Chronic Myeloproliferative Disorders

The effect of the *JAK2* V617F mutation on PRV-1 expression

$JAK2^{V617F}$, an acquired mutation of *JAK2*, is present in a majority of patients with polycythemia vera and to a lesser extent among patients with the other myeloproliferative disorders. We analyzed the effect of $JAK2^{V617F}$ on the expression of polycythemia rubra vera 1(PRV-1), using an *in vitro* model. Compared to wild-type *JAK2*, the presence of $JAK2^{V617F}$ increased both PRV-1 protein and mRNA levels in murine myeloid cells. A *JAK2* inhibitor eliminated the V617F-induced increase in PRV-1 expression.

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$JAK2^{V617F}$, a gain-of-function mutation in the pseudokinase domain of *JAK2*, has been detected in more than 80% of patient with polycythemia vera, in less than half of patients with essential thrombocythemia or agnogenic myeloid metaplasia, and infrequently in patients with atypical myeloproliferative disorders (MPD).[1-5] $JAK2^{V617F}$ is not the only molecular change detected with a higher frequency in MPD patients. High expression of PRV-1 has also been frequently found among patients with polycythemia vera[6] and was correlated with the presence of endogenous erythroid colonies in patients and with growth-factor-independent proliferation of heterologous cells.[7] There are several reports on the co-existence of $JAK2^{V617F}$ and high expression of PRV-1 in patients with polycythemia vera.[8-10] We studied the causal relationship between $JAK2^{V617F}$ and overexpression of PRV-1 in murine myeloid cells. We used FDCP-EpoR cells transduced with MSCV-GFP retroviral vector carrying either wild-type or mutant *JAK2* cDNA or the empty vector.[2] These cell lines were kindly provided by Dr. William Vainchenker (INSERM U362, Institut Gustave Roussy, Paris XI University, France). Cells were kept in RPMI medium enriched with 10% fetal calf serum and supplemented with 10 ng/mL of interleukin-3 (Sigma Aldrich). The cell fluorescence caused by the green fluorescent protein (GFP) was used as a surrogate marker for detecting the level of expression of *JAK2*. The surface level of PRV-1 was measured by flow cytometry using anti CD177 antibody (MEM166, BD Pharmingen) and a phycoerythrin-conjugated rabbit anti mouse secondary antibody (Zymed). After 1 hour of incubation at room temperature and subsequent washes, cells were analyzed by two-color flow cytometry, to detect PRV-1 and GFP simultaneously. The results showed that FDCP cells transduced with wild-type JAK2 (FDCP-$JAK2^{WT}$) expressed native PRV-1 detectable by MEM166 (Figure 1A). Expression of PRV-1 on FDCP cells transduced with empty vector (FDCP-Sham) was similar to that of the FDCP-$JAK2^{WT}$ cells. However, the presence of $JAK2^{V617F}$ instead of $JAK2^{WT}$ increased the expression of native PRV-1 on FDCP cells (Figure 1A and 1B). Additionally, we studied the effect of the *JAK2* mutation on the mRNA level of PRV-1 using a real-time reverse transcription polymerase chain reaction (RT-PCR). We designed oligonucleotides and a FAM-conjugated probe according to the sequence of the murine *PRV-1* gene. The murine *PRV-1* gene

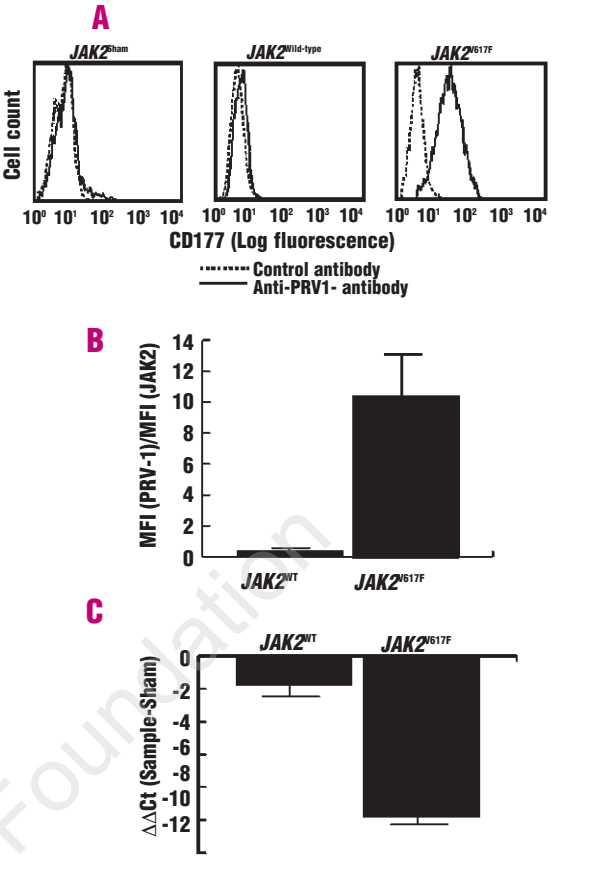

Figure 1. ***JAK2*** **mutation and expression of PRV-1. A. Expression of PRV-1 on FDCP-$JAK2^{V617F}$, FDCP-$JAK2^{WT}$, and FDCP-sham αIls was compared by flow cytometry. The results of one of the experiments are shown as histograms. Binding of a phycoerythrin-conjugated secondary antibody served as the negative control. B. Results of flow cytometry experiments are summarized as a bar graph. MFI (CD177)/MFI (GFP) ± s.e. for FDCP-$JAK2^{V617F}$ and FDCP-$JAK2^{WT}$ are 10.35±2.3 and 0.35±0.1, respectively (n=3, p=0.05) C. The relative expression levels of the *PRV-1* mRNA in FDCP-$JAK2^{V617F}$ and FDCP-$JAK2^{WT}$ were measured using real-time reverse transcription -PCR. ΔΔCt: ΔCt of either FDCP-$JAK2^{WT}$ or FDCP-$JAK2^{V617F}$-ΔCt of FDCP-Sham. The ΔCt of each cell line was calculated as Ct of *PRV-1* – Ct of 18S. A lower ΔΔCt means a higher expression of *PRV-1* mRNA.**

