

BMS-777607, a Small-Molecule Met Kinase Inhibitor, Suppresses Hepatocyte Growth Factor–Stimulated Prostate Cancer Metastatic Phenotype *In vitro*

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Abstract

Most prostate cancer–related deaths are due to advanced disease with patients with metastatic prostate cancer having a 5-year survival rate of only 34%. Overexpression of c-Met receptor tyrosine kinase has been highly associated with prostate cancer progression and metastasis. In the present studies, the effect of BMS-777607, a selective and potent small-molecule Met kinase inhibitor that has been advanced to clinical evaluation, on hepatocyte growth factor (HGF)–mediated cell functions and signaling pathways was evaluated in c-Met–expressing PC-3 and DU145 prostate cancer cells. BMS-777607 treatment had little effect on tumor cell growth but inhibited cell scattering activated by exogenous HGF, with almost complete inhibition at 0.5 $\mu\text{mol/L}$ in PC-3 and DU145 cells. This agent also suppressed HGF-stimulated cell migration and invasion in a dose-dependent fashion ($\text{IC}_{50} < 0.1 \mu\text{mol/L}$) in both cell lines. Mechanistically, nanomolar doses of BMS-777607 potentially blocked HGF-stimulated c-Met autophosphorylation and downstream activation of Akt and extracellular signal-regulated kinase. In addition, both wortmannin and U0126, but not dasatinib, attenuated cell scattering and migration induced by HGF, suggesting the involvement of the phosphoinositide 3-kinase and mitogen-activated protein kinase pathways, but not of Src or focal adhesion kinase, in HGF-mediated motogenic effects. Taken together, these data indicate that the downregulation of c-Met signaling by BMS-777607 treatment can significantly disrupt key steps in the metastatic cascade, suggesting that such a targeting strategy may hold promise for the treatment of advanced prostate cancer. *Mol Cancer Ther*; 9(6): 1554–61. ©2010 AACR.

Introduction

Prostate cancer has become the most prevalent cancer diagnosed and the second leading cause of cancer death for North American men (1). Despite improvement in early detection and more refined therapeutic modalities, metastatic prostate cancer remains largely untreatable. Whereas the 5-year survival rate of locally confined prostate cancer is ~100%, the rate drops to ~34% if distant metastases are detected at the time of diagnosis (2). The presence of metastases not only results in a drastic worsening of the prognosis but also leads to a substantial reduction of the quality of life. Key components of the metastatic process in biologically aggressive prostate tumors include proliferation, migration, invasion, and angiogenesis.

Met is a receptor tyrosine kinase that is expressed in epithelial and endothelial cells. In normal cells, c-Met is

activated by its ligand hepatocyte growth factor (HGF)/scatter factor that is produced by stromal cells such as fibroblasts, therefore generating a paracrine activation loop. At the molecular level, HGF stimulates c-Met autophosphorylation at tyrosine 1234 and 1235. Phosphorylated c-Met further triggers the activation of downstream signaling pathways including the Ras-mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinases (PI3K)-Akt pathway through the adaptor proteins Gab-1 and growth factor receptor binding protein 2 (3). In addition, nonreceptor Src tyrosine kinase has also been suggested as a downstream target molecule in c-Met signaling (4).

Under normal physiologic conditions, the pleiotropic effects of the HGF/c-Met axis are essential for embryogenesis and tissue homeostasis (5). In cancer, both HGF and c-Met have been closely linked to the regulation of the metastatic process (6). For example, c-Met is one of the most frequently abnormal signaling molecules in a variety of human malignancies (7). Furthermore, it is widely accepted that HGF functions as a mitogen, motogen, and morphogen that triggers multiple steps of cancer invasive growth including proliferation, scattering, migration, invasion, branching morphogenesis, and angiogenesis (8, 9). In glioma (10) and breast cancer cells (11), c-Met and HGF are coexpressed, thus generating

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an HGF/c-Met autocrine loop that renders tumor proliferation and invasion. c-Met can also be aberrantly activated in a ligand-independent manner, for example, by c-Met receptor mutation as has been reported in papillary renal cell carcinoma (12) and head and neck cancer (13). In addition, hyperactivation of c-Met signaling by gene amplification has been found in multiple human cancers including gastric (14) and metastatic colorectal cancer (15). In prostate cancer however, current studies suggest that c-Met receptor is neither mutated nor amplified (5). Although overexpression (due to gene upregulation, not amplification) of c-Met may exist in some prostate cancer cell lines, the activation of c-Met seems to be predominantly dependent on the ligand through a paracrine mode (16). Still, the expression of both c-Met receptor and HGF ligand has been correlated with prostate tumor aggressiveness in patient tissue samples (17, 18). In addition, increased serum level of HGF is an independent prognostic indicator in patients with advanced stage prostate cancer (19, 20). Finally, Met protein is found more often in metastatic lesions than in primary tumors (16), with 100% of prostate bone metastases being Met positive (21, 22).

