the fate of combination therapies using molecular targeted therapies together with immune-based manipulations, future experiments should be performed using spontaneous tumors in the nontransplantation setting or tumor systems with less intrinsic immunogenicity. Such combinations may enable the more frequent induction of long-term durable cancer regression.

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STATistical Power of Clonal Analysis: Differential STAT1 Pathway Activation Downstream of the *JAK2V617F* Mutation

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The biological basis of the phenotypic diversity observed in *JAK2V617F*-positive myeloproliferative neoplasms is poorly understood. In this issue of *Cancer Cell*, Chen et al. show that interferon and STAT1 signaling are activated in essential thrombocythemia but not in polycythemia vera. STAT1 promotes megakaryopoiesis and thus contributes to an essential thrombocythemia phenotype.

Myeloproliferative neoplasms (MPN) are clonal disorders of hematopoiesis and are characterized by an accumulation of mature blood cells. The *JAK2V617F*-activating mutation is the most common molecular abnormality in *BCR-ABL*-negative MPN and is present in approximately 95% of patients with polycythemia vera (PV) and approximately 50% of patients with essential thrombocythemia (ET) and myelofibrosis (Levine and Gilliland, 2008). JAK2 is a cytoplasmic tyrosine kinase that mediates intracellular signaling between cell-surface cytokine receptors and multiple downstream effector pathways. The V617F mutation is located in the negative regulatory pseudokinase domain of JAK2 and results in ligand-independent activation of JAK2 signaling and activation of STAT5 transcription (James et al., 2005). JAK2 was recently found to phosphorylate Tyr41 (Y41) on histone H3 and thus lead to the release of transcriptional repression from heterochromatin protein 1α (HP1α) (Dawson et al., 2009).

In this issue of *Cancer Cell*, Chen et al. (Chen et al., 2010) examine how the *JAK2V617F* mutation can lead to different diseases, PV and ET, with distinct clinical features. In part, PV is molecularly distinct from ET because of a higher *JAK2V617F* mutational burden in PV patients. Mitotic recombination of the distal part of chromosome 9p occurs in approximately one third of PV patients and results in *JAK2V617F* homozygosity, whereas mitotic recombination almost never occurs in ET patients (Kralovics et al., 2005). Chen et al. therefore focused on *JAK2V617F* heterozygous cells by comparing the transcriptional profiles of erythroid progenitors from patients with PV or ET to gain insight into the molecular differences in these diseases.

A key feature of this study is the use of clonally derived cells for molecular studies to overcome several technical challenges in the study of gene expression in MPN patients and in cancer samples more generally. The analysis of gene expression in MPN can be confounded by the effects of an aberrant microenvironment, variation in endogenous cytokine levels, different *JAK2V617F* mutational burdens between samples, genetic heterogeneity within a sample, and skewing of the differentiation state within a population of cells in a sample. Chen et al. addressed these challenges

by analyzing genetically defined clones of cells. The authors exploited the fact that *JAK2* mutant and wild-type progenitor cells coexist in the peripheral blood of *JAK2V617F*-positive MPN patients. They cultured peripheral blood mononuclear cells from *JAK2V617F*-positive MPN patients on methylcellulose media supplemented with 0.01 U/ml erythropoietin, a low concentration that the authors found maximally discriminated between the expression of PIM1 (a known *JAK/STAT* target) in *JAK2* mutant and wild-type colonies. In a real *tour de force*, they grew more than 5000 erythroid colonies from 36 *JAK2V617F*-positive MPN patients (20 ET and 16 PV) and then collected, genotyped, and pooled the colonies on the basis of the *JAK2V617F* mutational status. For each patient, a pool of *JAK2V617F* heterozygous erythroid colonies was compared to a pool of *JAK2* WT colonies via gene expression profiling.

