

Importance of genetics in acute myeloid leukemia

Papel de la genética en la leucemia mieloide aguda

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ABSTRACT

Acute myeloid leukemia (AML) comprises a biologically and clinically heterogeneous group of aggressive disorders that occur as a consequence of a wide variety of genetic and epigenetic abnormalities in hematopoietic progenitors. Despite significant advances in the understanding of the biology of AML, most patients will die from relapsed disease. Whole-genome studies have identified novel recurrent gene mutations with prognostic impact in AML; furthermore, it is likely that in the near future genome-wide sequencing will become a routine for newly diagnosed patients with AML. Therefore, future clinical trials should aim to identify genetically defined high-risk patients, and further research is necessary to identify effective agents and develop new individualized therapeutic strategies for the treatment of this deadly disease.

Key words. Acute myeloid leukemia. Genetic marker. Mutation. Whole-genome sequencing. Prognosis.

RESUMEN

La leucemia mieloide aguda (LMA) es una enfermedad clínica y molecularmente heterogénea, que surge como consecuencia de alteraciones genéticas y epigenéticas adquiridas en células progenitoras hematopoyéticas. A pesar de los avances realizados en el conocimiento de la biología de esta enfermedad, la supervivencia global de los pacientes sigue siendo muy baja debido principalmente a la alta tasa de recaídas. Los estudios de secuenciación masiva han permitido la identificación de nuevas mutaciones recurrentes y con impacto pronóstico en LMA; además, parece probable que en poco tiempo la secuenciación de todo el genoma sea una prueba diagnóstica de rutina, lo que permitirá el diagnóstico molecular de los pacientes. Por tanto, es necesario el desarrollo de dianas moleculares que abran nuevas perspectivas terapéuticas y permitan el tratamiento individualizado de los pacientes con esta enfermedad tan agresiva.

Palabras clave. Leucemia mieloide aguda. Marcador genético. Mutación. Secuenciación masiva. Pronóstico.

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INTRODUCTION

Acute myeloid leukemia (AML) comprises a biologically and clinically heterogeneous group of aggressive disorders that occur as a consequence of a wide variety of genetic and epigenetic abnormalities in hematopoietic progenitors. Despite significant advances in the understanding of the biology of AML, overall survival remains poor due chiefly to the high rate of relapse after achieving complete remission, as well as primary failure of induction chemotherapy¹.

Since it is predominantly a disease of older people, the therapeutic strategy offered for AML is determined by assessment of the patient's age and general fitness level. The standard of care for over 3 decades has been the combination of daunorubicin with cytarabine (Ara-C). The majority of younger patients achieve a complete remission (CR) (75%), and 40%-45% will have a chance of cure. This represents a steady improvement over the past 40 years, although much of this can be attributed to improvements in supportive care, much of which has been learned from the transplantation experience². A major challenge is the treatment of older patients, defined arbitrarily as over 60 years, who represent the majority of patients with this disease. Approximately 50% of older patients reach CR, but 85% of those entering remission will relapse, and the outcome from relapse in older patients is poor, with relatively few surviving. In addition, there are a substantial number of patients who are not considered suitable for intensive chemotherapy, for whom a palliative approach is usually offered².

Continuing research into the pathogenesis and heterogeneity of AML has resulted in the development of several potentially useful therapeutic agents. Notably, acute promyelocytic leukemia (APL) has much better prognosis due to the implementation of sensitive molecular diagnostic tools, and to the introduction of all-trans retinoic acid (ATRA) in combination with anthracycline into clinical practice. Clinical trials with patients with APL have reported complete

remission and long-term disease-free survival rates of approximately 90% and 85%, respectively³. Other therapies targeting specific molecular defects are being developed, such as small molecule inhibitors of FLT3 kinase in patients harboring the FLT3-ITD mutation, and all-trans retinoic acid in patients with the NPM1 mutation. However, despite some advances in the treatment of AML, the overall outcome is still dismal for most patients.

CYTOGENETIC AND MOLECULAR ABERRATIONS WITH PROGNOSTIC RELEVANCE IN AML

Several studies over the past decades have identified a large set of chromosome aberrations, mutations and overexpressed genes with prognostic relevance in AML, improving our understanding of AML pathogenesis and risk stratification. AML patients can be classified into three different prognostic subgroups according to presence or absence of distinct cytogenetic abnormalities. Moreover, in recent years, various molecular genetic markers have been identified, especially in normal karyotype (NK) AML, where screening for mutations in genes such as FLT3, NPM1, and CEBPA has allowed better prognostic prediction (Table 1)^{4,5}. At present, most patients can be categorized into clinicopathological subgroups based on the presence of genetic defects. Our increasing knowledge of AML biology led to the establishment of the 2008 World Health Organization classification, which considers morphologic features and recurrent cytogenetic and molecular abnormalities⁶. In addition, an international expert panel from the European LeukemiaNet (ELN) has also recently proposed new guidelines for the management and stratification of therapies based on the strongest prognostic factors identified to date such as cytogenetic or molecular defects⁷.

