Inhibition of the Janus Kinase Family Increases Extracellular Signal-Regulated Kinase 1/2 Phosphorylation and Causes Endoreduplication

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Abstract

The role of Janus-activated kinase (JAK) signaling in cell cycle transit and maintenance of genomic stability was determined in HL-60 myeloblastic leukemia cells. Inhibition of JAKs, all JAKs (JAK1, JAK2, JAK3, and tyrosine kinase 2), JAK2, or JAK3, caused a significant reduction in cell growth with a major G2-M arrest evident 24 hours after treatment. Targeting all JAKs also caused endoreduplication 48 and 72 hours after treatment. We discovered mitotic cells in both G2 (4N DNA) and G4 (8N DNA) subpopulations of cells treated with an inhibitor of all JAKs as detected by phosphorylated histone H3 expression. Treatment with inhibitors of just JAK2 or JAK3 drastically reduced such mitotic cells. We observed a complete blockage of IFN-γ and interleukin-6-induced signal transducer and activator of transcription (STAT)-1 and STAT-3 response when all JAKs were inhibited. At the same time, we found baseline phosphorylated extracellular signal-regulated kinase (ERK) 1/2 to be elevated by JAK inhibition, particularly when all JAKs were inhibited. The G2-M arrest and endoreduplication induced by JAK inhibitors were reduced in cells pretreated with PD98059 to inhibit ERK. PD98059 also increased back the expression of the M2D2 cell cycle checkpoint protein that was down-regulated during "all JAKs inhibitor"-mediated endoreduplication. These data suggest that JAK signaling is needed for G2-M transit with inhibition of ERK. (Cancer Res 2006; 66(18): 9083-9)

Introduction

In many cancers, disruption of cell cycle transit and genomic instability are involved in cell transformation. For many tumors, including leukemias, cytokine-regulated signaling through Janus-activated kinases (JAK) is aberrant. The function of such signaling is thus of significant interest in the pathology of cells whose growth and differentiation are governed by cytokines.

JAKs are associated with cytokine and growth factor receptors (1). Activation of these receptors via ligand binding induces activation of the receptor-associated JAKs through cross-phosphorylation. The tyrosine-phosphorylated sites become docking sites for Src homology 2 domains of signaling proteins. Prominent among these are the signal transducer and activator of transcriptions (STAT). Receptor-recruited STATs are phosphorylated by JAKs, homodimerize or heterodimerize, and rapidly translocate to the nucleus to induce target gene transcription (2).

Mammals have four members of the JAK family, JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2). JAK1, JAK2, and TYK2 are ubiquitously expressed, whereas JAK3 is predominantly expressed in hematopoetic cells and is highly regulated with cell development and activation (3). A large number of cytokines, such as interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21, are dependent on JAK1 and JAK3. JAK1 is also required for the signaling of receptors that share the gp130 subunit. Those include IL-6, IL-11, oncostatin M, leukemia inhibitory factor, ciliary neurotrophic factor, and granulocyte colony-stimulating factor (CSF). TYK2 is essential for hormone-like cytokines, such as growth hormone, prolactin, erythropoietin, thrombopoietin, as well as cytokines that signal through the IL-3 receptor (IL-3 and IL-5) and granulocyte-macrophage CSF. TYK2 is essential for IL-12 signaling (1). The JAK signaling pathways mediate both antiproliferative response following IFN stimulation, which is predominantly mediated by STAT1, and cellular proliferation in response to cytokines and growth factors, which is mediated by STAT3 and STAT5.

Constitutively active STAT3 and STAT5 have been shown in acute myelogenous leukemia (AML), with constitutive activation of STAT3 or STAT5 in 28% and 22% of 36 AML patients, respectively (4). In addition, constitutive activation of STAT3 was correlated with significantly shorter disease-free survival. Some patient samples, as well as the HL-60 human myeloblastic leukemia cell line used as a model for acute promyelocytic leukemia, show increased basal activities of all, STAT1, STAT3, STAT5, and STAT6 (5). Constitutive activation of STAT proteins in cancer can be caused by overexpression of growth factor receptors or their ligands, such as IL-6, epithelial growth factor and their receptors, the platelet-derived growth factor receptor, or ERB2. However, excessive JAK kinase activity in tumors is perhaps the most common mechanism for constitutive phosphorylation and activation of STATs (6).

