Reduced c-Met Expression by an Adenovirus Expressing a c-Met Ribozyme Inhibits Tumorigenic Growth and Lymph Node Metastases of PC3-LN4 Prostate Tumor Cells in an Orthotopic Nude Mouse Model

Sun Jin Kim, Marjorie Johnson, Kristen Koterba, Matthew H. Herynk, Hisanori Uehara, and Gary E. Gallick

Department of Cancer Biology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas [S. J. K., M. J., K. K., H. U.], and The Program in Cancer Biology, University of Texas Graduate School of Biomedical Sciences at Houston, Houston, Texas [M. H. H., G. E. G.]

Abstract

Purpose: The expression of c-Met, the receptor protein tyrosine kinase for hepatocyte growth factor/scatter factor, frequently increases during prostate tumor progression. However, whether reduced c-Met expression inhibits tumor growth and metastasis has not been ascertained.

Experimental Design: c-Met expression was reduced by infection of an adenovirus expressing a c-Met ribozyme into the highly metastatic human prostate cancer cell line PC3-LN4. In vitro, effects on c-Met, Akt, and extracellular signal-regulated kinase 1/2 expression and phosphorylation, Src expression and activity, and vascular endothelial growth factor expression were determined, as were effects on cell migration and invasion. Prostate tumor formation and metastasis to regional lymph nodes in nude mice were examined after both ex vivo and in vivo infection of cells.

Results: Infection of PC3-LN4 cells with the Ad-c-Met-expressing ribozyme decreased steady-state c-Met levels, decreased Src kinase activity, decreased vascular endothelial growth factor expression, and decreased migration and invasion versus the pU1 (control) virus. Significant inhibition of tumorigenicity (histologically confirmed tumors in only 1 of 10 mice) and consequent lymph node metastasis were observed upon ex vivo infection of Ad-c-Met. Similarly, gene therapy experiments led to complete inhibition of tumor growth in 7 of 8 mice.

Conclusions: Reduction in c-Met expression substantially inhibits both tumor growth and lymph node metastasis of PC3-LN4 cells in orthotopic nude mouse models. Therefore, targeting the c-Met signaling pathways may be important in controlling tumor growth and metastasis in human prostate cancers.

Introduction

Cancer of the prostate is the most common cancer in men in North America and ranks second in cancer-related deaths in this population (1, 2). Despite efforts to promote early detection, >50% of patients have metastatic disease at diagnosis and initiation of treatment (3). As a result, metastases of hormone-refractory cells develop and ultimately lead to mortality. Currently, conventional treatments such as taxane-based chemotherapy (4, 5) are ineffective in treating patients with advanced disease. Thus, the identification of novel targets and new treatment regimens are critical for future control of advanced prostate cancer. Inhibitors of RPTKs,2 often overexpressed in prostate cancer, may provide such targets for development of novel therapies.

One potential RPTK target in prostate cancer is c-Met. c-Met is expressed primarily in epithelial tissues (6). HGF, also known as SF, is the primary ligand of c-Met (6–8). Upon stimulation of HGF/SF, c-Met is tyrosine phosphorylated and initiates a cascade of signals that lead to activation of cellular behaviors such as mitogenesis, cell migration, matrix adhesion and invasion, angiogenesis, morphogenesis, survival, or combination of these events depending on the cell types stimulated. Mutations and aberrant overexpression of c-Met and HGF/SF have been correlated with disease progression and clinical outcome in a variety of cancers (9–14).

Aberrant signaling through c-Met has been associated with prostate tumorigenesis and progression in rodents and man. In the Nobel rat, overexpression of HGF and c-Met occurs during sex hormone-induced prostatic carcinogenesis (15). In the human disease, c-Met overexpression occurs as early as prostate intraepithelial neoplasia (16). Furthermore, strong correlative evidence also suggests a role for HGF/SF/c-Met signaling in the progression and metastasis of human prostate cancers. Analysis of prostate cancer surgical specimens demonstrates a direct

