Identification of c-Src as a Potential Therapeutic Target for Gastric Cancer and of MET Activation as a Cause of Resistance to c-Src Inhibition

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Abstract

Therapeutic strategies that target c-Src hold promise for a wide variety of cancers. We have now investigated both the effects of dasatinib, which inhibits the activity of c-Src and several other kinases, on cell growth as well as the mechanism of dasatinib resistance in human gastric cancer cell lines. Immunoblot analysis revealed the activation of c-Src at various levels in most gastric cancer cell lines examined. Dasatinib inhibited the phosphorylation of extracellular signal-regulated kinase (ERK) and induced G1 arrest, as revealed by flow cytometry, in a subset of responsive cell lines. In other responsive cell lines, dasatinib inhibited both ERK and AKT phosphorylation and induced apoptosis, as revealed by an increase in caspase-3 activity and cleavage of poly(ADP-ribose) polymerase. Depletion of c-Src by RNA interference also induced G1 arrest or apoptosis in dasatinib-responsive cell lines, indicating that the antiproliferative effect of dasatinib is attributable to c-Src inhibition. Gastric cancer cell lines positive for the activation of MET were resistant to dasatinib. Dasatinib had no effect on ERK or AKT signaling, whereas the MET inhibitor PHA-665752 induced apoptosis in these cells. The subsets of gastric cancer cells defined by a response to c-Src or MET inhibitors were distinct and nonoverlapping. Our results suggest that c-Src is a promising target for the treatment of gastric cancer and that analysis of MET amplification might optimize patient selection for treatment with c-Src inhibitors.

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Introduction

Gastric cancer is the second most frequent cause of cancer deaths worldwide (1). Chemotherapy has a beneficial effect on survival in individuals with advanced-stage gastric cancer, but overall survival is still usually <1 year (1, 2). Advanced gastric cancer is treated predominantly with the combination of fluoropyrimidine derivatives and platinum compounds, although a globally accepted standard regimen remains to be established. Improved therapy for affected individuals is thus urgently needed.

c-Src is a nonreceptor tyrosine kinase that plays key roles in intracellular signaling by interacting with and phosphorylating multiple proteins and protein complexes (3). Activation of c-Src has been found to contribute to the transformation, proliferation, survival, and motility of malignant cells as well as to tumor angiogenesis (3, 4). c-Src is highly activated in a wide variety of human cancers and clinical studies have shown that such aberrant activation is correlated with malignant progression (5). These properties have rendered c-Src a potential target for the treatment of solid tumors.

Dasatinib is an oral, multitargeted inhibitor of tyrosine kinases that inhibits the activities of c-Src, Bcr-Abl, and other kinases (6). It has been approved for clinical use in patients with chronic myelogenous leukemia or Philadelphia chromosome–positive acute lymphoblastic leukemia and it is currently under investigation as a potential therapy for solid tumors. Recent studies have shown that c-Src inhibitors induce apoptosis or arrest cell cycle progression in various cancer cell types (7–14). The activation of c-Src has pleiotropic effects that depend on cell type and context. Although c-Src activity has been found to be increased in most gastric cancers (15–18), the responses of gastric cancer cells to c-Src inhibition have not previously been characterized. We have therefore now examined the effects of c-Src inhibition by...
dasatinib on cell growth and signal transduction in human gastric cancer cell lines. Furthermore, we have investigated the mechanism of resistance to dasatinib in such cells. Our results provide a rationale for the clinical investigation of c-Src inhibition in individuals with gastric cancer.

Materials and Methods

Cell culture and reagents. The human gastric cancer cell lines SNU1, SNU5, Hs764T, and AGS were obtained from the American Type Culture Collection; MKN1, MKN7, MKN45, NUGC3, and AZ521 were from the Health Science Research Resources Bank; OKA/JMA, MKN28, and HSC39 were from Immuno-Biological Laboratories; and SNU216 was from the Korean Cell Line Bank. HSC58, 58As1, and 58As9 are established cell lines derived from human scirrhous gastric carcinoma as previously described (19). All cells were cultured under a humidified atmosphere of 5% CO2 at 37°C in RPMI 1640 (Sigma) supplemented with 10% fetal bovine serum, 1 mmol/L Na3VO4, leupeptin (1 μg/mL), and 1 mmol/L β-glycerophosphate, 1 mmol/L β-glycerophosphate, 1 mmol/L Na3VO4, leupeptin (1 μg/mL), and 1 mmol/L phenylmethylsulfonyl fluoride. The protein concentration of cell lysates was determined with a BCA protein assay kit (Thermo Fisher Scientific) and equal amounts of protein were subjected to SDS-PAGE on 7.5 or 12% gels defined by a mean HER2 copy number of >6 per cell, corresponding to the previous definition for HER2 amplification (20).

