Dasatinib Inhibits Migration and Invasion in Diverse Human Sarcoma Cell Lines and Induces Apoptosis in Bone Sarcoma Cells Dependent on Src Kinase for Survival


Abstract

Sarcomas are rare malignant mesenchymal tumors for which there are limited treatment options. One potential molecular target for sarcoma treatment is the Src tyrosine kinase. Dasatinib (BMS-354825), a small-molecule inhibitor of Src kinase activity, is a promising cancer therapeutic agent with p.o. bioavailability. Dasatinib inhibits Src activation in human sarcomas, we evaluated the effects of dasatinib in 12 cultured human sarcoma cell lines derived from bone and soft tissue sarcomas. Dasatinib inhibited Src kinase activity at nanomolar concentrations in these sarcoma cell lines. Downstream components of Src signaling, including focal adhesion kinase and Crk-associated substrate (p130CAS), were also inhibited at similar concentrations. This inhibition of Src signaling was accompanied by blockade of cell migration and invasion. Moreover, apoptosis was induced in the osteosarcoma and Ewing’s subset of bone sarcomas at nanomolar concentrations of dasatinib. Inhibition of Src protein expression by small interfering RNA also induced apoptosis, consistent with our previous findings of Src activation in sarcomas. Downstream components of Src signaling were also inhibited in similar concentrations. These results show that dasatinib inhibits migration and invasion of diverse sarcoma cell types and selectively blocks the survival of bone sarcoma cells. Therefore, dasatinib may provide therapeutic benefit by preventing the growth and metastasis of sarcomas in patients.

Introduction

Sarcomas comprise a relatively rare and diverse group of malignant tumors that arise from mesenchymal tissues, including bone, fat, and muscle. There are more than 50 different subtypes of sarcomas, with ~11,000 new cases diagnosed nationwide each year (1, 2). Although this represents a small fraction of all cancers diagnosed in the United States, sarcomas account for ~20% of newly diagnosed pediatric solid tumor malignancies and are among the cancers that pose the greatest risks of mortality and morbidity in children and young adults (3–6).

Histologically, sarcomas are divided into two main subgroups: soft tissue sarcomas and bone sarcomas (1). Genetically, sarcomas also fall into two subgroups: those with complex karyotypes characteristic of severe genomic instability and those with simple karyotypes that are near diploid. The documented defects of sarcomas with simple karyotypes usually include chromosomal translocations, such as those involving ETS family genes. Sarcomas with complex karyotypes have high frequencies of p53 and RB mutations as well as impairments in DNA repair and show chromosomal abnormalities. This latter group of sarcomas includes many of the more commonly diagnosed sarcomas. Sarcomas that fall into both histologic and genetic subgroups also frequently possess abnormalities in growth factor receptor signaling pathways. Protein tyrosine kinases make up the majority of effective signaling pathways in sarcomas, including platelet-derived growth factor receptor, c-KIT, c-MET, and insulin-like growth factor-I receptor (1).

