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Genetic basis of acute myeloid leukemia (AML): The most common molecular changes in patients with normal karyotype

Review

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Abstract

Acute myeloid leukemia (AML) is a clonal disorder that results from errors in proliferation and differentiation of bone marrow stem cells from myeloid lineage. According to the Gilliland "two-hit" model, genes of both groups related to proliferation (e.g., *FLT3*) and differentiation (e.g., *CEBPA*) must be mutated for full development of AML. The genetic background of AML is very complicated and varied, from single nucleotide mutations or changes in gene expression to cytogenetic aberrations. The DNA sequencing results enable identification of important gene alterations that occur first and may lead the whole leukemogenesis (driver mutations). Some of them have prognostic significance – that is, they are related to the overall survival (OS), complete remission rate, and event-free survival (EFS). The most common molecular changes in AML are mutations in *NPM1*, *CEBPA*, *FLT3,* and *DNMT3A*. Alterations in *NPM1* gene are associated with a good prognosis but simultaneous mutation in *FLT3* may change this prognosis. *DNMT3A* mutations are very often correlated with *NPM1* mutations and are associated with short OS.

Keywords

acute myeloid leukemia • prognostic factor • driver mutation • *NPM1* • *FLT3* • *CEBPA* • *DNMT3A*

1. Introduction

Acute myeloid leukemia is a very heterogenous disorder. Genetic changes occur in almost every patient. Many of them are very important in diagnosis and treatment of AML. In the normal karyotype acute myeloid leukemia (NK AML) group of patients, about 60% have a mutated *NPM1* gene. NPM1 is multifunctional shuttling protein that transfers preribosome between the nucleus and cytoplasm. It also takes part in cell cycle regulation. However, *NPM1* mutations are associated with a good prognosis [1]. Besides these changes, *FLT3*-ITD and *DNMT3A* mutations are the most common in NK AML [2]. Additionally, *FLT3* expression is linked to that of *CEBPA* [3, 4]. Due to these findings, *NPM1, FLT3, CEBPA,* and *DNMT3A* genes were chosen for this review.

2. Genetic basis of acute myeloid leukemia

2.1. The "two-hit model" in AML development

Acute myeloid leukemia is a disease characterized by genetic abnormalities in two groups of genes – those related to proliferation and those related to differentiation. These events lead to the overproliferation of an immature cell clone (Gilliland hypothesis). The genes responsible for cell proliferation and survival are mainly the ones coding for tyrosine kinases, such as *FLT3* (class I mutations). The activity strength of mutated *FLT3* is similar to other mutated tyrosine kinases (e.g., *BCR-ABL, ETV6-PDGFRB*). *FLT3* mutations lead to myeloproliferation, even in the absence of the FLT3 ligand (FL).

The impaired differentiation is caused by changes in the second group of genes, including transcription factor genes such as *CEBPA* and *RARA* (class II mutations). The coexistence of these two types of mutations, described as the "two-hit model", is essential for AML development (Fig. 1). Gene changes belonging to only a single class of mutations do not lead to the full development of AML [5]. Currently, mutations that can cause AML by affecting genome stability and DNA repair (e.g., *TP53*) or transcription (e.g., *TET2*, *IDH1/2, DNMT3*) that do not belong to the above two classes (class III mutations) are also taken into account [6].

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2.2. The genetic background of normal karyotype AML

The following changes may lead to the neoplastic transformation underlying AML:

1. chromosomal aberrations (e.g., translocations, deletions, inversions),
2. intragene aberrations:
   - short deletions, duplications or insertions, such as in the *FLT3, NPM1, CEBPA,* and *KMT2A* genes,
   - point mutations, such as in the *cKIT, N-RAS, K-RAS, IDH1/2, TET2* genes,
3. gene overexpression, such as of *BAALC, ERG,* and *MN1* genes,
4. abnormal function of microRNA [2, 6, 7, 8].

Approximately 55% of patients with acute myeloid leukemia have chromosomal aberrations in blast cells [9]. Mutations of genes representing particular classes may be present in both groups of patients, with normal karyotype (NK) or with chromosomal aberrations.

