally unique inhibitor of apoptosis (IAP) and might potentially play a key role in resistance to anti-cancer drugs. However, there is no information on the role of survivin in multidrug resistance (MDR) in the presence of Her-2 in cancer cells. HER2 is a member of the EGFR family, which is associated with poor chemotherapeutic response. In this study we set up HER2-overexpressing breast cancer cell lines (MCF7/HER2) and investigated the effects and mechanism of survivin on MDR by Her-2. The results showed that MCF7/HER2 cells had an increased expression of survivin mRNA and protein and the resistance index (IR) of this cells to Taxel, MMC, VP16, 5-Fu and Mit increased 21, 5.45, 3.12, 2.32, 2.87 folds than control cells, respectively. When HER2 was silenced the survivin expression decreased and the IR of aforementioned drugs decreased 14.3, 4.8, 5.64, 2.62 and 3.43 folds respectively. When survivin was silenced IR of MCF7/HER2 cells to the drugs decreased 6.48; 3.12; 2.18; 1.96 and 2.54 folds, respectively. HER2 could activates survivin by 2−6 fold with the increasing concentration of HER2 in cotransfection studies with survivin luciferase reporter, while tyrosine kinase inhibitor AG825 could inhibit the activity by 1.5 - 8 fold. HER2 also could activate NF-κB in cotransfection studies with NF-κB luciferase reporter. When IκBα were cotransfected with HER2, NF-κB activation was significantly inhibited. When treated with dexamethasome, a potent inhibitor of NF-κB, the expression of suvivin mRNA and protein in MEF7/HER2 cells were decreased. These results suggest that survivin took part in HER2- and NF-κB −mediated MDR of breast cancer cells and might be a potent therapeutic target in reversing MDR of tumor cells. This study was supported by grants from Basic Research Special Program of the Ministry of Science and Technology of China (2003CCC00700) and the Medicinal and Pharmic Science Foundation of Hunan Province, China (Z02-1).

ESTABLISHMENT OF A PYM-RESISTANT HUMAN TONGUE CARCINOMA CELL LINE TCA8113/PYM AND CLONING OF RESISTANCE-RELATED GENES

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Pingyangmycin(PYM) is an anti-cancer antibiotic frequently used in chemotherapy of tongue carcinoma before or after surgical. Innate and acquired resistance of tongue carcinoma to PYM interfere seriously the effects of PYM in chemotherapy. To probe into the resistant mechanism, we first set up a PYM-resistant human tongue carcinoma cell line Tca8113/PYM which showed an 55-fold higher resistance to PYM than parental cell (Tca8113) by incubation of the cells with increasing concentration of PYM. Tca8113/PYM cells did not present either significant differences in growth rate or cell cycle distribution, but were cross-resistant to a subset of clinically relevant anti-cancer agents, cDDP, VCR, THP, TAXEL and MMC, but not to ADM, VP-16 and 5-Fu in comparison with parental cells. Secondly, differential expressions of genes between Tca8113/PYM and parental cells were examined by cDNA microarray. To our surprise, no changes in MDR1, MRP or LRP mRNA expression were found, but a number of genes associated with a variety of cellular functions and EST showed differential expression. We cloned and identified two novel human sequences from an EST by bioinformatics binding RT-PCR methods and nominated as TCRP1 variant1 and variant2 (Genbank No. are EF197985 and EF362480). Further bioinformatics analysis indicates both splice variants encoding a predicted 129-amino-acid and a putative 235-amino-acid might be membrane proteins. Enforce expression of two full-length cDNA in parental Tca 8113 cells confers resistance of two cell lines to PYM and cDDP. The newly established Tca8113/PYM cell line and the accumulation of its gene expression data will provide potentially useful tools in gaining insights into the mode of action of PYM and elucidating the mechanisms of acquired resistance, as well as in investigating and developing methods to prevent and overcome resistance to this drug. This study was supported by grants from Basic Research Special Program of the Ministry of Science and Technology of China (2003CCC00700).

DOdFMG, A GENISTEIN DERIVATIVE, AS EFFECTIVE ANTI LUNG ADENOCARCINOMA CELL AGENTS *IN VITRO* AND *IN VIVO*

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It has aroused a general interest in the utilization of natural products, or their synthetic analogs to find new anti-cancer agents. Genistein, which is abundant in soybeans, has attracted more and more interest because of its ability to inhibit a number of kinds of tumor cell growth *in vitro*. However, genistein has not been considered for development as a chemotherapeutic drug because of its poor bio-availability and high rate of metabolism *in vivo*. To improve the

biological activities, in present study we synthesized a series of derivatives from genistein and found via extensive screening *in vitro* and *in vivo* that 5,4'-Di-n-octoxyl-7-gem-difluoromethylene-genistein (DOdFMG), one of these derivatives, could significantly suppress the proliferation of lung adenocarcinoma cells A549 in a dose-and time- dependent manner in vitro while it only had a weaker effect on normal cells (KMB-17), and inhibited tumor formation in nude mice xenografts by inoculating s.c. A549 cell. The results shown in further study were that cycle progression of A549 cells was blocked in G_1 phase and phosphorylation levels of Rb and Erk1/2 in A549 cells were markedly decreased in the protein after treated with DOdFMG. These results indicate that suppressing effects of DOdFMG on lung carcinoma cells in vitro and in vivo might be associated with its inhibiting on phosphorylation of Rb and Erk1/2 and, moving forward, blocking G1 phase progression. DOdFMG might become a potential chemotherapeutical agent for lung cancer and other human tumors. This study was supported by grants from Basic Research Special Program of the Ministry of Science and Technology of China (2003CCC00700).

VBMDMP EXHIBITS DISTINCT ANTIANGIOGENIC ACTIVITY MEDIATED BY INTEGRIN ALPHA5BETA3 AND DEATH RECEPTOR

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Tumstatin possesses an anti-angiogenic activity domain (amino acids 74–98) and an anti-tumor activity domain (amino acids 197–215). In previous studies, we constructed a fusion gene of human IgG3 upper hinge region and such two tumstatin-derived specific sequences, which is named vascular basement membrane derived multifunctional peptide (VBMDMP) and found that VBMDMP protein could specifically inhibit proliferation and induce apoptosis of HUVEC-12, and also inhibit tube formation of endothelial cells in C57BL/6 mice. However, the mechanisms involved are not clear. In the present study, we first used the Oligo GEArray for Human Extracellular Matrix and Adhesion Molecules containing 113 genes to examine VBMDMP-regulated pathways to analyze the alteration in gene expression after a 4hr treatment of HUVEC with 1.0 μmol/L of VBMDMP. The result in cDNA microarray showed a marked decrease in expression level of integrin αV mRNA in HUVEC. Similarly, the quantitative real-time PCR studies demonstrated substantial dose- and time-dependent downregulation of integrins αVβ3, which are important positive regulators of angiogenesis in tumors and normal tissue. Secondly, an antibody array containing 400 immobilized antibodies against well-studied signaling proteins was used after 30 min incubation of HUVEC with 1.0μmol/L VBMDMP and we found that VBMDMP significantly increased phosphorylation of integrin αVβ3; DR3; DR4; DR5 and caspase3 and dephosphorylation of FAK, PI3K and Akt. These results indicate that inhibitory effects of VBMDMP on proliferation of HUVEC in vitro and tube formation in vitro and in vivo may be associated with specific integrin-FAK/PI3K/AKT signaling pathways. Inducible apoptosis of HUVEC-12 by VBMDMP in vitro is probably associated with activation of Death Receptor/caspase-3 signaling pathway. These observations contribute significantly toward understanding of the therapeutic potential of the VBMDMP. This study was supported by grants from National Science Foundation of China (30472070).

DUAL REGULATION OF LMP1-AUGMENTED KAPPA LIGHT CHAIN EXPRESSION AND SECRETION IN NASOPHARYNGEAL CARCINOMA CELLS BY NFκB AND AP-1

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Though it was thought that immunoglobulin could only be generated by B cells, light chain Igκ was found in many human cancer cell lines of epithelial orgin, including nasopharyngeal carcinoma (NPC), yet the mechanism of this phenomenon is not clear. EBV encoded latent membrane protein 1 (LMP1), an oncogenic protein which plays an important role in the carcinogenesis of NPC, is known to induce gene expression via activation of several signal transduction pathways, including NFκB, AP-1 pathways. Our previous studies also indicated expression of κ light chain in NPC cells could be up-regulated by LMP1. A κB site within the intron enhancer (iEκ) of Igκ gene has been implicated as a key regulator of its expression, an AP-1 binding site upstream of the iEκ has also been found to play a role in the regulation of Igκ gene. Here in NPC cell lines, we demonstrated LMP1-increased Igκ chain expression together with its secretion through activation of the AP-1, NFκB signal pathways. This study provided us some hints of possible mechanism by which human cancer cells of epithelial origin produce immunoglobulin.

PRELIMINARY DOSIMETRIC INVESTIGATION OF PLANNING SCHEME FOR LOCAL ADVANCED NASOPHARYNGEAL CARCINOMA WITH C-SHAPED TARGET IN THREE-DIMENSIONAL TREATMENT PLANNING SYSTEM

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The aim of our study was to evaluate dosimetric characters of conventional post-auricular portal (PAP), three-dimensional conformal radiotherapy (3DCRT) and split-filling and simultaneous boost irradiation (SF-SBI) techniques in radiotherapy for nasopharyngeal carcinoma (NPC) with C-shaped extensive invasion of skull base, in order to provide optimal scheme for improvement of radiation treatment planning. One NPC patient with extensive invasion of skull base was eligible to study. The enhanced CT image of the patient was transmitted into 3 dimensional treatment planning system. After routine 50Gy irradiation?conventional PAP, 3DCRT and improved SF-SBI scheme were applied respectively. 1. Either PAP, 3DCRT and SF-SBI plan could obtain satisfied dose coverage of PTVnx 2. PAP plan had conspicuous high dose area, and V80 was 59.4%. Both 3DCRT and SF-SBI plans could improve dose homogeneity to some extent, especially V80 of 3DCRT plan with only 1.1 %. But more importantly, SF-SBI plan could give higher fractionation dose of 2.7Gy boost irradiation to skull base which was located in lower dose coverage with routine 50Gy irradiation. 3. Irradiation to brain stem in both PAP and 3DCRT plans increased obviously. V60 was 36.0 % and 30.2 % respectively, and D33 was 61.0 Gy and 57.0Gy respectively. However, in SF-SBI plan, V60 and D33 of brain stem were decreased to 15.1 % and 55.5Gy. The irradiated dose and volume to ipsolateral temporal lobe, temporo-mandibular joint and middle ear were lowest in SF-SBI plan. In conclusion, when planning for NPC with C-shaped target, SF-SBI technique could provide satisfied dose coverage and accepted dose homogeneity to PTVnx with significantly decreased irradiation to OARs. And this technique could increase fractionation dose and total effective dose to skull base area. It remains to be further investigated for the rational fractional dose, the optimized fields setup methods and whether to be applied at the planning beginning or not.

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HYPERTHERMIA HYPOTHERMIA INTEGRATION THERAPY (HIT): A POTENTIAL NEW ANTI-CANCER THERAPY

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Hyperthermia or hypothermia alone has shown promising results in killing cancer cells as monotherapy, or as adjunct or alternative therapy. We asked whether combination of hyper-, hypothermia therapy (HIT) worked better than single treatment on cancer cells. Murine colon cancer (CT-26) cells, human basal cell carcinoma (BCC) and human melanoma A375 cells were treated with HIT. Only 30 % of the cells survived after onc cycle of hyperthermia (45°C for 10 minutes) and hypothermia (-10°C for 1 second) treatment in all cell types. Five cycles HIT killed all cells. In addition, HIT enhanced the susceptibility of melanoma cell to chemotherapeutic agent dacarbazine (DTIC). These results suggest HIT might be a potential new anti-cancer therapy.

CLINICOPATHOLOGICAL SIGNIFICANCE OF NODAL EXPRESSION IN HUMAN GASTRIC CARCINOMA

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Because of its role in regulating tumor progression, the Nodal has been implicated in cancer metastasis. Previous evidence supports such a role of Nodal in human cancer, the study of Nodal in gastric cancer cells and cancer tissues, however, is lacking. To investigate Clinicopathological significance of Nodal expression in human gastric carcinoma, samples of paraffin-embedded sections from 113 gastric carcinoma patients were determined immunohistochemically for Nodal expression and the correlation of Nodal levels with prognosis was analyzed. 36 cases of normal gastric mucosa were served as controls. An obvious difference existed in the Nodal expression between carcinomas and normal mucosa. Among 113 cases of gastric carcinoma, the immunohistochemistry data indicated significant increase of Nodal expression levels in 72 cases. Importantly, the expression of Nodal was significantly correlated with the depth of penetration, lymph node metastasis, and clinical stage. Multi-variable Cox regression analysis revealed the Nodal expression level was an independent factor for prognosis. We conclude that Nodal may be involved in carcinogenesis and progression of gastric carcinoma. Nodal expression was elevated in gastric carcinoma tissues, which correlates with a poor prognosis, suggesting Nodal as a candidate prognostic marker of gastric carcinoma.

SCREENING AND IDENTIFICATION OF HUMAN ANTI-HEPATOMA SINGLE-CHAIN Fv FUSION PHAGE ANTIBODIES

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Hepatocellular carcinoma is one of the most frequent and malignant diseases worldwide. Most patients had lost the chance of operations and the drugs for chemotherapeutics frequently caused severe side effects. In this study, we tried to select the ScFv specifically binding to hepatocellular carcinoma. To construct, screen and identify fully human anti-hepatoma scFv fusion phage antibodies, peripheral blood mononuclear cells (PBMCs) of patients with liver cancer were sensitized *in vitro* and transformed by Epstein-Barr virus (EBV). VH and VL genes were amplified by RT-PCR and combined to single-chain fragment of variable region (ScFv) genes. ScFv genes were then cloned into vector fuse5 and transformed into E.coli MC1061 resulting a library with the size of 1.0×10^8. After three rounds of positive and negative cell panning and enrichment, 2798 phage clones were picked out and detected by phage-ELISA. Among these clones, the SA3 reacted strongly with $HepG_2$ but weakly with QSG-7701 and HUVEC cell lines. The binding specificity of phage clone SA3 with hepatoma carcinoma cells was confirmed by immunohistochemistry. The results of immunohistochemistry with cultured cells were conformed to the results of ELISA. The SA3 reacted specifically with the hepatoma cells in most human hepatoma tissue sections but in very few human liver tissue sections. There's statistical significance between the positive rate of human hepatoma tissue sections and human liver tissue sections. Based on these results, we concluded that fully human anti-hepatoma single-chain Fv fusion phage library was constructed by using phage antibody library technique in combination with *in vitro* immunization method and EBV transformation technique. By cell ELISA and immunohistochemistry, the clone SA3 was confirmed to specifically bind with hepatoma carcinoma cells. The ScFv fragment against hepatoma may be further developed and applied to clinical diagnosis and therapy of liver cancer.

THE ROLE OF PI3K/AKT/c-FLIP IN TRAIL-INDUCED APOPTOSIS IN COLON CANCER

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TRAIL can induce apoptosis of many kinds of cancer cells, while there are still a few cancer cells appear to be protected from its cytotoxic effects. To elucidate some of the critical factors that contribute to TRAIL resistance, we performed a genetic screen in the human colon carcinoma cell line SW480, HT29, HCT116 and found that HT29 was insensitive to TRAIL-induced apoptosis while SW480 and HCT116 were relatively sensitive to it. SW480 and HCT116 had high antitumor activity in a time and concentration dependent manner. PI 3-kinase has been shown to protect cells from apoptosis in a caspase-dependent manner. As a downstream molecule of PI3K/AKT pathway, c-FLIP could inhibit the activity of caspase 8 in a competitive inhibition way. In order to explore the role of PI3K/AKT/c-FLIP in resistance to TRAIL-induced apoptosis, we tested the expression of c-FLIP in SW480, HT29, HCT116 cell lines and found that it had the highest expression level in HT29 compared to others, indicating that a higher expression of c-FLIP might be correlated with resistance to TRAIL-induced apoptosis. To confirm this postulation, we incubated HT29 cell line with TRAIL after pre-treated with LY294002, specific inhibitor of PI-3K/Akt signaling pathway, for 1h. As a result, we found that c-FLIP decreased obviously while its apoptosis rate increased. Therefore we conclude that PI-3K/Akt/c-FLIP might involve in TRAIL resistance.

THE ROLE OF TGFβ1 IN MULTIDRUG RESISTANCE OF GASTRIC CARCINOMA CELLS AND ITS POSSIBLE MECHANISM

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Gastric cancer is one of the most common malignant tumors in China with a very high mortality. But nowadays it encounters a dilemma—multidrug resistance (MDR). In our earlier studies, we found that TGFβ1 could enhance the invasion and metastasis of gastric cancer and induce the expression of GST-π, indicating that TGFβ1 might involve in drug-resistance of gastric cancer. To test this hypothesis, we conducted MTT assay and found that

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pre-treatment of TGFβ1 could induce the drug-resistance of SGC-7901 to mitomycin. Meanwhile, we found no difference of apoptosis rate in TGFβ1-treated SGC-7901 with its counterpart, which excluded the influence of apoptosis induced by TGFβ1. Next, we tested the expression of MDR1, GST-π, two major MDR-related molecules, and found that they were up-regulated after treatment of TGFβ1, indicating that TGFβ1 induced drug-resistance might be correlated with MDR1, GST-π. At last, we used Smad4 siRNA and special inhibitor of MAPK signal pathway to explore the role of these signal pathways in this biological effect to investigate the mechanisms of TGFβ1-induced GST-π and MDR1. As a result, we found that expression of GST-π induced by TGFβ1 could be inhibited by Smad4 siRNA and PD98059 but not SB203580 and SP600125, indicating that TGFβ1 could induce expression of GST-π through Smad and ERK signal pathways. As to MDR1, we found that it could be induced through JNK and ERK signal pathway by TGFβ1. Based on our results, we conclude that TGFβ1 might induce the drug resistance in SGC-7901, which might correlate with GST-π and MDR1; TGFβ1 could induce the expression of GST-π through Smad4 and ERK signal pathway and the expression of MDR1 through JNK and ERK signal pathway respectively.

THE UBIQUITINATION OF P53 REGULATED BY EPSTEIN-BARR VIRUS ENCODED LATENT MEMBRANE PROTEIN 1

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EBV encoded latent memebrane protein 1 (LMP1), an oncogenic protein, plays an important role in the carcinogenesis of nasopharyngeal carcinoma (NPC). p53 protein accumulates but mutation of p53 gene is not common in NPC. The molecular mechanisms leading to the stabilization of p53 have not been completely elucidated. LMP1 regulated p53 phosphorylation on multiple sites via MAPK signaling pathway was determined previously, which is resulting in the increasing of p53 transcriptional activity, transactivation and stability. Ubiquitination is an emerging mechanism implicated in a variety of nonproteolytic cellular functions, which closely associated with protein phosphorylation. Here, we demonstrated that LMP1 induced Ub molecule Ub^{k63} binded with p53 via MAPK signaling pathway, which was associated with the p53 stability and activation. Among them, the regulation of p53 Ser20 via JNK pathway was more important than others. The phosphorylation of p53 could inhibit the combination of p53 to Ub molecule Ub^{k48}, involving in the p53 stability. These results suggest that Ub molecules Ub^{k48} and Ub^{k63} could bind p53 to play different functions, which provides a novel view for us to understand the mechanism of p53 accumulation in the carcinogenesis of NPC.

GENE THERAPY OF TUMOR METASTASIS BY NON-VIRAL VECTOR

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Metastatic disease represents one of the most difficult challenges in cancer therapy. Gene therapy provides modern medicine with new perspectives that were unthinkable before. Many non-viral vectors have been developed for therapeutic gene transfer for basic and clinical research. We previously reported a self-assembled non-viral gene vector, poly-L-lysine modified iron oxide nanoparticles (IONP-PLL), which is formed by modifying poly-L-lysine to the surface of iron oxide nanoparticles and could transfer exogenous DNA to cells in vitro and in vivo. In this study, experimental pulmonary metastasis model was formed by injecting B16 cells into C57BL/6 mouse via tail lateral vein and nm23-H1-GFP gene encoding nm23-H1 protein and green fluorescent protein was used as therapeutic gene. The inhibition efficiency of metastasis was evaluated by intravenous injection of IONP-PLL/nm23-H1-GFP complexes into mouse and the number of metastases in the lung was calculated. At the same time, the common therapeutic efficiency of gene therapy and chemotherapy was evaluated by treating mice with IONP-PLL/nm23-H1-GFP complexes and CTX. After intravenous injection, IONP-PLL transferred therapeutic gene nm23-H1-GFP to lung and significantly reduced the number of metastases. Furthermore, when used with CTX, the fewer number of metastases was observed. These results suggest that IONP-PLL, an efficient gene vector, appeared to have potential for metastatic disease in cancer therapy and might be a helpful additional way to current chemotherapy.

EBV-miR-BHRF1-1 CONTRIBUTES TO EPSTEIN-BARR VIRUS LYTIC CYCLE INDUCTION VIA INHIBITS P53

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Epstein-Barr virus (EBV) was the first human virus found to encode microRNAs (miRNAs), which are arranged in two clusters: 29 miRNAs are located in the introns of the viral BART gene while 3 are located adjacent to BHRF1. Here we demonstrated that ebv-miR-BHRF1-1, one of these miRNAs, was up-regulated after inducing EBV from latent into lytic cycle by treatment of TPA (12-O-tetradecanoylphorbol-13-acetate) in NPC cell lines. On the contrary, p53 protein level and mdm2 protein level, which is one of p53's downstream genes, were down-regulated after induction of the lytic cycle of EBV. And more interestingly, the percent of M-phase cells increased while apoptosis rate decreased accompanying with the down-regulation of p53 protein. Furthermore, bio-informatics discovery revealed that ebv-miR-BHRF1-1 could interact with the 3'-untranslated region of the p53 mRNA at two sites. These findings indicate that ebv-miR-BHRF1-1 contributed to EBV lytic cycle induction via inhibition of p53 in NPC cell.

LMP1 REGULATES Op18/STATHMIN SIGNALLING PATHWAY BY CDC2 MEDIATION IN NASOPHARYNGEAL CARCINOMA CELLS

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Op18/stathmin plays a crucial role in maintaining cell biological characters by regulating microtubule dynamics, especially entering into mitosis. The phosphorylated Op18/stathmin promotes microtubule polymerization to form the mitotic spindle which is essential for chromosome segregation and cell division. Cdc2 is a critical kinase to start M phase event in a cell cycle progression and a type of plus regulation factor of cell cycle. Latent membrane protein 1 (LMP1) is an EBV encoded oncogenic protein that is able to induce tumorigenesis by various mechanisms. Our studies focused on the regulation of Oncoprotein 18/stathmin (Op18/stathmin) signaling by LMP1 in nasopharyngeal carcinoma (NPC) cells, and showed that LMP1 could regulate Op18/stathmin signaling by cdc2 mediation. LMP1 up-regulated cdc2 kinase activity and Op18/sthamin phosphorylation at G2/M phase, promoted the interaction of cdc2 with Op18/stathmin and microtubule polymerization; Inhibition LMP1 expression attenuated the interaction of cdc2 and Op18/stathmin and promoted microtubule depolymerization; During mitosis, inhibition cdc2 kinase resulted in spindle disaggregation. All these results revealed a new pathway that LMP1 regulates Op18/stathmin signaling by cdc2 mediation. The new signaling pathway on LMP1 not only perfects the LMP1 regulation network, but also provides new insights for elucidating the molecular mechanism of LMP1 leading to tumorigenesis.

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PERIPHERAL PRIMITIVE NEUROECTODERMAL TUMOURS: A RETROSPECTIVE ANALYSIS OF 40 PATIENTS

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The clinical data of 40 patients (males:females, 2.08:1) with peripheral primitive euroectodermal tumors (PNET) from 1997 to 2007 at Cancer Center, Sun Yat-Sen University were reviewed, without any selection according to primary tumor site or disease extension. The age at diagnosis ranged from 2 to 72 years (median, 18 years). Primary sites were chest (n =7), pelvis (n = 8), retroperitoneum or abdomen (n = 7), limbs (n = 7), head and neck (n = 6), and vertebral canal (n = 5). At diagnosis, local extension was present in 7 patients, 5 patients had lymph node metastases, and 14 patients had distant metastases. Tumor size was greater than 10cm in 16 patients (40 %). There were 40 patients, 23 received multimodality treatment, 14 received only surgery or chemotherapy, 3 did not have any anticancer therapy. The median survival time was 23 months. The 1-, 2-, and 5-year overall survival (OS) rates of the 40 patients were 67.7 %, 49.9 % and 41.6 % respectively. Five-year OS was 57.9 % in patients received multimodality treatment, 13.1 % for the rest ($P = 0.000$), 25.1 % in those with tumor size $\geq$10cm, 48.5 % in those with tumor size $<$ 10cm (P=0.028), 50.8 % in those received complete resection, 41.7% in those underwent incomplete resection, 27.5 % in those received no surgery ($P < 0.05$), 45.9 % in those with nonmetastasis, and 35.7 % in those with metastasis ($P < 0.5$). Based on our experience and a review of the literature, we concluded that multimodality treatment was the main treatment of peripheral primitive euroectodermal tumors. Tumor size greater than 10cm, complete resection, and metastasis are prognostic factors for survival.

SMAD1 INACTIVATION CAUSED BY DECREASED EXPRESSION OF BONE MORPHOGENETIC PROTEIN RECEPTOR IB CONTRIBUTES TO GLIOMA AGGRAVATION

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In the present study, we first showed that the expression of phospho-smad1 and its upstream molecule bone morphogenetic protein receptor IB subunit (BMPR-IB) were decreased in most malignant glioma specimens and some human glioblastoma cell lines. Furthermore, the lower expression ratio of phospho-smad1 to smad1 significantly correlated with poor clinical outcome. Transient transfection of BMPR-IB to glioblastoma cells induced phosphorylation and nuclear localization of smad1 and activated downstream signal transduction as evidenced by BMP response element (BRE)-luciferase activation. FACS analysis showed that the proliferation index of the U251 and U87 cells transfected with BMPR-1B was decreased. AnnexinV staining showed the apoptosis portion was increased in cells transfected with BMPR-1B. Immunostaining with GFAP antibody indicated that overexpression of BMPR-1B induced glial differentiation of U251 and U87 cells. Along with the phenotypes, the expressions of p21, p27 and p53 proteins were upregulated by BMPR-1B overexpression. All the above effects could be blocked by co-transfection with the BMPs signaling pathway specific inhibitory smad6 plasmid. Our study suggests for the first time that the lower expression of BMPR-IB contributed to the inhibition of smad1 phosphorylation in malignant glioma development. And activation of BMPs/smad1 signaling pathway by BMPR-1B over-expression could induce growth arrest, apoptosis and differentiation of glioblastoma cells through up-regulating of p21, p27 and p53 proteins. This might provide a new molecular marker for glioma diagnosis and a new approach to glioblastoma (GBM) treatment.

ASPIRIN INDUCES LYTIC CYTOTOXICITY IN EPSTEIN-BARR VIRUS-POSITIVE CELLS

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Epstein-Barr Virus (EBV) infection in tumor cells is generally restricted to the latent forms of viral infection. Switching the latent form of viral infection into the lytic form may induce tumor cell death. High levels of nuclear factor (NF)-κB can inhibit EBV lytic replication, and aspirin has the ability to inhibit NF-κB activity. The aims of the current study were to determine the effects of aspirin on inducing EBV lytic infection, and thus to reveal the possibility of targeting EBV-positive cancer cells by aspirin. Our results showed that aspirin depleted NF-κB (p65) in the nucleus and reactivated EBV into lytic replication. Cells exhibited decreased viability in a dose- and time-dependent manner when incubated with aspirin. When ganciclovir was used in combination with aspirin to treat EBV-positive B95.8 cells and Raji cells, the cytotoxic effect of aspirin was amplified. We demonstrated that aspirin reduced the viability of EBV-positive B lymphocytes due to its ability to induce EBV lytic replication.

ASSOCIATION OF eIF3 p170 EXPRESSION WITH CHEMOTHERAPY RESPONSE OF LUNG CANCER AND ITS EFFECTS ON CISPLATIN SENSITIVITY IN LUNG CARCINOMA CELLS

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We were to investigate the expression of eIF3 p170 in lung cancer tissues and its association with chemotherapy response of lung cancer. In the present study, we collected 31 paraffin imbedded slices of lung cancer tissue from fiberscope diagnosis, 20 samples of benign lung tissues from inflammatory pseudotumor and bronchiectasis, and 10 samples of normal lung tissues from surgical operation. The protein expression of eIF3 p170 in lung-cancer tissues, benign lesion tissues, and normal lung tissues were determined by immunohistochemical staining. Our results showed that the positive ratio of eIF3 p170 expression in lung cancer tissues was higher than that in normal and benign tissues, and the lung cancer patients with a higher expression level of eIF3 p170 seemed to be more sensitive to chemotherapy. Here, we also found that down-regulation of expression of eIF3 p170 could inhibit the growth of human lung adeno-carcinoma cell A549 cells and decrease the sensitivity of A549 to cisplatin. Our data suggest that eIF3 p170 might be involved in the pathogenesis of lung cancer and it appeared to be associated with chemotherapy response of lung cancer.

RELATIONSHIP BETWEEN METHYLATION STATUS OF ERCC1 PROMOTER AND RADIO-SENSITIVITY IN GLIOMA CELL LINES

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Radio-sensitivity of gliomas determines radiotherapy efficacy. Evidence demonstrates that methylation of CpG island in the promoter region results in gene silencing. This study was designed to determine the relationship between methylation status of ERCC1 promoter and radio-sensitivity in glioma cell lines. Surviving fraction (SF2) was measured in 4 glioma cell lines (MGR1, MGR2, SF767 and T98G) by using colony forming experiment. Using bisulphate sequencing, we identified hypermethylation in the promoter region of ERCC1 in 4 gliomas cell lines. The result showed that ERCC1 gene promoter CpG islands were methylated in MGR1 and T98G cells, with a SF2 of 0.59 ± 0.09 and 0.70 ± 0.05 respectively. No methylamine of ERCC1 gene promoter CpG islands was found in MGR2 and SF767. SF2 of MGR2 and SF767 were 0.18 ± 0.05 and 0.32 ± 0.08 respectively. There was a statistical difference in the radio-sensitivity between glioma cells with or without methylamine of ERCC1 gene promoter CpG islands ($t = -4.437$, $P<0.05$). Our data indicate that methylation status of ERCC1 was associated with radio-sensitivity in gliomas cell lines. It could be used as a new biomarker for predicting the radiosensitivity of human gliomas.

LMP1-TARGET DEOXYRIBOZYME CAUSES S PHASE ARREST AND INDUCTION RADIOSENSITIVITY IN LMP1-POSITIVE CELLS

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This study was designed to determine the molecular mechanism underlying the radiosensitive effect of the LMP1-target deoxyribozyme on nasopharyngeal carcinoma cells (NPC), CNE1-LMP1, which constitutively expresses the LMP1.We observed active DNAzyme induced an S phase growth arrest correlated with reduction in Rb phosphrylation at S473, decrease in the levels of components of the cell cycle moleculars and suppression the interaction of CDK4 and cyclinD1. We showed that the DNAzyme down-regulated the expression of cyclinD1 and E, and sharply decreased in the level of E2F1. All experiments were done on CNE1 cells, which are LMP1-negative. There were no changes at all. These findings demonstrate that LMP1-target DNAzyme induced cell cycle blockade relied on modulated expression and activity of G1 and S phase associated regulatory proteins. Our results suggest that the DNAzyme induced S phase arrest may cause the LMP1-positive cells to be more sensitive, because when combined with radiation treatment, the DNAzyme significantly induced apoptosis in LMP1-positive cells. The DNAzyme may well be a clinical appealing new class of radio-sensitizing agent.

A TUMOR DOWN-REGULATED GENE NOR1 INDUCED BY HYPOXIA PROMOTE APOPTOSIS IN HELA CELLS

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Cobalt chloride could fluctuate the level of reactive oxygen species (ROS) and change the cytosol redox state. In this paper, we found the expression level of nitro oxidored reductase like gene (NOR1), which has the characteristic of reductase, a novel member of nitroreductases in cancer of cervix, nasopharyngeal, liver, stomach, colon and rectum, was significantly lower than those in their corresponding benign tissue shown with combination of tissue array and in situ hybridization (ISH) ($p<0.05$). However, we failed to indicate the correlation between tumor clinical pathology stage and the expression level of NOR1 ($p>0.05$). After induced by transition metal cobalt, NOR1 was up-regulated and the level of apoptosis of Hela was increased. Hence the effects of overexpression of NOR1 on Hela were investigated. Results showed that NOR1 was up-regulated after 12 hour with induction and maintained till 24 hour. NOR1 reduced proliferation of the Hela cells and promoted apoptosis. NOR1 also reduced the ability to form colonies in soft agar. The effects of overexpression of NOR1 in Hela were consistent with those treated by cocl2. Our results suggest that the NOR1 could suppress the tumor growth by promoting apoptosis and suppressing cell proliferation. This work was supported by National nature sciences foundation of china (No. 30400084, No. 30200312), Hunan province nature sciences foundation (No. 04JJ3096).

