IN THE SPOTLIGHT

Aberrant Cytokine Production by Nonmalignant Cells in the Pathogenesis of Myeloproliferative Tumors and Response to JAK Inhibitor Therapies

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Summary: Kleppe and colleagues use detailed cytokine profiling analyses to investigate the role of aberrant proinflammatory cytokine secretion in the pathogenesis of myeloproliferative neoplasms. Their analyses implicate constitutive activation of STAT3 in both malignant and nonmalignant bone marrow cell populations as a driver of aberrant cytokine secretion and as a cellular target mediating the therapeutic activity of ruxolitinib.
Cancer Discov; 5(3); 234–6. ©2015 AACR.

See related article by Kleppe and colleagues, p. 316 (7).

Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are BCR–ABL1-negative myeloproliferative neoplasms (MPN) driven by the constitutive activation of the JAK–STAT pathway (1, 2). Thus, more than 95% of PV cases show the *JAK2*^{V617F} activating mutation, which is also present in 60% of patients with ET and in around 50% of patients with PMF. In addition, rare *JAK2*^{V617F}–negative PV cases show alternative activating *JAK2*-mutant alleles or calreticulin mutations also implicated in activation of JAK signaling (1). Finally, about 5% of ET and PMF samples show activating mutations in the thrombopoietin receptor gene *MPL*, resulting in increased JAK2 activation (1). The central pathogenic role of constitutive JAK–STAT activation in MPNs provides a strong rationale for the development of JAK kinase inhibitors as targeted therapies for the treatment of these diseases. Among these, the JAK1- and JAK2-selective inhibitor ruxolitinib has shown remarkable therapeutic activity in patients with PV and PMF, with marked improvement in constitutional symptoms, reduction in spleen size, and amelioration of cytopenias and bone fibrosis (3, 4). In addition, patients with MPN frequently have constitutional symptoms as a result of increased levels of proinflammatory cytokines, a condition associated with adverse survival (5). Therapeutically, treatment with ruxolitinib leads to a rapid and sustained downregulation of cytokine levels, indicating that the JAK signaling pathway mediates this aberrant inflammatory cytokine profile (6). However, the specific molecular mechanisms underlying the aberrant expression of cytokines in patients with MPN and the role of the JAK–STAT axis in this context remain unknown.

In this issue of *Cancer Discovery*, Kleppe and colleagues (7) thoroughly characterize the cytokine profiles of PMF mouse models and patient samples to explore the mechanisms mediating the therapeutic activity of JAK inhibitors. Analysis of 32 different cytokines in the plasma of mice transplanted with hematopoietic progenitors expressing constitutively active MPL (MPL^{W515L}) and in the plasma of hematopoietic-specific JAK2^{V617F} knockin mice showed a proinflammatory cytokine profile similar to that present in patients with PMF. Moreover, treatment of diseased mice with ruxolitinib normalized the serum levels of cytokines, validating these models as useful for the study of the pathogenic role of cytokine production and as a therapeutic platform in PMF. In this context, increased levels of cytokines in bone marrow implicated this compartment as an important source of aberrant cytokine production in PMF. Single-cell profiling of cytokine expression in bone marrow cells further demonstrated an increased percentage of cytokine-secreting cells and increased absolute levels of cytokine production in PMF mice. In addition, these analyses showed the presence of marked functional heterogeneity and increased numbers of multifunctional cells coexpressing different cytokines. Moreover, single-cell cytokine profiling analysis of sorted mature myeloid cells and megakaryocyte/erythroid progenitors (MEP) in PMF mice showed an increased percentage of secreting cells and higher levels of cytokine production compared with controls. However, these two populations showed markedly different secretion profiles, with MEPs primarily producing IL6 and IL10 and more mature myeloid cells secreting CCL3 and TNFα.

Analysis of circulating granulocytes of patients with PMF compared with healthy controls validated these results, demonstrating increased percentages of cytokine-secreting cells, higher levels of cytokine production, and an overlapping pattern of cytokine secretion (6 of 8 factors) with that observed in MF mice. Overall, these results support that production of proinflammatory cytokines in MF is orchestrated by a heterogeneous group of cells with distinct and cooperative roles in the pathogenesis of the disease.