Cell growth inhibition assay. Cells were transferred to 96-well flat-bottomed plates and cultured for 24 hours before exposure to various concentrations of dasatinib or PHA-665752 for 72 hours. Tetra Color One (5 mmol/L tetrazolium monosodium salt and 0.2 mmol/L 1-methoxy-5-methyl phenazinium methysulfate; Seikagaku Kogyo) was then added to each well and the cells were incubated for 3 hours at 37°C before measurement of absorbance at 490 nm with a Multiskan Spectrum instrument (Thermo Labsystems). Absorbance values were expressed as a percentage of that for nontreated cells and the concentration of dasatinib resulting in 50% growth inhibition (IC50) was calculated.

Fluorescence in situ hybridization analysis. MET gene copy number per cell was determined by fluorescence in situ hybridization with the use of the LSI D7S522 (7q31) Spectrum Orange and chromosome 7 centromere (CEP7) Spectrum Green probes (Vysis; Abbott). Cells were centrifuged onto glass slides with a Shandon cyto-centrifuge (Thermo Electron) and were fixed by consecutive incubations with ice-cold 70% ethanol for 10 minutes, 85% ethanol for 5 minutes, and 100% ethanol for 5 minutes. Slides were stored at −20°C until analysis. Cells were subsequently subjected to digestion with pepsin for 10 minutes at 37°C, washed with water, dehydrated with a graded series of ethanol solutions, denatured with 70% formamide in 2× SSC for 5 minutes at 72°C, and dehydrated again with a graded series of ethanol solutions before incubation with a hybridization mixture consisting of 50% formamide, 2× SSC, Cot-1 DNA, and labeled DNA. The slides were washed for 5 minutes at 73°C with 3× SSC, for 5 minutes at 37°C with 4× SSC containing 0.1% Triton X-100, and for 5 minutes at room temperature with 2× SSC before counterstaining with an antifade solution containing 4’,6-diamidino-2-phenylindole. Hybridization signals were scored in 40 nuclei with the use of a ×100 immersion objective lens. Nuclei with a disrupted boundary were excluded from the analysis. Gene amplification was defined by a mean MET/chromosome 7 copy number ratio of >2.2 or by a mean MET copy number of >6 per cell, corresponding to the previous definition for HER2 amplification (20).

Cell cycle analysis. Cells were harvested, washed with PBS, fixed with ice-cold 70% methanol, washed again with PBS, and stained with propidium iodide–RNase staining buffer (BD Biosciences) for 15 minutes at room temperature. The stained cells were then analyzed by flow cytometry (FACSCalibur, BD Biosciences) and the Modfit software (Verity Software House).

Assay of caspase-3 activity. The activity of caspase-3 in cell lysates was measured with a CCF32/Caspase-3 Fluomimetric Protease Assay kit (Medical Biological Laboratories). Fluorescence attributable to the cleavage of the Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin (DEVD-AFC) substrate was measured at excitation and emission wavelengths of 390 and 460 nm, respectively.
Gene silencing. Cells were plated at 50% to 60% confluence in six-well plates or 25-cm² flasks and were incubated for 24 hours before transient transfection with small interfering RNAs (siRNA) for 48 or 72 hours with the use of the Lipofectamine RNAiMAX reagent (Invitrogen). siRNAs specific for human c-Src mRNA (5' CCACCUUUGU-GGCCCUATT-3') or human MET mRNA (5' ACAAGAUCGUACAAAATT-3') as well as a nonspecific siRNA (control) were obtained from Nippon EGT. The cells were then subjected to flow cytometry, immunoblot analysis, or assay of cell growth inhibition.

Statistical analysis. Data were analyzed by Student's two-tailed t test. A P value of <0.05 was considered statistically significant.

Results

Effects of dasatinib on the growth of gastric cancer cell lines. The baseline levels of total c-Src and activated c-Src (phospho-Y416) in 16 human gastric cancer cell lines were measured by immunoblot analysis. All the cell lines expressed detectable levels of total c-Src, whereas all lines with the exception of SNU1 and HSC39 manifested detectable (albeit different) levels of c-Src phosphorylation (Fig. 1A).