A common point of signal convergence downstream of many of the defective receptor tyrosine kinase pathways in sarcomas is Src kinase. Notably, Src is the oncprotein encoded in the first solid tumor virus to be reported, Rous sarcoma virus, which induces sarcomas in chickens (7–9). The cellular counterpart of viral Src, denoted as c-Src, was the first proto-oncogene to be identified (10–13). As one of nine members of the Src family kinases (SFK), Src was also the first protein tyrosine kinase to be described (7, 14). Src kinase is regulated by growth factors, cytokines, cell adhesion, and antigen receptor activation (15, 16) and is involved in controlling a myriad of fundamental cellular processes, including cell proliferation, migration, invasion, and survival (15). Among other critical cellular substrates, Src kinase phosphorylates and thereby activates focal adhesion kinase (FAK; refs. 17, 18). Significantly, overexpression and/or activation of Src and FAK correlate with cancer development and progression (19–21).
Dasatinib was originally selected as a Src kinase inhibitor and then shown to inhibit Bcr-Abl as well as other tyrosine kinases. There have been several studies showing the activity of dasatinib against Bcr-Abl–positive leukemic cell lines as well as epithelial tumor cell lines (22–27). In addition, early-phase clinical trials have established the safety and efficacy of dasatinib for treatment of imatinib-resistant chronic myelogenous leukemia (CML). However, the responses and mechanisms of action of dasatinib in mesenchymally derived tumor cell lines have not been described previously. We report that dasatinib inhibits Src and downstream FAK signaling at nanomolar concentrations, blocks cell migration and invasion in many diverse human sarcoma cell lines, and induces apoptosis in the bone sarcoma subgroup. Furthermore, knockdown of Src expression by small interfering RNA (siRNA) in bone sarcoma cells also induces apoptosis, suggesting that the observed response to dasatinib in these cells is conveyed through inhibition of Src-mediated signaling. Together, these findings indicate that dasatinib is a promising therapeutic agent for preventing growth and metastasis of a wide diversity of soft tissue and bone sarcomas.

Materials and Methods

Cells and reagents. SaOS-2, U-2 OS, MG-63, SK-ES-1, A673, RD, SK-LMS-1, and HT-1080 sarcoma cell lines were obtained from the American Type Culture Collection (Manassas, VA). The LM2 and LM7 osteosarcoma cell lines were provided by Dr. Eugenie S. Kleinerman (M. D. Anderson Cancer Center, Houston, TX). The TC-71 Ewing’s sarcoma cell line was provided by Dr. Timothy Triche (University of Southern California, Los Angeles, CA). The SaOs-2, MG-63, LM2, LM7, TC-71, SK-LMS-1, and HT-1080 cell lines were maintained in MEM supplemented with Eagle’s salts, 10% fetal bovine serum (FBS), 2-fold MEM vitamins, 1 mM/L sodium pyruvate, 1 mM/L nonessential amino acids, and 2 mM/L L-glutamine. The SK-ES-1 and U-2 OS cell lines were maintained in McCoy’s 5A medium supplemented with 10% FBS. The A673 cell line was maintained in DMEM supplemented with 10% FBS. The RD and RD18 cell lines were maintained in DMEM/F12 (1:1) supplemented with 10% FBS. All cells were maintained at 37°C in 5% CO₂.

Polyclonal antibodies to phosphorylated Src (Y419), phosphorylated FAK (Y576/Y577), phosphorylated p130CSK1 (Y410), and total FAK and poly(ADP-ribose) polymerase (PARP) proteins were obtained from Cell Signaling Technology (Cambridge, MA). Polyclonal antibodies to p130CSK1 and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibodies to total Src (clone GD11) and hILP/XIAP were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibodies to total β-actin and monoclonal, obtained from GE Healthcare Unlimited, Buckinghamshire, were used as loading controls. Densitometry was done on phosphorylated Src (Y419) Western blots done for dose responses in each of the cell lines and then analyzed using ImageQuant 5.2 (Molecular Dynamics, Sunnyvale, CA) software. Percentage inhibition of Src kinase activity as measured by Src (Y419) phosphorylation was determined by nonlinear regression analyses, and data were reported as the inhibitory concentration required to achieve 50% inhibition relative to control reactions (IC₅₀) in Table 1. Data are the averages of triplicate determinations.

Immunohistochemistry. Human tissues were obtained through the Moffitt Cancer Center Tumor Bank using Institutional Review Board–approved protocols. Tissues were fixed in formalin within 15 to 20 min from the moment of surgical excision to preserve the phosphorylation status of proteins, such as Src. Formalin-fixed, paraffin-embedded tissue sections of 3-μm thickness were deparaffinized by an initial warming to 60°C followed by two xylene changes of 10 min each, two series of 30 dips in absolute alcohol, 30 dips in 95% alcohol, and 20 dips in deionized water. Slides were placed for 5 min in TBS/Tween 20 and processed on a DAKO Autostainer using the DAKO LSAB+ peroxidase detection kit (DakoCytometry, CA). Endogenous peroxidase was blocked with 3% aqueous hydrogen peroxide followed by 20 dips in deionized water. The anti-phosphorylated Src (Y419) was applied at 1:100 dilution for 30 min after microwave antigen retrieval with 0.1 mol/L citrate buffer (pH 6.0; Emerson 1,100 W microwave, high to boiling, then 20 min on power level 5). The chromogen 3’-diaminobenzidine was used for detection. Counterstain was done with modified Mayer’s hematoxylin. Slides were dehydrated through graded alcohol, cleared with xylene, and mounted with resins mounting medium.

