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Hematopoietic transcription factor mutations and inherited platelet dysfunction

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Abstract

The molecular and genetic mechanisms in most patients with inherited platelet dysfunction are unknown. There is increasing evidence that mutations in hematopoietic transcription factors are major players in the pathogenesis of defective megakaryopoiesis and platelet dysfunction in patients with inherited platelet disorders. These hematopoietic transcription factors include RUNX1, FLI1, GATA-1, and GFI1B. Mutations involving these transcription factors affect diverse aspects of platelet production and function at the genetic and molecular levels, culminating in clinical manifestations of thrombocytopenia and platelet dysfunction. This review focuses on these hematopoietic transcription factors in the pathobiology of inherited platelet dysfunction.

Introduction

In most patients with inherited platelet dysfunction, the underlying molecular and genetic mechanisms remain unknown. Previous paradigms have focused on abnormalities in the 'end' responses of platelet aggregation and secretion studies and the investigation of postulated abnormal pathways and proteins. These approaches have been driven by existing knowledge of platelet mechanisms and come with limitations. At the genetic level, the focus has largely been on delineating mutations in the coding sequence of genes encoding the candidate proteins. Evidence is now available that in some patients with inherited platelet dysfunction the primary abnormality is a mutation in a hematopoietic transcription factor (TF), which can lead to altered downstream expression of numerous genes that affect diverse cellular pathways and can result in abnormalities in both platelet number and function [1,2].

TFs regulate lineage-specific gene expression through binding of cis-regulatory sequences. Major hematopoietic TFs include the Runt-related transcription factor 1 (RUNX1), friend leukemia integration 1 (FLI1), GATA-binding factor 1 (GATA-1), and growth factor independent 1B (GFI1B); these TFs act in a combinatorial manner to regulate hematopoietic lineage differentiation, megakaryopoiesis, and platelet production [3]. TF mutations may be more common in patients with inherited platelet dysfunction than previously considered. For example, Stockley and colleagues [2] recently reported results of next-generation sequencing studies in 13 unrelated patients suspected of having an inherited platelet defect from the UK Genotyping and Phenotyping of Platelets (UK-GAPP) study. Heterozygous *RUNX1* or *FLI1* mutations were uncovered in 6 of the 13 patients with excessive bleeding and impaired dense granule secretion and aggregation on activation; 5 of these patients also had thrombocytopenia. These findings highlight the importance of TF mutations in the pathogenesis of inherited platelet function defects. This review focuses on the TF mutations implicated in these disorders.

RUNX1

RUNX1—also known as core-binding factor subunit alpha-2 (CBFA2) and acute myeloid leukemia 1

(AML1)—is a critical hematopoietic TF required for definitive hematopoiesis encoded by the *RUNX1* gene located on chromosome 21 (21q22.12) [4]. In a murine model, generation of homozygous *RUNX1* mutants was lethal *in utero* because of hemorrhage [5]. In humans, heterozygous *RUNX1* mutation is associated with an autosomal dominant disorder, the familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML) (Mendelian Inheritance in Man [MIM] 601399), characterized by impaired megakaryopoiesis, quantitative and qualitative defects in platelet function, and over 40% risk of development of myelodysplastic syndrome (MDS) or AML at a median age of 33 years [6–9]. Several distinct *RUNX1* mutations, ranging from point mutations to deletional mutations, have been identified in patients with FPD/AML, and most are in the conserved Runt domain near the N-terminus, resulting in impaired binding of RUNX1 to cis-regulatory DNA sequences. In addition to the Runt domain, a mutation in the C-terminal transactivating domain (Y260X) has been identified [10]. Most *RUNX1* mutations result in haplodeficiency, whereas some mutations may produce dominant-negative activity that has been proposed to increase leukemia risk [7,10,11]. Interestingly, several syndromic cases of deletion of chromosome 21q22 including *RUNX1* have also been described, and affected individuals may have congenital thrombocytopenia and platelet dysfunction but develop MDS or AML at a much lower age (three cases ranging from 5 to 8 years) than observed in FPD/AML [7].

Numerous platelet abnormalities have been reported in patients with *RUNX1* mutation, including dense or α-granule storage pool deficiency (SPD) or both, impaired platelet responses of aggregation and secretion, reduced protein phosphorylation of myosin light chain and pleckstrin, and decreased activation of αIIbβ3 [1,9,10,12]. Platelet production of 12-hydroxyeicosatetraenoic acid and one specific protein kinase C isoform (PKC-θ) have also been shown to be decreased [12,13].

Platelet granule deficiency leading to impaired platelet function is an important abnormality associated with *RUNX1* mutations. In 1969, Weiss and colleagues [14] described one of the first families with inherited platelet dysfunction due to reduced platelet ADP and ATP, indicating a dense granule SPD. This affected family and some others described with SPD of dense or α-granules were later shown to carry *RUNX1* mutations [10,15]. Other studies have also shown decreased α-granule contents in association with *RUNX1* mutations [10,16]. In one patient, platelet albumin and IgG, two constituents of the α-granule, were decreased [12], which suggests a possible defect in uptake and storage of these proteins into α-granules because neither protein is synthesized by megakaryocytes (MKs).