In light of the role of c-Met signaling in cancer progression and metastases, both the c-Met receptor and the HGF ligand are considered potential targets for cancer therapy. Multiple approaches have been considered for targeting the HGF/c-Met axis including antibodies directed against the ligand or the receptor as well as small-molecule Met tyrosine kinase inhibitors (7, 23). The latter are able to block autophosphorylation of the c-Met kinase, thereby interrupting its downstream signaling pathways. Most Met tyrosine kinase inhibitors inhibit c-Met phosphorylation at doses in the nanomolar range (24). To date, a variety of these agents have been tested in preclinical settings and lead compounds have entered clinical trials (25). Although the function of Met tyrosine kinase inhibitors has been widely studied in a variety of cancer types, their effect on prostate cancer remains to be investigated.

BMS-777607 is a novel, selective, and orally available ATP-competitive Met kinase inhibitor that specifically targets several Met family members and potently binds to the c-Met active site with a K_i value of 3.9 nmol/L (26). In preclinical models, it potently blocked the autophosphorylation of c-Met with an IC_{50} of 20 nmol/L and impaired xenograft growth (26). BMS-777607 was evaluated in phase I clinical trials in patients with advanced or metastatic solid tumors including hormone-refractory prostate cancer. The present study assessed the effect of BMS-777607 on key functions associated with the metastatic cascade in HGF-stimulated human prostate cancer cells.

Materials and Methods

Reagents and antibodies

BMS-777607 and dasatinib were kindly provided by Bristol-Meyer Squibb. The powder was dissolved in

DMSO and stored as aliquots (10 mmol/L) at -20°C . Wortmaninn and U0126 were purchased from Calbiochem. Recombinant human HGF and anti-HGF neutralizing antibody were obtained from R&D Systems. Additional chemicals were purchased from Sigma unless otherwise indicated. Antibodies against phospho-c-Met (Y1234/1235), total c-Met, phospho-Akt (S473), phospho-ERK (T202/Y204), phospho-Src (Y416), and phospho-focal adhesion kinase (FAK; Y397) were purchased from Cell Signaling. Anti- β -actin (AC-74) antibody was obtained from Sigma.

Cell culture

Human prostate cancer cell lines PC-3 and DU145 were obtained from the American Type Culture Collection. PC-3 cells were maintained in Ham's F-12K supplemented with 10% fetal bovine serum. DU145 cells were maintained in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. Cells were cultured in a 5% CO_2 humidified incubator at 37°C .

Cell scattering

Cells were seeded in a six-well plate at a density of 2×10^3 cells per well and cultured for 7 days until colonies were formed. Cell colonies were incubated with serum-free medium overnight and pretreated with kinase inhibitors for 1 hour before stimulating with HGF (25 ng/mL). Cells were stained with crystal violet (0.1%) 24 hours after treatment. Scattered colonies were photographed.

Cell migration

Cell migration was determined using an *in vitro* "wound-healing" assay. Briefly, cells were seeded in six-well plates and grown for 48 hours to allow them to reach confluency. Cells then were serum starved overnight, pretreated with various kinase inhibitors for 1 hour, and stimulated with HGF (25 ng/mL). Before treatment, ~ 2 -mm-wide scratches were made in the confluent cell monolayer using a sterilized 1-mL tip. Cell migration into the denuded areas was assessed 24 hours after treatment by scoring 12 random fields (original magnification, $\times 100$). Relative migration was calculated as the ratio of migrated cells of treated to nontreated control plates.

Cell invasion

The invasive ability of cells was tested using a commercial Transwell insert (8 μm pore) preloaded with Matrigel (BD Biosciences). Inserts were incubated with serum-free medium with or without BMS-777607 at 37°C for 2 hours to allow the rehydration of Matrigel. Cells suspended in serum-free medium were then loaded onto the top chamber (10^4 /insert). HGF (final concentration of 25 ng/mL) was added to the lower chamber to act as a chemoattractant. After incubation for 24 hours, inserts were wiped with a cotton swab to remove Matrigel and were stained with crystal violet. Invading cells on the

underside of the filter were photographed and scored from eight random fields (original magnification, $\times 100$). Relative invasion was calculated from the ratio of invaded treated versus control tumor cells.

