MOLECULAR ASPECTS OF ACUTE MYELOID LEUKEMIA

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Leukemias are a group of heterogeneous neoplastic disorders, that differ significantly in terms of morhological, immunophenotypic, cytogenetic and molecular features of malignant cells. These specific features reflect the differences in the spectrum of underlying biological alterations involved in malignant transformation and/or variations in the level of hematopoietic stem cells hierarchy where the transforming events occur. During the last two decades a broad spectrum of sophisticated equipment and methods were implemented into the routine practice leading to a better understanding of the development of leukemias. This in term resulted in the invasion of new diagnostic criteria, definition of new entities and categories, introduction of novel therapeutic strategies, as well as improved monitoring of theatment effectiveness.

Acute myeloid leukemia (AML) develops as the consequence of a series of genetic changes in a hematopoietic precursor cell. These changes alter normal hematopoietic growth and differentiation, resulting in an accumulation of large numbers of abnormal, immature myeloid cells in the bone marrow and peripheral blood. These cells are capable of dividing and proliferating, but cannot differentiate into mature hematopoietic cells.

The recent developments of the genome analysis resulted into new and critical knowledge of genetic changes in leukemias and provided major insight into the pathobiology of AML.

The empact of genetic abnormalities

Specific cytogenetic abnormalities identified by karyotype analysis have considerable prognostic significance for patients with AML and affect treatment planning. Abnormalities in certain genes (eg, mutations in FLT3, nucleophosmin, KIT) as well as gene expression profiles confer prognostic significance in adult patients with AML. Even those patients without obvious abnormalities detected by karyotypic analysis or gene expression profiles have acquired copy number alterations that may help to identify genes important for the pathogenesis of AML

AML is characterized by a high degree of heterogeneity with respect to chromosome abnormalities, gene mutations, and changes in expression of multiple genes and microRNAs. Cytogenetic abnormalities can be detected in approximately 50% to 60% of newly diagnosed AML patients. The majority of AML cases are associated with nonrandom chromosomal translocations that often result in gene rearrangements. Many of these involve a locus encoding a transcriptional activator, leading to the expression of a fusion protein that keeps the DNA-binding motifs of the wild-type protein. Moreover, in many instances, the fusion partner is a transcriptional protein that is capable of interacting with a corepressor complex. A commonly accepted paradigm is that through aberrant recruitment of a corepressor to a locus of active transcription, the fusion protein alters expression of target genes necessary for myeloid development, thus laying the foundation of leukemic transformation. More than 700 chromosomal aberrations have been catalogued in AML so far. The frequencies of the 4 most common translocations (PML-RARα [t(15;17)(q22;q21)], AML1-ETO [t(8;21)(q22;q22)], CBFB-MYH11 [inv(16)(p13q22)/t(16;16)(p13;q22)] and 11q23/MLL-rearrangements) are between 3% and 10%, with remarkable geographic heterogeneity, while for others, the prevalence is significantly smaller such as for t(6;9)(p23;q34), DEK-CAN which in our series has been detected only in one case for a 10-years period. Approximately 40% to 50% of patients with AML

Table 1. Genetic variants in AML, grouped by prognostic category

Cytogenetic Variant	Single Gene Variant(s)	Frequency in AML	References
Favorable Prognosis			
t(8;21)		7%	Döhner et al., 2010
t(15;17)		13%	Grimwade et al., 2010
inv(16) or t(16;16)		5%	
Normal karyotype	Biallelic CEBPA mutation without FLT3-ITD	9% (regardless of FLT3-ITD)	Döhner et al., 2010 Döhner & Gaidzik., 2011
	IDH1 mutation	6%	Döhner & Gaidzik., 2011
	IDH2 mutation	9%	Patel et al., 2011
	NPM1 mutation without FLT3-ITD	26–64% (regardless of FLT3-ITD)	Döhner et al., 2010 Ding et al. 2012 Balatzenko et al, 2013
Intermediate Prognosis			
Isolated trisomy 8		10%	Döhner et al., 2010
~35% of t(8;21)	KIT mutation	3%	Li, Sun, & Wu 2008
~30% of inv(16) or t(16;16)	KIT mutation	~1.5%	Paschka et al. 2006
t(9;11)		1%	Döhner et al., 2010
Normal karyotype		41%	Grimwade et al., 2010
Normal karyotype	DNMT3A mutation	17%	Dohner & Gaidzik 2011
	FLT3-ITD	27–34%	Döhner et al., 2010 Patel et al. 2012 Estey, 2012 Spassov et al, 2013
Other cytogenetic abnormalities		N/A	Döhner et al., 2010
Poor Prognosis			
t(1;22)		< 0.5%	Grimwade et al., 2010
inv(3) or t(3;3)		1%	Grimwade et al., 2010
Monosomy 5 or 5q-		2%	Döhner et al., 2010
t(6;9)		1%	
Monosomy 7 or 7q-		5%	
t(9;22)		1%	
11q23, other than t(9;11)		3%	
Normal karyotype	TET2 mutation	16%	Dohner & Gaidzik 2011
Monosomal karyotype (any karyotype with ≥ 2 monosomies, or one monsomy and ≥ 1 additional structural abnormalities)		9.3%	Breems et al. 2008 Estey, 2013
Complex karyotype (≥ 3 clonal abnormalities)		27%	Grimwade et al., 2010 Döhner et al., 2010

have a normal karyotype and represent the largest subset of AML, yet, this group is quite heterogeneous in terms of treatment response. This is likely a result of the large variability in gene mutations and gene expression in this population.