Results from next-generation sequencing (NGS) demonstrate the complexity of the molecular basis of AML development. Whole genomic sequencing reported by the Cancer Genome Atlas Research Network identified 2315 somatic single nucleotide variants (SNV), 66% of which were missense changes. Somatic variants were found in 260 genes. A group of 23 genes with the highest frequency of pathogenic variants, including known mutations in genes related to the pathogenesis of AML, were identified [8].

According to genomic sequencing results, Metzeler et al. described driver mutations, which are probably the main cause of AML development. In the group of 664 patients with AML they analyzed, 2395 mutations were found in 59 genes. At least one mutation was found in 97% of patients. The ratio of mutant to normal alleles (variant allele frequency, VAF) in these genes was on average 0.36. VAF of heterozygous germline polymorphisms was about 0.5. For the somatic mutations of *TP53, IDH1, DNMT3A, CEBPA, TET2,* and *NPM1* genes, VAF was also about 0.5. However, this value was the lowest for the *FLT3, KIT,* and *NRAS* genes, which may indicate that their mutations were present only in subpopulations of the leukemic cells. In 47% of all AML patients, at least one mutation in the *NPM1*, *DNMT3A,* and *FLT3* genes was present and associated with the NK [2].

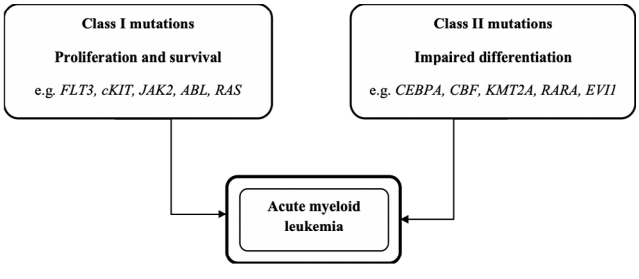


Fig. 1. "Two-hit model" according to Gilliland [5]

The relationship of mutations and aberrations with clinical (complete remission rate [CRR], overall survival [OS], disease-free survival [DFS], or event-free survival [EFS]) and hematological (such as WBC) data allowed for choosing those abnormalities which sort patients into the poor, good, and intermediate prognostic groups. Thus, among many of the detected changes some prognostic and predictive factors could be identified. Moreover, some mutated proteins have become therapeutic targets [7].

3. Risk stratification according to genetic changes

According to correlation of genetic abnormalities with AML outcome (WBC, percentage of blasts, OS, EFS, DFS) European LeukemiaNet (ELN) described 3 classification groups (favorable, intermediate, adverse) (Table 1). The most common mutations were taken into account in this classification. Additionally, allelic ratio of *FLT3-ITD* (VAF) was also an important factor categorizing patients into prognostic groups [7]. Recently, Herold et al. studied 1,116 newly diagnosed patients with AML and classified them into prognostic groups according to clinical outcome. The results confirmed ELN stratification [9].

4. Characteristics of selected genetic markers of acute myeloid leukemia

4.1. *NPM1*

Nucleophosmin, encoded by the *NPM1* gene, belongs to the group of chaperone proteins. The *NPM1* gene is located on chromosome 5 (5q35) and contains 11 exons. Two protein isoforms can be encoded; full-length (294 amino acids) with high expression, and shorter (259 amino acids) the expression of which in cells is low [10]. The NPM1 protein consists of 3 basic domains related to protein oligomerization, histone binding, and nucleic acid binding. It moves from the nucleus to the cytoplasm and back, carrying the components of the preribosomes. NPM1 plays a key role in the biogenesis of ribosomes. It is also involved in cell cycle control by regulating the duplication of centrosomes. It is involved in DNA repair, mainly of the HR (homologous recombination) type.

NPM1 is also associated with apoptosis by collaborating with the $p14^{ARF}$ (ARF) protein. In the cell nucleus NPM1 inhibits apoptosis by ARF binding. If NPM1 does not connect to ARF, ARF binds to the MDM2 protein. When MDM2 is bound, it does not inhibit the p53 protein, which may further initiate apoptosis. Nucleophosmin may also directly bind to the MDM2 protein and inhibit its activity, thus promoting apoptosis. Concluding, NPM1 protein promotes or inhibits apoptosis, depending on the protein with which it cooperates at the moment [11, 12, 13, 14].

