Fusion genes in malignant neoplastic disorders of haematopoietic system

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Objectives: The new World Health Organization’s (WHO) classification of haematopoietic and lymphoid tissue neoplasms incorporating the recurrent fusion genes as the defining criteria for different haematopoietic malignant phenotypes is reviewed. The recurrent fusion genes incorporated in the new WHO’s classification and other chromosomal rearrangements of haematopoietic and lymphoid tissue neoplasms are reviewed.

Methodology: Cytokines and transcription factors in haematopoiesis and leukaemic mechanisms are described. Genetic features and clinical implications due to the encoded chimeric neoproteins causing malignant haematopoietic disorders are reviewed.

Results and discussion: Multiple translocation partner genes are well known for leukaemia such as MYC, MLL, RARA, ALK, and RUNX1. With the advent of more sophisticated diagnostic tools and bioinformatics algorithms, an exponential growth in fusion genes discoveries is likely to increase.

Conclusion: Demonstration of fusion genes and their specific translocation breakpoints in malignant haematological disorders are crucial for understanding the molecular pathogenesis and clinical phenotype of cancer, determining prognostic indexes and therapeutic responses, and monitoring residual disease and relapse status.

Keywords: Fusion genes, Leukaemia, Translocations

Introduction
Genomic rearrangements through gene duplications, repositioning, mobile elements, and exon shuffling during human genome macro-evolution caused structural and functional changes on the chromosomes that resulted in the modern genes and genetic characteristics that endowed the human genome with greater fitness. For example, karyotypic G-band comparison revealed that simple end-to-end fusion of two small chromosomes in great apes resulted in metacentric chromosome 2 in the hominid lineage, and neuroglobin duplication and differentiation to blood globin genes further evolved to give rise to myoglobin. These examples illustrate how chromosomal segmental evolution contributed to the new molecular function in the human genome.

Many structural rearrangements, however, can affect gene expression and thereafter acquire neoplastic transformation and eventually progress to malignant transformation. The Philadelphia (Ph) chromosome in chronic myeloid leukaemia (CML) and MYC gene juxtaposition with the immunoglobulin heavy chain causing Burkitt’s lymphoma are some classic examples of pathological rearrangements. These pathological rearrangements are structural chromosomal aberrations present in the form of either translocations or inversions of genetic segments. In contrast, duplication and deletion are axioms of numerical genetic changes. Collectively these genetic changes mainly affect cytokines and growth factors that dysregulate the pluripotential stem cells and eventually cause neoplastic changes through increased and uncontrolled proliferation, maturation arrest, and/or failure of apoptosis.

With the advent of more sophisticated diagnostic and research tools and bioinformatics algorithms, exponential growth in fusion genes discoveries associated with various diseases has occurred. As of February 2014, the Mitelman database of chromosomal aberrations in cancer contained over 2000 recurrent fusion genes. The total number of recurrent balanced chromosomal rearrangements associated with FAB-AML-M1 (acute myeloblastic leukaemia without maturation) has reached 70, and the number of unbalanced abnormalities has reached over 200.
These observations suggest that most of these translocations are associated with haematological neoplasias. Other databases, such as the Sanger Cancer Genome Project and ChimerDB 2.0, also provide a comprehensive catalogue of translocations that have been well characterized. The latter is a knowledge base for fusion genes that has identified over 2699 fusion transcript using their new algorithm.

The goal of this review is to describe the common translocations and fusion genes observed in the broad and complex categories of leukaemias and lymphomas, and in non-neoplastic haemoglobinopathies.

Cytokines in normal haematopoiesis

The dynamic cell cycle of haematopoietic precursors involves quiescence, entering into cell division, and differentiating or cell death (apoptosis). Molecular mechanisms regulating these events are the concerted autocrine and paracrine signals provided by the marrow microenvironment and endocrine glands in the form of soluble growth factors known as cytokines. Cytokines of the haematopoietic system include stem cell factor (SCF: a ligand for the c-Kit receptor also known as SCF-R), Fms-like tyrosine kinase receptor 3 ligand (Flt3-L), colony stimulating factors (CSFs), erythropoietin (EPO: a ligand for the EPO receptor), thrombopoietin (TPHO: a ligand for myeloproliferative leukaemia receptor – cMPL), interleukin (IL)-3, IL6, IL11, and leukaemia inhibitory factor. They play an intricate role in the tight homeostatic balance between proliferation, differentiation, and survival of haematopoietic precursor cells.

Cytokines exert their effect by interacting with their cognate transmembrane receptor and causing either oligomerization of receptors or a conformational change. Upon interaction, the receptor homodimerizes and ligand, c-KIT tyrosine kinase receptor, directs the proliferation of myeloid and lymphoid haematopoiesis. Animal models have shown that SCF produced by the marrow stromal cells is also important for the normal erythropoiesis.

SCF is a haematopoietic growth factor, and its ligand, c-KIT tyrosine kinase receptor, directs the proliferation of myeloid and lymphoid haematopoiesis. Upon interaction, the receptor homodimerizes and phosphorylates the tyrosine residues at its intrinsic tyrosine kinase domain. These phosphorylated tyrosine residues serve as docking sites for a number of downstream signal transduction molecules containing SH2 domains, which are recruited to the receptor and activated several times. The end result is normal proliferation, survival, and adhesion in the primitive myeloid and lymphoid haematopoiesis.