Wound-healing assay for cell migration. Cells were plated in 24-well tissue culture plates, grown to confluency, and serum starved in 0.1% FBS overnight. Monolayer wounds were produced using a pipette tip scratched through the center of the well. Photomicrographs were taken of the initial wound for comparison. Cells were then treated with either DMSO alone as vehicle control or escalating doses of dasatinib and allowed to migrate into the denuded areas for 24 h. Following incubation, cells were briefly stained with Coomassie blue. Cell migration was visualized at ×10 magnification and digitally photographed. The distance of migration was measured as pixel units and compared with time 0. The average number of pixel units measured in the denuded area was determined from wound-healing assays for dose responses in each of the cell lines analyzed. Percentage inhibition of migration was determined by nonlinear regression analyses, and data were reported as the inhibitory concentration required to achieve 50% inhibition relative to control reactions (IC₅₀). Data are the averages of triplicate determinations.

Cell invasion assay. Cell invasion assays were done following the BioCoat Matrigel Invasion Chambers protocol obtained from BD Biosciences (Bedford, MA). Briefly, cells were trypsinized and washed once with 1× PBS and twice using serum-free medium. Cell suspensions were prepared at 5 × 10⁴ cells/mL in 0.5 mL containing DMSO alone as vehicle control or escalating doses of dasatinib in serum-free medium and added to the chamber insert. Chambers were incubated for 22 h at 37°C and 5% CO₂. Following incubation, noninvading cells were removed using a cotton-tipped swab. The cells that invaded to the lower surface of the membrane were stained using a Diff-Quick Staining kit from Fisher Scientific (Pittsburgh, PA), digitally photographed, and counted. Each experiment was completed in triplicate.

Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay. Apoptosis was detected by the terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay using the In Situ Cell Death Detection kit obtained from Roche Molecular Biochemicals (Indianapolis, IN) according to the manufacturer’s protocol. Cells were...
treated with either DMSO alone as vehicle control or escalating doses of dasatinib for 48 h.

siRNA transfections. siRNA directed specifically against c-Src and a nontargeting siRNA control were obtained from Dharmacon RNA Technologies (Chicago, IL). Cells were plated on 6-cm tissue culture plates in complete medium (5 x 10^5 per plate) and allowed to attach overnight. siRNA was transfected in escalating doses (50 and 100 nmol/L) using Oligofectamine obtained from Sigma-Aldrich. The transfection incubation time for the siRNA/Oligofectamine complexes was 24 h, and total incubation time before harvesting cell lysates was 72 h.

Statistical analysis. Descriptive statistics, such as mean values and SD, were calculated for the biological effects of dasatinib on invasion by dose levels (nmol/L). To determine statistical significance between pairwise dose levels, the exact Wilcoxon two-sample test was used, considering the small sample sizes. One-sided tests at a significance level of 0.05 were examined. All data were analyzed using the Statistical Analysis System software version 9.1 (SAS Institute, Cary, NC).