Cell proliferation and cell death

Cells were seeded in a 96-well plate at a density of 5×10^3 cells per well and exposed to serial dilutions of BMS-777607 for 1 hour. HGF (25 ng/mL) was then added and the cells were incubated (drug plus HGF) for a period of 96 hours. At the end of the treatment period, the tumor cells were exposed to WST-8 (MTT assay reagent) in a Cell Counting kit (Donjindo Molecular Technologies) according to the manufacturer's instruction, and absorbance was determined at 450 nm colorimetrically. Cell proliferation (%) was calculated as the ratio of absorbance from treated sample to the nontreated control. Cell death was examined by trypan blue exclusion, and positively stained (dead) cells were scored using a hemocytometer.

Western blot

Cells were harvested and disrupted in a radioimmuno-precipitation assay lysis buffer [50 mmol/L Tris-HCl (pH 8.0), 150 mmol/L NaCl, 0.1% SDS, 1% NP40, 0.25% sodium deoxycholate, and 1 mmol/L EDTA with protease inhibitor cocktail, 1 mmol/L NaF, and 1 mmol/L Na_3VO_4]. Total protein concentration was measured using a Bradford reagent (Bio-Rad), and equal amounts (50 μg) of whole-cell lysates were resolved by SDS-PAGE (Bio-Rad). After electrophoresis, samples were electrotransferred to a nitrocellulose membrane (Bio-Rad), probed with relevant primary antibodies at 4°C overnight, incu-

bated with horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch), and detected with an enhanced chemiluminescence substrate (Amersham).

Statistical analysis

Two-tailed Student's *t* test was used for analyzing data by GraphPad Prism 5.0. A threshold of $P < 0.05$ was defined as statistically significant.

Results

BMS-777607 inhibits HGF-induced prostate cancer cell scattering

Cell scattering is a complex process that is composed of cell-cell dissociation, cell spreading, and enhanced cell motility (8). It has been shown that cell scattering is a hallmark of cancer invasive growth and metastasis (27). To determine the effect of BMS-777607 on scatter behavior, cells were treated with HGF in the presence or absence of the agent. Cells pretreated with BMS-777607 exhibited inhibitory effect on HGF-induced cell scattering in PC-3 and DU145 cells. Compared with HGF alone, cell dissociation and movement ability was blocked by the addition of as little as 0.1 $\mu\text{mol/L}$ BMS-777607 (Fig. 1). At a dose of 0.5 $\mu\text{mol/L}$, this agent almost completely prevented cell spreading, a result comparable with that achieved with the anti-HGF neutralizing antibody (Fig. 1).

BMS-777607 suppresses HGF-induced cell migration

Enhanced cell migratory ability is involved in the cell scattering phenotype. To further examine the effect of BMS-777607 on cell motility, prostate cancer cells were

