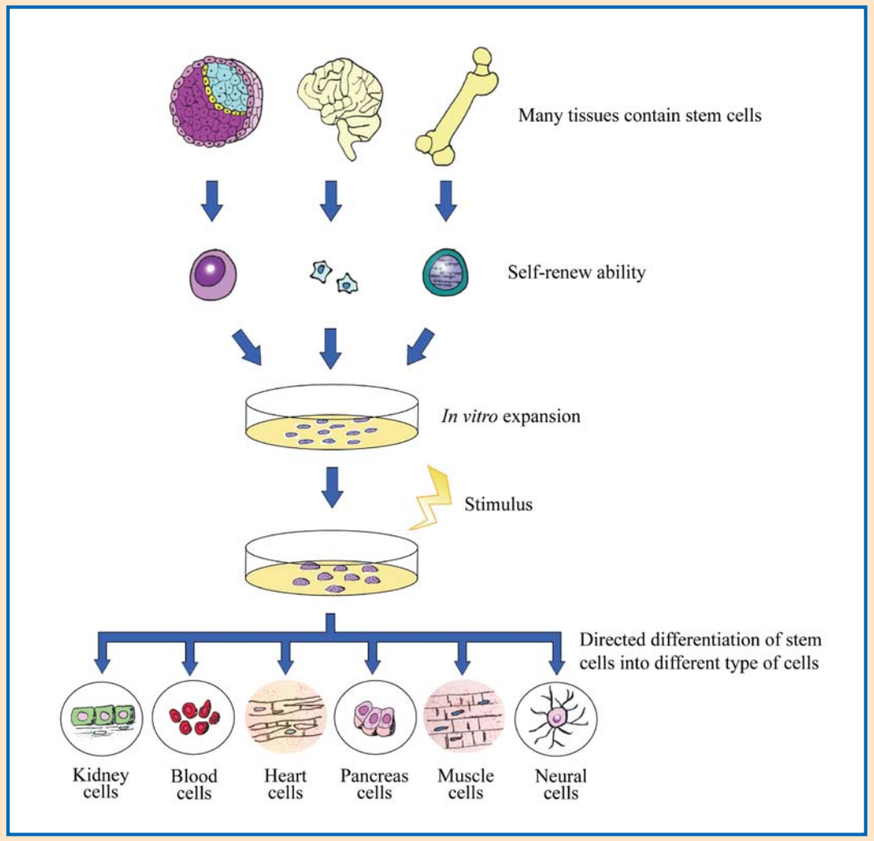

■ ***Fig. 1***
Schematic diagram showing the *in vitro* trans-differentiation of stem cells into different tissue cells.

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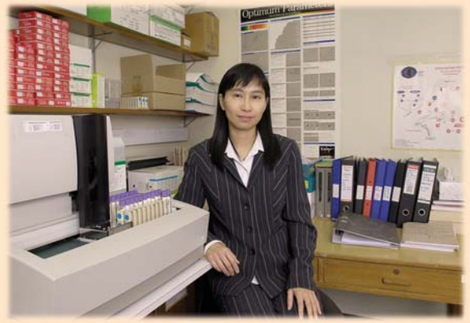
MOLECULAR PATHOLOGY Newsletter

Molecular Pathology Group, The Chinese University of Hong Kong

Molecular diagnosis of acute leukemia

Dr Margaret Ng



Acute leukemia is the commonest blood cancer that can affect people of all ages. Standard assessment by morphology, cytochemistry and immunophenotyping has been central to the diagnosis but provides little information for prognosis or to guide treatment decision. It has been increasingly recognized that within the broad groups of acute leukemias delineated normally by immunophenotyping, AML, B-lineage and T-lineage ALL, the data provided by cytogenetics or genotypes are major determinants to define prognostic subtypes for risk adapted therapy.[1] Up to date, more than 50 different consistently occurring translocations have been described to be specific for particular subtypes of leukemia.[2] A major advantage of conventional cytogenetic analysis is its ability to globally determine the presence of numeric and structural aberrations. However, as a result of the need for metaphase cells and good resolution of banding morphology, conventional cytogenetics suffers from limited sensitivity (only the cycling cells are examined), high labor with long turn-around-time and stringent requirements on fresh and good quality samples (the need for cell culture). Thus, molecular-based technologies with quick and sensitive results have emerged as the most useful tool for the detection of disease-defining genetic lesions and have provided important insights into the mechanisms of tumorigenesis.

Many genes involved in translocations found in acute leukemia are transcription factors. These translocations may alter the function or activities of cellular protooncogenes located at or near the breakpoint by two major mechanisms, first, by juxtaposition of a cellular proto-oncogene to the regulatory element of a tissue specific gene, e.g. IgH or TcR genes leading to inappropriate expression of the oncogene and second, by creating fusion genes coding for chimeric proteins with functional features different from the two parental proteins e.g. t(1;19) (q23;p13) and t(8;21)(q22;q22).[3] Molecular evaluation of most translocations in the routine diagnostic setting is based on reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR detection of the major leukemia associated translocations not only helps stratify prognostic groups for selection of treatment protocols (e.g. BCR/ABL or MLL/AF4 defines a high risk group and PML/RARA defines AML M3 with predicted response towards ATRA therapy), but also provides a sensitive marker for minimal residual disease detection (RQ-RT-PCR) during the course of therapy.