ARSENITE UPREGULATES EGR1 IN HaCaT CELL LINE

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Arsenite executes its carcinogenic effects by induction of signaling cascades. We analyzed whether arsenite had an impact on the biosynthesis of EGR1. Here we showed by Western blot that arsenite induced a transient synthesis of EGR1 in human keratinocyte HaCaT cell line. This arsenite triggered EGR1 biosynthesis was completely inhibited by the epidermal growth factor receptor-specific tyrosine kinase inhibitor and the mitogen-activated protein kinase inhibitor. These results indicate that activation of EGFR as well as stimulation of the mitogen activated/extracellular signal-regulated protein kinase was essential for arsenite-induced upregulation of EGR1. The fact that low dose of arsenite was sufficient to induce EGR1 biosynthesis suggests that EGR1 may be an integral part of arsenite-triggered signal transduction leading to tumor formation or cell death.

THE ASSOCIATION OF E-CADHERIN/CONNEXIN32 EXPRESSION WITH THE METHYLATION STATUS OF THE E-CADHERIN/CONNEXIN32 GENE IN Hep-G2 HEPATOCELLULAR CARCINOMA CELLS

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E-cadherin (E-cad)/Connexin32 (Cx32), a cell adhesion molecule, is regarded as an invasion-suppressor molecule and a prognostic marker in many types of human cancers. Loss of E-cad/Cx32 has been associated with progression and poor survival in hepatocellular carcinoma (HCC). This study was to clarify the role of methylation on E-cad/Cx32 inactivation in HCC cell line (Hep-G2). We examined 5' CpG island promoter methylation of E-cad/Cx32 in Hep-G2 cell line using methylation-specific PCR (MSP), and further studied the correlation of E-cad/Cx32 gene methylation with E-cad/Cx32 protein expression. We found that hypermethylation of E-cad/Cx32 was involved in Hep-G2 cell line; E-cad/Cx32 protein expression was lost in this cell line. Methylation, which was noted in this cell line, included fully methylated and partially methylated. The fully methylated cell line lacked E-cad/Cx32 protein expression; E-cad/Cx32 expression was reduced in a part of the methylated cell line but preserved in the other part. Treatment of E-cad/Cx32-negative carcinoma cells with the demethylating agent, 5-aza-2'-deoxycytidine, induced re-expression of the gene. These results suggest 5' CpG island methylation of E-cad/Cx32 gene may play an important part in the inactivation of E-cad/Cx32 in HCC; and the demethylation effect may contribute to enhancing cell adhesion through re-expression of E-cad/Cx32, which may be a potential therapeutic strategy for HCC.

METHYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISMS AND SUSCEPTIBILITY TO GASTRIC CANCER IN CHINESE POPULATIONS: A META-ANALYSIS

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Genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) gene are thought to have significant effects on folate metabolism thus on cancer risk but the reported results are not always consistent. In this meta-analysis we assessed reported studies of associations between polymorphisms of MTHFR susceptibility to gastric cancer in Chinese populations. A

computerized literature search was carried out in Chinese Biomedical Database (CBM) PubMed database to collect articles of case-control studies or cohort studies on associations between MTHFR polymorphisms susceptibility to gastric cancer up to October 2007. We also reviewed the reference lists of the relevant articles performed searching based on www.baidu.com and www.google.com. After data collection a meta-analysis was performed to assess heterogeneity combine results and evaluate variations. Different effect models were employed for the sensitivity analysis. Publication bias was examined by a funnel plot fail-safe number for P=0.05 (Nfs(0.05)). The meta-analysis for this study included 2319 cases 3316 controls from 12 studies. The pooled odds ratio (OR) for gastric cancer (all subsites) with polymorphisms of MTHFR C677T MTHFR A1298C were 1.38 (95% confidence interval (CI) 1.21 to 1.57) and 0.99 (95% CI 0.85 to 1.16) respectively. Subgroup analyses: the summary *OR* (95% CI) for cardia gastric cancer with polymorphisms of MTHFR C677T MTHFR A1298C were 1.18 (0.90−1.53) and 0.93 (0.73−1.18) respectively while for noncardia gastric cancer with polymorphisms of MTHFR C677T MTHFR A1298C were 1.21 (0.94−1.56) and 1.21 (0.59−2.48) respectively. The sensitivity analysis publication bias diagnostics confirmed the reliability stability of this Meta-analysis except the association between MTHFR C677T cardia gastric cancer in subgroup analyses. In Chinese populations polymorphisms of MTHFR might be a genetic risk factor for gastric cancer not a risk for cardia or noncardia gastric cancer. In the subgroup analysis more studies are required for definite conclusions since the number of studies is relatively small. This study was supported by Grants for Scientific Research of BSKY (xj2004007) from Anhui Medical University.

GLOBE GENE EXPRESSION CHANGES INDUCED BY NOR1 OVER-EXPRESSION IN HepG2 CELLS

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Our laboratory has previously cloned a novel gene NOR1 and showed its expression and down-regulation in carcinomas. To further investigate its downstream target genes and better understand its function, we established a NOR1 over-expressed HepG2 hepatoma cell line by gene transfection and identified globe changes in gene expressions by cDNA microarrays. We discovered 59 genes up-regulated in these cells compared with the original cells, including Grb2, HBP17, TNFRSF11B genes that have been implicated in numerous roles in tumorigenesis and cancer development. In addition, we also identified 103 genes down-regulated including genes encoding Bik, MAP2K6 and ZFP95 proteins. The expression patterns of certain genes identified by microarrays were validated by quantitative real-time PCR and showed consistent results from both methods. The quantitative real-time PCR results showed that the expression of Grb2, HBP17, TNFRSF11B in pcDNA3.1(+)-NOR1/HepG2 were respectively 4.87 ($F = 629.964$, $P<0.05$), 5.23 ($F = 2522.575$, $P<0.05$) and 6.33 ($F = 1455.404$, $P<0.05$) times higher than HepG2. There was no difference between pcDNA3.1(+)/HepG2 and HepG2 for mRNA expressions of the three genes (P_{all}?0.05). These data suggest that NOR1 may influence the biology and cancerous behaviors of HepG2 cells by exerting its effects on the expression of a set of genes involved in cell signal transduction, cell cycle regulation, transcription regulation and translation control related genes.

EFFECTS OF BONE MORPHOGENETIC PROTEIN-2 (BMP-2) ON THE SECRETION AND ACTIVATION OF MATRIX METALLOPROTEINASES IN HUMAN A549 LUNG CARCINOMA CELLS

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The present study was undertaken to investigate the action of BMP-2 on the secretion and activation of MMPs and TIMPs in A549 Cells. RT-PCR was used to detect BMP-2, BMPR-IA, BMPR-I and BMPR-II mRNA expression. MMP-2, MMP-9, TIMP-1 and TIMP-2 protein levels in cultured A549 cells media were detected by Western blot and ELISA analysis. Results from RT-PCR demonstrated the expression of BMPR-IA, BMPR-IB, BMPR-II and BMP-2 in A549 cells. Treatment with BMP-2 in different concentration (1 ng/ml, 10 ng/ml, 100 ng/ml), dose-dependently stimulated the secretion of MMP-2 and MMP-9 protein in A549 cells (vs. control group, $P<0.05$). When treated with BMP-2 (100 ng/ml) for different time durations (12h, 24h and 48h), the secretion of MMP-2 and MMP-9 protein from A549 cells increased time-dependently (vs. control group, $P<0.05$). Treatment with BMP-2 in different concentration (1ng/ml, 10ng/ml, 100ng/ml) did not change the secretion of TIMP-1 and TIMP-2 protein in A549 cells compared with control group ($P>0.05$). Furthermore, Western blot results also demonstrated that BMP-2 stimulated the secretion and activation of MMP-2 and MMP-9 in A549 cells, but had no effect on TIMP-1 and TIMP-2 protein secretion in the same cells. These data indicate that BMP-2 induced MMP-2 and MMP-9 secretion and activation in human lung carcinoma A549 cells. This work was supported by post-doctorate science foundation of People's Republic of China.

AUTOANTIBODIES AS POTENTIAL BIOMARKERS OF NASOPHARYNGEAL CARCINOMA (NPC)

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Autoantibody signatures, as new biomarkers, may improve the early detection of nasopharyngeal carcinoma. Therefore we constructed a NPC mixed tissues cDNA T7 phage library, and isolated 31 tumor-associated proteins using biopan enrichment techniques with NPC patient and normal sera. Sequence analysis showed that among the 31 phage-displayed proteins 22 had sequence identity with known or putative tumor-associated proteins. Immunochemical reactivity of patient sera with phage-expressed proteins showed enrichment on the number of immunogenic phage clones in the biopanning process and also confirmed that antibodies were present in patient sera but not in normal sera. Antibodies to four phage-expressed proteins HSP70, MAGE, fibronectin and CD44 were measured by ELISA to validate the concept that combinations had greater predictive value than any single antibody alone, and there was a positive correlation between HSP70, CD44, fibronectin and MAGE antibodies in normal sera and in patient sera ($r = 0.742$, $P<0.001$; $r = 0.835$, $P<0.001$; $r=0.851$, $P<0.001$ and $r=0.915$, $P<0.001$, respectively). Logistic regression analysis showed that combined measurements of four antibodies was more predictive of disease than any single antibody alone, the area under the curve was 0.8084 and the optimal predictive accuracy achieved was sensitivity 0.81 with specificity 0.82, underscoring the importance of identifying multiple potential markers. In the NPC group there was a significant correlation between HSP70, CD44, fibronectin or MAGE antibody levels and clinical stage ($P<0.001$), but no correlation with age or sex ($P>0.1$ for all comparisons). Our work shows autoantibodies against tumor-associated antigens derived from NPC tissue could be used as the basis for a screening test for NPC. An inventory of corresponding proteins may have significant relevance to tumor biology, novel drug development, and immunotherapy.

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THE EFFECT OF OVEREXPRESSED Daxx IN LIVER TUMOR CELLS ON THE APOPTOSIS INDUCED BY OXIDATIVE STRESS

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Death domain-associated protein (Daxx) can sensitize apoptosis of tumor cells through Fas-Daxx-JNK pathway, however, the impact of Daxx on the liver tumor cell line $HepG_2$ has not been reported. This experiment was to study the effects and mechanisms of Daxx overexpressed in $HepG_2$ on drug sensitivity in order to provide theoretic target for cancer chemotherapy. $HepG_2$ cells were transfected using lipofectamine 2000, and selected by treatment with G418. Stable cell lines were assessed for vector transfection by reverse transcriptase polymerase chain reaction (RT-PCR). The groups were as follows: (1) control group (non-transfected cells); (2) transfected with empty vector ($HepG_2$/GFP cells); and (3) transfected with pEGFP-C1-Daxx ($HepG_2$/GFP-Daxx cells). After incubation with hydrogen peroxide (H_2O_2) for 24 hours, cellular activity was analyzed by MTT; and cellular apoptosis was measured by flow cytometric analysis. Protein expression was detected by Western blot. The RT-PCR results showed that the cells transfected with pEGFP-C1-Daxx Daxx RNA was increased significantly compare with the $HepG_2$/GFP cells, and at the same time, Fluorescence microscopy showed that Daxx protein was localized in the nuclei. We used hydrogen peroxide to induce apoptosis of $HepG_2$ cells and observed that the hydrogen peroxide inhibited the activity of $HepG_2$ cells in a concentration-dependent way. The IC50 value of three groups cells (Normal cells, $HepG_2$/GFP cells and $HepG_2$/GFP-Daxx cells) were 0.72, 0.76, and 0.49 mmol/L respectively. The apoptotic ratio was significantly higher in $HepG_2$/GFP-Daxx cells when compared to the other groups. $HepG_2$/GFP-Daxx cells, which were incubated with hydrogen peroxide, showed a strong increase in the activation of caspase-3 and JNK compared with the other groups. These results suggest that over-expression of Daxx sensitized $HepG_2$ cells apoptosis induced by hydrogen peroxide. Furthermore, there may be a synergetic relation with apoptosis and increase of JNK and caspase-3 activity.

S100A11 IS HIGHLY EXPRESSED IN COLORECTAL CANCER TISSUES FOLLOWING THE DISEASE PROGESSIONS

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Early human colorectal cancers detection and treatment remain a challenge. Identification of new potential markers may help in the diagnosis of colorectal cancer. By comparative 2-dimensional gel electrophoresis of tumors and normal colorectal tissue, we identified a new protein, S100A11, which was highly expressed in colorectal cancer. In order to validate this finding and detect S100A11 expression in human colorectal cancer tissues at various stages of the tumor, Western blotting and immunohistochemical staining were employed in 89 specimens. S100A protein was expressed in the nuclei of normal tissue; however it was expressed in the nuclei and endochylema of colorectal cancer. S100A11 in colorectal cancer tissue was up-regulated following progression of the disease. These findings suggest S100A11 could be helpful in the pathological study of colorectal cancer, especially in the judgment of different stages in colorectal cancer. S100A11 could be considered as a potential marker assisting to diagnosis colorectal cancers. The result may have potential meaning in providing individualized treatment to the colorectal cancers patients.

THE ENHANCEMENT OF INHIBITION OF CISPLATIN WITH HYPERTHERMIA ON HUMAN BREAST CANCER CELL LINES OF DIFFERENT ER EXPRESSION

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Hyperthermia has been gradually added into a multimodality cancer treatment regimen to enhance the effects of radiotherapy or chemotherapy. The breast cancer is a systemic disease, so the comprehensive treatment has become the key to breast cancer treatment. According to estrogen receptor expression of tumor cells, breast cancer can be divided into hormone-dependent and hormone-independent. In clinical study, the status of estrogen receptor (ER) can suggest the prognostic information and guide endocrine therapy. Two human breast cancer cell lines were selected in this study including MCF-7 (ER-positive) and MDA-MB-453 (ER-negative). Cisplatin was administered at variable doses. Hyperthermia was administered at 43°C for 120 minutes using a thermostat-controlled water bath. The cancer cells response was assessed with the rate of growth inhibition by MTT assay and the rate of clone formation. Cisplatin combined with hyperthermia of each cancer cell line significantly inhibited the cell proliferation as compared with cisplatin alone or hyperthermia alone ($P < 0.001$), and the rate of clone formation of cisplatin combined with hyperthermia was lower than that of cisplatin alone or hyperthermia alone. The cisplatin dose required to control 50% of the tumors (IC50) was 31.91 μg/ml of MCF-7 and 32.78 μg/ml of MDA-MB-453; which was reduced to 22.55 μg/ml and 22.12 μg/ml respectively when combined hyperthermia was treated. The heat sensitivities of the two breast cancer cell lines with different ER expression status were not different statistically ($p=0.981$). These results suggested that Hyperthermia (at 43°C for 2 hours) could significantly enhance the anti-cancer effect of cisplatin irrespective of the ER status.

TGF β1-INDUCED GST-π MIGHT BE CORRELATED WITH SMAD4 AND ERK SIGNAL PATHWAY

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In our earlier studies, we found that TGFβ1 could enhance the invasion and metastasis of gastric carcinoma and induce the expression of GST-π, a well-known molecule correlated with multidrug resistance *in vitro*. To further explore the relationship between them, we tested their expression in gastric carcinoma tissue with immunohistochemistry assay. As a interesting result, we found that the expression of GST-π was correlated with stages of tumor, and promoted tumorigenesis and the expression of TGFβ1 positively *in vivo.* To investigate the mechanisms of TGFβ1-induced GST-π, we used Smad4 siRNA and special inhibitor of MAPK signal pathway to explore the role of these signal pathways in this biological effect. As a result, we found that expression of GST-π induced by TGFβ1 could be inhibited by Smad4 siRNA and PD98059 but not SB203580 and SP600125, indicating that TGFβ1 could induce the expression of GST-π through Smad4 and ERK signal pathway. We concluded that TGFβ1 might induce the expression of GST-π through Smad4 and ERK signal pathway and promote the invasion and metastasis of gastric carcinoma.

DIFFERENTIAL PROTEOMICS RESEARCH OF CAMPTOTHECIN ANALOG NSC606985 INDUCING APOPTOSIS IN U937 CELLS: PHOSPHORYLATION OF THREE KINDS OF CYTOSKELETON PROTEINS

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We reported previously that NSC606985, a camptothecin analog, induced apoptosis of acute myeloid leukemia (AML) cells through proteolytic activation of protein kinase Cδ. In order to explore further the molecular mechanisms of NSC606985 inducing AML cell apoptosis, the technology of differential proteomics was used to analyze the differential proteins expression in U937 cells treated with (36 hours) or without 50nM NSC606985 and 28 deregulated proteins were identified. Cytoskeleton proteins occupied a great proportion of these differential proteins. We used western blot and two-dimensional gel electrophoresis plus western blot to validate the changes of cytoskeleton proteins in 2D-GE. The results revealed that protein expressions of β-tubulin, β-actin and transgelin-2 were not changed. In the control group, these cytoskeleton proteins had several isoelectric points. After treated by NSC606985, the isoelectric points in the basic end transferred to the acid end and each of these cytoskeleton proteins had only one isoelectric point in the experimental group. Because phosphorylation can induce isoelectric points to move to left, we presumed that these cytoskeleton proteins were phosphorylated. When the total proteins of the experimental group were treated by alkaline phosphotase, the great mass of β-tubulin, β-actin and transgelin-2 transferred to the basic end. It was according with the characteristic of phosphorylated proteins, proving that these three cytoskeleton proteins were phosphorylated in the apoptotic process which was induced by NSC606985. Because the preliminary work showed that active cleaved PKCδ played a key role in the apoptotic process and all of these three cytoskeleton proteins had recognizing motif, we examined whether rottlerin (a specific inhibitor of PKCδ) pretreatment could inhibit the phosphorylation of these three cytoskeleton proteins. The results showed that rottlerin could totally inhibit the phosphorylation of β-tubulin and β-actin, but it was unable to inhibit the phosphorylation of transgelin-2.

THE RELATIONSHIP BETWEEN THE TNF-A AND NO LEVEL IN SERUM AND BIOLOGICAL BEHAVIOUR IN MALIGNANT TUMORS OF LARYNX.

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Laryngeal pre-cancer has the capability to canceration. For example, vocal fold leukoplakia, adults laryngeal papilloma, gradually developed from epithelium to carcinoma in situ. In this study, we tried to judge the degree of patients having cancer of larynx and Laryngeal pre-cancer to judge the index of their biological behavior. We detected the TNF-a and NO's level in healthy adult, Laryngeal pre-cancer (vocal fold leukoplakia, laryngeal papilloma) and cancer of larynx. The results show that the TNF-a and NO's level in cancer of larynx were obviously increased ($p<0.01$, $p<0.05$). The TNF-a and NO's level in cancer of larynx were decreased ten days postop, but compared with the vocal fold leukoplakia and laryngeal papilloma, there was no difference ($p>0.05$); 1 month postop, their levels obviously were back to normal. Our results indicate their levels in Laryngeal pre-cancer were all below the cancer of larynx's. Following improve the day's extension, the TNF-a and NO's level in cancer of larynx were obviously loss after the operation. In laryngeal lesions process, TNF-α and NO contents in Cancer of larynx sufferer were obviously higher than that of health adult and larynx pre-cancerous change sufferer. The formation and proliferation of tumor was related to some cell mediation factors in some research. TNF-α is a kind of soluble protein cytokine in certain condition that was secreted by mononuclear phagocyte system, NK cell and some tumor cell. Being a new kind of cell messenger NO becomes the key point of inflammation net and has the function of inflammatory mediator and cytotoxicity. The process from Laryngeal pre-cancer to cancer of larynx, the composition and releasing of the TNF-a and NO's level were increased. Detection of their levels can survey the change and prognosis in patients of Laryngeal pre-cancer and cancer of larynx, and evaluate the degree of laryngeal affection and the index of biological behavior.

INHIBITION OF LS-174T CELL GROWTH AND ACTIVITY OF TELOMERASE *IN VITRO* AND *IN VIVO* BY ARSENIC TRIOXIDE

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Arsenic trioxide (As_2O_3) shows a significant therapeutic effect upon acute promyelocytic leukemia (APL) and can induce the apoptosis of NB_4 cells, which attracts scholars' great attention. Especially, the therapeutic effect on solid carcinoma has been paid more close attention to. The present study was to evaluate the effect of As_2O_3 on human colorectal carcinoma cells (LS-174T cell) and the activity of telomerase *in vitro* and *in vivo*. This research made use of the electron microscope (EMS), PCR-Elisa, flow cytometry (FCM), MTT *in vitro* and *in vivo* (LS-174T xenograft model of nude mice). With increasing concentration of As_2O_3, the ratio of living cells to dead cells decreased significantly, and the IC_{50} value was 5.23 μmol/L; cells of the experimental groups could endure a series of morphological changes similar to the features of apoptosis. Apoptosis curve of FCM pictures appeared after 24h, and the cells showed the apoptosis in a time-dependent manner. As_2O_3 could inhibit the activity of telomerase of the cell extraction obviously in a concentration-dependent and time-dependent manner after 24 h. As to the inhibition impact of As_2O_3 on the xenograft model of nude mice in the two indexes, tumor volume and weight, there was a significant difference between As_2O_3 and the control group ($p<0.05$), and there was no difference between As_2O_3 and the 5-Fu group ($p>0.05$); in the group of peritoneal injections of As_2O_3, the cancer cells connected loosely with each other, nucleus changed markedly, and heterochromatin concentrated under the nucleus membrane. From the experiment *in vitro* and *in vivo*, we could see that As_2O_3 inhibited LS-174T cell growth mainly by inducing cell apoptosis, partly by inhibition of telomerase activity.

STAT3 INDUCED BY EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 CAUSES VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION AND CELLULAR INVASIVENESS VIA JAK3 AND ERK1/2 SIGNALING

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The principal EBV oncoprotein, latent membrane protein 1 (LMP1), has been suggested to contribute to the highly metastatic nature of Nasopharyngeal carcinoma (NPC). STAT3 is a master transcriptional regulator in proliferation and apoptosis and newly implicated in angiogenesis and metastasis, which, in turn, are likely to contribute to the highly metastatic character of NPC. The fundamental molecular mechanisms of LMP1-regulated STAT3 activation in NPC metastasis have not been completely elucidated. Here, we showed that LMP1 signaled through the Janus kinase 3 (JAK3) and extracellular signal-regulated kinase 1/2 (ERK1/2) pathway on the activation of Stat3. LMP1 stimulated STAT3 Tyr 705-dependent nuclear accumulation as well as STAT transcriptional activity. In addition, LMP1 induced vascular endothelial growth factor (VEGF) expression via the JAK/STAT and MAPK/ERK signaling pathway, whereas expression of Stat3 small interfering RNA or antisense oligonuceotides (ASO) reversed LMP1-induced VEGF expression. Moreover, LMP1-specific DNAzyme, JAK3-specific inhibitor WHI-P131, the MEK1 inhibitor PD98059 inhibited induction of VEGF by LMP1. Furthermore, these results suggest that STAT3 may play an important role in cancer cell invasion and induction of STAT3 by a human viral oncoprotein LMP1 may directly contribute to the metastatic nature of NPC.

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INHIBITORY EFFECT OF CARDIAC MUSCLE CELL CONDITIONED MEDIUM ON S-180 MICE-TRANSPLANTED TUMOR *IN VIVO*

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We set out to study the inhibitory effect of the cardiac muscle cell conditioned medium (CMCM) on S-180 mice-transplanted tumor *in vivo*. To establish the modal of S-180 mice-transplanted tumor, the experiment group CMCM was injected in 30 mg/kg, 60 mg/kg and 90 mg/kg, respectively, into tumor burden mice by i.p. once a day for 14 days. A negative control group was similarly treated with 0.9 % NS, and the positive control group was treated with cisplatin (DDP, 2mg/kg) once every other day. After 15 days, the mice were killed to observe the inhibitory effect of different groups on S-180 mice-transplanted tumor. The inoculation survival rate of S180 mice-transplanted tumor was 100%. The time of the tumor formation in DDP, CMCM 30 mg/kg, CMCM 60 mg/kg and CMCM 90 mg/kg were 7.70 ± 0.82d, 7.10 ± 1.10d, 7.20 ± 1.03d and 7.30 ± 0.82d. All of them were later than 5.90 ± 0.88d in 0.9 % NS group ($P<0.05$). The growth curve of S-180 mice-transplanted tumor went up sharply in 0.9 % NS group, and the others went up only a little. The weights of tumor and the inhibitory rate of tumor in DDP, CMCM 30 mg/kg, CMCM 60 mg/kg, CMCM 90 mg/kg group were 0.2705 ± 0.1102 g, 0.4812 ± 0.1235 g, 0.4493 ± 0.1168 g, 0.4496 ± 0.1080 g and 81.23 %, 66.61 %, 68.82 % and 68.80 %, respectively. The differences between every group mentioned and control group (1.4412 ± 0.4275g) in tumor weights were statistically significant ($P<0.001$). And there were not obviously dose dependence in CMCM groups. Compared with negative group, the growth of mice was inhibited in DDP group ($P<0.05$), and increased in CMCM groups ($P<0.05$). In conclusion, CMCM could inhibit the proliferation of S180 cells *in vivo* without side effect. It might be one of the mechanisms of the rarity of metastasis in cardiac muscle.

EXPRESSION AND CLINICAL SIGNIFICANCE OF DNA-PK IN NASOPHARYNGEAL CARCINOMA

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DNA double-strand break (DSB) is the main mechanism of the tumor death after ionizing irradiation. Homologous recombination (HR) and DNA nonhomologous end-joining (NHEJ) are the two important repair ways of DSB. DNA-PK is an essential protein of NHEJ, and plays a major role in the repair of DSB. In this study, we detected the expression of Ku70, Ku80 and DNA-PKcs (the three submits of DNA-PK) in Nasopharyngeal Carcinoma (NPC) by immunohistochemistry, and analyzed the correlations of expression of the three to the clinicopathologic and prognosis. We found that the overexpression rates of Ku70, Ku80 and DNA-PKcs were 64 %, 44 % and 37 %. The three proteins were positively related to each other ($P<0.01$). They all had significant correlations to TNM staging ($P<0.05$). Moreover, Ku70 and DNA-PKcs had significant correlations to T category ($P<0.05$), Ku70 and Ku80 had significant correlations to N category ($P<0.05$), and Ku80 and DNA-PKcs had significant correlations to M category ($P<0.05$). Kaplan-Meier survival analysis suggested that expression of Ku70 and DNA-PKcs had significant influence on the overall survival rate ($P<0.05$). Cox multivariate analysis indicated that TNM, T, N M category and the expression of DNA-PKcs were independent predictors of prognosis of the patients ($P<0.05$). These results suggest that DNA-PK was the important factor which affected the prognosis of NPC. Therefore, we propose that DNA-PK acts as a prognostic predictor of NPC.

MORPHOLOGICAL CHANGES AND MOLECULES EXPRESSIONS OF HEPATOCELLULAR CARCINOMA CELLS IN 3D CELL CULTURE MODEL

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Hepatocellular carcinoma (HCC) is a highly malignant tumor for being correlated with the breakdown of extracellular matrix by HCC cells, so the routine two-dimensional (2D) monolayer cell culture method cannot provide a well-defined microenvironment for HCC research. HAb18G/CD147, an HCC-associated antigen, plays important roles in HCC progression, migration and invasion. Here, we investigated whether HAb18G/CD147 enhanced the HCC migration and invasion through affecting the key molecules or enzymes such as focal adhesion kinase (FAK), matrix metalloproteinases (MMPs) and cytoskeleton proteins involving in the metastatic processes by using three-dimensional (3D) cell culture model. Results showed that expression of HAb18G/CD147 was significantly increased accompanying with the high production of MMPs ($P<0.001$), the enhanced expression and activation of FAK ($P<0.001$) and the changed distribution of F-actin in 3D cell culture model as compared with that in 2D cell culture model. In addition, the expressions of paxillin and E-cadherin, which enhance the adhesion and migration potentials, were also obviously increased in 3D cell culture model ($P<0.001$). All the results suggest that in the 3D reconstituted BM, enhanced expressions of HAb18G/CD147, MMPs, paxillin and FAK changed the distributions of cytoskeleton and increased the adhesion and invasion potentials of HCC cells. The 3D cell culture model provides a more defined microenvironment for HCC research.

EXPRESSION OF NPCM CORRELATES WITH RADIOSENSITIVITY AND METASTASIS IN NASOPHARYNGEAL CARCINOMA

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NPCM is a novel gene, whose structure is similar to uPAR. Many studies have shown that uPAR played an important role in metastasis and had close association with prognosis in many tumors. Dose NPCM have the same function as uPAR? It is still a question under discussion. In this study, we investigated the relationship of NPCM to the radiosensitivity and metastasis of nasopharyngeal carcinoma (NPC) with cellular experiments. With RT-PCR, We found higher expression of NPCM in nasopharyngeal carcinoma cell than in normal nasopharyngeal epithelial cell. With RT-PCR and western-blot, we compared expression of NPCM in different radiosensitivity nasopharyngeal carcinoma cell lines CNE1 and CNE2 (the radiosensitivity of CNE2 is higher than CNE1) and found that the expression of NPCM in CNE2 was higher than in CNE1, but there was no alteration of NPCM expression after irritated by different dose of X rays in either cell line. Similarly, with RT-PCR and western-blot, we compared expression of NPCM in different metastatic ability nasopharyngeal carcinoma cell lines 5–8F and 6–10B (the metastatic ability of 5–8F is higher than 6–10B) and found that the expression of NPCM in 6–10B was higher than in 5–8F, but there was no alteration of NPCM expression after irritated by different dose of X rays. These results suggest that NPCM correlated with radiosensitivity and metastasis in nasopharyngeal carcinoma. The other action of NPCM on nasopharyngeal carcinoma requires further investigation.

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INDUCTION OF DIFFERENTIATION AND APOPTOSIS IN MICE HEPATOCARCINOMA H22 CELLS INDUCED BY MICE EMBRYO EXTRACTIVE

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Differentiation induction is a therapy for cancer. Searching for non-toxic and natural origin substances that induce the differentiation of cancer cells is a key for anticancer therapy. We investigated the effect of mice embryo extractive (E) on proliferation, differentiation, and apoptosis of H22 cells. In order to observe morphologic change of H22 cells, Giemsa stain was used. The nuclear-cytoplasmic ratio and nuclear size of H22 cells were declined after treated with 2.5 % E contrast with control group and apoptosis cells were found. The result of MTT experiment showed that extractive (E) could effectively inhibit the proliferation of H22 cells in a dose-dependent and time-dependent manner. The activity of γ-GT decreased ($P<0.05$) and the activity of ALP increased ($P<0.05$) in the cells treated with 2.5 % E after 120 hours compared with control group, indicating that the H22 cells differentiation degree was higher than control group's. FCM analysis showed that apoptosis rate of E group was higher than control group's ($P<0.01$).

EFFECTIVE ANTI-TUMOR RESPONSES ON NUDE MICE MODEL OF BREAST CANCER BY IMMUNOTHERAPY USING BREAST TUMOR-LYSATE PULSED DENDRITIC CELLS

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Dendritic cells (DCs) loaded with breast tumor-lysate were injected into Balb/c female nude mice inoculated with MCF-7 breast tumor cells in order to investigate the effects of DCs' immunotherapy. A nude mice model of human breast cancer had been established by inoculating MCF-7 tumor cells in situ. Mononuclear cells (MNCs) were separated from health heparinized cord blood by Hyapque density gradient centrifugation, and MNCs were cultured in vitro by using RPMI1640 medium supplemented with GM-CSF, IL-4 and TNF-α. DCs were harvested at day 12, and cell counting and flow cytometry analysis were carried out. The capacity of DCs was assessed by the mixed leukocyte reaction (MLR). 65 tumor bearing nude mice models were randomized into 5 groups. Operations were carried out in group 1 to 4 to remove the solid tumors. Group 1 received DCs and CBMNCs, group 2 received CBMNCs, group 3 received DCs, group 4 received PBS, and group 5 received PBS only without operation. Tumor size was assessed by measuring the long (a) and short diameters (b) of tumors using calipers every three days and the tumor volume was calculated by $1/6лаb^2$. Totally 4 mice died of operation in group 3 and 4. After immunotherapy for 3 months, a high survival rate was shown in group 1 (93.33 %) which was injected with DCs and CBMNCs and it was statistically significant when compared to group 4 (60%) which was injected with PBS only ($P=0.026$, by a Chi-square test). In contrast, the tumor recurrence rate of group 1 (40%) was markedly lower than group 4 (77.8%) and group 3 (87.5%), and was statistically significant compared to group 4 ($P=0.027$,by a Chi-square test) and group 3 ($P=0.029$, by a Chi-square test). One-way ANOVA about the tumor volumes among these groups showed statistic significance ($P= 0.004$, by least-significant difference test) between group1 (516.66 mm^3) and group4 (5052.85mm^3). The tumor volume of group 5 (10285.73 mm^3) was the biggest and data showed statistic significance ($P<0.05$, by least-significant difference test) compared to other four groups. In conclusion, our results show that vaccination of DCs pulsed with tumor lysates could induce anti-tumor responses in nude mice model of breast cancer.

