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REVIEW

Molecular biomarkers in acute myeloid leukemia

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ABSTRACT

Acute myeloid leukemia (AML) is the most common acute leukemia in adults. The pathophysiology of this disease is just beginning to be understood at the cellular and molecular level, and currently cytogenetic markers are the most important for risk stratification and treatment of AML patients. However, with the advent of new technologies, the detection of other molecular markers such as point mutations and characterization of epigenetic and proteomic profiles, have begun to play an important role in how the disease is approached. Recent evidence shows that the identification of new AML biomarkers contributes to a better understanding of the molecular basis of the disease, is significantly useful in screening, diagnosis, prognosis and monitoring of AML, as well as the possibility of predicting each individual's response to treatment. This review summarizes the most relevant molecular (genetic, epigenetic, and protein) biomarkers associated with acute myeloid leukemia and discusses their clinical importance in terms of risk prediction, diagnosis and prognosis.

1. Introduction

Acute myeloid leukemia (AML) is a malignant clonal disorder characterized by alterations and low production of healthy hematopoietic cells; these alterations inhibit differentiation of cells and induce proliferation or accumulation of blasts [1]. Blasts replace normal hematopoietic tissue, triggering the appearance of cytopenias [2,3]. The accumulation of immature cells begins in the bone marrow, but in most cases quickly builds up in the blood, and sometimes spreads to other parts of the body such as the lymph nodes, spleen, liver, testes and the central nervous system [4,5]. Diagnosis of AML is based on bone marrow (BM) and peripheral blood (PB) analysis (complete blood count and blast count). Specific diagnosis is confirmed by immunophenotyping and cytochemistry, searching for myeloperoxidase activity in blasts, or by immunophenotyping surface type molecules like CD123, CD45, CD34, CD38, among others [6].

Due to the genetic origin of the disease, there are some common cytogenetic abnormalities that often occur in AML, such as t(8;21), t(15;17), inversion 16, trisomy 8, and deletions of parts, or all, of chromosomes 5 or 7. In some patients, chromosomal translocations are commonly found, related to rearrangements of critical regions of proto-oncogenes, which generate an abnormal fusion protein that is usually a transcription factor or a protein involved in intracellular cell growth and differentiation signaling pathways, which in turn increases the likelihood of malignant transformation. Some examples of mutated genes are core binding factor (CBF), retinoic acid receptor-α (RAR-α), HOX gene family, MLL, among others. Other oncogene activating mutations are those that affect FLT3, KIT, N-RAS, FES, FOS, GATA-1, JUN B, MPL, MYC, p53, PU.1, RB, WT1, WNT, NPM1 and CEPBA [6–8].

Despite extensive research that has been carried out to find prognostic biomarkers, AML is still a disease with a very variable prognosis and a high mortality rate: 5-yr overall survival is lesser than 50%, and in elderly patients only 20% will survive 2 years after diagnosis [9,10]. Currently, cytogenetic results and molecular abnormalities at diagnosis are considered the most important prognostic factors and are highly predictive of complete remission rates, disease-free survival, risk of relapse and overall survival [11–15]. Current clinical guidelines in AML recognize three groups of cytogenetic risk: favorable, intermediate and poor risk [16]. The favorable-risk group includes those patients who present any of the following abnormalities: t(8;21), t(15;17), inv.(16), and t(16;16), as well as patients with normal cytogenetics accompanied with NPM1 mutation in the absence of FLT3-ITD or isolated biallelic CEBPA mutation [17,18]. These patients have a complete remission rate of over 90%, and overall survival of 60%. The poor-risk group includes the following abnormalities in the karyotype: inv(3), t(3;3), t(6;9), −5, 5q-, −7, 7q-, or complex karyotypes. Within this group there are also patients who have normal cytogenetics with an FLT3-ITD mutation. These patients have a high treatment resistance rate during induction chemotherapy, with an increased likelihood of relapse, as well as low disease-free survival and overall survival, ranging between 5 and 15%. The last group of patients, which is the largest (about 45% of adult patients with AML), have a normal karyotype and are considered to be at intermediate risk. The optimal therapeutic strategies for these patients are still largely unclear and the outcome of treatment is heterogeneous. There is increasing evidence that it is

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possible to identify a subgroup of poor risk patients among those with normal cytogenic results. Molecular risk stratification for this last group may be possible, by means of molecular analysis of genes such as NPM1, FLT3, MLL, and CEBPα, as well as alterations in the expression levels of BAALC, MN1, ERG, and AF1q [9,10,19].

The focus of this review will serve to summarize the most relevant molecular (genetic, epigenetic, protein) biomarkers associated with acute myeloid leukemia and discuss their clinical importance in terms of risk prediction, diagnosis and prognosis (see Table 1). Prognostic biomarkers can give an estimate of the severity of the disease and predict long-term outcome for patients, while response biomarkers allow clinicians to monitor response to treatment and relapse.

2. Molecular biomarkers in AML

2.1. Genetic abnormalities in AML

From a clinical perspective, there are several important aspects to consider regarding the study of genetic alterations in AML. Firstly, according to current WHO classification it is necessary to search for genetic defects in AML patients, because each defect could define different clinical entities and pathological processes [20]. Subsequently, it is increasingly clear that some chromosomal abnormalities and specific molecular prognostic markers are important as they can be used as tools for risk stratification. In fact, recently Papaemmanuil et al. [21] published a study that involved more than 1500 patients with AML. They sequenced 111 important genes in the pathophysiological process of the disease, and were able to define a new classification system for AML based on the presence of certain somatic driver mutations. This genomic classification not only proved to have implications in prognosis, but also includes 85% of patients, as compared to only 52% with the current WHO classification. This classification system has important clinical implications; as it is based on the foundational mutations causing the disease, it is much more accurate as a potential tool for risk stratification.

