Published in final edited form as:

Leuk Lymphoma. 2017 November ; 58(11): 2540–2547. doi:10.1080/10428194.2017.1312370.

HiJAKing the Epigenome in Leukemia and Lymphoma

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Abstract

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway is central to signaling by receptors of diverse cytokines, growth factors, and other related molecules. Many of these receptors transmit anti-apoptosis, proliferation and differentiation signals that are critical for normal hematopoiesis and immune response. However, the JAK/STAT signaling pathway is deregulated in many hematologic malignancies, and as such is co-opted by malignant cells to promote their survival and proliferation. It has recently come to light that an alternative mechanism, wherein nuclear JAKs epigenetically modify the chromatin to increase gene expression independent of STATs, also plays an important role in the pathogenesis of many hematologic malignancies. In this review, we will focus on common genetic alterations of the JAK family members in leukemia and lymphoma, and provide examples in which JAKs regulate gene expression by targeting the cancer epigenome.

Keywords

leukemia; lymphoma; JAK; histone; phosphorylation; epigenetics

Over 60 cytokines have been shown to communicate through Janus kinase (JAK) signaling cascades to regulate an array of functional processes within a cell. Of the four JAK family members, JAK1 and JAK2 are responsible for regulating genes related to cellular growth, survival, and differentiation (1), while JAK3 and TYK2 primarily (though not exclusively) mediate genes involved in immunological responses (2). When JAKs non-covalently interact with specific cytokine receptors, and upon extracellular cytokine or growth factor binding, JAKs autoactivate via transphosphorylation. Activated JAKs induce receptor tyrosine phosphorylation, which creates binding sites for the signal transducer and activator of transcription (STAT) proteins. STAT binding leads to STAT phosphorylation, subsequent dimerization and translocation to the nucleus, where it regulates gene transcription (3, 4). Constitutive JAK signaling has been observed in a variety of cancers (2, 4) and is especially prevalent in hematologic cancers (5). Thus, understanding the multiple facets of JAK signaling mechanisms is essential to assessing potential therapies. Here, we focus on genetic alterations leading to constitutive JAK activation in leukemia and lymphoma, including gain-of-function mutations, chromosome amplification, and autocrine cytokine signaling. Further,

we will discuss the epigenetic mechanisms of transcriptional regulation by nuclear JAK1 and JAK2 in these diseases.

Genetic alterations of JAKs in leukemia and lymphoma

All JAK family members are comprised of four functional domains (6–8). The most N-terminal is the FERM domain (JH6 and JH7), which is responsible for interaction of JAK with an intracellular cytokine receptor. Adjacent to the FERM domain is the SH2 domain (JH3 and JH4), the function of which is not yet well understood. Between the SH2 domain and kinase domains is the pseudokinase domain (JH2), which has a sequence similar to that of the kinase domain, but lacks catalytic activity. The pseudokinase domain inhibits activation of the kinase domain in the absence of cytokine stimulation, and in this way is a major regulatory element in cytokine signaling. The kinase domain (JH1) is at the C-terminal end of the protein, and contains the catalytic residues necessary for activation (8). Different JAK mutations uniquely mediate subsequent signaling mechanisms, and as such, result in various disease states (2, 9). JAK activating mutations are primarily found in the pseudokinase domain, and likely abrogate the autoinhibitory role of the domain, allowing for unregulated autophosphorylation of the kinase domain independent of cytokine signaling (2, 5, 6, 10, 11).

JAK2 activating mutations

JAK2^{V617F} is the most common mutation in myeloproliferative neoplasms (MPNs), including 95% of polycythemia vera (PV) cases, 50%–60% of essential thrombocythemia (ET) cases and primary myelofibrosis cases (PMF) (12–16). PV patients who do not harbor the JAK2^{V617F} mutation often have a different mutation in exon 12 of JAK2 (17). Both JAK2^{V617F} and JAK2 exon 12 mutations are located in the JH2 pseudokinase domain, resulting in constitutive phosphorylation of the JAK2 kinase domain (5, 17, 18). Additional JAK2 mutations (R683 variants) in the JH2 pseudokinase domain are detected in 18%–28% of B progenitor acute lymphoblastic leukemia (ALL) patients with Down's syndrome (DS) (19, 20) and 7% of high-risk pediatric B lineage ALL patients (21).

