The JAK2V617F Mutation in Polycythemia Vera and Other Myeloproliferative Disorders: One Mutation for Three Diseases?

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The discovery of the JAK2V617F mutation has made the diagnosis of polycythemia vera (PV) much easier, but the pathogenesis of PV is still incompletely understood. In particular, it is not yet elucidated how a single mutation can be found in multiple myeloproliferative disorders (MPD) and myelodysplastic syndromes with ring sideroblasts and whether the sole JAK2V617F is sufficient to induce a MPD in humans. Several hypotheses are under investigation such as differences in the targeted hematopoietic stem cells (HSC), host modifier polymorphisms, intensity of JAK2V617F signaling, presence of other somatic mutations, or the presence of a pre-JAK2 event that may vary according to the MPD phenotype. Multiple studies have provided some evidence for and against each hypothesis, but it now seems possible to reconcile these hypotheses into a model that will need to be tested using newly developed tools. Recent investigations have also led to new treatment modalities that could benefit patients with PV.

Introduction

Polycythemia vera (PV) is a clonal and acquired stem cell disease characterized by an abnormal erythropoiesis, with some erythroid progenitors being erythropoietin (Epo)-hypersensitive and independent.[1] PV belongs to the family of chronic myeloproliferative disorders (MPD), which includes hematological diseases that share clinical and biological similarities, such as a hematopoietic stem cell origin: PV, essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic myeloid leukemia (CML), some types of hypereosinophilic syndrome (HES), systemic mast cell disease (SMD) and other rare disorders. The molecular characterization of PV came in 2005 with the discovery of the JAK2V617F mutation in about 90% of PV patients. While studying the mechanisms responsible for the Epo-independent growth characteristic of PV progenitors,[2] the team of William Vainchenker discovered the presence of a mutated form of the JAK2 protein, JAK2V617F.[3] Other groups came to the same conclusion after analyses of tyrosine kinome[4,5] or precise mapping[6] of the minimal 9p Loss of Heterozygosity region found in 30% of PV patients.[7] Analysis of different cell populations in PV patients demonstrated that the JAK2V617F mutation was acquired, clonal[3-5,7] and present in hematopoietic stem cells (HSC).[8] Experiments performed with murine cell lines showed that the mutated JAK2V617F was constitutively active, and able to activate the Epo receptor-signaling pathway without Epo.[3-5,7] Further confirmation on the role of JAK2V617F in the pathogenesis of PV came from animal models: retroviral transduction of JAK2V617F in murine HSC followed by transplantation into lethally irradiated mice led to the development of a PV phenotype rapidly evolving into secondary myelofibrosis,[9-13] thus recapitulating the polycythemic and the spent phases of PV. All these considerations undoubtedly demonstrate the crucial role of JAK2V617F in the pathogenesis of PV, and the story would be simple if stopped here. Indeed, the JAK2V617F mutation was not only found in almost all patients with PV but also in about 50% of patients with ET and PMF, in rare patients with CML and atypical MPDs,[14-16] in myelodysplastic syndromes with thrombocytosis[17] and even in hematologically normal patients with portal vein thrombosis.[18]

This intriguing feature raises the major and still unanswered question of how a unique mutation can cause different phenotypes. Several hypotheses are now under investigation and will be discussed in this review. On the one hand, the sole JAK2V617F mutation is sufficient to induce an MPD, and the MPD phenotype depends on the cell targeted by the mutation or the genetic background of the patients or the intensity of JAK2V617F signaling. On the other hand, JAK2V617F is an event secondary to a first hit that varies between the diseases. In this review, we will discuss these hypotheses separately for better clarity and then consider how it is possible to reconcile them. Finally, we will summarize the very recent data on the efficacy of targeted therapies in PV.