Results

Src kinase is activated in human sarcomas and sarcoma cell lines. Immunohistochemistry was done on human sarcoma specimens and cell lines for activated Src protein using antibodies to phosphorylated Src. Levels of phosphorylated Src on tyrosine residue 419 (Y419) due to autophosphorylation reflect Src kinase activities in intact cells and tissues. Results show activated Src in sarcomas of diverse subtypes (Fig. 1), including leiomyosarcoma (Fig. 1A), osteosarcoma (Fig. 1B), and liposarcoma (Fig. 1C). Src is activated in all but one (HT-1080) of the cell lines used for these experiments. Cell-free extracts were prepared from untreated cells grown in 10% FBS and immunoblotted with antibodies specific for phosphorylated Src (Y419, p-Src), total Src, or β-actin.

Table 1. Summary of cell line IC50 values and responses to dasatinib

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tumor type</th>
<th>Phosphorylated Src (Y419) expression</th>
<th>IC50 (nmol/L)</th>
<th>Phosphorylated Src (Y419) Migration</th>
<th>Induction of PARP cleavage (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SaOS-2</td>
<td>Osteosarcoma</td>
<td>++</td>
<td>46</td>
<td>65</td>
<td>30</td>
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<tr>
<td>LM2</td>
<td>Osteosarcoma</td>
<td>++</td>
<td>26</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>LM7</td>
<td>Osteosarcoma</td>
<td>++</td>
<td>68</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>U-2 OS</td>
<td>Osteosarcoma</td>
<td>+++</td>
<td>57</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>MG-63</td>
<td>Osteosarcoma</td>
<td>+++</td>
<td>28</td>
<td>58</td>
<td>N/R</td>
</tr>
<tr>
<td>SK-ES-1</td>
<td>Ewing's sarcoma</td>
<td>+++</td>
<td>11</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>TC-71</td>
<td>Ewing's sarcoma</td>
<td>+</td>
<td>3</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>SKLMS-1</td>
<td>Leiomyosarcoma</td>
<td>+++</td>
<td>46</td>
<td>44</td>
<td>N/R</td>
</tr>
<tr>
<td>HT-1080</td>
<td>Fibrosarcoma</td>
<td>−</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>A673</td>
<td>Rhabdomyosarcoma</td>
<td>++++</td>
<td>26</td>
<td>23</td>
<td>N/R</td>
</tr>
<tr>
<td>RD</td>
<td>Rhabdomyosarcoma</td>
<td>+</td>
<td>45</td>
<td>29</td>
<td>N/R</td>
</tr>
<tr>
<td>RD18</td>
<td>Rhabdomyosarcoma</td>
<td>+</td>
<td>50</td>
<td>277</td>
<td>N/R</td>
</tr>
</tbody>
</table>

NOTE: Summary of responses to dasatinib in human sarcoma cell lines. With the exception of one soft tissue sarcoma cell line (HT-1080), all of the cell lines examined respond to dasatinib by inhibition of Src phosphorylation on Y419 and migration at IC50 values consistent with Src kinase inhibition. A subset of bone sarcoma cell lines responds to dasatinib treatment by induction of apoptosis. Induction of apoptosis was not observed in the soft tissue sarcoma cell lines and in one osteosarcoma (MG-63) cell line. +/−/C0 depicts relative phosphorylated Src (Y419) expression.

Abbreviation: N/R, no response.
high-grade osteosarcoma (Fig. 1B), and liposarcoma (Fig. 1C). Because autophosphorylated Src was found in a majority of the human sarcomas examined, including diverse soft tissue and bone sarcomas (data not shown), we determined the level of Src activation in a panel of human sarcoma cell lines by Western blot analysis for phosphorylated Src (Y419) and total Src protein levels. Src was detectably activated in all but one (HT-1080) of the cell lines examined, albeit to different extents (Fig. 1D). Total Src protein expression does not correlate with levels of phosphorylated Src in every case, indicative of different levels of Src kinase activation among the sarcoma cell lines. In addition, the level to which Src kinase is activated (phosphorylated Src levels) does not correlate with specific sarcoma histologic subtypes (Fig. 1; Table 1).