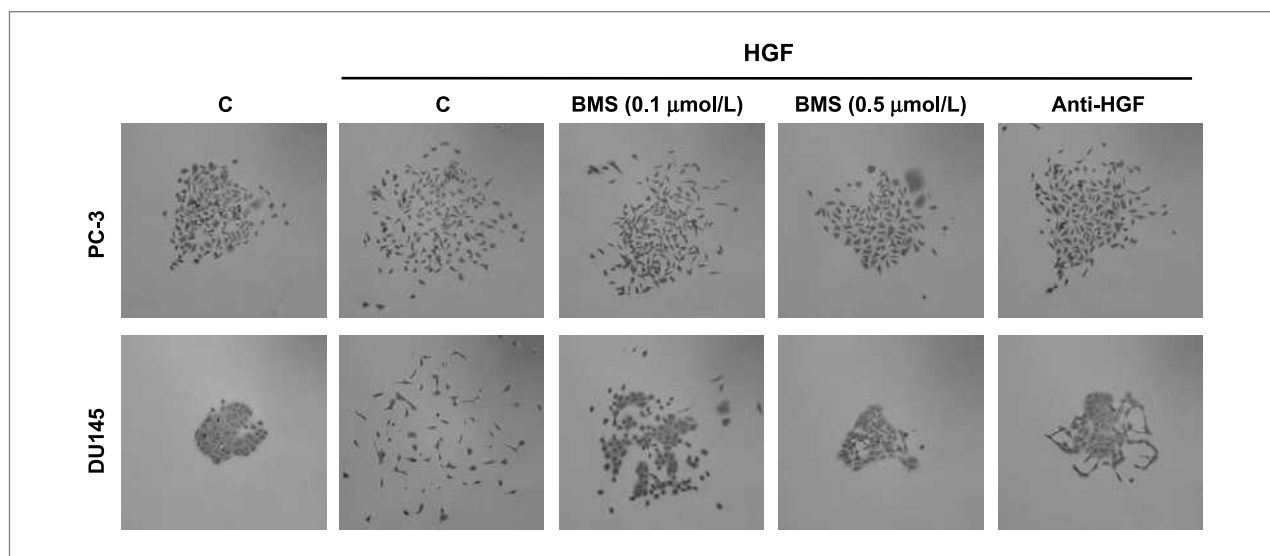


Figure 1. BMS-777607 inhibits HGF-induced cell scattering. PC-3 and DU145 cells were serum starved overnight and pretreated with different doses of BMS-777607 for 1 h before exposure to HGF (25 ng/mL) in the serum-free medium. Alternatively, HGF (25 ng/mL) was incubated with anti-HGF neutralizing antibody (10 $\mu\text{g/mL}$) in the serum-free medium at 37°C for 2 h, and the mixture was added to the cells. Cell scattering was monitored 24 h after the addition of HGF. Typical pictures of cell scattering for each sample are shown (original magnification, $\times 50$). Data represent one of three independent experiments. C, control; BMS, BMS-777607.

treated with exogenous HGF in the presence of various doses of BMS-777607 and migration was determined by an *in vitro* wound-healing assay. As shown in Fig. 2, BMS-777607 significantly suppressed HGF-induced cell motility at doses as low as 0.01 $\mu\text{mol/L}$ in both the PC-3 ($P < 0.01$) and DU145 ($P < 0.001$) cell lines. Greater than 50% inhibition of migration could be achieved with a dose of 0.1 $\mu\text{mol/L}$ ($P < 0.001$). A dose of 1 $\mu\text{mol/L}$ was sufficient to block the movement of most cells in both cell lines ($P < 0.001$), a result comparable with that achieved with 10 $\mu\text{g/mL}$ anti-HGF neutralizing antibody.

BMS-777607 impairs HGF-mediated cell invasion

Cell invasion is a critical step in the metastasis cascade that can be enhanced by HGF (28). The results of Fig. 3 illustrate that HGF increased PC-3 cell invasion 4.1 \pm 1.4-fold. BMS-777607 treatment significantly inhibited the HGF-induced cell invasion at doses in the

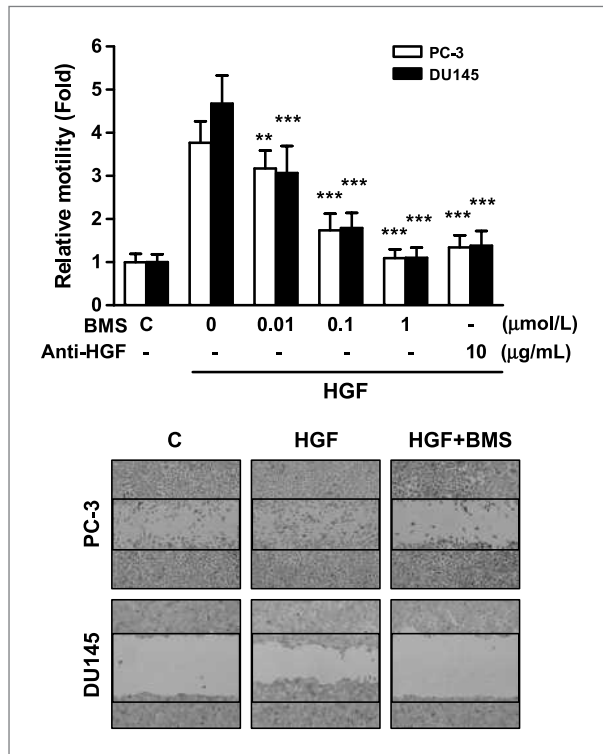


Figure 2. BMS-777607 suppresses HGF-induced cell motility. Confluent cells were serum starved overnight and scratched to generate artificial gaps. Cells were treated with different doses of BMS-777607 for 1 h followed by HGF (25 ng/mL) in the serum-free medium. Alternatively, HGF (25 ng/mL) was incubated with anti-HGF neutralizing antibody (10 $\mu\text{g/mL}$) in the serum-free medium at 37°C for 2 h, and the mixture was added to the cells. Cell motility was monitored 24 h after the addition of HGF, and cells migrated into denuded area were scored. Typical pictures of cell migration after treatment with the highest dose (1 $\mu\text{mol/L}$) of the agent are shown (original magnification, $\times 50$). Data are from three independent experiments. C, control; BMS, BMS-777607. Columns, mean; bars, SD ($n = 9$). **, $P < 0.01$; ***, $P < 0.001$.

