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Epigenetic Landscape of Acute Myelogenous Leukemia—Moving Toward Personalized Medicine

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ABSTRACT

Acute myeloid leukemia (AML) is an aggressive hematologic cancer that is characterized by accumulation of immature myeloid cells in the blood and bone marrow. The malignant cells in AML have reduced capacity to mature fully, and often exhibit chromosomal abnormalities, defects in cell signaling, and abnormal cell cycle control. Genetic and epigenetic changes are implicated in the onset and progression of AML. While progress has been made in using genetic and epigenetic changes as prognostic features of AML, these findings have not yet been effectively translated into novel treatment strategies. Disappointingly, rates of recurrence in AML remain high and overall survival is poor. Research strategies should focus on developing a comprehensive landscape of genetic and epigenetic changes in individual patients with AML to expand the clinicians' therapeutic armamentarium and to individualize and optimize treatment. J. Cell. Biochem. 115: 1669–1672, 2014.

KEY WORDS: NON-CODING RNA; DNA METHYLATION; HISTONE MODIFICATIONS; ACUTE MYELOID LEUKEMIA

THE LANDSCAPE OF THE CURRENT PROBLEM

AML accounts for about 25% of all leukemias and it is the most common acute leukemia in adults—it is a fast-growing cancer with an aggressive clinical course [Walter, 2011]. The American Cancer Society estimates that there will be approximately 18,860 new cases of AML and 10,460 deaths from AML in the United States in 2014 [Siegel et al., 2014]. AML is mainly a disease of the elderly, with a median age of 69 years in the white US population. Prognosis worsens with every decade of patient age over 30. In patients over age 60, population-based studies have reported 3- and 5-year survival rates of only 9–10% and 3–8%, respectively,. Even in younger patients, the 5-year survival rates have been reported to be only approximately 50% [Luger, 2010].

TREATMENT OVERVIEW

Once the diagnosis of AML is established, eligible patients undergo induction chemotherapy, with the goal of rapidly restoring normal bone marrow function. The induction regimen is highly toxic, primarily to the hematopoietic system, and has not changed significantly over the last two decades. The goal of induction therapy is to achieve complete remission by reducing the number of leukemic cells to an undetectable level, typically fewer than approximately 10^9 cells. Unfortunately, this does not mean the disease has been cured, but rather that it cannot be detected with conventional diagnostic methods. The likelihood of achieving and maintaining clinical remission depends on prognostic features of the original leukemia. It is generally assumed, however, that a

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substantial burden of leukemia cells persists undetected (i.e., presence of "minimal residual disease"), which for most patients will lead to relapse within weeks or months if no further therapy is administered [Cassileth et al., 1988]. Approximately 30–40% of patients require a second induction cycle to achieve complete remission [NCCN, 2013]. In select patients, additional treatment in the form of consolidation or maintenance chemotherapy, or stem cell transplantation may be utilized.

CURRENT PROGNOSTIC MARKERS IN AML

Response to treatment and overall survival in AML is variable, and depends on prognostic factors that include age and performance status, presence of additional comorbid disorders and antecedent hematologic disorders, and prior treatment with cytotoxic agents or radiation. In addition, treatment outcome is influenced by intrinsic characteristics of the AML cells, including morphology, immunophenotype, cytogenetics, and molecular markers. The single most important prognostic factor in AML, however, is the nature of chromosomal abnormalities observed by cytogenetic analysis. Approximately 60% of all AML patients have an abnormal karyotype (complement of chromosomes) and cytogenetics can be used to stratify patients into good, intermediate, and poor risk categories (Table I). In addition, molecular markers such as FMS-like tyrosine kinase (FLT3), Nucleophosmin 1 (NPM1), CCAAT/enhancer-binding protein alpha (CEBPA), and others confer variable prognostic value. For example, internal tandem duplications (ITDs) of FLT3 confer a poorer prognosis in AML [Schnittger et al., 2002]. Other prognostic molecular markers include mutations of isocitrate dehydrogenase 1 (IDH1), DNMT3A, Ten-Eleven-Translocation-2 (TET2), and alterations in the expression of ERG, WT1, meningioma 1 (MN 1), and brain and acute leukemia cytoplasmic gene (BAALC). Table II lists the impact of these mutations and molecular markers in AML patients. An outcome of research into the genetics of AML has been the development of tests that will help physicians prognosticate and, hopefully will translate into optimized, individualized therapy for a particular patient.