In addition to disrupting the regulatory role of the pseudokinase domain, JAK2^{V617F} binds protein arginine methyltransferase 5 (PRMT5) with a higher affinity than wild type JAK2 (18). JAK2 phosphorylates PRMT5, impairing its ability to symmetrically dimethylate nuclear histone proteins at H2A/H4 R3 (a repressive mark). Consequently, JAK2^{V617F} results in global upregulation of gene expression (18). Furthermore, the interaction between JAK2 and PRMT5 is detected in both the cytoplasm and nucleus of Ba/F3 leukemic cell line (18).

JAK1 activating mutations

Although uncommon, JAK1 mutations affect many hematological malignancies. For instance, the JAK1 homolog of JAK2^{V617F}, JAK1^{V658F}, results in constitutive activation of the kinase (13). In a study involving 186 adult leukemia patient samples, 4 JAK1 mutations were found, of which 75% were JAK1^{V658F} (22). JAK1 mutations were observed in 4 out of 45 T-cell prolymphocytic leukemia patients, including one patient harboring JAK1^{V658F}.

(23). Seven somatic heterozygous JAK1 missense mutations were found in ~18.4% of 38 T-ALL cases, but only 3.4% of 88 B-ALL cases (24). Like mutations in other JAK family members, most of these unique JAK1 mutants are in the pseudokinase domain, and are therefore thought to interfere with kinase autoinhibition and subsequent development of or progression to a lymphoid malignancy (24).

JAK3 activating mutations

A wide variety of JAK3 mutations have been discovered in many cell lines and patient samples. Mutations within the pseudokinase domain are most widely observed, and are associated with several leukemias and lymphomas, including T-cell adult lymphocytic leukemia (7%) (25), natural killer T-cell lymphoma (21%–35%) (26, 27), juvenile myelomonocytic leukemia (~10%) (28), and T-cell prolymphocytic leukemia (32%–42%) (29) (23). The homolog of JAK2^{V617F}, JAK3^{M592F}, does not result in constitutive activation of the kinase; in fact, JAK3 is the only JAK family member that does not become constitutively activated by this mutation (13). Mutations in the FERM domain are rare: loss of function mutations are associated with SCID, while gain-of-function mutations are associated with adult T-cell leukemia/lymphoma (30), Down's Syndrome (DS)-associated transient myeloproliferative disorder (30, 31), and non-DS-acute megakaryoblastic leukemia (32). Mutations within the kinase domain are associated with juvenile myelomonocytic leukemia (18%) (28). Kinase mutations are found to be secondary and therefore believed to be related to progression rather than initiation of these diseases (28).

TYK2 activating mutations

TYK2 activating mutations have been observed in both cell lines and patient samples. These mutations are common in cell lines. For example, in the Ba/F3 cell line, which typically depends on IL-3 cytokine signaling to survive, TYK2^{V678F} (the TYK2 mutant homolog of JAK2^{V617F}) is constitutively active in the absence of cytokines (13). Additionally, TYK2 variants were detected in 7/18 (38%) TALL cell lines. However, TYK2 mutations are rare in leukemia patient samples (2/93 T-ALL, 0/53 BALL, and 1/54 AML cases) (33). In general, the locations of TYK2 activating mutations span all 4 domains of the protein (33). Therefore, TYK2 mutations are proposed to not be a driver of the disease, but instead to be acquired during extensive cell culture (33).

