THE CONTROL OF NORMAL AND ABERRANT MEGAKARYOPOIESIS BY THROMBOPOIETIN AND ITS RECEPTRO, c-MPL

Radek C. Skoda

Introduction

Mature blood cells are short-lived and must be constantly replaced. A small number of multipotent hematopoietic stem cells (HSC) are the source of this regenerative process. Hematopoiesis is the best studied stem cell system and constitutes a model for other organs. HSC can either remain multipotent or differentiate into mature cells with very diverse functions and morphologies, as exemplified by lymphocytes, granulocytes, erythrocytes or megakaryocytes. Extracellular signals, such as cytokines and growth factors and their cell surface receptors, act in concert with cell intrinsic factors, e.g. components of the signal transduction machinery and transcription factors, to maintain a balance between production and destruction of blood cells. Given the complexity of the known regulatory pathways and the needs for adaptation to changing environmental factors, we should consider it surprising that blood cell disorders are not more common. Here I review the physiological role of a humoral factor called thrombopoietin and its receptor in the formation of megakaryocytes and platelets and I summarize our current understanding of disorders associated with an overproduction of platelets.

Thrombopoietin and its receptor, c-Mpl are the primary regulators of platelet production

Thrombopoietin (TPO) was first described as an biological activity in serum that stimulated the production of platelets in vivo (Kelemen et al., 1958). For more than 30 years this activity eluded all attempts of biochemical purification. TPO cDNA was finally cloned in 1994 by virtue of its high affinity receptor, which served as a bait for immuno-

affinity purification and expression cloning (Bartley et al., 1994; de Sauvage et al., 1994; Kaushansky et al., 1994). The TPO receptor was discovered as the cellular homologue of a retroviral oncogene. Experiments with Friend murine leukemia virus led to the isolation of a mutant virus called myeloproliferative leukemia virus (MPLV) (Wendling et al., 1986). MPLV caused a broad spectrum of myeloid malignancies in mice including erythroid, granulocytic, monocytic, megakaryocytic and mast cell leukemias (Wendling et al., 1989). This recombinant retrovirus carried a novel oncogene, v-mpl, that displayed sequence homology to members of the cytokine receptor superfamily. A rearrangement led to a fusion between sequences coding for the extracellular domain of the retroviral envelope protein and a truncated cytokine receptor, consisting of 40 amino acids of the extracellular domain, the transmembrane domain, and the entire cytoplasmic domain (Souyri et al., 1990). This suggested that signaling through the cytoplasmic domain of v-mpl was responsible for the proliferation and transformation of hematopoietic progenitors. Since I was interested in megakaryocyte formation and transformation, I began to study the properties of c-mpl, the cellular homologue of v-mpl, which at this time was an orphan receptor molecule. To demonstrate that c-mpl was capable of signaling for proliferation, I generated a chimeric receptor consisting of the extracellular domain of the interleukin-4 (IL-4) receptor and the transmembrane and cytoplasmic domain of c-mpl. When stimulated by IL-4, the cytoplasmic domain of the c-mpl protein was capable of providing a proliferative signal in factor-dependent cell lines (Skoda et al., 1993). The full-length c-mpl protein and a soluble extracellular domain was used by several laboratories to clone the mpl-ligand, which turned out to be the long sought for TPO (Bartley et al., 1994; de Sauvage et al., 1994; Kaushansky et al., 1994). TPO causes thrombocytosis in vivo (Kaushansky et al., 1994) and promotes proliferation and maturation of megakaryocyte precursors in vitro (Debili et al., 1995; Kaushansky et al., 1995). TPO is mainly synthesized in the liver and the control of TPO production provides an interesting paradigm for autoregulation (Kuter and Rosenberg, 1995). In support of this model (Figure 1), we found that TPO mRNA was not regulated during thrombocytopenia. Furthermore, isolated mouse platelets absorbed high amounts of bioactive TPO out of TPO-conditioned medium in a

dose-dependent fashion (Stoffel et al., 1996). Thus, serum TPO protein is being regulated directly through adsorption by platelets *(Figure 1)*. Consistent with this model, platelets deficient for c-mpl were unable to bind and absorb TPO (de Sauvage et al., 1996; Gurney et al., 1994).