However, a large number of AML patients lack any of these abnormalities and there remains significant heterogeneity in clinical outcome within currently classified

prognostic groups. These observations suggested that there may be additional biomarkers that can predict outcome in AML. In this regard, recent genome-wide studies have identified an increasing number of recurrent somatic mutations in AML patients, such as mutations in *ASXL1*, *DNMT3A*, *IDH1*, *IDH2*, *PHF6* and *TET2*⁴. Several of these newly identified genetic abnormalities have prognostic importance in AML. Moreover, the identification of mutations at diagnosis has served as a tool for minimal residual disease measurement. Interestingly, a recent study has provided a large-scale insight into the genetics of relapsed AML by performing whole-genome sequencing of 8 patients with relapsed AML⁸. In all patients there was a founding clone that was not ablated by chemotherapy and was still persistent at relapse; thus, prospective identification of this clone could be of great clinical utility.

MOLECULAR MARKERS IN NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA

Mutational and wide-genomic studies have been especially useful to increasingly refine prognosis in patients with intermediate-risk and/or NK-AML, who represent more than 45% of all AML cases. The 5-year survival rate of these patients varies from 24% to 42%, suggesting a genetic diversity within this subgroup. As indicated above, previous studies have shown that mutational analysis of *FLT3*, *NPM1*, and *CEBPA* can be used to risk-stratify these patients⁵. However, more extensive mutational analyses indicate that other genes should be included in the screening, and that mutations analysis of at least ten genes: *ASXL1*, *CEBPA*, *DNMT3A*, *FLT3*, *IDH1*, *IDH2*, *MLL*, *NPM1*, *PHF6*, and *TET2*, would represent a useful tool, discriminating intermediate-risk AML patients into robust, clinically relevant risk groups (Table 1)⁴.

About 20% to 25% of AML patients harbor internal tandem duplication (ITD) mutations in the RTK Fms-like tyrosine kinase-3 (*FLT3*), which leads to constitutive

phosphorylation of *FLT3* and activation of its downstream signaling cascades, resulting ultimately in aberrant proliferation and survival. *FLT3*-ITD mutations have been consistently found to be an adverse prognostic marker in NK-AML patients, associated with an increased risk of relapse and inferior overall survival⁹. This has led to clinical trials treating *FLT3*-ITD AML patients with small-molecule *FLT3*-selective tyrosine kinase inhibitors. To date, however, *FLT3* inhibitors used as a single agent or in combination with chemotherapy for *FLT3*-ITD AML have not demonstrated improved clinical efficacy¹⁰. Because *FLT3* can crosstalk with a network of various signaling pathways, identifying and analyzing the interplay of constitutively active *FLT3* with aberrant signaling pathways may lead to the identification of novel therapeutic targets for treatment of AML patients harboring constitutively active *FLT3*¹¹. Several studies have shown that risk associated with *FLT3*-ITD in AML may depend on mutational burden and its interaction with other mutations^{12,13}. In the subset of patients with *FLT3*-ITD-negative intermediate-risk AML, there are 3 distinct risk groups that are based on mutational status and have vastly different outcomes. *FLT3*-ITD-negative, *NPM1*/*IDH* mutant patients have outcomes that are better than patients with *inv(16)* or *t(8:21)*-positive AML, suggesting that this represents a favorable-risk AML subset defined by a specific mutational genotype. In contrast, *FLT3*-ITD-negative *NPM1* mutant patients without concurrent *IDH* mutations have a much less favorable outcome. Most importantly, the presence of poor-risk mutations, specifically *TET2*, *ASXL1*, *PHF6*, and/or *MLL-PTD*, is associated with very adverse overall survival for *FLT3*-ITD wild-type, intermediate-risk patients¹⁴. These data suggest that *NPM1* mutational status alone does not define a favorable subset of intermediate-risk AML, and that the presence or absence of additional disease alleles defines relapse risk in *FLT3*-ITD wild-type, intermediate-risk AML¹⁴. In addition, mutational studies have allowed for improved prognostication in *FLT3*-ITD-positive patients (Table 1)^{4,14}. These results

have important clinical implications, because patients with mutationally defined favorable risk have a better outcome with standard induction and consolidation than even patients with core binding factor-positive AML. In contrast, patients with muta-

tionally defined adverse-risk AML have an outcome similar to patients with adverse karyotypic risk, and standard therapies are not sufficient to offer curative intent to the majority of these patients^{4,14}.