Dasatinib inhibits Src kinase activity in human sarcoma cell lines. Dasatinib has previously been shown to directly inhibit the kinase activity of purified Src protein in vitro with an IC_{50} of 3 nmol/L (24). To evaluate the effect of dasatinib on Src kinase activity in intact sarcoma cells, we treated the above cell lines with escalating doses of dasatinib (30, 100, 300, and 1,000 nmol/L) for 6 h and Western blot analysis was done to evaluate phosphorylated Src levels. Dose-response results for the two representative cell lines (SaOS-2 and U-2 OS) are shown in Fig. 2A and B. The IC_{50} values for inhibition of phosphorylated Src by dasatinib range from 3 to 68 nmol/L for all cell lines analyzed (summarized in Table 1). Time course experiments were done to determine the kinetics at which Src phosphorylation is inhibited by dasatinib. As shown in representative results with U-2 OS cells, inhibition of Src phosphorylation is complete by 15 min following treatment with 100 nmol/L dasatinib and persists for at least 24 h (Fig. 2C). Interestingly, total Src protein expression was increased in a dose- and time-dependent manner in a subset of the cell lines treated with dasatinib. In particular, all but one bone sarcoma cell line (MG-63) produced increases in total Src protein expression, yet this effect was not observed in the soft tissue sarcoma cell lines (Fig. 2A–C; data not shown). These data suggest a positive feedback mechanism for compensation of Src kinase inhibition with increased levels of Src protein expression in the bone sarcoma cells.

Dasatinib selectively blocks Src downstream signaling. Src kinase has been shown to regulate cellular activities through several downstream signaling pathways. One such pathway is FAK, which is a nonreceptor tyrosine kinase that has been found to be increased in a variety of epithelial cancers, including prostate, cervical, and colon carcinomas (29–32). Furthermore, increased FAK expression is associated with tumor progression in a mouse model of skin carcinogenesis (33). FAK, in turn, has been implicated in the activation of Crk-associated substrate, p130^{CAS} (34). To investigate the effect of dasatinib on these Src downstream signaling pathways, sarcoma cell lines were treated in culture with escalating doses of dasatinib for 6 h. As representative results using SaOS-2 cells, Fig. 2D shows...
Western blot analysis done using antibodies to total FAK protein, phosphorylated FAK (Y397, Y576/Y577, and Y925), p130CAS, and phosphorylated p130CAS (Y410). The IC₅₀ values for inhibition of phosphorylated FAK (Y576/Y577 and Y925) and p130CAS were between 30 and 100 nmol/L, consistent with the IC₅₀ values for inhibition of Src kinase activity in these cells (Fig. 2; Table 1). FAK autophosphorylation (Y397) was not substantially inhibited until higher doses of dasatinib (1,000 nmol/L), indicating that dasatinib does not directly inhibit FAK kinase activity. Surprisingly, total p130CAS protein was diminished with dasatinib treatment, whereas total FAK protein was not affected (Fig. 2D), suggesting that p130CAS protein is subject to negative feedback regulation in these cells. By contrast, dasatinib did not inhibit signal transducers and activators of transcription 3 (STAT3) signaling in sarcoma cell lines (data not shown), another signaling pathway that has been shown to act downstream of Src in cells of other tumor types (35). Thus, dasatinib selectively blocks FAK and p130CAS signaling downstream of Src in sarcoma cell lines.

Dasatinib blocks cell motility and invasion by sarcoma cells. Both FAK and p130CAS activities are involved in regulating cell migration and invasion downstream of Src kinase. The effect of dasatinib on cell migration was evaluated using "wound-healing".
assays (by scratching cell monolayers with a pipette tip) and treating with drug. Cells were plated in 0.1% serum medium before inducing the wound to ensure that migration rather than cell growth was measured. The width of the wound was determined at $T_0$, and then, cells were treated with escalating doses of dasatinib in 0.1% serum medium for 24 h. Representative results are shown with the SaOS-2 cells, which were digitally photographed, and the width of denuded area in the wound was measured in pixels (Fig. 3A and B). Wound healing was dramatically inhibited by dasatinib in a dose-dependent manner, with detectable inhibition at 30 nmol/L dasatinib and substantial inhibition at 100 nmol/L dasatinib. To evaluate the effect of dasatinib on cell invasion, SaOS-2 and U-2 OS cells were treated with dasatinib in a dose-response manner for 22 h in Matrigel invasion chambers. Dasatinib significantly inhibited cellular invasion in both cell lines (Fig. 3C). The SaOS-2 cell line was more sensitive to inhibition of invasion by dasatinib compared with the U-2 OS cell line. The IC$_{50}$ values for inhibition of tumor cell invasion in this assay ranged from 30 to 100 nmol/L. These IC$_{50}$ values for blockade of cell migration and invasion are consistent with the IC$_{50}$ values for inhibition of Src kinase as well as downstream FAK and p130CAS signaling (compare with Table 1 and Fig. 2D).