The JAK/STAT pathways also have a role in cell cycle control as shown by the growth arrest induced by viral infection. Activation of JAK1 and TYK2 by IFN-α can delay G1-S to G2-M transition (7). However, the role and mechanism of JAKs in cell cycle control are not known. In the current study, we report that intact JAK signaling seems to be essential for proper progression through G2-M and for maintaining genomic stability. We also identified extracellular signal-regulated kinase (ERK) 1/2 as a possible mediator for the JAK inhibitor-mediated endoreduplication and down-regulation of the M2D2 G2-M checkpoint protein.

Materials and Methods

Cell culture. HL-60 human myeloblastic leukemia cells were grown in RPMI 1640 supplemented with 15% heat-inactivated fetal bovine serum (both were from Invitrogen, Carlsbad, CA) and 1× antibiotic/antimycotic (Sigma, St. Louis, MO) in a 5% CO2 humidified atmosphere at 37°C. JAK inhibitor I (targeting JAK1, JAK2, JAK3, and TYK2; referred to as "all JAKs inhibitor") --mediated endoreduplication and down-regulation of the M2D2 G2-M checkpoint protein.
inhibitor” throughout the article), JAK2 inhibitor II, and JAK3 inhibitor VI (Calbiochem, La Jolla, CA) were administered from stock solutions in DMSO with a final concentration of 2, 12.5, and 2 μM/L, respectively. Concentrations of the all JAKs and the JAK3 inhibitor were chosen as multiples for the in vitro IC₅₀ as provided by the manufacturer. The concentration for the JAK2 inhibitor was determined by the concentration for maximum inhibition (50 μM/L) as provided by the manufacturer and decreased to the maximum amount showing no apparent toxicity in our cell system (12.5 μM/L). ERK inhibitor PD98059 (10 or 20 μM/L Sigma) was administered from a stock solution in DMSO 1 hour before treatment with JAK inhibitors. Experimental cultures were initiated at a density of 0.2 × 10⁶ cells/mL and assayed 24, 48, and 72 hours after treatment. Viability was monitored by 0.2% trypan blue (Invitrogen) exclusion and routinely exceeded 95% before drug administration.

**Cell cycle and mitosis analysis.** Cells (0.5 × 10⁶) were collected from cultures, pelletted at 1,000 rpm in a microfuge for 5 minutes, and permeabilized with 1 mL of 90% methanol at −20°C for 20 minutes. Samples were washed 2× in 1 mL PBS and resuspended in 200 μL PBS containing 5 μg/mL 4',6-diamidino-2-phenylindole (DAPI; from a 0.2 mg/mL solution in double-distilled H₂O, Polysciences, Warrington, PA) and 5 μL/mL allopurinol (APC)–conjugated antibodies against Ser⁴⁰ phosphorylated histone H3 (pH3, Cell Signalling, Beverly, MA). Cells were incubated for 1 hour at room temperature and analyzed by flow cytometry. Doublets were identified by a DAPI signal width versus area plot and excluded from analysis. pH3-positive cells were identified as a subgroup of cells in G2 by a shift in fluorescence intensity in the APC channel. All flow cytometric analyses were done on a BD LSRIII flow cytometer (BD Biosciences, San Jose, CA).

**STAT activation assay.** Cells (0.5 × 10⁶) were collected in duplicates and pelletted at 1,000 rpm for 5 minutes. One set of duplicates was resuspended in 500 μL PBS (37°C) containing 0.5% bovine serum albumin (BSA) with 25 ng/mL IFN-γ or 40 ng/mL IL-6 (from a stock solutions in H₂O; Sigma); the control set was resuspended in 500 μL PBS containing 0.5% bovine serum albumin (BSA) and 5 μL/mL allopurinol (APC)–conjugated antibodies against Ser⁴⁰ phosphorylated histone H3 (pH3, Cell Signalling, Beverly, MA). Cells were incubated for 1 hour at room temperature and analyzed by flow cytometry. Doublets were identified by a DAPI signal width versus area plot and excluded from analysis. pH3-positive cells were identified as a subgroup of cells in G2 by a shift in fluorescence intensity in the APC channel. All flow cytometric analyses were done on a BD LSRIII flow cytometer (BD Biosciences, San Jose, CA).