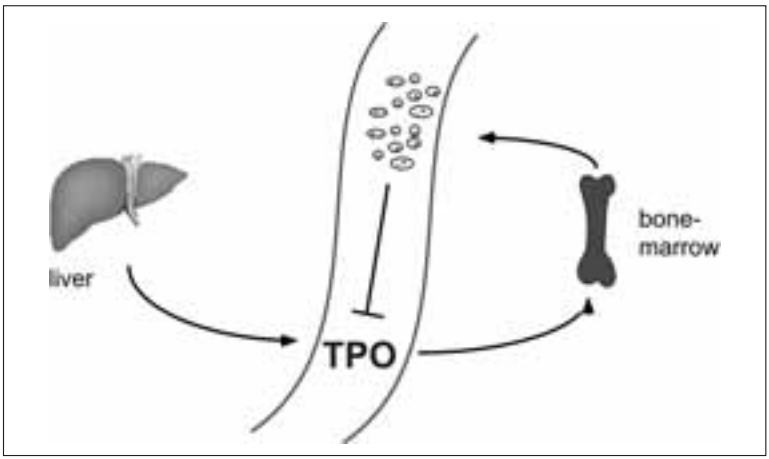

Figure 1:
Autoregulation of TPO serum levels. The liver produces constant amounts of TPO, which is transported to the bone marrow through the blood vessels. TPO leads to an increase of platelet production and results in a higher platelet count. Platelets express the TPO-receptor. They are able to bind and degrade circulating TPO protein and thereby exert a negative feedback.

One very interesting question is how specificity of the in vivo effects of TPO, i.e. the selective increase of platelets in the peripheral blood, can be explained. Since c-mpl is expressed on early hematopoietic progenitors and stem cells, one possibility is that signals generated by TPO and its receptor act by instructing the progenitors to commit to a specific hematopoietic lineage (instructive model). Alternatively, lineage specificity could be also achieved by restricting the expression of the receptor to cells of the appropriate lineage. According to this theory, the signals generated by the ligand-receptor interaction primarily permit the survival and proliferation of pre-determined progenitors through a

non-specific proliferative signal (permissive model). To address this question in vivo, we used homologous recombination to replace the c-mpl gene with a chimeric construct encoding the extracellular domain of mpl and the cytoplasmic domain of the granulocyte colony-stimulating factor receptor (G-CSFR) *(Figure 2)*. This chimeric receptor binds TPO, but signals through the G-CSFR intracellular domain (Stoffel et al., 1999). We found that, despite the absence of a functional mpl signaling domain, homozygous knock-in mice had a normal platelet count, indicating that in vivo the cytoplasmic domain of G-CSFR can functionally replace mpl signaling to support normal megakaryopoiesis and platelet formation *(Figure 2)*. This finding favors the permissive model, according to which cytokine receptors provide a non-specific survival or proliferation signal and argues against an instructive role of c-mpl or G-CSFR in hematopoietic cell fate decisions. Similar conclusions were reached in a knock-in model with a G-CSFR/EPOR chimera (Semerad et al., 1999).