THE PRELIMINARY RESEARCH ON THE RISK FACTORS OF THE IB LEVEL LYMPHATIC NODES METASTASIS IN RECURRENT NASOPHARYNGEAL CARCINOMA

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To find some predictable risk index for Ib level lymphatic nodes metastasis in recurrent NPC (nasopharyngeal carcinoma), 51 patients with pathologically confirmed recurrent NPC treated in the cancer center of Sun Yat-Sen University from 1995 to 1998 were studied. We selected factors as the local invasion and the expression levels of VEGF, P53, MDM2, $P21^{WAF1}$, $P21^{RAS}$ proteins were analyzed. There were 9 cases with Ib level lymphatic nodes metastasis. The Ib level lymphatic nodes metastasis rates had no significant difference between the different sex, T stages, N stages and TNM stages. The Ib level lymphatic nodes metastasis rate was 30.4 % (7/23) in the group with the nasal cavity invasion, only 7.1 % (2/28) in the group without nasal cavity invasion, but the difference was not significant in statistics ($P=0.061$). When we divided the patients into 2 groups of high and low expression level of VEGF protein, or P53, MDM2, $P21^{WAF1}$, $P21^{RAS}$ protein, the Ib level lymphatic nodes metastasis rates between high and low expression groups had no significant difference. When the NPC invaded the nasal cavity coordinated with high expression level of VEGF, the Ib level lymphatic nodes metastasis rate (37.5 %) (6/16) was significantly higher than others (8.6 %)(3/35) ($P<0.05$). When we used other index like P53, MDM2, $P21^{RAS}$, or $P21^{WAF1}$ protein to replace VEGF, the difference of Ib level lymphatic nodes metastasis rate were not significant statistically. We concluded when the NPC invaded the nasal cavity coordinated with high level expression of VEGF, the Ib level lymphatic nodes metastasis rate rose significantly in recurrent NPC.

HYPOXIA INDUCIBLE FACTOR-1 INFLUENCES SENSITIVITY TO PACLITAXEL OF HUMAN LUNG CANCER CELL LINES UNDER NORMOXIC CONDITIONS

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Paclitaxel (PTX) is an anticancer drug that is effective against a wide range of solid tumors. The effect of PTX on two human lung cancer cell lines, PC14PE6 and NCI-H441 cells, was examined in an orthotopically transplanted animal model with an in vivo imaging devise. Although PTX effectively suppressed tumor growth and improved survival rate in NCI-H441, it did not influence these in PC14PE6. *In vitro* experiments confirmed that PC14PE6 cells were resistant to PTX under normoxic conditions and that both cell lines were resistant to PTX under hypoxic conditions. It was found that the expression level of endogenous hypoxia inducible factor (HIF)-1α in PC14PE6 was much higher than that in NCI-H441 cells under normoxic conditions. Furthermore, sensitivity to PTX in these cell lines was reversed when HIF-1α expression was decreased by siRNA specific to HIF-1α in PC14PE6 and increased by overexpression of the exogenous HIF-1α gene in NCI-H441. These results suggest that HIF-1 influenced the PTX sensitivity of these cells. The authors further examined β-tubulin, a target molecule of PTX, with western blotting and immunohistochemical analysis in these cells. The expression level of β-tubulin was comparable in these cells under both normoxic and hypoxic conditions while the distribution of β-tubulin and cell morphology were changed according to HIF-1α expression levels, suggesting that HIF-1 influenced the conformation and dynamics of microtubules. These data support the potential development of HIF-1 targeted approaches in combination with PTX, where drug resistance tends to contribute to treatment failure.

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INFLUENCE OF IMBALANCED AMINO ACIDS ON TUMOR GROWTH IN TUMOR-BEARING RATS DURING CHEMOTHERAPY

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To investigate the effect of complex amino acids of arginine-enriched and leucine-enriched on tumor growth in tumor-bearing rats during chemotherapy, the SD rats were each given a catheterization of jejunostomy and an inoculation of Walker-256 carcinosarcoma cells subcutaneously. Thirty-six rats were randomized into 3 groups: Group A (balance amino acid+NS), Group B (balance amino acid + 5-Fu), and Group C (complex amino acids of arginine-enriched and leucine-enriched + 5-Fu). Tumor weight, tumor inhibitory rate, the expression of PCNA and apoptosis, cell cycle were investigated. In addition, rat's survival time was observed. The G0/G1 phase ratio, apoptosis index and survival time increased from group A to C. Conversely, The S phase ratio, PCNA index and tumor weight decreased from group A to C, respectively. Our results suggest that complex amino acids of arginine-enriched and leucine-enriched could inhibit tumor growth and enhance the anti-tumor effect of 5-Fu chemotherapy.

ARSENITE INTERFERES WITH P53 RESPONSE TO DNA DAMAGE

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Whereas it is well-known that arsenic compounds can act as co-carcinogens in promoting tumorigenesis, mechanisms underlying such a role of arsenite remain not fully understood. We reported here that arsenite exposure was associated with p53 accumulation in the cytoplasm. Through MAPK pathway, arsenite stimulated P1 promoter-mediated expression of Hdm2, which then induced p53 nuclear export. Significantly, arsenite impeded the p53 response to additional form of genotoxic stress, as evidenced by impaired p53 activation and apoptotic response to 5-Fu and UV radiation when arsenite was present. Taken together, our data indicate that arsenite exerted its co-carcinogenic effect by, at least in part, upregulation of Hdm2 and subsequent p53 functional inactivation.

IDENTIFICATION OF DISREGULATED CELL CYCLE PATHWAY IN NASOPHARYNGEAL CARCINOMA BY GENE SET ENRICHMENT ANALYSIS

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To characterize the globally affected pathways in nasopharyngeal carcinoma (NPC), we performed GSEA to analyze the gene expression profiles in 39 NPC and 13 non-cancerous nasopharyngeal epithelium (NPE) by the Human Genome-U133 Plus2.0 GeneChip. Through GSEA analysis, 164 gene sets were down-regulated in phenotype NPC and 751 gene sets were up-regulated in phenotype NPC. In the 751 up-regulated gene sets, Cell Cycle pathway was the most disregulated pathway in NPC (p=0.000, FDR q-value=0.007) by GSEA analysis. The aberrant expression of the Cell Cycle pathway components, such as p16, p27, p19, CDK4, Cyclin D1, Rb, CDK8 and DP2, were validated by the NPC tissue maicroarrays (TMA) which contains 174 non-cancerous NPE and 274 NPCs with different morphologic features. We found that overexpression of CDK4, Cyclin D1, Rb proteins and loss expression of p16, p27 and p19 were statistically significant in NPC tissues compared with non-cancerous NPE ($p<0.05$). The positive expression of EBER-1 hybridization signals in NPC had significant associations with overexpression of Rb (p=0.000), Cyclin D1 (p=0.002), CDK4 (p=0.003) and loss expression of p16 proteins (p=0.039). In the final logistic regression analysis model, the positive hybridization signals of EBER-1 and the abnormal expression of p16, Rb, Cyclin D1 and E2F6 were independent contributions for nasopharyngeal carcinogenesis. The data suggest that Cell Cycle pathway may be abnormally regulated in NPC, which provides insight into the molecular mechanisms of NPC. This work was supported by a grant from The National Key Project of Scientific Research Program (2006CB910502, 2006CB910504), The Key Program of The National Natural Science Foundation of China (30770825), The National Natural Science Foundation of China (30700469), New Century Excellent Talents in University (NECT 04 - 0761), a Foundation for the Author of National Excellent Doctoral Dissertation of PR China (200559), Hunan Provincial Natural Science Foundation of China (06JJ20013), the Special Funds of Science & Technology Departments of Hunan Province, China (05SK1001_1).

ANTINEOPLASTIC EFFECT OF SCUTELLARIAE DARBATAE D.DON EXTRACT IN EXPERIMENTALLY INDUCED TONGUE CARCINOMA

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This present study was to explore the antineoplastic effect of an extract from Scutellaria barbatae D.DON in Golden hamster tongue carcinoma induced by DMBA. 1 % DMBA solution dissolved in acetone was applied topically to the left margin of the tongue of Golden hamster 3 times per week for 6 weeks. After the last treatment of DMBA, an extract from Scutellaria barbatae D.DON was applied topically 2 times per day for 18 weeks. The extract from Scutellaria barbatae D.DON significantly decreased the oral visible tumor incidence and the squamous cell carcinoma (SCC) incidence. The extract from Scutellaria barbatae D.DON also decreased the number of visible tumors and the tumor volume as well as the numbers of SCC, dysplasic lesions, and papillomas respectively. From this study, we can suppose that constant application of extract from Scutellaria barbatae D.DON could inhibit the development of tongue carcinoma induced by DMBA in Golden hamster to some extent and such inhibitory effect seemed to be enhanced with time. The mechanism of the extract from Scutellaria barbatae D.DON inhibitory effect may be related to the inhibition of the proliferation of the tongue epithelium and the induction of apoptosis.

EXPRESSION OF Notch1, HIF-1, VEGF AND Notch1 mRNA IN HUMAN NON-SMALL CELL LUNG CANCER AND ITS SIGNIFICANCE

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To determine the expressions of Notch1, HIF-1, VEGF and Notch1 mRNA in human non-small cell lung cancer (NSCLC) and its clinical pathological significance, immunohistochemical SP method and in situ hybridization were used to detect the expression of Notch1, HIF-1, VEGF and Notch1 mRNA in 65 patients with NSCLC and 15 normal epithelial tissues of the lung, and the relationship between them and clinicpathological parameters were analyzed. The positive rates of Notch1, HIF-1, VEGF and Notch1 mRNA in NSCLC were 81.5 %; 96.9 %; 93.8 % and 73.8 % respectively, and were higher than that in normal epithelial tissues of the lung ($P<0.05$). Their positive expression levels were associated with tumor stage and lymph node

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metastasis (P<0.05). The expressions of Notch1, HIF-1 and VEGF protein were positively correlated with each other (P<0.05), and the expressions of Notch1 protein and Notch1 mRNA were positively correlated with each other. The enhanced expression of Notch1, HIF-1, VEGF protein and Notch1 mRNA in NSCLC suggest that they may play important roles in the pathway of carcinogenesis and progression of NSCLC, which may serve as important prognostic factors in invasion and metastasis of NSCLC.

STUDY ON THE REGULATION OF PKCα ON APOPTOSIS INHIBITED BY FGF-2 IN SMALL CELL LUNG CANCER

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Basic Fibroblastic Growth Factor (FGF-2) plays an important role in the development of tumor. But the mechanism remains to be determined. The NCI-H446 cells were treated with or without 50 ng/ml FGF-2 and pretreated with Calphostin C (special PKC inhibitor), then the surviving and PKCα in the whole protein and the PKCα in the membrane protein were detected by Western blot and the apoptosis was detected by flow and Hochest fluorescein stain. When NCI-H446 cells were treated with FGF-2, expression of survivin increased, expression of PKCα in total protein did not change and that in membrane protein increased, and apoptosis was reduced. When NCI-H446 cells were pretreated with PKC inhibitor, expression of survivin reduced, expression of PKCα in total protein did not change and that in membrane protein reduced, and the apoptosis became increased. FGF-2 can further activate PKC, increase the expression of survivin and inhibit the cells apoptosis in small cell lung cancer NCI-H446 cells. PKC may play an important role in this process. This work was supported by the grants from the Provinal Natural Science Foundation of Hunan (No. 06JJ2098).

PHOSPHORYLATION OF SURVIVIN THR34 BY P34CDC2 IN THE CARCINOGENESIS OF ORAL SUBMUCOUS FIBROSIS

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Oral squamous cell carcinoma (OSCC) is the eighth most common cancer worldwide. Oral submucous fibrosis (OSF) is a chronic precancerous condition, with major distribution in South-East Asia, India, Taiwan and Mainland China. Survivin, one of the inhibitors of apoptosis protein (IAP), is focused due to its unique prognostic and therapeutic potential. Up-regulation of survivin in the tissues of OSF and OSCC originated from OSF has already been demonstrated in our previous study. Survivin Thr34 phosphorylation is an important functional form involved in the inhibition of apoptosis. To determine the potential involvement of survivin phosphorylation in the carcinogenesis of OSF, 40 OSFs, 42 OSCCs originated from OSF and 10 normal tissues from surgical specimens were studied. Immunohistochemistry showed that the positive staining rate of the survivin phosphorylation on Thr34 in the OSCC originated from OSF group was significantly higher than that in the OSF group ($P<0.01$), and the normal oral mucosa specimens exhibited negative staining. The survivin phosphorylation on Thr34 was predominantly located in the nucleus, which could account for its function in apoptosis at cell division. Western blotting analysis further confirmed the phase expression of survivin Thr34 phosphorylation in the carcinogenesis of OSF. Furthermore, p34cdc2-cyclin B1 kinase was confirmed to phosphorylate survivin on Thr34 in the carcinogenesis of OSF by immunoprecipitation and immunoblot. These results suggest that phosphorylation of survivin on Thr34 critically regulated survivin and may play an important role during the malignant transformation of OSF, which will provide us an indication to early diagnosis and therapy in the carcinogenesis of OSF.

IDENTIFICATION OF CANDIDATE MOLECULAR MARKERS OF NASOPHARYNGEAL CARCINOMA BY MICROARRAY ANALYSIS OF SUBTRACTED cDNA LIBRARIES CONSTRUCTED BY SUPPRESSION SUBTRACTIVE HYBRIDIZATION

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Nasopharyngeal carcinoma (NPC) is a malignancy of epithelial origin, occurring a high incidence in southern China. Although evidence regarding to cytogenetic and molecular genetic alterations in NPC has been reported, the molecular events affecting the biologic characteristics of this tumor, including alterations of the gene expression profile, are largely unknown. To identify differentially expressed genes and scan candidate molecular markers in NPC, we constructed 4 subtracted cDNA libraries using suppression subtractive hybridization technique, and then randomly picked about 1200 colonies from the libraries to construct cDNA microarray. Through analyzing the gene expression profile in 19 NPCs, 3 NPC derived cell lines, and 10 chronic inflammation of nasopharyngeal mucosa tissue samples using the cDNA microarray, we found 37 highly expressed colonies and 68 poorly expressed colonies in NPC. Thirty-two known genes were identified by sequencing the 105 differentially expressed colonies in NPC. PLUNC and CDC37L1 genes had higher frequency than others in the 68 poorly expressed colonies in NPC. The frequency of STAT5A gene was the highest in the 37 highly expressed colonies in NPC, followed by RAB25 and SPARC genes. We used real-time quantitative reverse transcription-PCR and in situ hybridization techniques to confirm that our microarray results were reliable. The data suggest that PLUNC and CDC37L1 genes might be the putative molecular markers of NPC. This is the first report that there was a close relation between CDC37L1gene and NPC. This work was supported by National Key Project of Scientific Research Program (NO.2006CB910502, NO.2006CB910504); National Natural Sciences Foundation of China, Grant numbers: 30700469; the Special Funds of Science & Technology Departments of Hunan Province, China (05SK1001_1).

LTF, A CANDIDATE TUMOR SUPPRESSOR GENE IN METASTATIC NASOPHARYNGEAL CARCINOMA AT 3P21.3, INHIBITS PROLIFERATION OF NASOPHARYNGEAL CARCINOMA CELLS *IN VITRO* BY MODULATING THE MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY

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Using cDNA microarray analysis, the lactotransferrin gene, mapping close to a previously defined 3p21 nasopharyngeal carcinoma (NPC) critical region, was identified as showing consistent downregulated expression in NPC, as compared to non-tumor nasopharyngeal epithelial tissues. Utilizing a tissue microarray and immunohistochemical staining, 54.19 % of the NPC cases showed downregualated expression of LTF. The frequency of LTF downregulated expression in lymph node metastasis NPC was 58.42 %, which was significantly higher than that in primary tumor (46.36 %). Gene expression and protein analyses showed that LTF was downregulatedly expressed in NPC. In addition, the results of cell transfection and cell growth curve assays showed that overexpression of LTF negatively affected cell growth. These findings suggest that LTF was a good candidate tumor suppressor gene in NPC, which was significantly associated with lymphnode metastases. To investigate the mechanism of LTF to NPC, we studied underlying functions of LTF inhibited NPC cells *in vitro*. The results of flow cytometry for cell cycle analysis showed that LTF induced growth arrest at G0-G1 phase of the cell cycle in 5−8F, CNE1, and HNE1 cells. To assess proliferation-suppressing effect of LTF in NPC cell lines, we chose MTT assay to observe it. The results of MTT assay showed that LTF significantly inhibited 5−8F, CNE1, and HNE1 cell

proliferation after treated with lactoferrin (10 μmol/L) for 24 hours and 48 hours respectively, and the results of western blot showed that LTF inhibited proliferation of nasopharyngeal carcinoma cells in vitro by modulating the mitogen-activated protein kinase pathway. This work was supported by National Key Project of Scientific Research Program (NO.2006CB910502, NO.2006CB910504); National Natural Sciences Foundation of China, Grant numbers: 30700469; the Special Funds of Science & Technology Departments of Hunan Province, China (05SK1001_1); the national innovative experimental plan for undergraduate (YA07050).

EPITHELIAL FUNCTIONING AND RELATED DISEASES

EFFECT OF CALCITONIN GENE-RELATED PEPTIDE ON E-CADHERIN EXPRESSION IN HUMAN BRONCHIAL EPITHELIAL CELLS

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The function of the human bronchial epithelial cells (HBECs) is to act as barriers which can protect the underlying tissue against allergens, irritants, viruses, and microbial pathogens. The effect of the barriers relies on epithelial integrity which in turn is dependent on intercellular adhesion. Epithelial cadherin (E-cd) is an important adhesion molecule leading to adhesion between HBECs. Neuropeptide is constitutively expressed in normal lungs where it localizes to neuroendocrine cells. Among lung neuropeptides, Calcitonin gene-related peptide (CGRP) plays a prominent role for its multiple effects on tissue repair and anti-inflammatory actions. The objective of this study was to investigate the effects of CGRP on E-cd expression of HBECs *in vitro*. The experiments included following steps. First, the influence of CGRP on E-cd protein and mRNA expression were determined in both unstimulated and O_3-stressed HBECs using immunocytochemistry and RT-PCR analysis. Second, the signal transduction pathways of CGRP were observed by using PKC inhibitor H-7, calmodulin inhibitor W-7 and PKA inhibitor H-89. The results showed that E-cd was distributed on HBECs. Furthermore, O_3-stress decreased the membranous E-cd expression but increased the cytoplasm E-cd expression. We propose that the change of E-cd expression might mediate HBECs injuries induced by O_3-stress and CGRP produced a dose and time dependent increase of membranous E-cd and E-cd mRNA expression on both unstimulated and O_3-stressed HBECs probably via PKA, PKC and CaM pathways.

EXPRESSION OF CFTR IN MOUSE OOCYTES AND ITS INVOLVEMENT IN OOCYTE MATURATION

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The cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP dependent Cl^- channel, is recognized to play an important role in reproduction; however, its exact role in various reproductive events remains obscure. Our previous study has shown that CFTR is expressed in mouse ovary. The present study aimed to investigate the expression of CFTR in mouse oocytes and its involvement in oocyte maturation. The expression of CFTR in oocytes was determined by immunofluorecscence study and the results showed that CFTR was localized to the membrane of the oocyte in follicle. The involvement of CFTR in oocyte maturation was investigated by in vitro maturation (IVM). Cl^- channel blocker NPPB (100μM) significantly decreased the first polar body extrusion (PB) rate and germinal vesicle breakdown (GVBD) rate of the oocytes by 80.0% ($p<0.01$) and 46.6% ($p<0.05$) respectively. CFTR inhibitor Glibenclamide (100μM) also decreased the PB rate by 24.4% ($p<0.01$), but had no effect on GVBD. IVM result obtained from the occytes of CFTR knockout mice showed that PB rate of knockout homozygous and heterozygous oocytes was 48.5% and 76.1% of that obtained from normal C57BL/6 mouse oocytes ($p<0.001$), whereas no significant difference was found in GVBD rate among these three genotypes. It is concluded that CFTR is expressed in mouse oocytes and plays an important role in oocyte maturation, especially in first polar body extrusion.

JunD REPRESSES IMPORTIN-α1 TRANSCRIPTION THROUGH ITS PROXIMAL PROMOTER REGION TO REGULATE SUBCELLULAR LOCALIZATION OF HuR IN INTESTINAL EPITHELIAL CELLS

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In intestinal epithelial cells, our recent studies show that increased cytoplasmic levels of HuR stabilize mRNAs of p53, nucleophosmin, and ATF-2, thus inhibiting IEC proliferation. However, the mechanism underlying HuR trafficking between the nucleus and the cytoplasm remains elusive. JunD is an AP-1 transcription factor and is implicated in negatively regulating IEC proliferation. Importin-α1 is an adaptor protein that transports bound cargoes through the nuclear pore complex and is involved in mediating HuR nuclear import. This study determined if the subcellular localization of HuR is regulated by JunD through modulation of importin-α1 expression. Full-length importin-α1 promoter was cloned, and constructs of wild-type and various mutated importin-α1-promoter luciferase reporters were generated. Importin-α1 expression was examined by measuring its promoter activity and mRNA and protein levels. JunD overexpression was induced by transient transfection with the JunD expression vector under control of PCMV promoter in Caco-2 cells. Cytoplasmic and nuclear proteins were isolated for detecting HuR subcellular levels. JunD protein levels were increased by ~15-fold when Caco-2 cells were transfected with the JunD expressing vector for 48 and 72 h. Ectopic expression of JunD repressed importin-α1 gene transcription as indicated by decreased (~70%) importin α1-promoter activity and its mRNA and protein levels, but it did not affect expression of importin-β and transportin-1. Studies using deletion- and point-mutations of importin-α1-promoter revealed that JunD repressed importin-α1 transcription through CREB-binding sites that were located at its proximal promoter region. Reduction of importin-α1 by JunD overexpression increased cytoplasmic levels of HuR, although it failed to alter levels in total HuR. Levels of cytoplasmic HuR were ~3-fold the control value after JunD transfection. These findings indicate that JunD represses importin-α1 transcription through its proximal promoter region and that JunD-mediated inhibition of importin-α1 expression is crucial for the subcellular distribution of HuR.

L-ARGININE PROMOTES DNA REPAIR IN CULTURED BRONCHIAL EPITHELIUM WHEN EXPOSED TO OZONE: INVOLVEMENT OF ATM PATHWAY

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Nitric oxide (NO) has been identified as one of the most important mediators in various stress response, and reactive nitrogen species (RNS) participated in ataxia telangiectasia mutated (ATM) phosphorylation, an important switch for DNA repair, in normal condition. Our previous study demonstrated that marked increase of NO was induced by ozone exposure in human bronchial epithelial cells (HBECs), but the role of RNS under ozone stress remains unclear. The present study was designed to observe the effect of NO donor L-arginine on DNA wound repair and its possible medium in HBECs when exposed to ozone. Agarose gel electrophoresis and comet assay showed that ozone stress resulted in severe DNA breaks, while flow cytometry analysis found definite G1-phase cell cycle arrest at 12h after ozone stress. Pretreatment with L-arginine facilitated DNA wound repair in a time-dependent manner and increased G1-phase cell cycle arrest in the presence of ozone, whereas blockade of NO generation with NG-mino-methyl-L-arginine (L-NMMA) produced the opposite response. The results above supported that NO might participate in DNA breaks rejunction after ozone exposure. Interestingly, further study showed L-arginine (100–500 μg/ml) could up-regulate ATM kinase phosphorylation on ser1981 in a dose dependent manner when exposed to ozone. Moreover, L-NMMA could reduce the augmentation of p-ATM induced by L-arginine (500 μg/ml). These results suggest that NO donor L-arginine enhanced cell cycle arrest and DNA breaks repair induced by ozone in HBECs probably by promoting ATM kinase activity.

PROTEOMIC ANALYSIS OF TESTES IN MAN TREATED WITH INJECTABLE TESTOSTERONE UNDECANOATE

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In a recent study, we found that testosterone undecanoate (TU) treatment in Chinese men led to an increase in serum T and free T levels, and a decrease in serum LH and FSH levels resulting in a decrease in sperm concentrations (severe oligozoospermia or azoospermatism). In this study, we examined the underlying proteomic changes involved in TU-induced suppression of spermatogenesis at an early time period after TU treatment. Using a global proteomic analysis involving 2-D electrophoresis and MALDI-TOF/TOF, we investigated the differential protein expression in human testes comparing the results between the control and TU-treated groups. A network of these proteins and their related cellular processes showing changes after TU treatment were constructed by bioinformatics method, and key proteins among this network were selected and analyzed. Seventeen protein spots were found with differential expression between two groups and thirteen known proteins were identified. By PathwayStudio software analysis, eight proteins were observed to participate in the complex functional network after TU treatment. Among these eight proteins, four proteins (CALB2, SOD2, hnRNP K and PSMF1) were found to participate in most of cellular/molecular events induced by exogenous testosterone treatment, including the categories of assemble, cell survival, proliferation, and death. Spermatogenesis suppression by exogenous testosterone through reduction of intra-testicular T levels may act on events of cell assembly, cell survival, and apoptosis. The initial changes in testes induced by exogenous testosterone were the altered expression of proteins, among which four proteins (CALB2, SOD2, hnRNP K and PSMF1) may be candidate proteins and potential molecular targets responsible for suppression of spermatogenesis.

THE PROTECTIVE EFFECT OF CLERODENDRUM BUNGEI EXTRACTS ON AIRWAY INFLAMMATION IN MICE INDUCED BY OZONE EXPOSURE

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Clerodendrum bungei, a commonly used traditional Chinese herb, was reported to have some pharmacologic actions, including activating blood circulation to dissipate blood stasis, detumescence and odynolysis, promoting diuresis, detoxication, and the most important, anti-inflammation. Airway hyperresponsiveness (AHR) diseases, such as asthma and chronic obstructive pulmonary disease, are considered as the chronic airway inflammation, and commonly characterized as an excessive response of the airway to low level of antigens, slight challenge of environment factors and even endogenous bioactive substances under physiological condition. In order to explore the potential therapy value of Clerodendrum bungei on airway inflammation, in the present study, we established a mice AHR model by ozone exposure (0.5 ppm, 20 minutes one day , totally five days). In the experiment group, five hours after the ozone attacking, extracts of clerodendurm bungei was administrated by the way of intragastric. Meanwhile, we set up both normal control and positive control groups to be administrated with normal sodium and dexamethasone respectively. Buxco respiratory pattern measurement system was applied to detect the airway resistance of the mice, the inflammatory cells in bronchoalveolar lavage fluid were counted to calculate the quantity of inflammatory cell infiltration, and the pathological section of lung was made to observe the injury of lungs. We found that extracts of clerodendrum bungei could decrease airway resistance of AHR, reduce inflammatory cell infiltration at the local of airway, and mitigate lung injury. These results suggest that clerodendrum bungei might be a potential effective novel medicine aiming at AHR diseases by its anti-inflammation effects.

THE ROLE OF CAMP RESPONSE ELEMENT BINDING PROTEIN IN THE PROCESS OF WOUND REPAIR IN BRONCHIAL EPITHELIUM INDUCED BY VASOACTIVE INTESTIANL PEPTIDE

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We have verified that Vasoactive intestinal peptide (VIP) could accelerate the wound repair of the human bronchial epithelial cells (HBECs) via PKA, PKC, CAM and MAPK signal transduction pathways. In order to further reveal the signal molecular mechanism, the present study was designed to observe the role of cAMP response element binding protein (CREB) in the process of wound repair in HBECs induced by VIP. CREB is one of the most important nuclear factors, which influence cell stress, metabolism, growth and apoptosis by modulating expressions of multiple genes in response to various signal pathways such as PKC, PKA, CaM and MAPK. The wound repair index, chemotactic migration, proliferating cell nuclear antigen (Ki67) expression, S-phase cell fraction (SPF) and cell proliferation index (PI) were measured as wound repair indicators of HBECs, and antisense oligonucleotide (ASO) to CREB was used to observe the regulation of CREB on the above effects induced by VIP. The binding capability between CREB and DNA segment containing CREB sites was assayed by electrophoresis mobility shift assay. The expression of CREB and the phosphorylation of CREB were observed by western blot. The signal pathways involved in activation of CREB were also examined by using PKC inhibitor H-7, calmodulin inhibitor W-7, PKA inhibitor H-89 and ERK inhibitor PD98059. The results showed that the induced effects of VIP on wound repair index, chemotactic migration, Ki67 expression, SPF and PI of HBECs could be blocked by CREB ASO. VIP could upregulate the binding capacity between CREB and DNA segment containing CREB sites and promote the phosphorylation of CREB, which could be blocked by H-89, PD98059 and VIP receptor antagonist. In conclusion, CREB phosphorylation played an important role in the process of VIP promoting wound repair in HBECs, which was mediated by PKA and MAPK signal pathways. This study

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A COMPARATIVE MOROOLOGICAL OBSERVATION PREOPERATIVELY AND POSTOPERATIVELY OF NASAL MUCOSAL SECRETORY FUNCTION IN CHRONIC SINUSITIS TREATED WITH ENDSCOPIC SINUSM SURGERY

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To investigate morphologic changes of nasal mucosal secretory function both preoperatively and postoperatively in chronic sinusitis (CS) treated with endoscopic sinus surgery (ESS), light microscopic examination of nasal mucosa were taken preoperatively and postoperatively in 46 cases with CS and in 28 normal subjects as control. Scanning and transmission electron microscopy were performed to exam preoperatively and postoperatively the nasal mucosa in 10 cases with CS and in 2 normal cases as control. The pathological changes of nasal mucosa, such as subepithelial thickening, edema, squamous cell differentiation, polypoid formations and fibrosis were observed preoperatively. The number of inflammatory cells, goblet cells, submucosal glands and pathologic glands were obviously increased preoperatively. The number of inflammatory cells, thickening, edema, squamous cell differentiation, polypoid formations were significantly reduced ($P<0.01$) at four months after operation, and there was no significant difference compared with the controls ($p>0.05$). However, fibrosis, the number of inflammatory cells, goblet cells, submucosal glands and pathologic glands were not reduced ever after four months postoperatively. The examination of electron microscopy demonstrated that the ultrastructure of nasal mucosa was impaired preoperative. There was a decrease of ciliated cells, and an increase of goblet cells and opened glands. The damaged structure of nasal mucosa was greatly improved after ESS and almost completely recovered at four months postoperatively, but the number of goblet cells and opened glands were increased. The normal structure and secretory function of nasal mucosa in patients with CS were impaired preoperatively, and the impaired structure of nasal mucosa were greatly improved after ESS and almost completely recovered at four months postoperatively, but secretory function of nasal mucosa was not recovered completely. Our results show that the nasal mucosa was unstable at four months postoperative and secretory function of nasal mucosa may need longer period to recover.

CYCLIC CHANGES IN UTERINE BICARBONATE SECRETION AND THE UNDERLYING MECHANISMS

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Previous study in our laboratory has demonstrated that bicarbonate in the uterine fluid plays a central role in sperm capacitation and reduced female fertility probably due to defective bicarbonate transporting. However, the cellular mechanisms underlying the formation of bicarbonate-rich uterine fluid in the female reproductive tract remained largely unknown. Here the expression profile of candidate proteins, known to be involved in bicarbonate secretion throughout the estrous cycle was examined in mouse uterus by Western Blot. The results showed that Estrus stage exhibited maximum protein level of the cystic fibrosis transmembrane conductance regulator (CFTR), SLC26A6, Cabonic anhydrase (CA)2 and CA12. Using in vivo perfusion technique to monitor the luminal surface pH by fluorescent dye 5-N-hexadecanoylaminofluorescein (HAF), we further explored the physiological function of the bicarbonate secretion related proteins and underlying mechanisms in mouse uterus at Estrus and Diestrous stage respectively. Statistics analysis showed that the endometrial surface fluorescence ratio was inhibited significantly by CFTR blocker DPC, SLC26A6 inhibitor DIDS and nonspecific CA antagonist acetazolamide at Estrus compared with Diestrus stage. Under basal condition, the endometrium resting surface fluorescence ratio at Estrus stage was significantly higher than that in Diestrus ($P<0.01$). In summary, under physiological condition, the pH microclimate of uterine endometrium is constantly changing throughout the estrous cycle with the maximal expression of bicarbonate secretion related proteins and their function observed at Estrus stage. The finding suggests that the fluctuating of uterine bicarbonate secretion during the estrous cycle may be necessary for providing an optimal microenvironment that is important for a series reproductive events.

