Acute Myeloid Leukemia As a Genetic Disease
Review Article

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Abstract

The number of recurring genetic abnormalities recognized in acute myeloid leukemia (AML) has increased rapidly in recent years and at present, acute leukemia is probably the most extensively analyzed human cancer. Combined cytogenetic and molecular genetic studies have revealed that clonal chromosome abnormalities are present in the majority of patients with AML that are very closely, and sometimes uniquely, associated with distinct subsets of leukemia. Detailed analysis of these rearrangements indicates that in most instances chromosome rearrangements result in gene fusions leading to chimeric abnormal protein with oncogenic potential. Continued identification and characterization of genes involved in the development of leukemia has a major impact on our understanding of the molecular biology of cancer and in formulating of biologically based therapies.

Key Words

Acute myeloid leukemia, Oncogenes, leukemogenesis.

Introduction

Acute myeloid leukemia (AML) is a malignant neoplasm of hematopoietic cells characterized by an abnormal proliferation of myeloid precursor cells, decreased rate of self-destruction and an arrest in cellular differentiation. Current molecular studies demonstrate that AML arise from a single cell as a mutation in the genetic material [1,2]. The error is copied and passed on to subsequent generations of cells resulting in clonal expansion of the malignant clone.

All our cells are chronically faced with decision to divide, differentiate, or undergo programmed cell death. The normal cell regulation is balanced and delicately orchestrated by genes (parts of our DNA) promoting and suppressing cell growth. Acute myeloid leukemia is associated with a sequence of genetic changes that cause the cell cycle division, cell differentiation, or cell death processes to go out of control. Leukemia, as other cancer is a disease of genes.

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Genes involved in the pathogenesis

The genes involved in the pathogenesis of leukemia can be grouped into two general categories. The first group consists of genes that promote cell division and are referred to as oncogenes. Oncogenes are a small group of genes that have been highly conserved throughout evolution and it is generally presumed that they play essential roles in the coordination and regulation of the cell. The second general group of genes are genes, whose products normally provide negative control of cell proliferation and are called tumor suppressor genes or anti-oncogenes. Loss of function of both homologous copies of a tumor suppressor gene has a powerful growth-promoting effect. Other genes that may be altered include those involved in DNA repair or cause cell to die at the appropriate time [3,5].

The relevant genes can have their functions altered by a variety of mechanisms, which usually results in structural changes in the genome. The critical genetic alterations in gene function largely take the form of :

- point mutation (an example for point mutations leading to missense is RAS),
- gene amplification (a region of the genome is replicated numerous times within one

cell cycle leading to overexpression of genes located within the amplified region),

- gene deletion (loss of a segment of DNA, or the whole chromosome),
- inversion (a single chromosome is broken in two places and inverted)
- chromosomal translocation (a part of one chromosome becomes displaced and attached to another chromosome.)

Critical role of chromosomal translocations in leukemia

In most instances, leukemogenesis results from chromosomal translocation and specific chromosome translocations appear to be the most biologically and clinically significant karyotypic changes in human leukemias [5]. Molecular cloning of these chromosomal regions has confirmed that genes important for the regulation of cell growth and differentiation are often affected by such chromosome translocations. Some of these genes in these rearrangements are known oncogenes, others are genes that have been deduced to code for DNA binding proteins, growth factor receptors, protein kinases and other proteins [5,7]. There are two basic types of translocations seen in leukemia:

- *Translocations fusing two genes resulting in the creation of a new fused gene.* In this case, the chromosomal translocation disrupts the normal sequencing of the genes and brings two previously unlinked segments of the genome together. The resulting fused mRNA encodes a fusion protein made of parts of two independent proteins, which can be causally implicated in disease pathogenesis.
- *Translocations affecting gene regulation.* In this case, the proto-oncogene can be activated by the juxtaposition of the promoter and enhancer elements of a distinct gene. In this type of translocation a noncoding exon is usually removed from the gene, and the gene is placed in an abnormal environment with effects on the regulation of the gene's expression.

The principal consequences of both types of chromosomal translocations is that the genes involved often encode transcription factors, suggesting these are important in hematopoietic development and whose dysregulation plays a major role in leukemogenesis.

