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631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

JAK2V617F Activates IL-10R Signalling Potentially through Transactivation of JAK1

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Classical myeloproliferative neoplasms (MPNs) arise from somatic mutations in haematopoietic stem cells (HSCs) that lead to unregulated proliferation of mature myeloid cells. The most common mutation associated with MPNs is *JAK2* ^{V617F}, this causes constitutive JAK-STAT signalling leading to excessive production of mature myeloid cells. In humans, for MPN to arise, a *JAK2* ^{V617F} HSC must have a selective advantage over WT HSC. However, in mouse MPN models *Jak2* ^{V617F} HSCs do not display an overt selective advantage. This suggests that unique selective pressures may be present among those predisposed to acquire MPN that allow for the emergence of *Jak2* ^{V617F} clones.

We have identified defects in the IL-10R signalling pathway among MPN patients, with evidence that this defect is an intrinsic not acquired abnormality. In mice, we found that the blockade of IL-10R extends the cycling of WT but not *Jak2* ^{V617F}HSC in response to inflammatory stimuli and hastens proliferation-induced WT HSC exhaustion. We hypothesized that IL-10R blockade may create an environment that affords *Jak2* ^{V617F} HSC a selective advantage, indeed we found that IL-10R blocking antibody allowed *Jak2* ^{V617F} cells to outcompete WT cells in mouse transplant experiments.

From these mouse data, we hypothesized that *Jak2* ^{V617F}enhances IL-10R signalling. To test this hypothesis, we created Ba/F3 cell lines co-expressing IL-10Rα and Jak2 ^{V617F}. We found that Jak2 ^{V617F} enhances growth at limiting concentrations of IL-10. We also found that cytokine-independent IL-10Rα Jak2 ^{V617F} clones (but never IL-10Rα Jak2 ^{WT}or IL-10Rα cells) emerge at day 8-10 in culture after withdrawal of cytokines. We performed a limiting dilution assay to pinpoint the proportion of IL-10R Jak2 ^{V617F} cells capable of surviving IL-3 withdrawal. One in 126 IL-10Rα Jak2 ^{V617F} cells has transforming ability, whereas 1 in 1.4 MPL Jak2 ^{V617F} has transforming ability. Together, these data support our hypothesis that Jak2 ^{V617F} enhances IL-10R signalling and can activate IL-10Rα, however with weaker transformation potential compared to Jak2V617F's activation of MPL signalling. JAK2 canonically does not participate in the IL-10R pathway.

It has been previously shown that JAK1 can transactivate JAK2 when JAK2 ^{V617F} cells are under the stress of persistent JAK2 inhibitor exposure, and this is the purported mechanism of JAK inhibitor resistance among MPN patients. We hypothesized that JAK2 ^{V617F} can transactivate JAK1, leading to the activation of IL-10R. The same transfected BaF3 cells aforementioned were serum starved for 4 hours and stimulated with mIL-10 for 15 minutes to induce signalling. By way of Western Blot analysis, cytokine-independent Jak2 ^{V617F} IL-10Rα Ba/F3 cells showed constitutive activation of phosphorylated JAK1 and JAK2 as well as STAT3 and STAT5, consistent with transactivation. Studies to demonstrate direct interactions between JAK2 ^{V617F} and JAK1 are currently ongoing.

Disclosures Fleischman: *Pharmaessentia, CTI:* Speakers Bureau; *GSK, Incyte, CTI:* Consultancy.

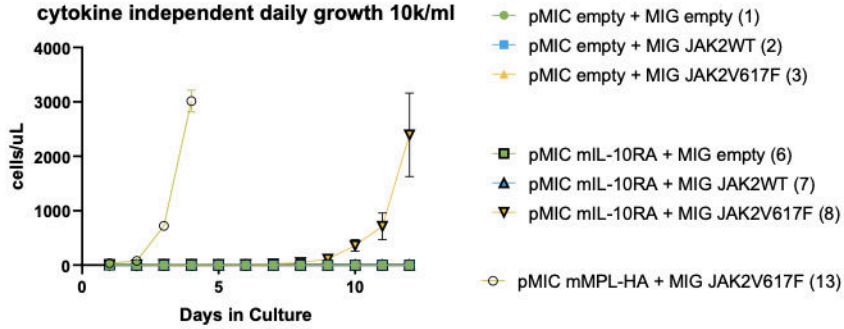

Figure 1. Cytokine independent transformation of JAK2V617F mutant BaF3 cells in presence of IL-10R.