2 The abbreviations used are: RPTK, receptor protein kinase; HGF, hepatocyte growth factor; SF, scatter factor; VEGF, vascular endothelial growth factor; FBS, fetal bovine serum; MOI, multiplicity of infection; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Erk, extracellular signal-regulated kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
association between increased c-Met expression and the formation of bone and lymph node metastases (17–19). More than 80% of all men who develop prostate cancer will develop metastatic disease in the bone, especially in the pelvis, femur and vertebrae and human bone stroma, a rich source of HGF/SF (20, 21). The possible role of HGF/SF in the preferential metastasis of prostate cancer to the bone was implicated by demonstrating the importance of this growth factor on the formation of prostate epithelial cell colonies on bone marrow stroma with epithelial cells from benign and malignant prostate tissues (22). HGF/SF and c-Met are also biomarkers of invasion of prostate cancer cells and progression of disease in latent and clinical prostate cancer, including prostatic intraepithelial neoplasia (23–29). Parr et al. (30) demonstrated that NK4, an HGF/SF variant, suppressed the HGF/SF-induced phosphorylation of paxillin, matrix adhesion, and invasion of prostate cancer cells. However, although substantial evidence implicates c-Met overexpression with prostate tumor progression, no studies have assessed the effects of reducing c-Met expression on tumor growth and metastasis.

To examine the role of c-Met in other tumor types in which it is overexpressed, the strategy of ribozyme-mediated-c-Met down-regulation has been used (31–33). In the glioblastoma cell lines U-373 (expressing c-Met only) and U-87 (expressing c-Met and HGF), Abounder et al. (32) demonstrated that stable ribozyme-expressing clones were reduced in c-Met expression by 95%. c-Met down-regulation in U-373 did not affect tumor growth or incidence. Reduction in tumor incidence and growth in the brain resulted only when an autocrine HGF/c-Met loop was present (32). Therapeutic treatment of orthotopically implanted U-87 tumors with adenovirus and/or liposomes containing the c-Met or HGF specific ribozyme increased the median survival of treated animals compared with untreated animals (31). Thus, in this model, c-Met down-regulation reduces tumorigenicity only when c-Met is activated by autocrine production of HGF. In two human breast cancer cell lines, MDA-MB-231 and MCF-7, ribozyme-mediated c-Met down-regulation significantly reduced HGF-induced migration and invasion (33) relative to a control (PU1) construct. Thus, expression of this c-Met ribozyme has proven successful in determining the biological effects of reducing c-Met expression in a highly specific manner. In this study, we report that reduction of c-Met expression by either ex vivo or in vivo infection with a defective adenovirus expressing a c-Met ribozyme of PC3P-LN4 prostate cancer cells, a metastatic variant of PC3P, inhibits tumorigenic growth and lymph node metastasis in orthotopic models in male nude mice. Previous studies have demonstrated that silencing through c-Met induces angiogenesis (34, 35). In accord with these results, we demonstrate that down-regulation of c-Met strongly down-regulates VEGF expression and, furthermore, that c-Src tyrosine kinase activity is, in part, responsible for reduced VEGF expression.

Materials and Methods

Cell Lines and Cell Culture Conditions. The PC-3 human prostate cancer cell line was originally obtained from the American Type Culture Collection (Manassas, VA). The PC-3M cell line was derived from a liver metastasis produced by parental PC-3 cells growing in the spleen of a nude mouse. PC-3M cells were implanted orthotopically into the prostate of nude mice, and after several cycles of in vivo selection through prostate and regional lymph nodes, the highly metastatic cell line to lymph node and distant organ, PC-3P-LN4, was isolated (36). The PC-3P-LN4 cell line was maintained as monolayer cultures in DMEM/F12 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% FBS, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM l-glutamine, a 2-fold vitamin solution (Life Technologies, Inc.) in the absence of antibiotics. Cell cultures were maintained and incubated in 5% CO2/95% air at 37°C. Cultures were free of Mycoplasma and the following murine viruses: reovirus type 3; pneumonia virus; K virus; Theliers’ encephalitis virus; Sendai virus; minute virus; mouse adenovirus; mouse hepatitis virus; lymphocytic choriomeningitis virus; ectromelia virus; and lactate dehydrogenase virus (assayed by M. A. Bioproducts, Walkersville, MD).

Animals. Male athymic nude mice (NCI-nu) were purchased from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research Facility (Frederick, MD). The mice were housed and maintained in specific pathogen-free conditions. The facilities were approved by the American Association for Accreditation of Laboratory Animal Care and meet all current regulations and standards of the United States Department of Agriculture, United States Department of Health and Human Services, and the NIH. The mice were used in accordance with Institutional guidelines when they were 8–12 weeks old.