Marcucci et al. [2004] reported the prognostic value of complete cytogenetic remission attained immediately following induction chemotherapy. Both overall survival and disease-free survival were significantly shorter for the abnormal cytogenetic group (ACR) at remission, compared with the normal cytogenetic group (NCR). At both three and five years, none of the patients in the ACR group were disease-free, compared with an estimated 33% for the NCR group. Notably, when considering disease-free survival only in the patient population having intermediate/unfavorable disease-risk at diagnosis, the ACR group still fared poorly compared to the NCR group.

Kronke et al. [2011] recently reported on monitoring of minimal residual disease in NPM1-mutated AML patients. Using a sensitive RNA-based real-time quantitative polymerase chain reaction (RQ-PCR) assay method, they observed that patients who were NPM1-mutation negative after induction therapy experienced a low cumulative incidence of relapse compared with patients who remained NPM1-mutation positive. This translated into statistically significant differences in overall survival. Multivariable analyses revealed that higher NPM1-mutated transcript levels were a significant factor for a higher risk of relapse and death.

These studies highlight the role of chromosomal and molecular markers in the onset and progression of AML as well as remission. Furthermore, these results point to a pressing need to establish an integrative landscape of genetic, epigenetic, and molecular markers that may enable earlier diagnosis, prediction of relapse or refractory disease, and/or personalized therapy. This is also likely to yield new targets for monitoring and treating the disease.

EPIGENETIC INDICATORS OF AML

Both the onset and progression of cancer are functionally linked with aberrant genetic (DNA-encoded) and epigenetic (non-DNA-encoded) mechanisms. Recent discoveries highlight the role of

TABLE I. Prognostication of AML Based on Cytogenetics and Molecular Markers

Risk category	Abnormality	Five-year survival (%)	Relapse rate (%)
Good	t(8;21), t(15;17), inv(16)	70	33
Intermediate	Normal, +8, +21, +22, del(7q), del(9q), abnormal 11q23, all other structural or numerical changes	48	50
Poor	−5, −7, del(5q), abnormal 3q, complex cytogenetics	15	78

From National Comprehensive Cancer Network Guidelines for AML. [cited 2013 August 27]. Available from: www.nccn.org.

TABLE II. Molecular Prognostic Markers in Development for AML With Normal Cytogenetics

Mutation	Description	Incidence[a] (%)	Prognostic impact	Reference
FLT 3–ITD+	Transmembrane tyrosine kinase receptor	20–30	Poor	Pawar et al. [2014]
NPM mutation	Nucleocytoplasmic shuttling protein	50	Good	Dohner et al. [2005]
CEBPA	CCAAT/enhancer binding protein alpha	10	Good	Green et al. [2010]
IDH 1	NADP+–dependent IDH found in the cytoplasm and peroxisomes	6–8	Poor	Schnittger et al. [2010]
DNMT3a	DNA methyltransferase	22	Poor	Ley et al. [2010]
TET	Tumor suppressor gene mutated in a variety of myeloid disorders	10	Equivocal	Chou et al. [2011]
WT1	Zinc-finger transcription factor	6–8	Poor	Hou et al. [2010]

[a]Approximate incidence in AML cases with normal cytogenetics.

non-coding RNA molecules, both long and short, in regulating gene expression epigenetically. For example, Croce and coworkers have carried out extensive profiling of leukemia and solid tumors to identify microRNA (miR) signatures of diagnostic value [Di Leva et al., 2014]. Similarly, Rowley and coworkers identified mRNA and miR signatures in a large cohort of AML patients. Other studies have shown alterations in genomic histone marks in AML patients as the disease progresses [Chen et al., 2010]. However, studies that comprehensively integrate gene expression profiles and epigenetic profiles with clinical outcomes in AML patients have not yet been conducted.