The impact of molecular abnormalities

The affected gene targets are involved in key pathways that regulate cellular survival, proliferation, and hematopoietic differentiation. These discoveries have led to the 2-hit model hypothesis in which the development of AML requires at least two types of genetic events. Type-I aberrations occur as mutations in hotspots of specific genes involved in signal transduction pathways (FLT3, KIT, NRAS, KRAS and PTPN11) which lead to uncontrolled proliferation and/or survival of leukemic cells. Type-II aberrations (ie, CEBPA, NPM1, and the recurrent chromosomal translocations/inversions described above) lead to impaired myeloid differentiation by

affecting genes involved in transcriptional regulation. These occur early during leukemogenesis and are stable throughout the disease course, generally do not coexist in AML and therefore have been proposed to be founder (initiating) mutations. Class I and class II mutations occur together in very specific patterns. For example, FLT3-ITD with concurrent NPM1 mutation is common and represents a collaboration of both enhanced proliferation (class I) and a block in differentiation (class II). Experimental data in mouse models provide additional confirmation that one aberration is not sufficient to induce leukemia, but that cooperative events are needed to develop overt leukemia. Knock-in of FLT3-ITD leads to the development of a myeloproliferative disorder but lacks the maturation arrest typical of acute leukemia, whereas co-expression with inv(16)(p13q22) or t(15;17)(q22;q21) resulted in AML.

Class I Mutations. FLT3 Gene Aberrations – an Unfavourable Risk Marker

Mutations in KIT, FLT3, and NRAS fall into the class I mutations. FMS-related tyrosine kinase 3 (FLT3) is a membrane-bound receptor tyrosine kinase that, when activated by its ligand, supports the proliferation and survival of hematopoietic progenitors. Internal tandem duplication mutations occur across a wide range of cytogenetic subsets, including about 30% of normal karyotype AML cases, and are strongly associated with poor outcome, including shorter relapse free and overall survival. The ITDs result from the duplication and tandem insertion of a small, variably sized (3–400 nucleotides) fragment of the gene. Mechanistically, this is a gain-of-function mutation that leads to ligand-independent constitutive activation of the receptor. In our hands, FLT3-ITD were detected in 33,3% of de novo AML, predominantly in the normal karyotype subgroup. Similarly to other study groups, a significant correlation with lower complete remission (CR) rate [15,4% vs 54,8%, p=0,021] and shorter disease free (DFS) [log rank test, p=0,001] and overall survival (OS) [log rank test, p=0,0001] was established in a cohort of Bulgarian patients. In addition, several observations support the idea that in AML, beside structural distortions in the FLT3 gene [ie. ITD; mutation in D835 tyrosine kinase domain], its overexpression is probably also of importance as an alternative mechanism of leukemogenesis. Our data showed significant overexpression of FLT3 gene in approximately 20% of AML patients, often in combination with structural abnormalities in the gene (FLT3-ITD, FLT3-TKD) and/or with other molecular aberrations (MLL-AF9, DEC-CAN, AML-ETO; EVI1 overexpression), characterised with resistance to the treatment, which determines its potential clinical significance.

Studies have shown that activating KIT mutations in approximately 30% to 40% of patients with inv(16) are associated with higher incidence of relapse and significantly lower survival. Mutations in NRAS and KRAS occur in approximately 10% and 5% of AML patients, respectively, and do not appear to have a significant impact on AML survival.

Class II Mutations. NPM1 Gene Mutations – a Favourable Risk Marker

In addition, mutations in MLL, Wilms tumor gene (WT1), CCAAT/enhancer-binding protein α (CEBPα), DNA methyltransferase gene DNMT3A and nucleophosmin 1 (NPM1) have also been observed in AML patients.

NPM1 is a multifunctional phosphoprotein that shuttles between nuclear compartments and the cytoplasm. In its normal state, NPM1 is predominately located in the nucleolus where, among other functions, it is implicated in ribosome assembly and regulation of ARF and p53 tumor suppressor function. NPM1 mutations are considered the most frequent AML-associated genetic lesion, reported with various incidence in different studies - about 30% of adult de novo cases to 50% to 60% of AMLs with normal cytogenetics, and the type “A” [NPM1-A] being the most frequent type. NPM1 mutation is associated with a good response to induction therapy and a favorable prognosis, however, the favorable impact of NPM1 mutation is highly dependent on FLT3-ITD status and only cases without FLT3-ITD (NPM1mut/FLT3-ITDneg) are associated with a favorable outcome. This has been recently confirmed also in a cohort of Bulgarian patients, where within the FLT3-ITDneg group the median OS of NPM1-unmut patients was 14 months while NPM1-Amut patients did not reach the median.