RUNX1 influences multiple genes involved in MK differentiation [3]. Platelet transcript profiling of a patient with *RUNX1* haplodeficiency has shown numerous genes relevant to multiple pathways to be downregulated [17]. Several of these genes with prominent roles in platelet structure and function have been shown to be direct transcriptional targets of RUNX1. These genes include *ALOX12* (12-lipoxygenase) [13], *PF4* (platelet factor 4) [16], platelet *MYL9* (myosin light chain) [18], and *PRKCQ* (protein kinase C-theta) [19]. Low expression of *c-MPL* (thrombopoietin receptor) in *RUNX1* mutation has been documented, providing an additional mechanism for thrombocytopenia in patients with FPD/AML [20]. More recently, *NF-E2*, which encodes a TF implicated in platelet granule development and αIIbβ3 signaling, has also been shown to be a transcriptional target of RUNX1 [9]. Thus, the defect in platelet number and function associated with *RUNX1* haplodeficiency may result from abnormalities in multiple mechanisms. Recently, Connelly and colleagues [8] showed that targeted *in vitro* correction of *RUNX1* mutation could recover the MK defects. The investigators differentiated induced pluripotent stem cells (iPSCs) from skin fibroblasts of two FPD/AML patients with Y260X mutation and showed reduced MK production and abnormalities in MK structure, such as abundance of vacuoles and deficiency of dense and α-granules. Gene targeting corrected the *RUNX1* mutation in two of seven cloned iPSCs. As compared with the patients with FPD/AML, the two corrected clones resulted in approximately 40% to 60% more $CD41^{+}CD42^{+}$ MKs with rescue of phenotypic features of abnormal MK differentiation. Gene expression profiling also showed significant upregulation of MK genes in the corrected clones as compared with one of the patients with FPD/AML, and *RUNX1* accounted for the differences. These studies constitute strong evidence that *RUNX1* mutation is the cause of defective megakaryopoiesis in patients with FPD/AML. The studies also raise the intriguing potential for gene-targeting therapy for these patients in the future.

It should be noted that, from a clinical standpoint, defects in platelet number and function in patients with FPD/AML can be heterogenous. Patients commonly have mild to moderate thrombocytopenia with normal-sized platelets and, despite the platelet dysfunction, a mild to moderate bleeding tendency [7,21]. Some individuals may lack bleeding symptoms and thrombocytopenia [7,21]. These features have important implications for treatment, as previously described pedigrees have documented recurrence of leukemia following hematopoietic

stem cell transplantation from an undiagnosed sibling donor with FPD/AML [21].

FLI1

FLI1 is part of the E-twenty-six (ETS) family of TFs that plays a major role in megakaryopoiesis through its influence on the expression of multiple genes, including *ITGA2B* (glycoprotein IIb) [22], *GP1BA* (glycoprotein 1b alpha chain) [22,23], *GP9* (glycoprotein 9) [22], and *c-MPL* (thrombopoietin receptor) [24]. Distal deletion of either the maternally or paternally derived chromosome 11 that includes the *FLI1* locus (11q23.3-24) is associated with a rare autosomal dominant disorder, the Jacobsen syndrome (MIM 147791), and its accompanying platelet disorder, the Paris-Trousseau syndrome (MIM 188025) [25–28]. The clinical features of Jacobsen syndrome include mental retardation, abnormal craniofacial appearance, and abnormalities in multiple organ systems [29,30]. The Paris-Trousseau syndrome is characterized by congenital macrothrombocytopenia with giant α-granules of 1 to 2 μm in diameter in a subpopulation of circulating platelets (1% to 5%) and bone marrow dysmegakaryopoiesis [30]. On the platelet function aspect, thrombin-induced platelet release of α-granule contents has been shown to be impaired. Platelet survival is normal, although there is a substantial expansion of bone marrow MKs because of arrested MK development [25]. A dimorphic population of normal and dysmorphic MKs is present as a result of only one of the two *FLI1* alleles being expressed in a single MK precursor in early development [27,28].

