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The diagnostic and clinical impact of genetics and epigenetics in acute myeloid leukemia

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SUMMARY

Acute myeloid leukemia (AML) is a complex disease, for which our understanding of the role of genetic and epigenetic changes has undergone significant advancements. Newer diagnostic and prognostic classifications have increasingly incorporated such information, and novel therapies have been developed to target specific genes, processes, and pathways based on this growing understanding. Given the rapid evolution of this field, it is critical for physicians and translational researchers to have a more in-depth understanding of this evolving landscape. Here, we review both genetics and epigenetics in acute myeloid leukemia from a practical standpoint.

INTRODUCTION

Acute myeloid leukemia (AML) is the most common acute leukemia in adults and second most frequent among children [1] and was first subclassified in 1976 with the French-American-British (FAB) classification, which relied heavily on morphologic and cytochemical assessment [2]. Newer classification algorithms and schemas have since emerged which have incorporated cytogenetic and molecular data: for example the World Health Organization (WHO) classification which has undergone several revisions since initial introduction [3], as well as clinical diagnostic and prognostic classifications such as those from the European LeukemiaNet group [4]. Most well-established and current AML classifications rely heavily on cytogenetic data, with minimal incorporation of somatic mutation information, while epigenetic changes and epigenetic gene modifiers have not yet become a part of the mainstream diagnostic and prognostic workflows, although newer efforts to incorporate multiple facets of more mutational and other molecular data have been undertaken [5–7]. Here, we focus on a genetic understanding of AML with incorporation of epigenetic modifiers largely using the WHO classification as a framework, additionally discussing prognostic and therapeutic implications within and outside this classification scheme (Figure 1).

CYTOGENETICS IN AML

The role of cytogenetic changes and more specifically, particular defined chromosomal translocations, in leukemogenesis has been fully embraced by pathologists,