Damage to these growth factor genes by exogenous or endogenous agents may result in loss of control of cell proliferation, survival, and apoptosis, which in turn may cause unrestrained cell growth and therefore become tumorigenic. Cells with genome injury, therefore, activate the cell cycle checkpoints at the G1/S, intra-S, or G2/M phases to repair the DNA lesion to avoid the genome instability and progression towards cancer due to unrepaired physical or chemical damages.

Haematopoietic cell cycle regulation

To stimulate cell division and become committed to the cell cycle, the mitogenic signalling pathways (for example Ras signalling) in a quiescent haematopoietic stem cell (HSC) must be activated. In response to this signal, a family of cyclins (-D, -E, -A, and -B) with their cyclin-dependent kinase (CDK) binding partners are activated to hyperphosphorylate retinoblastoma protein (pRb) and p130. These two proteins in non-dividing quiescent cells remain in a hypophosphorylated state and bind the E2F transcription factors that restrict entry into the cell cycle. However, when pRb and p130 are hyperphosphorylated, they relieve the E2F and transactivate the E2F responsive genes required for cells to enter S phase of the cell cycle. Admittedly, abnormalities in the CKI-CDK-pRB-
E2F pathway, therefore, may affect the balance between cell proliferation, differentiation, and apoptosis in haematopoietic malignancies.\textsuperscript{12}

**Transcription factors in haematopoiesis differentiation**

A body of evidence suggests that signals from growth factors/growth factor receptors may not be enough to mediate the differentiation of multipotent HSCs along a particular lineage and survival of daughter cells. Transcription factors are also required to drive the molecular mechanisms involved in regulation and generation of differentiated progeny from the HSCs. Different but integrated regulatory mechanisms may operate in different phases of the haematopoiesis cycle. For example, transcription factors govern the stem cell self-renewal and lineage commitment, whereas cytokines (growth factors) direct the decision by regulating the balance between cell survival, proliferation, and differentiation of haematopoietic precursors.\textsuperscript{13}

The growing literature from knockout mouse and mutant cell lines shows that a combination of transcription factors, such as SCL, GATA-2, Lmo-2, and RUNX1, is absolutely essential for normal survival and proliferation of HSCs.\textsuperscript{14,15} A separate group of transcription factors is essential for survival and terminal lineage-specific maturation of haematopoietic precursors. For example, Walters and Martin\textsuperscript{16} identified GATA-1 (which binds to the GATA motif), NF-E2 (which binds to an AP-1-like sequence), and ELK1 (which binds to the CACC motif) as important transcription factors for erythroid differentiation. Similarly, transcription factors PU.1, CBF\textsubscript{α}, and C/EBP transactivate most genes expressed in the myeloid cell lineage, whereas Ikaros, NF-κB, OCT-1, OCT-2, E2A, LIF-1, and TCF-1 bind to the regulatory elements of genes expressed in lymphoid lineage cells.\textsuperscript{17,18}

**Leukaemogenic mechanisms**

Proto-oncogenes and tumour suppressor genes that are ubiquitous in the human genome play the major integral roles in maintaining a homeostatic balance in erythropoiesis, leucopoiesis, and thrombopoiesis. This complex interplay by an array of transcription factors regulates the proliferation and differentiation of multipotential haematopoietic progenitors. High-resolution cytogenetic studies have shown that two classes of leukaemogenic changes can emerge: molecular changes that confer a proliferative and/or survival advantage to haematopoietic progenitors and aberrations that arrest haematopoietic differentiation. Transcription factor genes, therefore, are common targets for leukaemogenic aberrations.

Several \textit{in vitro} and \textit{in vivo} studies of malignant haematological disorders reflect altered patterns of transcription factor activity. Studies of numerous different target genes, mainly transcription factor genes, have revealed three main cytogenetic aberrations to be associated with haematological malignancies: deletions, translocations (t), and inversions (inv). Each of these processes, especially the latter two, has been observed at a large number of transcription factor genes that either dysregulate the target gene or form a fusion gene.

**Dysregulation of genes**

Dysregulation presents in different guises, including point mutations, deletions, and translocations. Point mutations in transcription factor genes can perturb their function. For example, a point mutation at the twelfth codon (GGC → GTC) of the \textit{RAS} proto-oncogene results in an amino acid substitution (Gly12Val) that dysregulates the normal function by remaining in its active GTP-bound form, continually activating the MAP kinase cascade by locking on and transforming it to a tumorigenic gene. Mutation in the \textit{RAS} gene is known to cause acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS). Another example of the effect of a deletion, the intact \textit{TAL1} gene is activated to express ectopically in T-cell progenitors as a result of a 90 kb deletion of \textit{TAL1} gene that places the powerful promoter of the \textit{STIL} gene into contiguity with \textit{TAL1}, t(1p32), causing T-ALL.\textsuperscript{19}

Dysregulated expression has also been observed as a consequence of recombination errors that result in the translocation of proto-oncogenes so that they are under the control of a highly powerful regulatory element of another gene, such as immunoglobulin heavy-chain gene promoter or T-cell receptor (TCR) gene. The former translocations are mostly associated with B-lineage leukaemias/lymphomas involving the upstream fusion partners of the band 14q32 heavy-chain gene, whereas Ikaros, NF-κB, OCT-1, OCT-2, E2A, LIF-1, and TCF-1 bind to the regulatory elements of genes expressed in lymphoid lineage cells.\textsuperscript{17,18}

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lymphoma, that juxtaposes CCND1 with the IgH locus, resulting in over-expression of cyclin-D1, and BCL2/IGH in primary follicular lymphoma (FL).21

**Fusion genes**

The second mechanism for generating chromosomal rearrangement is the formation of hybrid (fusion) genes as a result of two previously separate functional genes recombined illegitimately to encode chimeric neoproteins. This process can conveniently be classified into two broad groups: intrachromosomal and interchromosomal. The former results from terminal or interstitial deletion, inversion, or amplification of a large fragment of DNA, and the latter originates from translocation of a DNA segment from one chromosome to another.

