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AUTHOR'S VIEW

A haunted beast: Targeting STAT5B$_{N642H}$ in T-Cell Neoplasia

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ABSTRACT

The somatic hot spot mutation STAT5B$_{N642H}$ was found in many T cell leukemia/lymphoma patients. We generated and analyzed a transgenic mouse model with hematopoietic STAT5B$_{N642H}$ expression that caused aggressive T-cell leukemia/lymphomas. Herein, we discuss the scientific merit of our model and its relevance for pre-clinical studies.

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Janus kinase (JAK) and Signal transducer and activator of transcription (STAT) signaling is a major part of innate and adaptive immunity and many cancers are characterized by too much JAK/STAT3/5 signaling. Hyperactivation of STAT3/5 in hematopoietic malignancies is commonly caused by upstream oncogenic receptor and non-receptor tyrosine kinases such as JAK2$_{V617F}$, breakpoint cluster region protein (BCR) and Abelson murine leukemia viral oncogene homolog 1 (ABL) fusion protein (BCR/ABL) and FLT3-internal tandem duplication (ITD) mutation (FLT3-IDT).[1] STAT3 and STAT5 hyper-activation are typically associated with T-cell diseases. There are three mutational hotspots that were described in leukemia/lymphoma patients (STAT3$_{Y640F}$, STAT3$_{D661Y}$ or STAT5B$_{N642H}$), all located in the SH2 domain, the phospho-tyrosine (pY)-binding region essential for parallel STAT dimerization, efficient nuclear transport and gene regulation. Mutated STAT3 was mainly found in large granular lymphocytic leukemia but STAT5B$_{N642H}$ was reported in a variety of leukemia/lymphoma including T-cell prolymphocytic leukemia (T-PLL), T-cell acute lymphoblastic leukemia, gamma delta T-cell-derived lymphoma and enteropathy-associated T-cell lymphoma.[2] STAT5B$_{N642H}$ was shown to be hyper-activated, to exhibit prolonged activation and enhanced/stabilized dimerization. However, it remained unclear whether this mutation is sufficient to drive cancer or it only enhances common gamma chain (γ_c) cytokine receptor signaling.[3]

In our study published in the *Journal of Clinical Investigation*,[4] we investigated if STAT5B$_{N642H}$ can drive cancer. We characterized the STAT5B$_{N642H}$-driven disease model and we identified potential novel therapeutic avenues. We confirmed that STAT5B$_{N642H}$ is an activating mutation capable to transform. We generated a novel transgenic mouse model using the *vav1*-promotor that expresses STAT5B$_{N642H}$ in the hematopoietic system. We show that hSTAT5B$_{N642H}$ is a driver mutation for leukemia/lymphoma, providing a functional rationale for the frequent occurrence of this mutation in human patients. Transgenic mice expressing human wild/type STAT5B at a similar level were used as control. In agreement with previous *in vitro* observation, human STAT5B$_{N642H}$ displayed enhanced and prolonged STAT5 tyrosine phosphorylation in presence of a low dose of cytokines in mice. Animals expressing hSTAT5B$_{N642H}$ very rapidly developed lymphoma and leukemia. The proliferation of CD8$^+$ T-cells resulted in organ infiltration and death. Transplantation of whole bone marrow into immunocompetent recipient mice fully recapitulated the disease. Our experiments proof that the STAT5B$_{N642H}$ initiates and maintains proliferation of leukemic T-cells.

Analysis of the transcriptome and DNA methylation patterns revealed drastic changes in gene expression and specific alterations in DNA methylation patterns that correlated with the acceleration of cell cycle progression. Interestingly, mRNA and protein levels of Interleukin-2 (*Il-2*) receptor alpha chain (also called *CD25*), a target gene of STAT5 were elevated in neoplastic T-cells that further implicates its hyper-sensitivity toward IL-2 stimulation. In addition, we observed that DNA was less methylated at CpG islands adjacent to known Enhancer of zeste homolog 2 (EZH2) binding sites and EZH2 targets were highly upregulated in STAT5B$_{N642H}$ expressing T-cells. Among the upregulated EZH2 targets, Aurora kinase (AURK) posed great potential as a novel therapeutic target. Accordingly, we found that transcription and translation of AURKB were upregulated promoting increases AURKB signaling. However, AURKB inhibition only reduces AURKB activity but not STAT5B$_{N642H}$ phosphorylation. FDA-approved JAK1/2/3 inhibitors such as ruxolitinib and tofacitinib, on the other hand, could dramatically reduce STAT5B$_{N642H}$ activity *in vitro*