Hypothesis One: The Phenotype Depends on the Cell Targeted by the JAK2V617F Mutation

One way for a unique oncogenic event to give rise to different diseases is to target different cells. Given that X-linked clonality assays have demonstrated that PV,[19] a group of

ET[20,21] and PMF were clonal diseases, it is assumed that the cell targeted by a molecular event responsible for these diseases is a multipotent HSC. Because the phenotypes of ET, PV and PMF mainly differ by the extent to which the megakaryocytic, the erythroid or the granulocytic lineages are involved, one may hypothesize that the phenotype of these diseases is determined by the ability of the cells where the mutation first occurred to differentiate into these different lineages. For example, occurrence of the mutation in a self-renewing cell with high capacity to produce platelets but little ability to produce erythroid or granulocytic cells would essentially result in thrombocytosis. At the opposite, occurrence of the mutation in a stem cell having the ability to produce these three lineages together with ineffective hematopoiesis (probably due to an increase of TGFβ secretion in bone marrow) would result in a PMF-like phenotype. Various studies have shown that granulocytic, erythroblastic, megakaryocytic and lymphoid lineages contained JAK2V617F-positive cells both in PV[22,23] and PMF[22] but it cannot be excluded that the JAK2V617F targets different subsets of HSC, with different transcriptional programs, leading to different differentiation properties. Indeed, the group of Ron Hoffman reported that the HSC from PMF and from a fraction of PV patients had an abnormal differentiation program.[24,25] Moreover, our group recently reported other differences in the HSC compartment between PV and PMF patients.[26] Using a NOD/SCID mouse model, we reported that the HSC compartment of PMF patients is predominantly JAK2V617F-positive, whereas the majority of HSC in PV is JAK2-wild type. Working out the mechanism of this difference should be a productive avenue of future research for understanding how a unique mutation can explain different phenotypes.

Hypothesis Two: JAK2V617F is the Sole Event Responsible for MPD and the Phenotype Depends on the Genetic Background of the Patients

It may also be assumed that the same recurrent mutation occurs in a HSC in all MPD patients, but given the genetic background of the patient, the phenotype will be different. Knowing that some patients can evolve from ET to PV, this hypothesis may appear inadequate. Nevertheless, two arguments may indicate that the genetic background can modulate the phenotype. The first one comes from comparative analyses of diseases phenotypes of mice transplanted with JAK2V617F-transduced murine HSC in four studies.[9-12] When C57Bl/6 mice were transplanted with JAK2V617F-transduced cells, mice developed a PV-like disease characterized by a marrow trilineage hyperplasia, an enlarged spleen and a strong polycythemia associated with the presence of EEC.[9-11] Polycythemia was followed 3 months post-engraftment by a myelofibrotic stage.[9,11] Very interestingly, when Balb/C mice were used for the same experiments, the first step of the disease was not only a polycythemia but also a marked leukocytosis.[10-12] The second step of the disease was different as well, as the mice developed a more pronounced myelofibrosis. The second argument in support for a phenotype modulation by host genetic variation comes from the work by Pardanani and coworkers.[27] Genotyping of single nucleotide polymorphisms (SNP) in the genes encoding JAK2, the Epo-receptor (EpoR), the thrombopoietin-R (MPL) and the G-CSFR in PV, PMF and ET patients revealed that some SNPs in *JAK2* and *EpoR* were preferentially associated with some diseases. Nevertheless, no clear correlation between a particular haplotype and a phenotype could be unequivocally established.

Hypothesis Three: The Phenotype Depends on the Level of JAK2V617F Kinase Activity

The JAK2V617F mutation is found either at the heterozygous or homozygous state in MPD patients and the mechanism leading to homozygosity is in most cases mitotic recombination.[6,7,16] The presence of different copy numbers of the JAK2V617F allele led to the "dosage hypothesis" that implies that the phenotype diversity would depend on the level of kinase activity generated by the mutant protein, which would be crucial for the activation of certain signaling pathways. In this model, a low level of kinase activity would favor a megakaryocytic/ET phenotype and a high level an erythroid/PV phenotype. Depending on the level and duration of exposure, sustained kinase activity would ultimately lead to myelofibrosis.[28] In this model, ET, PV and PMF can be seen as a continuum of phenotypic variations that would depend on the level of JAK2V617F kinase activity. Therefore, JAK2V617F-positive MPD may be considered as one single disease with different stages/phenotypes that would depend on the level of JAK2V617F kinase activity.

