Analysis of Gene Mutations and Prognosis in Acute Myeloid Leukemia

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ABSTRACT
Acute myeloid leukemia (AML) is a malignant tumor that begins in the bone marrow and swiftly spreads throughout the body. Low erythrocyte counts, high leukocyte counts, and low thrombocyte counts are common symptoms of AML patients. Chromosomal abnormalities, cytogenetic deficiencies, and gene mutations are harbored by many people with AML. In recent years, the importance of cytogenetic deficiencies in prognosis and treatment has been proven by many studies. By understanding the gene mutations in AML, we can be provided with new insights of the pathogenesis of acute myeloid leukemia and we can better refine our treatment methods of AML. In this analysis, we can know that, without FLT3-ITD mutations, NPM1 mutations can improve the prognosis of CN AML patients, but NPM1with FLT-ITD mutation may result in bad prognosis. Also, FLT3 mutations are linked to a higher recurrence risk and a lower survival rate. RAS mutations were linked to a lower survival rate in AML patients undergoing induction chemotherapy. Patients with AML1/ETO showed a higher CR rate and survival rate.

Keywords: *gene mutations, acute myeloid leukemia, prognosis, occurrence, pathology*

1. INTRODUCTION

Acute myeloid leukemia (AML), an aggressive disease, is genetically and phenotypically miscellaneous and complex.To understand the way of leukemogenesis in AML, scientists have made major progress. AML is a hematological disorder caused by the accumulation of numerous acquired genetic defects in chromosomes. Recently, researchers have identified a number of mutational changes in DNA of AML patients. In this paper, I describe contributions of several gene mutations to prognosis and their clinical significance. These mutations provide valuable prognostic information as well as possible treatment targets. The prevalent subtype classification system and the system of prognostication are challenged by the more and more new insight into the AML genetic architecture. I will further explore NPM1, FLT3, KIT, RAS, AML-ETO mutations in this review by analyzing their pathology,occurrence in males and females and in different ages, phenotypes, prognosis, and interaction with other gene mutations. By understanding them, doctors can provide patients with more targeted treatment by using corresponding medicines.

2. NPM1 MUTATION

The NPM1 gene, found on 5q35 chromosome, is a partner in chromosomal translocations that lead to fused proteins containing the N-terminal region of NPM1 in leukemias and lymphomas. Mutations of somatic cells in exon 12 of the NPM gene are the most prevalent mutation in adult AML (NPM1). They are found in almost 60% of AML patients who have a normal karyotype. NPM1 mutations are found in around 25%~30% of patients with AML and approximately 60%~85% of CN patients with AML. [1] A multitude of biological processes are involved in the gene product, including ribosome biogenesis, genome stability, DNA duplication, chromatin remodeling, and transcriptional control[1]. NPM1 also regulates the ARF/p53 pathway via interacting with several proteins found in the nucleolus and spindle apparatus . NPM is a generated phosphoprotein that travels between the nucleus and the cytoplasm and plays important role in the process of leukemogenesis because it is involved in multiple tumor-associated chromosomal translocations[2]. It is also hypothesized to have a tumor-suppressor function and to control the p53 pathway by chaperoning. The loss of function of nuclear NPM due to mutation may obstruct the p53 pathway, which may result in

instability of genes; hence, NPM1 mutations appear to cause AML cells to develop more genetic defects.

Females (27 percent, 14/51) were found to have the NPM1 mutation more frequently than males (18 percent, 20/110)[3]. Adults were found to have the NPM1 mutation more commonly than children (26 percent versus 9 percent)[3]. Further age stratification revealed that the frequency of NPM1 mutation increases with age, with 38 percent (12/32) of AML patients over 45 years of age having the highest frequency[3]. In comparedison to patients who did not have any NPM1 or FLT3 mutations, patients with NPM1 mutations had considerably greater platelet counts[3].

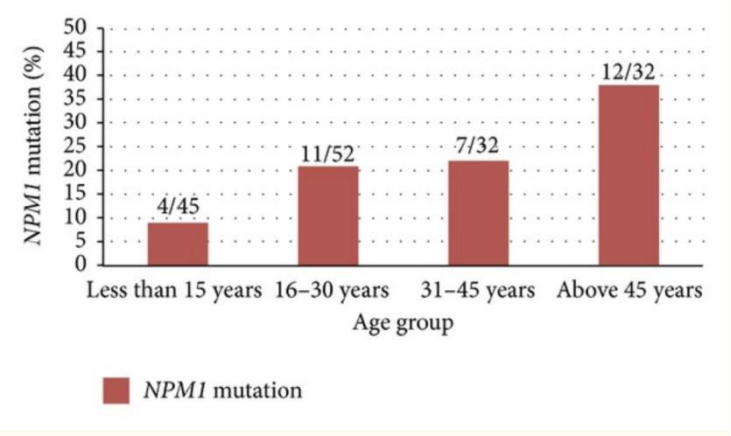

Figure 1 the incidence of NPM1 mutation changes with age[3]