**Dasatinib induces apoptosis of bone sarcoma cell lines.** To determine the effect of dasatinib on sarcoma cell survival, we did growth curve analyses in cell lines treated with increasing concentrations of dasatinib. These assays suggested that the subset of cell lines derived from bone sarcomas responded to dasatinib by induction of apoptosis in a dose-dependent manner (data not shown). To further validate the induction of apoptosis in this subset of cell lines, Western blot analysis was done for apoptotic markers. SaOS-2 and U-2 OS cells were treated with escalating doses of dasatinib for 72 h, and PARP cleavage and XIAP expression were evaluated in both cell lines (Fig. 4A and C). PARP cleavage, an indicator of apoptosis, is evident at 30 nmol/L dasatinib and increased with escalating doses of dasatinib. Furthermore, expression of XIAP, an inhibitor of apoptosis, was diminished by dasatinib treatment with IC$_{50}$ values ranging from 30 to 100 nmol/L (Fig. 4A and C). A time course analysis with 100 nmol/L dasatinib was done in the SaOS-2 cell line to determine when apoptosis was induced as measured by PARP cleavage. PARP cleavage is evident as early as 6 h following treatment with dasatinib and increased with time (Fig. 4B). Moreover, TUNEL assays done on SaOS-2 cells confirmed that increasing numbers of the cells were undergoing apoptosis by 48 h after treatment with escalating doses of dasatinib (Fig. 4D). Therefore, dasatinib induces apoptosis in the bone sarcoma subset with IC$_{50}$ values corresponding to those of inhibition of Src kinase and downstream signaling by dasatinib (Table 1).

**Src is required for survival of bone sarcoma cell lines.** To determine if inhibition of Src kinase by dasatinib is sufficient to induce apoptosis in the bone sarcoma cell lines, we transfected these cell lines with siRNA to c-Src. Two representative bone sarcoma cell lines, SaOS-2 and U-2 OS, underwent induction of apoptosis in a dose-dependent manner as measured by PARP cleavage in response to siRNA against c-Src but not to control.
siRNA. Src protein expression was inhibited by transfection with 50 and 100 nmol/L of siRNA against c-Src, corresponding to induction of PARP cleavage in both cell lines (Fig. 5A and B). MG-63, an osteosarcoma cell line that does not undergo apoptosis when treated with dasatinib, also does not undergo apoptosis when transfected with siRNA to c-Src (data not shown). These data show that a subset of the bone sarcoma cell lines relies on Src kinase for survival, indicating that inhibition of Src kinase activity by dasatinib is sufficient to induce apoptosis in these cells.

Discussion

After nearly a century since the discovery of the Rous sarcoma virus, which subsequently was shown to induce sarcomas by capturing and mutationally activating the cellular gene encoding the Src tyrosine kinase, targeted Src kinase inhibitors are now entering clinical trials for solid tumors. Sarcomas comprise a highly diverse set of human tumors that frequently occur among pediatric cancer patients and for which there are limited treatment options. Based on our observation of Src kinase activation in sarcoma clinical specimens, we sought to determine the action of dasatinib, a potent and p.o. bioavailable inhibitor of Src kinase, on human sarcoma cell lines.