Leukemogenesis may be caused by many factors, including mutated NPM1 protein, which mainly localizes in the cytoplasm,

Table 1. Risk stratification of AML according to ELN [7]

Risk category	Genetic abnormality
Favorable	• t(8;21)(q22;q22) *RUNX1-RUNX1T1*, • inv(16)(p13q22) or t(16;16)(p13;q22) *CBFB-MYH11*, • mutated *NPM1* without *FLT3-ITD* or with low allelic ratio *FLT3-ITD* (VAF<0,5), • biallelic mutated *CEBPA*.
Intermediate	• t(9;11)(p21;q23) *MLLT3-KMT2A*, • cytogenetic abnormalities not classified as favorable or adverse, • mutated *NPM1* with high allelic ratio *FLT3-ITD* (VAF>0,5), • wild type *NPM1* without *FLT3-ITD* or with low allelic ratio *FLT3-ITD*,
Adverse	• t(6;9)(p23;q34) *DEK-NUP214*, • t(v;11q23.3); *KMT2A* rearranged, • t(9;22)(q34;q11) *BCR-ABL1*, • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM* rearranged, • -5 or del(5q); -7; -17 or del(17p), • complex or monosomal karyotype, • wild type *NPM1* with high allelic ratio *FLT3-ITD* (VAF>0,5), • mutated *RUNX1*, *TP53* or *ASXL1*.

instead of shuttling between the cytoplasm and the nucleus [15]. The mutated NPM1 protein, after binding to the ARF, leads it to the cytoplasm, where it undergoes degradation, which then causes genome instability [16]. Papaemmanuil et al. identified *NPM1* changes as examples of the driver mutations in AML. They are present in approximately 27% of AML patients, most often those with NK (60% of NK AML cases) [17]. During disease relapse, the *NPM1* mutations are still present in 91% of patients who harbored them at diagnosis [18]. Mutations in *NPM1* are associated with a favorable prognosis. So far, 56 mutations of this gene have been described, all within exon 11. These are mainly insertions that change the reading frame (Table 2). The mutations may coexist with changes in other genes, such as with the *FLT3*-ITD mutation related to poor prognosis [19].

Mutations in *NPM1* are common in adult AML, thus they are a very good marker for minimal residual disease monitoring.In the future, the mutated *NPM1* or its product will probably be a suitable aim for treatment [1, 20]. Nowadays, AML patients with mutated *NPM1* are treated with intensive chemotherapy. The addition of gemtuzumab ozogamicin to treatment protocol improves therapy results. Depending on *FLT3*-ITD mutational status, allo-HSCT may be recommended. Other ongoing clinical trials are connected with NPM1 shuttling and its role in ribosome biogenesis, focusing mainly on proteins which cooperate with NPM1 in these processes. However, these approaches are not efficient enough to use in standard treatment [1].

4.2. *FLT3*

The *FLT3* gene, located on chromosome 13 (13q12 region), consists of 24 exons and encodes a receptor, tyrosine kinase, FLT3, which is expressed in bone marrow stem cells. It is composed of 4 domains: extracellular (ligand binding), transmembrane, and two intracellular domains with kinase activity. Under physi-