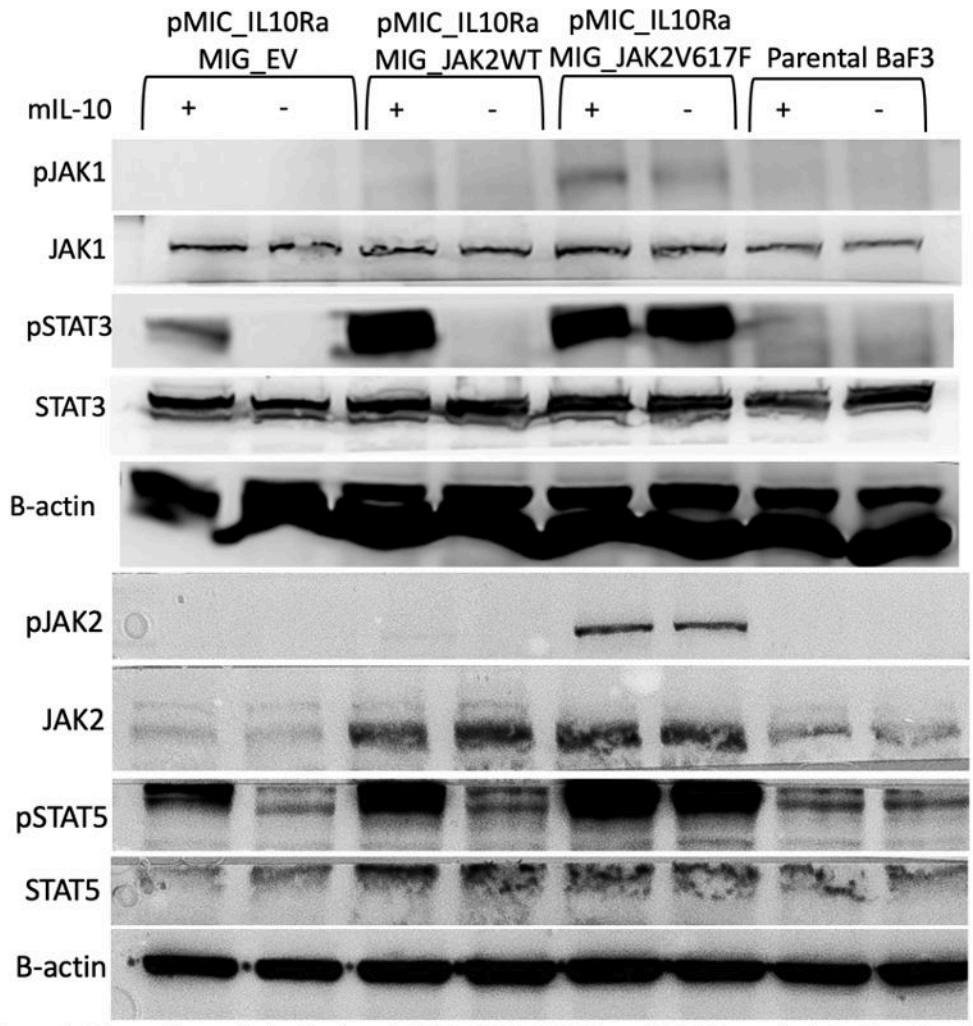

Figure 2. Western Blot analysis of activated JAK1, JAK2, STAT3, and STAT5 proteins in BaF3 cells under 5ng/mL of mIL-10 stimulation

Figure 1

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