Mechanistically, *in vitro* cellular assays showed increased STAT3 activation upon expression of MPN-associated *MPL*

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doi: 10.1158/2159-8290.CD-15-0095

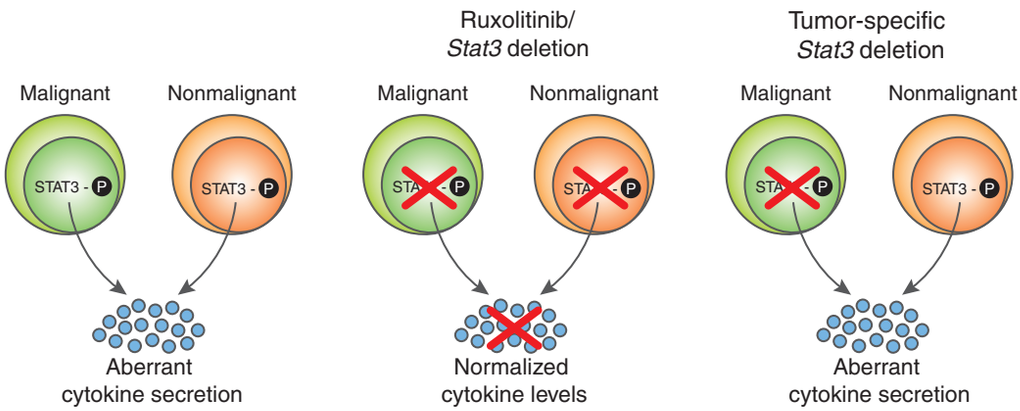

Figure 1. Constitutive activation of STAT3 in malignant and nonmalignant cells drives aberrant cytokine secretion in MPN. *Stat3* deletion in a MPN mouse model bone marrow cells normalizes the production of cytokines, recapitulating the therapeutic effects of JAK inhibition therapy with ruxolitinib. In contrast, selective tumor-specific deletion of *Stat3* fails to abrogate cytokine production, indicating that JAK inhibition in both malignant and nonmalignant populations is required for therapeutic efficacy.

and *JAK2* mutations, which was also present *in vivo* in splenocytes from Mpl^{W515L} PMF mice and in the bone marrow of patients with PMF. Moreover, genetic deletion of *Stat3* reduced disease severity and cytokine-mediated inflammation in the Mpl^{W515L} PMF model, recapitulating the effects of inhibiting JAK signaling by ruxolitinib. However, and most strikingly, transplantation of *Stat3*-deficient Mpl^{W515L}-expressing or $Jak2^{V617F}$ cells together with wild-type bone marrow hematopoietic progenitors fully recapitulated the PMF phenotype (both cytokine production and disease severity) in recipient animals, demonstrating that cell-intrinsic STAT3 signaling in *MPL*-mutant cells is dispensable for the pathogenesis of this disease.

Importantly, ruxolitinib treatment in this chimeric model effectively normalized cytokine levels in both malignant and nonmalignant cells. All together, these results support a cooperative model in which inhibition of STAT3-dependent cytokine secretion in both malignant and nonmalignant PMF cells is required for the therapeutic activity of ruxolitinib (Fig. 1).

Overall, the results presented by Kleppe and colleagues (7) unveil a complex landscape implicating a mosaic of functionally diverse malignant and nonmalignant cell populations in the pathogenesis of PMF and as targets of tyrosine kinase inhibition treatment. Moreover, these data provide important novel insights on the mechanism of action of JAK inhibitors in PMF and highlight the critical role of STAT3 activation in aberrant cytokine signaling as an essential factor for the pathogenesis of BCR–ABL1-negative MPNs.

It is also worth noting that some of the results shown here have important implications that may very well extend beyond the specific context of MPNs. First, the demonstration of a pathogenic role of non-tumor cells "instructed" by the tumor population to produce inflammatory cytokines essential for disease progression highlights the essential role of the tumor microenvironment in cancer progression. This adds to the well-established role of tumor stroma cells in invasion and metastasis, angiogenesis, and in the generation of immune-privilege environments protecting tumor cells from immune surveillance. Still, the specific mechanisms mediating the instructive signals by which MPN cells reshape the cytokine production profiles of nonmalignant bone marrow populations remain to be established. In addition, further studies will be needed to define the specific role of different non-tumor cell populations and specific cytokines as drivers of disease progression in MPNs.

The markedly heterogeneous pattern of cytokine production even within well-defined cell populations observed in single-cell analyses here depicts the tumor microenvironment as a complex and dynamic setting, which can be only partially recapitulated in *in vitro* studies. In this context, the need for improved understanding of the role of non-tumor cells as an essential component of the tumor ecosystem highlights the importance of studies using genetically engineered mouse tumor models as a tool to study the cancer ecosystem in an isogenic immunocompetent setting *in vivo*. Finally, a corollary of these studies is that host genetic variants and environmental factors influencing interaction of nonmalignant cells with the tumor may play a significant role in tumor initiation, disease progression, and response to therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Published online March 6, 2015.

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