Finally, there are novel therapies that specifically target certain genetic defects; therefore the identification of these genetic defects allows for an effective customized treatment for various AML subtypes [15,22].

Some of the genes in which mutations have been identified, and that are correlated with pathophysiological processes in AML or may have prognostic relevance, are described below. In this context, the molecular mechanisms involved in AML development are summarized in Fig. 1.

2.1.1. Molecular alterations that produce chromosomal rearrangements

2.1.1.1. MLL-AML. Chromosomal rearrangements at 11q23 are associated with pediatric, adult and therapy related leukemias and led to the discovery of the Mixed Lineage Leukemia (MLL) gene. MLL or Mixed Lineage Leukemia is a histone methyltransferase that has a role in the epigenetic regulation of transcription, and is critical to embryonic development and hematopoiesis. This gene belongs to the trithorax-group family which is involved in the methylation of histone H3 on lysine residue 4 (H3K4), that is associated with positive regulation of gene expression [23]. MLL is a large multi-domain protein ubiquitously expressed in hematopoietic cells, including stem and progenitor populations [24]. Current evidence suggests that, although MLL has domains which can bind DNA directly, this interaction could also occur via interactions with other DNA-binding proteins such as menin [23].

The MLL gene has a leukemogenic effect only after fusing to a wide array of partner genes, including AF4, AF9, ENL, AF10 and ELL [25,26]. MLL fusions are targeted to chromatin when MEN1 [Menin] and LEDGF bind to the N terminal of MLL, where they then activate genes such as HoxA9 and HoxA10, genes that are commonly upregulated in MLL linked leukemia. Thus, many MLL chimeras have the capacity to

Table 1
Summary of molecular biomarkers of AML described in this review.

	Biomarker	Clinical importance	References
Citogenetics	MLL-AML	Poor prognosis. Treated according to high-risk protocols. It is considered a marker which establishes diagnosis	Muntean and Hess, 2012 [27]; Muñoz et al., 2003 [185]
	CORE BINDING FACTOR	Establishes diagnostic of AML. Good prognosis. It predicts a good response to treatment	Vardiman et al., 2009 [186]; Ustun and Marcucci, 2015 [35]
	PML-RARA	Hallmark of APL. Favorable response to treatment. Overall survival improvement. Monitor marker to confirm molecular remission	De Braekeleer et al.,2014 [53]; Ma and Yang, 2015 [187]; Xin et al., 2007 [188]; Visser et al., 2012 [189]; Polampalli et al., 2011 [61]; Shigeto et al., 2016 [190]
Genetics	FLT3 (FLT3-ITD)	Most frequent mutation, predictor of poor prognosis.	Boissel et al., 2006 [48]; Gregory et al., 2009 [9]; Santos et al., 2011 [191]
	NPM1	Higher remission and survival rates.	Boissel et al., 2005 [82]; Thiede et al., 2006 [79]
	CEBPA	Improved prognosis, longer remission time and overall survival	Fröhling et al., 2004 [87]; Green et al., 2010 [88]
	DNMT3A	Decrease in overall survival.	Marková et al., 2012 [111]; Park et al., 2015 [116]
	TP53	Predictor of poor prognosis.	Ohgami et al.,2015 [99]; Cleven 2015 [97]
	IDH 1/2	Predictor of poor prognosis.	Abbas et al., 2010 [125]; Paschka et al., 2010 [124]
	α-Ketoglutarate-dependent dioxygenase TET Proteins	Adverse prognostic factor	Liu 2014 [143]
Epigenetics	Aberrant promoter CpGs hypermethylation[a]	AML subtypes classification, Clinical outcome predictors	Alvarez et al., 2010 [150]; Figueroa et al., 2010 [158]; Akalin et al., 2012 [156]
	Global Hypomethylation signature	Overall survival improvement	Deneberg and Gro 2010 [159]; Ozolinš, 2012 [192]
	5hmC levels	Increase rate of Clinical remission	Kroeze et al., 2014 [160]
	Hypomethylation of Repetitive regions (LINE-1, SINEs, LTR)	AML subtypes classification, 5-aza Clinical response predictors	Saied et al., 2012 [161]; Cross et al., 2013 [164]; Bujko et al., 2014 [162]
	Non-coding RNAs (miR21, miR155, miR125, miR191, miR199a, miR25)	AML subtypes classification, Clinical outcome and therapeutic predictors	Jongen-Lavrencic et al., 2008 [175]; Garzon et al., 2008 [176]; Bhise et al. 2015 [177]
Proteomics	Calgranulin A	Predictor of poor prognosis	Nicolas et al. 2011 [178]
	UBA1, FIBA and PF4	Predictors of poor prognosis and relapse	Bai et al. 2013 [180]
	BTG1	Predictor of good prognosis	Cho et al. 2004 [181]
	Heat shock proteins (HSPs)	Predictors of poor prognosis	Thomas et al. 2005 [182]
	Annexin I, glutathione transferase omega, and esterase D/formylglutathione hydrolase	Predictor of good prognosis	Kaźmierczak et al. 2013 [184]
	Gamma 1 actin	Predictor of resistance to therapy	Kaźmierczak et al. 2013 [184]

[a] Specific genes related to leukemogenesis: tumor suppressor genes, cell cycle, apoptosis and development/differentiation factors.