The Molecular Biology of Myeloproliferative Disorders

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The myeloproliferative disorders (MPDs) are a spectrum of clonal disorders of the hematopoietic system. The discovery of activating mutations of the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and primary myelofibrosis has lead to in vitro and animal model studies that promise to lead to therapeutic advances.

The myeloproliferative disorders (MPDs) are a spectrum of clonal disorders of the hematopoietic system (reviewed in Levine et al., 2007). The distinct clinical manifestations are dictated by the primary cell type affected, and thus chronic myeloid leukemia (CML) is a proliferation of mature granulocytes, polycythemia vera (PV) is an expansion or red blood cells, essential thrombocythemia (ET) results in an increase of platelets, etc. (Table 1). The natural history of MPDs is generally chronic in nature, and patients come to medical attention either by coincidence (abnormal blood findings during routine exam) or by signs and symptoms related to the expansion of the hematopoetic system (e.g., an enlarged spleen). Common to most MPDs is a small but finite risk of disease evolution to an acute leukemia, where hematopoetic development is blocked at an early stage of differentiation, leading to the accumulation of poorly functioning myeloid blasts at an expensive of critical depletion of normal white blood cells and platelets, leading to morbidity and mortality from infections and bleeding complications. If they do not progress to an acute leukemia, the natural history of MPDs often results in fibrosis of the bone marrow, migration of hematopoesis to other organs (spleen and liver), and eventual complications of this secondary organ involvement, as well as from decreased normal blood counts from marrow fibrosis.

A unifying theme in the pathogenesis of MPDs is the activation of tyrosine kinases. The "poster child" is CML, where the BCR-ABL translocation is found in all cases; the fusion BCR-ABL activates proliferative and antiapoptotic pathways; and most importantly, inhibition by tyrosine kinase inhibitors (TKIs) can markedly reverse the natural history of the disease. The molecular lesions responsible for PV, ET, and myelofibrosis (MF) were unknown until relatively recently. In 2005, a flurry of reports found that a point mutation in JAK2, resulting in a valine for phenylalanine substitution at codon 617 (JAK2V617F), occurred at a high prevalence in these disorders (Baxter et al., 2005; James et al., 2005; Kralovics et al., 2005; Levine et al., 2005). The mutation was found in roughly half of MF and ET cases and nearly all PV cases. Constitutive activation of JAK2 activates STAT and MAPK proliferative signaling pathways, leading to transformation of hematopoetic progenitors. Curiously, not all hematopoetic stem cells in cases with the JAK2V617F harbor the mutation. Moreover, the data suggested a differential dosage effect in the different diseases. Whereas in most cases the JAK2V617F is heterozygous with a normal JAK2 allele, in many cases of PV the mutation is homozygous (Kralovics et al., 2005) through the process of acquired uniparental disomy. Curiously, in vitro cultures of PV cases will often show homozygous JAK2V617F erythroid colonies, whereas similar colonies from ET patients are heterozygous for the mutation.

There has been a substantial body of work attempting to study the effects of the JAK2V617F in mouse models. Early reports focused on a bone marrow transplantation model, where mouse bone marrow cells harboring exogenous JAK2-V617F were transplanted into irradiated mice (Wernig et al., 2006). These models produced a syndrome of what appeared mostly like PV, but most failed to completely recapitulate the spectrum of leukocytosis, thrombocytosis, and myelofibrosis found in human disease. Transgeneic models followed, which again produced a spectrum of MPD disorders, with a suggestion of phenotype relating to the JAK2V617F expression levels (Tiedt et al., 2008). Very recently, several groups have created knockin systems placing a conditionally inducible JAK2V617F allele under control of the endogenous JAK2 promoter. This allows for control of the JAK2V617F expression in only hematopoetic tissues, getting one closer to replicating the disease experience of the human patient. Marty et al. found that the heterozygous expression of JAK2V617F produced a PV-like syndrome, not like human where heterozygousity is more often associated with ET (Marty et al., 2010). However, Akada et al. demonstrated that both heterozygous and homozygous JAK2V617F caused a PV syndrome, with a demonstration of a dose effect, as indicated by the fact that homozygous expressors had a greater manifestation of elevated blood counts and spleen size, compared to those mice with lower levels of JAK2V617F (Akada et al., 2010). In addition, Li et al. have produced a very provocative study in which a human JAK2V617F knockin was created (Li et al., 2010). This model produced a transplantable disease with some features of both ET and PV. Of interest is the finding that affected mice had reduced numbers of primitive hematopoetic cells that had evidence of impaired normal function (cell cycling, apoptosis, and DNA damage). Moreover, competitive marrow transplantation showed impaired hematopoetic stem cell function.