SILICA-INDUCED PAI-1 EXPRESSION IN HUMAN LUNG EPITHELIAL CELLS WAS INVOLVED IN THE ACTIVATION OF ERK, P38 KINASE AND AP-1

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Plasminogen activator inhibitor-1 (PAI-1) plays an important role in the silica-induced pulmonary fibrosis. However, the signal regulation mechanism for PAI-1 expression remains unclear. The expression of PAI-1 induced by silica in human lung epithelial cells (A549) was tested. We found that silica induced PAI-1 mRNA and protein expression in a time-dependent manner. Furthermore, the roles of extracelluar- regulated kinase (ERK), p38 kinase and activator protein-1 (AP-1) signaling pathways in silica–induced PAI-1 expression were determined. The results showed that silica treatment resulted in the activation of ERK, p38 kinase and AP-1. Moreover, c-Jun dominant negative mutant (TAM67) prevented silica-induced AP-1 activation and PAI-1 expression. The induction of PAI-1 by silica was suppressed by p38 kinase inhibitor (SB203580) and ERK inhibitor (PD98059). The results suggest that the PAI-1 expression induced by silica may be involved in the activation of ERK, p38 kinase and AP-1 signaling pathways in human lung epithelial cells.

INFLUENCE OF VASOACTIVE INTESTINAL PEPTIDE ON Muc1 mRNA EXPRESSION IN OZONE-STRESSED LUNG TISSUE OF RATS

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The present study was designed to investigate the influence of vasoactive intestinal peptide (VIP) on the ozone-stressed rats' lung tissue mucin gene Muc1 mRNA expression, and to probe the effects of VIP on the excessive mucus secretion in airway hyperresponsiveness. 15 SD rats were randomly divided into three groups including normal control group, ozone stress group and VIP plus ozone stress group. Ozone stress group and VIP plus ozone stress group inhaled mixed air including 2.0-ppm ozone for 1 h every day in four consecutive days. Then, VIP plus ozone stress group used intranasal VIP treatment from the fifth to eighth day and ozone stress group used normal saline alternative VIP treatment. The lung tissue was extracted at the eighth day and fluorescence quantitative PCR was used to detect the Muc1 mRNA expression. Results showed that the Muc1 mRNA expression increased obviously in ozone stress group (2.153 ± 0.001) when compared with normal group (0.755 ± 0.001, $P<0.01$). VIP treatment decreased obviously the Muc1 mRNA expression (1.654 ± 0.002) in ozone-stressed lung tissues. Our results indicate that VIP down regulated the Muc1 mRNA expression of ozone-stressed rats' lung tissue, which may have therapeutic effects on excessive mucus secretion of airway hyperresponsiveness.

PROTEOME ANALYSIS OF ROUND-HEADED SPERM BY TWO-DIMENSIONAL DIFFERENCE GEL ELECTROPHORESIS AND MASS SPECTROMETRY

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In this work, two-dimensional difference gel electrophoresis (2-D DIGE) coupled with mass spectrometry (MS) was used to investigate changes in the proteome of human round-headed sperm and normal sperm. For one gel, Cy5-labeled proteins from the round-headed sperm were combined with Cy3-

labeled proteins isolated from the normal human sperm and separated on the same 2-D gel along with a Cy2-labeled mixture of all samples as an internal standard. For the other gel, we labeled the sample reversed. Over 61 protein spots were analyzed in each paired normal/abnormal comparison, and using DIGE technology with the mixed-sample internal standard, 36 protein spots were identified by ms/ms as showing significant changes (paired t-test, $p<0.05$) in the level of expression between normal and round-headed. 9 proteins were up-regulated and 27 proteins were down-regulated in round-headed. These proteins seem to play important roles in a variety of pathways including spermatogenesis, cell signaling, cell skeleton and metabolism.

DOWN-REGULATION OF INTEGRIN BETA-4 ON HUMAN BRONCHIAL EPITHELIAL CELLS LEADS TO INHIBITION OF PROLIFERATION, WOUND REPAIR AND RESTRICTED ANTI OXIDATIVE DAMAGE

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The epithelia of asthma patients have damaged structures and abnormal physiological functions, such as the shedding of epithelial cells and irregular repair, which can be found clearly in both asthma models and asthma patients. The β4 integrin is a structural and functional adhesion molecule expressed on the basal surface of the airway epithelial cells. And the main function of integrin β4 is to maintain the integrity of airway epithelia. Our previous work found that the expression of integrin β4 decreased significantly in the epithelia of asthma model. Further work by J. Dowling et al showed that integrin β4 knock out mice died quickly after birth, having severe respiration deficiency and shedding of mass epidermal tissues. However, whether the decrease of integrin β4 is an inducement of epithelium cells desquation, the cause of the physiological function turbulence of the airway epithelial cells, or even the integrin β4 has some connection to the occurrence of asthma remain largely unknown. Therefore in this work, we first detected the expression of integrin β4 on the blood leukocytes and airway epithelia of asthma patients. Then three siRNA were designed to transfect the human epithelium bronchial cells (hBECs), and the functional changes of the effective transfected cells were detected. We found that the expression of the integrin β4 fell obviously in the blood leukocytes and airway epithelia in asthma patients. The integrin β4 silenced hBECs showed a remarked inhibition on the proliferation and wound repair. The cell cycle also was restrained by the integrin β4 silence. And after ozone stress the catalasis activity was restricted in the integrin β4 silenced hBECs. Therefore we conclude that integrin β4 was required for the basic physiological function of hBECs. And the integrin β4 was also engaged in the resistance of oxidative damage. These results will have a tight connection to the asthma pathology and hint some new gene target to the asthma pathopoiesis.

EFFECTS OF CALCITONIN GENE-RELATED PEPTIDES AND VASOACTIVE INTESTINAL PEPTIDE ON MATRIX METALLOPROTEINASES PRODUCTION BY ALVEOLAR MACROPHAGES IN RATS

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Alveolar macrophage-derived matrix metalloproteinases (MMPs) play critical roles in the lung, such as lung branching morphogenesis, asthma and chronic obstructive lung disease. In many studies, it has been reported that the proinflammatory cytokines IL-1, IL-6, and TNF-α upregulate MMPs expression, but little is known about the endogenous factors regulating alveolar macrophages (AM) MMP-2 and MMP-9 production. Calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP) are two important neuropeptides with potent anti-inflammatory actions in airway and can inhibit proinflammatory cytokines production by macrophage. The gel zymography and quantitative RT-PCR data show that (1) LPS administration dramatically increased MMP-2 and MMP-9 activity as well as mRNA levels after 6h in a time and dose dependent manner, major MMP-9, minor MMP-2; (2) VIP and CGRP administration simultaneously with LPS decreased the LPS-induced increasing in MMP-9 production (at 10 nM), while the effect on MMP-2 was not significant. The inhibition of VIP was more obvious. The results indicate that physiological concentrations of neuropeptides can modulate MMP-9 production in AMs; (3) The effects of VIP and CGRP were diminished by using PKC inhibitor H-7 and calmodulin inhibitor W-7. These results suggest that VIP and CGRP could attenuate the augmentation of MMP-9 induced by LPS via PKC and CaM pathways in rat AMs. The observation that VIP and CGRP inhibited excessive AMs functions suggests neural regulation of inflammatory responses in airway and possible clinical application perspective.

VASOACTIVE INTESTINAL POLYPEPTIDE PROMOTED CFTR PROPERTIES IN HUMAN BRONCHIAL EPITHELIAL CELLS

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Vasoactive intestinal polypeptide (VIP) plays an important role in airway wound healing and its receptors are widely distributed in nerve fibers and airway epithelium, but its effects on Cl^- channel remain unclear. This study was designed to investigate the effect of VIP on the expression of cystic fibrosis transmembrane conductance regulator (CFTR) and CFTR Cl^- currents. Activation of CFTR in human bronchial epithelial cells (HBECs) was studied. We found VIP ($10^{-11} - 10^{-6}$ mol/L) evoked a high expression level of CFTR protein in a dose-dependent manner as assessed by western blot and in vitro phosphorylation. Whole-cell patch clamp showed glibenclamide-sensitive and DIDS-insensitive CFTR Cl^- currents were consistently observed after stimulation with forskolin (a positive control). VIP ($10^{-11} - 10^{-6}$mol/L) increased CFTR Cl^- currents in a dose-dependent manner. The augmentation of CFTR Cl^- currents enhanced by VIP (10^{-8} mol/L) was reversed, at least in part, by protein kinase A (PKA) inhibitor, H-89 and protein kinase C (PKC) inhibitor, H-7, suggesting PKA and PKC participated the VIP-promoted CFTR Cl^- currents. Moreover, we demonstrated that VPAC1 antagonist (10^{-6} mol/L) could inhibit VIP-increased CFTR Cl^- currents. Further studies showed that intracellular chloride concentration and basal chloride efflux of VIP (10^{-8} mol/L) treated cells increased significantly compared with untreated cells. These results show that in HBECs stimulation of CFTR-dependent chloride secretion following activation by VIP of $VPAC_1$ receptors were mediated by PKA and PKC signaling pathways.

DOWN-REGULATED EXPRESSION OF CFTR IN HUMAN BRONCHIAL EPITHELIAL CELLS UNDER OZONE STRESS

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To investigate abnormalities of CFTR expression in chronic inflammatory airway diseases and its regulation mechanism, we stressed the cultured human bronchial epithelial cells (HBEC) with ozone, on which the expression of CFTR, CFTR chloride current and the possible relevant signal pathways were primarily explored by using real-time PCR, immunofluorescence, Western blot and whole cell patch-clamp. Results demonstrated that ozone stress down-regulated CFTR expression and whole cell patch-clamp recorded an ozone-repressed CFTR chloride current in HBEC. Two signal pathways, Nrf2 and STAT1, were checked to investigate the signaling mechanism. It was found that pretreatment with Nrf2 antisense oligonucleotide aggravated the down-regulated effect of ozone stress on CFTR expression, while STAT1 inhibitor attenuated the effect of ozone stress. We also observed that ozone stress accelerated nuclear translocation of Nrf2 and STAT1

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phosphorylation in HBEC, which could be influenced by some signaling molecules related to the early transduction of cellular stress. Furthermore, using reactive oxygen species inhibitors N-acetylcysteine and nitric oxide synthase inhibitor aminoguanidine, we observed increased expression of CFTR directly. In conclusion, ozone stress could down-regulate the expression of CFTR and decrease CFTR chloride current in HBEC, the signaling mechanism of which included early oxidative stress signal transmission molecules, and subsequently transcription modulator Nrf2 and STAT1.

EFFECT ON THE HUMAN LIVER CELL PROLIFERATION AFTER CAVEOLIN-1 GENE KNOCK-DOWN

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Caveolin-1 (Cav-1) is a major functioning protein of non-clathrin, flaskshaped invaginations named caveolae (little caves), and acts as scaffolding protein to regulate signal transduction. In this experiment, we used gene clone technology to design and synthesize three shRNAs (short hairpin RNAs) based on the sequence of CAV-1 gene which were named CAV3, CAV6 and CAV7. They were separately subcloned to the plasmid psiRNA-hH1zeo G2 containing H1 promoter. The eukaryotic expression vectors specific to CAV-1 were constructed, which were verified by sequencing. The vector CAV7 was transferred into the Chang liver cells with Lyovec *in vitro*. The positive cell clones transfected were obtained after being screened with 100μg/mL zeocin for 2 weeks. CAV3 or CAV6 was transfected transiently the same way. Expressions of Cav-1 and other signaling proteins were detected by Western blot, while the proliferation rate was examined by MTT. The results show that after Cav-1 expression was down-regulated by RNA interference (RNAi), the transfected cells increased faster at first (72 hours after transfection, $p<0.05$; 96 hours after, $p<0.01$), and then the rate got normal and even slower. Consistent with this, PI3K-Akt and Erk signaling pathway that related with cell growth and proliferation were activated after Cav-1 down-regulated (96 - 120 hours after transfection), and then became inactivated. The results indicate that caveolin-1 correlated with liver cell proliferation, and might regulate it by interacting with PI3K and growth factor receptors on the membrane surface and influencing the cellular signaling afterwards; caveolin-1 may both maintain the natural growth of hepatocytes and control them from excrescence.

INVOLVEMENT OF EPITHELIAL SODIUM CHANNEL IN THE RELEASE OF PGE2 FROM CULTURED ENDOMETRIAL EPITHELIAL CELLS OF MICE

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Prostaglandins, including PGE2, have been demonstrated to play crucial roles in processes of implantation, especially in mediating the cross-talk between uterine epithelial cells and stroma cells during decidualization. Our previous studies in implantation model of mice have shown that amiloride, the inhibitor of ENaC, could reduce the implanted embryo number indicating the involvement of ENaC. In addition, serine proteases, which have been reported to activate ENaC, are also known to be among those proteases required for trophoblast invasion during implantation and shown to promote PGE2 production in many other epithelial cells. Thus, we hypothesized that ENaC may be involved in the initiation of PGE2 release in endometrial epithelial cells leading to decidualization. In the present study, endometrial epithelial cells of mice were primarily cultured and grown on semipermeable membranes for forming polarized monolayers. Trypsin, a serine protease, and amiloride were added to the culture medium, and the release of PGE2 to basolateral compartment was detected using an ELISA kit. The results showed that, incubating with trypsin (2 μg/mL) for 20–30 min could significantly enhance the PGE2 level (12.90 ± 3.64 ng/mL) in the treated cells as compared to those under control conditions (5.92 ± 1.71 ng/mL). While treatment together with amiloride (10 μM) and trypsin (2 μg/mL) reversed the effect of trypsin to 4.89 ± 1.39 ng/mL. In addition, amiloride (10 μm) alone was found to reduce the PGE2 level to 3.68 ± 0.33 ng/mL as well. These results have demonstrated the involvement of ENaC in the release of PGE2 in endometrial epithelial cells indicating its potential role in decidualization and thus essential to implantation.

EFFECT OF L-NAME ON SPERMATOGENIC CELL APOPTOSIS IN RATS EXPERIMENTAL ORCHIDOPEXY

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The mechanisms of spermatogenic apoptosis are very complex when the temperature was heightened. In order to investigate effect of L-NAME on spermatogenic cell apoptosis in rat's experimental orchidopexy, we designed the following experiments. Seventy-five immature male Sprague-Dawley rats (22 day-old) were randomly divided into two groups: sham operation and cryptorchid. Unilateral cryptorchidism was surgically induced in the rats. On the 12th day after operation, the cryptorchid rats were randomly divided into four groups: cryptorchid, orchidopexy, orchidopexy + normal saline, and orchidopexy + L-NAME. Unilateral orchidopexy was surgically induced in the rats. The rats of orchidopexy +L-NAME group were injected with L-NAME accordingly. The blood samples were collected on the 12th day. The rats were sacrificed on the 24th day after the second operation. We found that the level of spermatogenic cell apoptosis, serum NO concentration and the activity of NOS in rats cryptorchidism were higher than the rats orchidopexy ($P<0.01$). Administration of L-NAME could decrease serum NO concentration and spermatogenic cell apoptosis in rats experimental orchidopexy ($P<0.01$). The activity of NOS was attenuated significantly by L-NAME. The protein expression level of eNOS and iNOS in the testes of the sham operation group was the lowest ($P<0.01$). These results suggest that: 1. NO is the important biology active gene of excess apoptosis in spermatogenic cell in rats experimental orchidopexy. 2. L-NAME can reduce the production of NO and spermatogenic cell apoptosis, and the inhibitor of NOS can promote the enhancement of spermatogenic capability in rats experimental orchidopexy.

BICARBONATE TRANSPORT AND LUMINAL pH IN A CF BRONCHIAL CELL LINE AND ITS CORRECTED CELL LINE

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Cystic fibrosis (CF) is a recessive disease caused by mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which accounts for the cAMP-modulated chloride conductance. The chronic lung infection and inflammation is the chief cause of morbidity and mortality in CF patients. While reduced chloride secretion characterized the most common mutation in CF, the fatal consequences of CF have been difficult to rationalize solely in terms of this defect. CFTR-mediated bicarbonate secretion has been identified and CFTR mutations could thus alter airway surface fluid (ASL) pH, which in turn could alter a number of innate defense processes. The aim of this study was to compare the bicarbonate transport and luminal pH between IB3-1, a CF bronchial epithelial cell line, and its CFTR-corrected C38 cell line. Polarized cells grown on permeable filter supports were utilized in this study. We examined the luminal pH by loading a membrane bound pH-sensitive dye to the apical compartment of the two cell lines and observe differences in the fluorescent ratio (490/440nm), which could be converted into pH unit with careful calibration. The luminal pH of C38 cells was found to have an averaged alkaline pH of 9.165 ± 0.394 (n=6) as compared to a relatively neutral pH of 7.560 ± 0.236 (n=6) observed in IB3-1 cells ($p<0.01$). We then compared the expression levels of transporters and enzymes that are known to be involved in bicarbonate transport or production, including carbonic anhydrase (CA) CA-2, and SLC26A6, the sixth member of the solute carrier 26 gene family, between the two cell lines using western blot analysis. The results

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showed that there was no difference in the expression level of SLC26A6 between the two cell lines, while the expression level of CA2 was higher in C38 cells than that in IB3-1, which may significantly contribute to the higher luminal pH observed in the CFTR-corrected C38 cells.

Huoxiang-Zhengqi LIQUID STIMULATES cAMP-INDEPENDENT HCO_3^- SECRETION IN PORCINE DISTAL AIRWAY EPITHELIUM

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The short-circuit current technique was used to examine the HCO_3^- secretory effect of cAMP evoking agents forskolin/IBMX and a Chinese medicinal formula Huoxiang-Zhengqi Liquid (HZL) by intact porcine distal airway epithelium. The freshly isolated airway epithelial tissue displayed a transepithelial basal current of 94.9±8.2 (μA/cm^2), which was partially inhibited by amiloride (epithelial Na^+ channel blocker) and NPPB (CFTR Cl^- channel blocker), respectively. Application of forskolin/IBMX (10μM/100μM) stimulated an I_{SC} increase of 13.8±1.9 (μA/cm^2) which could be blocked by Cl^- channel inhibitor DPC. With Cl^- and Cl^-/HCO_3^- substitution, forskolin/IBMX evoked HCO_3^--dependent, DPC-inhibitable I_{SC} (I_{HCO3}) of 7.3±0.5 (μA/cm^2). Noticeably, basolateral application of Chinese medicine HZL (10μl/ml) in normal KH solution evoked an I_{SC} of 15.9±2.4 (μA/cm^2). The EC_{50} of this I_{SC} was 6.1±1.4 (μl/ml). When substituting Cl^-, HZL stimulated a HCO_3^--dependent, DPC-inhibitable I_{SC} of 7.4±1.9 (μA/cm^2), suggesting HZL-induced HCO_3^- secretion. Pretreating the epithelial tissues with forskolin/IBMX in Cl^--free KH solution, application of HZL induced a further I_{HCO3} increase of 8.4±0.9 (μA/cm^2), and pretreating tissues with HZL did not significantly affect the I_{HCO3} response of subsequent forskolin/IBMX, indicating that HZL-induced I_{HCO3} responses appeared to be cAMP-independent and most likely mediated via different cellular mechanisms. Our results suggest that HCO_3^- can be secreted by porcine distal airway epithelium under stimulation of HZL which is likely to be a hopeful new agonist of therapeutic significance.

A NOVEL ANIMAL MODEL OF AIRWAY HYPER-RESPONSIVENESS INDUCED BY OZONE EXPOSURE

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Airway hyper-responsiveness (AHR) is characterized by increased sensitivity of airways to various constrictor agonists. Damage of the bronchial epithelium associated with leukocyte infiltration and increased airway responsiveness are consistent features of asthma. Therefore, in the present study we constructed an animal AHR model by damaging the airway epithelium with ozone stress, and investigated in particular the roles of airway epithelium in airway hyperresponsiveness. BALB/c mice were exposed to 2 ppm ozone 30min/d. 4 days of ozone exposure caused a small but significant increase in pulmonary resistance (R_L) and decrease in dynamic lung compliance (Cdyn). Aerosolized histamine challenge elicited a dose-dependent change in R_L and Cdyn in ozone-exposed mice ($P < 0.05$). Mice exposed to ozone showed a significant increase in total protein and total cell numbers in bronchoalveolar lavage fluid (BALF) compared with the control group, especially the number of macrophages, neutrophils and eosinophils increased obviously. Typical inflammatory pathology changes were observed in pulmonary tissue slides, including neutrophils and eosinophils infiltration, mucus exudation and bronchial epithelial cells shedding. In conclusion, our results indicate that this ozone exposed animal model mimiced the airway obstruction, airway inflammatory response, and increased airway responsiveness observed in human AHR disease, indicating the success of AHR formation. Compared with the traditional allergic animal model, our model was focused on the central role of airway epithelium in airway immune response, airway inflammation, and airway remodeling. The detailed understanding of the role of bronchial epithelial cells in the process of AHR will provide new insight into the cellular and molecular mechanisms underlying AHR diseases and therefore possible new targets for treatments.

THE AGE-DEPENDENT EXPRESSION OF CFTR AND CARBONIC ANHYDRASE II IN RAT PROSTATE

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Bicarbonate plays an important role in maintaining the physiological function in human body. It is well known that the function of CFTR and carbonic anhydrase II (CAII) in the epithelium are involved in the bicarbonate transport and production. Previous study has demonstrated that the expression of CAII changes with age in rat brain and fundus. However the age-dependent expression profile of these two genes has not been shown in the prostate where the age is the strongest risk factor for some prostate diseases. Thus, this study was carried out to test whether there is correlation between gene expression and age. The CFTR and CAII expression was detected in the epithelium of rat ventral prostate by RT-PCR, western blot and immunostaining. The expression of both CFTR and CAII was decreased remarkably with increase in the age which suggested that the prostate secretion of bicarbonate may decrease with ageing. The physiological significant of the decrease in bicarbonate secretion with the age and its possible involvement in the pathogenesis of prostate diseases await further investigation.

EFFECT OF PRESSURE ON ION TRANSPORT ACROSS THE COLONIC EPITHELIUM IN RAT WITH EXPERIMENTAL AGANGLIONOSIS

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The present study aimed to establish a rat model of experimental aganglionosis for mesenchymal stem cells (MSCs) transplantation, and investigate the effect of pressure created by retention of bowl contents on ion transport across the colonic epithelium in the rat *in vitro*. Eighty Sprague Dawley rats, 8–9 weeks old, were randomly divided into two groups: treatment group (n=40) and control group (n=40). All animals were operated upon under Ketamine anesthesia. In treatment group, 0.1% benzalkonium chloride (BAC) was applied onto the serosa for 40 minutes, and then the colon was washed well with 0.9 % normal saline. For the control group, 0.9% saline (nature saline, NS) was applied using the same method. Macroscopic and microscopic observations, contrast roentgenogrphy of the colon, colon manometry and histologic examination were performed at an interval of 1w, 2w, 3w, 4w and 8w after operation. Detection of the effect of pressure, which was created by adding and removing Kreb's solution on the mucosal compartment, on ion transport of colonic epithelium was monitored by the short-circuit current at 1w and 2w after operation. The results show that one week after BAC treatment, the rats had abdominal distention. Roentagenographic examination and autopsy revealed a narrowed segment accompanied by distended proximal colon filled with massive feces, and the longer the duration after treatment, the more serious distention. Manometry showed the abolition of reflex contraction in colonic smooth muscle. Histologic examinations revealed transparent reduction and vacuolation of ganglion cells 1 week after BAC treatment with complete disappearence of 3 weeks after BAC treatment. As for the short-circuit current (*Isc*) across colonic epithelium of BAC/NS treatment segment and proximal segment, in control group, the pressure-induced *Isc* response was significantly greater than that of the treatment group, indicating desensitization in the treatment group to pressure. In addition, transepithelium electrical resistance (TER) showed no difference between treatment and control group. The results suggest that the animal model for experimental aganglionosis was successfully established with normal epithelial barrier function in aganglionosis rat colonic epithelium, while the ion transport was disordered with no response to pressure stimulation. The results further suggest that the problem of constipation

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in rats with aganglionsis is not only due to abnormal smooth muscle and sphincter function but also to a disorder of ion transport in the colon. Supported by ShanXi Province Natural Science Fund Commission (No.20051110) and Li Ka Shing Institute of Health Sciences of the Chinese University of Hong Kong.

THE EFFECTS OF DIFFERENT KI INTAKE ON H_2O_2 CONTENT, CALCIUM CHANNEL AND ANTI-OXIDATIVE ABILITY IN FRTL CELLS

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To explore the effects of iodine on function and anti-oxidative ability 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 observed the number and the morphology of cells, specific genes expression and NIS protein, the content of H_2O_2, intracellular free calcium concentration ($[Ca^{2+}]_i$), calcium current by whole-cell patch clamp technique, and the anti-oxidative ability of FRTL cells. The more dosage of KI was given to FRTL cells, the fewer cells survived, and the more H_2O_2 were detected. Furthermore, if we extended incubation period, these tendencies were more obvious. NISmRNA, TPOmRNA, and TGmRNA had no difference between high iodine groups and control group. However, high iodide decreased the levels of NIS protein. After given by different dosage of KI, ranging from 10^{-6} mol/L to 10^{-3} mol/L, the calcium current and $[Ca^{2+}]_i$ were increased. There were no difference in GPxmRNA and SODmRNA among control, 10^{-4}mol/L, 10^{-3} mol/L and 10^{-2} mol/L KI groups, however, the SOD activity and MDA content of cells and culture medium were both on inclined with the dosage of KI. In conclusion, after administration by 10^{-6} mol/L KI, there was no obvious effect on FRTL cells, but administration of 10^{-5} mol/L KI induced oxidative stress. Furthermore, 10^{-4} mol/L KI and 10^{-3} mol/L KI would induce oxidative damage on cells, and 10^{-2} mol/L KI would make a strong oxidative injury effect on FRTL cells.

EFFECTS OF KANAMYCIN SULFATE ON THE EXPRESSION OF PRESTIN IN COCHLEAR OUTER HAIR CELLS IN MICE OTOTOXICITIAL MODELS

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Aminoglycoside antibiotics are among the long-sought remedies for some serious infectious diseases and the most frequently used drugs due to their high efficacy, especially against gram-negative bacteria and low cost. Its side effects causing renal and auditory toxicity, however, have led to a decline of their use in most countries since last two decades. Nevertheless, aminoglycoside antibiotics are still the most commonly used antibiotics worldwide, particularly in developing countries. We, therefore, set out to investigate the mechanism underlying aminoglycoside-induced ototoxicity using the mouse ototoxicitial model induced by kanamycin sulfate, one of the aminoglycoside antibiotics. Auditory Brain-stem Response (ABR) was used to evaluate the degree of hearing loss. First, we investigated the effect of kanamycin sulfate on several strains of mice, including CBA, BALB/c, KunMing, trying to select a strain that is most sensitive to kanamycin sulfate in terms of hearing loss. Our results showed that after kanamycin sulfate treatment the thresholds of all three strains of mice shifted upward in dose- and time- dependent manner. However, the amplitudes of the threshold shift were significantly different among the three strains with the following order: BALB/c>CBA>KunMing. Therefore, we used BALB/c strain for subsequent studies. It is generally accepted now that the acute sensitivity and frequency discrimination of mammalian hearing requires active mechanical amplification that stems from somatic electromotility of the outer hair cells (OHCs) attributable to the motor protein prestin. Our immunoflurorescent staining results showed that the expression pattern of prestin in cochlear OHCs was affected by kanamycin sulfate, raising the possibility that prestin may be involved in mediating the kanamycin sulfate-induced hearing loss. Future studies will elucidate the molecular mechanisms underlying prestin's role in kanamycin sulfate- induced hearing loss, which will shed new light on prevention and cure of aminoglycoside antibiotics induced ototoxicity.

DELAYED WOUND HEALING IN CD-9 NULL MICE

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Cell-matrix interaction has been implicated in the process of wound healing; however the underlying regulatory mechanisms remain unclear. Here we report that mice lacking CD9 showed delayed wound healing with impaired keratinocyte migration due to elevation of MMP-9 in leading epidermis through JNK pathway. Selective inhibition of MMP-9 either *in vivo* or *in vitro* rescued wound healing. Notably, elevation of MMP-9 in CD9-null wound resulted in over-degradation of collagen IV in the new formed basement membrane, which accounted for the impaired migration of keratinocyte. These data indicate that CD9 is a proximal signal molecule involved in the metabolisms of extracellular matrix by regulation of MMP-9, which in turn affects the migration of keratinocyte and wound repair.

MECHANISM UNDERLYING THE INHIBITORY EFFECT OF MAGNOLOL ON SMOOTH MUSCULAR SPONTANEOUS CONTRACTION IN RAT COLON

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Magnolol, as a main component of magnolia bark, is well known to be effective in treating gastrointestinal diseases. However the mechanism of magnolol effect is poorly understood. In the present experiment, inhibition of the spontaneous contraction induced by magnolol, which could be blocked by TEA, was demonstrated in a does-dependent manner in both intact and de-epithelium rat colon strips. Measuring the intracellular calcium concentration of both intestinal SMC (smooth muscle cell) and ICC (Interstitial Cajal Cell), magnolol could induce a transient increase of intracellular Ca^{2+} concentration in both normal Ca^{2+} and Ca^{2+}-free solutions. But magnolol was found to inhibit store-operated calcium entry, which could be diminished by TEA. Membrane potential was dropped to 30% of the resting value after the addition of magnolol in both cultured ICC and SMC which could be abolished by TEA and Iberiotoxin (BK_{Ca} inhibitor). Using whole cell patch-clamp technique, magnolol was also found to open K^+ channel in ICC. Our results indicate that magnolol stimulates Ca^{2+} releasing from intracellular stores, which in turn activates K^+ channel in ICC. Subsequently, on one hand the K^+ flowing out of ICC hyperpolarizes the pacemaker and on the other hand, the hyperpolarization observed in smooth muscle cell may block extracellular Ca^{2+} influx. The present study may provide more knowledge for using magnolol in clinical treatment on IBS (irritable bowel syndrome) and other gastrointestinal hypermotility diseases.

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P2Y RECEPTOR-MEDIATED ANION SECRETION IN RABBIT CORNEA BY EXTRACELLULAR ATP

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Adenosine-5'-triphosphate (ATP) plays a pivotal role in various tissues as an extracellular signaling molecule. The present study explored whether purinegic receptors were expressed and involved in the regulation of Cl^- and HCO_3^- secretion across rabbit cornea by extracellular ATP. The corneal tissue responded to apical application of ATP (1-100uM) with transient increase in short circuit current (Isc) in a concentration dependent manner. UTP, agonist of P2Y receptor, had the similar effect. When ATP was added after UTP, or vice versa, a second Isc response wasn't observed, indicating that ATP and UTP shared the same P2Y receptors. While α-β-Methylene ATP, a potent P2X agonist, was found to be ineffective. Suramin and reactive blue 2, effective P2Y receptor blocker, inhibited the Isc increase of ATP respectively. Ion substitution experiment showed the rise in Isc was due to Cl^- and HCO_3 secretion. Pretreatment with glibenclamide and DIDS, two Cl^- channel blockers, attenuated the ATP response, but amiloride, an ENaC blocker, had no effect. And the response could be significantly impaired by other Cl^- channel inhibitors, NPPB and DPC, too. In patch clamp study, ATP stimulated Ca^{2+}-activate Cl channel which was blocked by DPC. K^+ channel and NKCC of endothelium in corneal tissue were involved in the rise of Isc, for basolateral application of Ba^{2+} and bumetanide in corneal tissue could reduce the response. The Isc was inhibited by U73122, indicating PLC was also selective for ATP mediated anion secretion signaling axis, and it was abolished by BAPTA/AM, demonstrating the requirement for the involvement of intracellular calcium $[Ca^{2+}]i$. In conclusion, extracellular stimulation of ATP could stimulate the anion secretion through P2Y receptor to activate PLC and $[Ca^{2+}]_i$ mobilization in rabbit cornea.