The structural rearrangements that generate fusion genes are thought to be due to two main processes: micro-homology-mediated break-induced replication and double strand breaks (DSBs).22 A close spatial association between the two chromosomes in the form of micro-homology has been identified at several rearrangement breakpoints in a variety of solid tumours.23–25 Mouse embryonic stem cell studies conducted to understand the role of DNA damage as a source of chromosomal translocation have provided further evidence that two DSBs, one on each chromosome, are sufficient to promote frequent reciprocal translocations.26

Numerous fusion genes have been implicated in leukaemogenesis. Recurrent translocations studied in acquired myeloid malignancies have provided further evidence that two DSBs, one on each chromosome, are sufficient to promote frequent reciprocal translocations.26

### Table 1 Molecular characterization of chromosomal rearrangement in haematological malignancies

| Genes | Translocations | Leukaemia/lymphoma disease phenotype | Functional domains |
|-------|----------------|--------------------------------------|--------------------|
| Oncogenes juxtaposed with Ig loci | t(8:14) (q24;q32) | B-cell acute lymphoblastic leukaemia; non-Hodgkin’s lymphoma (Burkitt’s lymphoma) | HLH domain |
| c-MYC | t(2;8) (p12;q24) | | |
| | t(8:22) (q24;q11) | | |
| IGH-CCND1 | t(11:14) (q13;q32) | B-cell chronic lymphocyte leukaemia; MCL; plasma cell leukaemia | PRADI-GI cyclin |
| IGH-MALT1 | t(14:18) (q32;q21) | Malt lymphoma | Inner mitochondrial membrane |
| IGH-BCL3 | t(14:19) (q32;q13.1) | B-cell CLL | CDC10 motif |
| IGHL3 | t(5:14) (q31;q32) | Pre-B-cell acute lymphoblastic leukaemia | Growth factor |
| Oncogenes juxtaposed with TCR loci | t(8:14) (q24;q11) | T-cell acute lymphoblastic leukaemia | HLH domain |
| TRA-c-MYC | t(7:19) (q35:p13) | T-cell acute lymphoblastic leukaemia | HLH domain |
| TRB-LYL1 | t(1:14) (p32;q11) | T-cell acute lymphoblastic leukaemia | HLH domain |
| TRB-TAL1 | t(7:9) (q35:p34) | T-cell acute lymphoblastic leukaemia | HLH domain |
| TRB-TAL2 | t(11:14) (p15;q11) | T-cell acute lymphoblastic leukaemia | LIM domain |
| LMO2 | t(11:14) (p13:q11) TRD-LMO2 | T-cell acute lymphoblastic leukaemia | LIM domain |
| LMO2 | t(7:11) (q35:p13) TRB-LMO2 | T-cell acute lymphoblastic leukaemia and non-Hodgkin’s lymphoma | Homeodomain |
| TRB-NOTCH1 | t(11:14) (q35:q32.1) TRB-TCL1A | T-prolymphocytic leukaemia; ataxia telangiectasia | Notch homologue |
| TCL1A | t(14:14) (q11:q32.1) TRA/TRA-TCL1A | | |
| Translocation involving transcription factors | RUNX1-RUNX1T1 | t(8:21) (q22;q22) | AML2 |
| RUNX1-RUNX1T1 | t(10:14) (q24;q11) | | |
| CBFβ-MYH11 | inv(16)(p13;q22) | AML-M4Eo | |
| ETV6-RUNX1 | t(12:21)(p13;q22) | Precursor B-cell ALL | |
| PMI-RARA | t(15:17)(q22;q21) | AML-M3; treatment-related AML | |
| TCF3-PBX1 | t(1:19)(q23;p13) | Precursor B-cell ALL | |
| MYC | t(8q24) | B-cell ALL | |
| TAL1 | t(1p32) | T-cell ALL | |
| ETV6 | t(12p13) | AML, ALL | |
| HOX genes | Variable | AML | |
| Signal transduction | BCR-ABL | t(9;22)(q34;q11)- | CML, ALL-precursor B |
| Chromatin modulation | KMT2A | t(11q23) | AML-M4/5, secondary AML, childhood acute leukaemia |
| RBFM15/MKL1 | T(1;22)(p13;q13) | AML-M7 | |
| CAT6A-REBBP | T(8;16)(p11;p13) | AML including M4, M5, and treatment-related AML | |

AML, acute myeloid leukaemia; HLH, helix-loop-helix domain; HMG, high mobility domain.