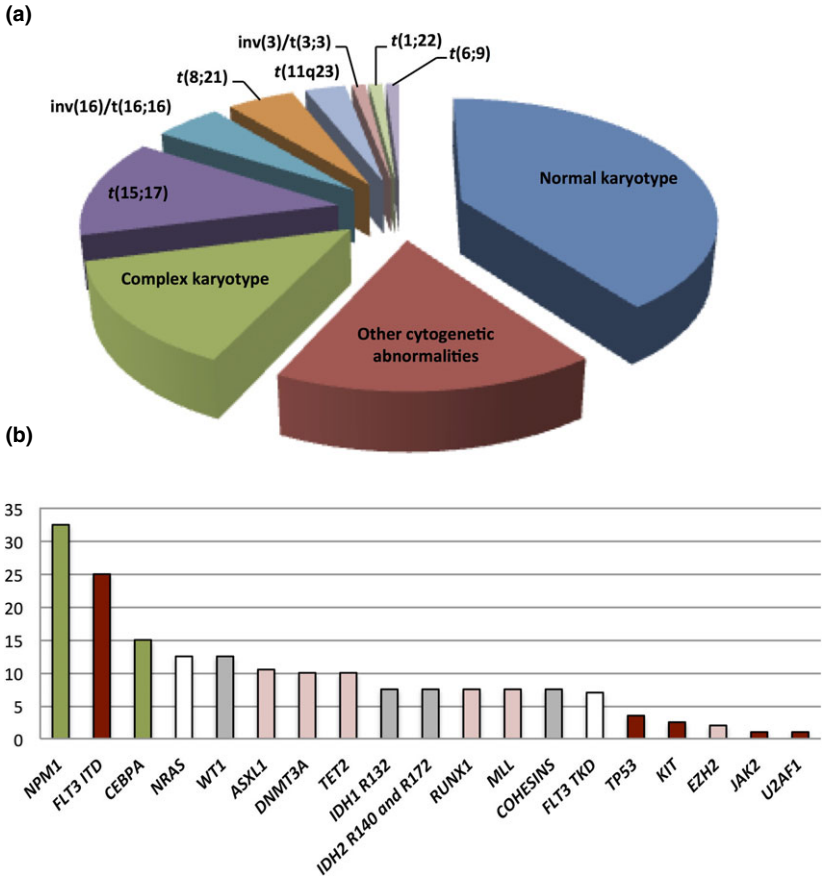

Figure 1. Genetic data (a) Frequencies of cytogenetic findings in acute myeloid leukemia. The proportion of acute myeloid leukemia cases with characteristic cytogenetic changes are shown in this wheel diagram. (b) Mutational frequencies of genes in acute myeloid leukemia. Colors correspond to prognosis: green bar indicates association with good prognosis, white bar indicates neutral prognosis, gray bar indicates prognosis is poorly defined, pink bar indicates association with probable poor prognosis, red bar indicates association with poor prognosis.

clinicians, and researchers. Since the discovery by Nowell and Hungerford of the *BCR-ABL1* Philadelphia chromosome translocation in chronic myelogenous leukemia [8], it has become evident that certain repetitive chromosomal abnormalities are associated with particular hematopoietic diseases. Such is the case in AML where chromosomal rearrangements may result in distinct biologic, diagnostic, and prognostic phenotypes. Currently, the WHO classification has identified a subgroup of AMLs, under the designation AML with recurrent genetic abnormalities, where chromosomal translocations are present and result in such characteristic clinicopathologic features [9]. We review critical features of each of these subgroups below as well as a subset of other cytogenetic alterations that additionally deserve attention in the diagnosis and prognosis of AML (Figure 1 and Table 1) [4, 10–13].

AML with t(8;21)(q22;q22); *RUNX1-RUNXT1*

AML with t(8;21)(q22;q22); *RUNX1-RUNXT1* correspond to 5–10% of all AMLs [12]. This translocation, which involves the *RUNX1* protein component of the core binding factor (CBF) alpha transcriptional subunit, ultimately generates a dominant negative mutant protein that represses transcription at CBF DNA-binding sites. This genetic alteration results in characteristic morphologic, immunophenotypic, and clinical features: (i) blasts with perinuclear hofs and 'salmon-colored' granules, (ii) aberrant immunophe-

notypic expression of B-cell markers (CD19, PAX5, and CD79a), and (iii) improved overall survival with standard induction and high-dose consolidation chemotherapy.

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* account for 5–10% of all AMLs [12] and are the result of fusion of the CBF-beta subunit to the coiled-coil region of smooth muscle myosin heavy chain (MYH11) which effects repression of specific gene transcription in a dominant negative fashion. This genetic alteration also results in characteristic morphologic, immunophenotypic, and clinical features: (i) increased mature eosinophils with promyelocytes and myelocytes with abnormally large basophilic granules and (ii) better overall survival in patients treated with standard induction and high-dose consolidation chemotherapy.

AML with t(15;17)(q24.1;q21.1); *PML-RARA*

Perhaps the best known translocation in AML is t(15;17)(q24.1;q21.1); *PML-RARA* which is seen in 10–20% of AMLs. This translocation fuses the retinoic acid receptor alpha (*RARA*) with the nuclear regulatory factor gene, promyelocytic leukemia (*PML*), generating an oncofusion protein that represses transcription of genes involved in myeloid differentiation, apoptosis, and proliferation. Treatment with all-trans retinoic acid (ATRA) results in differentiation of abnormal promyelocytes/blasts, and prognosis is excellent for patients with this AML; however, rarer variant translocations such as t(11;17)(q23;q21); *ZBTB16-RARA* or t(17;17)(q21;q21); *STAT5B-RARA* may result in resistance to ATRA. Additionally, three different breakpoint regions in t(15;17)(q24.1;q21.1); *PML-RARA* can be seen: break-point cluster region (bcr) 1, 2, or 3 which correspond respectively to long, variable, and short isoforms. The bcr3 isoform (short isoform) is often associated with the hypo- or microgranular variant of this AML [14–16]. While the classic hypergranular variant is frequently CD34 negative and HLA-DR negative, the hypogranular variant is often CD2 positive and can be CD34 and HLA-DR positive [17].