sequence and its intron-exon boundaries were determined by using the reported murine *PRV-1* mRNA (NM_026862) and the NCBI Map Viewer website. Total RNA was extracted from the transduced FDCP cells, reverse transcribed to double-stranded DNA using a cell to cDNA kit (Ambion), and used as a template in the real-time PCR. We used the 18S RNA as an internal standard and normalized all the real-time PCR results to that of the FDCP-Sham cells. FDCP-$JAK2^{V617F}$ cells had significantly higher amounts of *PRV-1* mRNA than had FDCP-$JAK2^{WT}$ cells (Figure 1C; ΔΔCt±s.e. of -9.8±0.3 and -1.7±0.8, respectively; n=9, p=0.0006). To investigate whether the *JAK2* mutation is the cause of PRV-1 overexpression, we studied the effect of a JAK2-specific tyrosine kinase inhibitor on PRV-1 expression. This inhibitor, 1,2,3,4,5,6-hexabromocyclohexane (HBCH), was obtained from the Drug Synthesis and Chemistry Branch, developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute. About 10^5 FDCP

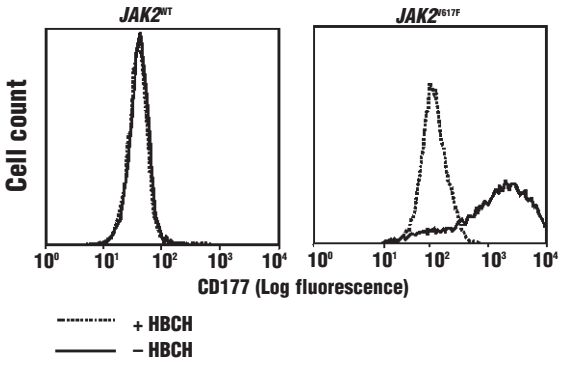

Figure 2. *JAK2* inhibitor and expression of PRV-1: FDCP cells transduced with the wild-type or mutant variant of the JAK2 cDNA were incubated with 5μM of hexabromocyclohexane (HBCH), a *JAK2*-specific tyrosine kinase inhibitor, for 24 hrs. Cells were harvested and analyzed for expression of PRV-1 using flow cytometry. The results are shown as histograms.

cells in culture media supplemented with the necessary growth factors were incubated in the presence of different concentrations of HBCH (0, 1, 5, 20, and 40 μM) for 24 hours. HBCH (particularly at concentrations ranging from 1 to 5 μM) decreased PRV-1 expression on FDCP-*JAK2*^{V617F} cells (Figure 2), but did not significantly alter the expression of PRV-1 in FDCP-*JAK2*^{WT} cells. Our results show that mutation in *JAK2* could be responsible for an increase in the PRV-1 expression in MPD. One intriguing question is whether PRV-1 overexpression is only a phenotypic change or whether it has any functional consequence. We have previously shown that overexpression of *PRV-1* in a heterologous cell line can promote proliferation in the absence of growth factors.[7] Considering our observations, we put forward the following hypothesis: in MPD, *JAK2*^{V671F} is the cause of PRV-1 overexpression and the latter increases cell proliferation. This hypothesis should be tested by studying the effect of selective inhibition of PRV-1 (in the presence of *JAK2*^{V671F}) on cell proliferation.

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References

1. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Cancer Genome Project. Lancet 2005;365:1054-61.
2. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 2005; 434:1144-8.
3. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352:1779-90.
4. Zhao R, Xing S, Li Z, Fu X, Li Q, Krantz SB, et al. Identification of an acquired JAK2 mutation in polycythemia vera. J Biol Chem 2005;280:22788-92.
5. Steensma DP, Dewald GW, Lasho TL, Powell HL, McClure RF, Levine RL, et al. The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both "atypical" myeloproliferative disorders and myelodysplastic syndromes. Blood 2005;106:1207-9.
6. Temerinac S, Klippel S, Strunck E, Roder S, Lubbert M, Lange W, et al. Cloning of PRV-1, a novel member of the uPAR receptor superfamily, which is overexpressed in polycythemia rubra vera. Blood 2000;95:2569-76.
7. Mnjoyan Z, Li J, Afshar-Kharghan V. Expression of polycythemia rubra vera-1 decreases the dependency of cells on growth factors for proliferation. Haematologica 2005;90:405-6.
8. Goerttler PS, Steimle C, Marz E, Johansson PL, Andreasson B, Griesshammer M, et al. The Jak2V617F mutation, PRV-1 overexpression, and EEC formation define a similar cohort of MPD patients. Blood 2005;106:2862-4.
9. Tefferi A, Sirhan S, Lasho TL, Schwager SM, Li CY, Dingli D, et al. Concomitant neutrophil JAK2 mutation screening and PRV-1 expression analysis in myeloproliferative disorders and secondary polycythaemia. Br J Haematol 2005;131:166-71.
10. Kralovics R, Teo SS, Buser AS, Brutsche M, Tiedt R, Tichelli A, et al. Altered gene expression in myeloproliferative disorders correlates with activation of signaling by the V617F mutation of Jak2. Blood 2005;106:3374-6.