Two major arguments sustain this "dosage hypothesis." The first one comes from the correlation between the JAK2V617F burden in granulocytes and the patients' phenotypes. JAK2V617F-positive ET were called "forma frustra" of PV after the observation that they displayed many features of PV.[29] In addition, PV patients with a high JAK2V617F burden (>50%) display significantly higher hemoglobin levels and higher rate of fibrotic transformation than heterozygous PV patients,[30] and progression from PV to post-PV MF (PPVMF) is associated with an increase in the JAK2V617F burden.[31] Collectively, these data support the model of a continuum of phenotypes depending on the level of JAK2V617F. When erythroid progenitors were analyzed separately, allowing the precise analysis of the JAK2V617F allelic status at the single cell level, a striking difference was seen between ET and PV: homozygous erythroid colonies were rarely detected in patients with ET, whereas they were in the vast majority of patients with PV,[32,33] supporting the "dosage hypothesis." The second argument came recently with studies on transgenic mice

expressing human JAK2V617F in hematopoietic cells.[13,34] Tiedt and coworkers clearly demonstrated the link between the JAK2V617F/JAK2 wild-type ratio and the MPD phenotype. These authors showed that a weak JAK2V617F expression in hematopoietic cells was associated with a phenotype resembling ET, whereas a higher expression led to a PV phenotype.[13]

If we assume that modulation of JAK2V617F kinase activity level is responsible for the diversity of phenotypes, what are the mechanisms responsible for these modifications? Homozygosity following mitotic recombination is obviously the first step, as it not only leads to the *JAK2*-V617F duplication but also to the disappearance of the wild-type allele. Indeed, the JAK2V617F protein seems to compete with the wild-type JAK2 protein[3] and disappearance of the wild-type *JAK2* would lift the competition. Another way to modify the kinase activity level is to decrease the activity of proteins that negatively regulate the JAK2 signaling pathway, such as suppressors of cytokine signaling 3 (SOCS3), SOCS1 and SHP1. It is noteworthy that a recent study has reported epigenetic inactivation of SOCS3, SOCS1 and SHP1 in MPDs, but no correlation was made with the presence of JAK2V617F.[35,36] Besides, the SOCS3 protein did not decrease JAK2 activity but, on the contrary, enhanced the proliferation of murine cell lines expressing JAK2V617F.[37] It thus remains to be investigated whether an abnormal regulation by SOCS3 is present in MPD patients and whether differences exist between the JAK2V617F MPD.

How can the level of kinase activity modulate the phenotype? The clue comes from the structure of the JAK2V617F protein. The JAK2 protein is mutated in its pseudokinase domain, which normally exerts a negative control on the kinase domain. The V617F mutation is thought to prevent the pseudokinase domain from inhibiting the kinase domain, thus resulting in a constitutively active state of the protein. The FERM (band 4.1, ezrin, radixin, moesin) domain of JAK2, which interacts with cytokine receptors, is intact in the JAK2V617F protein. This domain was recently demonstrated to be crucial for the activity of JAK2V617F.[38] The mutant protein also requires the presence of dimeric receptors on the cell surface to be fully oncogenic.[39,40] JAK2V617F thus binds to EpoR, MPL and granulocyte-colony stimulating factor receptor (G-CSFR), as does wild-type JAK2. Complexes between JAK2V617F and these receptors probably explain cytokine hypersensitivity and independence in MPD. To understand how variation in the amount of a unique mutant protein can lead to different phenotypes, we have to remember that EpoR, MPL and G-CSFR are differently expressed in progenitors. MPL is expressed at high levels in megakaryocyte precursors, suggesting that a small amount of JAK2V617F would be sufficient to induce MPL signaling and thus megakaryocyte proliferation and platelet production, as seen in ET. EpoR is, on the contrary, expressed at low levels on erythroid progenitors, implying that a higher amount of JAK2V617F would be necessary to induce EpoR signaling and erythroid hyperplasia leading to a PV phenotype. MPL oversignaling by excessive Tpo stimulation was shown to lead to myelofibrosis,[41] suggesting that a high amount of JAK2V617F leading to strong MPL signaling in megakaryocytes would be responsible for myelofibrosis. Another way by which different levels of JAK2V617F kinase activity could modulate the phenotype involves the trafficking of the receptors and thus their expression. Such a mechanism may explain the low cell surface expression of MPL reported in MPD platelets and megakaryocytes.[42,43] Lastly, recent findings indicate the existence of cross-talks between JAK2V617F and tyrosine kinase receptors like the insulin-like growth factor 1 (IGF1) receptor[44] or c-kit.[45] These data suggest that JAK2V617F may have effects on myeloid progenitors that are not restricted to Epo, Tpo and G-CSF.