AML with t(9;11)(p22;q23); *MLLT3-MLL*

Depending on the age group, adult or pediatric, AML with t(9;11)(p22;q23); *MLLT3-MLL* may account for either 2% of AMLs or 5–10% AMLs, respectively. The mixed-lineage leukemia (*MLL*) gene, also known as lysine (K)-specific methyltransferase 2A (*KMT2A*) gene, is a histone-lysine N-methyltransferase. This translocation may result in sustained transactivation of *MLL* and leukemogenesis. More than 50 alternate partners for the *MLL/KMT2A* gene beyond *MLLT3* have been identified, each conferring unique biologic properties to the *MLL* gene, although the most common alternative partners are *MLLT1* (*ENL*), *MLLT10* (*AF10*), *MLLT4* (*AF6*), and *ELL*. The prognosis of AML with t(9;11)(p22;q23); *MLLT3-MLL* is intermediate although other *MLL* variants may have variably worse prognostic outcomes.

AML with t(6;9)(p23;q24); *DEK-NUP214*

Cases of AML with t(6;9)(p23;q24); *DEK-NUP214* are rare, and account for <2% of AMLs. The translocation results in a fusion of *DEK* with *NUP214* generating an oncoprotein that may act to increase global protein translation. The morphologic features of this AML are unique with increased basophils and trilineage dysplasia; such patients have generally poor overall survival.

AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*

AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1* represents <2% of all AMLs. Translocation of the *RPN1* gene enhancer close to *EVI1* results in increased *EVI1* expression and ultimately promotes cellular proliferation. Other cytogenetic abnormalities such as those of chromosome 5 or 7 are also common in this AML. From a clinical and morphologic standpoint, patients with this AML frequently have an increased platelet count and multilineage dysplasia, as well as characteristically numerous small megakaryocytes with hypolobate nuclei. Prognosis of this AML is poor.

AML with t(1;22)(p13;q13); *RBM15-MKL1*

AML with t(1;22)(p13;q13); *RBM15-MKL1* is generally only seen in infants and young children. This

gene rearrangement results in fusion of the megakaryocyte leukemia-1 (*MKL1*) gene with the RNA-binding motif gene (*RBM15*). The morphology in this leukemia is characteristic with numerous small and larger megakaryoblasts which are positive for CD41 and or CD61. The prognosis is considered poor but may be overcome with higher intensity chemotherapy.

De novo AML with t(9;22)(q34;q11); *BCR-ABL1* not arising from CML

In recent years, a subset of patients with AML and t (9;22)(q34;q11); *BCR-ABL1*, but no prior history of chronic myelogenous leukemia (CML) have been reported, and may represent a distinct subcategory of AMLs: so-called Ph+ AML [18]. While somewhat controversial as a distinct entity, regardless, it is clear that these AMLs show similarly poor prognosis as AMLs arising from CML.

Other cytogenetic changes in AML

Other cytogenetic changes can be seen in AML which confer specific prognostic properties. Although not translocations resulting in new fusion protein partners, deletions of chromosome 5, 5q, 7, 17, or 17p are frequently seen in AML and individually any of these abnormalities confer worse prognosis. While the genes responsible for conferring the adverse clinical phenotype in the cases of deletion of chromosomes 5, 5q, or 7 are uncertain, in the case of chromosome 17 and 17p, it is clear that *TP53*, a tumor suppressor, is a critical gene affected by deletion.

While individual cytogenetic abnormalities noted above can confer worse prognosis, various permutations of cytogenetic abnormalities can also result in poor prognosis. AMLs with ≥3 cytogenetic abnormalities form a clinical subgroup known as AMLs with complex karyotype (AML-CK) that are associated with poor prognosis [19]; however, AML-CK is not a specific WHO diagnostic group. AML-CK is frequently associated with one of two WHO subgroups: AML with myelodysplasia-related changes or therapy-related AMLs. Another clinical prognostic group identified in recent years is monosomal karyotype AMLs (MK-AML), which refers to those with at least two autosomal monosomies or a single autosomal monosomy with any additional structural cytogenetic abnormality [20]. However, the vast majority of MK-AML overlap with AML-CK, and the definition of a monosomal karyotype is considered controversial by many cytogeneticists.