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**Saleem and Yusoff**

Fusion genes in haematological malignancies
haematological malignancies. Fusion proteins, also sometimes referred as oncproteins, contain structural and functional domains from two different oligopeptides – 5' of one gene and 3' of the other – hybridized together with transformed activity. The precise function of a fusion protein obviously depends on the functional domains of the fused proteins, and it may target distinct steps in common signalling transduction pathways. Functionally, these transcription factors may acquire abnormal DNA binding capacity, abnormal transactivation (normal DNA binding but abnormal transactivation), and sequestration by protein–protein interaction or they may exhibit ectopic expression driven by the promoter of the fusion gene’s upstream component.

RUNX1, the α subunit of core binding factor (CBF) protein, heterodimerizes with CBFβ protein and is functionally essential for the physiological definitive

**Table 2  2008 WHO classification of AML (adopted from Campo et al.)**

| AML with recurrent genetic abnormalities |
|-----------------------------------------|
| AML with t(8;21)(q22;q22) (RUNX1-RUNX1T1) |
| AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22) (CBFB-MYH11) |
| Acute promyelocytic leukaemia with t(15;17)(q22;q12) (PML-RARA) |
| AML with t(9;11)(q22;q23) (MLL3-MLL) |
| AML with t(6;9)(p23;q34) (DEK-NUP214) |
| AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) (RUN1-MECOM) |
| AML (megakaryoblastic with t(1;22)(p13;q13) (RB-M5-MKL1) Provisional entry: AML with mutated NPM1 Provisional entry: AML with mutated CEBPA AML with myelodysplasia-related changes Therapy-related myeloid neoplasms AML, not otherwise specified (NOS) AML with minimal differentiation AML without maturation AML with maturation Acute myelomonocytic leukaemia Acute monocytic/myelomonocytic leukaemia Acute erythroid leukaemia Pure erythroid leukaemia Erythroleukaemia, erythroid/myeloid AMKL Acute basophilic leukaemia Acute panmyelosis with myelofibrosis (acute myelofibrosis, acute myelosclerosis) Myeloid sarcoma (extramedullary myeloid tumour; granulocytic sarcoma; chloroma) Myeloid proliferations related to Down syndrome Transitional abnormal myelopoiesis (transient myeloproliferative disorder) AML associated with Down syndrome Blastic plasmacytoid dendritic cell neoplasm Acute leukaemias of ambiguous lineage Acute undifferentiated leukaemia Mixed phenotype acute leukaemia with t(9;22)(q34;q11.2) BCR-ABL1 Mixed phenotype acute leukaemia with t(1;19)(q23;p13) MLL-rearranged Mixed phenotype acute leukaemia, B/myeloid, NOS Mixed phenotype acute leukaemia T/myeloid, NOS Provisional entry: natural killer cell lymphoblastic leukaemia/lymphoma

Fusion genes in haematological malignancies

Haemoglobin fusion genes are good examples of intrachromosome aberrations; most non-alpha globin gene fusions are non-homologous, whereas the a globin gene fusions are paralogous crossovers. The best studied of these haemoglobinopathies is the pathological non-homologous crossover (unequal crossover) between part of the δ locus and part of the β locus to form the hybrid Lepore gene and anti-Lepore gene. The Lepore gene is produced by the fusion of the amino-terminal of the β globin gene (HBB) and the carboxy terminal of the δ globin gene (HBD). The reciprocal of this event forms anti-Lepore gene (i.e. a fusion gene produced by unequal crossing-over that has the N terminal of HBB and the C terminal of HBD). Another example, haemoglobin Kenya, results from fusion of more distinctly located genes: the amino-terminal sequence of the γ δ (HBB) gene and the carboxyl terminal sequence of the HBD gene. The Hb Lepore fusion gene produces a β thalassaemia phenotype that ranges from thalassaemia major to thalassaemia intermedia, and the Hb Kenya fusion gene produces a hereditary persistent of foetal haemoglobin phenotype with 5–10% Hb F in the heterozygous state. Most cases of Hb Kenya appear to originate from east Africa, whereas Hb Lepore is more widespread in individuals from Mediterranean countries to Southeast Asia.
factors linked to the characteristic morphologic, immunophenotypic, and clinical features to define the distinct subtypes of AML and heterogeneous and ambiguous categories of disease.31 Karyotyping, therefore, has become essential in all cases of AML for proper classification. In this updated classification (Table 2), new definitions for lymphoid neoplasias, including chronic lymphocytic leukaemia (CLL), plasma cell neoplasm, and Waldenström macroglobulinaemia, were also adopted.

**Recurrent fusion genes in AML**

Since 2001, the WHO’s classification of tumours of haematopoietic and lymphoid tissues has used recurrent fusion genes as defining criteria to cluster seven subtypes of AMLs into a broad general category called *AML with recurrent genetic abnormalities*. With improved understanding of their biology, this classification system was updated in 2008 (Table 2).32 All of these translocations have a specific association with a unique neoplastic type and play a crucial role in its pathogenesis and therapeutics.33 They are reviewed below.