Adenovirus Ribozyme Construct Targeting Human c-Met. A plasmid expressing the c-Met ribozyme was constructed by Abounder et al. (32). Briefly, pZeoU1EcoSpE parent vector was derived from wild-type U1snRNA, which is an essential component of the spliceosome complex and is stable and abundant in the nucleus. The U1 promoter was cloned in a BamHI site of a modified pZeo-EcoSpE vector, which is zeocin resistant. A hammerhead ribozyme that recognizes and cleaves the GUC sequence at position 560 of human c-Met was synthesized, and resulting cellular lysates were subjected to double CsCl gradient centrifugation. Concentrated virus was dialyzed, aliquoted, and stored at −80°C. Adenovirus titer represented as optical particles units was determined by optical absorbance as described previously (28). Infectious viral titers were determined by quick cytopathic effect assays. Viral particles/ml were calculated by using the following formula: [(number of cells/well) × (dilution with cytopathic effect, 10^5) × (10 virus/cell)]/0.3 ml viral
sample/well. For infection of PC3-LN4 cells, cultures at 70% confluency were infected with at the indicated MOI. MOI was determined by plaque forming units in 293 s cells after serial dilution.

**Immunoblotting.** Cells (1 × 10⁶) were plated in monolayer on a 10-cm dish and infected with either Ad-PU1 or Ad-c-Met for 24 h. Cells were washed twice with Ca²⁺-, Mg²⁺-, free ice-cold PBS [135 mM NaCl, 1.5 mM KH₂PO₄, 8.0 mM Na₂HPO₄ (pH 7.4), and 2.5 mM KCl] and lysed on ice in lysis buffer [50 mM HEPES (pH 7.0), 150 mM NaCl, 1.5 mM MgCl₂, 1 mM EGTA, 10 mM Na PP₃, 10% glycerol, 1% Triton X-100, and 1 mM Na₂VO₄] plus 1 tablet/10 ml lysis buffer consisting of Complete Mini, EDTA-free protease inhibitor mixture tablets (Roche) for 10 min before clarification by centrifugation at 15,000 rpm at 4°C for 15 min. Protein concentrations were quantitated by using the Detergent-Compatible Protein Assay C for 15 min. Protein concentrations were determined by plaque forming units in 293 s cells after serial dilution.

**Immunoblotting.** Cells (1 × 10⁶) were plated in monolayer on a 10-cm dish and infected with either Ad-PU1 or Ad-c-Met at MOIs of 25 and 50. Cells were trypsinized, and viable cells were counted using a hemacytometer after trypan blue staining. One thousand cells for each line were plated in triplicate each on a 96-well plate. Every 24 h, 50 μl of MTT (Sigma-Aldrich) were added to each well. After 2 h of incubation, all media were removed, and the cells were solubilized with the addition of 200 μl of DMSO (Fisher Scientific). The absorbance readings were performed on a spectrophotometer at 750 nm. The optical densities readings were divided by the reading at time 0 to obtain the percent increase in growth and plotted on a log scale versus time.

**Migration and Invasion Assays.** Migration assays were performed using a modified Boyden chamber system with a filter containing 8-μm pores (Control Inserts; Fisher Scientific). Invasion chambers (Biocoat Matrigel Invasion Chambers; Fisher Scientific) are identical to the migration chambers, with the addition of Matrigel layered on the top of the insert. For both assays, the bottom chamber was filled with 500 μl of conditioned media and then the insert was placed in the conditioned media. Conditioned media were obtained by growing NIH 3T3 fibroblasts to 80% confluency at 72 h in DMEM/Ham’s F-12 + 10% FBS. After 72 h, the media were removed from the cells and filter sterilized. For the migration and invasion assays, equal numbers of cells in a volume of 500 μl for each cell line were suspended in their growing media and placed in the top chamber. The chamber was placed at 37°C for the length of the assay. To assess the cells that had migrated, the cells on the top of the filter were removed with a cotton swab. The cells that migrated through and adhered to the bottom of the filter were fixed and stained with the Hema 3 Stain Set (Fisher Scientific) according to the manufacturer’s instructions. The stained cells on each insert were counted in five ×200 fields and averaged to give n = 1/insert. The sample size was therefore the number of inserts.

