Prognostic significance of mutated genes in megakaryocytic disorders

Ali Amin Asnafi, Mohammad bagher Mohammadi, Hadi Rezaeeyan, Nader Davari, Najmaldin Saki

Thalassemia and Hemoglobinopathy Research Center, Research Institute of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Abstract

Megakaryopoiesis is a process during which platelets that play a major role in hemostasis are produced due to differentiation and maturation of megakaryocytic precursors. Several genes, including oncogenes and tumor suppressor genes, play a role in the regulation of this process. This study was conducted to investigate the oncogenes and tumor suppressor genes as well as their mutations during the megakaryopoiesis process, which can lead to megakaryocytic disorders. Relevant literature was identified by a PubMed search (1998-2019) of English language papers using the terms 'Megakaryopoiesis', 'Mutation', 'oncogenes', and 'Tumor Suppressor'. According to investigations, several mutations occur in the genes implicated in megakaryopoiesis, which abnormally induce or inhibit megakaryocyte production, differentiation, and maturation, leading to platelet disorders. GATA-1 is one of the important genes in megakaryopoiesis and its mutations can be considered among the factors involved in the incidence of these disorders. Considering the essential role of these genes (such as GATA-1) in megakaryopoiesis and the involvement of their mutations in platelet disorders, study and examination of these changes can be a positive step in the diagnosis and prognosis of these diseases.

Introduction

Megakaryopoiesis is as a process in which bone marrow (BM) progenitors are progressively differentiated into megakaryocytes and finally platelets. Several genes, including oncogenes and tumor suppressors, are involved in the process of megakaryopoiesis. Oncogenes and tumor suppressors exert their effects via regulating the expressions of cell surface receptors, growth factors, cytokines, signaling molecules, transcription factors, as well as controlling the cell cycle and apoptosis. Oncogenes are the genes that increase cell proliferation and decrease or even inhibit cell apoptosis, so that any mutation and dysfunction in these genes may lead to excessive proliferation of malignant cells and potentiate the activity of these cells. In contrast, tumor suppressors are the genes that reduce proliferation of cells, increase their differentiation and induce apoptosis in a normal situation, which can lead to elimination or decreased activity of tumor cells. However, any disturbance in the expression or function of these genes (e.g., mutation) may interfere with the production of various cells, including hematopoietic cells. Impaired production of hematopoietic cells disrupts the generation of cells differentiated from them, including erythrocytes, leukocytes, and platelets. It is worth noting that mutations occur in each of these tumor suppressor genes, which contribute to the diversity of their function as well as effect on the process of megakaryopoiesis, predisposing people to certain diseases. In this review, we examine the mutations in genes effective upon megakaryopoiesis, their role as tumor suppressors or oncogenes, and their effect upon platelet production process and related diseases.