Deregulated Gene Expression – Potential Risk Markers

Deregulated expression of certain genes, such as EVI1, ERG, MN1, BAALC, NFκB genes has been also subjected to extensive studies in regard to the impact to leukemogenesis as well as to clinical

behaviour and possibe therapetic targeting. One of these is the Wilms' tumor gene (WT1), which encodes a zinc-finger DNA binding protein. Since it might act as tumor suppressor gene or as oncogene, functional duality is assumed as it could either be involved in transcriptional activation or in suppression of differentiation. WT1 is highly expressed in a considerable proportion of AML patients - 72,8% according to our data, associated with lower CR rate and poorer DFS and OS. The BAALC (brain and acute leukemia, cytoplasmic) gene encodes a protein with no homology to any known proteins or functional domains. It is overexpressed in different hematologic malignancies. High expression of the BAALC, ERG, MN1 and EVI1 (ecotropic virus integration-1) genes was found to correlate in normal karyotype AML with a negative prognosis, so these genetic markers might as well be candidates for risk assessment.

Patterns of relationships between molecular events in aml

Though many molecular aberrations that contribute to the pathogenesis of AML have been identified, the relationships between patterns of mutations and epigenetic phenotypes are not yet clear. In an attempt to elucidate the possible interactions, an international research team has reported the findings from a multiplatform genome analysis of de novo AML recently. Data from 200 AML patients' samples demonstrated that the genomic landscape of AMLs includes at least one potential driver mutation in nearly all AML samples organised in nine functionally related categories of genes that are almost certainly relevant for pathogenesis, including transcription-factor fusions (18% of cases), the gene encoding nucleophosmin (NPM1) (27%), tumor-suppressor genes (16%), DNA-methylation-related genes (44%), signaling genes (59%), chromatin-modifying genes (30%), myeloid transcription-factor genes (22%), cohesin-complex genes (13%), and spliceosome-complex genes (14%). Interestingly, in contrast to other adult malignancies, AML genomes have fewer mutations. This lack of complexity is relative, however. A complicated interplay of genetic events contributes to AML pathogenesis in individual patients. Patterns of interaction among mutations, including cooperation (i.e. mutations seen together more commonly than would be expected statistically, such as FLT3 with DNMT3A and NPM1) and mutual exclusivity (i.e., mutations seen together less frequently than predicted by overall prevalence, such as mutations in FLT3 and in genes encoding other tyrosine kinases), suggest elaborate biologic relationships that deserve additional studies.

Translating genetic findings into clinical applications

With the increasing discovery of genes associated with AML pathogenesis continuing at a high speed, the challenge is to integrate this knowledge into the current clinical understanding of AML and to translate this findings into patient-oriented routine clinical applications. Improved molecular characterization of AML not only allows a more detailed subclassification and more exact prognostic predictions in many patients, but also provides the basis for future therapeutic approaches, that will be based on genetic and epigenetic targets that will be individually defined for each patient (Table 2).

Table 2. Genotype-specific treatment in AML

Target	Compound
PML-RARα	All-trans retinoic acid (ATRA) Arsenic trioxide (AS203)
FLT3 mutations	Unspecific FLT3-inhibitors [indoline tyrosine kinase inhibitors (SU5416; sunitinib - SU11248); small molecular compounds (PKC412)] Specific FLT3-inhibition [tandutinib (MLN518), CEP701]
KIT mutations in CBF leukemias	Imatinib, dasatinib
NPM1mut/FLT3neg	ATRA (in addition to cytotoxic therapy)
PRAME over-expression in PML-RARα(-)	ATRA and/or immunotherapeutic approaches
RAS mutated AML with constitutive activation of MAPK	Farnesyltransferase inhibitors [tipifamib (R1157777), ionafarnib]
DNA hypermethylation	Demethylating agents [5-azacytidine, decitabine]
Deacetylase activity	Histone deacetylase (HDAC) inhibition [valproic acid]

The monitoring of minimal residual disease (MRD) as determined by RT-PCR detecting leukemia-specific targets (eg, gene fusions, gene mutations, overexpressed genes) remains also an active field of investigation.

Conclusion

Acquired genetic lesions in AML are being increasingly recognized as relevant markers whose identification improves diagnostic refinement, classification and prognostic assessment in this heterogeneous disease. Discrete AML entities requiring specific therapeutic targeting approaches could be better identified based on the detection of these alterations as well as the treatment can be better monitored and tailored based on molecular MRD monitoring. As a consequence, genetic characterization of all AML patients is nowadays regarded as mandatory to determine treatment choices and should always integrate first level diagnostic studies based on morphology, cytochemistry and immunophenotype.

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