Hypothesis Four: JAK2V617F Is an Event Secondary to an Unknown Pre-JAK2 Event Determining the Phenotype

Another way to explain that one unique mutation gives rise to different phenotypes is to assume that it is not the sole event responsible for the pathogenesis of the diseases and that another molecular event would occur before the JAK2V617F mutation. This molecular event would be different depending on the disease, thus explaining the different phenotypes. There are now several arguments that strongly suggest that a preceding event occurs before JAK2V617F in some patients, although it is not known whether this first hit would be different between the MPD.

The first argument is the discrepancy in some patients with PV or PMF, but in most with ET, between the JAK2V617F burden in granulocytes and the clonality data obtained with X-inactivation studies,[46-48] as some female patients had clonal granulopoiesis with a JAK2V617F/JAK2 total of only 2% to 25%. Similar results were obtained when clonality was assessed using the 20q deletion as an autosomal clonality marker.[48] These studies suggest that a first hit would be responsible for a clonal expansion within the hematopoietic compartment and that the JAK2V617F mutation would occur as a second hit in an already clonal hematopoiesis. Another evidence for the presence of a pre-JAK2 event comes from the analysis of leukemic transformation in patients with a JAK2V617F-positive MPD. Most of them transform to a JAK2V617F-negative acute myeloid leukemia[49-51] independently from the treatment previously received, suggesting either a *de novo* origin of the AML or a common clonal origin of JAK2V617F MPD and AML. SNP arrays analysis will be a valuable tool to address this question.[51] Over the last two years, many groups described some patients with another molecular abnormality associated with JAK2V617F, such as BCR-ABL,[52,53] *MPL* muta-

tions[54] or another *JAK2* mutation.[55,56] Together, these data suggest a common clonal origin of both clones, given the low probability that two different MPDs would occur *de novo* in normal HSC.

Nussenzveig and coworkers, who recently found JAK2 wild-type EEC together with JAK2V617F-positive EEC in JAK2V617F-positive PV patients,[57] gave another evidence for the presence of a first hit in some patients. This unexpected result suggests that the unknown PV pre-JAK2 event would not only be responsible for the development of a clonal disease but would also promote erythropoietin-independent differentiation, raising the question of the additional role of JAK2V617F.

Lastly, observation of familial cases of MPD is the most striking evidence of the presence of a pre-JAK2 event in some patients.[58,59] The first remarkable feature of these families is the occurrence of different MPD within the same families, such as JAK2V617F-positive ET, JAK2V617F-positive PMF, JAK2 wild-type mastocytosis and BCR-ABL CML. This suggests the presence of an unknown germline event that would be a predisposing factor common to multiple MPDs. The second interesting feature comes from the observation of families with only JAK2V617F-positive MPD, where the absence of the JAK2V617F mutation in the patients' T and B cells clearly demonstrates that the JAK2V617F is an acquired event as in sporadic MPD. Linkage analysis is a powerful tool for pointing out the unknown common molecular event responsible for the development of MPD in these families. Identification of this event would be of great interest in sporadic MPD.

Is It Possible to Reconcile All These Hypotheses?

The different hypotheses were summarized separately for better clarity. However, it now seems that none of them is the absolute right one, but in fact all of them are. Even if JAK2V617F transgenic mice reproduce ET, PV and post-PV phenotypes, suggesting that the sole JAK2 mutation is sufficient to induce a MPD, we should keep in mind that this model might not be perfect to understand the human pathology, as the mouse and human diseases are very different in terms of clonality. Indeed, the disease induced in transgenic mice is not clonal as all HSC are JAK2V617F-positive, which contrasts with PV where the disease arises from one unique mutated HSC. At this time, it is not known whether the sole occurrence of the *JAK2* V617F mutation in HSC could confer a sufficient proliferative advantage to develop a monoclonal disease. Long-term serial analysis of X-chromosome inactivation patterns and JAK2V617F mutant levels in polyclonal ET patients showed that small mutant clones can remain stable for many years, suggesting that JAK2V617F does not confer a strong proliferating advantage to HSC.[60] Another event present in HSC may thus be necessary for a JAK2V617F monoclonal disease to develop. As described previously, it is very likely that a first hit occurs in an HSC that gives a proliferative advantage leading to a monoclonal hematopoiesis. Such an hypothesis would explain the discrepancies sometimes observed between clonality data using X chromosome inactivation studies and JAK2V617F quantification.[47] This pre-JAK2 stage would therefore be pre-leukemic, explaining JAK2V617F-negative transformation of JAK2V617F-positive MPD[50] and MPD with two oncogenic events.[53-56] The JAK2V617F would then target a pre-leukemic HSC and induce an MPD. The phenotype of the disease would depend on the intensity of JAK2V617F signaling, which could vary either through host genetic modifiers[27] (polymorphisms in *JAK2*, cytokine receptors), the amount of mutant JAK2V617F protein (mitotic recombination, trisomy) or the regulation of JAK2V617F signaling (SOCS, cross talks with other receptors).