**VEGF Expression.** To determine expression of VEGF protein, conditioned medium from PC3-LN4 control and infected cells were harvested after 48 h from cells growing in 1% FBS-containing medium. The VEGF concentration was determined using an ELISA kit for human VEGF (Biosource) as we have previously reported (38, 39) [the ELISA kit recognizes the VEGF121 and VEGF165 isoforms of the protein (40)].

**Ex Vivo Infection and Orthotopic Implantation of PC3 LN-4 Cells.** Tumor cells (1 × 10⁶) were infected for 24 h with either Ad-PU1 or Ad-c-Met at an MOI of 25. Cells were harvested after a brief exposure to 0.25% trypsin:0.1% EDTA

```
Clinical Cancer Research 5163
```

Downloaded from clincancerres.aacrjournals.org on July 14, 2017. © 2003 American Association for Cancer Research.
Nembutal (Abbott Laboratories, North Chicago, IL) and placed previously (36). In brief, nude mice were anesthetized and resuspended in Ca\(^{2+}\)/Mg\(^{2+}\)-free HBSS at 5 \(\times\) 10\(^5\) cells/50 \(\mu\)l. Orthotopic injection was done as described previously (36). In brief, nude mice were anesthetized with Nembutal (Abbott Laboratories, North Chicago, IL) and placed in a supine position. A low midline incision was made, and the prostate was exposed. Fifty \(\mu\)l of HBSS containing 5 \(\times\) 10\(^5\) cells were injected into a lateral lobe of this prostate. The wound was closed with surgical metal clips. A total of 10 mice/group was injected.

**Gene Therapy Studies.** Subconfluent cultures of PC3-LN4 cells were harvested by trypsinization. Cells were counted in sterile Ca\(^{2+}\)/Mg\(^{2+}\)-free HBSS at 5 \(\times\) 10\(^5\) cells/50 \(\mu\)l. Cells were then implanted in the prostate of nude mice as described above, and the wound was closed with surgical metal clips. Tumors were treated at 7 and 14 days after implantation. Tumors were exposed by the same procedure by low midline incision and exposure of the prostate. Tumors were injected with PBS or 1.5 \(\times\) 10\(^5\) cells migrating upon infection with an MOI of 50 (a 97% decrease in migration rate).

**Mutation Analysis.** Mutations in the c-Met gene were analyzed in the PC3-LN4 cell line. The method used was PCR followed by direct sequencing. The results showed that mutations in this region were observed in c-Met in PC3-LN4 cells. To examine the effects of c-Met down-regulation, PC3-LN4 cells were mock infected or infected with PU1 and Ad c-Met adenoviruses. As shown in Fig. 1, infection with pU1 (control) virus did not change the expression of c-Met at MOIs as high as 50. In contrast, infection with MOIs of 25 and 50 decreased c-Met expression by 95% relative to PU1 infection at similar MOIs and corrected for loading by GAPDH expression.

**Inhibition of Cellular Proliferation of PC3-LN4 Cells by Ad-c-Met Infection.** To determine the in vitro growth rates of c-Met down-regulated cells, MITT analysis was performed at 24, 48, 96, and 120 h after infection. The results are shown in Fig. 2. Infection with pU1/control adenovirus did not affect cellular proliferation of PC3-LN4 cells, but c-Met ribozyme adenovirus significantly inhibited the cellular proliferation of PC3-LN4 cells. Growth inhibition rates (percentage) at 24, 48, 72, 96, and 120 h were 33.0 \(\pm\) 7.3, 52.5 \(\pm\) 3.0, 64.5 \(\pm\) 5.2, 82.8 \(\pm\) 0.9, and 87.2 \(\pm\) 0.8 with 25 MOI and 44.6 \(\pm\) 2.7, 62.1 \(\pm\) 4.4, 73.1 \(\pm\) 1.0, 82.3 \(\pm\) 1.4, and 92.4 \(\pm\) 0.8 with 50 MOI, respectively (P \(<\) 0.01, paired Student’s t test). There was no significant difference in inhibitory effect of cellular proliferation between the two different MOIs. These results suggest that c-Met expression is important to proliferation and/or survival of PC3-LN4 cells.