To assess the effects of dasatinib on cell growth, we exposed the gastric cancer cell lines to various concentrations of the drug and then measured cell viability. Seven cell lines were responsive to dasatinib with IC₅₀ values ranging from approximately 40 to 540 nmol/L, whereas nine cell lines remained resistant to dasatinib at concentrations up to 5 μmol/L (Table 1; Fig. 1B). SNU1 and HSC39 cells, both of which seemed to lack activated c-Src, were resistant to dasatinib. For the remaining cell lines positive for phosphorylated c-Src, there was no apparent correlation between the antiproliferative effect of dasatinib and the baseline phosphorylation level of c-Src (Fig. 1A).

Figure 1. Phosphorylation of c-Src or MET and growth-inhibitory effects of c-Src or MET inhibitors in gastric cancer cell lines. A, the indicated gastric cancer cell lines maintained in a medium containing 10% serum were lysed and subjected to immunoblot analysis with antibodies to phosphorylated (p-) or total forms of c-Src or MET or to β-actin (loading control). B and C, gastric cancer cell lines were cultured in medium containing 10% serum for 72 hours in the presence of various concentrations of dasatinib (B) or PHA-665752 (C), after which cell viability was assessed as described in Materials and Methods. The number of viable cells is expressed as a percentage of the value for nontreated cells. Black and red lines, dasatinib-responsive and dasatinib-resistant cells, respectively. Points, mean of values from three independent experiments; bars, SD.
MET activation is associated with dasatinib resistance in gastric cancer cell lines. The level of c-Src phosphorylation was thus not sufficient to distinguish dasatinib-responsive from dasatinib-resistant cells. Redundancy of tyrosine kinases has been shown to contribute to de novo resistance to tyrosine kinase inhibitors (21, 22). Given that amplification of MET is frequent in gastric cancer (23–25), we examined whether resistance to dasatinib in gastric cancer cell lines positive for c-Src activation might be due to MET activation. We first determined the abundance and activation status of MET in the gastric cancer cell lines. Immunoblot analysis revealed that all dasatinib-resistant cells positive for phosphorylated c-Src manifested high levels of both MET expression and baseline activation, as reflected by phosphorylation of tyrosine residues 1234/1235 (Fig. 1A) and tyrosine-1349 (data not shown). In contrast, cells categorized as responsive to dasatinib had undetectable levels of phosphorylated MET. We next examined the gastric cancer cell lines for MET amplification by fluorescence in situ hybridization analysis. Six of the seven cell lines positive for MET activation showed evidence of MET amplification. In contrast, all dasatinib-responsive cell lines as well as SNU1 and HSC39 were resistant to PHA-665752 (Fig. 1C). These results thus suggested that the subsets of gastric cancer cells defined by the response to c-Src or MET inhibitors are distinct and nonoverlapping.

Dasatinib inhibits ERK or AKT signaling in dasatinib-responsive gastric cancer cell lines but not in dasatinib-resistant cells. The effects of dasatinib on cell signaling were evaluated in the gastric cancer cell lines with activated c-Src. Cells were exposed to various concentrations of dasatinib and then subjected to immunoblot analysis of phosphorylated and total forms of c-Src, ERK, and AKT (Fig. 2). Dasatinib induced marked inhibition of c-Src phosphorylation in all cell lines tested. In dasatinib-responsive cells, dasatinib also inhibited AKT phosphorylation in SNU216, AGS, and MKN1 cells. In contrast, dasatinib exhibited no substantial inhibitory effect on the phosphorylation of ERK or AKT even at a concentration of 300 nmol/L in dasatinib-resistant cells. These findings indicated that the antiproliferative effect of dasatinib in gastric cancer cells correlates with the inhibition of ERK or AKT signaling.