**AML with t(8;21)(q22;q22): (RUNX1/RUNXIT1)**

The balanced translocation t(8;21)(q22;q22) results in the splicing of RUNX1 (a CBF previously known as *AML1*), located on chromosome 21, and *RUNXIT1* (a transcription factor also known as *ETO*), located on chromosome 8. The *RUNX1* gene breaks within introns 1a or 1b, and the *RUNXIT1* gene breaks within intron 5, which then forms the *RUNX1/RUNXIT1* fusion. This is observed in 5–12% of AML, most common in younger children; most correlates with FAB-AML-M2 and few reported with AML-M1 and AML-M4.34 The runt-related transcription factor 1 gene encodes for alpha subunits of haematopoiesis; the transcript of *RUNX1* encodes for CBF DNA binding sites. Loss of sex chromosome and partial deletion of 9q22 are frequent observations along with t(8;21)(q22;q22). This translocation has been reported to occur at low frequencies as a preleukaemic anomaly in some normal individuals.

Despite its dominant negative effect on definitive haematopoiesis, the chimeric protein is poorly leukaemogenic on its own and requires an additional ‘second-hit’ (commonly known as two-hit model of leukaemogenesis) for transformation.35 A transgenic mouse model with *RUNXI–RUNXIT1* fusion resulting from nearly the full length of *RUNXIT1* in its amino-terminal has been shown to require additional mutations to be leukaemogenic.36 Similar mouse models also have shown that deficiency of GM-CSF signalling compliments to the leukaemogenesis, which also suggests that GM-CSF has a tumour suppressor role in patients with t(8;21)(q22;q22).37 The c-Kit mutation in patients with 8;21 translocation complicates the clinical outcome and is known to be a poor prognostic indicator.38

Blast cells in this balanced translocation are usually CD34+ and are co-expressed with CD19 and CD33. A subset of blast cells and mature monocytes during long-term haematological remission may show mRNA of *RUNXI–RUNXIT1* on reverse-transcription polymerase chain reaction (RT-PCR) analysis: therefore, quantitative RT-PCR was found to be useful in monitoring residual disease.

**AML with inv(16)(p13.1q22)/t(16;16)(p13.1q22) (CBFB–MYH11)**

The pericentric inversion of chromosome 16, inv(16)(p13.1q22), or the less frequent t(16;16)(p13.1q22) accounts for nearly 10% of adults with AML and 6% of children with AML, and it belongs to class II of the two-hit theory of leukaemogenesis. Its characteristic morphology is nearly pathognomonic of the FAB M4Eo (acute myelomonocytic leukaemia with abnormal eosinophils) subset of AML; less frequently it resembles the M2 and M5 subtypes.39 The aberration is characterized by at least 10 *CBFB–MYH11* variant transcripts (A–J), and transcript type A is the most frequent.40,41 Like t(8;21)(q22;q22), AML with inv(16)(p13.1q22)/t(16;16)(p13.1q22) (*CBFB–MYH11*) disrupts the beta subunit of CBF that is normally involved in the regulation of haematopoiesis.42 These two AMLs collectively are referred to as CBF leukaemias and have a favourable prognosis. A study conducted by the German–Austrian AML study group demonstrated that secondary genetic lesions, such as trisomy 22, trisomy 8, and mutations in *RAS, Kit*, and *FLT3*, define the clinical heterogeneity of inv16 or t(16;16) AML patients (in particular, aberrations in the latter three represent adverse prognostic markers).43 Molecular screening for chimeric fusion genes in CBF leukaemias is important as the overt cytogenetic lesion is not detected in nearly 10% of cases due to cryptic or more complex rearrangements.44

**Acute promyelocytic leukaemia with t(15;17)(q22;q12): (PML–RARA)**

The 2008 WHO classification requires demonstration of the presence of the t(15;17)(q22;q12) or *PML–RARA* transcript to diagnose acute promyelocytic leukaemia.31 This translocation has been renamed t(15;17)(24;q21) since the specific reciprocal translocation involves the retinoic acid receptor α (*RARA*) at chromosome band 17q21 (a transcription regulating
protein), spliced with the promoter and upstream sequence of the zinc finger binding promyelocytic leukaemia (PML) gene at 15q24 that encodes a transcription factor. Genomic variability and alternative splicing has resulted in three isoforms of PML-RARA chimeras; short (S), long (L), and variable (V). The PML-RARA fusion protein arrests the maturation of myeloid cells at the promyelocytic stage thereby leading to their accumulation. Acute promyelocytic leukaemia with t(15;17)(q24;q21) (PML-RARA) translocation has a favourable prognosis if detected early, and it is sensitive to trans-retinoic acid treatment. Both the fusion products PML-RARA and its reverse RARA-PML can be detected in leukaemic cells. Detection is important to identify those likely to benefit molecularly targeted therapies such as all-trans retinoic acid (ATRA) and arsenic trioxide. Immunophenotypic characteristics of promyelocytes show homogeneous expression of CD33 and heterogeneous expression of CD13 positivity, and HLA-DR and CD34 are usually absent.

Few patients presenting with morphologic and immunophenotypic promyelocytic leukaemia without classical PML-RARA translocation have been diagnosed with t(11;17)(q23;q21) which fuses the RARA gene with ZTBT16 (previously known as the PLZF – promyelocytic leukaemia zinc finger) gene. The ZTBT16-RARA fusion oncoprotein contains the transcription repression POZ-domain of ZTBT16 and the DNA binding domains of RARA, which explains its leukaemogenic effect. Other rare cases with variant RARA translocations are NUMAI at chromosome region 11q13, NPM1 at 5q35, and STATB5 at 17q11.2. Clinical studies have shown that this group of patients is mostly refractory for ATRA treatment and predicts a dismal prognosis.