TWO HERBAL COMPOUNDS AS POTENTIATORS FOR CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) Cl^- CHANNELS

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CFTR is a cAMP activated Cl^- channel that provides the major pathway for Cl^- transport across the apical membrane of various epithelia. Mutations in the gene encoding CFTR result in cystic fibrosis, a multisystem genetic disease that manifests in the lung, intestine, pancreas, sweat glands and reproductive tracts. Tremendous efforts have been invested in finding CFTR potentiators to circumvent the defective Cl^- transport through CFTR channels. We have recently purified two compounds, designated as CFTR-P1 and CFTR-P2, from traditional Chinese medicine. Using the short-circuit current (Isc) measurement on Caco-2, a human colonic epithelial cell line with abundant CFTR expression, we found that both compounds, applied apically, induced Isc response that could be blocked by CFTR channel blocker NPPB. In addition, CFTR-P1 and CFTR-P2 could further potentiate the Isc response induced by forskolin, a well-known CFTR activator. To investigate whether CFTR-P1 and CFTR-P2 exert their effect by directly binding to CFTR, we performed molecular docking, using the newly developed CFTR NBD dimer model and a novel molecular docking method, and successfully docked CFTR-P1 and CFTR-P2 to the NBD dimer, suggesting that both CFTR-P1 and CFTR-P2 may bind directly to the CFTR dimer interface to increase CFTR channel activity, a mechanism shared by many known CFTR potentiators, such as genistein. Future site-directed mutagenesis will further identify the binding sites for these two compounds. The outcome of this study can have potential therapeutic implications for CFTR related diseases, such as cystic fibrosis, constipation, secretory diarrhea, and polycystic kidney disease.

GLOBULAR ADIPONECTIN: A NEW PRO-PROLIFERATIVE AND PROTECTIVE FACTOR AGAINST OXIDANT INJURY ON HUMAN BRONCHIAL EPITHELIA CELLS

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Epidemiologic data indicate an increased incidence of asthma in the obese individuals whose serum levels of the adipokine adiponectin are always reduced. It has been reported that exogenous adiponectin can attenuate allergic airway responses in mice, but the mechanism is still not clear. Here we report that a proteolytic cleavage product of adiponectin, known as globular adiponectin (gAd), worked as a protective regulator of human bronchial epithelial cells (HBECs). By RT-PCR we found that HBEC expressed adiponectin and its receptors especially AdipoR1, the high-affinity receptor for gAd. Cells exposure to ozone (1.5ppm) for 2 hours decreased the AdipoR1 mRNA expression by 39 %. Flow cytometry and confocal laser scanning microscopy showed that treatment with gAd(5ug/ml) inhibited basal and ozone-induced production of reactive oxygen species, The increased release rate of lactate dehydrogenase (LDH) activity induced by ozone was also reversed significantly by gAd. In normal HBECs, gAd (1.25-5 μg/ml) incubation for 24 hours showed a dose-dependent upregulatory effect on proliferation, with 3- (4, 5-dimethylthiazol-2 -yl) -2, 5-diphenyl tetrazolium bromide (MTT) method. These data indicate that airway epithelia were the target of gAd which plays an important role in protecting HBECs from oxidant injury, and it may have therapeutic implications in the treatment of asthma.

INFECTION AND IMMUNE DEFENSE

EXPRESSION OF HUMNAN β-DEFENSIN IN NASAL MUCOSA OF DIFFERENT TYPE IN CHRONIC RHINOSINUSITIS

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To observe the expression level of β-defensin in nasal mucosa of different type in chronic rhinosinusitis and normal control and to investigate the effect of hBD-1 and hBD-2 in pathogenesis of different type in chronic rhinosinusitis, HBD-1mRNA expression and hBD-2mRNA expression from the 4 groups above were detected by reverse transcription polymerase chain reaction (RT-PCR). First, no significant difference between different types in chronic rhinosinusitis and control group tissue of the hBD-1mRNA expression in nasal mucosa was found. Second, there was significant difference between different types in chronic rhinosinusitis and control group tissue of the hBD-2mRNA expression in nasal mucosa. Moreover, the hBD-2mRNA expression of type I and II was higher than type III and control group ($p<0.05$). In conclusion, hBD-1 and hBD-2 might play an important role in nasal mucosa defensins, which may be related with the prognosis of chronic rhinosinustis.

TH1 AND TH2 CYTOKINES IN MICE INFECTED WITH ECHINOCOCCUS GRANULOSUS AND IMMUNIZED WITH Eg95 GENETIC VACCINE

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To observe the change of the cytokines in mice infected with Echinococcus granulosus, the mice were injected by abdominal inoculation of Echinococcus protoscoleces. The level of Th1 cytokines (IL-2, IFN-r, TNF-a) and Th2 cytokines (IL-4, IL-5, IL-10) were determined by ELISA during infection from 1

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day to 300 days. Compared with uninfected control, the level of all cytokines was significantly increased in infection. The level of IL-2 and TNF-a reached peak at 120 days post infection and sharply went down on 200day post infection. Highest level of IFN-r was detected after 160 day and decreased slowly after 240 days post infection. The levels of IL-4, IL-5, IL-10 were lower before 120 days and increased quickly after 160day and reached peak at 240 days post infection. These results suggest that mice immune types were affected by infection of Echinococcus granulosus (E.g) and elicited both Th1 and Th2 cell activation: Th1 played an important role at early stage of infection and Th2 played an important role in late stage of infection. The Th1 response benefited the host, whereas the Th2 response benefited the parasite. Thus Th1/Th2 polarization played an important role in the development of echinococcosis. To study the mechanism of immune protection against challenge infection with E.g protoscoleces in mice induced with Eg95 genetic vaccine, the mice were immunized 4 times with Eg95 genetic vaccine and then infected with protoscoleces. Compared to the infected group, the results showed the levels of Th1 cytokines (IL-2, IFN-r, TNF-a) were significantly enhanced and the levels of Th2 cytokines (IL-4, IL-5, IL-10) were slightly declined after vaccination with Eg95 genetic vaccine at 200day post infection. The results indicated that the balance of Th1/Th2 cytokines in murine sera infected with protoscoloces could be modulated by Eg95 genetic vaccine. Thus we deduced that protection against echinococcosis induced with Eg95 genetic vaccine was associated with predominate Th1-like responses.

EFFECT OF ANTIFLAMMIN-1 ON CYTOKINES RELEASE FROM LIPOPOLYSACCHARIDE-STIMULATED RAT ALVEOLAR MACROPHAGES

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Antiflammin-1 (AF-1) is a synthetic nonapeptide with similar sequence to the conserved sequence of CC10 secreted by lung Clara cells. Studies suggested that it is a potent inhibitor for inflammation. We investigated the effect and the possible mechanism of AF-1 on LPS-induced alveolar macrophages (AMs) activation in vitro. AMs harvested from BALF of Sprague Dawley (SD) rat were used in this study. Cells were treated with various concentration of AF-1 simultaneously or after LPS stimulated. To further investigate the possible mechanism by which AF-1 modulates the expression of the cytokines, cells were pretreated with anti-IL-10 antibody. The concentrations of cytokines IL-1β, IL-6, and IL-10 in supernatants were detected by Enzyme-Linked Immunosorbent Assay. The mRNA expression levels of these cytokines in AMs were analyzed using quantitative RT-PCR. Toll-like receptor-4 (TLR-4) expression on the cell surface was also detected using flow cytometry. The results showed that AF-1 suppressed the mRNA expression and protein production of IL-1β and IL-6 while promoted IL-10 expression on LPS stimulated AMs in a dose dependent manner. Those inhibitory effects of AF-1 were decreased when endogenous production of IL-10 was blocked. AF-1 also showed an effect on down-regulate TLR-4 expression in LPS stimulated AMs. Our data revealed for the first time that AF-1 could modulate AMs response to LPS via regulating TLR-4 expression, and up-regulating IL-10 secretion. It could be another important mechanism for AF-1 inhibiting inflammation.

DIFFERENTIAL PROTEOMICS ANALYSIS FOR HUMAN NASAL POLYPS AND POLYPOSIS TISSUE

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In the present study, we set out to establish a two-dimensional polyacrylamide gel electrophoresis (2-DE) map from human nasal polyps and polyposis, and identify differential expression proteins of 2-DE map. Samples of human nasal polyps and polyposis, (each sample group containing 7 cases) were obtained. The total proteins were extracted and separated by immobilized pH gradient (IPG)-based 2-DE. The silver-stained 2-DE were scanned with digital Image Scanner and analyzed with ImageMaster 2-DE Elite 4.01 software. We then obtained peptide mass fingerprint (PMF) of differential protein spots with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The peptide mass fingerprints were searched in Swiss-Prot and TreMBL database by PeptIdent software, and differential expression proteins were identified. First, 2-DE for a randomly selected 1 sample from each of the 2 groups was repeated 3 times respectively to analyze the reproductive of the method. The image analysis showed that for the polyps tissues the average proteins spots of three 2-DE maps were 825 ± 78 spots which matched with the average matching rate of 82.7 % for the human nasal polyposis tissues. The average proteins spots of three 2-DE maps were 891 ± 67 spots which matched with the average matching rate of 86.1%. The average deviations of matched spot position were 1.13 ± 0.16 mm in IEF direction and 1.45 ± 0.21 mm in SDS-PAGE direction, respectively. Comparing the average electrophoresis profiles of the two tissues, each sample containing 7 cases obtained. The proteins spots of nasal polyps and nasal polyposis tissues were 1458 and1532, respectively. A total of 1201 proteins spots were matched between the two tissues average electrophoresis profiles. Second, twenty differential expression protein spots were incised from sliver staining gel randomly and digested in gel by TPCK-Trypsin. 11 differential expression proteins were identified. In this study, the well-resolved, reproducible 2-DE map of human nasal polyps and polyposis were established. Certain differential proteins were identified.

THERAPEUTIC EFFECTS OF ANTI-B7-1 ANTIBODY ON MURINE ASTHMATIC MODEL INDUCED BY OVALBUMIN

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Allergic asthma is a chronic inflammatory disorder of airways, which is characterized by attacks provoked exposure to so-called asthma triggers, such as pet dander, second-hand tobacco smoke, dust mites, and mold spores. One of the most widely studied co-stimulatory molecules involved in asthma was B7-1 (CD80). Previous studies have demonstrated that B7-1 played key roles in regulating allergen-induced T cell activation in asthma, probably by T cell recruitment and Th cell differentiation on allergen provocation. The present study was designed to test the hypothesis that anti-B7-1 antibody might have therapeutic effects in asthma by blocking B7-1/CD28 pathway. Asthma was induced by ovalbumin (OVA) sensitization and challenge on female Balb/c mice. One hour after the last induction, mice were sacrificed. Whole lung lavage was conducted. Cells concentrations of BALF were counted. Numbers of cells in BALF were calculated. Expressions of IFN-γ and IL-4 in supernatant were measured by enzyme-linked immunosorbent assay method. Sedimental cells smears were stained with Wright's-Gimsa mixed coloring method. B7-1 expression was detected by immunohistochemistry method on frozen sections. The anti-B7-1 antibody treatment could alleviate asthmatic syndromes induced by OVA. The number of recoverable eosinophils in BALF in the anti-B7-1 antibody treated group was significantly less compared to the asthmatic control group ($P<0.01$). In anti-B7-1 treated asthmatic mice, histological evaluation revealed marked suppression of eosinophils peribronchial infiltration. Treatment of anti-B7-1 antibody could obviously increase the expression level of IFN-γand decrease the expression level of IL-4 compared to asthmatic control group ($P<0.01$). These results suggest that inhibition of B7-1 by anti-B7-1 antibody could alleviate asthmatic syndromes, suppress eosinophils peribronchial infiltration, increase IFN-γ expression and decrease IL-4 expression in BALF of murine asthmatic models induced by OVA. Thus, we concluded that the anti-B7-1 antibody approach might provide a novel therapy for allergic asthma.

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HUMAN METAPNEUMOVIRUS INFECTION IN YOUNG CHILDREN IN SUZHOU, CHINA: EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS

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In June 2001, a new respiratory virus, human metapneumovirus (hMPV), was identified in the respiratory aspirates of children with respiratory tract disease. We sought to determine whether hMPV was circulating in Suzhou area and to determine the epidemiological and clinical features associated with hMPV infection in comparison with those of RSV infection. In this study, samples were collected from 1,932 young children hospitalized for acute respiratory tract infections. We tested respiratory aspirates for the presence of hMPV using reverse-transcription polymerase chain reaction (RT-PCR). These specimens were also screened for respiratory syncytial virus, influenza A and B, parainfluenza viruses 1, 2, 3 and adenovirus by direct immunofluorescence assay. The results of RT-PCR revealed that 128 (6.6 %) of the 1,932 patients screened showed evidence of hMPV infection. Among them, single hMPV infection was identified in 123 cases, while 5 (3.9 %) had coinfection with other respiratory virus (hMPVco). The majority of hMPV-positive children were detected during the winter seasons. The mean age of patients with hMPV infection was 22.17 months. hMPV was identified in patients with either upper or lower respiratory tract infection or both. The infected children were diagnosed as having upper respiratory tract infection (1.6 %), laryngitis (3.3 %), bronchiolitis (41.5 %), pneumonia (47.1 %), and asthma exacerbation (6.5 %). Main clinical manifestations included fever (55 %), cough (99.2 %), rhinorrhea (27.1 %), wheezing (47.3 %) and abnormal chest radiographs (93 %). Clinical symptoms associated with hMPV infection were similar to those associated with RSV infection; however, dyspnea, feeding difficulties and hypoxemia were more frequently observed in RSV infected children. These results suggest that: (1) hMPV infection accounted for a small but significant proportion of respiratory tract diseases in infants and children; (2) hMPV prevailed predominantly in the winter time; (3) clinical manifestations of hMPV infection did not differ much from other respiratory tract viruses; and (4) coinfection of hMPV with other respiratory viruses was rare and clinically similar to single infections.

SINTEGRON IN GRAM-NEGATIVE PATHOGENS ISOLATED FROM CLINICAL INFECTION OF LIVER TRANSPLANTATION

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Integrons are gene sequences located in bacterial genome or plasmid and play an important role in the spread of antibiotic resistance. Integrons are efficient recombination and expression systems able to capture alien movable gene cassettes through site specific recombination. Postoperative infection of liver transplantation is the main cause of operation failure commonly due to multidrug-resistance (MDR) bacillus. The clinical isolates after liver transplantation have been observed for half a year. In order to investigate the integron-associated MDR in clinical isolates of liver transplantation, we investigated twenty-seven strains of Gram-negative bacilli collected from patients after liver transplantation. Antibiotics susceptibilities were determined using standard agar diffusion. Integron and inserted gene cassettes were amplified by PCR and analyzed by RFLP, and the gene cassettes were detected by sequencing. The results showed that all of the isolates displayed multidrug resistance, and highly resistant to Cefotaxime (100 %), Aztreonam (100 %), trimethoprim /sulfamethoxazole (88.8 %) and Ciprofloxacin (81.5 %). The positive rate of class Iintegron was 51.9 %. Class II and III integron were not detected in any of the isolates studied. Integron cassettes with sizes of 750 bp, 1.0 kb, 1.8 kb and 2.0 kb were found. Nucleotide sequencing of the PCR products revealed four variable regions: blaVIM-2-aacA-Ib-aadA2-qacE, dfrA12-aadA2-qacE-blaCTX-M-2, dhfrXII-orfF- aadA2-agr-dfs, and dhfrX-orfF-aadA2-sul1-Dbp-dfs. The MDR were widely associated with the class I integron. AadA2 and dhrfXII cassettes were predominant, cassette combinations dhfrXII-orfF-aadA2 was most frequently found. Gene cassettes and phenotype showed roughly conformity which may be due to the selective expression of the bacterial genome. Our results indicate that screening for integron played an important role in prevention of hospital onset of infection and could improve the infection-control measure of liver transplantation.

ROLES OF MAPKS/AP-1 PATHWAYS IN THE PATHOGENESIS OF SILICOSIS: IN VITRO STUDY

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Macrophages play a fundamental role in silicosis in part by removing silica particles and producing cytokines in response to silica. Among the cytokines secreted by lung macrophages, tumor necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1) have been shown to play a critical role in the pathogenesis of silicosis, but the underlying mechanism remains to be determined. The aim of the present study was to investigate the role of mitogen-activated protein kinase (MAPKs) /activator protein-1 (AP-1) signaling pathways in silica-induced TNF-α and TGF-β1 expression in murine macrophage cells (RAW264.7). We found that silica activated p38 kinase and extracellular signal-regulated kinase (Erk) in RAW264.7 cells. The induction of TNF-α and TGF-β1 by silica was suppressed by Erk inhibitor (PD98059), but not by p38 kinase (SB203580). AP-1 inhibitor curcumin also inhibited silica-induced TNF-α and TGF-β1 expression. In addition, dominant negative mutant c-Jun (TAM67) inhibited silica-induced AP-1 DNA binding activity and downregulated the TNF-α and TGF-β1 expression. In addition, PD98059 but not SB203580 inhibited the AP-1 DNA binding activity induced by silica. Based on these findings, we conclude that Erk/AP-1 signaling pathways are responsible for the TNF-α and TGF-β1 expression induced by silica in murine macrophage.

AN ATYPICAL HYPERACIDIC Grp78/BiP ISOFORM IS UPREGULATED BY TLR2 AGONISM OR BACTERIAL INFECTION BUT NOT BY ENDOPLASMIC RETICULUM STRESS

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We have used an *in vitro* protein phosphorylation assay to detect changes in the levels of phosphorylatable proteins of low abundance with sensitivity higher than that of silver, Sypro Ruby or Pro-Q Diamond staining. THP-1 macrophage-like cells were infected with *Mycobacterium avium*, *Borrelia burgdorferi* or heat-killed *E. coli,* or treated with agonists of TLR2 and TLR4 for different times. We screened for proteome differences induced by the different agents by comparing with non-infected, untreated cells. One of the proteins up-regulated after infection or treatment with TLR2 agonists was identified as a novel Grp78/BiP isoform. This *in vitro* phosphorylated isoform (haGrp78) was less abundant and had a very low isoelectric point compared with the typical, more basic isoforms of Grp78 (bGrp78) that are not phosphorylated *in vitro*. haGrp78 up-regulation was apparent from 6 hours after infection and peaked at 24 hours regardless of the bacterial type or the TLR2 agonist. LPS did not affect the level of haGrp78, nor did 2-deoxyglucose-induced ER stress or thapsigargin-induced UPR. At day 4 post-infection, haGrp78 levels were higher when macrophages had ingested live as opposed to heat-killed *M. avium*. This suggests that live, intracellular *M. avium* continuously synthesizes TLR2 agonistic molecules while residing and proliferating in the phagosome. All forms of Grp78 were found both in cytosolic and

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membrane cell fractions. The up-regulation of haGrp78 has the potential to become a novel and useful marker of delayed and sustained effects of TLR2 agonists and of bacterial infections.

RELATIONSHIP BETWEEN THE Th1/Th2 CYTOKINES IMBALANCE AND AUTOIMMUNE THYROID DISEASES

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Autoimmune thyroid diseases (AITD) are group organ-specific autoimmune diseases with unknown etiology. Th1/Th2 cellular immune responses play an important role for the pathogenesis of AITD. To clarify the relationship between serum Th1/Th2 cytokines levels and autoantibodies against thyroid, and explore the role of Th1/Th2 cellular immunity imbalance in the pathogenesis of autoimmune thyroid diseases (AITD), we examined the serum level of Th1/Th2 cytokines and autoantibodies in 21 patients with Graves' disease (GD), 18 cases with Hashimoto's thyroiditis (HT), 17 cases with non-toxic nodular goiter (NTNG) and 20 healthy subjects in this study. The serum concentrations of their Th1 cytokines (IFN-γ, IL-2) and Th2 cytokines (IL-4, IL-10) were assayed by ELISA. The serum levels of their thyrotropin receptor antibodies (TRAb), thyroglobulin antibodies (TGAb) and thyroid peroxidase antibodies (TPOAb) were measured by routine methods. The relationship between the serum Th1 and Th2 cytokines levels and serum TRAb, TGAb and TPOAb levels were analyzed. Our results showed that the serum levels of IL-4 and IL-10 in patients with GD were significantly higher than those in patients with HT, NTNG and healthy subjects ($P<0.01$), while the serum levels of IFN-γ and IL-2 in patients with HT were significantly higher than those in patients with GD, NTNG and healthy subjects ($P<0.01$), and serum IL-4 level was significantly lower than that in healthy subject as well ($P<0.05$). In GD patients, the serum levels of both IL-4 and IL-10 were positively associated with serum TRAb titer, respectively ($r = 0.683, 0.579$; $P < 0.05$). In HT patients, the serum levels of both IFN-γand IL-2 were positively associated with serum TGAb and TPOAb titers, respectively (IFN-γ: $r = 0.542, 0.650$; IL-2: $r = 0.517, 0.602$; $P<0.05$). These results suggest that the patients with GD or HT had a cellular immunity imbalance, with a Th2 cell immune response dominant in GD patients and a Th1 cell immune response dominant in HT patients.

THE PROTECTIVE ROLE OF HIGH-DENSITY LIPOPROTEIN ON LPS-INDUCED ACUTE LUNG INJURY ON MICE

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High-density lipoprotein (HDL), an abundant plasma lipoprotein, has been thought to be anti-inflammatory in both health and infectious diseases. It binds lipopolysaccharide (LPS) and neutralizes its bioactivity. This study was aimed to investigate the potential role of HDL, which can be separated from human serum, in LPS-induced acute lung injury in mice. Kunming mice (18-22 g) were treated with either HDL (70mg/kg, via tail vein) or saline 30 min after LPS administration (10mg/kg, intraperitoneally) and were decapitated 6 h after LPS challenge. Artery blood was collected and analyzed for blood gas variables (PaO_2, PaO_2/ FiO_2, pH, and $PaCO_2$). Bronchoalveolar lavage fluid (BALF) samples were analyzed for the concentration of total protein, lactate dehydrogenase (LDH) activity, and white blood cell count (WBC). Lung samples were taken for histologic evaluation and for determination of wet-to-dry (W/D) lung weight, malondialdehyde (MDA) level, myeloperoxidase (MPO) activity and TNF-alpha levels. (1) Arterial blood gas analysis showed that after LPS challenge, HDL treated mice showed a higher PaO_2, PaO2/FiO2, and pH, but a lower $PaCO_2$ relative to saline controls ($P <0.01$); (2) LPS-induced increases in the concentration of total protein and the numbers of WBC and LDH activity in BALF were significantly decreased in HDL-treated mice ($P <0.01$). HDL treatment also resulted in a significant protection in lung tissue tested against LPS induced acute lung injury via decreasing W/D ratio, MPO activity, MDA level and via decreasing levels of the pro-inflammatory cytokine TNF-alpha ($P <0.01$ and $P <0.05$, respectively); (3) Histological examination revealed that HDL treatment resulted in significantly lower scores of acute lung injury induced by LPS with reduced hemorrhage, intra-alveolar edema, and neutrophilic infiltration ($P<0.01$). We conclude that HDL plays a protective role in attenuating LPS-induced lung injury in mice.

RESEARCH ON TRANSFECTION OF CULTURE CELLS FROM LARVAL WORMS OF *SCHISTOSOMA JAPONICUM* USING HUMAN TELOMERASE REVERSE TRANSCRIPTASE (hTERT) GENE

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A cell line from *Schistosoma japonicum* (*S. japonicum*) larval worms would be a useful source of the parasite material for immunologic, protein biochemical and enzymatic studies. Despite the immense value such a cell line would have, until now no cell line has ever been produced from *S. japonicum*. Our previous research had developed a kind of 1640-40 defined medium which was capable of promoting growth and propagation of some cells from 12-day-old schistosomula and immunization of mice with these cells could provide high level of immunoprotection against schistosomiasis. However, the cells cultured in such a medium could not be passaged successively. To obtain immortalized cell lines from *S. japonicum*, in this study, cells from *S. japonicum* larval worms were cultured in 1640-40 defined medium, and steadily transfected by a retroviral vector containing human telomerase reverse transcriptase (hTERT) gene, which expressed in some hTERT-negative mammalian cells leads to telomere length maintenance and further to immortalization by bypass of senescence and crisis. The presence, transcription and translation of transgene were confirmed by polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR) and Western-blotting analysis, respectively. Subsequently, the telomerase activity and the telomere lengths of transfected cells at different population doublings (PDs) or the primary culture cells were identified. The results showed that hTERT gene was successfully transferred into culture cells from *S. japonicum* larval worms and expressed in transduced cells. Whereas, the transduced cells neither expressed telomerase activity nor divided vigorously, and the telomere elongation was not observed. It is suggested that transfection of plasmids containing the cloned hTERT gene is not sufficient to establish immortalized cell lines from *S. japonicum* and, probably, the ectopic expression of hTERT gene in combination with other genes involved in immortaliztion is required for immortaliztion.

mRNA EXPRESSION OF LTC4 SYNTHESIS ENZYMES ARE REGULATED VIA NF-κB SIGNALING PATHWAY IN HEPATIC I/R INJURY RATS

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Recently, we reported that LTC4 synthesis enzymes including leukotriene C4 synthase (LTC_4S), microsomal glutathione S-transferase (mGST) 2 and mGST3, could all conjugate LTA4 and reduced glutathione (GSH) to form LTC4, and were related to hepatic ischemia-reperfusion (I/R) injury. However, the gene expression mechanism of LTC4 synthesis enzymes during early hepatic I/R is largely unclear. Adult male Sprague-Dawley rats were divided into 2 groups: sham group (Sham), and ischemic-reperfusion group (I/R).

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Liver was subjected to 60 min of partial hepatic ischemia followed by 5 h of reperfusion. We examined the mRNA levels of LTC4 synthesis enzymes, iNOS and eNOS in rat liver and the protein expressions of NF-κB p65, p50 and IκBα in liver cell lysates and nuclear extracts. The levels of serum NO2- and liver tissue GSH, MDA and SOD activity were also detected. Increases of the mRNA expressions of LTC4S and iNOS and decreases of the mRNA levels of mGST2, mGST3 and eNOS were significant after hepatic 5h reperfusion in rats when compared with Sham ($P<0.05$), liver MDA and serum NO2- elevation, hepatic GSH and SOD activity decline. In addition enhancement of the protein expression of NF-κBp65 and p50 in nucleus extract in I/R group were also observed ($P<0.05$). Immunohistochemistry revealed that strong cytoplasmic and nuclear staining exhibited in I /R rat liver while Sham liver displayed negative staining. But IκBα protein level in liver tissue remained unchanged in both groups. Our results lend support to the idea that hepatic I/R up-regulated LTC_4 S mRNA but down-regulated the mRNA expressions of mGST2 and mGST3 via NF-κB activation pathway independent of IκBα degradation. Moreover, NF-κB activation may be associated with ROS, lipid peroxidation, GSH depletion and NO elevation.

V-PYRRO/NO REDUCED LEUKOTRIENE C4 PRODUCTION VIA REGULATING EXPRESSION AND ACTIVITY OF LEUKOTRIENE C4 SYNTHASE IN HEPATIC ISCHEMIA/ REPERFUSIO INJURIED RAT

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We recently reported that hepatic ischemia-reperfusion (I/R) injury was related to the cysteinyl leukotriene (LT), which was regulated by Sodium Nitroprusside. However, the effects of a liver specific nitric oxide (NO) donor on LTC4 generation during hepatic I/R injury are largely unclear. This experiment was designed to assess whether a liver specific NO donor (V-PYRRO/NO) affected LTs generation in hepatic I/R injury rats. Sprague-Dawley rats were divided into 3 groups: sham group (Control), I/R group and V-PYRRO/NO (1.06 μmol/kg/h) +I/R group. Liver was subjected to 60 min of partial hepatic ischemia followed by 5h of reperfusion, saline or V-PYRRO/NO (1.06 μmol/kg/h) administered intravenously. Protein expressions of the LTC4 synthesis enzymes including LTC4 synthase (LTC4S), microsomal glutathione S-transferase (mGST2) and mGST3 were detected with western blotting, and the activities of the LTC4 synthesis enzymes and LTC4 content were measured by RP-HPLC. Tissue injuries were assessed by serum ALT and AST levels and histological changes. Serum NO2- and liver tissue GSH were also examined by biochemical methods. Compared with I/R group, V-PYRRO/NO (1.06 μmol/kg/h) markedly decreased LTC4 content, LTC4S expression and the activities of the LTC4 synthesis enzymes ($P<0.05$). The decline in serum ALT, AST and NO2- levels ($P<0.05$) together with hepatic GSH elevation ($P<0.05$) in V-PYRRO/NO+I/R group were also observed compared to I/R group. The LTC4S expression on hepatocytes and sinusoidal endothelial cells in V-PYRRO/NO+I/R group was lower than that in I/R group. But no significant differences in the protein expressions of mGST3 and mGST2 existed in control, I/R and V-PYRRO/NO (1.06 μmol/kg/h) +I/R group ($P>0.05$). These results demonstrated that decreased LTC4 production by V-PYRRO/NO treatment during hepatic I/R could be mainly due to V-PYRRO/NO up-regulating the protein expression of LTC4S rather than mGST2 or mGST3 and inhibiting the activity of the LTC4S.

INNATE IMMUNE RESPONSES OF EPIDIDYMAL EPITHELIAL CELLS TO *STAPHYLOCOCCUS AUREUS* INFECTION

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The epithelium is an active participant in the host response to infection. We hypothesized that epididymal epithelia play a role in the innate immune responses by sensing the presence of pathogens, expressing and secreting inflammatory cytokines that recruit inflammatory cells in response to invading pathogens. Our results indicated that TNF-α and IL-1β could be secreted by the primary cultured rat epididymal epithelia infected with *Staphylococcus aureus* (S. aureus). Epididymal epithelia induced nitrite oxide synthase (iNOS) expression was up-regulated after *S. aureus* infection and nitrite oxide (NO) was also found to be produced significantly. NF-κB inhibitor BAY11-7082 inhibited TNF-α secretion completely and p38 Mitogen-activated protein kinases inhibitors SB203580 decreased TNF-α secretion partly, indicating that NF-κB and p38 signal pathways were involved in this inflammation response. Toll-like receptor 2 (TLR 2) and - 4 were shown to be expressed in primary cultured rat epididymal epithelia. After infection the expression level of TLR2, but not TL4, was up-regulated. These results demonstrated that epididymal epithelia play an innate immune response through activation of p38 MAPK and NF-κB after *S. aureus* infected the TLR2.

OTHER PAPERS

EFFECT OF SALVIA MILTIORRHIZA ON GENTAMICIN-INDUCED ACTIVITY OF NF-κB IN THE COCHLEA OF GUINEA PIG

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Recent reports have suggested that nuclear factor-kappa B (NF-κB) participated in physiology and physiopathology of mammalian. Our earlier work had indicated that salvia *miltiorrhiza (SM)* could protect cochlea from gentamicin (GM)-induced ototoxicity. In this study, we investigated the effect of *SM* on gentamicin-induced activity of NF-κB in the cochlea of guinea pig. Cochlea from animals treated for control, GM, GM plus *SM* and *SM* were subjected to immunostaining for assaying the activity of NF-κB p50 and p65. Auditory brainstem response was also applied. We found that expression of NF-κB p65 and p50 were observed in the cochlea in normal guinea pigs. After treatment with GM, immunostaining for NF-κB p50 was present in nuclei of spiral ganglion and outer hair cells of GM, threshold of auditory brainstem response increased simultaneously. However, concomitant injection of *SM* had no nuclear immunostaining for p50. Threshold of auditory brainstem response diminished compared with GM group. These results suggest that after treatment with GM, NF-κB was activated and hearing was injured. *SM* could effectively attenuate GM ototoxicity by inhibiting the activity of NF-κB induced by GM, which might be one of the molecular mechanisms by which *SM* could protect against GM ototoxicity.

ANTIOXIDANT TREATMENT PROTECTS CELL APOPTOSIS IN COPD

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We aimed to study the relationship between the antioxidant N-acetylcysteine (NAC) and the alveolar septal cell apoptosis in smoking-induced COPD rats. Wistar rats (n=48) were randomly assigned into normal group, COPD group, sham-interfered group and NAC-interfered group. All rats except in the normal group were exposed to cigarette smoking daily for 80 days. Rats in the NAC-interfered group and the sham-interfered group were instillated with NAC and saline solutions respectively. $FEV_{0.3}$/FVC and PEF in the COPD group were lower than the normal group and the NAC-interfered group. The MLI in the COPD group was higher than those in the normal group and the NAC-interfered group, whereas the MAN in the COPD group was lower than those in the normal group and the NAC-interfered group. Coincide with the VEGF expression in lungs by Western blot, the contents of VEGF in BALF in the normal group were higher than those in the COPD group, the sham interfered group and the NAC-interfered group. The contents of VEGF in the NAC-interfered group were also higher than those in the COPD group and the sham-interfered group. The AI of alveolar septal cells in the normal group was lower than those in the COPD group, the sham-interfered group and the NAC-interfered group, and the AI in the NAC-interfered group was significantly lower than those in the COPD group and the sham-

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interfered group. There was a negative correlation between the contents of VEGF in BALF and the AI of alveolar septal cells. Our results indicate that antioxidant protects lung function, ameliorates pulmonary emphysema and decreases alveolar septa apoptosis by partly reversing the decrease in VEGF secretion in smoking-induced COPD rats. This work was supported by National Research Fund of the Ministry of Education, China (200050533023) and Research Fund for Reform of Postgraduate Education in Hunan Province (06B07).