DNA METHYLATION AND HISTONE MODIFICATIONS IN AML

It is well established that epigenetic changes such as DNA methylation and post-translational histone modifications play a critical role in the regulation of normal hematopoiesis as well as in the onset and progression of AML [Itzykson and Fenaux, 2014]. Several studies have identified signatures for both of these epigenetic changes in AML patients [Neff and Armstrong, 2009], and drugs targeting the enzymes responsible for these epigenetic modifications are at various stages of development and clinical evaluation [Dawson and Kouzarides, 2012]. The epigenetic signatures of AML identified by various studies, however, are often not comparable, making it difficult to identify and implement a unified therapeutic approach for treatment of AML based on epigenetic disease indicators. Furthermore, the enzymatic machinery involved in these processes has additional, wide-ranging functions in normal cells. Treatment with inhibitors that block activity of these enzymes inevitably also results in off-target effects.

NON-CODING RNA MOLECULES AS BIOMARKERS AND THERAPEUTIC AGENTS

Non-coding RNA molecules that include long non-coding RNAs (LncRNAs) and microRNAs (miRs) are emerging as a key component of epigenetic mechanisms that control cellular processes such as cell cycle and lineage commitment by regulating gene expression [Ling et al., 2013]. miRs are small non-coding RNA molecules that regulate protein levels by targeting their cognate messenger RNA (mRNA) for degradation and/or inhibition of translation [Ameres and Zamore, 2013]. miRs are a focus of extensive research because of their potential as biomarkers for the onset and progression of disease, and as potential therapeutic agents that can specifically and selectively repress translation of their targets.

Many miRs are differentially expressed between AML blasts and normal cells [Mi et al., 2007; Garzon et al., 2008; Jongen-Lavrencic et al., 2008; Cammarata et al., 2010]. Additionally, changes in miR expression have been shown to define lineage-specific leukemia. Distinctive patterns of increased and/or decreased expression of multiple miRs have been associated with specific categories of AML defined by their cytogenetic and molecular-marker profiles [Garzon and Croce, 2008]. Furthermore, some miRs have been shown to promote cancer progression, whereas others appear to inhibit it [Esteller, 2011].

Levels of both single miRs and multiple miRs (i.e., miR profiles or expression signatures) may offer valuable prognostic information that can complement the information gained from cytogenetics, gene mutations, and altered gene expression. Moreover, anti-leukemic effects have been achieved in vitro by modulating miR expression with pharmacologic agents, such as synthetic miRs that are designed to augment endogenous levels miRs with tumor suppressor function, or antisense oligonucleotides (antagomirs) to effectively reduce levels of leukemogenic miRs [Ling et al., 2013].

A major shortcoming of the existing datasets, however, is that a total concordance has not been achieved among the miR signatures from separate studies. This is probably due to the lack of standardization of the analytic methods used by different groups. This currently precludes the use of miR-expression profiles as a clinically useful diagnostic criterion. Nevertheless, miR profiling has the potential to become a valuable diagnostic tool because miRs are more stable than coding mRNAs, and may prove to have greater diagnostic accuracy.

THE POTENTIAL OF PERSONALIZED MEDICINE IN AML

As a result of the extensive efforts to define genetic and epigenetic signatures of AML, patterns with both diagnostic and therapeutic value are emerging. Some of these will prove to be important for patients, but the challenge is to delineate how these different classes of biomarkers can be used to formulate multi-parameter indicators of AML subtype and prognosis.

The strategy of personalized medicine, that is, obtaining comprehensive genetic, epigenetic, and expression data from individuals patients and identifying trends that correlate to disease onset, progress, and remission, can provide the ground work needed to improve AML treatment based on individual patient characteristics. It is imperative that collaborative teams of physician–scientists and basic cancer researchers combine their expertise to enable clinical trial strategies that move innovative translational cancer research from bench to bedside.

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