**MAD2 expression.** Cells were collected, fixed, and permeabilized as described above. Cells were incubated with 1 μL MAD2 antibody (BioLegend, San Diego, CA) per 200 μL PBS for 1 hour, washed once with PBS, and incubated with a goat anti-rabbit secondary antibody conjugated with R-phycocerythrin (Southern Biotechnology Associates, Birmingham, AL) for 1 hour. MAD2 expression was determined by flow cytometry reading the PE channel. Cells that received secondary antibody only were used to set the threshold for identifying MAD2-positive cells.

**Fluorescence microscopy.** Cells (0.5 × 10⁶) were collected 72 hours after treatment with all JAKs inhibitor or vehicle. Cells were permeabilized in 1 mL of 90% methanol, washed, and stained with DAPI as described above. MAD2 staining was done as described above. Cells were transferred onto a cover slide and visualized using the blue and red excitation filter of the fluorescence microscope (Olympus BX 41, Tokyo, Japan), respectively. Magnification was ×400.

**Statistical analysis.** Statistical analyses were done with the analysis Toolpak add-in to Microsoft Office Excel 2003. ANOVA analyses were used to test for overall significance within one data set. Paired sample Student’s t tests were used to compare two treatment groups at a time. Ps lower than 0.05 in a two-tailed test were considered to be significant.

**Results**

Inhibition of JAKs reduced cell growth and induced G2 arrest. The JAK/STAT pathway is implicated in both enhancing cell growth, mediated by STAT3 and STAT5, as well as in suppressing cell growth, mediated by STAT1. Because JAKs can signal through multiple STATs, we determined the effect of JAK inhibition on cell proliferation. Cell cultures were initiated at 0.2 × 10⁶ cells/mL in the absence or presence of JAK inhibitors that targeted either JAK2, JAK3, or all JAKs (JAK1, JAK2, JAK3, and TYK2). At 24, 48, and 72 hours of treatment, cell numbers were determined by hemocytometer counts. All of the JAK inhibitors used dramatically suppressed cell growth when compared with vehicle-treated control cells (Fig. 1A). Because cells treated with the JAK inhibitors appeared enlarged compared with vehicle-treated cells, DNA content was determined by flow cytometric evaluation of DAPI-stained cells. Flow cytometric analysis of DAPI-stained cells revealed a G2 arrest in cells with JAK2 inhibited and more dramatically in cells with JAK3 inhibited (Fig. 1A). For JAK3 inhibitor-treated cells, cells in S phase was drastically reduced and cells in G2 showed greater DNA content than G2 control cells (data not shown). Inhibiting all JAKs (JAK1, JAK2, JAK3, and TYK2) caused multinucleation as detected by fluorescence microscopy (Fig. 1B-E). Flow cytometric DNA histograms showed endoreduplication resulting in a second S phase (S2) and an 8N DNA population (G4) by 24 hours of treatment (Fig. 2A). Treatment with 2 μM/L of all JAKs inhibitor for 24 hours caused an enlarged G2 peak (Fig. 2D). At 72 hours, endoreduplication resulted in 8N DNA (G4) and 16N DNA (G8)
cells (Fig. 2E). JAKs were thus required for cell growth and cell cycle progression through G2-M. Inhibition of all JAKs resulted in failure of cytokinesis with endoreduplication causing 8N (G4) and 16N (G8) DNA subpopulations. Genomic stability thus seems dependent on JAKs.

Effect of JAK inhibitors on mitosis. Because cells treated with the inhibitors of all JAKs did not multiply yet continued growing through endoreduplication, we measured the mitosis marker pH3 to determine whether endoreduplicating cells continued to undergo mitosis. Figure 3 shows the dual-variable flow cytometric plots (Fig. 3A-D) of pH3 versus DNA from which the percentage pH3 of G2 or G4 subpopulations was calculated (Fig. 3E). In control cells, 12.5 ± 1.4% (SD) of the G2 cell subpopulation stained positive for pH3 in 24-hour cultures. In parallel cultures, inhibiting JAK2 or JAK3 caused a reduction in pH3-positive cells. Cells (5.8 ± 0.84% and 0.2 ± 0.29%) in G2-M were pH3 positive after JAK2 or JAK3 inhibition, respectively. Inhibiting all JAKs, however, resulted in an overall increase in mitotic cells identified within the G2 DNA as well as the G4 subpopulations. G2 (4N DNA) cells (12.6 ± 1.78%) and G4 (8N DNA) cells (14.2 ± 5.9%) were pH3 positive. Inhibiting JAK2 or JAK3 thus caused a G2-M block with inhibition of mitosis. However, inhibiting all JAKs caused cells to initiate mitosis with subsequent endoreduplication.

