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REVIEW

Transcription factor defects causing platelet disorders

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ABSTRACT

Recent years have seen increasing recognition of a subgroup of inherited platelet function disorders which are due to defects in transcription factors that are required to regulate megakaryopoiesis and platelet production. Thus, germline mutations in the genes encoding the haematopoietic transcription factors RUNX1, GATA-1, FLI1, GFI1b and ETV6 have been associated with both quantitative and qualitative platelet abnormalities, and variable bleeding symptoms in the affected patients. Some of the transcription factor defects are also associated with an increased predisposition to haematologic malignancies (*RUNX1*, *ETV6*), abnormal erythropoiesis (*GATA-1*, *GFI1b*, *ETV6*) and immune dysfunction (*FLI1*). The persistence of MYH10 expression in platelets is a surrogate marker for *FLI1* and *RUNX1* defects. Characterisation of the transcription factor defects that give rise to platelet function disorders, and of the genes that are differentially regulated as a result, are yielding insights into the roles of these genes in platelet formation and function.

1. Introduction

Inherited platelet disorders, accompanied by normal or reduced platelet counts, account for a significant proportion of bleeding diatheses and have been defined historically according to whether the major feature was a quantitative abnormality affecting the platelet count, or a qualitative abnormality affecting platelet function. Over the last 15 years, however, characterisation of the underlying genetic causes of many platelet disorders has allowed better discrimination between these disorders, and classification based on whether the abnormality affects platelet adhesion, platelet receptor signalling, platelet secretion, the platelet cytoskeleton, platelet procoagulant activity and, or platelet production [1,2]. The application of next generation sequencing technology for whole exome or genome analysis is facilitating identification of further underlying genetic defects in patients with previously unexplained platelet disorders [3]. Indeed, this approach has been used in the investigation of an interesting group of platelet disorders caused by defects in transcription factors which are required to regulate megakaryopoiesis, and platelet production, and which can result in both quantitative and qualitative platelet abnormalities [4]. Notably, the number of transcription factor defects identified in patients with platelet function disorders is growing and these appear to account for a significant number of cases.

In this review, the processes involved in megakaryocyte development and platelet production will be outlined briefly before focusing on those transcription factors that are required to regulate megakaryopoiesis and which have been shown to harbour mutations in patients with platelet disorders.

2. Megakaryopoiesis and platelet production

Like other blood cells, megakaryocytes are derived from pluripotent haematopoietic stem cells (HSCs) that reside mainly in the bone marrow and undergo differentiation through discrete steps to give rise to increasingly committed progenitors, including the bipotent megakaryocyte-erythroid progenitors (MEPs) which are the precursors of cells of both the erythroid and megakaryocytic lineages [5]. Under the regulation of thrombopoietin (TPO), early megakaryocytes undergo a proliferative stage during which progression through the cell cycle is identical to that of other HSCs. This is followed by several rounds of endomitosis where the diploid promegakaryocytes undergo DNA replication without cell division, to accumulate a DNA content of 4N up to 128N in a multilobular nucleus [6]. Following nuclear polyploidization, further maturation of the megakaryocytes is accompanied by formation of an extensive invaginated membrane system, which is continuous with the plasma membrane and permeates the cytoplasm and is thought to act as a membrane reservoir that supports proplatelet formation [6,7]. It is also characterised by the development of alpha (α) and dense (δ) storage granules in the cytosol and by the expression of cell surface receptors that mediate platelet adhesion and aggregation [8]. Thus, megakaryocytes mature into large cells of 150 μ or more in diameter which contain all the necessary cellular machinery for functioning platelets. It is thought that endomitosis occurs during megakaryopoiesis in order to support the protein and membrane synthesis required for production of platelets, through functional

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amplification of the genome [9], and indeed there is some evidence for a correlation between the extent of ploidy of megakaryocytes and the number of platelets that they produce [10].

The precise mechanism by which platelets are derived from megakaryocytes is not fully understood, but the widely accepted model is one in which the megakaryocytes develop multiple, long, branching processes, known as proplatelets, which are decorated along their lengths by platelet-sized, bead-like swellings that are connected by cytoplasmic bridges [8]. The proplatelets extend into the sinusoidal blood vessels of the bone marrow, where the blood flow results in release of their terminal buds into the circulation as platelets. In this way, each megakaryocyte is estimated to release in the region of 10 platelets into the circulation [11], with the average human producing approximately 10 platelets daily [12].

The formation of proplatelets is a microtubule-driven process, and normal microtubule function is essential to support elongation of the proplatelet branches and accumulation of platelet granules and other cellular components in the proplatelet buds. The importance of microtubules to thrombopoiesis is evidenced by the block in proplatelet production that occurs when megakaryocytes are treated with drugs that disrupt microtubule assembly [13]. Similarly, mutations in the genes encoding tubulin [14–16], or affecting myosin IIa activity are associated with macrothrombocytopenia [17,18].