SOMATIC MUTATIONS IN AML

Next-generation sequencing (NGS) has resulted in a recent explosion of information and data regarding somatic gene mutations in AML. In some instances, this information has come through whole-genome sequencing or exome sequencing [5]; however, it has become more common and accessible to perform targeted sequencing of smaller numbers of genes (10s–100s) in recent years [21]. While we do not review such technologies here, it behooves the reader to become familiar, if not already so, with these technologies [22].

The current WHO classification incorporates some somatic mutational data in the diagnosis of AML. However, only two genes are provisionally and currently recognized in the subclassification of AML: mutations in *NPM1* and *CEBPA* [9]. However, mutations in other genes have been identified as important for prognostication or other reasons in AML and are also reviewed here (Figure 1 and Table 2) [21].

NPM1 mutations

Nucleophosmin (*NPM1*) is a gene whose protein functions as a nuclear phosphoprotein and nuclear chaperone [23]. Mutations in this gene can be seen in over 50% of normal karyotype AMLs. In nearly 80% of AMLs with mutated *NPM1*, a characteristic 4 base nucleotide duplication (TCTG) is present, designated mutation A. This mutation interferes with localization of NPM1 and chaperoned partners such as p19Arf (tumor suppressor) and NF-kappaB to the nucleus. A characteristic morphologic change may be seen in the blasts with *NPM1* mutated, so-called, blasts with a cuplike nucleus, and a majority of cases are CD34 negative. Currently, the WHO classification recognizes this as a provisional but distinct subgroup of AMLs, and clinically, patients with normal karyotype and *NPM1* mutations without *FLT3-ITD* mutations show better prognosis with standard induction and consolidation chemotherapy [24].

CEBPA mutations

The CCAAT/enhancer-binding protein alpha (*CEBPA*) is a single exon gene encoding for a basic region leucine zipper transcription factor that forms a homodimer to regulate granulocytic differentiation. Mutations result in increased cellular proliferation and inhibition of differentiation. The two most frequent types of mutations are N-terminal frame-shifts and C-terminal in-frame insertions or deletions generating a dominant negative protein, or affecting DNA-binding function, respectively. Approximately 10–20% of all AMLs will have a mutation in *CEBPA*, and 70% of these cases will have a normal karyotype. Of these, many cases contain bi-allelic mutations (40–50% of CEBPA mutated cases) and it is the bi-allelic combination that results in better prognosis for patients [25–27]. Single allelic mutations have not been shown to be associated with better prognosis in multivariate analysis and frequently are associated with *FLT3* (30–35% of cases) or *NPM1* mutations (30–35% of cases). Among cases of AML with a *CEBPA* mutation, 5–10% of these have been identified to be germline mutations. Currently, the WHO classification recognizes AML with mutated *CEBPA* as a provisional but distinct subgroup of AMLs.

Other gene mutations of significance

FLT3 mutations

Fms-like tyrosine kinase-3 (FLT3) is a tyrosine kinase receptor whose activation by homodimerization results in a phosphorylation cascade involving STAT, AKT/PI3 kinase, and MAP kinase pathways. The most common mutation is an internal tandem duplication (ITD) within the juxtamembrane domain (exons 14 and 15), and in most cases, the size of the ITD falls in the range of 15–180 base pairs (the insertion is always a multiple of 3 base pairs), resulting in auto-dimerization and autophosphorylation and constitutive activation [28, 29]. Missense mutations in the tyrosine kinase activation domain (TKD) also are seen with the most frequent occurring at amino acid D835, which results in ligand-independent activation. Mutations of *FLT3*-ITD are seen in 20–30% of all AMLs, and point mutations of in the TKD are seen in 5–10% of all AML cases. *FLT3-ITD* mutations are associated with leukocytosis, normal karyotype AMLs and confer worse prognosis even in the presence of *NPM1* or *CEBPA* mutations. The morphologic features of cases with *FLT3*-ITD may overlap with *NPM1*-mutated AMLs; blasts may show cuplike nuclei. Targeted therapies against FLT3 have been developed such as sorafenib and quizartinib, midostaurin, and pacritinib, but others are also in development and testing. While not an official subgroup of AMLs, in the WHO classification, the importance of *FLT3* as a poor prognostic marker is recognized.