*Table 2. The most common mutations in the **NPM1** gene [15]*

No	Mutation type/name	DNA sequences: wild-type (black) and mutated (red)							
1.	No mutation (wt)	gatctct	-	g	-	gcagt	-	ggagg	aagtctctttaagaaaatag
2.	A	gatctct	-	g	TCTG	gcagt	-	ggagg	aagtctctttaagaaaatag
3.	B	gatctct	-	g	CATG	gcagt	-	ggagg	aagtctctttaagaaaatag
4.	C	gatctct	-	g	CGTG	gcagt	-	ggagg	aagtctctttaagaaaatag
5.	D	gatctct	-	g	CCTG	gcagt	-	ggagg	aagtctctttaagaaaatag
6.	E	gatctct	-	g	-	gcagt	CTCTTGCCC	-	aagtctctttaagaaaatag
7.	F	gatctct	-	g	-	gcagt	CCCTGGAGA	-	aagtctctttaagaaaatag
8.	G	gatctct	-	g	-	gcagt	GCTTCGCC	-	aagtctctttaagaaaatag
9.	H	gatctct	-	g	-	gcagt	GTTTTTCAA	-	aagtctctttaagaaaatag
10.	J	gatctct	-	g	-	gcagt	CTCTTTCTA	-	aagtctctttaagaaaatag
11.	L	gatctct	CCCG	g	-	gcagt	-	-	aagtctctttaagaaaatag
12.	K	gatctct	-	g	-	gcagt	CCCTTTCCA	-	aagtctctttaagaaaatag
13.	M	gatctct	-	g	TAGC	gcagt	-	ggagg	aagtctctttaagaaaatag
14.	N	gatctct	-	g	CCAC	gcagt	-	ggagg	TCTCTTTaagaaaatag

Legend: the sites of possible insertions or deletions are written in red

ological conditions, the association of the FLT3 receptor with its ligand results in the induction of intracellular signals regulating cell proliferation and differentiation. *FLT3* gene expression disappears as cells differentiate. As presented by Gilliland, changes in this gene may be one of the main bases for the development of AML [5]. Increased expression of normal *FLT3* in AML blast cells is found in approximately 70–100% of patients. However, in about 30% of patients the gene is mutated. Mutations in *FLT3* are mainly internal tandem duplications (ITD) of various lengths (*FLT3*-ITD, approximately 22% of patients) or missense mutations leading to amino acid changes, mainly at position 835 (*FLT3* TKD, *FLT3* tyrosine kinase domain) [5]. They often coexist with other genetic changes, such as the *NPM1* mutations described earlier.

The persistence of *FLT3*-ITD until disease relapse may indicate that this mutation is one of the driving ones [21]. *FLT3*-ITD, first described by Nakao et al., is the result of the amplification of repetitive sequences of 3 to 400 bp in the exon 14 or 15 [22]. The *FLT3*-ITDs are located in the transmembrane domain, while the *FLT3* TKD mutations occur in the kinase domain (exon 17). The latter ones most often result in asparagine replacement by tyrosine, histidine, valine, glycine, or glutamic acid [4]. Both mutation types lead to the activation of the FLT3 receptor without the presence of the FLT3 ligand and increase cell proliferation. Thus, they are considered as one of the main causes of leukemogenesis. Moreover, Sallmyr et al. showed that *FLT3*-ITD may be the basis for further changes leading to genome instability. *FLT3* duplication can initiate the signal transducer and activator of the transcription-5 (STAT5) signaling pathway changes that increase reactive oxygen species (ROS) production. High levels of ROS lead to accelerated DNA double-strand breaks (DSBs) and DNA repair errors [23]. The assessment of *FLT3* mutational status, especially *FLT3*-ITD/*FLT3* allelic ratio, is essential for AML treatment and prognosis [24].

Due to very poor prognosis associated with the *FLT3-ITD* mutation (short overall survival, high relapse rate, high white blood cell count, and high percentage of blasts [4]), new therapies have been searched for. FLT3 inhibitors (e.g.,midostaurin, sorafenib), that restrain FLT3 kinase activity, applied during induction and consolidation, improve treatment results in clinical trials. Due to the introduction of these therapies, patients with *FLT3*-ITD could be reclassified from the adverse risk group into a favorable risk group (patients with *NPM1* mutation with low allelic ratio <0.5) [25].