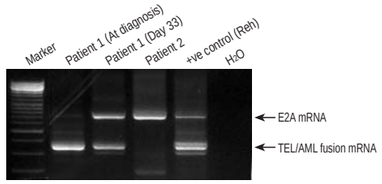
The main barrier to bring the PCR platform up-front in the diagnosis of acute leukemia is the large number of fusion genes and breakpoint variants that may need to be screened. To overcome this shortfall, the Denmark group of researchers has successfully developed a multiplex RT-PCR protocol to detect 29 translocations/chromosomal aberrations in patients with acute leukemias by 8 parallel multiplex PCR reactions.[3] Subsequently, the launching of this platform commercially as the Hemavision-7 system has greatly enhanced its routine application in the diagnostic laboratory, and the value of this application has also been validated recently by the American group.[4] The beauty of this platform is its flexibility of application and the little amounts of tissues required. The different combinations of reactions applied may yield different information as appropriate to the clinical needs.

In our Molecular Hematology Laboratory at the Prince of Wales Hospital, for routine diagnostic assessment and as part of the initiative for the I-BFM international study on childhood ALL, we have adopted the use of this system to detect multiple diagnostic or prognostic relevant molecular markers for risk stratification. Depending on the information required, the 8 rounds of reaction can be strategically designed **(Table 1 & Figure 1)**. In our experience, the molecular platform is easy to apply and standardize and is a good and powerful adjunct to conventional cytogenetics. The results are normally available within a few days. As the reference center for the I-BFM study, we have tested in particular, the *BCR/ABL, TEL/AML1* **(Figure 1)** and *MLL/AF4* for all childhood ALL patients from five major hospitals in the territory. Many other major leukemia associated translocations such as *CBF/MYH11* [inv(16)(p13q22)], *AML/ETO* [(t8;21)(q22;q22)], *PML/RARA* [t(15;17)(q21;q22)] **(Figure 1)**, *E2A/PBX1* [t(1;19)(q23;p13)] **(Table 1)** have also been tested for specific diagnostic and clinical needs in our daily practice. From an academic point of view, it is important to emphasize that the molecular data generated from the successful application of this molecular platform is also a great asset for correlation with other laboratory or experiment data in future research endeavors or collaborations.

Chromosomal Alteration	Genes Involved	Fusion Gene	Multiplex No.
t(X;11)(q13;q23)	*MLL* (11q23); *AFX* (Xq13)	*MLL/AFX*	R1
t(6;11)(q27;q23)	*MLL* (11q23); *AF6* (6q27)	*MLL/AF6*	R1
t(11;19)(q23;p13.1)	*MLL* (11q23); *ELL* (19p13.1)	*MLL/ELL*	R1
inv(16)(p13q22)	*CBF* (16q22); *MYH11* (16p13)	*CBF/MYH11*	R1
t(1;11)(p32;q23)	*MLL* (11q23); *AF1p* (1p32)	*MLL/AF-qp; MLL/AF-1p*	R2
t(10;11)(p12;q23)	*MLL* (11q23); *AF10* (10p12)	*MLL/AF10*	R2
dup MLL (11q23)	*MLL* (11q23); *MLL* (11q23)	*MLL/MLL*	R2
t(11;17)(q23;q21)	*MLL* (11q23); *AF17* (17q21)	*MLL/AF17*	R2
TAL1D	*SIL* (1p34); TAL1 (1p34)	*SIL/TAL1*	R3
t(1;19)(q23;p13)	*E2A* (19p13); *PBX1* (1q23)	*E2A/PBX1*	R3
t(12;21)(p13;q22)	*TEL* (12p13); *AML1* (21q22)	*TEL/AML1*	R3
t(17;19)(q22;p13)	*E2A* (19p13); *HLF* (17q22)	*E2A/HLF*	R3
t(3;21)(q26;q22)	*AML1* (21q22); *MDS1* (3q26) (EVI1) (3q26)	*AML1/MDS1/(EVI1)*	R4
t(8;21)(q22;q22)	*AML1* (21q22); *ETO* (8q22)	*AML/ETO*	R4
t(16;21)(p11;q22)	*TLS* (16p11); *ERG* (21q22)	*TLS/ERG*	R4
t(1;11)(q21;q23)	*MLL* (11q23); *AF1q* (1q21)	*MLL/AF1q*	R5
t(9;11)(p22;q23)	*MLL* (11q23); *AF9* (9p22)	*MLL (11q23)*	R5
t(11;19)(q23;p13.3)	*MLL* (11q23); *ENL* (19p13.3)	*MLL/ENL*	R5
t(4;11)(q21;q23)	*MLL* (11q23); *AF4* (4q21)	*MLL/AF4*	R5
t(5;12)(q33;p13)	*TEL* (12p13); *PDGFR* (5q33)	*TEL/PDGFR*	R6
t(9;12)(q34;p13)	*TEL* (12p13); *ABL* (9q34)	*TEL/ABL*	R6
t(9;22)(q34;q11)	*BCR* (22q11); *ABL* (9q34)	*BCR/ABL*	R6
t(6;9)(p23;q34)	*DEK* (6p23); *CAN* (9q34)	*DEK/CAN*	R7
?t(9;9)	*SET* (9q34); *CAN* (9q34)	*SET/CAN*	R7
t(11;17)(q23;q21)	*PLZF* (11q23); *RARA* (17q21)	*PLZF/RARA*	R8
t(15;17)(q21;q22)	*PML* (15q21); *RARA* (17q21)	*PML/RARA*	R8
t(2;5)(p23;q35)	*NPM* (5q35); *ALK* (2p23)	*NPM/ALK*	R8
t(3;5)(q25.1;q34)	*NPM* (5q35); *MLF1* (3q25.1)	*NPM/MLF1*	R8
t(5;17)(q35;q22)	*NPM* (5q35); *RARA* (17q21)	*NPM/RARA*	R8