KIT mutations

c-kit is another tyrosine kinase which rarely may be mutated in AML; when mutations are seen, they are frequently present in either AML with t(8;21)(q22; q22); *RUNX1-RUNXT1* (approximately 20% of cases) or AML with inv(16)(p13.1q22) or t(16;16)(p13.1; q22); *CBFB-MYH11* (20–30% of cases) [30]. Mutations are seen in exons 8 and exon 17 (frequently amino acid D816) and result in activation of the kinase domain and constitutive activation in the absence of ligand; when seen in these subgroups of AMLs, these mutations may confer worse prognosis. While *KIT* mutations can be seen in other nonhematopoietic neoplasms, for instance, gastrointestinal stromal cell tumors (GISTs) or melanomas, the mutations seen in AMLs, are generally unique from those in GISTs (exon 9 and exon 11) and melanomas (exon 11 and exon 13). Treatment using multikinase inhibitors such as imatinib may not be effective against the most common mutations seen at D816 [31, 32].

JAK2 mutations

Janus-kinase-2 (JAK2) is a nonreceptor tyrosine kinase involved in cell growth and differentiation, responsible for phosphorylating STAT proteins, which are then translocated to the nucleus. While *JAK2* is well known to be mutated in myeloproliferative neoplasms, it also can be rarely mutated in de novo AMLs and may be seen in cases of AML with t(8;21)(q22; q22); *RUNX1-RUNXT1* and AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* AMLs. Mutation in cases of AML with t(8;21)(q22; q22); *RUNX1-RUNXT1* confers poorer prognosis. Ruxolitinib is a JAK inhibitor undergoing testing in refractory acute leukemias, and other JAK2/FLT3 inhibitors

Table 1. Cytogenetic abnormalities in AML

AML subtype	Cytogenetic abnormality	Molecular background	Other clinical and pathologic features
AML with t(8;21)(q22;q22); *RUNX1-RUNXT1*	t(8;21)(q22;q22); *RUNX1-RUNXT1*	Translocation generates fusion protein of core binding factor, inhibits transcription of multiple genes	Blasts with perinuclear hofs and cytoplasmic salmon-colored granules, good prognosis
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	Core binding factor beta (*CBFB*) to smooth muscle myosin heavy chain (*MYH11*) fusion protein results in dominant negative protein and repression of transcription	Increased eosinophils, myeloid precursors with basophilic granules, good prognosis
AML with t(15;17)(q24.1;q21.1); *PML-RARA*	t(15;17)(q24.1;q21.1); *PML-RARA*	Fusion of retinoic acid receptor alpha (*RARA*) to promyelocytic leukemia protein (*PML*) represses differentiation, apoptosis	Abnormal promyelocytes/blasts with folded nuclei and prominent eosinophilic granules, good prognosis
AML with t(9;11)(p22;q23); *MLLT3-MLL*	t(9;11)(p22;q23); *MLLT3-MLL*	Mixed-lineage leukemia (*MLL*) encodes a histone methyltransferase and may be fused to MLLT3 resulting in increased MLL activity	Intermediate prognosis
AML with t(6;9)(p23;q24); *DEK-NUP214*	t(6;9)(p23;q24); *DEK-NUP214*	This fusion product may lead to increased protein translation	Basophilia and multilineage dysplasia are common, poor prognosis
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*	Results in increased EVI1 expression and cellular proliferation	Increased platelets and micromegakaryocytes, poor prognosis
AML with t(1;22)(p13;q13); *RBM15-MKL1*	t(1;22)(p13;q13); *RBM15-MKL1*	Results in novel fusion protein of MKL1 with RBM15	Rare and essentially exclusive to infants and children, megakaryocytic blasts, poorer prognosis
De novo AML with t(9;22)(q34;q11); *BCR-ABL1* not arising from CML	t(9;22)(q34;q11); *BCR-ABL1*	Results in novel tyrosine kinase fusion protein	Must be de novo and not arising from CML. Poor prognosis.
Monosomal karyotype AML	At least two autosomal monosomies or a single autosomal monosomy with any additional structural cytogenetic abnormality	Controversial clinical subgroup; most cases overlap with AML with complex karyotype	Most cases are either AML with myelodysplasia-related changes or therapy-related AMLs, frequently multilineage dysplasia is seen
AML with complex karyotype	At least 3 cytogenetic abnormalities	May reflect more profound genomic instability. Variably defined, in some instances ≥5 cytogenetic abnormalities required for complex karyotype	Most cases are either AML with myelodysplasia-related changes or therapy-related AMLs, frequently multilineage dysplasia is seen