The predictive value of NPM1 is influenced by the existence of other genetic changes. NPM1 mutations are frequently found in combination with other AML-related mutations, such as IDH1 and IDH2 (isocitrate dehydrogenase 1 and 2 (NADP+)), FLT3 (fms-related tyrosine kinase 3), and DNMT3A (DNA (cytosine-5-methyltransferase alpha). [1] Without concomitant FLT3 gene internal tandem duplications, mutations in the NPM1 gene may lead to a better prognosis in cytogenetically normal AML (ITD).[4]NPM1 mutations are linked to a better consequence and outcome for CN AML patients, especially in those over 60 yrs old, when FLT3-internal tandem duplication (ITD) mutations are absent. [1].Despite their different prognostic effects, with FLT3-ITD mutations, NPM1 mutations appear to promote AML cell proliferation, resulting to greater leukemia cell counts and indicating poor prognosis and C-KIT mutations are associated with bad outcomes in patients with t(8;21).

3. FLT-3 MUTATION

A mutation in FLT3, which is found in around one-third of newly diagnosed AML patients, is the most common genetic change. FLT3 internal tandem duplication mutations (FLT3-ITD) have been associated to a higher likelihood of recurrence and a shorter survival time.

FLT3 is a tyrosine kinase that belongs to the class III receptor tyrosine kinase family and generally expressed on the surface of hematopoietic progenitor cells. FLT3 and its ligand play a crucial role in multipotent stem cell survival, proliferation,and differentiation[5]. FLT3 is encoded by a gene that is found on 13q12 chromosome and contains 24 exons.Internal tandem duplication mutations in FLT3 (FLT3-ITD) are linked to a higher recurrence risk and a shorter survival time. FLT3-TKD mutations are typically point mutations at codon D835 or deletions of codon I836 in the activation loop of FLT3[6]. They are thought to be gain-of-function mutations because they cause constitutive tyrosine phosphorylation, which activates the receptor tyrosine kinase.

FLT3-ITD is a mutation that causes AML patients to have a significant leukemic load and a poor prognosis. The prognostic significance of a FLT3 mutation in the tyrosine kinase domain (FLT3-TKD), which occurs in a smaller percentage of AML cases (7 – 10%), remains unknown[7]. Patients with FLT3-ITD mutations have higher percentages of blood and bone marrow blasts, higher leukocyte counts, and a higher likelihood of being diagnosed with denovo rather than secondary AML before therapy[7]. The FLT3-ITD mutation is linked to a bad prognosis, a shorter remission time, and a higher recurrence risk.

FLT3-TKD mutations are found in 10% of all AML patients, both adults and children. Constitutive tyrosine kinase activation is also a result of these alterations. At codon 835, the most common TKD mutation occurs, changing aspartic acid to tyrosine (D835Y). D835V, D835E, and D835H variants are also observed[1]. In terms of physiologically transforming potency, TKD mutations differ from ITD mutations in FLT3. The influence of FLT3-TKD mutations on prognosis is still debated.

4. C- KIT MUTATION

The most common genetic abnormalities linked to gastrointestinal stromal tumors are mutations in the KIT gene (GISTs). GISTs are gastrointestinal tumor that most usually develops in the stomach or small intestine. C-kit expression can be found in myeloblasts and is found in 60~80% of AML patients. In addition, c-kit activation mutations are found in 12.8~46.1% of CBF leukemia adult patients. Exon 8 or 17 mutations are found in 20 – 25 percent of t(8;21) and 30 percent of inv(16) instances, respectively[8]. C-Kit is expressed by myeloblasts in 60-80 percent of patients, and c-Kit mutations or internal tandem duplications are the most often found activating RTK mutations in AML (second to FLT3), with a 17 percent overall incidence[8].

In adults with CBF-AML, Kit mutations may lead to recurrence and the growth of leukocyte. Mutations in exons 8 and 17 of the c-kit gene have been linked to a higher relapse rate and a poor prognosis[9]. In children, c-Kit mutations were found to be more strongly linked to a poor prognosis than FLT3 ITD[10]. c-Kit has a remarkable level of conservation across species, with some exons sharing up to 100% amino acid sequence homology. Besides the AML mutations, five c-Kit single nucleotide polymorphisms (SNPs) are well-known, with only a few of them causing amino acid sequence alterations[10]. Different kinds of further studies strengthened C-KIT mutations are associated to adverse outcomes in patients with t(8;21) but not in inv(16)/t(16;16) AML. [11] t(8;21) and inv(16) AML with KIT mutations are considered a moderate risk by the National Comprehensive Cancer Network (NCCN). Overexpression of wild-type c-Kit is a bad prognostic indicator for the survival rate in adults, according to Advani et al. However, how c-Kit polymorphisms, epigenetics, and aberrant expression influence AML pathology and prognosis is still unknown.