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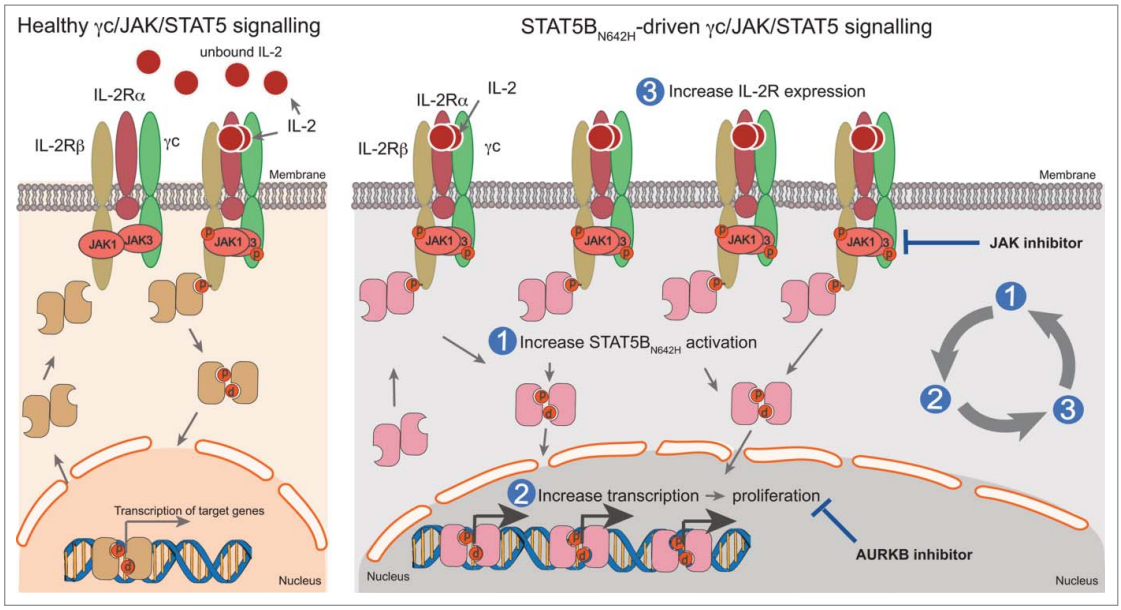

Figure 1. STAT5B$_{N642H}$-driven proliferation in T-cell leukemia/lymphoma via common γ-chain (γ_c)-receptor signaling. STAT5B$_{N642H}$ is activated by Janus kinase (JAK) upon cytokine stimulation such as interleukin 2 (IL-2). Subsequently, phosphorylated STAT5B$_{N642H}$ translocates into the nucleus and initiates transcription. (1) STAT5B$_{N642H}$ exhibits prolonged activation and enhanced DNA binding leading to (2) increased transcription of STAT5 target genes and enhanced proliferation. Increased transcription of cytokine receptor chains leads to (3) stronger STAT5B$_{N642H}$ action that accelerates and amplifies cytokine receptor signaling, e.g. through direct Interleukin 2 receptor alpha (IL-2Rα) (CD25) upregulation that further promotes hyper-sensitivity to common gamma chain (γ_c) cytokine stimulation. This vicious cycle leads to uncontrolled T-cell proliferation and leukemia/lymphoma initiation and progression. Neoplastic T-cell proliferation can be targeted using JAK and Aurora kinase B (AURKB) inhibitor in combination therapy approach. Alternatively, future direct SH2 domain blockers of STAT5 could become clinically available to tailor targeted therapy against too much STAT5 action.

as well as *in vivo* and strikingly, inhibition of AURKB together with JAK presented a greater effect on diseased T-cell survival. We concluded that targeting AURKB together with JAK kinase represents a potential novel therapy approach for leukemia/lymphoma patients carrying hSTAT5B$_{N642H}$.

In the context of therapy for T-cell lymphoma, JAK/STAT and the associated γ_c-receptor cytokine signaling (Fig. 1) have become important targets and ruxolitinib has entered phase two clinical trial for T-cell leukemia.[5] In T-PLL, STAT5B$_{N642H}$ was not found together with *JAK* or *IL2RG* mutation and shown to have resistance to JAK1/3 inhibitors.[6] Resistance to JAK inhibition was also acquired in patients without development of a secondary mutation and a combinatorial treatment of ruxolitinib and an AURK inhibitor could be promising. AURK promotes cell cycle progression and currently, there are more than 30 different AURK inhibitors in different clinical trials for solid tumors as well as blood cancer.[7] AURKB activation is enhanced as the result of IL-2 stimulation and IL-2-induced T-cell proliferation can be inhibited by AURK inhibitors.[8] Although AURK inhibitors are used mainly against myeloid neoplasia, trials for lymphoid neoplasia are on the horizon.

JAK/STAT mutations were found in 71% of T-PLL patients and point mutations in tumor protein p53 (TP53, best known as p53) or high mammalian target of rapamycin (mTOR) signaling accompanied by hyper STAT5 activity are closely associated presumably driving T-PLL.[6] Interestingly, mTOR signaling was reported to have a positive stimulus on AURKB activity as Survivin, a target of mTOR is required for AURKB function.[2,6,8] Due to STAT5 hyper activation in many cancers, inhibiting STAT5 is an attractive strategy not only for STAT5B$_{N642H}$-driven leukemia/lymphoma but also for many cancers with hyper-activation of JAK/STAT5. In this respect, our model serves as an effective preclinical model for testing different STAT5 inhibitors.[9]

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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