Oncogenes in normal and mutated situation and their effect on megakaryopoiesis

GATA-1

GATA-1 is an important transcription factor in maturation and differentiation processes of different hematopoietic lineages, especially megakaryocytes. It has two zinc finger motifs, one at C-terminal and the other at N-terminal. The latter plays a role in GATA-1 binding to DNA, as well as in the interaction with Friend Of GATA-1 (FOG-1) cofactor and the formation of GATA-1: FOG-1 complex. FOG-1 is a protein with zinc finger motif interacting with GATA-1 to induce the maturation and differentiation of megakaryocytic series. Therefore, the defective function of GATA-1 or FOG-1, i.e., impaired interaction of these two molecules can be a function of mutation in either of these two factors (especially GATA-1), leading to megakaryocytic disorders such as X-linked thrombocytopenia. Several variants occur due to a
mutation in GATA-1, each somehow affecting GATA-1 function and thus platelet function, including V205M that results from a missense mutation in C-terminal of GATA-1 (Val205Met).11 Val205 is a highly conserved amino acid, which plays an essential role in binding and interaction of GATA-1: FOG-1; therefore, mutation in this amino acid reduces GATA-1 binding affinity to FOG-1 and eventually impairs the production, maturation, and differentiation of megakaryocytic lineage via inhibiting the normal function of GATA-1. V205M mutation also leads to platelet disorders such as macrothrombocytopenia, which indicates the important role of GATA-1: FOG-1 complex in the production of platelets.10,11 Another variant, namely D218G that leads to macrothrombocytopenia is a function of mutation in C-terminal GATA-1, which reduces GATA-1 affinity to FOG-1 but does not inhibit GATA-1: DNA binding.12,13 Asp218 amino acid may be converted to Tyr due to mutation, which leads to D218Y variant and is quite similar to D218G in terms of mutation site. The difference between the two mutations lies in the severity of clinical symptoms among patients, so that the severity of clinical complications is higher in D218Y because GATA-1 affinity to FOG-1 is lower in D218Y than D218G but is similar to V205M. It is worth mentioning that macrothrombocytopenia resulting from D218Y is more severe than D218G.11,13 The next variant, which is a function of mutation in codon 208 of C-terminal GATA-1, is called G208S and leads to the conversion of Gly to Ser.7 Following this mutation, the interaction of FOG-1: GATA-1 is also disrupted due to the reduction of GATA-1 affinity to its cofactor because Gly208 plays a crucial role in binding to FOG-1 and is therefore essential for the production of normal platelets.9 This mutation also leads to macrothrombocytopenia.14 Another mutation may occur in codon 208 converting Gly to Arg and resulting in the incidence of G208R. The difference between G208S and G208R mutations is that the latter interferes with and reduces GATA-1: DNA binding in addition to decreasing GATA-1 affinity to FOG-1. It follows from the above explanations that the clinical symptoms of patients harboring G208R mutation are more severe than those of G208S, and these patients will be afflicted with severe thrombocytopenia of macrothrombocytopenia type.7 R216Q is another variant of GATA-1 that is derived from a missense mutation in C-terminal GATA-1 in which Gln replaces Arg.8,15 Unlike previous mutations, in this case GATA-1: FOG-1 interaction is normal and only GATA-1 binding to its site on DNA is disrupted. Patients bearing this mutation have beta-thalassemia as well as macrothrombocytopenia.11 Indeed, R216Q mutation is associated with X-Linked Thrombocytopenia with Thalassemia (XLT).16 The binding mechanism of GATA-1: FOG-1 from a molecular perspective is as follows. In normal conditions, the amino acid of interest (e.g. Val205) is phosphorylated by AKT (a serine-threonine kinase), GATA-1 binding affinity to FOG-1 is increased, and megakaryocytic lineage is normally proliferated. However, following a mutation in GATA-1 (such as V205M), the amino acid is not phosphorylated by AKT, resulting in disrupted formation of GATA-1: FOG-1 complex17 (Table 1).35 As discussed, GATA-1 plays a central role in megakaryopoiesis, and mutation in GATA-1 can lead to the incidence of megakaryocytic disorders. The question that arises here is whether GATA-1 can be used for the diagnosis or prognosis of megakaryocytic disorders (Figure 1).

Myeloproliferative leukemia protein

Myeloproliferative leukemia protein (MPL) is a member of Cytokine Receptor Superfamily, which plays an essential role in platelet production by inducing proliferation and maturation along with its ligand of Thrombopoietin (TPO).18,19 To date, a majority of MPL gene mutations have affected the amino acids in Transmembrane or Extracellular Domains (such as Ser or Arg). W508S is a new MPL variant, which, unlike what stated up to now, is the result of a mutation in Intracellular Domain leading to Ser substitution for Trp. Intracellular signaling pathways like JAK-STAT and PI3K-AKT-Bad are activated because of this mutation.18 As JAK-STAT (JAK2 and STAT-3 and STAT-5) signaling pathway is involved in the induction of proliferation and maturation of megakaryocytes,20 the occurrence of W508S mutation may lead to increased production of platelets and thrombocytosis. Megakaryocytic progenitors harboring MPL-W508S are only able to proliferate but cannot differentiate.19 S505N is another variant resulting from a point mutation converting Ser into Asn. The incidence of this variant relaxes the inhibitory effect on TPO gene and hence increases the translation and expression of TPO gene, leading to increased serum TPO and thus enhanced platelet production.19 R102P is another mutation with a negative inhibitory effect on megakaryopoiesis, which has been identified in Congenital Amegakaryocytic Thrombocytopenia21 (Table 1).

