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JAK/STAT pathway directed therapy of T-cell leukemia/lymphoma: Inspired by functional and structural genomics

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ABSTRACT

Abnormal activation of the γc cytokine JAK/STAT signaling pathway assessed by STAT3 or STAT5b phosphorylation was present in a proportion of many T-cell malignancies. Activating mutations of STAT3/STAT5b and JAK1/3 were present in some but not in all cases with constitutive signaling pathway activation. Using shRNA analysis pSTAT malignant T-cell lines were addicted to JAKs/STATs whether they were mutated or not. Activating JAK/STAT mutations were not sufficient to support leukemic cell proliferation but only augmented upstream pathway signals. Functional cytokine receptors were required for pSTAT expression. Combining a JAK1/2 inhibitor with a Bcl-xL inhibitor navitoclax provided additive/synergistic activity with IL-2 dependent ATLL cell lines and in a mouse model of human IL-2 dependent ATLL. The insight that disorders of the γc/JAK/STAT system are pervasive suggests approaches including those that target gamma cytokines, their receptors or that use JAK kinase inhibitors may be of value in multicomponent therapy for T-cell malignancies.

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Contents

1. Introduction

The common gamma (γc) receptor-dependent cytokines and their signaling pathways play critical roles in T-cell immunity (Rochman et al., 2009; Waldmann, 2006; O'Shea et al., 1997). Activation of this pathway can be identified by the nuclear presence of pSTAT3 or pSTAT5. Recently activation of this γc/JAK/STAT system was identified in virtually all forms of T-cell leukemia/lymphoma (Waldmann and Chen, 2017). Most studies of T-cell lymphoma have focused on JAK/STAT mutations; however activation of this

Abbreviations: ATLL, adult T-cell leukemia/lymphoma; γc, common gamma receptor dependent cytokine; HTLV-1, human T-cell lymphotropic virus-1; IL-2/IL-2R, interleukin 2/interleukin 2 receptor; JAK, Janus kinase; JAK3i, Janus kinase 3 inhibitor; PBMCs, peripheral blood mononuclear cells; pSTAT3, phospho-STAT3; STAT, signal transducers, activators of transcription.

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pathway is a unifying feature that is more pervasive (Waldmann and Chen, 2017). The demonstration of activation of the JAK/STAT pathway in a major proportion of T-cell malignancies suggests JAKs as potential therapeutic targets–an approach that may revolutionize multiagent therapy of patients with pSTAT expressing T-cell malignancies.

2. JAK/STAT activation in T-cell lymphoma

JAK/STAT activation was assessed by phospho-STAT3 and STAT5 expression and nuclear localization and by monitoring the negative effects of JAK inhibitors. Such JAK/STAT activation was shown to be present from rare to 86% of patients with most forms of T-cell malignancy. In particular, γc cytokine, JAK1/3, STAT3/5 pathway activation was demonstrated in NK/TCL (Bouchekioua et al., 2014; Coppo et al., 2009; Koo et al., 2012) ALK positive and ALK- ALCL, (Crescenzo et al., 2015; Chiarle et al., 2005; Khoury et al., 2003), large granular lymphocytic leukemia (LGL) (Koskela et al., 2012) prolymphocytic leukemia (PLL) (Kiel et al., 2014; Bergmann et al., 2014; Bellanger et al., 2014), Sézary Syndrome (SS) and mycosis fungoides (MF) (Eriksen et al., 2001; Zhang et al., 1996; Choi et al., 2015), as well as in smoldering and chronic ATLL (Chen et al., 2008, 2010; Ju et al., 2011; Kataoka et al., 2015; Elliott et al., 2011).

3. STAT mutations in T-cell lymphoma

In light of the frequent activation of the JAK/STAT system in T-cell malignancies, these leukemias were examined for activating mutations of STAT proteins (Odejide et al., 2014; Koskela et al., 2012; Crescenzo et al., 2015; Coppo et al., 2009; Kücük et al., 2015; Nicolae et al., 2014; Choi et al., 2015; Zhang et al., 2012; Kataoka et al., 2015). Many T-cell malignancies manifested STAT3/STAT5b mutations that were focused predominantly in their Src homology 2-(SH2) domain – a site that is involved in docking of phosphorylated tyrosine residues, transient binding to cytokine receptors and binding to other STAT proteins that mediate dimerization and nuclear localization (Koskela et al., 2012).