Table 2. Genes recurrently mutated in AML

Gene	Molecular background	Other clinical and pathology features
NPM1	Nucleophosmin (NPM1) protein, functions as nuclear protein chaperon with insertion mutation TCTG most commonly resulting in altered nuclear signal transduction	Mutations associated with good prognosis in absence of *FLT3-ITD*; blasts can show cuplike nuclei
FLT3	Receptor tyrosine kinase; internal tandem duplication mutations or point mutations in juxtamembrane domain are seen and result in activation	Mutations associated with worse prognosis; blasts can show cuplike nuclei as well
CEBPA	CCAAT/enhancer binding protein alpha (*CEBPA*) transcription factor. Two most frequent mutations are N-terminal frame-shift or C-terminal in-frame indel	Bi-allelic mutations associated with improved prognosis; single allelic mutation shows no prognostic significance
KIT	Receptor tyrosine kinase with mutations frequently in the kinase domain at D816	May be mutated in AML with t(8;21)(q22;q22); *RUNX1-RUNXT1* or AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11.* Most common *KIT* mutation D816 shows no response to imatinib
Cohesins	Several genes (*STAG1, STAG2, SMC1A, SMC3,* and *RAD21*) involved in sister chromatid separation	Mutated in 5-10% of AMLs overall, but >50% of AMLs associated with Down syndrome
WT1	Wilms Tumor protein (WT1), zinc finger transcription factor	Clinical significance is uncertain
JAK2	Janus-kinase-2 (JAK2), nonreceptor tyrosine kinase frequently mutated in myeloproliferative neoplasms; mutations result in activation which is independent of upstream cytokine signaling	Rare mutations in de novo AML. Associated with AML with t(8;21)(q22;q22); *RUNX1-RUNXT1* or AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
NRAS	Membrane associated signal transduction GTPase; activating mutations increased cellular proliferation and decrease apoptosis	Mutations may not have an impact on clinical prognosis
U2AF1	Component of spliceosome complex, mutations result in abnormal splicing of genes involved in myeloid differentiation and proliferation	Mutations confer poor prognosis and may be associated with multilineage dysplasia
TP53	Tumor suppressor protein, inactivating point mutations and indels associated with complex karyotype and therapy-related AMLs	Mutations associated with poor prognosis
DNMT3A	DNA methyltransferase 3A (*DNMT3A*), inactivating point mutations most commonly at amino acid D882	Mutations associated with poor prognosis in some studies but a prognostic impact is not seen in others
MLL	Histone methyltransferase that also interacts with RUNX1 to effect cellular functions. Mutations are frequently partial tandem duplications	Prognostic impact still under study but may confer poorer prognosis
TET2	Involved in hydroxymethylation of DNA to reverse methylation effects on DNA	Mutational impact still under study, some studies point to poorer prognosis
IDH1 & IDH2	Isocitrate dehydrogenase -1 and -2 (IDH1, IDH2) are enzymes that convert isocitrate to alpha-ketoglutarate in the Krebs cycle; mutations result in production of 2-hydroxyglutarate which inhibits hydroxymethylation of DNA	Mutations of unknown prognosis, clinical trials with IDH targeted inhibitors are ongoing
ASXL1	Additional sex combs-like gene (ASXL1) is a chromatin-binding protein likely involved in methylation of histone proteins	Mutations associated with poor prognosis in some studies
EZH2	Enhancer of zeste homologue 2 (*EZH2*) is the catalytic component of the PRC2 complex and functions to trimethylate histone tails	Rarely mutated in AML, mutations associated with poor prognosis in some studies

such as pacritinib and lestaurtinib are also being evaluated in clinical trials.