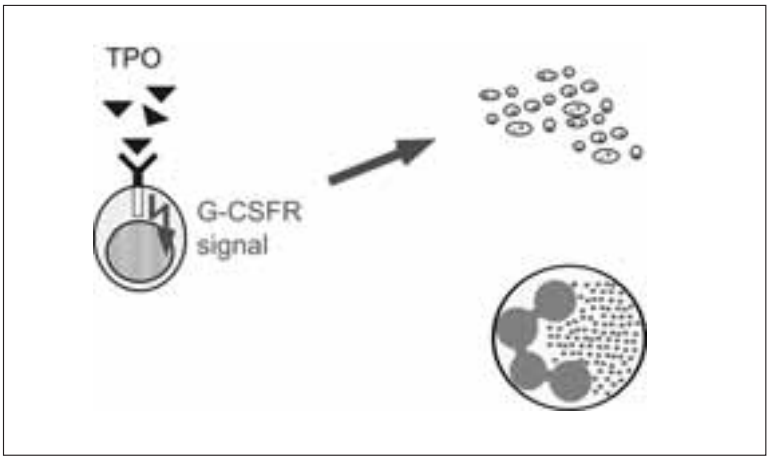

Figure 2:
Summary of the conclusions reached from the analysis of homozygous mpl/G-CSFR knock-in mice. The chimeric mpl/G-CSFR binds TPO but signals through the G-CSFR cytoplasmic domain. The resulting signal does not re-program the progenitor cells to become granulocytes, instead this signal is capable of promoting megakaryopoiesis and platelet production in vivo.

Since mice deficient for mpl are viable, they can be used as a genetic background to introduce mutant receptors and assess their capability to complement the platelet deficiency in vivo. We have introduced transgenes encoding c-mpl mutants into c-mpl-deficient mice and found that these mutants change platelet levels, but not platelet function or the balance between cells of different hematopoietic lineages (Coers et al., in preparation). These results are again consistent with the predictions of the permissive model.

Recently, we have studied the function of a truncated mpl receptor isoform (mpl-tr), which results from alternative splicing (Skoda et al., 1993). The mpl-tr variant is the only alternate mpl isoform conserved between mouse and humans, suggesting a relevant function in regulating mpl signaling. Despite the presence of a signal peptide and the lack of a transmembrane domain, mpl-tr is retained intracellularly. Our results provide evidence that mpl-tr exerts a dominant-negative effect on thrombopoietin-dependent cell proliferation and survival (Coers et al., 2004). We demonstrated that this inhibitory effect is due to down-regulation of the full-length mpl protein. The C terminus of mpl-tr, consisting of 30 amino acids of unique sequence, is essential for the suppression of TPO-dependent proliferation and mpl protein down-regulation. Cathepsin inhibitor-1 (CATI-1), an inhibitor of cathepsin-like cysteine proteases, counteracts the effect of mpl-tr on mpl protein expression, suggesting that mpl-tr targets mpl for lysosomal degradation. Together, these data suggest a new paradigm for the regulation of cytokine receptor expression and function through a proteolytic process directed by a truncated isoform of the same receptor. This example highlights the many levels of fine-tuning nature invented to obtain a robust regulatory system for maintaining stable numbers of circulating blood cells.

Aberrant megakaryopoiesis in hereditary forms of myeloproliferative disorders.

Myeloproliferative disorders (MPD) are a heterogeneous group of diseases characterized by increased hematopoiesis leading to elevated

numbers of non-lymphoid cells and/or platelets in the peripheral blood. In addition to thrombotic and hemorrhagic complications, leukemic transformation can occur. The classical definition of MPD included four disease entities, which were thought to be related (Damashek, 1951): chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (IMF). The Philadelphia chromosome and the bcr/abl fusion transcripts established CML as a separate entity, allowed developing diagnostic PCR tests and made it possible to derive a specific tyrosine kinase inhibitor (Imatinib/ Glivec), which is used successfully in the treatment of CML patients. The cause of the three remaining forms of MPD remains unknown, but progress has been made in defining the functional and molecular characteristics of MPD. The investigation of inherited disorders resembling MPD complements these studies and provides important clues to the hierarchy of events in the pathogenesis of MPD.