JAK2 chromosome amplification

Amplification of a several megabase region of the chromosome band 9p24 is observed in ~44% of primary mediastinal B-cell lymphoma (PMBL) (34, 35) (36, 37), ~33% of Hodgkin lymphoma (HL) (36, 38), 11% of activated B-cell like diffuse large B-cell lymphoma (ABC DLBCL), and 7% of germinal center B-cell like (GCB) DLBCL patients (39). In PMBL and HL, JAK2 amplification upregulates JAK2 expression (40) and promotes cell survival and proliferation (see further discussion below) (37).

Autocrine cytokine signaling

Autocrine cytokine signaling that provides self-sustaining growth has been demonstrated in lymphomas. In addition to JAK2 amplification, autocrine production of IL-13 induces

STAT6 activation in HL (41, 42) and PMBL (43, 44). Another example is ABC DLBCL, in which STAT3 is activated by IL-6 and IL-10 (39, 45). Secretion of these cytokines results from high NF- κ B activation, a hallmark of the disease, and the gain-of-function MYD88^{L265P} mutation, which was found in ~30% of the ABC DLBCL cases (46).

Chromatin modification by nuclear JAKs

Discovery of the non-canonical JAK signaling pathway

A decade ago, a non-canonical mechanism for JAK signaling in gene regulation was discovered by a drosophila genetic study (47). A loss-of-function in *Hopscotch*, the drosophila JAK results in overexpression of HP1, an essential component of heterochromatin that is required for heterochromatin-mediated gene silencing. Conversely, a *Hopscotch* gain-of-function mutation precludes heterochromatin formation by decreasing H3K9 methylation, leading to a reduction in transcriptional repression and subsequent expression of normally repressed genes that are not necessarily STAT target genes (47). The study suggests that disruption of heterochromatic gene silencing is a non-canonical mechanism by which JAK overactivation induces hematopoietic tumors (47). Further, the molecular mechanisms underlying epigenetic regulation in gene expression by JAK signaling have been revealed in human leukemia cells (Figure 1) (48). JAK2 or JAK2^{V617F} are localized in both the cytoplasm and nucleus of the cancer cells. Nuclear JAK2 directly phosphorylates tyrosine 41 on the histone H3 (H3Y41). This epigenetic modification precludes specific interaction between the modified histone region and the chromo-shadow domain of the HP1 α . The resulting euchromatic state leads to the upregulation of disease specific genes, most of which are not direct targets of STAT5 (48, 49).

Epigenetic gene regulation by JAK2 in primary mediastinal and Hodgkin lymphoma

JAK2-mediated chromatin modification has also been observed in PMBL and HL, both of which share common biological and molecular features (37, 40, 50). As discussed above, autocrine IL-13 production and chromosome 9p24 amplification both lead to JAK2 activation (Figure 2). Using an RNA interference library screen, we found that knockdown of either JAK2 or the co-amplified JMJD2C, a gene encoding histone demethylase, is lethal to the cancer cells (37). Notably, these two genes function cooperatively to regulate gene expression to promote cell survival and proliferation. The underlying mechanism of this oncogenic cooperation is H3Y41 phosphorylation by JAK2 along with H3K9 demethylation by JMJD2C, a coordinated chromatin modification process that releases HP1 suppression and consequently activates chromatin for gene transcription (37, 51). Genome-wide mapping of phosphorylated H3Y41 has revealed ~2,000 target genes regulated by JAK2 through this epigenetic mechanism. In addition to well-defined oncogenes such as MYC, JAK2 upregulates expression of several genes, including JAK2 itself, which forms positive feedback loops in this signaling pathway (Figure 2) (37). JAK2 also induces expression of the other two co-amplified genes PD-L1 and PD-L2, both of which encode ligands for the inhibitory PD-1 receptor on T cells. Malignant cells take advantage of high expression levels of PD-L1 and PD-L2 to escape immune surveillance in their T-cell rich microenvironment (52). Indeed, the antibody nivolumab, which targets PD-1 to restore tumor immunity, has been effective in the treatment of relapsed or refractory Hodgkin lymphoma (53).