Table 1. Relevant molecular markers in normal karyotype or intermediate-risk cytogenetic AML (adapted from Patel et al., 2012¹⁴)

Overall Risk Profile
FAVORABLE
FLT3-ITD negative Mutant NPM1 and IDH1 or IDH2
INTERMEDIATE
FLT3-ITD negative Wild-type ASXL1, MLL-PTD, PHF6 and TET2
FLT3-ITD negative or positive Mutant CEBPA
FLT3-ITD positive Wild-type MLL-PTD, TET2 and DNMT3A and trisomy 8 negative
UNFAVORABLE
FLT3-ITD negative Mutant TET2, MLL-PTD, ASXL1 or PHF6
FLT3-ITD positive Mutant TET2, MLL-PTD, DNMT3A, or trisomy 8, without mutant CEBPA

CLINICAL IMPACT OF GENETIC ABERRATIONS IN ACUTE MYELOID LEUKEMIA

With the discovery of novel genes associated with AML pathogenesis continuing at a high speed, the challenge is to integrate this knowledge into the current clinical understanding of AML⁴. Nevertheless, in the clinical setting, most AML centers use cytogenetic abnormalities and a relatively small set of gene-based tests to assign risk in AML and to determine post-remission therapy. As Patel and Levine indicate, the relative paucity of clinically used biomarkers is due to several factors. First, most biomarker studies focus on a specific genetic lesion and its prognostic relevance without considering the complete set of known mu-

tations in parallel to determine which mutations predict outcome independently in AML. Second, most studies consider each mutant allele as a distinct variable without considering complex genotypes in which the presence/absence of multiple disease alleles has different effects on outcome than individual mutations by themselves. Third, many studies have focused on mutational “hotspots” or have used less sensitive techniques to identify loss-of-function mutations in large tumor suppressors. Finally and most importantly, until recently, most studies of AML were relatively small in size and/or were not derived from clinical trial cohorts in which the effects of treatment on outcome can be controlled and investigated. Thus, given the increasing number of genetic abnormalities that

have been identified in AML patients, it has become important to determine the prognostic relevance of all known recurrent genetic abnormalities in a uniformly treated AML patient cohort⁴.

CONCLUSIONS AND FUTURE PERSPECTIVES

Recent advances in the research of AML, especially the identification of novel genetic mutations, have enabled us to stratify this heterogeneous disease entity into distinct subtypes beyond the scopes of cytomorphology and cytogenetics. However, to date, most studies have failed to identify robust predictors that modify outcome in patients with favorable or unfavorable cytogenetic risk, suggesting that chromosomal lesions remain the best predictor of outcome for the 40% of AML patients with favorable or unfavorable karyotype⁴. Thus, there is a common trend to characterize better AML subtypes as soon as the diagnosis is made. This is exemplified by the subgroup of patients with the monosomal karyotype, who have a dismal outcome with standard treatment, including transplantation¹⁵. However, in the group of intermediate AML the identification of mutations with impact on outcome has clinical and biological importance, and molecular screenings help to refine the treatment strategies. Therefore, it is important to determine specific mutations or combinations of mutations in this subgroup of patients, although the main goal of future mutational studies should be to inform and improve prognostic algorithms in AML. Moreover, validation of these findings in other large, homogeneously treated patient cohorts is of utmost clinical importance⁴.

As discussed above, application of whole-genome sequencing to AML has already yielded important discoveries, including the identification of common gene mutations with prognostic impact in AML. In the near future, it is likely that whole genome sequencing will become a routine part of the diagnostic workup of patients with AML¹⁶. Therefore, future clinical trials

should aim to identify genetically defined high-risk patients to offer them novel therapies early in their disease course, in an effort to reduce relapse and increase cure⁴.

Finally, although hundreds of different genetic lesions have been described in AML, this disease shares common programs of self-renewal and transformation downstream of leukemia-associated oncogenes¹⁷. These findings argue for the presence of common mechanisms of leukemia cell survival, and suggest that mechanistically common therapeutic approaches to AML are likely to be possible¹⁸. Further research is thus necessary to identify effective agents and develop new individualized therapeutic strategies for the treatment of this deadly disease.

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