The finding that TPO and its receptor exert strong lineage selective effects on platelet production suggested that mutations in TPO or c-mpl could play a role in disorders with increased or decreased megakaryopoiesis. Inherited disorders are particularly suited to test such a hypothesis, because the involvement of a candidate gene locus can be confirmed or excluded by genetic linkage analysis. In a family with autosomal dominant thrombocytosis resembling ET, we were able to locate the disease-causing gene to the TPO locus on chromosome 3q and to find a mutation in the TPO gene (Wiestner et al., 1998). The mechanism of how this mutation causes elevated TPO serum levels was at first quite difficult to understand. Affected family members carry a point mutation in the +1 position of the splice donor of intron 3. This mutation causes exon-skipping and results in loss of exon 3, including the nucleotides encoding the first 4 amino acids of the TPO protein. This mutation appeared to cause a loss of function of the TPO allele, thus, the opposite of what we had expected. However, more detailed analysis revealed that a novel N-terminus is created by fusion with an upstream open reading frame and we could show that the extended N-terminus behaves as a functional signal peptide. Importantly, the mutation results in TPO overproduction due to removal of sequences contained within exon 3 that normally inhibit the translation of TPO

mRNA into protein. A simplified description of the mechanism, which leads to TPO overproduction in family members that carry the mutation is shown in *Figure 3*.

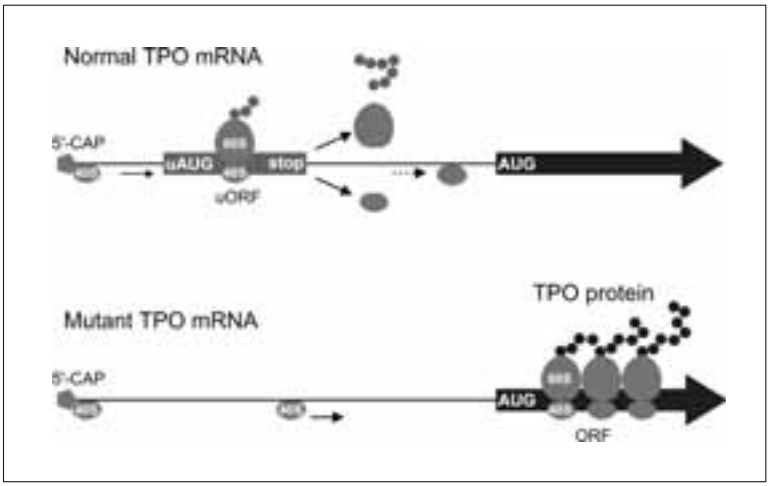

Figure 3:
Mechanism of increased TPO protein production in family members carrying a TPO mutation. Physiologically, the translation of TPO mRNA is strongly inhibited by the presence of an upstream translational start codon (uAUG) located in the 5'-untranslated region (5'-UTR) of the TPO mRNA (Ghilardi et al., 1998). The ribosome (40S and 60S subunits) initiates synthesis of a short peptide and dissociated from the mRNA after encountering a stop codon. Therefore, under normal circumstances, production of TPO protein is very inefficient. In affected family members, exon-skipping removes the uAUG and allows efficient translation (Wiestner et al., 1998). Thus, more TPO protein is synthesized from the same amount of mRNA, leading to increase TPO serum levels. Directed mutagenesis of the uAUG in the TPO mRNA restored full translational efficiency, demonstrating that translational inhibition of TPO biosynthesis is entirely mediated by uORF (Ghilardi et al., 1998).

To date, 3 additional mutations in the TPO gene have been described (Ghilardi et al., 1999; Jorgensen et al., 1998; Kondo et al., 1998). In all cases reported, the inheritance is autosomal dominant and the phenotype is already manifest in childhood or at birth. The clinical findings in these families consist of a high platelet count, markedly elevated

serum TPO levels, but normal red blood cell and normal leukocyte counts. The penetrance is usually 100%, the clinical course is mild with occasional thrombotic or bleeding complications, but without leukemic transformation. In all cases studied, overproduction of TPO is caused by a mechanism of increased translational efficiency for the mutant TPO mRNA (Ghilardi and Skoda, 1999; Ghilardi et al., 1999; Wiestner et al., 1998). These mutations constitute paradigms of translational pathophysiology and are examples of a novel molecular mechanism of human diseseas (Cazzola and Skoda, 2000). Mutations in TPO have not been found in patients with sporadic MPD (Harrison et al., 1998; Taksin et al., 1999). Therefore, thrombocythemia due to gain of function mutations in the TPO gene constitutes a phenocopy of sporadic ET.