**Suppression of Migration and Invasion by Ad-c-Met Infection.** One of the principal functions of c-Met is promotion of migration, a process also important in metastasis. To examine the effects on migration of reduced c-Met expression in PC3-LN4 cells, single cells were seeded in the upper chamber of a standard two-chamber migration assay (Boyden chamber), and cells migrating to the bottom of the insert were counted as described in “Materials and Methods.” As shown in Fig. 3A, PC3-LN4 cells were substantially reduced in migratory capacity when infected with Ad c-Met but not Ad-Pu1, with almost no cells migrating upon infection with an MOI of 50 (a 97% decrease in migration rate).
Tumorigenicity and lymph node metastases induced by PC3-LN4 cells after ex vivo infection of adenovirus or mock infection

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor incidence</th>
<th>Tumor size (g)</th>
<th>Lymph node involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock</td>
<td>8/8</td>
<td>0.58 (0.31–1.01)</td>
<td>8/8</td>
</tr>
<tr>
<td>Ad-Pu-1</td>
<td>9/9</td>
<td>0.47 (0.01–2.45)</td>
<td>9/9</td>
</tr>
<tr>
<td>Ad-c-Met</td>
<td>1/10</td>
<td>0.07</td>
<td>0/1</td>
</tr>
</tbody>
</table>

"PC3-LN4 cells were mock infected, or infected with the indicated adenovirus at an MOI of 50, and 5 × 10^5 cells were injected into the prostates of nude mice, as described in “Materials and Methods.”

Number of mice with tumors/number of mice surviving surgery for prostate tumor cell implantation.

Number of mice with lymph node metastases/number of mice with tumors.

Effects of Intraprostatic Injection of Adenoviruses into Preestablished PC3-LN4 Tumors. To determine whether reduced c-Met expression could affect the growth of PC3-LN4 cells growing in the prostate, gene therapy experiments were performed by allowing the tumor cells to grow in the prostate for 7 days, then injecting Ad-c-Met, Ad-Pu-1, or sham-injecting mice in the prostate 7 and 14 days later as described in “Materials and Methods.” The results of these experiments are shown in Table 2. In 7 of 8 mice infected with Ad-Pu-1 and 8 of 8 sham-injected mice, tumors developed in the prostate. In contrast, only 1 of 8 mice injected with Ad-c-Met developed a tumor and that tumor was substantially smaller than tumors forming in control mice (Table 2). These results suggest that c-Met is performing an important survival function in PC3-LN4-induced tumors, and c-Met reduction is incompatible with development of macroscopic tumors.

Decreased c-Met Decreases VEGF Expression and Src Kinase Activity. Ample evidence has implicated c-Met signaling in angiogenesis and especially expression of VEGF (34, 35, 41, 42). To determine whether decreased VEGF expression along with decreased proliferation rates might explain, in part, the growth inhibition of tumors after decreased c-Met expres-

...
sion, we examined VEGF expression in the media of PC3-LN4 cells 48 h after infection with ribozyme-expressing (Ad-c-Met) or control (PU-1) virus. The results are shown in Fig. 6. Significant reduction in VEGF expression (per 10^6 cells as measured by ELISA) was observed in PC3-LN4 cells in Ad-c-Met-infected cells relative to Ad-PU1 (control)-infected cells.

To begin to examine signaling pathways that might be affected by c-Met expression and are important to VEGF expression, we determined the status of Erk1/2, Akt, and Src after down-regulation of c-Met by Ad-c-Met. The activity of Src (Src autophosphorylation and phosphorylation of the exogenous substrate enolase) in the immune complex kinase assay is reduced in PC3-LN4 cells infected with Ad-c-Met, with >50% reduction observed in autophosphorylation at an MOI of 25 and >80% reduction of autophosphorylation observed at an MOI of 50, relative to uninfected controls (Fig. 7). Phosphorylation of the exogenous substrate enolase was also reduced relative to uninfected controls in a dose-dependent manner, although to a lesser extent than autophosphorylation. Only a small reduction in Src activity was observed upon infection with high MOIs of the

Fig. 4 Tumor formation in nude mice following: mock infection (A); ex vivo infection with Ad-PU-1 control adenovirus (B); or ex vivo infection with Ad-c-Met (C). PC3 LN-4 cells were mock infected or infected with given adenoviruses at an MOI of 50, after which cells were implanted into the prostate of nude mice as described in “Materials and Methods.” Mice were killed 4 weeks later and examined for presence of tumors. C displays the only mouse implanted with PC3 LN-4 Ad c-Met-infected cells with macroscopic evidence of tumor.