Genetic markers in AML

The presence of genetic alternations in human leukemias has been recognized for decades. Due to technical improvements and increased level of experience in detection of subtle structural aberrations, a number of specific cytogenetic abnormalities has been recognized and continue to be discovered. The most important fact to emerge from cytogenetic studies is the realization that these aberrations are strictly nonrandom, i.e, the breakpoints occur within the same, relatively small segments of DNA and different chromosomes and chromosomal regions are preferentially involved.

Many of these genetic alterations, such as chromosomal translocations, inversions and deletions of large chromosomal regions can be recognized as a cytogenetic change in the chromosomal region where the gene is located. Cytogenetic studies have revealed that clonal abnormalities are present in the majority of successfully karyotyped patients in AML [5,6]. More importantly, such chromosome abnormalities, especially translocations and inversions are tightly linked to specific clinical syndromes and tend to be characteristic of particular stages of differentation; for example, the t(15;17) is tightly linked to the M3 subtype and can be detected in virtually 100% of patient with AML-M3 [Table 1].

Prognostic significance of genetic markers

Due to the increased use of modern technologies, the study of chromosomal re-arrangements has much importance in terms of the management of the disease and the patient. The characterization of these subtypes is most closely related to prognosis and make it possible to define groups with different prognosis and it could be shown, that most of these abnormalities are an independent prognostic factor, which can be used for stratification of therapy. Whereas

Chromosomal Abnormality	Genes Involved	Disease association
Translocations		
t(1;3)(p36;q21)	MEL1/RPN1	M1, M4
t(1;22)(p13;q13)	OTT/MAL	M7
t(3;3)(q26;q21)	Ribophorin1/EV11	AML
t(3;5)(q21;q31)	Unknown	M6
t(3;5)(q25;q34)	MLF1/NPM	AML
t(3;21)(q26;q22)	EAP/AML1	AML, CML, MDS
t(5;17)(q35;q21)	NPM/RARA	M3
t(6;9)(q27;q23)	AF6/MLL	M4,M5
t(6;9)(p23;q24)	DEK/CAN	M1, M2, M4
t(7;11)(p15;p15)	HOXA9/NUP98	M2
t(8;21)(q22;q22)	AML1/ETO	M2, rarely M1 or M4
t(8;16)(p11;p13)	MOZ/CBP	M4, M5
t(9;22)(q34;911)	ABL/BCR	M1, M2, CML
t(9;11)(q21;q23)	AF9/MLL	M5
t(10;11)(p12;q23)	AF10/MLL	M4, M5
t(11;17)(q13;q21)	NuMA/RARA	Atypical M3
t(11;17)(q23;q21)	PLZF/RARA	M5
t(11;17)(q13;q21)	NuMA/RARA	M3
t(11;17)(q23;q21)	PLZF/RARA	M3
t(15;17)(q21;q21)	PML/RARA	M3
t(16;16)(p13;q2)	MYH11/CBF-B	M4
t(16;21)(p11;q22)	FUS/ERG	M1 ,M2 ,M4, M5
t(16;21)(q24;q22)	MTG16/AML1	M2, M4
Inversions		
inv(3)(q21;q26)	Ribophorin1/EV11	M4, M6
inv(16)(p13;q22)	MYH11/CBF-B	M4 with eosinophilia
Deletions		
del(2)(p23)		AML
del(5)(q13q33)		AML, MDS
del(5)(q31q35)		AML, MDS
del(7)(q22q34)		AML, MDS
del(7)(q32q34)		AML, MDS
del(9)(q13q22)		AML, MDS
del(11)(q23)		AML, MDS
Numeric Anomalies		
+8		AML, MDS
+21		AML, MDS
-5/-7		AML, MDS

Table 1: Recuarring Chromosomal Anomalies and Genes Involved in AML

t(9;22), and the aberrations of the long arm of chromosome 11 have a poor prognosis, t(15;17), t(8;21) and inv(16) indicate a favorable outcome. These patients are generally younger and have a good response to chemotherapy and ultimately longer survival than patients with unfavorable cytogenetics. Classification of hematologic malignancies according to the types of chromosomal abnormalities can be helpful to redefine criteria for the assignment

of patients to "poor-risk" or "favorable-risk" groups and to modify therapy accordingly [7,9]. Therefore, the detection of specific chromosomal rearrangements in AML provides both diagnostic and prognostic information and genetic testing become increasingly important in the clinical management of patient [Table 2].