Novel Therapies for PV Based on Pathogenetic Concerns

After the discovery of the JAK2V617F mutation many firms rapidly started to develop anti-JAK2 therapies and promising results were reported at the 2007 meeting of the American Society of Hematology. We can cite AZ-01,[61] XL019,[62] TG101348,[63] INCB 018424, AT 9283, and CEP 701. The efficacy of these drugs against JAK2V617F was usually proven by using murine cell lines, but a few were shown to be active in murine models.[61,63] Clinical trials are now ongoing to test the efficacy and toxicity of these molecules, and the latest results are detailed in a review by Levine and Heaney in this volume.[64] Despite the promising future of these JAK2 inhibitors, three major concerns are now raised: firstly, they all target JAK2V617F but also JAK2 wild-type raising concerns about their potential toxicity. Secondly, the JAK2V617F mutation was shown to be present in HSC,[8,26] implying that the anti-JAK2 therapy will have to be effective against malignant HSC in order to be curative. Thirdly, the more we understand the pathogenesis of MPD, the more we question whether inhibiting a mutated protein, which may not be the founding pathogenic event, will be useful. An alternative to anti-JAK2 therapies has recently emerged with the demonstration of the efficacy of less specific tyrosine kinase inhibitors, such as imatinib,[45] AEE 788[65] and erlotinib[66] in JAK2V617F cell lines and in human PV progenitors. Interestingly, JAK2V617F-positive cells showed greater susceptibility to these inhibitors than their negative counterparts, suggesting that these drugs could be used for treatments of JAK2V617F-positive MPD. Despite the initial enthusiasm following the discovery of the JAK2V617F mutation in 95% of PV patients, there is no denying that the miracle drug for PV is not yet born. Moreover, a specific anti-JAK2V617F molecule able to target HSC may not be ideal since there is incontestable evidence that a pre-JAK2 event exists, at least in some patients.

Conclusions and Prospects

Although CML is undoubtedly the better-characterized MPD, the study of PV pathogenesis has also led to important breakthroughs in our understanding of MPD: the description of endogenous erythroid colonies more than 30 years ago, the discovery of the JAK2V617F mutation 3 years ago and more recently evidences for the existence of a pre-JAK2 event, at least in some patients. Even if the presence of the JAK2V617F mutation is now of great help in the diagnostic work up of PV,[67,68] the presence of this mutation is neither specific for this disease nor necessary for the development of PV. Indeed, a small fraction of PV patients are negative for the V617F mutation but display a mutation in the exon 12 of *JAK2*.[69] Despite the link between PV and JAK2 mutations, PV stays a heterogenous disease when studied in detail at the cellular level, and analysis of this heterogeneity will probably provide new insights into the pathogenesis of MPD. Another way to progress in the understanding of the complexity of MPD pathogenesis is to study the effects of JAK2V617F in human cells by using newly developed lentiviral vectors that can efficiently transduce human normal cells.[70] This tool will demonstrate whether the introduction of JAK2V617F in normal human cells is sufficient to recapitulate the various abnormalities observed in PV, such as the Epo-independence. If yes, it would mean that a sole JAK2V617F is sufficient to induce PV; future work will be focussed on understanding how one event can cause different phenotypes. If no, this would be a major argument in favor of the "pre-JAK2" hypothesis, and multiple strategies could be undertaken to characterize this/these event(s) such as linkage analysis in familial MPD.

Disclosures

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