In studies with ALK- T-cell lines using shRNA loss-of-function analysis our group demonstrated that cell lines that manifested pSTAT3 were addicted to STAT3 whether or not the STAT elements were mutated suggesting the importance of STAT3 activation in leukemic T-cell survival (Chen and Waldmann, unpublished results). Kücük and coworkers 2015 demonstrated that STAT5b mutations provided a growth advantage. Nevertheless, they demonstrated that such activating STAT mutations were not sufficient for leukemic cell proliferation but only enhanced upstream signals from the cytokine-cytokine receptor, JAK/STAT pathway. Following IL-2 withdrawal pSTAT expression disappeared in 1 h after transient IL-2 stimulation of wild-type STAT5b transduced cells, whereas it persisted for more than 6 h with STAT5b N642H mutant-transduced cells. In accord with this view that upstream signals were required, the growth promoting activity of the mutant was partially inhibited by the addition of the upstream JAK1/2 inhibitor AZD1480.

4. JAK mutations in T-cell lymphoma

Since STAT activating mutations were not sufficient but only enhanced upstream signaling, activations of JAKs were explored using shRNA loss-of-function analysis with JAK1 shRNA. JAK1 was required in phospho-STAT3 positive ALK- cell lines whereas its loss had no effect on phospho-STAT3 negative cell lines (Chen and Waldmann, unpublished results). Given the requirement for activated JAKs, especially JAK1 and JAK3 for the activation of the gamma cytokine JAK/STAT pathway, leukemic cells were examined for JAK mutations. Many mutations were found especially in the pseudokinase domain that was reported to function as a protein kinase that phosphorylates two residues that negatively regulate JAK kinases to suppress their activity (Saharinen and Silvennoinen, 2002). It is clear from our JAK1 knockdown studies that JAK1 was required in cells that manifest activation of the cytokine receptor JAK/STAT system whether mutations were present or not as with STAT mutations JAK mutations appear to be incapable of initiating but rather augment responses initiated by upstream signals.

5. Activation of the JAK/STAT pathway required functional cytokine receptors

In studies by Lu et al., 2005, and Hornakova et al., 2009 and their coworkers even with activating JAK and STAT mutations there was a requirement for expression of a functional cytokine receptor that played two roles; first as a scaffold for cross-activation of JAK kinases and second as a docking site for recruitment of STAT transcription factors. In addition to JAK/STAT mutations, mutations were present in select cytokine receptors for example the IL-7 receptor in childhood ALL (Shochat et al., 2011; Zenatti et al., 2011).

6. Disorders of cytokine expression in T-cell lymphoma

Although JAK3 FERM domain mutations were only rarely observed in patients with smoldering and chronic HTLV-1 associated ATLL, the gamma-c JAK/STAT pathway was usually activated (Migone et al., 1995; Chen et al., 2008). We demonstrated that the HTLV-1 encoded Tax protein transactivated two autocrine (IL-2/IL-2R alpha, IL-15/IL-15R alpha) and one paracrine (IL-9) loop in such patients (Tendler et al., 1990; Azimi et al., 1998; Chen et al., 2010). These cytokine-cytokine receptor loops led to activation of the JAK1/3 STAT signaling pathway and were associated with spontaneous proliferation of ATLL cells, a phenomenon that could be inhibited by the addition of the pan-JAK inhibitor tofacitinib (Ju et al., 2011). In further examination of cytokine disorders there was evidence supporting a role for the gamma-c cytokine IL-15 in cutaneous T-cell lymphoma (Döbbeling et al., 1998) and for IL-21 in patients with anaplastic T-cell lymphoma (Jain et al., 2015). In summary, disorders of the gamma cytokine JAK/STAT signaling pathway are pervasive in T-cell malignancy suggesting novel molecular targets and therapeutic opportunities that may be of value in the multicomponent treatment of these tumors.

7. Gamma cytokine JAK/STAT system as a target in the treatment of T-cell malignancies

The demonstration of activation of the JAK/STAT system in T-cell malignancies provided the rationale for diverse therapeutic approaches including those that targeted the gamma-c cytokines directly, those that blocked cytokine-receptor interactions and especially JAK kinase inhibitors. The best biomarker suggesting JAKs would be a rational target is the presence of pSTAT3 or pSTAT5 and their nuclear translocation rather than the less frequent mutations affecting this system. JAK inhibitors inhibited the proliferation of cytokine dependent cell lines and *ex vivo* leukemic cells from patients with smoldering and chronic ATLL who manifest activation of the JAK system (Ju et al., 2011). To translate these observations a clinical trial of ruxolitinib is underway in patients with smoldering and chronic ATLL (Conlon and Waldmann, 2017). However, therapy with ruxolitinib is clearly not optimal. With rare exceptions T-cell leukemia/lymphomas were associated with activation of the JAK1/JAK3 signaling but did not involve JAK2. However, ruxolitinib and tofacitinib inhibit the off-target JAK2 kinase, therefore interfering with the cell signaling mediated by