NRAS mutations

Mutations in the NRAS GTPase proto-oncogene can be seen in 5–15% of AMLs. RAS activating mutations may contribute to leukemogenesis through effects on cellular proliferation; however, the prognostic significance of these mutations is overall uncertain.

Other gene mutations

Numerous other somatic mutations are seen in AMLs, some which can confer growth advantage, increased proliferative properties, inhibit apoptosis, or affect cellular differentiation. Six of these genes or families of genes, frequently mutated, are discussed here, while others that impact epigenetic pathways are discussed below. Wilms tumor protein is encoded by the *WT1* gene and is a zinc finger transcription factor; mutations in this gene are frequent, seen in 10–15% of AMLs. Although initially thought to be significant and related to poorer prognosis, currently, it is unclear what role such mutations have in leukemogenesis, progression or prognosis. Inactivating *RUNX1* mutations are seen in 5–10% of AMLs. Interestingly unlike AML with t (8;21)(q22;q22); *RUNX1-RUNXT1*, somatic point mutations and indels in *RUNX1* may be associated with worse overall survival. *U2AF1*, a component of the spliceosome complex, is mutated in a subset of AMLs as well as myelodysplastic syndrome patients. Mutations in this gene are associated with aberrant splicing of multiple transcripts, including those involved in epigenetic regulation such as *ASXL1*; overall such mutations are associated with poor prognosis in AML [21]. *TP53*, among the most famous and well-studied tumor suppressors, is mutated in a subset of AMLs and most frequently those with a complex karyotype and/or history of chemo- or radiotherapy. Mutations in this gene are associated with poor prognosis. Mutations in genes of the cohesin complex *(STAG1, STAG2, SMC1A, SMC3, and RAD21)* are also seen in 5–10% of AMLs but more frequent in AMLs associated with Down syndrome, >50% of cases. The role of the cohesin complex in normal cellular physiology is during sister chromatid separation during mitosis, but the effect of mutations in AML is less clear.

MUTATIONS OF EPIGENETIC MODIFIERS IN AML

Epigenetic changes are those that affect the transcription of genes or their expression, without modifying the genetic code. The effect of epigenetic changes in AML may be seen through one of three mechanisms: (i) DNA methylation, (ii) DNA hydroxymethylation, or (iii) histone modification. While the role of epigenetics has not yet become a part of current diagnostic WHO categorization, in recent years, these molecular changes have been heavily studied in leukemogenesis. In many cases, epigenetic modifiers have been shown to be important in the pathogenesis and prognostication in AML (Figure 1 and Table 2).

DNA methylation

DNA methylation controls gene expression through methylation of cytosine residues at CpG sites, which can be clustered in groups (CpG islands), or appear individually and relatively dispersed from other CpG sites. DNA methylation of CpG islands within promoter regions typically results in recruitment of transcriptional repressors and ultimately downregulation of gene expression.

While CpG island methylation, particular to single genes, has been identified as important in some instances in the prognostication of AML, global changes in such DNA methylation have also been described in AML and may subclassify cases into clinically relevant prognostic categories [5, 33, 34]. These global methylation signatures also correlate with WHO AML subtypes further supporting the merits of the WHO classification from a biologic point of view. In addition, particular genes that are the enzymatic components of the DNA methylation machinery can be altered. Here, we focus predominantly on genes involved in epigenetic modification in AML.