Our findings show that dasatinib inhibits Src kinase activity, as measured by autophosphorylation at Y419, in a dose-dependent manner in sarcoma cells. Furthermore, in 11 of 12 sarcoma cell lines examined, dasatinib inhibits cell migration and invasion. The single cell line that did not respond to dasatinib (HT-1080) was also the only one that lacked detectable Src kinase activity in this panel (Table 1). Moreover, suppression of cell migration and invasion was associated with inhibition of downstream Src signaling through FAK and p130CAS, proteins known to be involved in mediating these cellular processes (17, 36–38). The IC_{50} values for inhibition of Src/FAK/p130CAS signaling as well as migration and invasion are all in the range of approximately 30 to 100 nmol/L regardless of histologic type (Table 1). Taken together, our findings suggest a model for the mechanism of dasatinib action in which blockade of Src and downstream signaling suppresses migration and invasion of sarcoma cells (Fig. 5C).

Significantly, dasatinib induces apoptosis in the majority of bone sarcoma cell lines, including osteosarcoma and Ewing’s sarcoma, but not in any of the soft tissue sarcoma cell lines in our panel. Genetic inhibition of Src using siRNA also induced apoptosis in bone sarcoma cell lines that respond to dasatinib with apoptosis but not in the only osteosarcoma cell line (MG-63) in which dasatinib did not induce apoptosis. Thus, dasatinib induces apoptosis in bone sarcoma cell lines dependent on Src kinase for survival. A major Src signaling pathway involved in preventing apoptosis in other cellular contexts is STAT3 (39, 40). Whereas most of the sarcoma cell lines in this study harbor activated STAT3, with the sole exception of SK-ES-1, dasatinib did not inhibit STAT3 activation, indicating that this pathway is not involved in the dasatinib-mediated apoptosis response in sarcomas. On the other hand, the IC_{50} values for induction of apoptosis by dasatinib are in the same range required for blockade of FAK and p130CAS signaling in these cell lines. Because FAK and p130CAS have been implicated

Unpublished data.
in tumor cell survival, in addition to cell migration and invasion, it is possible that these pathways are involved in dasatinib-mediated apoptosis in sarcoma cells. It is notable that levels of Src activation do not correlate with IC_{50} values of dasatinib responses in terms of cell migration, invasion, or apoptosis (Table 1). This finding may be explained by the possibility that low levels of Src kinase activation are sufficient to induce these biological properties. Alternative explanations are the possibilities that other SFK members or unidentified targets are involved in the responses to dasatinib. Similar results have been observed for other molecular-targeted therapeutic agents, such as Iressa, where clinical response to this epidermal growth factor receptor (EGFR) inhibitor is not correlated with levels of EGFR expression or activation (27). In the specific case of Iressa, EGFR mutations have been shown to influence response to Iressa; however, mutations in the c-Src gene are extremely rare in human cancers (41). Thus, selection of patients for dasatinib treatment based on Src expression or activation levels may not predict the optimal clinical responses. It remains to be determined whether any of the known genetic subtypes of sarcomas are more sensitive to dasatinib than others.

Earlier preclinical laboratory studies pointed to the possibility that dasatinib in the treatment of Gleevec-resistant CML, a prediction that has been borne out in clinical trials (26, 42–47). Based on more recent preclinical laboratory studies, several human solid tumor sites have shown promise for clinical trials, including prostate, lung, pancreatic, and head and neck cancers (23, 24, 27). We have established that Src is activated in a wide variety of human sarcoma clinical specimens, including soft tissue and bone sarcomas. Furthermore, our data show that dasatinib inhibits Src kinase and downstream signaling, leading to blockade of cell migration and invasion of sarcoma cell lines of diverse origins. In the subset of bone sarcomas, dasatinib also induces apoptosis. Taken together, our results suggest that dasatinib will provide clinical benefit to soft tissue and other sarcomas by preventing metastasis, which may be further augmented in bone sarcomas by induction of apoptosis.

Acknowledgments

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References


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