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The JAK2 Inhibitor AZD1480 Potently Blocks Stat3 Signaling and Oncogenesis in Solid Tumors

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SUMMARY

Persistent activation of Stat3 is oncogenic and is prevalent in a wide variety of human cancers. Chronic cytokine stimulation is associated with Stat3 activation in some tumors, implicating cytokine receptor-associated Jak family kinases. Using Jak2 inhibitors, we demonstrate a central role of Jaks in modulating basal and cytokine-induced Stat3 activation in human solid tumor cell lines. Inhibition of Jak2 activity is associated with abrogation of Stat3 nuclear translocation and tumorigenesis. The Jak2 inhibitor AZD1480 suppresses the growth of human solid tumor xenografts harboring persistent Stat3 activity. We demonstrate the essential role of Stat3 downstream of Jaks by inhibition of tumor growth using short hairpin RNA targeting Stat3. Our data support a key role of Jak kinase activity in Stat3-dependent tumorigenesis.

INTRODUCTION

The signal transducer and activator of transcription (Stat) proteins comprise a family of transcription factors that mediate cytokine and growth factor responses (Akira et al., 1994; Darnell, 1997; Darnell et al., 1994). Persistent activation of Stat3 is oncogenic (Yu and Jove, 2004) and is prevalent in a wide variety of human cancers, including breast, prostate, head and neck, and ovarian cancers, among other solid and hematologic tumors (Bromberg et al., 1999; Catlett-Falcone et al., 1999; Dhir et al., 2002; Garcia et al., 2001; Grandis et al., 2000; Levy and Inghirami, 2006; Silver et al., 2004; Yu et al., 2007). Aberrant Stat3 activation is required for the survival of some types of human cancer cells by promoting the overexpression of genes that encode antiapoptotic proteins, cell-cycle regulators, and angiogenic factors (Bowman et al., 2000, 2001; Grandis et al., 2000; Niu et al., 2002b).

Stat3 is activated by phosphorylation of Tyr705, promoting cytosolic dimerization, nuclear translocation, and DNA binding (Darnell et al., 1994). Stat activation by cytokines is mediated through the Janus family kinases (Jak), which include four family members, Jak1, Jak2, Jak3, and Tyk2 (Schindler and Darnell, 1995). Jak1, Jak2, and Tyk2 are ubiquitously expressed, whereas expression of Jak3 is primarily restricted to the lymphoid lineage (Johnston et al., 1994). Jak family kinases associate with the large hematopoietin subfamily of cytokine receptors that lack intrinsic kinase activity and are dependent on Jak catalytic activity for signal transduction (Leaman et al., 1996). In addition, Stat3 can be phosphorylated by activated growth factor receptors such as c-MET and EGFR (Boccaccio et al., 1998; Quesnelle et al., 2007). Src family kinases have also been implicated in Stat3 activation (Bowman et al., 2000).

A growing body of evidence has documented an important role for autocrine and/or paracrine cytokine loops in driving aberrant activation of Stat3 in human cancer. In particular, interleukin-6 (IL-6) signaling has been implicated in tumorigenesis (Catlett-Falcone et al., 1999; Grivennikov et al., 2009; Hodge et al., 2005; Hong et al., 2007). Recent studies in breast (Berishaj

SIGNIFICANCE

Development of small molecule inhibitors of Jak2 for the treatment of myeloproliferative neoplasms provides an opportunity to assess the role of persistent Jak/Stat activation in solid tumors. Chronic cytokine stimulation is associated with constitutive Stat3 activation in many types of tumors, contributing to growth and survival. Using the Jak2 inhibitor AZD1480, we demonstrate the central role of Jak family kinases in Stat3 activation and growth of human solid tumor xenografts. Our data provide support for the further development of Jak2 inhibitors for treatment of solid tumors.

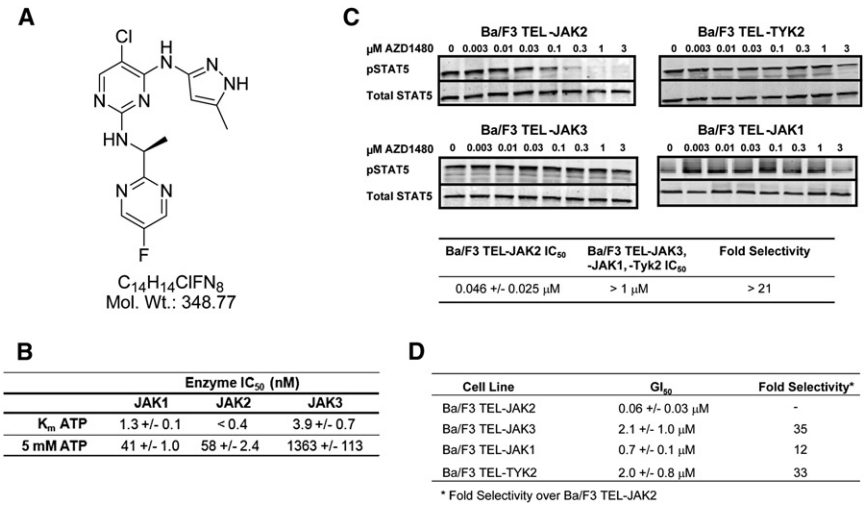

Figure 1. Janus Kinase Family Selectivity of AZD1480

Jak family kinase selectivity was determined using both enzymatic assays and Ba/F3 cells engineered to express constitutively active Jak kinases by fusing the kinase domain of Jak1, Jak2, Jak3, and Tyk2 with the dimerization domain of TEL.