Epigenetic gene regulation by JAK1 in diffuse large B-cell lymphoma

A recent study on mouse embryonic stem cells demonstrated that JAK1 is also present in the nucleus and can directly phosphorylate H3Y41(18). In line with this observation is our investigation into JAK1 signaling in ABC DLBCL in which JAK1 is activated by the cytokines IL-6 and IL-10 (54) (Figure 2). JAK1 bears a nuclear localization signal (NLS, KRKK) between the FERM and SH2 domain, which is essential for nuclear translocation of JAK1 (55). In ABC DLBCL, JAK1 but not JAK2 is responsible for phosphorylation of STAT3 and H3Y41. Genome-wide mapping of phosphorylated H3Y41 revealed ~3,000 potential JAK1 target genes, including those that are central to ABC DLBCL biology, such as MYC, IRF4 and MYD88. Given that gain of function MYD88 mutations result in autocrine production of IL-6 and IL-10, JAK1 and MYD88 form a positive feedback loop to promote cell survival and proliferation. Some JAK1 targets such as the AP1 transcription factor JUN contribute to tumor dissemination by modulating the tumor microenvironment (54). Notably, this JAK1 mechanism for gene regulation is distinct from the canonical JAK1/STAT3 pathway, since there is no STAT motif enrichment within phosphorylated H3Y41 peaks and more than 90% of corresponding genes do not bear STAT motif in their promoter region.

Inhibition of JAKs in leukemia and lymphoma

JAK inhibitors suppress oncogenic growth and activate apoptosis in cells that depend on JAK signaling for their proliferation and survival. Lestaurtinib, for example, is a tyrosine kinase inhibitor that targets many kinases, including JAK2 (56). It decreases cell viability in HL cell lines and primary patient samples by inhibiting growth and activating apoptosis (57). Like Lestaurtinib, Fedratinib inhibits JAK2, and is particularly effective in cells with high 9p24.1/JAK2 copy number. Fedratinib decreases cell proliferation and increases apoptosis in HL and PMBL cell lines. Further, it diminishes tumor growth and prolongs survival in murine xenograft models of HL and PMBL lymphoid tumors (58). Both JAK1/JAK2 inhibitors AZD1480 (59) and Ruxolitinib (60) are effective in decreasing viability in ABC DLBCL, but not GCB DLBCL, cell lines (54). CHZ868 was developed as a type II JAK inhibitor (meaning that it traps JAK in the inactive conformation) that suppresses tumor growth and improves the survival of transgenic mice with human or murine B cell acute lymphoblastic leukemias with CRLF2 rearrangements (61).

Several clinical trials are underway to evaluate JAK inhibitors in leukemia and lymphoma. Patients with relapsed mantle cell lymphoma, indolent lymphoma, or HL were evaluated in a phase I clinical trial after treatment with the JAK2 inhibitor Pacritinib (SB1518) (NCT00741871). Ruxolitinib is currently being tested in a pilot study as a molecular therapy in relapsed or refractory HL and PMBL patients (NCT01965119). Ruxolitinib is also currently involved in two phase II clinical trials for the treatment of patients with relapsed or refractory DLBCL or peripheral T-cell non-Hodgkin lymphoma (NCT01431209) and HL (NCT02164500).

Although the effect of JAK inhibitors alone is minimal, it has great promise as an agent in a combinatorial regimen (62). AZD1480 synergizes with MEK inhibitors on HL cell lines to decrease both cell proliferation (by inducing G2/M cell cycle arrest) and immune system