5. RAS MUTATION

RAS mutations are found in 12~27% of AML patients and improve cytarabine sensitivity in vitro. RAS mutations are commonly acquired during the development of MDS to AML and are linked to a poor prognosis[12]. Induction chemotherapy leads to a higher clearance rate in patients with RAS mutation, according to next generation sequencing (NGS) at diagnosis and during complete remission[12].

Several investigations have found that the RAS mutation has no value in the prognosis of CN AML patients. RAS mutations, on the other hand, have been linked to a bad prognosis by certain studies[1]. In the favorable risk population of pediatric AML, activation of NRAS mutations usually occur in tandem with mutation of NPM1. RAS mutations are more likely to arise with NPM1 mutations, whereas RAS mutations in the presence of CEBPA, WT1, or FLT3-ITD mutations appear to be less prevalent[1].

RAS mutations were linked to a lower survival rate in AML patients who were undergoing induction chemotherapy[12]. Patients with AML-MRC and past myeloid neoplasms were more likely to have RAS mutant AML, which was linked to a lower survival rate[12]. Chemotherapy treatment resulted in a high incidence of RAS mutation clearance in responders, which lasted until the disease relapsed[12]. Because RAS mutant AML has a poor prognosis despite RAS mutation clearance, new therapies other than chemotherapy are needed to improve outcomes in this high-risk population[12].

6. AML-ETO

One of the most common AML chromosomal abnormalities is the chromosomal abnormality t(8;21) (AML). An AML1-ETO fusion transcription factor is created when the ETO gene on chromosome 8 and the AML1 gene on chromosome 21 are translocated. Although reverse transcriptase polymerase chain reaction can only detect the AML1-ETO transcript transcribed from the derivative 8 chromosome, the t(8;21) mutation can result in the expression of two fusion genes, AML1-ETO and ETO-AML1 (RT-PCR)[14]. The most prevalent aberration that leads to AML1-ETO fusions is simple reciprocal translocation, however the fusion can also be caused by variant rearrangements. Insertion can also result in AML1-ETO fusion, and both ins(21;8) and ins(21;8) (8;21)[15].

By its zinc finger domain, ETO have interactions with a conserved domain of the corepressors NCoR and SMRT in the context of the AML1/ETO fusion protein, thereby recruiting the histone deacetylase (HDAC) complex in vivo [13]. The C-terminus of ETO is deleted, which prevents NCoR binding and HDAC recruitment, as well as AML1/ability ETO's to inhibit hematopoietic differentiation[13]. The 31 amino acids lacking from the AML1/ETO fusion protein's N-terminus are not known to be part of a functionally important protein domain[13]. Between AML1 exon 5 and ETO exon 2, the t(8;21) translocation creates a conventional genomic breakpoint[13].

AML1/ETO fusion transcripts were found in 22.0 percent of AML patients and 44.8 percent of AML-M2 patients[16,17]. In patients with AML1/ETO positive, the morphologic findings of bone marrow revealed a greater incidence of Auer rods, large blasts with prominent golgi, and aberrant granules.

	AML			AML-M2		
	positive (n=13)	negative (n=46)	*p*	positive (n=13)	negative (n=16)	*p*
Age (median)	42	45	0.60	42	47	0.38
Sex (M:F)	8:5	24:22	0.55	8:5	9:7	0.77
Laboratory (mean)						
WBC ($\times 10^3$/L)	19.2	50.0	0.34	19.2	20.7	0.30
Hemoglobin (g/dL)	6.0	7.0	0.12	6.0	7.1	0.16
Platelet ($\times 10^3$/L)	45.3	58.0	0.15	45.3	76.1	0.18
Blast % in BM (median)	58%	80%	0.06	58%	60%	0.65

Figure 2: Comparison of age,sex, WBC, hemoglobin, platelet and blast in BM between AML1-ETO positive and negative group[17]

Clinically, AML patients with t(8;21) is more common in young adults and has a better prognosis than other AML patients. Patients with AML1/ETO showed a higher CR rate, a longer overall survival and progression-free survival than those without AML1/ETO[17], AML-M2 patients with AML1/ETO rearrangement had a trend for longer overall survival and progression-free survival, despite the fact that there was no significant statistical difference between the two groups. As a result, AML1/ETO rearrangement is frequently found in AML, particularly M2, and is a good prognostic indicator[18].

7. CONCLUSION

Acute myeloid leukemia (AML) is a varied group of neoplastic illnesses with a wide range of therapeutic responses, as well as a genetic and molecular basis. There has been major progress made to comprehend the leukemogenesis and genetic architecture in AML. By analyzing the pathophysiology of each mutation, scientists can get more important prognostic information and potential therapeutic targets. Different mutations induce and affect pathology of AML in different ways. Some mutations indicate higher CR rate and a higher survival rate in contrast to other mutations. However, there are is still some mutations' pathology which we haven't figure out.

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