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Review Paper

PREVALENCE OF FLT-3 GENE MUTATION IN ACUTE MYELOID LEUKEMIA

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Abstract

Acute myeloid leukemia is a cancer of the myeloid line of blood cells, characterized by rapid growth of abnormal white blood cells that accumulate in the bone marrow and consequently interfere with the production of normal blood cells. FLT3 gene is the most frequently mutated gene in AML. Prevalence of internal tandem duplication (ITD) of the juxtamembrane domain-coding sequence and a missense mutation of D835 within the kinase domain of the FLT3 gene is associated with acute myeloid leukemia (AML) in adults, and vary from 20-35% and 4-10% respectively. Other types of mutations, though less common, such as point mutations, deletions, and insertions have also been found in the juxtamembrane domain and in other codons within the kinase domain. The studies conducted so far in patients with AML have shown that FLT3 mutations are strongly associated with poor prognosis and a high leukemia cell count, suggesting that FLT3 mutations are involved in disease progression.

Key words: Leukemia, prevalence, FLT3 mutation, internal tandem duplication, prognosis.

INTRODUCTION

Acute myeloid leukemia also known as acute myelogenous leukemia, is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age. Although AML is a relatively rare disease, accounting for approximately 1.2% of cancer deaths in the United States [1]. Acute myeloid Leukemia is characterized by an increase in number of myeloid cells in the bone marrow and an arrest on their mutation. These immature myeloid cells are called blasts, accumulate and rapidly replace bone marrow, resulting in decreased production of red cells, white cells and platelets [2]. Acute myeloid leukemia (AML) is a heterogeneous malignant hematopoietic disorder characterized by clonal expansion of immature myeloid cells in the bone marrow, blood or other organs. The affected cells underlay an uncontrolled proliferation and impaired differentiation program. Typically, the cells are blocked at various maturation steps and are resistant to cell death [3].

FLT3 gene: The human FLT-3 gene is over 1,000 kilobases in length and is composed of 24 exons located on chromosome 13 (13q12).The FLT3gene encodes a 993-amino acid protein that is observed as a major 140-kDa band and a minor 160-kDa band because o N-linked glycosylation and a 130-kDa band when unglycosylated and not membrane bound [4]. FLT3 plays an important role in proliferation, differentiation and apoptosis of normal hematopoietic cells. In normal cells, expression of FLT3 occurs mainly in early myeloid and lymphoid progenitorsal and not in erythroid cells, megakaryocytes or mast cells [5].

FMS-LIKE tyrosine kinase receptor: The extracellular signal proteins that act through receptor tyrosine kinases consist of a large variety of secreted growth factors and hormones. The human genome as currently sequenced, is thought to contain 90 tyrosine kinase genes, of which 58 are of the receptor type. Receptor tyrosine kinases can be classified into more than 20 structural families, each dedicated to its complementary family of protein ligands. One such family is the Class III receptor tyrosine kinases, members of which have been have been found to be mutated or overexpressed in the patients with AML. This class includes FMS, KIT, FLT3, PDGFRα and PDGFRβ [6]. Structurally, the RTK family, is composed of an extracellular domain that consists of five immunoglobulin-like repeats, a trans-membrane domain (TM), a juxtamembrane domain (JM), two intracellular tyrosine kinase domains (TK1 and TK2) divided by a kinase insert domain (KI) and a C-terminal domain. FLT3 is primarily expressed in haematopoietic stem cells, where it is thought to play an important role in haematopoiesis. Ligand binding to the receptor causes its dimerisation and activation, leading to receptor autophosphorylation, which is followed by induction of multiple intracellular signaling pathways, which are involved in cell proliferation and activation. Hence alteration to the structure and/or expression of RTKs can result in tumorigeneis [7, 8].

FLT3 mutations in AML: FLT3 (FMS-related tyrosine kinase 3) is a receptor tyrosine kinase class III that is expressed on by early hematopoietic progenitor cells and plays an important role in hematopoietic stem cell proliferation, differentiation and survival. Two different types of functionally important FLT 3 mutations have been identified. The high frequency of the FLT3 proto-oncogene mutations in acute myeloid leukemia AML suggests a key role for the receptor function [9]. FLT3 mutations have been reported to be the most frequent mutation in acute myeloid leukemia (AML) [10]. About 30 to 35% of patients have either internal tandem duplications (ITDs) in the juxtamembrane domain or mutations in the activating loop of FLT3 [11]. FLT3 is expressed at high levels in a spectrum of hematologic malignancies including 70% to 100% of acute myelogenous leukemia (AML) of all FAB subtypes, B-precursor cell acute lymphoblastic leukemia (ALL), a fraction of T-cell ALL, and chronic myelogenous leukemia (CML) in lymphoid blast crisis [12]. Both FLT3 over expression and activating mutations in the FLT3 gene can be found in patients with AML. Three distinct activating mutations of FLT3 in haematological malignancies have been reported: point mutations (FLT3-JM-PM) and FLT3-internal tandem duplications (FLT3-ITD) found in the JM domain are present in 2% and 20-25% of AML patients respectively, whereas, mutations in the tyrosine-kinase domain (FLT3-TKD) represent 7-10% of AML patients [4]. The prevalence of internal tandem duplication (ITD) of the juxtamembrane domain-coding sequence and a missense mutation of D835 within the kinase domain of the FLT3 gene is 15-35% and 5-10% of adults with acute myeloid leukemia (AML), respectively [13]. In addition, point mutations, deletions, and insertions have been found in the juxtamembrane domain and in the other codons within the kinase domain, though these are less common. Several large-scale studies in well-documented patients published to date have demonstrated that FLT3 mutations are strongly associated with a poor prognosis and a high leukemia cell count in patients with AML, suggesting that FLT3 mutations are involved in disease progression [13].