PIRFENIDONE ATTEUNATES PULMONARY VASCULAR REMODELING VIA INHIBITING RhoA/ROCK PATHWAY

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RhoA/Rho kinase (ROCK) pathway plays a key role in vascular remodeling and was recently thought as a novel therapeutic target for pulmonary hypertension (PH). Pirfenidone (Pir) is an effective anti-fibrosis drug and can inhibit bleomycin-induced pulmonary fibrosis. The aim of the present study was to investigate the involvement of RhoA/ROCK pathway in the inhibitory effects of Pir, on pulmonary vascular remodeling. In the rat model of monocrotaline-induced PH, oral treatment with Pir (125, 250 and 500 mg/kg) for 4 weeks could concentration-dependently attenuate pulmonary arterial remodeling and decrease plasma level of transform growth factor-β1 (TGF-β1), concomitantly with decreasing ROCK protein expression of pulmonary artery. In cultured primary rat pulmonary arterial smooth muscle cells (PASMCs), treatment with angitension II could markedly upregulate both protein expression and activity of ROCK, increased TGF-β1 production and induced proliferation, which was markedly attenuated by treatment with Pir ($10 - 100$ μM) in a concentration-dependent manner. These results suggest that Pir could inhibit pulmonary vascular remodeling via inhibiting Rho/ROCK pathway and reducing TGF-β1 production.

ACUTE HIGH INTRAOCULAR PRESSURE INCREASES SYNAPTOGENESIS IN THE OUTER PLEXIFORM LAYER OF RAT RETINA

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In response to injury, synapse alteration may occur earlier than changes in cell body of neurons. Retinal ganglion cell death and thinning of the inner part of retina were found after acute high intraocular pressure (HIOP), while the structural and functional changes of synapses in the retina remain unknown. In the present study, we investigated the protein and mRNA expression of synaptophysin (SYN), an important molecule closely related to synaptic activities, synaptogenesis and synaptic plasticity, and also observed the ultra-structural changes of the retinal synapses. Our data showed that besides the out plexiform layer (OPL) and inner plexiform layer (IPL), where SYN is distributed in normal condition, SYN immunoreactivity was also found in the inner part of the outer nuclear layer (ONL) and the band of SYN positive products in the OPL and ONL were expanded and reached the maximum at the 7^{th} day following HIOP. The pattern of SYN immunoreactivity at the 14^{th} day was similar to that in normal rats. At the same time, SYN mRNA level was gradually increased until the 7^{th} day following HIOP and then decreased. The change of number of synapses in the OPL exhibited the similar pattern. These results suggest that trans-synaptic plasticity: new synapses formation in the OPL and synaptic activities enhancement may occur in rat retina at the early stage following HIOP.

VANADIUM IMPROVES THE SPATIAL LEARNING AND MEMORY BY ACTIVATION OF CAVEOLIN–MAPK-CREB PATHWAY IN DIABETIC MICE

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Vanadium, a required trace element of human body, is reported to reduce the blood glucose values of glycemia animals and has an effect on the treatment of diabetes complications. To investigate the role of vanadium in the pathogenesis of diabetic cognitive function impediment, Kunming mice were divided into control group, diabetes group and vanadium-treatment group. Diabetic mice were induced by intraperitoneal injection of 200mg/kg of alloxan and in vanadium-treatment group diabetic mice were treated by intraperitoneal injection for three weeks with 5mg/kg of VOpz'(TP')$(SCN)_3$, a new vanadium complex with pz (SCN) ligand and low toxicity we synthesized. The three groups were trained by Morris water maze and then the expression of proteins related to learning and memory in the hippocampus of mice were examined by western blot. The results showed that (1) the latency to find platform was longer ($p>0.05$) and the percent time in target quadrant was lower ($p<0.05$) in diabetes group compared with that in control group. The learning and memory score of vanadium-treatment group was obviously higher than that of diabetes group ($p<0.05$) and equivalent to that of control group; (2) the phosphorylation level of p42/p44MAPK protein was remarkably decreased in diabetes group and then increased after vanadium treatment ($p<0.05$). Furthermore, caveolin-1 expression was remarkably reduced and CREB2 expression was higher in diabetes group while after vanadium treatment caveolin-1 expression was significantly increased ($P<0.05$). These results suggest that vanadium could improve the learning and memory ability of diabetic mice and its mechanism may be involved in the activation of Caveolin–MAPK–CREB pathway in the neuron.

EFFECTS OF CHRONIC ZINC DEFICIENCY ON ERYTHROPOIESIS

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Zinc is one of the important micronutrients. It was been considered that iron deficiency disorder (ID) and vitamin A deficiency disorder (VAD) were the major factors of anemia, and there were not relationship between zinc and erythropoiesis thought by many researchers for a long time. Nevertheless, recently, some research papers support that zinc deficiency (ZD) might induce anemia. To identify the relation between chronic ZD (ChrZD) and erythropoiesis, we fed young male SD rats (n=27) with zinc adequate diet (zinc 30 mg /kg, ZA group, n=10) or ChrZD synthetic diet (zinc 4.5 mg /kg, ChrZD group, n=17) respectively for 9 weeks. At the end of 9^{th} week, hemoglobin (Hb) was measured and bone marrow slides were differential counted. Hb of ZA group and ChrZD group were 165.33 ± 9.24 g/L and 139.56 ± 12.56 g/ L ($P<0.05$, variance analysis). Erythroid cells in bone marrow slides were 24 ± 7.79 % and 19.79 ± 6.4 % in the two groups ($P<0.05$, variance analysis). These results suggest that ChrZD can induce rats' erythropoiesis to decline.

THE EXPRESSION OF SLC26A3 IN THE TESITIS AND SPERM AND ITS ROLE IN SPERM CAPACITATION

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Our previous study has demonstrated the chloride dependencdence and the involvement of Cystic fibrosis transmembrane conductance regulator (CFTR) in transporting bicarbonate into sperm necessary for the capacitation, an activation process by which sperm acquire their ability to fertilize the egg; however, whether its involvement is direct or indirect remains unclear. Using mice and

guinea pig as a model, the present study investigated the possibility of a Cl^-/HCO_3^- exchanger, solute carrier family 26A3 (SLC26A3), operating with CFTR during capacitation. Depleting Cl^-, even in the presence of HCO_3^-, abolished sperm capacitation and vise versa, indicating the involvement of both anions in the capacitation process. Sperm capacitation, could be reduced by antibodies of SLC26A3 with a concentration dependent manner. Similarly, HCO_3^--dependent increase in intracellular pHi and cAMP level as well as another capacitation-associated event, sperm hyperactivated motility, were also inhibited by SLC26A3 antibody. The RT-PCR results showed that the SLC26A3 was expressed in the mice testis and sperm. Also the expression and localization of SLC26A3 in guinea pig sperm were demonstrated using immunostaining and westernblot. Taken together, our results indicate that Cl^- is required for the entry of HCO_3^- necessary for sperm capacitation, implicating the involvement of the Cl^-/HCO_3^- exchanger SLC26A3 in transporting HCO_3^- , in addition to the previously reported CFTR.

GnRH EXPRESSION AND EFFECT OF ELECTRO-ACUPUNCTURE IN RATS AND RABBITS AT DIFFERENT DEVELOPMENT STAGES

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To investigate sexual development, central GnRH expression, electrophysiological characteristics in arcuate nucleus (Arc), as well as the effect of electro-acupuncture (EA) in animals at different developmental stages. EA (3Hz) at the same acupoints was performed for 20 min per day in Sprague–Dawley rats for 10 days. GnRH expression in the hypothalamus was determined using RT-PCR and real-time PCR. Testosterone (T) and sperm count in male rabbits were reduced by repeated EA ($P<0.01$). GnRH expression in rats of the early pubertal group (EPG) and adult group (AG) were significantly depressed by EA at acupoints ($P<0.01$). EA reduced significantly sperm count at puberty ($P<0.01$), while didn't influence body weight ($P>0.01$) and structures of the gonadial tissues. The repeated EA is a good option that can be considered for regulating the function of the hypothalamus-pituitary-gonad (HPG) axis during puberty.

SCREENING AND IDENTIFICATION OF A HUMAN ScFv ANTIBODY FRAGMENT AGAINST FOLLICLE-STIMULATING HORMONE BETA (FSH-β)

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The aim of this study was to screen and identify scFv antibody fragment against FSH-β from a human scFv antibody library. Using synthetic FSH-β peptide as coating antigen, the scFv antibody against FSH-β was screened by four times' combining-eluting-amplifying. The positive antibody was identified by ELISA. The specificity of soluble antibody was identified by ELISA. The affinity of soluble antibody was measured by non-competitive ELISA. The scFv antibody screened was specific for FSH-β. The soluble antibody was also specific for FSH−β, with low cross-reaction with some analogs. Its molecular weight was about 30 kD by SDS-PAGE and its affinity constant was about 2.64×10^{-7} mol/L. The screened scFv antibody is specific for FSH-β and has low cross-reaction with analogous molecules. It would be further used in specific treatments of immunological contraception and sexual precocity.

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) CONTRIBUTES TO THE PAIN HYPERSENSITIVITY FOLLOWING SURGICAL INCISION IN THE RATS

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The pathogenic role of brain derived neurotrophic factor (BDNF) in the incisional pain is poorly understood. The present study explores the role of the BDNF in the incision-induced pain hypersensitivity. Mechanical allodynia was developed rapidly and sustained three days in the hind-paw incision model in the rats. After hind-paw incision, dramatic upregulation of BDNF was observed in the ipsilateral DRG and spinal cord in the lumbar segments as determined by immunohistochemistry. Double-labeling immunofluorescence showed that the increased BDNF in the spinal cord was mainly localized in the neurons but not microglia or astrocytes. Sciatic nerve blockade with lidocaine prevented the increase of BDNF in the DRG and spinal cord. Intrathecal (IT) injection of BDNF antibody greatly inhibited the mechanical allodynia whereas intra-peritoneal (i.p) administration had only marginal effect. Taken together, the present study showed that incision induced the upregulation of BDNF in the DRG and spinal cord through somatic afferent nerve transmission, and the upregulated BDNF contributed to the tactile allodynia in the incisional pain.

REMIFENTANIL MEDIATES BRADYCARDIA AND HYPOTENSION WITH DISTINCT MECHANISMS

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Remifentanil (REM), a short-acting opioid, has been well known to cause much more significant bradycardia and hypotension compared with other opioids such as fentanyl. However, it is still poorly understood and controversial about the mechanism of the REM-mediated bradycardia and hypotension. The present study was aimed to explore the mechanism of REM-mediated bradycardia and hypotension in the rabbit model and the patients for cardiac surgery. Here, we showed that remifentanil caused very short but significant bradycardia (15% decrease of heart rate) which recovered within 1 min. In contrast, remifentanil led to the fall of the blood pressure as much as 40% and the hypotension sustained for more than 5min. The non-specific opioid receptor antagonist, naloxone, could entirely prevent the REM-mediated hypotension but only marginally reverse REM-mediated bradycardia. The REM-mediated bradycardia could not be reversed by the vagotomy or sympathetic block. In contrast, the hypotension induced by REM could be slightly inhibited by sympathetic nerve block but not by vagotomy. In addition, REM also caused the fall of blood pressure during the period of cardiopulmonary bypass (CPB) in the patients for cardiac surgery. Taken together, the present study showed that REM induced bradycardia and hypotension with distinct mechanisms. The REM-mediated-bradycardia is likely independent of opioid receptor and autonomous system. In contrast, REM-mediated-hypotension is likely through acting on the opioid receptor in the sympathetic nervous system indirectly and those in the blood vessels directly.

EFFECT OF MK-801 ON THE CHANGE OF CYTOKINES IN HYPEROXIA-INDUCED LUNG INJURY IN NEONATAL RAT

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The aims of this study were to observe both the change of the releasing of TNF-α, IL-1β, IL-6, MIP-2 and the effect of glutamate NMDA receptor antagonist MK-801 on hyperoxia-induced lung injury in neonatal rat. The 22-day gestation full term rats in 12 hours after birth were randomly divided into four groups: air control group, air+MK-801group, hyperoxia group and hyperoxia+MK-801 group. Hyperoxia group and hyperoxia+MK-801 group were exposed to > 95 % O_2 for 7 days. Air control group and air+MK-801 group were placed in room air. Air+MK-801 group and hyperoxia+MK-801group were received MK-801 intraperitoneally every day. Air control group and hyperoxia group received the same volume of saline. After 7 days of exposure, the neonatal rats were sacrificed and the content of TNF-α, IL-1β, IL-6, MIP-2 in the homogenate of lung tissues were measured by enzyme linked immunosorbent assay (ELISA). The contents of TNF-α, IL-1β, IL-6 and MIP-2 in hyperoxia group were higher than those in air group (124.89 ± 14.86 pg/mgprot vs. 78.98 ± 8.14 pg/mgprot, 81.56 ± 10.17 pg/mgprot vs 55.11 ± 11.10 pg/mgprot, 69.46 ± 9.37 pg/mgprot vs. 33.82 ± 14.27 pg/mgprot, and 96.27 ± 11.95 pg/mgprot vs. 60.75 ± 10.70 pg/mgprot, for TNF-α, IL-1β, IL-6 and MIP-2 respectively, $P<0.05$). MK-801 could inhibit the increase of TNF-α, IL-1β, IL-6 and MIP-2 induced by hyperoxia. The contents of TNF-α, IL-1β, IL-6 and MIP-2 in the hyperoxia+MK-801 group were lower than those in the hyperoxia-group (90.59 ± 6.44 pg/mgprot vs. 124.89 ± 14.86pg/mgprot, 66.76 ± 11.32 pg/mgprot vs. 81.56 ± 10.17 pg/mgprot, 40.82 ± 8.74 pg/mgprot vs. 69.46 ± 9.37pg/mgprot, 73.05 ± 15.78 pg/mgprot vs. 96.27 ± 11.95 pg/mgprot, respectively, $P<0.05$) and were not different from air group and air+MK-801 group. The releasing of TNF-α, IL-1β, IL-6, MIP-2 had increased in neonatal rats which were exposed to high concentration of oxygen for seven days. Our results show that NMDA receptor antagonist MK-801 could inhibit TNF-α, IL-1β, IL-6 and MIP-2 released increased induced by hyperoxia.

A COMPARISON STUDY ON THE RESPONSES OF UMBILICAL ARTERIES AND THORACIC AORTS TO THE ADRENERGIC RECEPTOR AGONISTS

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Adrenergic receptor agonists are widely used to treat hypotension during spinal anesthesia in the cesarean section of obstetrics operations. However, the blood supply to the fetus through the umbilical artery is uncertain because little is known about whether adrenergic receptor agonists contract both the maternal artery and umbilical arteries while they increase the blood pressure. In this in vitro study, the effects of phenylephrine (PE), ephedrine, metaraminol (MET) and dopamine (DOP) on the human umbilical artery (HUA) and the pregnant (21 days after pregnancy) or non-pregnant rat thoracic aorts (RTA) were investigated. In the HUA study, cumulative administration of PE, ephedrine, MET and DOP (10^{-9}–10^{-4} M) did not affect the vasomotion of rings in basal tension or preconstricted with KCl (6×10^{-2} M) or 5-HT (10^{-6} M). In the RTA study, PE significantly increased the tension of RTA both in the pregnant and non-pregnant rat at the same concentration range. Moreover, MET and DOP caused a significant concentration-dependent vasoconstriction on RTA. But ephedrine did not change the tension of RTA neither in the pregnant nor in the non-pregnant rat at the same concentration range. From all the above results, we concluded that these popular used adrenergic receptor agonists did not contract umbilical artery while they caused vasoconstriction to increase the maternal blood pressure. Although the mechanism of their effects on increasing the maternal blood pressure is quite different from each other and remained to be explored, the usage of PE, ephedrine, MET and DOP to treat hypotension in obstetrics operations is safe for fetus.

GUINEA PIG AS A RESEARCH MODEL IN THE NEUROENDOCRINE REGULATION OF LUTEINIZING HORMONE SECRETION

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Among many research models, animal studies have always been tempting in the investigation of the neuroendocrine regulation of the reproductive function. It is indeed true that the *in vivo* studies have many features, such as the study of the feedback effects, of the interactions between different systems, of the side effects of the tested agents, that can not be replaced by the *in vitro* studies. In the past, rat, rabbit and monkey have been the most often used research models in the *in vivo* studies in the neuroendocrine regulation of reproduction. Guinea pig as a research model, however, has become increasingly popular. That is because that several aspects of its reproductive function are more close to those of the primate. For instance, its reproductive cycle is composed of a true luteal phase and the distribution of the hypothalamic GnRH neurons is more similar to that of the primate. On the other hand, guinea pig also has some remarkable special features in its endocrine axes and metabolism pathways. Therefore, comparing them with those in other species may generate useful information. The amino acid sequence of GnRH is highly conserved among different species. The dominant form of GnRH in the brain of all mammals is the same except in the guinea pig. The amino acids His in position 2 and Leu at position 7 in GnRH in all other mammals are replaced by Tyr and Val, respectively, in the guinea pig. The immunoreactivity of the synthetic guinea pig GnRH was different compared to that of mammalian GnRH. The guinea pig GnRH was much weaker in stimulating LH release in both guinea pig and rat *in vivo* and *in vitro*, which may be due to their differences in their affinity to the receptors.

THE ROLE OF HYPOTHALAMIC STEROID METABOLITES IN THE REGULATION OF LH SECRETION IN THE RAT

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Lines of evidence have been accumulated in the past years showing that the central nervous system can synthesize steroids *de novo*, and the brain can also metabolize steroids resulting in a variety of metabolites. These steroids are now collectively called neurosteroids. Evidence also demonstrated that some neurosteroids were involved in the regulation of luteinizing hormone secretion. The aims of the current study were to evaluate the possible role of the neurosteroids resulted from progesterone metabolism in the regulation of gonadotropin secretion by comparison of the progesterone metabolites and the gonadotropin levels. The metabolites of progesterone through 5alpha-reductase and 3alpha-oxidoreductase in the hypothalamus of the male rats were measured by *in vitro* incubation of ^{14}C-labeled-progesterone with hypothalamic tissue of the male rats of different age and after castration/adrenalectomy. The endogenous levels of progesterone levels were also measured. The blood levels of testosterone, progesterone, luteinizing hormone and follicle stimulating hormone were also assessed simultaneously. The levels of the 5alpha-reduced and 3alpha-oxidoreduced metabolites were highest in the hypothalami of the young (18 day-old) rates as compared to the mature rats. Adrenalectomy and/or castration performed on the 6th day of life did not change the pattern of levels of the 5alpha-reduced and 3alpha-oxidoreduced metabolites observed in the intact rates. Blood LH and FSH levels were lower in the prepubertal rates as compared to those in the mature rates. Castration resulted in an increase in gonadotropin level. In conclusion, the current data do not allow us to make a conclusion that the hypothalamic 5alpha-reduced and 3alpha-oxidoreduced metabolites were involved in the regulation of gonadotropin secretion.

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N-METHYL-D, L-ASPARTIC ACID STIMULATING LH SECRETION *IN VIVO* IN THE ADULT MALE GUINEA PIG PARTIALLY VIA NON-GnRH RECEPTOR PATHWAY

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Guinea pig as a research model has some advantages. For example, several aspects of its reproductive function are more close to those of primate, i.e. its reproductive cycle is composed of a true luteal phase and the distribution of the hypothalamic GnRH neurons is more similar to that of the primate. However, guinea pig also has some particularities in its endocrine axes and metabolism pathways, which by comparison with other species may provide new insight. Previous studies in rats and monkeys showed that N-Methyl-D,L-Aspartic Acid (NMA) stimulated luteinizing hormone release via the hypothalamus. In the male guinea pig we observed that NMA could induce strong LH secretion, which could be blocked by the NMDA receptor antagonist, DL-2-amino-5-phosphonovaleric acid (AP5). Administration of Cetrorelix, an GnRH receptor antagonist, before introducing NMA could only partially block the LH release induced by NMA, whereas it could completely block the LH secretion induced by GnRH. It is concluded that NMA induced LH secretion in the male guinea pig was only partially mediated by GnRH receptor.

EFFECT OF TMP ON BURN INJURY PAIN MEDIATED BY $P2X_3$ RECEPTOR

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Peripheral administration of P2X agonists rapidly causes nocifensive behavior in experimental animals and pain sensation in humans. Most of the nociceptive response to peripheral ATP is mediated by $P2X_3$ receptor. The effects of tetramethylpyrazine (TMP) on rat burn injury pain mediated by $P2X_3$ receptor was investigated. First degree and superficial second degree burn injury models were adopted. Mechanical withdrawal threshold and thermal withdrawal latency were measured and the $P2X_3$ receptor expressions of nerve terminal in burn injury skin were detected by immunohistochemistry. After burn, the mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL) in group IIIA (first degree foot burn +NS group) and group IIIB (superficial second foot burn +NS group) were lower than that in group I (sham foot burn), lasting for 24 and 96 hours respectively ($p<0.01$). After hour 24, there was no difference in MWT and TWL between group IIA (first degree foot burn +TMP group) and group I ($p>0.05$). However, there was difference between group IIB (superficial second foot burn +TMP group) and group I ($p<0.01$) until hour 72 ($p>0.05$). At day 3 post burn, the $P2X_3$ receptor expressions at the burn injury skin nerve terminal in group VIA (first degree back burn +NS group) and group VIB (superficial second back burn +NS group) were significantly increased compared with other groups ($p<0.05$). Post-treated with TMP, the $P2X_3$ receptor expressions at the nerve terminal in group VA (first degree back burn + TMP group) and VB (superficial second back burn +TMP group) were markedly decreased. These results suggest that TMP may alleviate burn injury pain mediated by $P2X_3$ receptor. This work was supported by the National Natural Science Foundation of China (No. 30260030).

PROTECT ACTION OF PUERARIN TO INTESTINE AND EXTRAINTESTINAL ORGAN IN INTESTINAL ISCHEMIA-REPERFUSION INJURY

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The aims of this study were to study the protect action of Puerarin to the lesion of intestine and extraintestinal organ, and to monitor concentration changes of cellular signal transductive factor-NO and cellular factor receptor-TNF-α in serum by intestinal ischemia-reperfusion injury. 30 rats were divided into three groups, 10 per group. The control group was injected N.S in abdomen. Animal model groups were injected Puerarin in abdomen with the dose of 100 mg/kg, 200mg/kg once daily, for 5 days. On the sixth day, 30 minutes after inject of medicine, rats were anesthetized to build the model of intestinal ischemia-reperfusion injury (IIRI). 45 minutes after superior mesenteric artery was closed, blood flow was recovered for 90 minutes, and then at the end of experiment, blood was obtained to detect the concentration of TNF-α and NO in serum. Part of liver, intestine, lung and kidney were used to observe the pathological diversity. We found Puerarin could relieve the pathological lesion of intestine and extraintestinal organ and increase the concentration of NO ($P<0.05$), and decrease the concentration of TNF-α ($P<0.05$). These results suggest that Puerarin have preserve action to the lesion of intestine and extraintestinal organ - liver, lung and kidney in IIRI in rats, which may related to concentration changes of cellular signal transductive factor NO and TNF-α in a dose-dependent manner.

PROTECTIVE EFFECT OF TELMISARTAN ON PANCREATIC β CELLS OF TYPE 2 DIABETIC RATS

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The aim of this study was to investigate whether Telmisartan had a protective effect on structure and function of pancreatic β cells of type 2 diabetic rats induced by high fat and fructose diet. The normal Sprague-Dawley (SD) rats were randomly divided into three groups: Standard Chow Diet (SCD) group was kept on a standard diet (4 % [w/w] fat, 51 % carbohydrate and 19 % protein); High Fat and Fructose Diet (HFFD) group was kept on a special diet (13 % [w/w] fat, 65 % D-fructose, 10 % protein); and High Fat and Fructose Diet with Telmisartan (HFFD + Tel) group was kept on the same special diet and treated with Telmisartan. Fasting Serum Glucose (FSG) and Fasting Serum Insulin (FSI) were measured every two weeks. Intraperitoneal injection Glucose Tolerance Test (IGTT) was performed to analyze insulin sensitivity. Insulin Release Test (IRT) was performed to evaluate function of pancreatic β cells. Body weight and pancreatic tissue weight of SD rats were measured, and islet morphology was assessed by haematoxylin and structure of pancreas was observed by eosin staining and immunohistochemistry. Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed to test the level of Glucose Transporter 2 (Glut 2) mRNA in pancreatic tissue. SD rats on high fat and fructose diet developed diabetes (blood glucose>9.0 mmol/l) due to insulin resistance and selective destruction of pancreatic β cells associated with severe loss of immunoreactivity of insulin and decrease of Glut 2 mRNA. In contrast, diabetic SD rats on treatment with Telmisartan remained normoglycaemic, and exhibited normal pancreatic islets and appropriate ability of insulin secretion. Our results indicate that Telmisartan had a protective effect on structure and function of pancreatic β cells of type 2 diabetic rats.

EFFECTS OF SINOMENINE ON CO/NO-cGMP SIGNALING CASCADE IN THE CEREBELLUM AND SPINAL CORD OF MORPHINE-DEPENDENT AND WITHDRAWAL MICE

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The CO/NO-cGMP signaling cascade plays an important role in morphine dependence and withdrawal. To explore the effect of sinomenine on the CO/NO-cGMP signaling cascade in the cerebellum and spinal cord of morphine -dependent and morphine-withdrawal mice, mice were subjected to injection of morphine with an increasing dose for 5 d (d1: 10 mg/kg, d2: 20 mg/kg, d3: 30 mg/kg, d4: 40 mg/kg, d5: 50 mg/kg, s.c.), and then were treated with sinomenine (40 mg/kg, i.p.) for another 5 d. Naloxone (4 mg/kg, i.p.) was used to develop acute withdrawal, and the withdrawal syndromes (including body weight, teeth chattering, twisting, straightening, sneezing, and ptosis) were investigated. The mRNA levels of HO2, nNOS, sGCα1 and sGCα2 in the cerebellum and spinal cord were determined by semi-quantitative RT-PCR, respectively. The results obtained were as follows: (1) Sinomenine restored the decrease of body weight and alleviated the signs of withdrawal in mice. (2) Sinomenine reduced the increase of mRNA level HO2, nNOS, sGCα1, and sGCα2 in the cerebellum and spinal cord resulting from morphine dependence. (3) Administration of sinomenine only did not develop physical

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dependence in mice. The results obtained indicate that sinomenine may attenuate morphine addiction and significantly alleviate morphine withdrawal symptoms, and that the molecular mechanism may be associated with the effect of sinomenine on the CO/NO-cGMP signaling cascade in the cerebellum and spinal cord.

CALCIUM BINDING-PROTEINS EXPRESSION IN THE VENTRAL HORN FOLLOWING HEMISECTION OF RAT SPINAL CORD

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Previous studies suggest that the spontaneous recovery of locomotion may concern with interneurons after subtotal spinal cord injury. Calcium binding-proteins have the effects on neuronal function activity by mediating intracellular-free calcium, which are extensively distributed in interneurons of nervous system. Therefore we hypothesized that alteration of expression of calcium binding-proteins in the interneurons might contribute to this spontaneous recovery of locomotion after subtotal spinal cord injury. The aim of this study was to seek for the expression pattern of calcium binding-proteins following T10 spinal cord hemisection in rats. SD rats were randomly divided. The L5 spinal segments were taken for detection of expression of calcium binding-proteins (calbindin, CB; parvalbumin, PV; Calreticulin, CR) by immunohistochemistry. We found that CB-, PV- and CR − immunoreactions were mainly distributed in small or medium interneurons of laminel VII, VIII in the ventral horn of normal control group and experimental groups. Interestingly, a few PV-ir large motoneurons were observed only on 12h post injury. Furthermore optic density analysis showed that CB-, PV- and CR expressions in the injured lateral were upregulated transiently after spinal cord hemisection, but the highest expression of CB occurred at 12h post injury whereas PV or CR appeared on day 7. Subsequently, the expression levels of three calcium binding-proteins decreased and returned to normal levels on day 14 post injury. However, there was no significant difference of CB-, PV- and CR expressional quantities between normal control and the injured contralateral. The data demonstrate the spatiotemporal patterns of the expression of CB, PV and CR in the interneurons of ventral horn following spinal cord hemisection, which were related to different modulation of intracellular-free calcium, implying that calcium binding-proteins in the interneurons may participate in spontaneous recovery of locomotion after subtotal spinal cord injury.

EXPRESSION OF P-TrkB RECEPTOR IN RAT RETINA FOLLOWING ACUTE HIGH INTRAOCULAR PRESSURE WITH BDNF PRE-TREATED

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To investigate the expression of p-TrkB receptor in rat retina following acute high intraocular pressure (HIOP) with BDNF pre-treated, seventy-two adult rats were randomly divided into acute HIOP group, BDNF pre-treated HIOP group and vehicle pre-treated HIOP group. The left eyes of rats in BDNF pre-treated HIOP group and vehicle pre-treated HIOP group were injected with BDNF or vehicle respectively 2 days before HIOP. The intraocular pressure of all left eyes was increased until b wave of flash electroretinogragh (fERG) disappeared and such pressure maintained for 60 minutes. All the right eyes were served as normal control group. The rats were sacrificed after 1, 3, 7 or 14 days, and immunohistochemistry for detecting the expression of p-TrkB was used. We found that compared to the normal control group, the expression of p-TrkB was decreased significantly ($P<0.05$) during reperfusion in the acute HIOP group. The expression of p-TrkB during reperfusion in vehicle control group was similar to that in acute HIOP group. In the BDNF pre-treated HIOP group, the expression of p-TrkB was also decreased, but significantly higher than that in the acute HIOP group at all time points. The results indicate that down-regulation of p-TrkB following HIOP was relieved by exogenous BDNF, which may be involved in the protection role of BDNF to the injured retina following HIOP.

EFFECT OF INTRATHECAL INJECTION OF MK-801, *L*-NNA AND MORPHINE ON THE NOCICEPTIVE BEHAVIORAL REACTION DURING INFLAMMATORY PAIN

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In order to explore the changes of nociceptive response by intrathecal injection of NMDA receptor antagonist MK-801, NOS inhibitor *L*-NNA and Morphine in tail flick test and formalin test, SD rats were randomly distributed into formalin test group (intrathecal injection of saline), MK-801 group, *L*-NNA group and Morphine group. All groups were treated with 5% formalin 100 μl injections in the right hind paw of rats. MK-801, *L*-NNA and Morphine groups were treated with intrathecal injection of 10, 20,40 nmol/L respectively before 20 min of injecting formalin. The nociceptive behavioral reaction was recorded. Also, the change of the tail flick latency (TFL) of rats in heat tail-flick test was observed. The nociceptive reaction induced by injection of formalin in hind paw exhibited two phases. The weighted pain scores of second phase were significantly reduced by intracecal injection of MK-801, *L*-NNA and Morphine. The TFL of rats was significantly prolonged by intrathecal injection of MK-801, while the TFL of rats were more significantly prolonged by intrathecal injection of Morphine. However, in the heat tail-flick test, the TFL and maximum percent effect (MPE) did not change significantly when the lower concentration of *L*-NNA was used, and the hyperalgesia happened when the higher concentration of *L*-NNA was used. These results suggest that MK-801, *L*-NNA and morphine had significant analgesic effect. But the analgesic effect of MK-801 and *L*-NNA was weaker than that of Morphine. NMDA receptor and NO play an important role in the pain transduction and modulation in the spinal cord. This study was supported by the Youth Science and Technology Special Foundation of Heilongjiang Province (QC06C060), Medical Basic Subjects Youth Science Foundation of Harbin Medical University (060014) and Science and Technology Foundation of Heilongjiang Provincial Health Department (2006-472).

DEVELOPMENTAL POTENTIAL STUDIES OF HEXAPLOID EMBRYOS PRODUCED BY BLASTOMERES FUSION OF DIPLOID AND TETRAPLOID EMBRYOS AT 2-CELL STAGE

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The polyploid mouse embryos are important models for understanding the cleavage and preimplantation development mechanism in mammalian. Here we report the produce of the Kun Ming (KM) hexaploid embryos by exchanging and electrofusing blastomeres from diploid and tetraploid KM mouse embryos at 2-cell stage. Firstly, the tetraploid embryos were made by inducing two blastomeres fusion at 2-cell stage. About 20 hours after fusion, tetraploid embryos could develop to 2-cell stage. At this moment, one blastomere was taken out and transferred to a normal diploid 2-cell stage embryo, in which one blastomere had been taken out already. Electrofusion was performed in 0.28 M mannitol solution when the micromanipulation was done. The assessment of the hexaploid embryos which derived from the 2n/4n embryonic fusion was evaluated by *in vitro* culture, karyotype analysis, nuclear number count, cytoskeleton and Oct4 immunofluorescence. In results, hexaploid embryos were able to develop to the blastocyst stage at 72.7 % which was lower than that of normal diploid embryos (98.0 %, $P<0.05$) but no significant difference with tetraploid blastocyst development (86.2 %). However, the cell number in hexaploid blastocyst was less than that in diploid or tetraploid blastocyst (12.3 ± 2.0 vs 52.2 ± 7.2, 18.4 ± 3.5). Karyotype analysis confirmed that the number of chromosomes in hexaploid embryos were 120. Furthermore, β-tubulin and Oct-4 immunofluorescence indicated that hexaploid

blastocysts were nearly absent of inner cell mass (ICM) but some blastomeres did show Oct4 positive expressions.