**AML with t(6;9)(p22;q34) (DEK-NUP214)**

Approximately 1% of patients (both adults and children) diagnosed with morphological AML have the (6;9)(p23;q34) translocation. This translocation has been renamed as t(6;9)(p22;q34) because DEK sits in 6p22.3. DEK protein, which contains several Asp/Glu-rich domains, is involved in chromosome organization by binding to cruciform and super helical DNA and in DNA damage response signalling, whereas NUP214 is a member of phenylalanine-glycine-repeats containing nucleoporins. The fusion of the two results in a class II protein of the two-hit theory of leukaemogenesis and induces leukaemogenesis in HSCs frequently in association with FLT3 mutations. This cytogenetic change is observed in AML preceded by an episode of MDS and therapy-related AML. AML with t(6;9) (p22;q34) (DEK-NUP214) is now formally recognized as a distinct disease category that requires molecular diagnosis rather than a descriptive morphologic diagnosis. DEK-NUP214 fusion occurs when the 5′ part of DEK splices with the 3′ portion of NUP214 (also known as CAN), and the fusion protein transcribes into a 165-kDa chimeric protein.

**AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) (RPN1-MECOM)**

These two chromosome anomalies are variants of each other. The RPNI gene encodes an integral membrane protein of the rough endoplasmic reticulum and forms a part of the regulatory subunit of the 26S proteasome, and the MECOM gene encodes a transcriptional regulatory protein that is not normally expressed in haematopoietic cells. Leukaemogenic expression of RPNI-MECOM in HSCs therefore is driven by promoter sequence of RPNI. It is observed in 1–2% of de novo adult AML or after history of MDS with no difference between sexes. The translocation most commonly observed in adults and rarely in children is often associated with monosomy 7 or 5q deletion. The 3q26 rearrangement is difficult to demonstrate in conventional cytogenetics analysis but readily detectable using FISH. Secondary karyotypic changes are reported to be common in this group of patients. Morphologically characteristics display a multilineage dysplasia with features of AML without maturation, acute myelomonocytic leukaemia, or acute megakaryoblastic leukaemia (AMKL).

**AML (megakaryoblastic) with t(1;22)(p13;q13): RBMI5-MKL1**

AML (megakaryoblastic) with t(1;22)(p13;q13) is rare but distinctively associated with AMKL that presents almost exclusively in infants younger than 6 months.
old\textsuperscript{52} with an unbalanced female predominance. RNA binding motif 15 (RBM15), as its name suggests, contains RNA recognition motif consensus, and is a member of the split-end family of proteins, and it has a repressor function in multiple signalling pathways. MKL1 protein interacts with the transcription factor myocardin. The exact leukaemogenesis mechanism of \textit{RBM15-MKL1} (also known as OTT-MAL) is not well understood. However, the chimeric protein retains almost all of the putative functional domains of RBM15 and MKL1, thus this oncoprotein is believed to be involved in extracellular signalling pathways, modulation of chromatin organization, \textit{HOX}-induced differentiation, and anti-proliferation. Studies of a knock-in mouse model revealed that \textit{RBM15-MKL1} deregulates the transcription of Notch receptor target.\textsuperscript{53}

\textbf{Fusion genes with other myeloid lineages}\n\textit{t}(8;16)(p11;p13): \textit{KAT6A-CREBBP}  
The (8;16)(p11;p13) translocation is a hallmark of AML and typically is associated with FAB M4/M5 subtypes. The majority of AML with this translocation are secondary to therapy related, and therefore are t-AML. Typical cytological features are monocytoid or myelomonocytic differentiation, erythrophagocytosis from bone marrow blasts, positive myeloperoxidase and non-specific esterases, and disseminated intravascular coagulation. \textit{KAT6A-CREBBP} disrupts \textit{RUNXI} regulated transcription and therefore arrests myeloid differentiation. This fusion is characterized by upregulation of \textit{MEIS1}, \textit{HOXA9}, and \textit{HOXA10}; over-expression of \textit{RET} and \textit{PRL}; and down-regulation of \textit{CCND2}, \textit{WT1}, and \textit{STAT5}).\textsuperscript{54} At least six variants of \textit{KAT6A-CREBBP} fusion transcripts have been reported and all contain MYST3 domain.\textsuperscript{55}  
Type I (\textit{KAT6} exon 16-\textit{CREBBP} exon 3) is the most frequent in adults. Reciprocal \textit{CREBBP-KAT6A} has also been identified, although its pathological role in leukaemogenesis is not well understood. Recently, a simplified RT-PCR method was developed to molecularly confirm the presence of the type I \textit{KAT6A-CREBBP} chimeric transcript.\textsuperscript{56}

\textit{t}(3;5)(q25;q34): \textit{NPM1-MLF1}  
\textit{NPM1} translocation \textit{t}(3;5)(q25;q34) with \textit{MLF1} is a rare chromosomal aberration detected in MDS and all AML, with the exception of FAB subtype M3. The highest incidence occurs in AML-M6. The chimeric \textit{NPM-MLF1} gene encodes a fusion for which the first 175 amino acids from the N terminal of NPM1 are spliced with the MLF1 protein starting from the sixteenth amino acid until its carboxyl end. The exact mechanism for the leukaemogenesis of this oncprotein remains obscured. However, because \textit{MLF1} (myelodysplasia/myeloid leukaemia factor 1) is not normally expressed in haematopoietic tissue, myelodysplasia or AML may be the result of ectopic expression of \textit{MLF1} in the haematopoietic tissue. An \textit{in vitro} study showed that \textit{MLF1} expressed in erythroleukaemia cells restricts biological and morphological maturation in response to EPO by preventing cells from exiting the cell cycle through suppression of \textit{CDKN1B} expression.\textsuperscript{57} A different study showed that this fusion protein promotes apoptotic cell death, which was shown to be counter-balanced by anti-apoptotic BCL2 protein. These two observations suggest why \textit{t}(3;5)(q25;q34) is most frequent in AML-M6 and ineffective haematopoiesis in MDS, respectively.

