Molecular basis of thrombocytosis

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The bone marrow of a normal individual produces 1×10^{11} platelets per day,[1] i.e., about half of the number of red cells (2×10^{11} per day). Platelets derive from fragmentation of megakaryocytes through a biogenetic process that involves long cytoplasmic extensions called proplatelets.[2] Megakaryocytes develop from committed progenitors referred to as colony-forming unit megakaryocyte (CFU-MK), a heterogeneous population of cells that are capable of proliferation.[3] At a certain stage, CFU-MK stop proliferating and enter endomitosis, thus becoming megakaryoblasts or immature megakaryocytes. The process of endomitosis involves DNA replication without cellular division, and gives rise to a polyploid cell with a single polylobulated nucleus, the typical pattern of a mature megakaryocyte.[4]

Megakaryocytopoiesis and platelet production are mainly regulated by thrombopoietin although cytokines such interleukin-11 and interleukin-6 may also play a role.[5] In particular, these latter cytokines would allow the progenitors to relocate to a microenvironment that is permissive and instructive for megakaryocyte maturation and platelet production.[6]

The effect of thrombopoietin on megakaryocytopoiesis is mediated through its receptor, c-Mpl (CD110),[7] which is found on both megakaryocytes and platelets, although at low levels on these latter. Since thrombopoietin is produced at a constant rate by the liver, its concentration is regulated – at least in part – by binding to its receptor on circulating platelets. When the platelet count decreases (e.g., as a result of antibody-mediated destruction), increased plasma levels of thrombopoietin expand megakaryocytopoiesis and platelet production. Conversely, when the platelet count rises, more thrombopoietin binds to platelet c-Mpl receptors and less ligand is available for megakaryocyte receptors, leading to slowed megakaryocytopoiesis. A number of clinical observations, however, suggest that other regulatory mechanisms may operate. In an elegant study,[8] Skoda and co-workers showed that the translation of thrombopoietin mRNA is physiologically almost completely inhibited by the presence of uAUG codons in the 5'-untranslated region. They also found that a splice variant was more efficiently translated than the two regularly spliced isoforms, suggesting that regulation of alternative splicing may serve as an additional control mechanism for thrombopoietin production.

The platelet count may range from 100×10^9/L up to 400×10^9/L in healthy individuals, and only occasional apparently normal subjects show values between 400 and 450×10^9/L. A platelet threshold count of 450×10^9/L appears useful for making a diagnosis of thrombocytosis in clinical practice, while values between 350 and 450×10^9/L require follow-up with sequential evaluations.

Thrombocytosis can be classified into three major categories as shown in Table 1: i) hereditary or familial thrombocytosis; ii) thrombocytosis associated with myeloproliferative and/or myelodysplastic disorders (clonal thrombocytosis), and iii) reactive (secondary) thrombocytosis.[9]

Most patients with thrombocytosis have reactive thrombocytosis. Indeed, in a retrospective German study,[30] 643/732 (88%) patients with a platelet count of more than 500×10^9/L were found to have secondary thrombocytosis, mainly related to inflammatory conditions. Primary thrombocytosis is mainly found in myeloproliferative disorders, and particularly in essential thrombocythemia (Table 1). Somatic mutations of *JAK2* or *MPL* lead to more efficient translation of the thrombopoietin signal and platelet production.

Interestingly, an activating germline mutation of *MPL* has been found in families with essential thrombocythemia.[15,16] This mutation [*MPL* (S505N)] has also been found as an acquired somatic mutation in patients with essential thrombocythemia or primary myelofibrosis.[21] This reinforces the concept that this is indeed a causative mutation responsible for dysregulated platelet production, irrespective of its congenital or acquired nature.

In other families with a similar condition, however, the molecular mechanism is a gain-of-function mutation in the thrombopoietin gene (*THPO*) that leads to increased thrombopoietin production.[10-13] The *THPO* mutations identified so far alter the 5'-untranslated region of the *THPO* mRNA, which contains upstream open reading frames (uORF) that inhibit mRNA translation. All mutations remove the inhibitory uORF and lead to increased translation of the *THPO* mRNA, causing overproduction of thrombopoietin and platelets. This represents a remarkable example of a novel molecular mechanism of disease that we defined "translational pathophysiology".[31]

In this issue, Liu and co-workers[14] describe studies on a large Polish family with hereditary thrombocythemia associated with a *de novo* splice donor mutation in *THPO* involving increased thrombopoietin production. Interestingly, megakaryocyte morphology in these patients differs from that in patients with sporadic essential thrombocythemia associated with *JAK2* or *MPL* mutations. These patients have symptoms of impaired microcirculation and require aspirin therapy, but do not require cytoreductive therapy for prevention of thrombosis. Likely, they lack the increased platelet and leukocyte activation which is found in essential thrombocythemia associated with *JAK2* (V617F).[32] Indeed, activating *THPO* mutations have not been detected in patients with sporadic thrombocythemia,[33] and are, therefore, unlikely to be causative somatic mutations.

Table 1. Conditions associated with thrombocytosis.

Familial thrombocytosis associated with germline mutations of *THPO* or *MPL*

- Hereditary thrombocythemia associated with germline activating mutations in the thrombopoietin (*THPO*) gene (autosomal dominant disorder)[10-14]
- Familial essential thrombocythemia associated with a germline activating mutation in the thrombopoietin receptor (*MPL*) gene [*MPL* S505N - autosomal dominant disorder)[15,16]
- Thrombocytosis associated c-Mpl Baltimore (functional *MPL* K39N polymorphism unique to individuals of African-American descent, conforming to a pattern of autosomal dominance with incomplete penetrance)[17]

Thrombocytosis associated with myeloproliferative and/or myelodysplastic disorders (clonal thrombocytosis associated with somatic mutations of *JAK2*, *MPL* and additional currently unknown genes)

- Essential thrombocythemia [associated with the somatic *JAK2* (V617F) mutation in 50 to 60% of patients,[18] somatic mutations of *MPL* (W515L, W515K, S505N)[19-21] in less than 5% of patients, and unknown mutant genes in the remaining patients]
- Polycythemia vera [mainly associated with low burdens of the somatic *JAK2* (V617F) mutation, rarely found in association with somatic mutations of *JAK2* exon 12 mutations][22,23]
- Primary myelofibrosis [associated with the somatic JAK2 (V617F) mutation in 50 to 60% of patients, somatic mutations of MPL (W515L, W515K, S505N)[19-21,24] in 5% of patients or more, and unknown mutant genes in the remaining patients]
- Refractory anemia with ringed sideroblasts associated with marked thrombocytosis [somatic mutations of *JAK2* and *MPL*][25-28]
- Myelodysplastic syndrome with isolated del(5q) (5q- syndrome whose hematologic features are likely related to haploinsufficiency of several genes of chromosome 5q31-q32[29]
- Chronic myeloid leukemia

Reactive (secondary) thrombocytosis

- Acute and chronic inflammatory and infectious disorders, including malignancy (associated with excessive endogenous cytokine production)
- Iron deficiency
- Acute blood loss
- Post-splenectomy
- Hemolytic anemia

As indicated by Skoda and Prchal,[34] studies of families with myeloproliferative disorders can contribute greatly to our understanding of the molecular basis of sporadic conditions. However, we must clearly distinguish between familial disorders associated with germline mutations (as those reported in Table 1) and familial myeloproliferative disorders associated with somatic mutations of *JAK2* or *MPL*.[35] What is inherited in these latter is not the *JAK2* or *MPL* mutation, but rather a genetic predisposition to its acquisition, as remarkably indicated by the two families described by Pietra and co-workers.[36]

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