Favorable
Normal karyotype , t(8;21), t(15;17), inv(16), del(16q), del(9q), +8, 20q
Intermediate
+8, -Y, +6, del(12p), normal karyotype
Unfavorable
Complex karyotypes, -7/del(7q), -5/del(5q), t(9;22), t(6;9), abnormal 3q ,11q

Table 2: Prognostic Significance of Genetic Markers in AML

Molecular genetics of acute myeloid leukemia

Analysis of the molecular basis of chromosomal rearrangements in leukemia has contributed a great deal to our understanding of the biology of tumor cells. At present, more than 70 different chromosomal abnormalities, including translocations, inversions and deletions have been cloned and characterized. The transforming genes involved in chromosomal translocations fall into several functional classes, including serine or tyrosine protein kinases, cell surface receptors, and growth factors, however, in most cases these aberrations target transcription factors that are important in hematopoietic development [1,4]. Molecular cloning of the breakpoints involved in these chromosomal re-arrangements has led to the discovery of many genes and in fact, the study of translocations provided some of the best evidence in support of the genetic basis for cancer.

t(8;21)(q22;q22)

The presence of the balanced translocation between chromosomes 8 and 21, is one of the most frequent recurrent cytogenetic abnormalities in AML. The abnormality is generally restricted to patients with a diagnosis of acute myeloblastic leukemia with maturation (FAB M2 type) and is found in 38% of all chromosomally abnormal patients with this disorder. The result of the translocation is the fusion of the AML1 gene on chromosome 21 to the ETO gene on chromosome 8. The product of the AML1 gene is the core binding factor–A (CBFA), which complexes with another protein, core binding factor-B (CBFB), to form a transcriptional factor [4,8]. CBF alpha/CBF beta which is responsible for the coordinated expression of more than 50 genes that are important in hematopoietic development. This transcription factor regulates the expression of a number of genes that are critical to myeloid cell growth and differentiation or function. The AML1/ETO fusion protein produced in cells with the t(8;21) is able to bind to the regulatory regions of these genes but is unable to activate their expression; it also acts as a dominant negative inhibitor of residual normal CBFA/B transcription factor [Table 3].

t(15;17)(q22;q21)

Another clinical-cytogenetic association involves acute promyelocytic leukemia (APL)

GENE NAME	ETO	AML1
Location	8q22	21q22
Hybrid Gene	5'AML1-3'ETO	
Fusion Protein	N-term runt domain from AML1 fused to the 577 C-term from ETO; DNA binding role; negative dominant with the normal AML1	
Oncogenesis	AML1 is a member of the runt family of transcription factors and possesses DNA binding, altered transcriptional regulation of normal AML1 target genes; AML1 /ETO binds DNA and interacts with CBFB; AML1/ETO fusion protein is a dominant negative inhibitor of transcription	

Table 3: Genes Involved and Proteins

and the translocation between chromosomes 15 and 17. The associated chromosomal translocation, (15;17) is found virtually in 100% of APL cases. The affected gene on chromosome 17 is the retinoic acid receptor-alpha (RARA) gene at band q21, and the gene on chromosome 15 is called PML. The RARA gene has been found to be involved in five different translocations, each of which is associated with acute promyelocytic leukemia, however, the most frequently observed fusion partner for RARA is the PML gene. RARA is a ligand-dependent transcription factor that regulates many genes, whereas PML is a tumor suppressor that plays a role in programmed cell death. The translocation results in a chimeric gene with the 5' region derived from PML and the 3' region from RARA including its DNA binding and retinoic acid response elements. The fusion gene is formed on the re-arranged chromosome 15, and the corresponding t(15;17) results in an in-frame PML-RARA fusion protein [10,12].

The retinoic acid receptor gene is involved in normal hematopoietic differentiation. The presence of abnormal retinoic acid receptor with dominant effect over RARA results in an aberrant differentiation, arresting cells as promyelocytes through its ability to attract co-repressor complexes. The PML/RARA fusion inhibits transcription, but unlike the normal RARA gene, it is not activated by physiologic doses of retinoid acid. This can be reversed using very high doses of all-trans-retinoic acid (ATRA) [1]. On exposure to ATRA, the PML-RARA fusion is released and may then activate retinoic acid responsive gene transcription, resulting in differentiation of APL cells [Table 4].

inv(16)(p13q22) and t(16;16)(p13;q22)

Inv(16) is one of the most frequent chromosomal re-arrangement found in AML representing approximately 16 % of documented karyoptypic abnormalities [13,14]. The cytogenetic abnormalities inv(16) and t(16;16) have been recognized as a non-random abnormalities in myelomonocytic leukemia with abnormal eosinophils (AML-M4). These anomalies both result in the fusion of the core binding factor B subunit (CBFB) at 16q22 to the smooth muscle myosin heavy chain gene (MYH11) at 16p13 [Table 5]. CBFB is the heterodimeric partner of the human AML1 (CBFA gene) involved in the t(8;21) suggesting there may be common pathways which lead to the leukemic phenotype in t(8;21), t(3;21) and inv(16) associated leukemias [1,4].