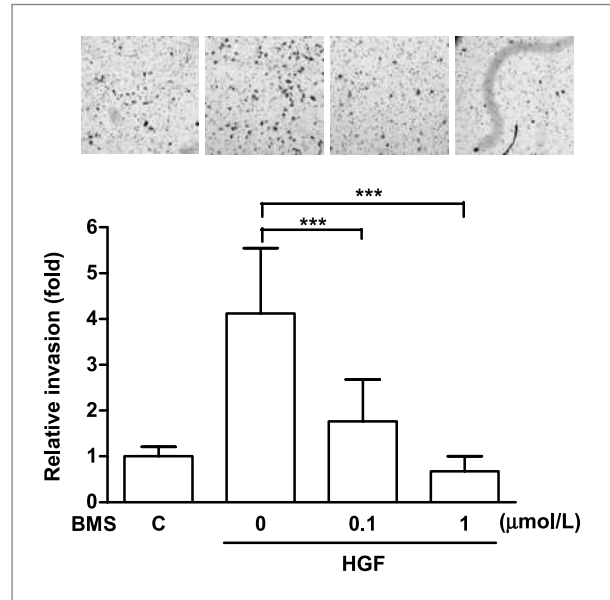


Figure 3. BMS-777607 inhibits HGF-mediated cell invasion. PC-3 cells were seeded into Matrigel-coated Transwell inserts (10^4 /insert) in the presence or absence of BMS-777607. HGF (25 ng/mL) was loaded on the bottom chamber as a chemo-attractant. The underside of the filter was stained 24 h after cell loading. Invading cells were scored from eight random fields. Typical fields of cell invasion are shown (original magnification, $\times 100$). Data are from three independent experiments. C, control; BMS, BMS-777607. Columns, mean; bars, SD ($n = 6$). ***, $P < 0.001$.

submicromolar range (Fig. 3). For example, doses of 0.1 and 1 $\mu\text{mol/L}$ BMS-777607 resulted in a $60.0 \pm 24.0\%$ and $85.0 \pm 8.9\%$ inhibition of invasion of PC-3 cells, respectively (Fig. 3).

BMS-777607 reduces cell proliferation increased by HGF

In addition to enhancing motogenesis, HGF also has been reported to increase cell proliferation as a mitogen (29). To test whether BMS-777607 treatment could affect prostate cancer cell proliferation, PC-3 cells were incubated with BMS-777607 and HGF for a period of 4 days. The results (Fig. 4) showed that HGF moderately increased cell growth ($P < 0.01$) in this cell line. BMS-777607 treatment had no significant effect on cell growth at doses ≤ 1 $\mu\text{mol/L}$ ($P > 0.05$). Doses of 3 and 10 $\mu\text{mol/L}$ did result in significant ($P < 0.001$) reductions in the HGF-induced cell proliferation (Fig. 4) without apparent cytotoxicity (data not shown).

BMS-777607 blocks HGF-stimulated c-Met downstream signaling pathway

To show the effect of BMS-777607 on c-Met signaling pathways, cells were treated with serial dilutions of the compound followed by HGF stimulation. In prostate cancer cell lines, BMS-777607 inhibited HGF-triggered c-Met autophosphorylation (Y1234/1235) with an IC_{50} value of

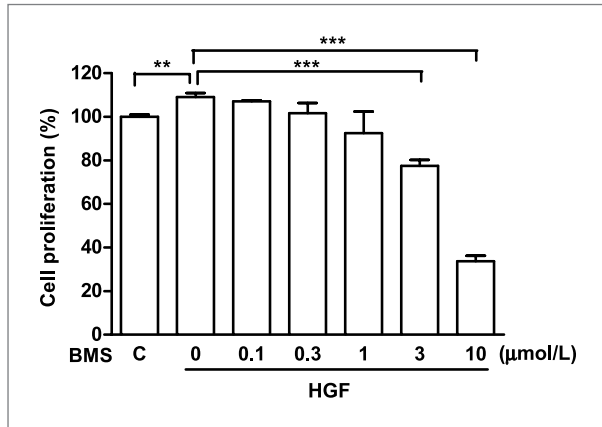


Figure 4. BMS-777607 reduces HGF-induced cell proliferation. PC-3 cells were seeded into a 96-well plate and treated with various doses of BMS-777607 before exposure to HGF (25 ng/mL). Proliferated cells were determined by MTT assay after 96 h of incubation. Data are from at least three independent experiments. C, control; BMS, BMS-777607. Columns, mean; bars, SD ($n = 9$). ***, $P < 0.001$.