Friend leukemia virus integration-1

Friend leukemia virus integration-1 (FLI-1) is a transcription factor and member of E-twenty-six (ETS) (11q24.3) family.22,23 Normal expression of FLI-1 plays an important role in the production and evolution of megakaryocytes; in other words, it induces the expression of some genes involved in megakaryocyte maturation and differentiation, including GP6, c-mpl, GP9, and ITGA2B.24,25 Although a heterozygous mutation has been recently discovered in FLI-1 leading to thrombocytopenia,22 most disorders causing the impairment and defective function of FLI-1 are a function of a homozygous mutation or deletion in 11q chromosome. This heterozygous mutation is a missense mutation, which commonly occurs in codon970 of the gene and leads to the conversion of Arg to Trp in FLI-1 DNA-Binding domain.26 As a result, the transcriptional activity of FLI-1 target genes such as GP6, c-mpl, GP9, and ITGA2B decreases and subsequently leads to impaired production of platelets.24 In general, mutation in FLI-1 causing impairment or loss of its function leads to Inherited Thrombocytopenia, especially Jacobson Syndrome and Paris-Trousseau Syndrome. As noted above, FLI-1 is located on 11q chromosome23 and most patients with Paris-Trousseau Syndrome lack FLI-1;24 therefore, to achieve targeted therapy for differential diagnosis of this syndrome or to find the genetic defect of this disease, chromosome 11 (especially its long arm) can be assessed, or this molecule can be considered as a diagnostic factor of the disease.

Other oncogenes

Myeloblastosis (myb) is a gene downregulating the platelet production process.22 Also, thrombopoietin (TPO) gene induces megakaryopoiesis, which leads to platelet production in physiologic conditions.23 One of the effective protooncogenes in megakaryopoiesis is C-myc, which downregulates megakaryopoiesis and any mutation in this gene can lead to platelet disorders such as thrombosis.24 The last protooncogene we discuss is LNK (SH2B3) that inhibits cell proliferation by downregulating cytokine signaling25 (Table 1).

Tumor suppressor genes in normal and mutated situation and their effect on megakaryopoiesis

P53

P53 is an essential tumor suppressor transcription factor, which
is activated in conditions such as stress or hypoxia in the cell, leading to the cessation of cell cycle, inhibition of cell proliferation, and finally apoptosis. The activity of P53 is increased in later stages of megakaryocyte differentiation, leading to endomitosis arrest via suppression of polyplody and thus the onset of apoptosis. Therefore, no megakaryocytic apoptosis occurs due to mutation in the gene of this transcription factor, which can lead to aneuploidy or polyploidy.\textsuperscript{27-29} Therefore, it may be concluded that the absence or dysfunction of P53 results in unchecked production and proliferation of platelets, which can lead to platelet disorders.

**ETS variant 6**

ETS Variant 6 (ETV6) is a transcription factor that inhibits the proliferation, differentiation, and maturation of megakaryocytes by controlling the activity of ETS, including FLI-1. Mutation in ETV6 results in impaired platelet function and problems such as thrombocytopenia or megakaryocyte hyperplasia.\textsuperscript{24,25,30} In one study, after examining three groups of patients with thrombocytopenia, a missense mutation was observed in ETV6, which led to three types of amino acid translocations in ETS binding domain of ETV6 gene, including Arg399Cys, Arg369Gln, and Pro214Leu. In the first two variants, amino acids playing a role in DNA binding change and thus interfere with the binding and interaction of ETV6 with DNA, and in the third one, Internal Linker Domain involved in DNA binding is disrupted. In other words, this mutation in ETV6 impairs its binding to DNA and reduces the effect of trans-

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**Table 1. Common mutated genes that lead to platelet disorders.**

