"THE THROMBOPOIETIN RECEPTOR SUPPORTS PROLONGED JAK2 V617F DIMERIZATION AND ACTIVATION AND IS MORE SENSITIVE TO LOW JAK2 V617F LEVELS DUE TO LONG HALF-LIFE WHEN COMPARED TO EPO AND G-CSFR"

Gryshkova, Vitalina ; Balligand, Thomas ; Najjar, Salwa ; Constantinescu, Stefan N.

ABSTRACT

Background: The activating mutation V617F in the pseudokinase domain of JAK2 is prevalent in myeloproliferative neoplasms (MPN) such as Polycythemia Vera (PV), Essential Thrombocytosis (ET) and Myelofibrosis (MF). Low JAK2 V617F allele burdens in ET together with evidence from transgenic and retroviral mouse models established that low expression of mutated kinase specifically associates with an ET phenotype. The mechanism for this effect and how this single mutation promotes three distinct diseases still remains elusive. Aims: Our aim was to investigate the differences in the interaction between JAK2 V617F and dimeric cytokine receptors for thrombopoietin (Tpo), erythropoietin (Epo) or granulocyte-colony stimulating factor (G-CSF) and to assess biochemical reasons to any possible distinctions in these interactions. Methods: Gaussia princeps luciferase protein-fragment complementation was utilized to study interaction between JAK2 V617F and the cytokine receptors as well as dimerizat...

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Aims: Our aim was to investigate the differences in the interaction between JAK2 V617F and dimeric cytokine receptors for thrombopoietin (Tpo), erythropoietin (Epo) or granulocyte-colony stimulating factor (G-CSF) and to assess biochemical reasons to any possible distinctions in these interactions.

Methods: Gaussia princeps luciferase protein-fragment complementation was utilized to study interaction between JAK2 V617F and the cytokine receptors as well as dimerization of JAK2 V617F kinase co-expressed with these receptors. Signaling assays in transiently transfected HEK293 and γ2A cells as well as stably transduced Ba/F3 cells were used to validate the results of protein-fragment complementation assay.

Results: We have shown stronger interaction between JAK2 V617F and TpoR compared to EpoR or GCSFR at gradually decreasing dosages of the JAK2 V617F. Also, dimerization of JAK2 V617F appeared to be higher in presence of TpoR compared to other receptors tested. Interaction of the JAK2 V617F kinase with the cytokine receptors and its dimerization correlated with the half-lives of the receptors. The more stable TpoR supported prolonged JAK2 V617F and STAT5 activation compared to the less stable receptors EpoR or GCSFR. We have identified the intracellular domain in these receptors responsible for the distinction in their half-lives and made a shorter-lived TpoR and a longer-lived EpoR mutant by adding or removing a lysine, which triggers ubiquitination and degradation. As expected, the stability of the receptors was crucial for inducing pathologic signaling from JAK2 V617F with the less stable TpoR mutant losing its sensitivity to the low levels of JAK2 V617F, and, conversely, the more stable EpoR mutant becoming more sensitive to low JAK2 V617F levels.

Summary/Conclusion: Our results suggest that due to its natural long half-life in the cell and at the cell-surface, TpoR could support prolonged JAK2 V617F dimerization and activation even at the lower level of kinase expression explaining the \textit{in vivo} JAK2 V617F gene dosage effect, where low JAK2 V617F levels are associated with thrombocytosis.

Keywords: c-mpl, Erythropoietin receptor, G-CSF receptor, Myeloproliferative disorder