DNMT3A mutations

DNA methyltransferase 3A (*DNMT3A*) is gene encoding an enzyme, which is involved in de novo methylation of CpG dinucleotides. Mutations in this gene

can occur in 10–25% of AMLs. The most common mutation occurs at R882 and results in increased cellular proliferation. Mutations in this gene have been shown to affect methylation patterns in AML [35]. While recurrently mutated in AML, a deep understanding for how mutated forms of *DNMT3A* are functioning to affect gene expression in AML is still lacking. The mutations in *DNMT3A* have been shown in most studies to be associated with poor prognosis although some have shown no effect on prognosis at all [35–37]. It is possible that mutations in epigenetic modifiers such as *DNMT3A* are permissive for leukemogenesis but not sufficient to initiate transformation alone.

DNA hydroxymethylation

The hydroxylation of methylated cytosines is one mechanism by which DNA CpG methylation, as discussed above, may be reversed. Hydroxymethylated DNA cannot bind DNA repressor proteins, effectively reversing repression. Several genes that encode for proteins directly involved in DNA hydroxymethylation are recurrently and frequently mutated in AML; we review those genes and their significance.

TET2, IDH1 and IDH2 mutations

Tet methylcytosine dioxygenase-2 (*TET2*) is a gene whose protein product is involved in the conversion of 5-methylcytosine to 5-hydroxymethylcytosine. A total of 5–25% of AMLs have somatic mutations in *TET2*; these mutations have been shown to result in loss of function. *TET2* mutations may be involved in leukemogenesis as they are seen early during disease detection, and murine models have shown that loss of TET2 function results in increased myeloproliferation and expansion of myeloid precursors. However, the relationship between prognosis is somewhat uncertain, although the majority of studies show an association with poorer prognosis.

Isocitrate dehydrogenase-1 (*IDH1*) and -2 (*IDH2*) are genes encoding for NADP+ enzymes which convert isocitrate to alpha-ketoglutarate in the Krebs cycle. Mutations individually in both these genes in AML occur in a total of 15–30% of AMLs and interestingly result in a new enzymatic function allowing the aberrant-accelerated conversion of alpha-ketoglutarate to 2-hydroxyglutarate (2-HG). It is the accumulation of the metabolite 2-HG which has been shown to inhibit TET2 function, thus ultimately inhibiting hydroxymethylation of DNA. While the prognostic impact of IDH mutations in AML is controversial and remains uncertain, clinical trials using inhibitors relatively specific for IDH1 and IDH2 are ongoing.

Histone modification

Histone proteins can be modified by methylation, acetylation, phosphorylation, ADP-ribosylation, and ubiquitination, all which affect the chromatin structure and accessibility of DNA. Although some evidence for the importance of this fundamental molecular mechanism in AML exists, incorporation into the WHO classification has not been made. Mutations in three genes in this molecular pathway are reviewed.

EZH2, MLL, and ASXL1 mutations

Enhancer of zeste homologue 2 (*EZH2*) is a gene encoding for the catalytic enzyme in the PcG repressor complex 2 (PRC2) and involved in methylation of histone tails; increased activity of this protein ultimately leads to increased chromatin condensation and suppression of gene expression. Mutations in EZH2 are rare in AML <5% and may be associated with poor prognosis, although because these mutations are so rare, it has been difficult to definitively parse out their significance. Mixed-lineage leukemia protein (MLL) is an epigenetic modifier of histones through methylation. Discussed previously as the fusion protein in AML with t(9;11)(p22;q23); *MLLT3-MLL*, the MLL protein participates in binding target promoter regions to control expression of genes; in particular, HOX genes involved in cell proliferation/differentiation. Mutation of *MLL* occurs in 5–10% of AMLs, and these mutations are frequently partial tandem duplications; these mutations may be associated with poorer prognosis. Finally, the additional sex combs-like gene (*ASXL1*) is chromatin-binding protein, which may function in pathways to methylate histone lysines; *ASXL1* is mutated in 5–30% of AMLs. Mutation in this protein has been associated with poor prognosis in several studies and may also be associated with a specific WHO subcategory of AMLs, AML with myelodysplasia-related changes.

CONCLUSION

The genetic and epigenetic landscape of AML is ever shifting requiring continued study and re-evaluation of old and possible new criteria. As our understanding of the biology of AML grows so too will our incorporation of novel mechanisms and genetic mutations in our classification.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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