Using this approach, the authors found that the gene expression profiles of *JAK2V617F* mutant colonies were more closely related to *JAK2* wild-type colonies from the same patient than to mutant colonies from other patients. This suggests that despite the known effects of JAK2 on histone phosphorylation, endogenous heterozygous *JAK2V617F* expression has modest effects on global gene expression. Nevertheless, Chen et al. demonstrate that a gene expression signature of interferon (IFN) signaling is preferentially activated in *JAK2V617F* heterozygous erythroid progenitors from ET patients relative to PV patients.

The authors go on to show differential STAT1 phosphorylation, a known effector of IFN signaling, in erythroid colonies from ET patients versus PV patients. They demonstrate that STAT1 activation promotes megakaryocytic differentiation, consistent with the known role of STAT1 in megakaryopoiesis. STAT1 plays a critical role in the differentiation and polyploidization of megakaryocytes (Huang et al., 2007). It has been demonstrated that ectopic expression of STAT1 can rescue the defects in megakaryocytic maturation seen in Gata1-deficient murine megakaryocytes, suggesting that STAT1 functions downstream of GATA1 (Huang et al., 2007). Interestingly, enforced expression of *JAK2V617F* did not promote megakaryocytic differentiation or enhance STAT1 phosphorylation in a GATA1-null cell line, suggesting that GATA1 is required for *JAK2V617F*-mediated activation of STAT1 (Huang et al., 2007).

Although Chen et al. report differential IFN and STAT1 signaling in *JAK2V617F* heterozygote ET versus PV erythroid progenitors, the molecular basis for this finding remains to be elucidated. The authors suggest that the development of divergent phenotypes in *JAK2V617F*-positive MPNs occurs as a result of competition between STAT5 and STAT1 activation; the former would create a bias toward the development of a PV phenotype, and the latter would create a bias toward an ET phenotype. The observation that differential signaling occurs in cells grown under the same experimental conditions suggests a cell-intrinsic mechanism, such as the presence of other acquired genetic or epigenetic alterations in addition to *JAK2V617F* or the presence of inherited genetic modifiers. Multiple novel somatic genetic mutations have been described in MPN patients in the last few years (Tefferi, 2010), and host genetic variation has been associated with differences in MPN phenotype (Pardanani et al., 2008).

The use of gene expression profiling in clonally derived cells to compare genotypically matched populations might shed light on other aspects of MPN biology and the effects of other mutations in cancer. For example, an important and still unresolved issue in the MPN field is the contribution of *JAK2V617F* gene dosage to phenotype. *JAK2V617F* homozygous progenitors occur in the majority of PV patients, whereas almost all progenitors in ET patients are *JAK2V617F* heterozygous (Scott et al., 2006). Gene expression profiling of PV homozygote erythroid progenitors for comparison with *JAK2V617F* heterozygote clones from the same patient could be highly informative. It is also notable that ET patients who are *JAK2* wild-type have higher platelet counts and lower hematocrit than *JAK2V617F* mutant ET patients (Campbell et al., 2005), and expression profiling of these patients might also be informative. Identifying reliable gene expression signatures of kinase mutations in cancer has often proved to be challenging. The strategy reported by Chen et al. has the potential to refine these studies by controlling many aspects of context dependency, pathway activation by cytokines, and differentiation state of the cells profiled.

The finding of a previously unrecognized role for IFN and STAT1 signaling in MPN offers an explanation for the phenotypic diversity observed between PV and ET. The therapeutic implications of this finding remain to be determined. As the authors note, the clinical efficacy of IFNα in both PV and ET patients presents an apparent paradox with a role for STAT1 activation in ET. It is possible, however, that the therapeutic efficacy of IFNα is due to its effects on the bone marrow microenvironment or on pathways other than STAT1 or that pharmacologic doses of IFNα cause exhaustion of the MPN clone. Differential utilization of STAT pathways in MPN could result in distinct molecular dependencies and novel therapeutic opportunities in MPN. Regardless, transcriptional profiling of clonally derived cells provides a powerful tool to interrogate the consequences of defined molecular abnormalities in MPN and in human malignancies.

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