Table 1. IC50 values of dasatinib for inhibition of the growth of gastric cancer cells in vitro

<table>
<thead>
<tr>
<th>Dasatinib response</th>
<th>Cell line</th>
<th>Dasatinib IC50 (μmol/L)</th>
<th>MET activation</th>
</tr>
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<tbody>
<tr>
<td>Resistant</td>
<td>SNU5</td>
<td>&gt;5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hs746T</td>
<td>&gt;5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>MKN45</td>
<td>&gt;5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HSC58</td>
<td>&gt;5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>58As1</td>
<td>&gt;5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>58As9</td>
<td>&gt;5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>OKAJIMA</td>
<td>&gt;5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SNU1</td>
<td>&gt;5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HSC39</td>
<td>&gt;5</td>
<td></td>
</tr>
<tr>
<td>Responsive</td>
<td>AGS</td>
<td>0.54 ± 0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MKN28</td>
<td>0.50 ± 0.11</td>
<td></td>
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<tr>
<td></td>
<td>NUGC3</td>
<td>0.45 ± 0.16</td>
<td></td>
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<tr>
<td></td>
<td>MKN7</td>
<td>0.42 ± 0.26</td>
<td></td>
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<tr>
<td></td>
<td>MKN1</td>
<td>0.28 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Highly responsive</td>
<td>AZ521</td>
<td>0.06 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SNU216</td>
<td>0.04 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Data are means ± SD of triplicates from experiments that were repeated a total of three times with similar results.
Dasatinib induces G1 arrest or apoptosis in dasatinib-responsive gastric cancer cell lines. To investigate the mechanism by which dasatinib inhibits gastric cancer cell growth, we first analyzed the cell cycle profile by flow cytometry after exposure of cells to the drug for 0, 24, or 48 hours. We chose a dasatinib concentration of 300 nmol/L for these experiments because it approximated the IC50 values for dasatinib-responsive cell lines. Dasatinib increased the percentage of cells in G0-G1 phase of the cell cycle and decreased the percentage of those in S phase in a subset of dasatinib-responsive cell lines, including AZ521, MKN28, NUGC3, and MKN7 (Fig. 3A). The other dasatinib-responsive cell lines, including SNU216, MKN1, and AGS, in which dasatinib inhibited both ERK and AKT phosphorylation, showed an increase in the sub-G1 cell population on exposure to dasatinib, indicative of the induction of apoptosis (Fig. 3A). In dasatinib-resistant cells with MET activation, dasatinib had minimal effects on cell cycle distribution (Supplementary Fig. S1A). We also examined the effect of dasatinib on the abundance of the cyclin-dependent kinase inhibitor p27, which contributes to the regulation of G1-S progression. Dasatinib induced the upregulation of p27 in the four dasatinib-responsive cell lines in which it induced G1 arrest (Fig. 3B), but not in cell lines in which it did not trigger such arrest (Supplementary Fig. S1B). As a further test for apoptosis in SNU216, MKN1, and AGS cells, we measured the activity of caspase-3 and probed for cleavage of PARP. Dasatinib increased caspase-3 activity (Fig. 3C) and induced PARP cleavage (Fig. 3D) in these three cell lines. These findings thus indicated that induction of G1 arrest or apoptosis underlies the antiproliferative effect of dasatinib in responsive cells. On the other hand, PHA-665752 was previously shown to induce apoptosis in gastric cancer cells with MET amplification (27). Consistent with these previous results, we showed that PHA-665752 induced a substantial increase in the frequency of apoptosis, as revealed by an increase in caspase-3 activity and PARP cleavage, in dasatinib-resistant cells with MET activation, whereas PHA-665752 had minimal effects on apoptosis in dasatinib-responsive cells (Fig. 3C and D).

**Effects of c-Src depletion in dasatinib-responsive gastric cancer cell lines.** To verify that the inhibitory effect of dasatinib on cell growth is indeed mediated by c-Src inhibition rather than by nonspecific inhibition of
Figure 3. Effects of dasatinib on cell cycle distribution and apoptosis in gastric cancer cell lines. A, cells were incubated in medium containing 10% serum for 0, 24, or 48 hours in the presence of 300 nmol/L dasatinib, after which they were fixed, stained with propidium iodide, and analyzed for cell cycle distribution by flow cytometry. All data are means of triplicates from experiments that were repeated a total of three times with similar results. B, cells were incubated in a medium containing 10% serum for 24 hours in the absence or presence of dasatinib (300 nmol/L). Cell lysates were then subjected to immunoblot analysis with antibodies to p27. C, cells were incubated in medium containing 10% serum for 48 hours in the absence or presence of dasatinib (300 nmol/L) or PHA-665752 (500 nmol/L). Cell lysates were then assayed for caspase-3 activity. Columns, mean of values from three independent experiments; bars, SD. *, P < 0.05 versus the corresponding value for control cells. D, cells were incubated in medium containing 10% serum for 72 hours in the absence or presence of either dasatinib (300 nmol/L) or PHA-665752 (500 nmol/L). Cell lysates were then subjected to immunoblot analysis with antibodies to PARP.
other kinases such as the platelet-derived growth factor receptor, c-Kit, or Bcr-Abl (6), we transfected dasatinib-responsive cells with an siRNA that targets c-Src mRNA. Similar to the effects of dasatinib, depletion of c-Src resulted in G1 arrest (Fig. 4A), accompanied by accumulation of p27 (Fig. 4B), in AZ521, MKN28, NUGC3, or MKN7 cells. Moreover, also similar to the effects of dasatinib, depletion of c-Src in SNU216, MKN1, or AGS cells triggered apoptosis as revealed by an increase in the sub-G1 cell population (Fig. 4A) and PARP cleavage (Fig. 4C). These results thus indicated that the effects of dasatinib on cell growth or survival in gastric cancer cell lines are mediated by inhibition of c-Src.