Reduction of STAT1 and STAT3 phosphorylation by JAK inhibitors. To show the differential specificity of different JAKs for the activation of different STATs, STAT activation following inhibition of JAK2, JAK3, or all JAKs was determined. Following treatment with JAK inhibitors for 48-hour cultures, either IFN-γ or IL-6 were added to cells for 10 minutes to activate STAT1 and STAT3, respectively. Cells were harvested and assayed for STAT1 or STAT3 activation using flow cytometry to quantify cellular expression of phosphorylated (Y701) STAT1 or phosphorylated (Y705) STAT3. The fluorescence signal was divided by the forward compensation signal to calculate the ratio of pSTAT1 and pSTAT3.

Figure 1. Inhibiting JAKs results in reduced cell number but increased size in HL-60 cells. A, cell counts at 24, 48, and 72 hours after drug administration following culture initiation at 0.2 x 10^6. X axis, targets of the inhibitors used. All JAKs includes JAK1, JAK2, JAK3, and TYK2. Columns, mean of three independent experiments; bars, SD. *, statistically significant differences from the respective control. Phase-contrast (B and D) and fluorescence (C and E) microscopy of DAPI-stained cells receiving either vehicle (B and C) or all JAKs inhibitor (D and E). Original magnification, >400.
angle light scatter signal to normalize out any effects of changing cell size. Using control cells, basal levels of STAT1 and STAT3 activation were derived against which treated cells were compared. From the fluorescence histogram from control cells, a gate capturing 95% of cells was defined and the percentage of cells exceeding it was defined as positive cells exceeding basal levels for treated populations. Inhibition of JAK2 or JAK3 alone was not sufficient to suppress STAT1 phosphorylation induced by IFN-γ, whereas inhibiting all JAKs resulted in a complete loss in pSTAT1 response to IFN-γ (Fig. 4A). In addition to the loss of responsiveness to IFN-γ, inhibiting all JAKs also resulted in a 19.2% reduction of the mean basal pSTAT1 level (data not shown).

JAK inhibition also reduced the effect of IL-6 on STAT3. The all JAKs inhibitor and the JAK3 inhibitor significantly suppressed STAT3 activation by IL-6 (Fig. 4B). This verifies that the pharmacologic inhibitors used had the anticipated effect of inhibiting STATs and shows the differential dependence of STAT activation on different JAKs. A Western blot of pSTAT3 confirms the flow cytometric data on JAK-dependent activation of STAT3 by IL-6 (Fig. 4C).

JAK inhibition caused increased levels of pERK. Because JAK/STAT signaling has been found to be directly connected with both positive and negative regulation of ERK, the effect of JAK inhibition on ERK was determined. Levels of phosphorylated (T202 and Y204) ERK were determined by flow cytometry for control cells and cells treated with inhibitors of all JAKs, JAK2, or JAK3. The percentage of cells exceeding basal levels comparing control and treated populations is reported as for the STATs. Inhibiting all JAKs caused an increase in the flow cytometrically detected pERK compared with basal levels in control cells: 17.2 ± 6.57% were now positive. Inhibition of either JAK2 or JAK3 also caused an increase of total

Figure 3. Effect of JAK inhibition on mitosis marker pHH3. Dot plots, cells costained for pHH3 and DNA generated by flow cytometry. X axis, increasing values represent increased DNA content throughout the cell cycle phases; y axis, increasing values represent cells staining positive for mitosis marker pHH3 as subgroups of cells in G2, G4, and G8, respectively. A, vehicle-treated control cells; B, cells treated with all JAKs inhibitor for 48 hours; C, cells treated with JAK2 inhibitor for 48 hours; D, cells treated with JAK3 inhibitor treated for 48 hours; E, percentages of G2-M and G4-M cell population staining positive for mitosis marker pHH3 24 hours after drug administration. Columns, mean of three independent experiments; bars, SD. *, statistically significantly differences from the respective control.