Aberrations of 11q23

Fascinating topic is connected to the aberrations of the long arm of chromosome 11. The gene located at 11q23 is the MLL gene involved in over 30 translocations and therefore the MLL translocations are unique in that a large number of partner genes [15]. The MLL group of translocations has a number of variants, but the 11q locus is invariant suggesting the function of the fusion partner is not important

GENE NAME	PML	RARA
Location	15q22	17q12-21
Chimeric Gene	5'PML -3'RARA	
Fusion Protein	N-term PML with DNA binding and dimerization domains fused to most of RARA with DNA and retinoid binding regions	
Oncogenesis	RARA is a ligand-dependent transcription factor that interacts directly and regulate many genes; whereas PML is a tumor suppressor that plays a role in apoptopic pathway; abnormal retinoic acid receptor with a dominant effect over RARA antagonizing differentiation; translocation results in an aberrant differentiation arresting cells as promyelocytes; normal PML protein is present in discrete multiprotein complex, possible that disruption of these bodies could be an important factor in the development of acute promyelocytic leukemia	

Table 4: Genes Involved and Proteins

GENE NAME	MYH11	CBFb
Location	16p13	16q22
Hybrid Gene	5'CBFb-3'MYH11	
Fusion Protein	N-term: the first 165 amino acids of CBFb fused to the tail of muscle myosin heavy chain gene MYH11 with its multimerization domain	
Oncogenesis	CBF is disrupted by the inv(16) and the t(16;16); the fusion protein seems to distinguish the quantity of active CBF and to complete with it; there is an accumlation of CBF-MYH11/CBFa multimeres in the nucleus; interference with the normal transcriptional transactivation	

Table 5: Genes Involved and proteins

GENE NAME	MLL	variable genes
Location	11q23	
Hybrid Gene	5'MLL -3' partner; highly variable breakpoints on the partner	
Fusion Protein	MLL encodes a 431-kd protein that contains N-term AT hook, 2 central zinc finger domains, a region with homology to DNA methyl transferase and a C-termal region that shows homology to Drosophilia trithorax protein	
Oncogenesis	MLL is a developmental regulator and is structurally altered in translocations with different chromosomes; altered expression of various down stream target genes.	

Table 6: MLL re-arrangements Genes Involved and proteins.

and only the disruption of the MLL gene is of importance, possibly with dominant negative effects on the normal MLL allele. However, all of the observed translocations result in in-frame fusion products, leaving the exons preserved, suggesting the fusion partner does supply critical elements to the fusion [Table 6]. Current evidence supports this hypothesis, because all of these translocations result in fusion proteins, which have lost the MLL activation domain but retain the DNA-binding and repression domains; thus they may alter expression of various downstream target genes [16,17].

Discussion

During the past decade, dramatic advances in molecular genetic techniques have focused attention on cancer as a genetic disorder. Leukemia is a model disease for cancer and in fact, the study of chromosomal aberrations in leukemia cells provided some of the best evidence in support of the genetic basis for cancer.

Numerous recurrent karyotypic aberrations have been and continue to be discovered in acute myeloid leukemia and our understanding of the molecular basis has increased considerably. Advances in our understanding of the pathogenesis offer exciting prospects. It has been recognized, that chromosomal translocations in leukemia can result in new association between genes that lead to altered function. More importantly, these particular chromosomal translocations tend to be closely associated with a particular morphologic or phenotypic subtype of leukemia. The detection of chromosomal abnormalities, therefore, can be helpful in establishing the correct diagnosis and has a great clinical relevalence for classification, treatment, and outcome of the patients.

Many questions about the pathology of leukemia remain to be answered and much remains to be learned. The development of leukemia is a multistep process and the sequence of events can be extremely complex and heterogeneous. Identification and characterization of genes involved in the process of leukemogenesis contribute to increase in our understanding and may result in novel therapeutic intervention [18].

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