(A) Chemical structure of AZD1480.

(B) Jak1, Jak2, and Jak3 enzymatic assays were carried out in triplicate at K_m levels of ATP and 5 mM ATP. Ranges depicted represent ± SD.

(C) Inhibition of Stat5 phosphorylation in Ba/F3 TEL-Jak2, TEL-Jak3, TEL-Jak1, and TEL-Tyk2 cells. TEL-Jak cells were treated with the indicated concentrations of AZD1480 for 1 hr and the levels of phospho-Stat5 were determined by western immunoblotting. Signal intensity was quantified using Li-Cor Odyssey software. IC_{50} values were calculated from a minimum of three independent experiments. Ranges depicted represent ± SD.

(D) Inhibition of Jak1, Jak2, Jak3, and Tyk2 kinase-driven cellular proliferation in engineered Ba/F3 cell lines. TEL-Jak cells were plated in 96-well plates, treated 24 hr later with AZD1480, and incubated for 48 hr. Cell proliferation was determined using the Alamar blue assay. GI_{50} values were calculated from a minimum of four independent experiments. Ranges depicted represent ± SD.

et al., 2007), lung (Gao et al., 2007), and diffuse large B cell lymphoma (Lam et al., 2008) cancer cell lines have demonstrated a central role of Jak family kinases in mediating IL-6 signaling in these cells. These observations provide a molecular basis for constitutive Stat3 activation in solid tumor types and highlights Jaks as potential targets for cancer therapy.

The recent identification of an acquired Jak2 mutation in myeloproliferative neoplasms has led to the rapid development of selective Jak2 small-molecule inhibitors (Levine and Gilliland, 2008; Morgan and Gilliland, 2008). These reagents provide a means of testing the involvement of Jaks in Stat3-dependent tumorigenesis. We have used the Jak2 inhibitors AZ960 (Gozgit et al., 2008) and AZD1480 to determine whether Jak2 is a central mediator of constitutive and inducible Stat3 activation in tumor cells and if inhibition of this signaling axis could suppress the growth of solid tumor xenografts.

RESULTS

In Vitro Characterization of AZD1480

The pyrazolyl pyrimidine AZD1480 is a potent ATP competitive inhibitor of Jak2 kinase, with an inhibition constant (Ki) of 0.26 nM (Figure 1A and Figure S1 available online). To evaluate Jak family selectivity of AZD1480, we carried out Jak1, Jak2, and Jak3 enzymatic assays at K_m levels of ATP and 5 mM ATP, the high end of ATP concentrations in cells (Figure 1B). AZD1480 demonstrated significant Jak2 selectivity over Jak3, in particular at high ATP concentrations and marginal selectivity over Jak1 at K_m ATP.

To evaluate the cellular selectivity of AZD1480 between the Jak family of kinases, we tested a panel of isogenic Ba/F3 cell lines driven by the JH1 catalytic domains of Jak1, Jak2, Jak3, or Tyk2 fused to the oligomerization domain of TEL (Gozgit et al., 2008; Lacronique et al., 2000). AZD1480 inhibited the phosphorylation of Stat5 with an IC_{50} of 46 nM in TEL-Jak2 cells, whereas little or no inhibition of Stat5 phosphorylation was observed in the TEL-Jak3, TEL-Jak1, or TEL-Tyk2 cells at or below 1 μM AZD1480 (Figure 1C). In these same cells, AZD1480 potently inhibited the growth of the TEL-Jak2 cell line with a GI_{50} of 60 nM. Proliferation of Ba/F3 cell lines bearing the other Jak family members was inhibited at much higher GI_{50} values in line with the selectivity observed in enzyme and/or pStat5 assays (Figure 1D).

To assess the overall kinase selectivity, we evaluated AZD1480 against a panel of 82 kinases (Millipore Corporation) at or near K_m for ATP with three drug concentrations (0.01, 0.10, and 1.0 μM). The kinases represent the diversity of the kinome based on kinase binding site similarity and the gatekeeper residue, a major determinant of small molecule kinase selectivity. Eleven out of 82 kinases, including Jak2, were inhibited by greater than 50% at 0.10 μM (Figure S2).

Jaks Are Central Mediators of Stat3 Signaling in Solid Tumor Cells

Screening of a panel of cell lines manifesting constitutive or inducible Stat3 tyrosyl phosphorylation demonstrated that in virtually all (17/18) of the lines $pStat3^{Tyr705}$ was dependent on Jak kinase activity (Figures 2A and 2B). Stat3 is activated downstream of Src family kinases and activated growth factor receptors, therefore the impact of Src, EGFR, and Met kinase inhibitors was also tested. Notably, neither inhibition of Src (15 cell lines tested) nor EGFR (seven cell lines tested) resulted in modulation of $pStat3^{Tyr705}$ in this panel of cell lines, despite complete inhibition of pSrc and pEGFR (Figure S3). Only c-Met inhibition in the gastric cell line MKN45 showed Jak2-independent inhibition of $pStat3^{Tyr705}$. These data indicate a central role of Jak family kinases in mediating Stat3 activation in solid tumor cell lines.

To further investigate the role of Jak kinases in modulating Stat3 activity, we used a murine embryonic fibroblast (MEF) cell