Figure 4. Differential effect of JAK inhibition on STAT1 and STAT3 activation. Forty-eight hours after treatment with JAK inhibitors, 0.5 × 10⁶ cells were added to IFN-γ (A) or IL-6 (B and C) for 10 minutes. Stimulated and unstimulated cells were stained with APC-conjugated antibodies specific for pSTAT1 (A) or pSTAT3 (B) and analyzed by flow cytometry. Unstimulated control cells were arbitrarily set to be 95% negative and used as standard to quantify percentage cells responsive to 25 ng/mL IFN-γ (A) or 40 ng/mL IL-6 (B), respectively. Columns, mean of three independent experiments; bars, SD. *, statistically significant differences from the respective control. C, STAT3 phosphorylation and β-actin loading control by Western blot confirming data obtained by flow cytometry. Left to right, vehicle-treated control cells, control cells treated with IL-6 for 10 minutes, all JAKs inhibitor-treated cells added to IL-6, JAK2 inhibitor-treated cells added to IL-6, and JAK3 inhibitor-treated cells added to IL-6.
pERK to 10.23 ± 3.71% and 9 ± 3.61% positive, respectively (Fig. 5). These data suggest an inhibitory effect of JAKs on ERK with increased ERK baseline phosphorylation following JAK inhibition. A Western blot confirms activation of ERK by inhibition of all JAKs (Fig. 5B). The results are consistent with previously reported negative regulation of ERK by JAK/STAT signaling and motivate interest in its significance to the endoreduplication we observed.

ERK inhibition by PD98059 ameliorated the G2 arrest and endoreduplication induced by JAK inhibition. To test the significance of the induced ERK activation on the endoreduplication induced by JAK inhibition, we inhibited ERK with PD98059 before inhibiting JAKs. PD98059 is a MAPK/ERK (MEK) inhibitor shown previously to inhibit ERK in these cells (8). DNA histograms of the control and treated populations after 48 hours are shown in Fig. 6. When ERK was inhibited by 10 or 20 μmol/L PD98059 1 hour before treatment with JAK inhibitors, the G2 arrest and endoreduplication mediated by JAK inhibition was reduced in a dose-dependent manner (Fig. 6A-L). In addition, the ERK inhibition normalized signs of aneuploidy, such as the increased DNA content of cells in G2 following JAK2 inhibition (Fig. 6D, H, and L) and the peaks detected between G1 and G2 and between G2 and G4 in cells treated with the all JAKs inhibitor (Fig. 6B, F, and J). Hence, ERK activation seems to be mediating the endoreduplication caused by JAK inhibition. The results indicate a role for MAPK signaling in promoting genomic instability.

MAD2 expression is decreased in endoreduplicating cells. To test whether the endoreduplication was associated with changes in cell cycle checkpoint proteins, we determined the expression of G2-M regulator MAD2. Cells treated with the all JAKs inhibitor showed decreased levels of the G2-M checkpoint protein MAD2 48 hours after treatment (Fig. 7B). JAK2 or JAK3 inhibition did not cause any detectable change in MAD2 expression (Fig. 7C and D) paralleling effects on endoreduplication. When ERK was inhibited with PD98059, MAD2 expression was increased in all treatment groups (Fig. 7E-I, N, and P). This coincided with a decrease in G2-M arrest and endoreduplication. JAK inhibition and consequential ERK activation thus seem to down-regulate expression of the G2-M checkpoint regulator MAD2, which normally inhibits the APC. When ERK was inhibited by PD98059, MAD2 expression was increased resulting in an inhibition of anaphase and subsequent cytokinesis. This disruption of normal APC regulation may thus contribute to the endoreduplication observed.

Discussion

In summary, we find that inhibition of JAKs, which results in compromised STAT but enhanced ERK activation with decreased expression of the MAD2 G2-M checkpoint protein, causes accumulation of G2-M cells and subsequent rounds of endoreduplication. Blocking the ERK enhancement prevented the endoreduplication indicating the functional significance of the enhanced ERK activation. The results point to a need for signaling through JAKs to prevent G2-M genomic instability and polyploidy.

When JAK2, JAK3, or all JAKs were inhibited, we observed a severe G2-M arrest with significant reduction in cell proliferation. The G2-M arrest seems to be a sign of a common downstream facilitator. STAT3, for example, was inhibited by all JAKs inhibitors.