**Mechanism of dasatinib resistance in gastric cancer cells with MET activation.** Given the association of activated MET with resistance to dasatinib, we examined whether the depletion of MET might affect dasatinib cytotoxicity in dasatinib-resistant cells with MET activation. Immunoblot analysis revealed that transfection of 58As9 or OKAJIMA cells with an siRNA specific for MET mRNA resulted in the marked depletion of the corresponding protein (Fig. 5A). Such depletion of MET restored the sensitivity of these dasatinib-resistant cells to the inhibition of cell growth by dasatinib (Fig. 5B). These results thus indicated that activated MET indeed contributes to dasatinib resistance in gastric cancer cells.

To examine the mechanism by which MET activation gives rise to dasatinib resistance, we determined the effects of PHA-665752 or dasatinib on the phosphorylation of MET, c-Src, ERK, and AKT in gastric cancer cells with MET activation. PHA-665752 inhibited the phosphorylation of MET, c-Src, AKT, and ERK in such cells (Fig. 5D). In contrast, dasatinib had no effect on either MET phosphorylation or downstream signaling by AKT or ERK in these cells (Fig. 5D). These data thus suggested that MET activation results in AKT and ERK phosphorylation in a c-Src–independent manner, although c-Src is activated at least in part by increased MET signaling in dasatinib-resistant cells with MET activation.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Effects of c-Src depletion on cell cycle distribution and apoptosis in gastric cancer cell lines. A, cells were transfected with nonspecific (control) or c-Src siRNAs for 48 hours, fixed, stained with propidium iodide, and analyzed for cell cycle distribution by flow cytometry. All data are means of triplicates from experiments that were repeated a total of three times with similar results. B and C, cells were transfected as in A for 48 hours (B) or 72 hours (C), after which cell lysates were subjected to immunoblot analysis with antibodies to the indicated proteins.
Discussion

We have shown that c-Src is activated at various levels in most human gastric cancer cell lines, consistent with previous studies showing the upregulation of the kinase activity of c-Src in most gastric cancers examined (15–18). We therefore investigated the potential utility of c-Src as a molecular target for treatment of gastric cancer. Dasatinib has been developed as a multikinase inhibitor with activity against c-Src, Bcr-Abl, and several receptor tyrosine kinases (6). We examined the effects of c-Src inhibition by dasatinib on cell growth in gastric cancer cell lines, finding that dasatinib inhibited the growth of a subset of such cell lines exhibiting c-Src phosphorylation. No relation was apparent between the response to dasatinib and the level of c-Src phosphorylation in cell lines with activated c-Src, whereas SNU1 and HSC39, both of which did not manifest detectable c-Src phosphorylation, were resistant to dasatinib, consistent with previous observations (7, 8, 14, 28, 29). These findings suggest that c-Src promotes cell proliferation and survival in a subset of gastric cancer cell lines positive for c-Src activation but not in those without c-Src activation.