evasion (63). Synergism is also observed between AZD1480 and the BTK inhibitor Ibrutinib in ABC DLBCL cell lines (54). The dual Syk/JAK inhibitor Cerdulatinib (PRT062070) has been found to be toxic to many types of leukemia and lymphoma. In ABC and GCB DLBCL, this inhibitor has broad anti-tumor activity (53). Cerdulatinib stopped cell proliferation in 60 chronic lymphocytic leukemia (CLL) Ibrutinib-resistant primary patient samples (64). Cerdulatinib induces apoptosis of CLL cells by interrupting signaling in the BCR and microenvironment, and synergism is observed with the BCL2 inhibitor ABT-199 in these cells (65). Patients with B-cell malignancies (aggressive B-cell NHL, HL, and CLL) were evaluated in a phase I clinical trial on the PI3Kdelta inhibitor INCB040093 in combination with the JAK1 inhibitor Itacitinib (INCB039110) with the hope of providing synergistic efficacy (NCT01905813). A phase I/II clinical trial of Itacitinib in combination with Ibrutinib in relapsed or refractory DLBCL (NCT02760485) was initiated very recently. It is hopeful that these combinatorial drug strategies will improve clinical outcomes of patients with leukemia or lymphoma.

Conclusion

Over the last two decades, the JAK/STAT signaling pathway and its role in hematologic malignancies has intensively been studied using basic molecular strategies combined with genetic and phenotypic analysis. The discovery of genetic alterations in this pathway has led to better pathogenetic insights, diagnostic advances, and new therapeutic options. The members of the JAK/STAT pathway have emerged as effective drug targets; for example, patients with MPNs bearing the JAK2^{V617F} mutation have been successfully treated with small molecule inhibitors of JAK2 (4). Clearly, much work remains in elucidating the biological significance of chromatin modification by JAKs in hematologic malignancies and dissecting the relationship between this epigenetic mechanism and the canonical STAT-dependent pathway. A better understanding of the oncogenic mechanisms of these two pathways will aid in the development of therapeutic agents to treat JAK-dependent hematologic malignancies.

Acknowledgments

This review was supported by the National Cancer Institute (1R01 CA187299) to LR, and by the UW-Madison T32 Hematology Training Award (T32 HL07899) to ACD.

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Figure 1. Canonical and non-canonical JAK signaling in leukemia

In many types of leukemia, STAT5 and/or STAT3 are constitutively activated by JAK2 signaling from receptors of diverse cytokines/growth factors, or directly by JAK2^{V617F}, a hallmark of myeloproliferative neoplasms (MPN). In addition to this canonical pathway, both JAK2 and JAK2^{V617F} can activate chromatin by phosphorylation of tyrosine 41 of the histone H3 (H3Y41) and displacement of the inhibitory protein HP1 from chromatin. Target genes in the canonical JAK/STAT pathway, along with those through the epigenetic mechanism, are persistently activated in transformed cells to promote cell survival and proliferation, as well as to alter differentiation programs. GM-CSF: granulocyte-macrophage colony stimulating factor; LIF: leukemia inhibitory factor; EPO: erythropoietin; TPO: thrombopoietin.

Figure 2. Canonical and non-canonical JAK signaling in lymphoma

In primary mediastinal B-cell lymphoma (PMBL) and Hodgkin lymphoma (HL) (top panel), autocrine IL-13 signaling and chromosome 9p24 amplification result in JAK2 constitutive activation. In addition to STAT6 phosphorylation, JAK2 can also phosphorylate tyrosine 41 of the histone H3 (H3Y41) and subsequently leads to euchromatic gene transcription. JAK2 target genes that are induced for expression through this non-canonical mechanism include JAK2 itself, the other 9p24 amplicon genes JMJD2C, PD-L1 and PD-L2, and many oncogenic genes such as MYC. Thus, persistent expression of these genes promotes cancer cell survival and proliferation or evades tumor immunity. In activated B cell-like diffuse large B-cell lymphoma (ABC DLBCL) (bottom panel), JAK1 is constitutively activated by autocrine production of IL-6 and/or IL-10, which results from MYD88 mutations (mainly L265P) and other NF- κ B activation mechanisms. Activated JAK1 regulates gene expression through both the canonical STAT3 pathway (Li Lu, unpublished data) and the non-canonical chromatin modification mechanism to promote cell survival and proliferation, as well as modulate the tumor microenvironment (see details in the text).