Figure 5. Increased levels of pERK1/2 in cells treated with JAK inhibitors. Baseline pERK levels were determined 24 hours after treatment with JAK inhibitors by staining with APC-conjugated antibodies specific for pT202/Y204 ERK1/2. A, vehicle-treated control cells were defined to be 95% pERK negative and used to set the threshold for determination of percentage cells staining positive (i.e., in excess of basal levels) for pERK in JAK inhibitor-treated cells. Columns, means of three independent experiments; bars, SD. * cells treated with the all JAKs inhibitor were significantly different from control cells. B, data obtained by flow cytometry were confirmed by Western blotting. Left to right, vehicle-treated control cells, all JAKs inhibitor-treated cells, JAK2 inhibitor-treated cells, and JAK3 inhibitor-treated cells. β-Actin is shown as a loading control.
and ERK was shown with IFN-coimmunoprecipitate (15). The antagonistic behavior of STAT1 suggested previously. ERK and STAT1 have been reported to baseline pERK. A negative cross-talk of STAT1 and ERK has been only with a complete block of STAT1 but also with an increased regulation (14).

mediated by cyclin D3 down-regulation and p21(WAF1) up-
treatment caused a significant reduction of cells in M and S phase (13). Hence, early mitotic events appeared to be intact in mitosis during chromosome condensation initiating early pro-

survivin (9). Although these molecules are related to G 1 activation has been identified as promoter of proliferation and constitutively active in leukemias and other tumors. STAT3 is considered to be growth inhibitory as it induces the classic IFN response genes, such as IFN response factor-1 (IRF-1), which is a mediator of reducing growth and protein synthesis in response to viral infection (12). It is plausible that the STAT1/IRF-1 pathway could be involved in regulating cell cycle progression, such as preventing mitosis. When all JAKs were inhibited with PD98059, thus indicating that ERK might be involved in the protection of tumor cells from apoptosis by IFN-α, also indicating a negative cross-talk between ERK and STAT1. MAPKs have also been reported to interact with STAT3 and STAT5 (16–18).

ERK has been implicated in controlling genomic instability. Treatment of HeLa cells with fumarylacetoacetate, a substance causing multinucleation, resulted in sustained activation of ERK. When ERK was inhibited using PD98059, micronuclei with signs of mitotic instability and spindle disturbance were significantly reduced (19). Another group reported that tyrosine-phosphorylated ERK relocates from the nucleolus to the Golgi complex during G2 and M and is associated with Golgi vesicles in proximity to mitotic spindles (20).

In our current study, we found an increased expression of the G2-M cell cycle checkpoint protein MAD2 following treatment with PD98059, thus indicating that ERK might be involved in driving G2-M transition. In fact, in cells treated with all JAKs inhibitor, elevated ERK levels coincided with decreased expression of MAD2 (Fig. 7B). The resulting endoreduplication in cells with low levels of MAD2 expression might be linked to the absence of p53 in HL-60 cells used in the current study. Burds et al. (21) reported that cells with deletion in both MAD2 and p53 were extremely vulnerable to chromosomal instability. Loss of p53 as it occurs in many cancer cells, including HL-60 cells, is frequently linked to chromosomal instability by creating a cellular environment that is more forgiving of DNA or chromosomal damage (22).

To explore the possibility that the endoreduplication following inhibition of all JAKs could be a sign of megakaryocytic differentiation, we measured megakaryocytic cell surface markers CD41, CD62, and van Willebrand factor. All those markers were absent in endoreduplicating HL-60 cells (data not shown). Because HL-60 cells are extremely unlikely to differentiate into megakaryocytes, we also examined K562 cells, which are known for their ability to differentiate into megakaryocytic cells. Although these cells showed similar endoreduplication in response to the all JAKs inhibitor, megakaryocytic cell surface markers were also not expressed (data not shown).
The results of this study provide evidence for an important role of the JAK/STAT pathway in G2-M cell cycle control and maintaining genomic stability. STATs seem to be signaling molecules able to integrate multiple layers of signaling events resulting in a finely tuned output signal. However, to further untangle the JAK/STAT pathway, future work needs to target specific STATs and JAKs, such as JAK1 and TYK2, for which no specific inhibitors are currently commercially available.

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References

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