THE INCIDENT OF MORPHINE ADDICTION AND WITHDRAWAL IN PARENTS PRIOR TO MATING PRODUCES ANXIETY-LIKE BEHAVIOR IN ADULT OFFSPRING RATS

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Exposure of the developing foetus to drugs of abuse during pregnancy may lead to persistent abnormalities of offspring brain function and morphology. It is unknown, however, whether drugs abuse prior to mating affect the behavior of offspring. Here we assessed the effects of morphine addiction and withdrawal prior to mating to the emotional behavior in adult offspring rats. Experimental male and female adult Sprague-Dawley rats were subjected to injection of morphine intraperitoneally twice per day for 10 consecutive days. The incremental dose was 5 mg/kg per day from 5 mg/kg on day 1 to 50 mg/kg on day 10. 21 days after morphine withdrawal, rats were mated. Rats without the morphine injection, as the control group, were given saline treatment with the same procedure as the above. The emotional behavior was assessed in offspring at 10 weeks of age. Our results showed that compared with control group, offspring in experimental group spent significantly less time in the center zone as well as a lower ratio of center versus periphery time in the open field test ($p<0.05$). Similarly, in the elevated plus maze, offspring in experimental group exhibited significantly decreased duration in open arm ($p<0.05$). In the dark/light apparatus, offspring in experimental group showed longer latencies to enter the light side, spending the majority of their time on the dark side ($p<0.05$). These findings suggest that parents experiencing morphine addiction and withdrawal incident could result in the offspring's anxiety-like behavior, which might be possibly relative to the changes of gene phenotype in the next generation.

ENDOGENOUS BRAIN-DERIVED NEUROTROPHIC FACTOR MEDIATE ASCENDING TRACT REGENERATION INTO SPINAL CORD IN MODEL OF SELECTIVE MOTOR NERVE INJURY AFTER SPINAL CORD INJURY

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Lumbar 5 ventral root transection (L5 VRT) induces neuropathic pain and triggers upregulation of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF). We hypothesized that VRT could enhance the regeneration of injured ascending sensory neurons. Sprague Dawley rats, anaesthetized by Isofluorane, were subjected for L5 VRT one week earlier and then for the dorsal column cut. Regenerating neurons were retrogradely traced by Fast Blue. After L5 VRT, BDNF mRNA and protein were highly expressed in the dorsal horn, motor neurons of the spinal cord and dorsal root ganglia (DRG). Ventral root transection resulted in a significant number of Fast Blue+ neurons in the ipsilateral DRG after dorsal column cut. This combined injury increased BDNF-like immunoreactivity in the dorsal column caudal to the lesion site and increased the expression of $p75^{NTR}$ in the glia but had no effect on the expression of $p75^{NTR}$ in sensory neurons in the dorsal root ganglia. Most regenerating sensory neurons were surrounded by p75+ glia and expressed BDNF and trkB but not p75NTR, indicating physiological overexpression of BDNF in sensory neurons could override the p75NTR-mediated inhibitory signals. Taken together, our results demonstrate that L5 VRT could promote regeneration of dorsal root into the spinal cord, most likely by increasing BDNF levels in the spinal cord and DRG and by overriding the activation of neuronal $p75^{NTR}$.

POSTNATAL VITAMIN A SUPPLEMENT CANNOT FULLY REPAIR OFFSPRINGS' LEARNING AND MEMORY IMPAIRMENT CAUSED BY MARGINAL VITAMIN A DEFICIENCY IN PREGNANCY

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Our previous study discussed retinoic acid receptors (RARs) expression in embryonic brain of rats and found the changed quantity of RARβ played a critical role in the embryonic brain development. Hence, we investigated the location and quantity of RARs expression in rat hippocampus during postnatal development. Wistar rats were sacrificed by decapitation at birth, 8th week and 44th week. The hippocampus sections were used for immunoflouresent reaction, and other tissue was frozen for real-time PCR. Location of RAR receptors (RARα, RARβ and RARγ) in hippocampal cell was detected by Laser Scanning Confocal Microscope. Quantity of RARα, RARβ and RARγ expression were analyzed through real-time PCR. RARα was expressed mainly in hippocampal cell nucleus at birth, cytoplasm at 8 and 44 weeks old. However, the location of RARβ was detected mainly in hippocampal cell nucleus at birth and 8-year-old, and endochylema expression was found when at 44th week. Meanwhile, quantity of RARα and RARβ expression decreased with aging. RARγ expression could hardly be observed in postnatal hippocampus development. Location and quantity of RARα and RARβ expression in hippocampus were programmed during the rat postnatal life, which may be closely related to the postnatal hippocampus development, function and some neurodegenerative processes.

ASYMMETRIC DIMETHYLARGININEA-ACTIVATING RhoA/ROCK PATHWAY CONTRIBUTES TO VASCULAR REMODELING IN PULMONARY HYPERTENSION

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Asymmetric dimethylarginine (ADMA) is a major endogenous inhibitor of nitric oxide synthase, plasma levels of which were significantly elevated in various animal models of pulmonary hypertension (PH) and patients with PH. However, the mechanistic linking between the elevated level of ADMA and the pathogenesis of PH still remains unknown. The activation of RhoA/Rho-Kinase (ROCK) pathway contributes to pulmonary vascular remodeling, which plays a critical role in the development of PH. The aim of the present study was to investigate the role of ADMA in pulmonary vascular remodeling and its relationship to RhoA/ROCK pathway. In the rat model of monocrotaline-induced PH, the medial and intimal hypertrophy of pulmonary arteries was observed, concomitantly with elevating plasma concentrations of both ADMA and transform growth factor-β1 (TGF-β1). The expression and activity of ROCK in pulmonary artery were significantly enhanced. In cultured primary pulmonary arterial smooth muscle cells (PASMCs), treatment with ADMA (3-30 μM) concentration-dependently upregulated both the protein expression and activity of ROCK, increased TGF-β1 production and induced cell proliferation reflected by the results of MTT and cells cycles, which could markedly attenuated by Y-27632, an inhibitor of ROCK. In summary, these results suggest that elevated level of ADMA contributes to vascular remodeling via activating RhoA/ROCK pathway in PH.

EFFECT OF FERULIC ACID ON PRIMARY SENSORY AFFERENT OF NEUROPATHIC PAIN MEDIATED BY $P2X_3$ RECEPTOR

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Tissue damage, inflammation or injury of the nervous system may result in chronic neuropathic pain characterized by sensitivity to painful stimuli (hyperalgesia), the perception of innocuous stimuli as painful (allodynia) and spontaneous pain. The $P2X_3$ receptors play a crucial role in facilitating pain transmission at primary sensory afferent. Ferulic acid (FA) is one of the alkaloids contained in Ligustrazine which has been used in traditional Chinese medicine as an analgesic for injury. The present research investigated the effects of FA on the primary sensory afferent transmission induced by $P2X_3$ receptor in neuropathic pain states. Chronic constriction injury (CCI) model was adopted. Sprague-Dawley male rats were randomly divided into normal saline (NS) group (I), sham group (II), CCI group (III), CCI + FA group (IV), and FA group (V). Mechanical withdrawal threshold and thermal withdrawal latency were measured and $P2X_3$ protein in L4/L5 dorsal root ganglion (DRG) was detected. The mechanical withdrawal threshold and thermal withdrawal latency in group III were lower than group I, II, IV and V ($p<0.05$), while the expression of $P2X_3$ protein in L4/L5 DRG of group III was higher than group I, II, IV and V ($p<0.01$). The mechanical withdrawal threshold, thermal withdrawal latency and $P2X_3$ protein of L4/L5 DRG in group IV showed no significant difference compared with group I, group II or group V ($p>0.05$). The amplitude of the currents in group III (CCI) was much larger than those obtained in other groups after application of the same concentration ATP ($P<0.01$). α, β-MeATP (a selective agonist of $P2X_3$ receptor) activated current in DRG neurons of CCI rats was more evident than those obtained in other group rats ($P<0.01$). The results showed that FA could inhibit the primary sensory afferent transmission of neuropathic pain induced by $P2X_3$ receptor. This work was supported by the National Natural Science Foundation of China (No. 30260030) and the National Natural Science Foundation of Jiangxi Province (No. 0640062).

MICRORNA-375 PROMOTES 3T3-L1 PROADIPOCYTE DIFFERENTIATION

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MicroRNAs (miRNAs) represent a class of short (~22 nt), noncoding regulatory RNAs which are involved in multiple biological processes such as energy homeostasis, sugar and lipid metabolism, and cell differentiation by negatively regulate gene expression at the post-transcriptional level. To investigate the effect of microRNA-375 on differentiation of 3T3-L1 preadipocyte (3T3-L1 cell), 3T3-L1 cells were cultured and transfected with microRNA-375 plasmids, and were induced to differentiation using with 0.5 mmol/L 3-isobutyl-1-methyxanthine, 1 mg/L insulin, and 1μmol/ L dexamethasone. Oil red O staining was performed and marker genes of preadipocyte differentiation were detected by using real-time PCR. Results showed that the microRNA-375-transfected 3T3-L1 cells contained many more small lipid droplets than did non-transfected 3T3-L1 cells before induced. Following induction, differentiation in the microRNA-375-transfected 3T3-L1 cells was dramatically promoted. The expression of adipocyte differentiation marker genes such as lipoprotein lipase (LPL), adipocyte fatty acid-binding protein (aP2) and peroxisome proliferator-activated receptor gamma (PPARgamma) were upregulated in the microRNA-375-transfected cells, whereas expression of preadipocyte factor-1 (Pref-1), an inhibitor of preadipocyte differentiation, was downregulated. These results suggest that microRNA-375 could promote 3T3-L1 preadipocyte differentiation, indicating microRNA-375 may be of potential use in the treatment of obesity and obesity-induced insulin resistance.

APPLICATION OF A BACTERIAL TWO-HYBRID SYSTEM FOR THE ANALYSIS OF BRS-3 INTERACTED PROTEINS

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Protein–protein interactions play an important role in virtually all cellular processes including translation and modification of proteins, living things respondent reaction, the signal transduction and a lot of metabolic process. The bacterial two-hybrid (BTH) system has become a widely used tool for determining such interactions in vivo. Since there is very little known about the biological function, we have screened the interacted proteins of BRS-3 by using BTH technology. The pBT/BRS-3 bait plasmid was constructed and verified by double digestion and sequencing. Western blot analysis confirmed that the molecular weight of recombinant fusion protein was about 70 kDa, which was unanimous with a predicted molecular weight. Since the bait protein alone cannot activate the transcription of reporter gene, it can be used for screening of fetal brain library. Interactions were determined to be positive as measured by growth on "Selective Screening Medium" and validated by growth on "Dual Selective Screening Medium". To identify the proteins encoded by the target DNAs, the nucleotide of the target DNA was sequencing and compared to nucleotide databases to identify related proteins. All together 9 kinds of proteins and 4 kinds of new genes were identified. The findings of the BTH screen were confirmed by the glutathione S-transferase (GST)-pull down assay. These results showed that BRS-3 could interact with GRP, CNTF, SERPINB5, SERPINE1, HPN, PTK6, CSNK2A1, PRKCB1 and BIRC5. These proteins have a wide range of functions including cell growth, differentiation, anti - apoptosis, cytoskeleton construction, and the tyrosine kinase activities.

PROTECTIVE EFFECT OF INTRATRACHEALLY ADMINISTERED ANTIFLAMMIN-1 ON BLEOMYCIN-INDUCED LUNG FIBROSIS

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Antiflammin-1 (AF-1, MQMKKVLDS) is a nonapeptide which has powerful anti-inflammatory effect. It acts as its original full length protein-Clara Cell Secretory Protein (CCSP) and may display potently clinical application value. The previous studies indicate that bleomycin-injured mice down-regulate the expression of CCSP, and our further study has suggested Clara cell of the terminal bronchioles can significantly inhibit the collagen deposition and fibroblast proliferation in bleomycin-induced pulmonary fibrosis. In this study we investigated whether the AF-1 had a protective role against fibrosis in lung. To establish the fibrotic model, mice were subjected to intratracheal administration of bleomycin (5 mg/kg), and then received AF-1 (7.5 mg/kg) by intratracheal administration immediately. ELISA revealed that TNFα, IL-1β, TGFβ levels in lung homogenates of bleomycin-injured mice with AF-1 treated decreased significantly than those of only bleomycin-administered mice at 3, 7, 14 days after bleomycin administration. The collagen deposition was quantified by Masson staining and hydroxyproline content, the fibroblast proliferation was evaluated by immunohistochemical analyses for α-Smooth muscle actin at 28 days after bleomycin administration, and these results all showed that AF-1 could reduce the degree of lung damage and fibrosis. In conclusion, Antiflammin-1 has protective effect on bleomycin-induced lung fibrosis and it might prove useful as an add-on therapy for the treatment of fibrotic disorders of the lung such as idiopathic pulmonary fibrosis, a disease that still represents a major challenge to medical treatment.

EFFECTS OF LIVER APOPTOSIS AND PROLIFERATION ON SIMULATED 4000M HILO RATS

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In this research we tried to provide research data for a medium and practice science application about hypoxia. 60 male SD rats (Hunan Agricultural University supplied, license number: XIANG scxk 2003-003) were randomly divided into six groups: control (C), 8 hours hypoxic exposure (8hHE), 12 hours hypoxic exposure (12hHE), purely training (T), 8hHilo and 12hHilo. C did not hold hypoxia and training. HE and Hilo were held in hypoxia chamber (as a altitude of 4000 m) with 8 hours and 12 hours each day. T and Hilo were trained at the speed of the 25 m/min for 1 hour each day, 5 days per week for 4 weeks.

Apoptosis was detected in paraffin sections by the TUNEL technique. Expression of PCNA protein was examined by immunohistochemistry. As compared with group C, the indexes of apoptosis increased significantly in other groups ($p<0.01$). The indexes of apoptosis occurred in groups Hilo more than groups HE and T ($p<0.01$). Compared with group C, PCNA protein expressed slightly ascend in spite of groups HE, group T and groups Hilo. And compared with groups HE, it showed that there was prominent significance in groups Hilo ($p<0.01$). In conclusion, there were liver apoptosis in purely training, hypoxic exposure and Hilo. This research demonstrated that apoptosis occurred significantly in different hypoxic time. There were liver proliferation in hypoxic exposure, purely training and Hilo. It showed there was different appearance with different hypoxia time in hypoxic exposure and Hilo. Proliferation increased significantly after training intervention. Training avail liver cells to renewing. It is important to keep a dynamic equilibrium between liver apoptosis and proliferation.

IN VITRO CULTIVATION OF *SCHISTOSOMA JAPONICUM* GERMINAL CELLS

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The possibility of cultivating *schistosoma japoncum* germinal cells in vitro by using modified human primordial germ cells (MHPGC) medium was evaluated in this study. Cells from 12-day-old juvenile worms and 36-day-old adult worms of *S. j* were selectively cultured by using MHPGC medium. Growth characteristics, general morphology, ultrastructures, alkaline phosphatase (AKP) staining, chromosomes karyotype and proliferations of cultured cells were observed, respectively. Biological identification of cultivation of cells from larva worms displayed that the cells grew in a semi-floating or accumulative way, and were round with slick-surface and clear-circumsciption after cultured for 8 hours. Ultrastructure showed that most of the cells revealed normal shape and structure with integrity cell envelope, clear caryotheca and chromatospherite during the period of 30 days. Chromosome karyotype analysis of the cells cultured for 60-day showed diploid, a typical character of blood-fluke. The growth features of the cells from adult worms were semi-floating and accumulative, too. Cells grew faster and had great morphological differences in initial 4 weeks. BrdU incorporation assay was carried out in 4th week. The result revealed that the DNA synthesis in cells was athletic. Ultrastructure showed that most cells cultured for 5 weeks possessed the normal morphology with numerous vacuoles in different sizes in cytoplasm, caryon became bigger and caryotina became puffer, the characteristics of germinal cells. Chromosome karyotype of the cells cultured for 5 weeks showed the characters of the diploid and haploid. The cells cultured for 6 weeks were strong positive for AKP staining. It could be concluded from our research that some types of cells from *S. j* possessed some characteristics of germinal cells, and could be successfully propagated *in vitro* by using MHPGC medium.

PROTECTIVE ROLE OF A NEW VANADIUM COMPLEXES IN DIABETIC NEPHROPATHY

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Vanadium is an early first-row transition metal and forms colorful compounds in its many different oxidation states. The catalytic and material properties of vanadium compounds and their effects in biological systems have long provided the impetus and fuel to studies of vanadium science. We previously synthesized three kinds of vanadium complexes and tested the cytotoxicity by MTT *in vitro*, which showed that VOpz' (TP')$(SCN)_3$ was more feasible to cure the diabetes. Here we focused on fundamental biological studies *in vivo* to investigate the role of vanadium in the pathogenesis of diabetic nephropathy. Diabetic mice were induced by alloxan and in vanadium-treatment group diabetic mice were treated by intraperitoneal injection for three weeks with 5mg/kg of VOpz'(TP')$(SCN)_3$. The results indicated that the glucose level was decreased significantly in the vanadium-treatment mice, compared with diabetic mice, which protected from progressive glomerular damage by diabetes. Furthermore, the expression of caveolin-1, the marker protein of caveolae that related to insulin signaling, was decreased significantly in the kidney after the treatment. Also, the protein expression of in PI3k-Akt pathway was decreased in Vanadium treated group. These results suggest that VOpz ' (TP')$(SCN)_3$ had a better effect of decreasing of glucose level and curative effect to pathology changes of glomerulus, indicating potential use of VOpz' (TP')$(SCN)_3$ in the treatment of diabetes. Cav-1 may play an important role in the process of insulin signal transduction.

THE RESEARCH OF SERUM KYNURENINE AND TRYPTOPHAN IN CHRONIC RENAL FAILURE PATIENTS

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The aim of this study was to explore the clinical significance of serum kynurenine (KYN) and tryptophan (TRP) determined in chronic renal failure (CRF) patients. The concentrations of serum KYN, TRP, creatinine (Cr), urua and basical blood parameters in chronic glomerulonephritis (CG) patients and CRF patients were determined, and the clinical significance of serum KYN and TRP determined in different progresses of CRF was explored. Compared with healthy subjects, there were significantly increased concentrations of serum KYN and significantly decreased concentrations of TRP in the patients with CRF and CG. Compared with the patients with CG, the altered concentration of serum KYN and TRP in the patients with CRF were significant. It showed the levels of serum KYN and TRP may be correlated with the development of the renal function or blood parameters. It showed there was severe unbalance of kynurenine pathway in peripheral tissues in CRF patients, and it suggested that accumulation of some KYN metabolites in CRF patients blood may somewhat be responsible for symptoms.

EXPRESSION OF UTEROGLOBIN-BINDING PROTEIN IS UPREGULATED IN MURINE LUNGS AND IN NIH 3T3 FIBROBLAST FOLLOWING FIBROTIC STIMULI

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As a small, secreted, multifunctional protein, uteroglobin (UG) plays important roles in preventing pulmonary fibrogenesis. Recent studies have suggested that UG-binding protein, a protein with nine transmembrane domains, is a putative receptor of UG, and mediates some cellular functions of UG on several cell types. In this study, we demonstrated the expression of mouse UG-binding protein (mUGBP) in bleomycin-induced pulmonary fibrosis in mice as well as its induction by profibrotic TGF-β1 in murine fibroblast NIH 3T3 cells. Real-time PCR showed that mUGBP mRNA in whole lung homogenates began to increase gradually from day 14 to day 28 following bleomycin challenge. In situ hybridization revealed that increased mUGBP positive labeling was preferentially observed in interstitial sites of bleomycin-injured lungs. In vitro studies further showed that treatment of mouse NIH 3T3 cells with TGF-β1 for 48 hours led to increased expressions of mUGBP mRNA and protein. TGF-β1 stimulated procollagen 1 (I) (pro-collα1(I)) expression in NIH 3T3 cells was inhibited by 10μM of antiflammin-1 (AF-1, MQMKKVLDS), a known UG-derived bioactive fragment. However, this UG-bioactive fragment did not change the expression levels of pro-collα1 (I) under basal condition without TGF-β1. Antisense oligonucleotides (AS-OND) of mUGBP partly blocked the inhibition of TGF-β1-stimulated pro-collα1(I) protein expression by AF-1. These results pointed to the likelihood that the induction of mUGBP was necessary for AF-1 to inhibit of procollagen I expression,

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and its induction was protective during fibrogenesis. Further elucidation of the molecular mechanisms involved might yield novel insights into potential roles of mUGBP during pulmonary fibrosis and help develop new therapeutic strategies. This study was supported by Grants 30400190 and 30670770 from National Natural Science Foundation of China.

STUDY ON IDENTIFYING GENOTYPES OF *ECHINOCOCCUS GRANULOSUS* BY MICROSATELLITE MARKERS

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This research used Sca, Emsk and C106 microsatellite sequence of *Echinococcus granulosus* as typing markers and identified the genotypes and heterozygosity of *E.granulosus* isolated from cystic echinococcosis (CE) patients in different areas of Xinjiang. Two different fluorescent dyes of FAM (carboxy fluorescein) and HEX (hexacho rofluorescein) were used as markers for three pairs of primers of microsatellite sequences. After amplification of satellites by PCR, amplified products were based on capillary electrophoresis by using 310 auto DNA analysis machine, and then basic number of amplified products was calculated by Genescan 2.1 software. Our result showed that 66 isolated strains from 44 CE patients were homozygote of *E. granulosus*, in which 65 isolated strains from 43 CE patients were identified as G1 genotype by PCR and microsatellite markers, and 1 isolated strain from 1 CE patient was identified as G6 genotype by PCR and microsatellite markers. The results of genotypes identified by microsatellite markers were consistent with the result of DNA sequence analysis. Mixing infection and heterozygote of G1 (sheep strain) and G6 (camel strain) genotypes were found in the dog intestines, but this research did not find *E.granulosus* heterozygote. It may indicate that *E.granulosus* was autofertilization, and therefore, few chance of heterozygote takes place in the patient. Microsatellite DNA markers can be used to finely and quickly identify the genotypes and heterozygosity of *E.granulosus* at genetic level, and possessed the important value in study of genetic polymophosim, epidemiology and pathogenicity of *E.granulosus*.

DETERMINATION OF PHENYLALANINE AND TYROSINE IN PERIPHERAL BLOOD AND ITS APPLICATION IN PHENYLKETONURIA

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Several methods have been reported for determination of tyrosine (Tyr) or phenylalanine (Phe) in neonatal blood, but the simultaneous determination of Tyr and Phe in peripheral blood by reverse phase high-performance liquid chromatography (RP-HPLC) by no derivatization has not been reported. Here we describe a simple, accurate and reliable RP-HPLC assay for measurement of Tyr and Phe in peripheral blood of newborn infants and patients with phenylketonuria (PKU). Supernatant fluid of peripheral blood precipitated with perchloric acid was isocratically eluted using a base-deactivated C_8 column with 5 % acetonitrile in water as the mobile phase. Untraviolet detector worked at 210nm. The retention time of tyrosine (Tyr) and phenylalanine (Phe) were 5.88min and 8.43min respectively. The effects of various aspects on operation and determination were examined to establish optimal assay conditions, such as precipitator, anticogulant, means of collecting and storing samples. Phenylalanine and tyrosine in finger blood of 102 healthy enfant were (67.7 ± 15.4) μmol/L and (62.2 ± 13.9) μmol/L respectively. The ratio of Phe/Tyr was 1.15 ± 0.27. There was no obviously difference between males and females. Phenylalanine and tyrosine in heel blood of 32 healthy neonatal were (66.2 ± 20.5) μmol/L and (59.5±18.8) μmol/L respectively. The ratio of Phe/Tyr was 1.12 ± 0.24. The levels of phenylalanine and tyrosine in whole-blood which anticogulated by $EDTA \cdot K_2$ are consistent with those of anticogulating by heparin. The levels of phenylalanine and tyrosine in peripheral blood were significantly lower than those in plasma, but showed a good correlation. The means of collecting and storing sample affect the levels of phenylalanine and tyrosine in whole-blood.

THE MECHANISMS OF ALCOHOL-INDUCED MYOCARDIUM INJURY AND THE EFFECTS OF NIMODIPINE AND ERIGERON BREVISCAPUS

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The changes of cardiac troponin I (cTnI), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) contents in serum after acute alcohol administration, and the possible mechanisms of myocardium injury induced by alcohol and the protective effect of Nimodipine and Breviscapus were investigated. 40 Wistar rats were divided to 5 groups randomly. The rats in the control group were given physiological saline (1.5 ml/100g). The rats in the alcohol group were given 40% alcohol, 1.5 ml/100g by administration. The rats in the Nimodipine, Breviscapus and the united group were given corresponding medicine beforehand, and then given alcohol one hour later. All rats were administrated 14 days continuously. To detect the contents of cTnI, MDA, GSH-Px in the serum, blood was collected before alcohol administration, and 24 hours, 48 hours, 7 days and 14 days after. Compared to the control group, the content of cTnI in serum was increased significantly after alcohol administration and was higher than that before alcohol administration ($p<0.05$), Nimodipine or Breviscapus significantly decreased the increase of the cTnI content induced by alcohol. The changes of MDA content were similar to that of cTnI content. The content of GSH-Px in alcohol group was significantly decreased after 48 hours alcohol administration. However, in the Nimodipine, Breviscapus and the united group, the content of GSH-Px in serum was not obviously reduced. cTnI is one of the structural protein of myocardial cell. This experiment manifested that heavy alcohol consumption induced the increase of cTnI concentration in serum, which indicated that the myocardium was injured. The content of MDA was increased significantly in serum, indicating that oxidative damage might be one of the mechanisms of alcohol toxic effects. Our results show that administrated Nimodipine and Breviscapus can inhibit the increment of cTnI and MDA contents induced by alcohol administration, indicating that Nimodipine and Breviscapus have the protective effect on alcohol-induced myocardium injury.

ISOFLURANE MEDIATES RENAL ARTERY SMOOTH MUSCLE CONTRACTION THROUGH Ca^{2+}-INDEPENDENT PKC SIGNALING

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To study the direct effect and potential mechanisms of isoflurane on renal artery smooth muscle, rabbit renal artery strips were treated with 3% saponin to permeate the cellular membrane. The calcium stored in endoplasmic reticulum (ER) was removed by caffeine. Then the renal artery strips were equilibrated in 10^{-7} M Ca^{2+}-EGTA buffer. Steady tension was obtained when the strips were soaked in submaximal calcium concentration buffer. Then the submaximal calcium concentration EGTA buffer with isoflurane of various concentration or 10uM BIM was used, and the tension variety was collected and calculated by computer. 1 %; 3 % and 5 % isoflurane could all contract rabbit renal artery strips in the equilibrate buffer with a concentration-dependent manner. This effect of isoflurane on smooth muscle could be inhibited by BIM, an inhibitor for PKC, but not inhibited by Gö6976, a Ca^{2+}- dependent inhibitor for PKC. In conclusion, isoflurane could contract rabbit renal artery smooth muscle with a concentration-dependent manner, which may be mediated by Ca^{2+}-independent PKC signaling.

DETERMINATION OF KYNURENIC ACID AND TRYPTOPHAN IN SERUM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY- FLUORESCENCE DETECTION

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The aim of this study was to establish a method for simultaneous determination of Kynurenic acid (KYNA) and tryptophan (Trp) in serum by high performance liquid chromatography - fluorescence detection (HPLC-FLD). We employed a Hypersil C8 column and a mobile phase consisted of 0.5 mol/L zinc acetate, 50 mM sodium acetate with 6 % (v/v) acetonitrile at a flow rate of 1.5 ml/min. The fluorescence excitation and emission wavelengths were operated at 344 nm and 404 nm respectively at the beginning of the run, and 9.5 minutes later, and the excitation wavelength was changed to 254 nm. Serum samples were precipitated by 5 % perchloric acid solution and centrifuged to remove protein residuals and then assayed by HPLC. The retention time of KYNA was 8.1min, the linearity of the assay was from 1.5nmol/L to 2093 nmol/L, and the detection limits was 0.05 μmol/L. The average recovery of KYNA was 101.19 %, and the intraday and interday coefficients of variations were 3.20 %and 4.27 %, respectively. The retention time of Trp was 11.3 min, and the linearity of the assay was from 0.49 μmol/L to 196 μmol/L, the detection limits was 0.001μmol/L. The average recovery of Trp was 104.43 %, the intraday and interday coefficients of variations were 3.31 % and 4.14 %, respectively. These results indicated these five substances had no interfering effect to the method. The serum KYNA and Kyn and TRP levels in 50 healthy people were 24.25 ± 9.11 nmol/L and 49.05 ± 11.67 μmol/L, respectively. The method is simple, fast, accurate and convenient, and suitable for routine analysis.

CHANGES OF NMDA-INDUCED TUMOR NECROSIS FACTOR −α PRODUCTION IN RAT ALVEOLAR MACROPHAGES

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Hyperoxia-induced lung injury is one of the most serious problems following treatment of the neonate with high oxygen concentrations. N-methl D-aspartate receptors (NMDAR) are expressed in the rat lung and in the alveolar macrophage (AM). Recently, we have reported NMDAR plays an important role in hyperoxia-induced lung injury and NMDA receptor antagonist MK-801 ameliorates hyperoxia-induced lung injury in neonatal rats. The purpose of the study was to determine whether NMDAR plays an important role on tumor necrosis alpha (TNF-α) production in rat AM and to explore the cell source of the production of pro-inflammatory cytokines of hyperoxia-induced lung injury. AMs were divided into four groups: control group, NMDA group, MK-801 group and NMDA+MK-801 group. While pretreated with MK-801 for half hour AMs were treated with NMDA, and the culture supernatants were collected 6 hours after the treatment with NMDA to determine the production of TNF-α by ELISA. The content of TNF-α in NMDA group was significantly higher than that in control group and in MK-801 group. MK-801 could inhibit the increase of TNF-α by NMDA. The content of TNF-α in NMDA?MK-801group was markedly lower than that in NMDA group. There were no difference among the control group and MK-801 group and NMDA?MK-801 group. These results provided evidences that exterior NMDA could promote the production of TNF-α in rat alveolar macrophages and MK-801 could antagonize this effect. It suggests that NMDAR plays an important role in the secretion of AM and in the pathogenesis of chronic inflammation-related disease such as hyperoxia-induced lung injury. It also suggests that alveolar macrophage may be one of cell sources of the production of pro-inflammatory cytokines.

ACTIVATION OF N-METHYL-D-ASPARTATE RECEPTOR INDUCE ACUTE LUNG INJURY IN MICE

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Excitatory amino acid toxicity, resulting from overactivation of N-methyl-D-aspartate (NMDA) glutamate receptors, is a major mechanism of neuronal cell death in acute and chronic neurological diseases. NMDA receptors are widely expressed in lungs while the function is largely unknown. Elevated plasma glutamate concentrations have been observed in the pulmonary vein of septic rats. NMDA could trigger high-permeability edema on perfused, ventilated rat lungs. These findings suggest that glutamate caused NMDA receptor activation might be an important mechanism of acute lung injury. To investigate the possible injury caused by activation of NMDA receptor, the lungs of mice receiving glutamate (500 mg/kg) or NMDA (50 mg/kg) by intraperitoneal injection were tested. Eight hours' insult of glutamate and NMDA increased lung wet /dry weight ratios (W/D), lactate dehydrogenase (LDH) and protein leakage in the bronchoalveolar lavage fluid (BALF), lung tissue myeloperoxidase (MPO) activity. Inflammation characterized by neutrophils recruitment was proved by pathologic examination again. The increase of W/D, a golden index of acute lung injury, caused by glutamate and NMDA was nearly abolished when pre-treating with MK801 (0.1 mg/kg, ip), a specific blocker of NMDA receptor. NMDA decreased proSP-C, a mark protein of ATII, and CCTα, the rate-limiting enzyme and regulator of biosynthesis of phosphatidylcholine (PC), a major component of lipids of PS in lung tissue. These changes were converted by pretreatment with MK-801, too. The depletion of neutrophils by pretreatment with vinblastine (5 mg/kg, iv) for 4 d would result in relief of increase of W/D caused by NMDA. Thus we conclude that activation of NMDA receptor can cause lung injury, which was associated with neutrophils accumulation and dysfunction of alveolar type II cells. This study was supported by Grants 30370531 from National Natural Science Foundation of China.