<1 nmol/L (Fig. 5). At 0.1 $\mu\text{mol/L}$, BMS-777607 completely suppressed induced c-Met phosphorylation, but had only minimal effect on total c-Met. Phosphorylation of Akt (S473) and extracellular signal-regulated kinase (ERK; T202/Y204) also was blocked by BMS-777607, but autophosphorylation of c-Src (Y416) and FAK (Y397) remained unaffected in the presence of either HGF or BMS-777607. In PC-3 cells, a much higher dose of BMS-777607 was required to suppress induced Akt than ERK activity (IC_{50} , 0.1 – 1 $\mu\text{mol/L}$ and 0.01 – 0.1 $\mu\text{mol/L}$ for p-Akt and p-ERK, respectively). In contrast, the reverse effect was observed in DU145 cells (IC_{50} , <0.001 $\mu\text{mol/L}$ and 0.01 – 0.1 $\mu\text{mol/L}$ for p-Akt and p-ERK, respectively; Fig. 5).

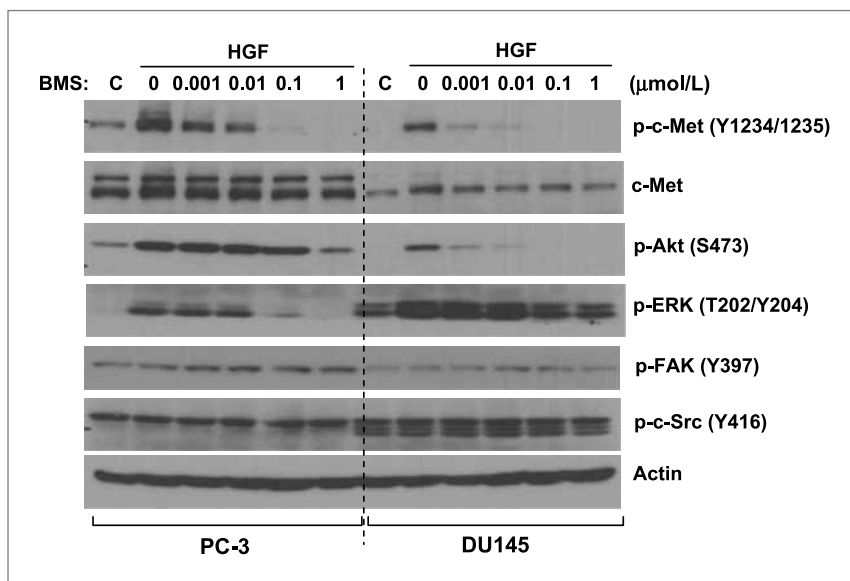


Figure 5. BMS-777607 blocks the HGF-stimulated c-Met signaling pathways. Cells were serum starved overnight, pretreated with BMS-777607 for 1 h, and stimulated with HGF (25 ng/mL) for 10 min. Whole-cell lysates were processed for Western blot analysis. Membranes were probed with respective primary antibodies. Actin is used as a loading control. C, control; BMS, BMS-777607.

Both PI3K and MAP/ERK kinase are required in the motogenic effect triggered by HGF/c-Met axis

For comparison with BMS-777607 and to further elucidate the role of PI3K (upstream of Akt) and MAP/ERK kinase (MEK; upstream of ERK) in c-Met-mediated cell motogenesis, cells were treated with wortmannin (PI3K inhibitor), U0126 (MEK inhibitor), or dasatinib (Src inhibitor) followed by HGF stimulation (Fig. 6). In DU145 cells, U0126 largely blocked HGF-induced cell scattering, whereas wortmannin showed some inhibition that was clearly less dramatic than that seen with U0126 (Fig. 6A). In contrast, dasatinib had no effect on the scattering behavior (Fig. 6A). Using doses that barely affected cell migration in the absence of exogenous HGF, wortmannin and U0126 significantly inhibited HGF-stimulated cell motility in both cell lines (Fig. 6B). In PC-3 cells, wortmannin ($37.4 \pm 7.8\%$, $P < 0.001$) showed a stronger inhibition than U0126 ($14.9 \pm 3.8\%$, $P < 0.05$), whereas U0126 ($40.0 \pm 9.9\%$, $P < 0.001$) prevented more DU145 cell motility than did wortmannin ($15.0 \pm 5.9\%$, $P < 0.01$). In contrast, BMS-777607 reduced the migration of PC-3 and DU145 by $61.2 \pm 6.6\%$ and $68.7 \pm 4.4\%$ ($P < 0.001$), respectively.