| Gene     | Chro. | Variant       | Function                              | Disease                  | Method        | Ref.             |
|----------|-------|---------------|---------------------------------------|--------------------------|---------------|------------------|
| GATA-1   | Xp11.23 | V205M         | GATA-1:FOG-1 interaction              | Macrothrombocytopenia    | Nested PCR    | 6,7,10,11,13,14,16 |
|          |       | D218G         |                                       |                          | PCR           |                  |
|          |       | D218Y         |                                       |                          | PCR           |                  |
|          |       | G208S         |                                       |                          | PCR           |                  |
|          |       | G208R         |                                       |                          | PCR           |                  |
|          |       | R216Q         | GATA-1-DNA binding                    |                          | PCR           |                  |
| MPL      | 1p34.2 | W508S         | Activates the signal transduction pathways | Thrombosis              | PCR           | 18,19,21         |
|          |       | S505N         | Removes the inhibition on TPO         | Excessive platelets production | GMT           |                  |
|          |       | R102P         | Decreases megakaryopoiesis           |                          |               |                  |
| Myb      | 6q23.3 | -             | Downregulation of megakaryopoiesis    | -                        | RT-qPCR       | 32               |
| TPO      | 2p25.3 | -             | Upregulation of megakaryopoiesis      | Thrombocytopenia         | -             | 33               |
| C-myc    | 8q24.21| -             | Downregulation of megakaryopoiesis    |                          | RT-PCR        | 34               |
| LKN (SH2B3) | 12q24.12 | R262W       | Downregulation of cytokine signaling and cell proliferation | Excessive platelets production | RT-qPCR       | 35               |

**Figure 1.** GATA-1 is an important transcription factor in megakaryopoiesis. It stimulates MKs maturation and differentiation through binding to its cofactor called FOG-1. GATA-1:FOG-1 interaction causing multipotent progenitor cell differentiation to committed megakaryocyte progenitor cell and then platelet production. Several variants occur due to mutation in GATA-1, each somehow affecting GATA-1 function and thus platelet function, including V205M, D218G, D218Y, G208S, G208R and R216Q. The first five variants cause macrothrombocytopenia and the last one cause XLTT. MKs, megakaryocytes; FOG-1, friend Of GATA-1; V, valine; M, methionine; D, aspartic Acid; G, glycine; Y, tyrosine; S, serine; B, arginine; Q, glutamine; XLTT, X-linked thrombocytopenia with thalassemia.
scriptional inhibitors, leading to disruption of hematopoiesis (Figure 2).  

Discussion

Several genes control the process of megakaryopoiesis, some of which have oncogenic properties (such as GATA-1 and MPL) and others are tumor suppressive (e.g., P53 and ETV-6). However, any mutation in such genes leading to increased or decreased expression of them affects megakaryopoiesis and thus platelet production. GATA-1 is one of the most important genes involved in megakaryopoiesis, which interact with FOG-1 to induce megakaryocytic differentiation and maturation, so that any mutation and emergence of different variants upset megakaryopoiesis and platelet production. V205M, G208R, G208S, and R216Q are among these variants. V205M is a missense mutation decreasing GATA-1 affinity to FOG-1, G208S variant disrupts the formation of GATA-1:FOG-1, whereas G208R decreases GATA-1 binding to DNA in addition to the reduction of GATA-1:FOG-1 binding, and finally R216Q variant leads to platelet disorders through disruption in GATA-1:DNA binding. It is notable that V205M, G208S and G208R cause X-Linked Thrombocytopenia (XLT) with increased Mean Platelet Volume (MPV) and that R216Q causes X-Linked Thrombocytopenia with Thalassemia (XLTT). MPL is another gene that plays an essential role in megakaryopoiesis. In contrast to GATA-1, mutations of MPL cause disorders associated with increased platelet counts (thrombosis). Considering the content discussed in this paper, it is likely to hypothesize that since the variants of genes involved in megakaryopoiesis (especially GATA-1) play a crucial role in the incidence of platelet disorders, the study of these variants in patients (even when the patient has other symptoms such as erythroid disorders as well as platelet disorders) is useful and contributes to the diagnosis and prognosis of these diseases.

Conclusions and future perspective

The current study reveals the importance of detection and investigation of genes mutations such as oncogenes (e.g., GATA-1 and MPL) and tumor suppressor genes (e.g., P53 and ETV-6) for diagnosis and prognosis of platelet disorders. Thus, according to what we discussed in this paper, we may take a few steps forward in diagnosis and treatment of these patients; however, further studies are need to reach that goal.

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Figure 2. Oncogenes and tumor suppressor genes can up- or downregulate the process of platelet production. Some of them are called oncogenes, which stimulate and increase the production of platelets such as GATA-1, MPL, and FLI-1, while the tumor suppressor genes like P53 and ETV-6 regulate and decrease platelet production. MPL, myelo proliferative leukemia protein; FLI-1, friend leukemia virus integration-1; ETV-6, E-twenty-six variant 6.
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