We found that the level of ERK phosphorylation was reduced by dasatinib in all responsive cell lines but not in resistant cell lines, suggesting that the inhibition of ERK might correlate with the antiproliferative effects of c-Src inhibitors in gastric cancer cells. In addition, inhibition of both ERK and AKT phosphorylation by dasatinib was associated with the induction of apoptosis in SNU216, MKN1, and AGS cells. To confirm that the effects of dasatinib on cell cycle progression and apoptosis are indeed attributable to c-Src inhibition in dasatinib-responsive gastric cancer cells, we specifically depleted the cells of c-Src by RNA interference. In cells in which dasatinib induced G1 arrest, depletion of c-Src also triggered G1 arrest accompanied by the upregulation of p27. Similarly, in cells in which dasatinib induced apoptosis, c-Src depletion also

Figure 5. Mechanism of dasatinib resistance in gastric cancer cells with MET activation. A, cells were transfected or not with nonspecific (control) or MET siRNAs for 48 hours, after which cell lysates were subjected to immunoblot analysis with antibodies to the indicated proteins. B, cells transfected as in A were cultured in a medium containing 10% serum and various concentrations of dasatinib for an additional 48 hours, after which cell viability was assessed. The number of viable cells is expressed as a percentage of the corresponding value for cells not exposed to dasatinib. Points, mean of values from three independent experiments; bars, SD. C and D, cells were incubated in medium containing 10% serum for 0, 24, or 48 hours in the presence of 500 nmol/L PHA-665752 (C) or 300 nmol/L dasatinib (D). Cell lysates were then subjected to immunoblot analysis with antibodies to the indicated proteins.
elicited apoptosis, as revealed by the detection of PARP cleavage. These data thus provide more definitive support for the notion that c-Src signaling promotes the proliferation and survival of dasatinib-responsive gastric cancer cell lines. A c-Src inhibitor was previously shown to induce G1 arrest in prostate cancer cells (11). On the other hand, dasatinib was found to induce apoptosis in non-small cell lung cancer cells harboring an epidermal growth factor receptor gene mutation (8). Sensitive cells thus exhibit different responses to c-Src inhibitors. The mechanisms underlying the cellular decision to undergo G1 arrest or apoptosis in response to such inhibitors remain unclear but may be related to differences in cell type.

Gastric cancer cell lines positive for MET activation were resistant to dasatinib, despite the activation of c-Src apparent in these cells. We showed that dasatinib sensitivity was restored in such cells by the depletion of MET, suggesting that MET activation contributes to resistance to c-Src inhibitors. The MET inhibitor PHA-665752 suppressed c-Src, ERK, and AKT phosphorylation in cells with activated MET, whereas dasatinib had minimal effects on either ERK or AKT phosphorylation. These findings suggested that MET-c-Src and MET-ERK/AKT pathways operate independently of each other in such cells. We found that PHA-665752 inhibited the growth of cells with MET activation, with this effect being accompanied by the induction of apoptosis. Consistent with our findings, MET amplification was previously shown to identify a subset of gastric cancers likely to respond to MET inhibitors (27). These results suggest that cells with MET activation may have switched their cell growth dependence from the c-Src-ERK/AKT pathway to the MET-ERK/AKT pathway, although the precise mechanism of the altered signal transduction remains unknown. We further found that the combination of dasatinib and PHA-665752 manifested an additive to synergistic inhibitory effect on the growth of gastric cancer cells with MET activation but not on that of cells without MET activation (Supplementary Table S1 and Fig. S2). These data suggest that the survival of cells with MET activation depends at least in part on activated c-Src in the presence of a MET inhibitor. Further studies are required to determine the mechanism of dasatinib resistance in gastric cancer with MET activation. Amplification of MET is often responsible for the activation of MET signaling, with such amplification occurring most frequently (10-20%) in gastric cancer (23-25). Our data therefore suggest that the analysis of MET amplification might optimize patient selection for gastric cancer treatment with c-Src or MET inhibitors.

The recent success of molecularly targeted agents seems to depend on the identification of drug targets and patients who are likely to benefit from these agents. In the present study, we show that c-Src is a promising target for the treatment of gastric cancer. Moreover, our results indicate that testing to exclude the possibility of MET amplification should be done in consideration of c-Src inhibitors for the treatment of gastric cancer. Dasatinib inhibited the growth of SNU216 and AZ521 cells with IC50 values in the low nanomolar range (40 and 60 nmol/L, respectively); however, the IC50 values for most of the responsive cell lines were greater than the maximum achievable plasma concentration (100 nmol/L) of dasatinib (30, 31). Novel c-Src inhibitors with increased potency are currently under development (32) and might prove beneficial for the treatment of gastric cancer. Our results provide a rationale for future clinical investigation of the therapeutic efficacy of c-Src inhibitors in individuals with gastric cancer as well as for the selection of patients likely to benefit from such treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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