Discussion

Dysregulation of cellular c-Met activity is a frequent event in cancer that has been strongly implicated in metastasis. In prostate cancer, c-Met activation is HGF dependent, and increased *MET* expression due to transcriptional upregulation is most frequently associated with a metastatic phenotype and poor prognosis (30). Typically, c-Met receptors on prostate cancer epithelial cells are phosphorylated by exogenous HGF that is released from stromal cells (31). Such a paracrine mode highlights the critical role of the tumor

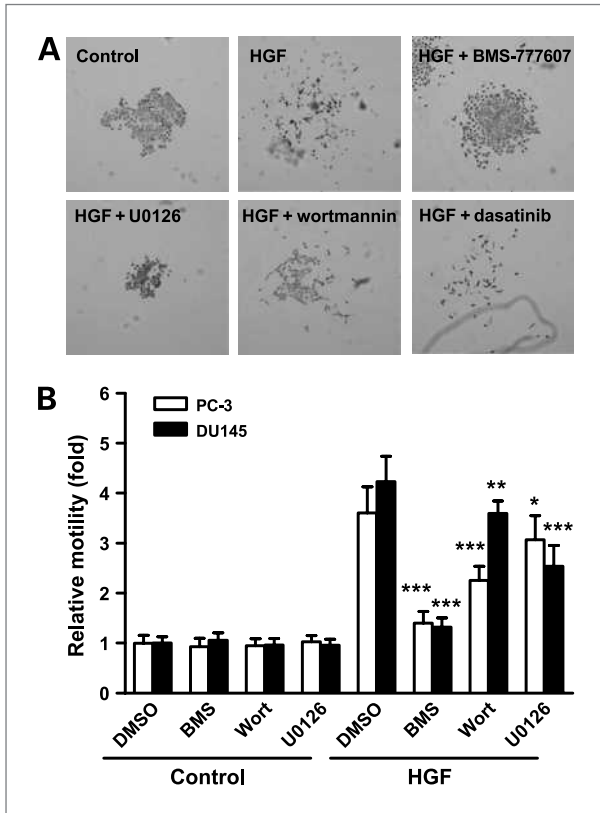


Figure 6. Both PI3K and MEK are required for the HGF-induced motogenic effect. **A**, DU145 cell colonies were serum starved overnight and pretreated with BMS-777607 (0.1 $\mu\text{mol/L}$), U0126 (1 $\mu\text{mol/L}$), wortmannin (0.1 $\mu\text{mol/L}$), or dasatinib (0.05 $\mu\text{mol/L}$) for 1 h before exposure to HGF (25 ng/mL) in the serum-free medium. Cell scattering was monitored 24 h after the addition of HGF. Typical pictures of scattered cell colony are shown (original magnification, $\times 50$). Data represent one of two independent experiments. **B**, confluent cells were serum-starved overnight and pretreated with BMS-777607 (0.1 $\mu\text{mol/L}$), U0126 (1 $\mu\text{mol/L}$), or wortmannin (0.05 $\mu\text{mol/L}$ for PC-3 and 0.1 $\mu\text{mol/L}$ for DU145, respectively) for 1 h before exposure to HGF (25 ng/mL) in the serum-free medium. Cell motility was monitored 24 h after the addition of HGF, and cells migrated into denuded area were scored. Data are from three independent experiments. BMS, BMS-777607; Wort, wortmannin. Columns, mean; bars, SD ($n = 9$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

microenvironment in mediating c-Met activation in prostate cancer (32).

The present data show that HGF significantly drives a variety of biological and biochemical functions in c-Met-expressing prostate cancer cells and that neutralizing HGF activity almost completely abolishes the cells' motogenic function (scattering and motility). Interestingly, a dose of HGF (25 ng/mL), which strongly promotes migration and invasion (approximately 3- to 5-fold) in PC-3 and DU145 cells (Fig. 2), results in only a modest (10%) or no increase in proliferation in PC-3 (Fig. 4) and DU145 (data not shown) cells, respectively. These results suggest that motogenesis is a predominant outcome rather than HGF-mediated mitogenesis in these cancer cell lines. The less dramatic effect of HGF on proliferation

of prostate cancer cells than what has been reported in other cancer models (33, 34) implies that the HGF/c-Met axis may be less critical in the growth of primary prostate tumors. However, its importance in motogenesis suggests that targeting the c-Met pathway may offer a possible therapeutic strategy for affecting metastatic prostate disease.