Mutations in the FLT3 gene have also been shown in several studies to be a strong prognostic factor in both pediatric and adult AML. The most common is the internal tandem duplication, a head-to-tail duplication of 20 to 300 base pairs in the exons coding for the juxtamembrane region of the molecule. This structural change alters the biology of the FLT3 receptor from normally signaling through ligand-dependent dimerization to that of ligand-independent

activation. Tyrosine kinase activation of FLT3 also occurs in 5% to 10% of patients through point mutations in the activating loop of the kinase domain of the molecule [14]. An in-frame internal tandem duplication (ITD) mutation in the JM domain of the FLT3 receptor correlates with the highest frequency of FLT3 related AML cases. Clinical studies identify the FLT3-ITD mutation in 17%–26% of AML cases [15]. The ITD was first identified when screening primary leukemia specimens for FLT3 expression, through reverse transcriptase polymerase chain reaction (PCR), produced amplification products that were longer than expected in multiple patients. DNA sequencing revealed that the insertional mutation occurs within exon 14 and varies in length from 3 to 400 base pairs [16]. Internal tandem duplication of the FLT3 gene (FLT3/ITD) has been discovered in myelodysplastic syndrome (MDS) with frequency of 3–15%, respectively.The FLT3/ITD is a somatic event since no mutation was found in normal hematopoietic cells or matched remission samples of AML patients harboring FLT3/ITD. The function of FLT3/ITD has not been fully elucidated. A recent study demonstrated that elongation of the JM domain causes ligand-independent dimerization of the mutant FLT3 receptor, resulting in a constitutive activation [17].

The exact mechanism for the formation of ITDs is unknown, both slippage of the replication machinery and a failure in a mismatch repair mechanism has been proposed [18]. FLT3 internal tandem duplication (FLT3/ITD) is a common somatic mutation in acute myeloid leukemia (AML) with significant variation in the position, length, and number of duplications of the FLT3 gene [19]. Most newly diagnosed AML with FLT3 dual mutations had monocytic differentiation and a normal karyotype. Over the disease course, changes in FLT3 mutation status are seen in 89% of cases, and are associated with cytogenetic changes. FLT3 dual mutations occur rarely in AML, and appear to be related to clonal evolution [20]. The presence of the mutation in AML is associated with adverse prognosis in terms of increased risk of disease relapse. The majority of mutations (25–35% of AML patients) occur as internal tandem duplication (ITD) within the juxtamembrane region. A further 3-7% of patients have a mutation within the tyrosine kinase domain (TKD) of FLT3. The type of mutation, and its population size, may have prognostic implications. In this study the ITD in 19.9% of the 291 AML samples was detected, with the size of the ITD varying from 15–212 bp (median 41 bp) [21]. Internal Tandem Duplication (ITD) of the juxtamembrane domain is one of the most common genetic alterations in acute myeloid leukemia (AML) and in some FAB subgroups seems to represent an unfavorable prognostic factor. Thus, its correct identification is critical. In this study 261 AML cases were analyzed to individuate FLT3-ITD by RT-PCR and compared different techniques (agarose and poly acrilamide gel electrophoresis, sequence and Gene scan of PCR products) to define FLT3-ITD presence, length and number. Gene scan analysis was used to confirm the presence and the length of the ITD and to study the rate between ITD/WT transcripts. In their series they found 20% of positive cases, 7.5% of those lacked FLT3 wild-type transcript and 13.2% showed two different FLT3-ITDs. In addition they identify 2 cases carrying 2 FLT3-ITD with the same length but different nucleotide sequence [22]. In acute myeloid leukemia (AML), two clusters of activating mutations are known in the FMS-like tyrosine kinase-3 (FLT3) gene: FLT3-internal tandem duplications (FLT3-ITDs) in the juxtamembrane (JM) domain in 20% to 25% of patients, and FLT3 point mutations in the tyrosine-kinase domain (FLT3-TKD) in 7% to 10% of patients, respectively [23].

Conclusion: Mutation in the FLT3 gene plays a very important role in the progression of acute myeloid leukemia. Several types of point mutations, deletions and insertions have been reported in AML. The prevalence of internal tandem duplication varies from 20 to 35%, whereas point mutations vary from 4 to 10% in acute myeloid leukemia patients.

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