Whereas mutations in TPO account for only approx. 20% of familial thrombocytosis (Kunishima et al., 1998; Wiestner et al., 2000), the disease-causing gene still remains unknown in all other cases. Thus, mutations in one or several additional genetic loci can also cause thrombocytosis. Genetic analysis in these families will allow us to identify new components that regulate megakaryopoiesis. The phenotype in some of the families resembles sporadic forms of MPD. We found clonal hematopoiesis in the affected individuals, indicating that a somatic mutation acts in synergy with a predisposition inherited through the germline. The search for the mutated gene in families, which display a phenotype very closely resembling sporadic MPD, appears particularly worthwhile and we expect that the mutated gene will also be of relevance for patients with sporadic MPD. In one large pedigree with MPD-like phenotype, we have located the gene to chromosome 9q and are now searching for the mutation in genes that lie within the co-segregating interval (Kralovics et al, in preparation). Our studies suggest that at least two hits are necessary for phenotypic manifestation of MPD: a pre-disposing mutation in a gene on chromosome 9q and a somatic mutation in a second locus *(Figure 4)*.

Studies on sporadic MPD revealed a number of new molecular markers, such as decreased expression of c-MPL protein in platelets (Moliterno et al., 1998) and PRV-1 mRNA in granulocytes (Temerinac et al., 2000). These alterations in the expression pattern could be valuable as

diagnostic tools or might hold keys to the understanding of the pathogenesis. We compared several of these markers simultaneously in a cohort of patients with MPD and found that the correlation between the markers is lower than reported (Kralovics et al., 2003). In particular, the decreased expression of c-MPL protein was present in less than half of patients with PV or ET and was also found in members of a family with a mutation in the TPO gene. This indicates that decreased c-MPL protein is not involved in the pathogenesis, but rather represents a consequence of high platelet count. Comparing patients with sporadic MPD with family members carrying a known molecular alteration proves to be very helpful for distinguishing primary alterations from secondary events.

Future directions

Genome wide expression studies comparing mature blood cells or progenitors from patients with MPD and healthy controls will undoubtedly improve our picture of the changes that occur during the evolution of MPD. We have performed a high-density microarray expression analysis on RNA from MPD patients, healthy controls and familial MPD-like syndromes and are currently analyzing the data. Methods for screening for subtle chromosomal alterations, such as genome wide microsatellite analysis and chip based comparative genomic hybridization, will complement these studies. One result on which we are following up is the finding of loss of heterozygosity on chromosome 9p (9pLOH), which occurs in 30% of patients with PV and constitutes the most frequent chromosomal aberration in MPD (Kralovics et al., 2003; Kralovics et al., 2002). This somatic mutation could act synergistically with a putative mutation of a gene in chr. 9q. Finally, an interesting aspect of MPD is the suppression by the MPD clone of normal hematopoiesis from stem cells not carrying the MPD mutations. We hypothesize that inhibitory signals, such as the transforming growth factor beta (TGFβ) family of signaling molecules, could be mediating this effect *(Figure 4)*. We will test this hypothesis by inactivating the TGFβ receptors on hematopoietic cells using mouse knockout models or by applying siRNA in human hematopoietic progenitors (Schomber et al.,

2004). By the same approach we will assess the functional consequences of inactivating candidate genes identified in our studies of 9pLOH and familial form of MPD-like disorders. The ultimate goal is to identify the primary molecular defect(s) in MPD and secondary mutations that are associated with disease progression. This information will be used to improve clinical management of disease in these patients and will open possibilities for finding new effective treatments.