Consistent with previous reports of Met kinase inhibitors in other tumor models (35, 36), the present studies show that BMS-777607 treatment can impair key steps in the metastatic cascade including scattering, migration, and invasion in prostate cancer cells stimulated by HGF. Interestingly, doses of BMS-777607 that completely abrogated cell migration and invasion had little or no effect on cell proliferation. Only when micromolar doses were applied, i.e., doses ~ 100 -fold higher than those affecting motogenesis, did this agent begin to impair HGF-mediated PC-3 cell proliferation. The lack of effect on cell proliferation is somewhat inconsistent with prior reports that other Met inhibitors with c-Met Ki values similar to that of BMS-777607 could not only significantly reduce cell growth but also induce apoptosis (36, 37). Perhaps these differences could be explained by differences of cellular context rather than characteristics of the Met inhibitors. If for example, cells are dependent on c-Met to survive, then it might be anticipated that blocking this signaling pathway would have severe consequences that might be reflected in growth inhibition or even cell death. Conversely, the lack of an effect on these parameters as was observed in the present investigation might imply that in prostate cancer cells, c-Met signaling is not critical to cell proliferation.

Compared with other prostate cancer cells, PC-3 cells have been reported to overexpress c-Met due to transcriptional upregulation (16). The present data not only confirm that the total c-Met level of PC-3 cells is higher than that of DU145 cells but also indicate that the matured form of phosphorylated c-Met (p145) can be detected in the former under serum-deprived conditions but not in the latter. This finding suggests that PC-3 cells may possess a basal level of activated c-Met that maintains cellular function during tumor progression even in an environment that lacks HGF. How such constitutive c-Met activation is acquired remains elusive. In some studies, the existence of a HGF/c-Met autocrine loop has been suggested (36, 38), but others indicate that PC-3 cells do not express HGF (16, 39). Clearly, elucidating the mechanism of c-Met activation in the absence of exogenous ligand in the PC-3 model would provide important information about the potential therapeutic application of Met inhibitors in cancer patients whose tumors possess cells with constitutively activated c-Met.

In prostate cancer cells, PI3K and MAPK, two primary downstream molecules of c-Met (40, 41), are activated by HGF stimulation (Fig. 5; refs. 42, 43). However, unlike what has been observed in other cancer types (44–46), Src and FAK, two kinases that are widely documented to be involved in cell migration and invasion, remained

unaltered by HGF treatment (Fig. 5). This observation suggests that Src and integrin-FAK signaling functions independently of c-Met signaling in these prostate cancer models. Consistent with these molecular observations, at the cellular level, inhibitors of Met, PI3K, and MEK, but not Src, attenuated HGF-induced cell scattering and migration (Fig. 6). The results further show that the cell functions impaired by Met inhibition are dependent on PI3K and MAPK. Indeed, HGF-induced DU145 cell scattering is dependent on the activity of Rho family GTPases and actin cytoskeleton reorganization mediated by the PI3K pathway (47).

When comparing the two prostate cancer cell lines used in the present investigations, it is clear that both Akt and ERK are phosphorylated by HGF. However, PC-3 cells express high basal levels of phospho-Akt, whereas high constitutive phospho-ERK expression is noted in DU145 cells. The implication is that PC-3 cells may have a greater reliance on Akt (probably due to the documented loss of PTEN) than ERK signaling to maintain cellular functions, whereas the opposite seems to be the case for DU145 cells. This hypothesis is supported by evidence indicating that (a) at the highest dose that completely blocks both constitutive and induced c-Met phosphorylation, BMS-777607 only suppresses induced but not constitutive phospho-Akt and phospho-ERK in PC-3 and DU145 cells, respectively; (b) the IC₅₀ value for inhibition of induced phospho-Akt is much higher than that for phospho-ERK in PC-3, whereas the opposite is the case for DU145 cells; (c) blocking PI3K in PC-3 cells achieves better functional inhibition than blocking MEK, whereas in DU145 cells, inhibiting MEK is more effective. Yet despite these differences in downstream signaling, both cell lines exhibit similar c-Met kinase inhibition when treated with BMS-777607. Furthermore, more dramatic cell function suppression

results from inhibiting the c-Met receptor than its downstream pathways. Taken together, these findings suggest that even if the pathways mediated by the HGF/c-Met axis vary in their dominance depending on their cellular context, targeting c-Met per se may prove to be a promising approach to interfering with HGF-stimulated metastasis.

In summary, the small-molecule Met kinase inhibitor BMS-777607 was found to be capable of inhibiting c-Met signaling and suppressing HGF-stimulated prostate cancer cell behaviors that included scattering, migration, invasion, and proliferation in prostate cancer cells. Although these results await further testing in experimental metastatic models *in vivo*, the present findings do support the notion that Met kinase inhibition may offer a novel treatment strategy for advanced metastatic prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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