3. Role of platelets in primary haemostasis

Once released into the circulation, platelets have an average lifespan of 9 days and do not interact significantly with the endothelium within the intact vasculature. However, when blood vessel damage leads to exposure of the subendothelial matrix, platelets rapidly accumulate at the site of injury to mediate formation of a fibrin-rich platelet plug that stems bleeding into the surrounding tissue [19]. The initial interactions between platelets and the subendothelium are determined to a large extent by the location of the injury. In areas of low shear, such as in veins and larger arteries, platelet adhesion is mediated primarily by direct interaction of platelet receptors with exposed collagen and other adhesive proteins. In areas of high shear, such as in small arteries and the microvasculature, the platelet glycoprotein (GP) Ib-IX-V receptor complex mediates tethering of platelets to the site of injury through von Willebrand factor (VWF) which is bound to collagen in the extracellular matrix. This interaction allows more stable adhesion of platelets through engagement of their GPVI and integrin $\alpha_2\beta_1$ collagen receptors, triggering intracellular signalling pathways that lead to changes in intracellular free calcium, remodelling of the platelet cytoskeleton, and aggregation of platelets through crosslinking of the activated integrin $\alpha_{IIb}\beta_3$ receptors on different platelets via fibrinogen [19]. Fusion of the intracellular secretory granules of the platelets with the outer platelet membrane and the controlled release of their contents into the local environment also occur mediating further platelet activation and responses with other cells [20].

4. Transcription factors are key regulators of platelet formation

Transcription factors and their co-activators regulate lineage-specific gene expression during haematopoiesis by binding to *cis*-regulatory elements located upstream of the promoter of specific target genes to either activate or repress transcription. Knowledge of the key regulatory role of transcription factors in the lineage decisions involved in megakaryopoiesis and platelet production has expanded rapidly in recent years largely as a result of the development of procedures that allow the growth and differentiation of megakaryocytes from HSCs *in vitro*, and also characterisation of the roles of specific transcription factors by mutating the corresponding genes in mice. More recently, the identification of patients with defects in transcription factor genes that result in disturbances in platelet number and function have provided insights into their role in platelet formation and function. Thus, germline mutations affecting the haematopoietic transcription factors RUNX1, GATA-1, FLI1, GFI1b and ETV6 have been shown to result in platelet dysfunction, thrombocytopenia and variable bleeding symptoms in patients. The roles of these five transcription factors in haematopoiesis will be briefly described (Fig. 1) [21], and the germline defects that have been identified in these genes will be summarised in the remainder of this review.

4.1. RUNX1

4.1.1. Structure and function of RUNX1

Runt-related transcription factor-1, or RUNX1 (also known as core binding factor alpha, CBFα, or acute myeloid leukaemia 1, AML1) is one of three RUNX family members seen in mammals, which binds CBFβ to form the heterodimeric core binding factor (CBF) transcription complex. In humans, heterozygous germline defects in *RUNX1* are associated with Familial Platelet Disorder with predisposition to Acute Myeloid Leukaemia (FPD/AML; MIM 601399). The RUNX1 gene, *RUNX1*, is located at chromosome 21q22.12 and comprises eight exons. Alternative splicing gives rise to at least three RUNX1 variants which differ in size. The largest and least abundant variant, RUNX1c, comprises 480 amino acids and differs from RUNX1b by having a distinct 27 amino acid N-terminal region. There is no apparent difference in function between RUNX1b and RUNX1c. The smallest variant, RUNX1a, comprises 250 amino acids and has a similar N-terminal region to RUNX1b, but differs in the C-terminal region [22–24]. For ease of reference, amino acid residues in RUNX1 are numbered here according to the RUNX1b sequence (Fig. 2). All three isoforms share a well characterised 128 amino acid Runt domain, which is necessary for binding to CBFβ and also to the consensus sequence 5′-PyGPyGGTPy-3′ in DNA [25]. The structure of the Runt domain has been solved and the regions that interact with CBFβ and DNA have been shown not to overlap. The interaction with CBFβ increases DNA binding of RUNX1 by stabilising the Runt domain in a high affinity DNA binding conformation. The C-terminal regions of RUNX1b and RUNX1c also encompass a transactivation domain which harbours a nuclear matrix-targeting signal and is essential for *in vivo* function, an inhibitory domain, a proline rich PY motif that allows interaction with WW domain-containing proteins and a C-terminal VWRPY motif that it is thought to be involved in transcription repression [26].

RUNX1 is critical for definitive haematopoiesis, and knockout of the mouse *Runx1* gene is lethal in utero. Conditional deletion of the gene

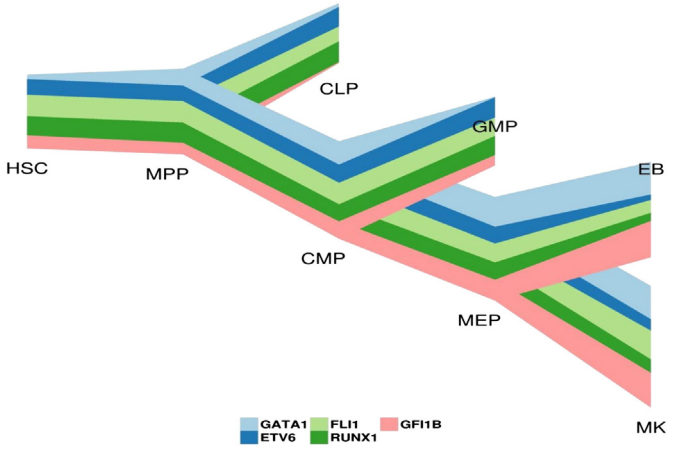

Fig. 1. Relative expression of the transcription factors *GATA1*, *ETV6*, *FLI1*, *RUNX1* and *GFI1B* across eight cell types derived from haematopoietic stem cells (HSC) in the bone marrow. MPP multipotent progenitor; CMP common myeloid progenitor; CLP common lymphoid progenitor; GMP granulocyte-monocyte progenitor; EB erythroblast; MEP megakaryocyte erythrocyte progenitor; MK megakaryocyte. The riverplot was generated using the Blueprint Progenitors dataset and the online tool available at http://blueprint.haem.cam.ac.uk/ [21].

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