\textit{t}(9;22)(q34;q11): \textit{BCR-ABL} fusion  
CML is the most frequent malignant neoplastic disorder of the haematopoietic system. This reciprocal translocation \textit{t}(9;22)(q34;q11), commonly known as the Ph chromosome which involves breakpoints at the tyrosine kinase \textit{c-ABL1} oncogene on chromosome 9q34 and the guanosine triphosphate-binding protein \textit{BCR} on chromosome 22q11, is seen in all CML. The result is a constitutively active oncogenic \textit{BCR-ABL} fusion gene made up of regulatory elements from the gene on chromosome 22 and the majority of open reading frames of the proto-oncogene from chromosome 9. This translocation originates and transforms a pluripotent HSC to drive clonal expansion of terminally differentiated myeloid cells.

The tyrosine kinase activity of the \textit{BCR-ABL} chimeric oncprotein is elevated by several orders of magnitude compared to its normal native protein, and it is known to activate a number of cell signalling pathways, including \textit{RAS} and \textit{MAPK}, \textit{JAK-STAT}, \textit{PI3}, and the \textit{Myc} pathways.\textsuperscript{58–61} A recent comprehensive micro-array analysis using long non-coding RNA (lncRNA) cDNA on human CML cells provided compelling evidence that silencing of the tumour suppressor lncRNA-BGL3 is required for \textit{BCR-ABL}-mediated malignant cellular transformation.\textsuperscript{62}  
A growing number of preclinical data have shown that mutations on \textit{BCR-ABL1} gene affect the therapeutic response. For example, \textit{BCR-ABL1} c.944C>T (T315I) mutation confers reduced efficacy and increased resistance to tyrosine kinase inhibitors dasatinib, nilotinib, and bosutinib.\textsuperscript{63}  
The National Comprehensive Cancer Network Practice Guideline and WHO 2008 classification requires \textit{BCR-ABL1} fusion genes or their transcripts be identified to make a diagnosis of CML.\textsuperscript{64} The absence of Ph chromosome is not CML. Routine karyotyping detects the translocation \textit{t}(9;22)(q34.1;q11.21) in 90–95\% of patients, but for the remainder detecting the presence of the Ph chromosome requires the use of FISH or RT-PCR.
Although CML requires demonstration of the presence of the Ph chromosome, it is not specific to this myeloproliferative disorder. The translocation is observed in 15–30% of adults and 5% of children with B-ALL and it also is found in some cases of mixed phenotype acute leukaemias, for which it carries a poor prognosis.65 The breakpoints on the BCR gene in most cases of CML are different from the breakpoints observed in nearly two-thirds of Ph-positive ALL transcripts of BCR-ABL1. This finding explains the observation that the BCR gene has three distinct breakpoints while the ABL gene breakpoint is largely invariant; this means that the fusion transcripts encode to either 190-, 210-, or 230-kDa fusion proteins. Molecular analysis shows that the BCR-ABL1 expressed in CML and approximately 70% of BCR-ABL1+ ALL transcribe a fusion protein with a molecular weight of 210 and 190 kDa, respectively.66

Two landmarks studies using the RT-PCR method showed that the Ph chromosome is also present in about 30% of the general population.67,68 In many instances, these BCR-ABL transcripts have aberrant junctions and thus encode non-functional truncated proteins, but some true leukaemia types have also been demonstrated. These findings provide strong evidence that oncogenicity of BCR-ABL is not, in itself, sufficient for malignant clonal expansion. Alternatively, it could be postulated that the translocation occurs in relatively mature precursors that are rapidly lost upon normal differentiation or cell death.

**Oncogenes juxtaposed with immunoglobulin loci**

**t(14;18)(q32;q21): IGH-BCL2**

The BCL2 gene encodes a mitochondrial membrane protein in resting B and T lymphocytes but not in the normal germinal centre cells. Over-expression selectively blocks the apoptotic cells under growth factor deprivation in a variety of cell systems.69 Translocation (14;18)(q32;q21), which juxtaposes the BCL2 gene under the influence of the highly active IGH promoter on chromosome 14, is the most common clonal rearrangement seen in human follicular B-cell lymphomas, accounting for 70–90% of cases. Nearly 20% of diffuse large B-cell lymphoma also shows BCL2 gene rearrangement with the same translocation, thus it may have transformed from primary FL. In rare cases of FL, the BCL2 gene is placed with the promoters of light chain on chromosome 2, thereby forming the t(2;18)(p11;q21) (IGK-BCL2). Although, in vitro studies have shown that t(14;18)(q32;q21) results in over-expression of BCL2 protein, which confers a survival advantage for B-cells by inhibiting apoptosis, this rearrangement was also reported in many healthy individual blood lymphocytes; this finding indicates that this rearrangement by itself is not enough for malignant transformation. However, use of a constellation of molecular techniques showed that no other abnormality other than t(14;18)(q32;q21) was detected in nearly 10% of FLs. A subset of patients with primary cutaneous marginal zone lymphoma may have this translocation, but those with primary cutaneous follicle centre lymphoma typically lack it.