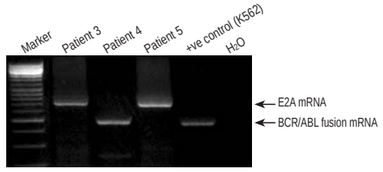
■ ***Table 1.*** 29 chromosome alterations detected by the Multiplex RT-PCR at our Laboratory

TEL/AML1 gene fusion multiplex RT- PCR



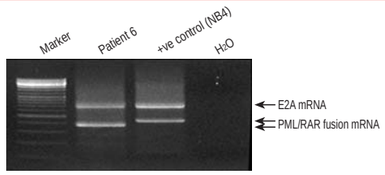
Patient 1: positive for TEL/AML1 gene fusion
Patient 2: negative for TEL/AML1 gene fusion

BCR/ABL gene fusion multiplex RT- PCR



Patient 3: negative for BCR/ABL gene fusion
Patient 4: positive for BCR/ABL gene fusion
Patient 5: negative for BCR/ABL gene fusion

PML/RAR gene fusion multiplex RT-PCR



Patient 6: positive for PML/RAR gene fusion

■ ***Figure 1***

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Making Stem Cells Differentiate into Neurones

Ming Li, Pang-Chui Shaw and Kent Tsang

■ Picture of the collaborators from left to right : Li Ming MPhil, Pang-Chui Shaw PhD and Kent Tsang PhD.

Stem cells are either embryonic or adult, depending on the developmental stage from which they are obtained. Embryonic stem cells (ESC) are harvested from the inner cell mass of blastocysts of pre-implantation embryos. Apart from self-renewal, embryonic stem cells also have infinite capacity for differentiation and can give rise to cell types of three dermal layers. Adult stem cells, in contrast, are stem cells at the postnatal stage and are harvested from tissues of the endoderm, mesoderm or ectoderm. Examples of adult stem cells are hepatic stem cells, haematopoietic stem cells of the bone marrow and neural stem cells found in the hippocampus of the brain. For a long time, differentiation of adult stem cells was thought to be unidirectional and irreversible. Recent studies have questioned this dogma and have shown that that adult stem cells can contribute to cell types of different tissue lineages *in vitro* (Figure). Besides, different types of stem cells have been noted to engraft to damaged organs *in vivo*. The extraordinary plasticity and differentiating potentials of stem cells bring new hopes and strategies to the treatment of diverse diseases related to cell death or degeneration such as myocardial infarction, diabetes, stroke, Parkinson's disease and spinal cord injury.

Embryonic and haematopoietic stem cells can be made to undergo neural differentiation and such differentiated cells hold promise for future therapeutic use for neurological diseases.[1,2] Haematopoietic stem cells (HSC) are well characterized and are not difficult to be transplanted. Adult stem cells too from bone marrow, umbilical cord blood and umbilical cord can trans-differentiate *in vitro* into neurones.[3,4] In patients who have undergone a haematopoietic stem cell transplant, donor cell-derived neurones have been detected in the hippocampus of the brain. This exciting finding has led to many more investigations into the possibility of using stem cells for the treatment of neurological diseases.[5]

Traditional Chinese medicine such as; ginseng, ginkgo and red sage, have been shown beneficial to the nervous system by inducing neuronal differentiation and promotion of neurite outgrowth.[6] Recent reports showed that ginsenoide-Rd, total *Panax notoginseng* saponin and ginkgolides can significantly induce differentiation of neural stem cells into astrocytes and dopaminergic neurons.[7,8] At the Chinese University of Hong Kong, we are from the Departments of Anatomical & Cellular Pathology, Biochemistry and Neurosurgery. We are exploring the applicability of traditional Chinese medicine-derived phytochemicals on the trans-differentiation of haematopoietic stem cells into neural cell lineage and elucidating the signalling pathways involved. The primary goals are: 1) to induce human bone marrow-derived HSC into a high yield of quality neural lineage cells by the phytochemicals, 2) to investigate the signalling pathways involved in the trans-differentiation, and 3) to study the therapeutic efficacy of cell therapy in animal models. It is anticipated that novel regulatory mechanisms will be uncovered and further developed for treatment of neural injury and degeneration.