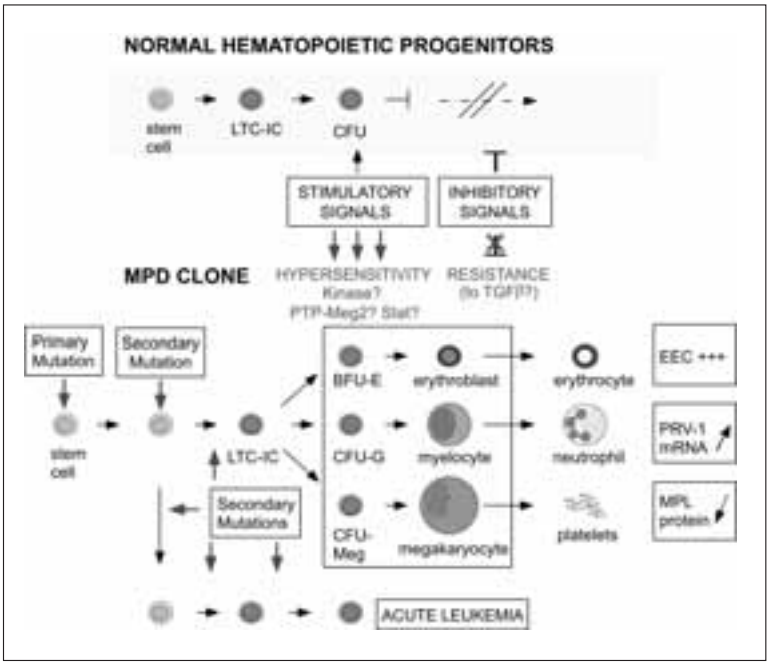

Figure 4:
Hypothetical model for the pathogenesis of myeloproliferative disorders. At least two mutations are needed for phenotypic manifestation of MPD. These mutations convey to cells of the MPD clone hypersensitivity to incoming stimulatory signals and resistance to inhibitory signals. The latter may explain why only blood cells descending from the MPD clone are found in peripheral blood (clonal dominance).

Acknowledgements

I wish to express my deep appreciation and gratitude to the Prof. Dr. Max Cloëtta Foundation and its Board for the recognition of the work of my research team. I am indebted to the talented graduate students, postdoctoral fellows and technicians, past and present, for their essential contributions and for making it a pleasure and privilege to continue our scientific endeavors. I could not have succeeded without the advice and support by my scientific mentors (in chronological order): Peter Sonderegger, Philipp Christen, Urs. A. Meyer and Philip Leder. I am also very grateful to the Swiss National Science Foundation for providing a base for beginning my work as an independent investigator by awarding me a SCORE-A fellowship 1993–1999 and for supporting my research throughout my career. I am equally thankful for the grant support by the Swiss Cancer League/Oncosuisse, the Krebsliga beider Basel, the Juliläumsstiftung der Rentenanstalt and the Stiftung für Hämatologische Forschung and to the University Hospital Basel for the opportunity to continue our work. Finally, my very special thanks go to my beloved wife Regula Saner Skoda and my children Raphael and Michael.

REFERENCES

1. Bartley, T.D., Bogenberger, J., Hunt, P., Li, Y.S., Lu, H.S., et al. (1994) Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor mpl. *Cell*, **77**, 1117–1124.

2. Cazzola, M. and Skoda, R.C. (2000) Translational pathophysiology: a novel molecular mechanism of human disease. *Blood*, **95**, 3280–3288.

3. Coers, J., Ranft, C., and Skoda, R.C. (2004) A Truncated Isoform of c-Mpl with an Essential C-terminal Peptide Targets the Full-length Receptor for Degradation. *J Biol Chem*, **279**, 36397–36404.

3. Damashek, W. (1951) Some speculations on the myeloproliferative syndromes. *Blood*, **6**, 372.

4. de Sauvage, F.J., Carver-Moore, K., Luoh, S.M., Ryan, A., Dowd, M., Eaton, D.L. and Moore, M.W. (1996) Physiological regulation of early and late stages of megakaryocytopoiesis by thrombopoietin. *J Exp Med*, **183**, 651–656.

5. de Sauvage, F.J., Hass, P.E., Spencer, S.D., Malloy, B.E., Gurney, A.L., Spencer, S.A., Darbonne, W.C., Henzel, W.J., Wong, S.C., Kuang, W.J., Oles, K.J., Hultgren, B., Solberg, L.A., Goeddel, D.V., and Eaton, D.L. (1994) Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-mpl ligand. *Nature*, **369**, 533–538.