**t(9;14)(p13;q32): PAX5-IGH**

The paired box 5 (PAX5) is a B-lineage-specific activator protein expressed during early B-cell differentiation and involved in CD19 gene regulation. Chromosomal aberrations involving PAX5 gene cause acute lymphoblastic leukaemia. The translocation juxtaposes the E-mu enhancer of the IGH locus in the close vicinity of the IGH gene 9(p13) promoter, which, without creating a fusion gene, causes deregulated transcription of intact PAX5 and contributes to leukaemogenesis. The t(9;14)(p13;q32) translocation was previously thought to be present in about 50% of low-grade B-cell lymphomas characterized by plasmacytoid differentiation. However, with the recent classification of mature B-cell neoplasms and improvement in the definitions of different entities, this fusion is now believed to be associated with large B-cell lymphoma instead.71

**t(11;14)(q13;q32) CCND1-IGH**

t(11;14)(q13;q32) is present in virtually all cases of MCL, 15–18% of plasma cell leukaemia, (15–20%) of multiple myeloma,72 and nearly 40% of amyloidosis.73 This translocation juxtaposes the immunoglobulin heavy-chain locus at chromosome 14q32 to a region on 11q13 designated cyclin D1 (CCND1), causing over-expression of cyclin-D1, leading to deregulation of the cell cycle. Cyclin-D1, a G1 glycine involved in normal regulation of the mitotic cell cycle, is not synthesized in the normal lymphocytes and myeloid cells, but it is pathognomonic in MCL. The mechanism for the translocation in the lymphoid precursors is due to an illegitimate V–D–J recombination and that for the myeloid is in a switch in the recombination process.74 MCL is a B-cell neoplasm with small lymphoid cells arising from naive B-cells, and it develops in the inner mantle zone of secondary lymphoid follicles.

**Diagnostics approaches for detecting the presence of fusion genes**

The new WHO classification of tumours of haematopoietic and lymphoid tissues requires karyotyping for recurrent fusion genes to define distinct subtypes of AML and lymphoid neoplasias. Identification and characterization of fusion genes involved in the
fusion transcripts are crucial for understanding the pathogenesis of haematological malignancies, determining prognostic indexes and therapeutic responses, and monitoring residual disease and relapse status. Chromosome banding, FISH, Southern blotting, and RT-PCR are tools commonly used to detect gene rearrangements that result from chromosomal translocations. Visible cytogenetic alterations occur in a substantial majority of haematological malignancies. Southern blotting in haematopathology has been widely used and still is in use in many laboratories as a standard tool to visualize the translocations and clonal rearrangements in several genes including IGH, TCR, BCL2, CCND1, and BCL6 in B-cell neoplasms; TCR, RUNX1, RARA, MYC, RAS, and others in acute leukaemias and myeloproliferative disorders.

The advent of new technologies, such as interphase FISH, spectral karyotyping, multiplex FISH (which utilizes whole-chromosome painting probes specific for each of the 22 autosomes as well as for X and Y chromosomes), array-based comparative genome hybridization, single-nucleotide polymorphism genotyping, and array-based gene expression profiling, to study the transcriptome has provided adjunct tools to study fusion genes and their transcripts in a large variety of cells in haematological malignancies with a low mitotic yield with great precision.

High-resolution tools and biotechnological advances currently are being sought to identify new translocations in haematological neoplasias. The advent of next-generation high-throughput DNA sequencing using the paired end resequencing has facilitated the interrogation of molecular fusion genes in unprecedented detail. Furthermore, high-throughput transcriptome sequencing of cancer cells has recently been used to identify novel fusion gene transcripts resulting from cryptic chromosomal rearrangements with clear precision. To evaluate this wealth of information, new bioinformatics algorithms implemented to target align against artificial exon–exon junctions and spliced alignment to the genome have been developed and shown to be effective in identifying transcription-induced chimera events in human solid cancers. Similar bioinformatics and dynamic programmes, such as FusionSeq, deFuse, and FusionQ, have been developed to identify novel fusion genes. More recently, simple and rapid flow cytometry immunobead assays have been developed for the detection of fusion genes such as BCR-ABL and PML-RARA.

Conclusion

Understanding the molecular basis for gene fusion provides remarkable insight into the repertoire of fusion genes responsible for the pathological process of many neoplastic disorders. As the molecular basis of these processes becomes better characterized, better selective therapeutics can be developed.

In addition to the usual cytogenetic analysis for the detection of fusion genes, mutation analysis in a variety of genes is becoming important for the proper classification of haematological neoplasms, especially the broad category of AML with recurrent genetic abnormalities. NPML, CEBPA, and FLT3 are recommended, especially for all cyogenetically normal AMLs, while mutation in JAK2 should be sought in BCR-ABL negative MPNs. Mutation analysis of KIT, NRAS, PTPN11, and others should be performed as clinically indicated.

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