Recently in this journal, Mullally and colleagues make critical observations

Table 1. Classification of MPD and Examples of Activating Mutations

Myeloproliferative Disorders	Primary Cell Involved	Activating Mutations
Chronic myeloid leukemia	neutrophils	BCR-ABL (100%)
Polycythemia vera	red blood cells	JAK2 (>90%)
Essential thrombocythemia	platelets	JAK2 (~50%)
Primary myelofibrosis	monocytes?	JAK2 (~50%)

that add substantially to the impressive work summarized above (Mullally et al., 2010). Similar to the approaches above, the authors used a conditional JAK2-V617F expression model to yield physiological levels of the mutated allele. The phenotype in the mice resembled much of the cellular biology and clinical features of human PV, and it was serially transplantable with great efficiency. Separation of the bone marrow into immature Lineage$^-$ SCA-1$^+$ c-Kit$^+$ (LSK) and more mature myeloid erythroid progenitor (MEP) and granulocytic monocytic progenitor (GMP) subpopulations demonstrated that the "MPD-initiating" JAK2-V617F cell capable of transplantation resided in the LSK population, but not in the committed myeloid MEP or GMP progenitors. Surprisingly, several studies showed that mutant cells in the LSK compartment were quite similar to wild-type cells in regard to cell cycle status, STAT signaling, and gene expression (though JAK2V617F cells showed enrichment of the erthyroid, myeloid, and megakaryocytic differentiation pathways). Similar to the Li et al. paper, competitive transplantation experiments showed that mutant cells had at best a minor competitive edge compared to wild-type, and a small number of mutated cells nonetheless causes a PV phenotype. Lastly, the authors demonstrated that the MPD-initiating cell was not killed by JAK2 inhibition. Mice treated with the inhibitor had a dramatic decrease in spleen size and a reduction of erthyroid precursors in the marrow, but LSK cells from treated mice were able to cause the PV phenotype in subsequently transplanted mice.

These studies in total offer an increased understanding of the MPD that may usher in a new era of therapy, much like what occurred in the study of CML. Similar to CML, these studies suggest the initiating cell resides in the primitive compartment but is genetically and phenotypically quite similar to its normal complement. Like CML, mutated cells in the stem cell compartment appear resistant to kinase inhibition. However, as we move toward better therapies for MPD, these findings have implications in the feasibility of "stem cell" therapy, because there may not be a large therapeutic window to selectively kill MPD "stem cells." In addition, a limitation of murine systems is that however eloquent, they are still only models of human disease. For example, human MPD may well have additional genetic lesions contributing to initiation and progression, and mouse models cannot easily recapitulate this complexity. In this regard it is interesting that several other mutations have recently been discovered in MPD (e.g., TET2, ASXL1, IDH1, and IDH2); TET2 mutations have been found in JAK2V617F-positive and -negative clones from the same patient, suggesting that TET2 mutations may be a relatively early event in MPD. Moreover, if tumor initiation and progression is influenced by interactions with a host's innate immunological system, then disease in the mouse model might be expected to be very different than in humans. Nonetheless, the work presented by Mullally and others are quite significant, and provide us with powerful tools to better understand disease and test new agents of therapy.

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