6. Debili, N., Wendling, F., Katz, A., Guichard, J., Breton-Gorius, J., Hunt, P., and Vainchenker, W. (1995) The Mpl-ligand or thrombopoietin or megakaryocyte growth and differentiative factor has both direct proliferative and differentiative activities on human megakaryocyte progenitors. *Blood*, **86**, 2516–2525.

7. Ghilardi, N. and Skoda, R.C. (1999) A single-base deletion in the thrombopoietin (TPO) gene causes familial essential thrombocytosis through a mechanism of more efficient translation of TPO mRNA [letter]. *Blood*, **94**, 1480–1482.

8. Ghilardi, N., Wiestner, A., Kikuchi, M., Oshaka, A., and Skoda, R.C. (1999) Hereditary thrombocythemia in a Japanese family is caused by a novel point mutation in the thrombopoietin gene. *Br. J. Haematol.*, **107**, 310–316.

9. Ghilardi, N., Wiestner, A., and Skoda, R.C. (1998) Thrombopoietin production is inhibited by a translational mechanism. *Blood*, **92**, 4023–4030.

10. Gurney, A.L., Carver-Moore, K., de Sauvage, F.J., and Moore, M.W. (1994) Thrombocytopenia in c-mpl-deficient mice. *Science*, **265**, 1445–1447.

11. Harrison, C.N., Gale, R.E., Wiestner, A.C., Skoda, R.C., and Linch, D.C. (1998) The activating splice mutation in intron 3 of the thrombopoietin gene is not found in patients with non-familial essential thrombocythaemia. *Br J Haematol*, **102**, 1341–1343.

12. Jorgensen, M.J., Raskind, W.H., Wolff, J.F., Bachrach, H.R., and Kaushansky, K. (1998) Familial thrombocytosis associated with overproduction of thrombopoietin due to a novel splice donor site mutation. *Blood*, **92, supplement 1**, 205a (abstract).

13. Kaushansky, K., Broudy, V.C., Lin, N., Jorgensen, M.J., McCarty, J., Fox, N., Zucker-Franklin, D., and Loftonday, C. (1995) Thrombopoietin, the mpl ligand, is essential for full megakaryocyte development. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 3234–3238.

14. Kaushansky, K., Lok, S., Holly, R.D., Broudy, V.C., Lin, N., Bailey, M.C., Forstrom, J.W., Buddle, M.M., Oort, P.J., Hagen, F.S., Roth, G.J., Papayannopoulou, T., and Foster, D.C. (1994) Promotion of megakaryocyte progenitor expansion and differentiation by the c-mpl ligand thrombopoietin. *Nature*, **369**, 568–571.

15. Kelemen, E., Cserhati, I., and Tanos, B. (1958) Demonstration and some properties of human thrombopoietin in thrombocythemic sera. *Acta Haematologica*, **20**, 350–355.

16. Kondo, T., Okabe, M., Sanada, M., Kurosawa, M., Suzuki, S., Kobayashi, M., Hosokawa, M., and Asaka, M. (1998) Familial essential thrombocythemia associated with one-base deletion in the 5'-untranslated region of the thrombopoietin gene. *Blood*, **92**, 1091–1096.

17. Kralovics, R., Buser, A.S., Teo, S.S., Coers, J., Tichelli, A., Van Der Maas, A.P., and Skoda, R.C. (2003) Comparison of molecular markers in a cohort of patients with chronic myeloproliferative disorders. *Blood*, **102**, 1869–1871.

18. Kralovics, R., Guan, Y., and Prchal, J.T. (2002) Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. *Experimental Hematology*, **30**, 229–236.

19. Kunishima, S., Mizuno, S., Naoe, T., Saito, H., and Kamiya, T. (1998) Genes for thrombopoietin and c-mpl are not responsible for familial thrombocythaemia: a case study. *Br J Haematol*, **100**, 383–386.