GATA-1

GATA-1 is a member of the GATA TF family that binds to the GATA sequence on DNA. GATA-1 is an important regulator of both MK and erythroid development, and the encoding gene is located on the short arm of the X chromosome (Xp11.23) [30]. Two mutations in *GATA-1* (V205M and D218G) have been connected to an X-linked syndrome consisting of macrothrombocytopenia and dyserythropoiesis with or without anemia (MIM 300367) [31,32]. Such mutations have resulted in impaired GATA-1 interaction with the essential co-factor friend of GATA-1 (FOG1) [31,32]. Multiple platelet defects have been described in this syndrome, including selectively impaired responses to ristocetin and collagen owing to glycoprotein Ib and glycoprotein VI abnormalities, respectively. There is also reduced expression of platelet GαS mRNA and protein suggestive of incomplete maturation of MKs [32,33]. A sex-linked form of the gray platelet syndrome (GPS), a congenital platelet disorder characterized by macrothrombocytopenia and deficiency of α-granules, in association with *GATA-1* R216N mutation has also been described [34]. This entity had been referred to as X-linked thrombocytopenia with β thalassemia. The R216N mutation is unique in that it results in decreased affinity between GATA-1 and its palindromic site rather than disrupting interaction with its co-factor FOG1 [35]. Another identified *GATA-1* mutation involves a splice site (332G-C, V74L) that produces a truncated variant of GATA-1 and has been associated with the X-linked syndrome of anemia with or without neutropenia or platelet abnormalities or both (MIM 300835) [36].

GFI1B

GFI1B is a TF, which functions as a transcriptional repressor, that has been shown to be essential for MK and erythroid development. The encoding gene is located on the long arm of chromosome 9 (9q34.13) [37,38]. Two recent studies [37,38] have implicated mutations in the zinc finger 5 DNA-binding domain region of *GFI1B* in autosomal dominant platelet disorders characterized by dysmegakaryopoiesis, macrothrombocytopenia, α-granule deficiency, and variable bleeding tendency through distinct genetic mechanisms that produce a dominant-negative effect. The first study [37] identified a single nucleotide insertion in exon 7 (c880-881insC) that predicts a frameshift mutation in the fifth zinc finger DNA binding domain of *GFI1B* in a family with a bleeding disorder originally described in 1976 [37,39]. The family members were also found to have evidence of red cell anisopoikilocytosis, impaired platelet aggregation responses, and decreased platelet P-selectin, fibrinogen, glycoprotein Ibα, and glycoprotein IIIa [37]. The second study [38] uncovered a truncating mutation (c.859C>T), also within the fifth zinc finger DNA binding domain of *GFI1B*, in a family originally reported in 1968 and subsequently diagnosed with GPS [38,40]. The family members also had reduced platelet factor 4 and β thromboglobulin as well as evidence of bone marrow myelofibrosis and emperipolesis (intact cell within cytoplasm of another cell) [38]. Identification of autosomal dominant genetic mechanisms in GPS is particularly noteworthy as previously described pedigrees in GPS have primarily been autosomal recessive, and three groups have reported biallelic mutations in the *NBEAL2* gene, which encodes a BEACH protein involved in vesicular trafficking [41–43]. Interestingly, it was recently demonstrated in a murine knockout model that *NBEAL2* deficiency results in loss of α-granules from platelets after initial formation and proinflammatory MKs, which may drive GPS features including myelofibrosis, splenomegaly, and emperipolesis, with α-granule loss also leading to protection from cancer metastasis [44]. Alpha-granule deficiency due to mutations in the gene encoding the VPS33B protein (a member of the Sec1/Munc18 protein family) and the *VPS16B* gene in the arthrogryposis multiplex congenita, renal dysfunction, and cholestasis (ARC)

syndrome has also been described [45–47]. These studies highlight the heterogeneous mechanisms that can lead to α-granule deficiency and GPS, including TF mutations involving *RUNX1* [10,16], *GATA-1* [34], and *GFI1B* [37].

From a different perspective, it is clear that these TF mutations are generally associated with a combination of thrombocytopenia and defects in platelet function, although in some instances there are associated abnormalities in red cells as well, as is the case for mutations in *GATA-1* [30] and *GFI1B* [37].

Conclusions

In summary, evidence is now available that in some patients with impaired platelet aggregation and secretion responses on an inherited basis the primary genetic defect may be in a TF. TF mutations may be more common in such patients than generally considered. Most, but not all, of these patients have a variable degree of thrombocytopenia. The abnormalities in platelet number and function arise because of alterations in multiple pathways regulated by the TF. Some of the TF mutations have prognostic and treatment implications beyond the platelet defect, such as the association of myeloid malignancies with mutations in *RUNX1* and donor selection for hematopoietic stem cell transplant [6,7,21].

Abbreviations

AML, acute myeloid leukemia; FLI1, friend leukemia integration 1; FOG1, friend of GATA-binding factor 1; FPD, familial platelet disorder; GATA-1, GATA-binding factor 1; GFI1B, growth factor-independent 1B; GPS, gray platelet syndrome; iPSC, induced pluripotent stem cell; MDS, myelodysplastic syndrome; MIM, Mendelian Inheritance in Man; MK, megakaryocyte; RUNX1, Runt-related transcription factor 1; SPD, storage pool deficiency; TF, transcription factor.

Disclosures

The authors declare that they have no disclosures.

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