4.3. *CEBPA*

The *CEBPA* gene, located on chromosome 19 (19q13.1), consists of only one exon. It encodes a transcription factor involved in myelopoiesis which activates genes related to granulocyte cell line maturation. The CEBPA protein has two transactivation domains, TAD1 and TAD2, the base region and the leucine zipper, which together form the bZIP domain. CEBPA may occur in two forms: as a full-length 42kDa protein, which works as a CEBPA transcription factor, and a shorter, 30kDa protein, which may inhibit the function of the 42kDa protein [21]. The 30kDa form lacks the TAD1 domain and therefore does not activate transcription as efficiently as the 42kDa. The *CEBPA* gene mutations that occur in patients with AML are most often insertions and deletions, and less frequently point mutations. They mainly concern N and C ends of the protein. Pabst et al. showed that mutations in the DNA fragment that encodes the N-terminus of a full-length 42kDa protein shortened this isoform to 20kDa. This reduced to 19% of normal activity the binding of CEBPA to the promoter of the *CSF3R* gene, which encodes the receptor for the granulocyte colony stimulating factor and is associated with the production, differentiation, and function of granulocytes. The lack of adequate amounts of the normal 42kDa protein is compensated for by the increase in amount of the 30 kDa isoform, which activity, however, is not sufficient for the proper functioning of CEBPA [26].

CEBPA gene mutations are an important prognostic factor in AML. Simultaneous mutations at both ends of the protein, C and N, in a different allele each, qualify patients for the good prognostic group, while a single mutation qualifies them to the intermediate prognostic group only [26]. It has been proved that in leukemia cells, expression of *CEBPA* with N- and C-terminal mutations is higher than *CEBPA* with a single N-terminal mutation [21].

CEBPA mutated AML subtype is very sensitive to standard chemotherapy (high complete remission rate). Hematopoietic stem cell transplantation in the group of patients with biallelic *CEBPA* mutations improves relapse-free survival. However, currently there is no therapy targeted to CEBPA protein [27].

4.4. *DNMT3A*

The *DNMT3A* gene encodes a methyltransferase that is involved in the binding of methyl groups to CpG islands. *DNMT3A* mutations occur in approximately 20-25% of AML patients and are associated with poor prognosis. The most common missense mutation, at codon 882, results in a loss of methyl transfer capacity, which results in methylation errors. Its presence is associated with an increased number of white blood cells in AML patients. *DNMT3A* may also undergo nonsense protein-shortening mutations, causing frame shift or incorrect splicing of exons. The gene expression profile examined in patients with *DNMT3A* mutations revealed reduced levels of gene methylation, such as oncogenes [28]. According to Papaemmanuil et al., mutations in the *DNMT3A* gene are one of the first changes that initiate neoplastic transformation, even before mutations in *NPM1*. This confirms that they are also the driver mutations [17]. At diagnosis, they occur in 64% of *NPM1*mutated patients, and in 95% of them they remain during relapse of the disease [18].

DNTM3A mutations are associated with the adverse prognosis and currently there is no therapy targeted to DNMT3A mutated protein. However, an experimental treatment aiming to in-

hibit histone 3 lysine 79 (H3K79) methyltransferase (DOT1L) was introduced. *DOT1L* is overexpressed when *CEBPA* is mutated (loss of function mutation) and inhibition of this expression leads to induction of apoptosis, cell cycle arrest and terminal differentiation of *DNMT3A* mutated AML cell line [17]. Except *DNMT3A* mutated AML, DOT1L inhibitor can be also used in other leukemias, such as *MLL-AF4* mutated ones [29].

5. Conclusions

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder with the presence of various genetic abnormalities in hematopoietic myeloid stem cells that lead to clonal proliferation of immature cells. Many of them impact the clinical outcome and help in patient stratification into prognostic groups. Mutations in *NPM1* and *CEBPA* genes are associated with favorable outcome, while mutations in *FLT3* or *DNMT3A* may change outcomes into intermediate or adverse ones. There are many clinical trials aiming at mutated genes or their products; however, their efficacy still requires many trials on large groups of patients to be fully proved.

Abbreviations

AML – acute myeloid leukemia, **DFS** – disease free survival, **EFS** – event free survival, **NGS** – next generation sequencing, **NK** – normal karyotype, **OS** – overall survival, **ROS** – reactive oxygen species, **SNV** – single nucleotide variant, **VAF** – variant allele frequency, **wt**– wild type.

Authors' Contribution

K.M.B.: writing – original draft preparation, literature review; **A.B.K.:** writing – review and editing, literature review; **O.H.:** final proofreading and approval of the version for publication, funding acquisition.

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Conflict of Interest

The authors have no potential conflicts of interest to declare.

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