20. Kuter, D.J. and Rosenberg, R.D. (1995) The reciprocal relationship of thrombopoietin (c-mpl ligand) to changes in the platelet mass during busulfan-induced thrombocytopenia in the rabbit. *Blood*, **85**, 2720–2730.

21. Moliterno, A.R., Hankins, W.D., and Spivak, J.L. (1998) Impaired expression of the thrombopoietin receptor by platelets from patients with polycythemia vera. *N Engl J Med*, **338**, 572–580.

22. Schomber, T., Kalberer, C.P., Wodnar-Filipowicz, A., and Skoda, R.C. (2004) Gene silencing by lentivirus-mediated delivery of siRNA in human CD34$^+$ cells. *Blood*, **103**, 4511–4513.

23. Semerad, C.L., Poursine-Laurent, J., Liu, F., and Link, D.C. (1999) A role for G-CSF receptor signaling in the regulation of hematopoietic cell function but not lineage commitment or differentiation. *Immunity*, **11**, 153–161.

24. Skoda, R.C., Seldin, D.C., Chiang, M.K., Peichel, C.L., Vogt, T.F., and Leder, P. (1993) Murine c-mpl: a member of the hematopoietic growth factor receptor superfamily that transduces a proliferative signal. *Embo J.*, **12**, 2645–2653.

25. Souyri, M., Vigon, I., Penciolelli, J.F., Heard, J.M., Tambourin, P., and Wendling, F. (1990) A putative truncated cytokine receptor gene transduced by the myeloproliferative leukemia virus immortalizes hematopoietic progenitors. *Cell*, **63**, 1137–1147.

26. Stoffel, R., Wiestner, A., and Skoda, R.C. (1996) Thrombopoietin in thrombocytopenic mice: evidence against regulation at the mRNA level and for a direct regulatory role of platelets. *Blood*, **87**, 567–573.

27. Stoffel, R., Ziegler, S., Ghilardi, N., Ledermann, B., de Sauvage, F.J., and Skoda, R.C. (1999) Permissive role of thrombopoietin and granulocyte colony-stimulating factor receptors in hematopoietic cell fate decisions in vivo. *Proc Natl Acad Sci U S A*, **96**, 698–702.

28. Taksin, A.L., Couedic, J.P., Dusanter-Fourt, I., Masse, A., Giraudier, S., Katz, A., Wendling, F., Vainchenker, W., Casadevall, N., and Debili, N. (1999) Autonomous megakaryocyte growth in essential thrombocythemia and idiopathic myelofibrosis is not related to a c-mpl mutation or to an autocrine stimulation by Mpl-L. *Blood*, **93**, 125–139.

29. Temerinac, S., Klippel, S., Strunck, E., Roder, S., Lubbert, M., Lange, W., Azemar, M., Meinhardt, G., Schaefer, H.E., and Pahl, H.L. (2000) Cloning of PRV-1, a novel member of the uPAR receptor superfamily, which is overexpressed in polycythemia rubra vera. *Blood*, **95**, 2569–2576.

30. Wendling, F., Varlet, P., Charon, M., and Tambourin, P. (1986) MPLV: a retrovirus complex inducing an acute myeloproliferative leukemic disorder in adult mice. *Virology*, **149**, 242–246.

31. Wendling, F., Vigon, I., Souyri, M., and Tambourin, P. (1989) Myeloid progenitor cells transformed by the myeloproliferative leukemia virus proliferate and differentiate in vitro without the addition of growth factors. *Leukemia*, **3**, 475–480.

32. Wiestner, A., Padosch, S. A., Ghilardi, N., Cesar, J. M., Odriozola, J., Shapiro, A., and Skoda, R. C. (2000) Hereditary thrombocythaemia is a genetically heterogeneous disorder: exclusion of TPO and MPL in two families with hereditary thrombocythaemia. *Br J Haematol*, **110**, 104–109.

33. Wiestner, A., Schlemper, R. J., van der Maas, A. P., and Skoda, R. C. (1998) An activating splice donor mutation in the thrombopoietin gene causes hereditary thrombocythaemia. *Nature Genet*, **18**, 49–52.