EFFECTS OF EXERCISES WITH DIFFERENT INTENSITY AND DIFFERENT PATTERN ON PLASMA ET, CGRP AND THEIR RELATIONSHIP WITH CARDIOPULMONARY FUNCTION

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This research is to explore the laws of effects of exercise with different intensity and different pattern on plasma endothelin (ET) and calcitonin gene related peptide (CGRP). The correlation of cardiac endocrine and cardiopulmonary function is also discussed after endurance training. The contents of the peripheral plasma ET and CGRP in 41 male athletes and 11 college students were determined by applying specific radio- immunoassay after exercises of different intensity and different pattern. The results showed that the greater the exercise intensity was, the more the plasma ET concentration was raised. The concentration of plasma endothelin was increased remarkably after exhaustive exercise on the treadmill, but did not remarkably induce the change of plasma ET response in exercises with constant workload and exhaustive exercise on bicycle ergometer. The level of plasma ET in endurance training group was obviously higher than that in control group at rest ($P<0.001$). The concentration of the plasma ET was increased significantly in both groups and the increments of the ET in endurance training group were significantly greater than that in control group after exhausting exercise. The contents of the plasma CGRP were not significantly changed after exercises of different intensity and different pattern. It prompts that exercise is the cause of inducing the increase of plasma ET concentration. The degree of the increase depends on the training intensity and the exercise pattern. The endurance training results in the increments of the plasma ET in both rest and after exhaustive exercise on the treadmill. The changes of the plasma

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ET relates significantly to the cardiopulmonary function. It suggests that these changes may be related to changes of adaptability of cardiac endocrine after endurance training.

ENDOTHELIUM-DEPENDENT AND DIRECT RELAXATION INDUCED BY AURICULARIA AURICULAJUDAE POLYSACCHARIDE IN RAT THORACIC AORTA

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The aims of the present study were to investigate the direct vascular effects of auricularia auriculajudae polysaccharide (AAP) and the underlying mechanisms on the rat thoracic aorta. It was demonstrated that cumulative administration of AAP (2-20 μg/mL) did not affect the vasomotion of aortic rings with endothelium either in basal tension or preconstricted by KCl (6×10^{-2} M). However, AAP caused a significant concentration-dependent and time-dependent relaxation on endothelium-intact rings precontracted with phenylephrine (PE, 10^{-6} M). Endothelium removal or incubation of the aortic rings with nitric oxide synthase inhibitor N(ω)-nitro-L-arginine-methyl ester (L-NAME, 10^{-4} M) or methylene blue (10^{-5} M) significantly attenuated the acute vasorelaxation induced by AAP. Cyclooxygenase inhibitor indomethacin (10^{-5} M) did not influence the vasodilator effect of APP. Moreover, the expression of nitric oxide synthase (NOS) in aortic rings was markedly higher after incubated with APP (10 μg/mL), which had a peak at time point of 10 minutes. These results suggest that AAP has endothelium-dependent relaxation effect, in which the activation of NO and cGMP-mediated pathway may probably be involved.

DETERMINATION OF KYNURENINE IN SERUM BY HIGH-PERFORMANCE LIQUID WITH ON-COLUMN FLUORESCENCE DERIVATIZATION

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Kynurenine (KYN), 2-amino-4- (2-aminophenyl)-4-oxobutanoic acid, is one of the most important metabolic products of tryptophan (TRP), a human essential amino acid. It is further transformed to kynurenic acid (KYNA), which is an antagonist of *N*-methyl-d-aspartate (NMDA) and α7 nicotinic acetylcholine receptors. We established a method for determination of kynurenine (KYN) in serum by high performance liquid chromatography - fluorescence detection (HPLC-FLD) with on-column derivatization. It employed a Hypersil C8 column (300mm×6.0mm i.d., 10μm) and a mobile phase consisted of 250 mmol/L zinc acetate, 50 mmol/L acetate with 3 % (v/v) acetonitrile at a flow rate of 1.5 ml/min. The fluorescence excitation and emission wavelengths were 365 nm and 480 nm, respectively. Serum samples were precipitated by 5 % perchloric acid solution and centrifuged to remove protein residuals, then assayed by HPLC-FLD with on-column derivatization. The retention time of KYN was 8.3 min, the linearity of the assay was from 0.098 μmol/L to 98 μmol/L, and the detection limit was 0.04 μmol/L. The recoveries of KYN were 90.82 - 96.17 %, and the intraday and interday variations were 3.82 % and 4.63 %, respectively. Tryptophan (TRP), 5-hydroxytryptamine (5-HT), kynurenic acid (KYNA), phenylalanine (PHE), tyrosine (TYR) and creatinine (CRE) had no interfering effects to the method. The method is simple, fast, accurate and convenient, and suitable for routine analysis.

EFFECTS OF PRENATAL STRESS ON VOLUNTARY ETHANOL INTAKE AND BRAIN GLUCOCORTICOID RECEPTOR IN ADULT FEMALE RAT OFFSPRING

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Previous researches show that Prenatal stress (PS) can affect the adult female offspring by mediating the functions of hypothalamic-pituitary-adrenal axis, changing glucocorticoid receptor expression. These changes are closely related to the ethanol addiction. We hypothesized that PS might affect the voluntary ethanol intake (EI) of adult female offspring. Six pregnant SD rats were randomly divided into PS (n=3) and control (CON) group (n=3). During the late gestational periods, pregnant rats in PS group were subjected to restraint stress and those in CON group were left undisturbed. The adult female offspring in PS group were randomly divided into PS plus stress (PS-S group, n=8) and PS without stress group (PS-NS group, n=8). The adult female offspring in CON group were randomly divided into stress (CON-S group, n=8) and no stress group (CON-NS group, n=8). The rats in PS-S group and CON-S group were subjected to restraint stress, and three weeks later ice-water stress. Our results showed that the EI of PS-NS group at the 1st, 4th, 5th and 6th week was significantly less than that of CON-NS group ($P<0.05$). Compared to the PS-NS group, the EI of PS-S group at the 1st week after restraint stress increased by 95.4 %. In contrast, compared to the CON-NS group, the EI of CON-S group at the 1st week decreased by 40.4 %. In the 2nd and 3rd week, the EI of PS-S group significantly decreased, and CON-S group significantly increased. Similar EI changes in PS-S group and CON-S group were observed after ice-water stress. Glucocorticoid receptor expression in hippocampus and amygdala in PS group was significantly higher than that in CON group. These results suggest that PS can affect the alcohol preference of adult female rat offspring, which possibly is related to the increase of glucocorticoid receptor expression in brain.

THE RELATIONSHIP BETWEEN THE SELECTIVE GANGLION CELL DEATH AND LOCAL BLOOD SUPPLY IN RAT RETINA FOLLOWING ACUTE HIGH INTRAOCULAR PRESSURE

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The mechanism of selective retinal ganglion cell (RGC) death in glaucoma remains elusive. Previous researches have shown that the blood supply of glaucoma retina decreased and it was closely related to the ganglion cell death. Thus we hypothesize that selective RGC death in glaucoma is related to the differential changes of local blood supply. In this study, we detected the local blood supply changes of the central, middle and peripheral rat retina by gelatin-ink infusion and microsphere injection at the 3 hours, 6 hours, 12 hours, 1 day, 3 days, 7 days and 14 days after reperfusion following acute high intraocular pressure (AHIOP). We also detected the RGC survival by fluorogold retrograde labeling during the reperfusion. Our results show that during the first 6h of reperfusion following AHIOP, the increased amplitude of blood supply in the central and middle retina was significantly higher than that of the peripheral. In contrast, the decreased amplitude in the central and middle retina was lower than that of the peripheral during the following reperfusion time. Additionally, the death rate of RGCs in the peripheral retina was significantly higher than that of the central and middle during the reperfusion, especially at the early stage of reperfusion. Further analysis showed that there was significantly positive correlation between the local RGC death rate and the corresponding local blood supply changes during the reperfusion following AHIOP, especially at the early stage of reperfusion. The correlation coefficient between the local RGC death rate and blood supply changes in the peripheral retina was significantly bigger than that of the central and middle retina during the whole reperfusion. These data suggest that the selective RGC

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death is significantly positively correlated with differential changes of local blood supply during the reperfusion following AHIOP.

EXPRESSION OF CALPAINS IN THE KANAMYCIN-DAMAGED GUINEA PIG COCHLEA

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Apoptosis may play an important role in the mechanism of aminoglycoside ototoxicity. Calpains are regarded to be essential for mediation and promotion of cell death in the inner ear. Thus in this study we investigated the expression of calpains in the kanamycin-poisoned guinea pig cochlea, and to explore the role of calpains on the parmanent hearing loss induced by kanamycin. Immunohistochemistry and imaging analysis technique were used to detect the expression and localization of μ-calpain and m-calpain, and auditory thresholds were measured by evoked auditory brainstem response (ABR). We demonstrated that μ-calpain and m-calpain were co-localized in hair cells, in cells of stria vascularis and in spiral ganglion cells. Lower expression was seen in saline-injected control cochlear tissue. After treatment with kanamycin for 14 days, immunostaining for μ-calpain and m-calpain became more intense, and auditory threshold shifts were significantly elevated about 30 dB. These results suggest that calpain may be responsible for apoptosis induced by aminoglycoside. This indicates that calpain inhibitor may be of potential therapeutic value in protecting from aminoglycoside-induced hearing loss.

ROLE OF BRAIN ANGIOTENSIN AT1 RECEPTOR IN CARBACHOL-INDUCED NATRIURESIS AND EXPRESSION OF THYROSINE HYDROXYLASE IN THE ROSTRAL VENTROLATERAL MEDULLA

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Central administration of angiotensin II type 1 (AT1) receptor antagonist losartan effectively inhibited the increase in blood pressure and drinking response induced by cholinergic agonist carbachol. However, whether the angiotensin AT1 receptor is related to the natriuresis induced by brain cholinergic stimuli is still unclear. The purpose of this study was to investigate the role of brain angiotensin AT1 receptor in the carbachol-induced natriuresis and expression of thyrosine hydroxylase (TH) in the rostral ventrolateral medulla (RVLM) in Sprague Dawley (SD) rats. The results showed that after intracerebroventricular (ICV) injection of carbachol (0.5 μg), the urinary sodium excretion increased at 20 min, reaching the peak [(0.548 ± 0.049) μmol/min·100g] at 40 min. Immunohistochemistry showed that carbachol induced an increase of thyrosine hydroxylase-immunoreactivity (TH-IR) in the RVLM. After pretreatment with 20 μg of losartan, the urinary sodium excretion reduced to (0.249 ± 0.067) μmol/min·100g, and TH-IR in the RVLM induced by carbachol was also reduced. The results suggest that brain AT1 receptor was involved in carbachol-induced natriuresis and the increase in activity of adrenergic neurons in the RVLM. Consequently, we provided the new evidence that brain angiotensinergic pathway and adrenergic pathway contributed to renal changes in the natriuresis to brain cholinergic stimuli and thus played an important role in the regulation of fluid homeostatic.

ROLE OF GLUTAMATE AND ITS NMDAR IN HYPEROXIA-INDUCED LUNG INJURY

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Previous study in our laboratory showed that NMDA receptor antagonist MK-801 could decrease the hyperoxia lung damage. The purpose of the study was to demonstrate the role of intrinsic Glu and NR2D in hyperoxia-induced lung injury. 22-day gestation full term rats in 12 hours after birth were randomly marked with a different number and assigned to four groups: air control group, air+MK-801 group, hyperoxia group and hyperoxia+MK-801 group. The content of LDH was significantly higher after having 1d exposure. After 3 days, content of protein and LDH, the counts of inflammatory cell, and W/D were much higher than air group. After 7 days, LDH, protein, W/D were much higher than air group. Our data show that MK-801 had no influence on the content of LDH in BALF after 1d exposure, and also no effect on the content of protein after 3 days. The hyperoxia+MK-801 group showed less of W/D and LDH than the hyperoxia-group after 3 days and 7 days. The content of Glu was significantly higher after having 1d exposure. After having 3 days exposure, NR2D mRNA expressions were much higher than air group ($P<0.05$). After having 7 days of exposure, NR2D mRNA expressions were much higher than the 1 d and 3 days, and also higher than air group. The content of Glu in BALF had a dramatic decrease; it was not lower than 3 days, but lower than air group. In conclusion, hyperoxia could induce the release of intrinsic Glu in lung and enhance the expression of NMDA receptor. NMDA receptor antagonist MK-801 can alleviated hyperoxia-induced lung injury in neonatal rats in 3 days - 7 days hyoeroxia exposured time. Glu and NMDA receptors may play an excitotoxicity role in the pathogenesis of hyperoxia-induced lung injury.

3, 4, 5, 6-TETRAHYDROXYXANTHONE PROTECTS AGAINST HYPOXIA/ REOXYGENATION-INDUCED APOPTOSIS OF PC12 CELLS: ROLE OF THE DDAH/ADMA PATHWAY

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Our previous studies showed that 3,4,5,6-tetrahydroxyxanthone (Xan), a synthesized polyphenolic compound, protected against myocardial ischemia/reperfusion (I/R) injury. Endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA), which is hydrolyzed by dimethylarginine dimethylaminohydrolase (DDAH), was reported to induce apoptotic death in various kinds of cells. In the present study, we observed the inhibitory effect of Xan on hypoxia/reoxygenation (H/R)-induced apoptosis in PC12 (pheochromocytoma) cells and investigated the relationship between anti-apoptotic action of Xan and the DDAH/ADMA pathway. Three-hour hypoxia (1 % O_2) and consequent 24-hour reoxygenation significantly increased the apoptotic death of PC12 cells as evidenced by increases in Hochest staining-positive cell number, concomitantly with increasing intracellular reactive oxygen species (ROS) production and caspase-3 activity, which was attenuated by pretreatment with Xan (3 − 30 μM) and caspase-3 inhibitor DEVD-CHO as well as antioxidant PDTC. Also, the decrease in DDAH activity and the increase in ADMA level were also observed after H/R treatment. Furthermore, incubation with exogenous ADMA (1 − 10 μM) could concentration-dependently increase caspase-3 activity and induce the apoptosis of PC12 cells. Pretreatment with Xan could markedly attenuate the decrease in DDAH activity and the increase in ADMA level and inhibit ADMA-induced caspase-3 activation and apoptosis of PC12 cells. In summary, the present data suggest that Xan protects against H/R-induced apoptosis via inhibiting caspase-3-dependent apoptotic signaling pathway, which may be related to improving the DDAH/ADMA pathway by reduction of oxidative stress.

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EFFECTS OF MARGINAL VITAMIN A DEFICIENCY ON RAT'S LUNG MATURATION AND THE EXPRESSION OF RETINOIC ACID RECEPTORS FROM PRENATAL TO ADULT STAGE

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Lung morphogenesis is a highly regulated process that could be impaired by nutrition deficiency and recent research suggests that disturbed early lung development may pave the way for later illness and accelerated senescence. Vitamin A (retinol) and their derivatives (retinoic acids, RA) are known key developmental regulators that bind and activate retinoic acid receptors (RARs). To evaluate whether marginal vitamin A deficiency (MVAD) begun from pregnancy alters the lung structure and extracellular matrix, we monitored lung morphology, collagen and elastin fiber at postnatal day 1, week 2 and adulthood (week 8) on MVAD and control group. In addition to morphological change, lung RA receptor (RARα, ß, and γ) expression was analyzed by immunofluorescence, whereas mRNA levels were measured using RT-PCR. We demonstrate that MVAD begun from pregnancy resulted in lower lung weight, reduced numbers of alveoli and total alveolar surface area, in addition to increased alveoli septa thickness till to adulthood. Exposure to MVAD also resulted in increased collagen deposits and decreasing elastin fiber at postnatal week 2 and 8 in an unorganized manner. Over the normal course of development, total protein and mRNA levels for the RARs declined, but immunofluorescence and RT-PCR demonstrate that exposure to MVAD during the pregnancy period resulted in an immediate and durative increase in RARs levels from postnatal day 1 to adulthood, especially at postnatal week 2. In summary, this study demonstrates that developing lungs are sensitive to MVAD and this effect is permanent throughout the life of the animal and may be mediated in part through augmentation of transcriptional signals in the retinoid pathway. Thus, we hypothesize that durative pregnancy MVAD could impact lung development and result in the permanent impairment which may underlie at least some susceptibility to adult-onset chronic lung disease.

GLUTAMIC ACID MODIFIED MAGNETIC NANOPARTICLES AS VECTOR FOR GENE DELIVERY

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Due to the growing concerns over the toxicity and immumogenicity of viral gene delivery systems, gene delivery via nonviral routes has become more desirable and advantageous. In this work, we developed a self-assembled nonviral gene carrier, glutamic acid modified magnetic nanoparticles (GMMN), which were formed by modifying glutamic acid to the surface of $Znfe_2O_4$ nanoparticles. Transmission electromicroscopy results indicated that these particles were 30 nm and below in diameter with a narrow size distribution. Zeta potential demonstrated that the GMCN had 12.8 mV positive surface charges due to the exposed amino groups outward on the surface of magnetic nanoparticles. Gel retardation assay and co-sedimentation assay showed a high affinity of GMMN for DNA under physiological conditions. The DNA encapsuled inside the GMMN was protected from the external DNAase environment. The cell culture experiments showed that the GMMN were internalized into human hepatocyte QSG7701 cells and exhibited higher efficiency of intracellular uptake than bared magnetic nanoparticles. Furthermore, the GMMN-DNA complex had no obvious cytotoxicity for QSG7701 cells, while the liposome-DNA complex had certain cytotoxicity. After intravenous injection, GMMN transferred reporter gene EGFP-C2 to liver, lung, spleen and kidney. Therefore, the GMMN show potential as viable vector candidates for safer and cost-effective DNA delivery.

DISCUSSION ON TRANSLATIONAL MEDICINE: WHO WILL BE A TRANSLATOR, RESEARCHER OR DOCTOR?

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The clinical medicine has been always facilitated by the fruits of other disciplines, especially the basic researches of medicine. With the development of modern techniques and methods, clinical medicine shows an increasing dependency on breakthroughs of basic researches. There is a huge amount of research achievements. However, only few truly valuable test-tube results can be applied to practical world. What is the barrier that prevents the development of translational medicine? Who will be the translator to push it forward, doctor or scientist? We think that the radical cause is that micros and macros are not well matched in this field. Although clinical medicine shares the same goal as the basic researches as to helping healthy life, they have different research systems that are not corresponding in the same level. As we all know, the diagnosis and treatments of many disorders can make use of the micro techniques since the limited macro methods are available. However, many solutions of health problems are still on the traditional way. For example, antibiotics developed in laboratories can kill the pathogens under microscope, but they cannot resolve the abscess with thick walls; we still need a macro method-surgery-to remove it. Therefore, it is obvious that there is no marked border between basic researches and clinical medicine. The realization of translational medicine should be based on the bedside needs of patients and the close communications and cooperations between researchers and doctors that have a foundation of traditional ways of researches, diagnosis and treatments.

THE FUNCTIONAL ROLE OF CFTR IN SPERMATOGENESIS

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Although the expression of CFTR in the testis has been demonstrated for more than a decade, whether CFTR is functional in the process of spermatogenesis remains obscure. Soluble adenylyl cyclase (sAC) is a newly defined cytoplasmic adenylyl cyclase and is responsible for the production of cAMP in sperm; and thus a major player in sperm function. In this study we first examined the expression of CFTR and sAC in germ cells and somatic cells in the testis by RT-PCR, Western blot and immunostaining. The possible function of CFTR was also studied using primary cell culture system and the results showed that CFTR in Sertoli cells is linked to bicarbonate transport, as shown by the inhibition of the bicarbonate-induced intracellular pH increase by CFTR blocker. The function of CFTR in spermatogenesis was further studied in CF mice model. Compromised spermatogenesis in CF mice is demonstrated by the decreased testis weight, reduced daily sperm production (DSP), and high percentage of abnormal sperm morphology as well as reduced expression of downstream molecules of sAC-mediated pathway. Taken together, our study for the first time has demonstrated the physiological function of CFTR in spermatogenesis. The CFTR-linked sAC pathway may represent a novel signal cascade involved in the process of spermatogenesis.

THE BONE CANCER PAIN INDUCED EXPRESSION OF NR1 AND NOS ON THE DORSAL HORN OF MURINE SPINAL CORD

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The present study was designed to investigate the role of NR1 (NMDA-R1) and NOS in the generation and maintenance of bone cancer pain in the C57BL/6 mice. The result of the test immunohistochemistry at the 23rd days after surgery and inoculation showed a significant difference between test group (inoculated with Lewis lung cancer cell $2x10^6$) and control groups (inoculated with same amount PBS), including blank control group (only surgery, no inoculation), but no significant difference between control group and blank control group. The result of the spinal NOS exam at the same days also showed a significant difference between test group and control groups, including blank control group, while no significant difference between control group and blank control group was observed. These results indicate that the expression levels of NR1 and NOS are increased in the mice with cancer induced pain, which means NR1 and NOS in the spinal cord may participate and mediate in transmitting algesthesia message and forming of hyperalgesia in the cancer induced pain.

ASYMMETRIC DIMETHYLARGININE IS INVOLVED IN THE DEVELOPMENT OF INSULIN-RESISTANCE IN 3T3-L1 ADIPOCYTES

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To investigate whether asymmetric dimethylarginine is involved in the impairment of glucose utility and the development of insulin-resistance in adipocytes, 3T3-L1 adipocytes were treated with asymmetric dimethylarginine. Then the effects of asymmetric dimethylarginine on the expression of insulin receptor substrate-1 (IRS-1), the expression and translocation of glucose transporter-4 (GLUT4) and glucose uptake were examined. To further explore whether oxidant stress is involved in the action of asymmetric dimethylarginine, the production of reactive oxygen species (ROS) was measured and Vitamin E was used as both anti-oxidant agent and positive control. We also examined the nitric oxide (NO) and tumor necrosis factor alpha (TNF-a) level in adipocytes. We found that asymmetric dimethylarginine could dose and time dependently enhance reactive oxygen species production, while significantly inhibit IRS-1 expression and decrease the expression and translocation of glucose transporter-4, which concomitantly with an impairment of 2-deoxy-[^{3}H] glucose uptake in adipocytes. Vitamin E at a concentration of 30μM could partly reverse the role of asymmetric dimethylarginine. Nitric oxide level was decreased while tumor necrosis factor alpha production was enhanced after treatment with asymmetric dimethylarginine. In conclusion, asymmetric dimethylarginine may participate in the development of insulin resistance in adipocytes and the role of asymmetric dimethylarginine in oxidant stress may be involved in its mechanism.

ANTAGONISM OF PHOSPHATIDYLCHOLINE FROM SWINE LIVER ON ALCOHOL INDUCED IN HUMAN HEPTAIC CELL LINE

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In the present study, we discussed the antagonism of phosphatidylcholine from swine liver on alcohol induced in human hepatic cell line, which would provide a theoretical and experimental basis for the prevention of alcohol-induced hepatic injury and the development of phosphatidylcholine reagents. Phospholipids from swine liver were separated and purified by the Folch method and treated by the acetone deoiling. High-yield and high-purity hepatic phosphatidylcholines were acquired by Al_2O_3 column chromatography. Subsequently, the hepatocytes were pre-treated with phosphatidylcholine of different doses (12.5 - 100 μmol/L) in different time (0 - 24 hours) followed by sensitive 100 mmol/L alcohol exposure for 9h in vitro. The AST activities in the supernatants of hepatocytes, MDA and GSH contents in hepatocytes were determined. Results showed there were significant changes of AST, MDA and GSH between ethanol group and normal control group. However, the pre-treatment of phosphatidylcholine at the dose of 50 μmol/L for 24 hours could significantly prohibit the release of AST, the rise of MDA level and the decline of GSH content of hepatocytes exposed to alcohol ($P<0.01$). In conclusion, phosphatidylcholine could antagonize human hepatic cell line from alcohol-induced oxidative damage probably by reducing GSH consumption, improving antioxidant enzyme activity and inhibiting lipid peroxidation reaction in a dose-dependent and time-dependent manner. The underlying mechanism may be related to the special structure and the synthesis pathway of liver PC.

THE STUDY OF THE DIFFERENTIAL EXPRESSION OF RATS' SKELETAL MUSCLE PROTEOME AFTER ACUTE HIGH-INTENSITY EXHAUSTIVE EXERCISE

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The purpose of this study was to analyze the differential expression of SD rat's skeletal muscle proteome after acute exhaustive exercise. Ten male SD rats were randomly divided into exercise and rest groups according to their weight. The exercise group was sent to bout intensive exhausted treadmill training and was executed by decollation at third hour after exhaustive exercise. The rest group was the same doing like exercise group. The lower limb gastrocnemius protein was extracted and subsequently separated by 2D electrophoresis. The result showed that 572 ± 28 protein spots were found in the exercise group and 667 ± 35 in the rest group. There were a total of 282 protein spots which were differentially expressed. 88 protein spots were disappeared, 32 spots were new appeared, 12 spots increased to twice the volume, and 10 spots decreased to half the volume after exercise. Six kinds of protein and one unknown protein which were expressed differentially after exercise were identified by MALDI-TOF − TOF. Myosin (light polypeptide 3) and phosphoglucomutase 1 were down-regulated. The unknown protein was up-regulated, glyoxylase 1 was newly appeared, and H^+-transporting two-sector ATPase, acyl- Coenzyme A dehydrogenase and glyceraldehyde 3-phosphate-dehydrogenase were disappeared after exercise. We concluded that there had been qualitative and quantitative changes in protein expression after high intensity and exhaustive training, and it laid solid foundation for further research on exercise-induced fatigue.

HERBAL EXTRACT ECBRC-AG AND ITS ACTIVE INTGREDIENT REDUCES HPA AXIS FUNTION BY INHIBITING PVN NEURONS ACTIVITY THROUGH T-TYPE CALCIUM CHANNELS

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Resent studies have revealed the functional links between stress, depression and insomnia. The hypothalamic-pituitary-adrenal (HPA) axis plays important roles in the interactions among these mental disorders. Stress and depression induced hyperactivity of HPA axis is one of the major cause of insomnia, especially primary insomnia. Chronic insomniacs also showed higher cortisol levels during late night, indicating higher activity of HPA axis. In previous studies, we showed herbal extract ECBRC-AG and its active ingredient CpdB, had sedative effects on rats which decrease sleep latency and increase NREM sleep. However, the mechanisms are still unknown. In this study, we found CpdB enhanced GABAergic transmission in rat PVN neurons and this effect could be blocked by CaM-KII inhibitor KN-62. In a calcium imaging study, CpdB increased intracellular Ca^{2+} levels in the primary culture of rat hypothalamus neurons and this Ca^{2+} increase was blocked by co-perfusion of T-type calcium channels blocker mibefradil. The inhibitory effect of

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CpdB on the PVN neurons may be mediated by the phosphorylation of $GABA_A$ receptors through CaM-KII and this process was initiated by Ca^{2+} influx through T-type calcium channels. We found that oral administration of ECBRC-AG and CpdB reduced plasma corticosterone levels in rats during light period, which indicates the down regulation of HPA axis functions. Our data suggested that the HPA axis is involved in the sedative effects of ECBRC-AG and CpdB, and T-type calcium channels may play important roles in mediating the inhibitory effects of CpdB on the PVN neurons.

ASTROCYTES EXPRESS FUNCTIONAL N-METHYL-D-ASPARTATE RECEPTORS

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Increasing evidence indicates that astrocytes can respond to the chemical transmitter glutamate, since they express the ionotropic and the metabotropic glutamate receptors. However, the N-methyl-D-aspartate (NMDA) receptor, one of the ionotropic glutamate receptors broadly expressed in neurons, has still not been identified in astrocytes in vivo. Using hippocampal slices from adult GFAP-GFP transgenic mice, we found that a stimulus-evoked current in astrocytes could be inhibited 38.1 ± 5.1 % and 82.7 ± 2.0% by the selective NMDA receptor antagonist D-AP5 in Mg^{2+}-containing and in Mg^{2+}-free extracellular solution, respectively. Furthermore, the mRNAs of the NMDA receptor subunits NR1, NR2A and NR2B were detected in astrocytes using single cell rt-PCR analysis. Finally, to investigate the functional significance of these NMDA receptors, we subjected astrocytes to tetanic stimulation and found, as in neurons, tetanic stimulation induced LTP-like responses in astrocytes, which were blocked by D-AP5, but not by the selective AMPA receptor antagonist DNQX or by a K^+ channel inhibitor Cs^+. These results suggest that functional NMDA receptors are expressed in hippocampal astrocytes of adult mice. And astrocytic NMDA receptors may contribute to enhanced astrocytic responses to neuron activity. Supported by National Science Foundation of China (Grant No.30600169).

EFFECT OF ESTROGEN ON OXYGEN-GLUCOSE DEPRIVED INJURY OF ASTROCYTES

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The aim of this study was to explore the effect of estrogen on oxygen-glucose deprived injury and apoptosis of astrocytes. Primary culture of the rat cerebral cortical astrocytes with a cell damage model was induced by oxygen-glucose deprivation. Astrocytes were pretreated with estrogen at various final concentration of 1-100nmol/L. The change of cell morphology was observed by Giemsas staining. The cell damage and viability were evaluated by 3- [4,5-dimehyl-2-thiazolyl] -2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction assay and lactate dehydrogenase (LDH) released rate, and the detection of apoptotic cells was determined by the flow cytometry. Oxygen-glucose deprivation decreased the cell viability, increased the LDH release rate and increased the number of apoptotic astrocytes. While with the estrogen pretreatment at the same condition, the cell viability increased, the LDH release rate decreased, and the percentage of apoptotic cells decreased ($p<0.05$). The maximal protective dose was 20 nmol/L. Our results show that estrogen could protect primary cultured astrocytes from oxygen-glucose deprivation injury, and attenuate the apoptosis of astrocytes in a dose-dependent manner.

THE INFLUNCE OF THE STRUCTURE OF RIBOZYME, M1GS, ON ITS ACTIVITY

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To investigate the rule of the varying structure of M1RNA with guide sequence (M1GS) on its activity *in vitro*, we constructed a specific ribozyme, M1GS-T7, which targeted the mRNA segment of deoxyribonucleic acid polymerase gene, unique long 54 gene (UL54), in human cytomegalovirus (HCMV). We simulated the secondary structure of M1GS-T7 under different temperature by Vienna RNA Package. Meanwhile, we tested its activity in relevant temperature. The simulation showed that if the change of temperature resulted in minor diversification, then the activity varied but existed; if that led to failing in formation of catalytic center, meanwhile the activity lose. We made the conclusion that the formation of ribozyme's catalytic center was necessary for its activity, while flexibility of catalytic center led to the change of its activity degree.

AN ENHANCED METHOD FOR OPTIMAL MMPASE STAINING ON PARAFFIN EMBEDDED OF AS SECTIONS

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Matrix metalloproteinase (MMPs) are a group of >20 zinc containing endopeptidases that are either secreted or expressed at the cell surface of all of the main vascular cell types. Intervention studies in animal models have been used to investigate the potential roles of MMPs in cardiovascular disease. In standard immunohistochemical procedures, We incubated formalin-fixed and paraffin-embedded atherosclerotic lesion sections of rabbit with a spectrum of routinely employed MMP-1, 2, 3, 7, 8 and 9 antibodies and used unmask the antigens methods within incubation heating. But the immunohistochemical staining of these MMPase showed weak signal in section of formalin fixation and paraffin embedded. Tyramide signal amplification is an enzyme-mediated detection method that utilizes the catalytic activity of horseradish peroxidase to generate high-density labeling of target protein. The yield in terms of target amplification has allowed three to five thousands fold dilution of primary antibodies. This study evaluated a Tryamide-enhanced immunoassaying method on atherosclerotic lesions sections of paraffin embedded. Results, compared with the standard immunoassaying technique, Tyramide-enhanced immunostaining, showed superior signal intensity on these tissue specimens. This method is highly sensitive and obtained optimal staining of MMPase without a loss of specificity or increment of background staining. Tyramide signal amplification provides an excellent morphology result and a powerful tool.

THE CORRELATION OF INTEGRIN BETA 4 EXPRESSION AND ASTHMA

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Adhesion molecules expressed on leukocytes play a vital role in airway hyperresponsiveness or airway inflammation. In order to probe the relationship between imbalance of expressions of adhesion molecules and asthma pathogenicity, we designed experiments to screen differential expression adhesion molecules by using cDNA microarray. The results showed that there were various adhesive molecules with abnormal expressions and expressions of integrin β4 were down-regulated in all 6 asthma patients, which was further verified by real-time PCR. In order to verify the correlation of integrin beta 4 expression and asthma, the 5'flanking region of integrin β 4 from 100 asthma patients, age from 50 to 60 with COPD and 20 normal adults were amplified and sequenced. The expression of integrin beta 4 was also assayed by real-time PCR. The result showed that there were three variation sites in 5'flanking region of integrin β4 at −1106, -1143 and -1162 from A to G in asthma patients which were correlated with downregulated expression of integrin beta 4. This present study demonstrated that Integrin β4 was highly correlated with asthma. Down-regulated integrin β4 expression was associated with the variation in 5'flanking region.

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