Fig. 5 H&E staining of nude mouse prostate tumors after orthotopic injection of mock, Ad-PU 1 control virus, or Ad-c-Met-infected PC3-LN4 cells. Primary tumor (A) and lymph node metastasis (B) from mice with mock-infected cells; primary tumor (C) and lymph node metastasis (D) from PU-1-infected cell; H&E of possible primary tumor from Ad-c-Met-infected cells (D); normal prostate (E).
control virus, and no obvious changes in Src expression were observed at the MOIs used for control or ribozyme-expressing virus (Fig. 7). These results demonstrate that down-regulation of c-Met down-regulates Src activity. In contrast, adenovirus infection (i.e., Ad-c-Met or Ad-PU1) appeared to increase expression of Akt, but no differences in phosphorylation were observed between control (Ad-PU1) and ribozyme-expressing (Ad-c-Met) viruses (Fig. 8). Erk1/2 expression appeared somewhat decreased by infection with Ad-c-Met, but this decrease was <2-fold and likely reflects the slower proliferation rate of these cells. Thus, decreased VEGF expression observed after c-Met down-regulation correlates with decreased c-Src activity.

**Discussion**

Despite surgical removal of organ-confined lesions, the biological unpredictability of prostate cancer does not guarantee patient survival. As with most solid tumors, patients that succumb to prostate cancer almost invariably do so because of metastatic spread of the disease. Metastasis of androgen-independent prostate cancer to the bone causes devastating symptoms such as intractable bone pain, nerve compression and pathological fractures, and at this stage, tumors rarely respond to conventional chemotherapies (4, 5). Therefore, development of new treatment modalities inhibiting progression and metastasis of the disease are clearly needed. Inhibition of receptor protein tyrosine kinases has shown considerable promise in the treatment of some tumors. In prostate tumors, aberrant expression of RPTKs is relatively common. For example, members of the ErbB family, specifically HER1, HER2, HER3 (43, 44) but not HER4 (45), are frequently overexpressed. This overexpression is associated with androgen-independent growth of prostate tumor cells, suggesting that overexpression of this gene family promotes tumor progression. One of the most consistently overexpressed RPTKs in prostate cancer is c-Met. Analysis of clinical specimens has linked c-Met overexpression to prostate tumor progression (16–18, 23, 24, 26); however, no studies have examined the cause/effect relationship between c-Met tumor growth and metastasis in an orthotopic model.

In this study, we used PC3-LN4 cells, a variant of PC3-P
cells that by passage through nude mice is increased in metastatic potential to the lymph nodes. Reduction of c-Met expression was achieved by an adenovirus expressing a highly specific c-Met ribozyme as described by Abounader et al. (32). The approach of using adenoviruses expressing ribozymes has been highly successful in specifically reducing the expression of diverse gene products such as HER2/neu (46) and Bcl-2 (47). The selectivity of this approach ensures that c-Met alone is targeted, thus allowing specific conclusions to be derived with respect to c-Met function. In our studies, >90% reduction of c-Met expression resulted from adenovirus infection at an MOI of 50, and inhibition of c-Met alone was sufficient to affect biological functions of prostate tumor cells. In colon tumor cells, we have demonstrated that reductions of only ~30% in c-Met expression are sufficient to decrease the ability to form tumors in the livers of nude mice (48). In contrast, in glioblastoma cells, Abounader et al. (32) required inhibition of both c-Met and its ligand, HGF, to affect tumor growth. Thus, overexpression alone in PC3P-LN4 cells appears to be sufficient to activate signaling pathways important to the biological properties of these cells. Furthermore, similar in vitro effects were observed with PC3P cells (data not shown), suggesting that c-Met overexpression is not important only to selected variants of high metastatic potential.

The main focus of this study was to examine the effect of c-Met down-regulation in tumor cells on in vivo growth and metastasis of tumor cells. Therefore, we compared the tumor growth in orthotopic site and development of metastases to the regional lymph nodes. We used two approaches for these studies: ex vivo infection of adenoviruses into PC3-LN4P cells followed by orthotopic implantation and orthotopic implantation of tumor cells followed by in vivo injection of adenoviruses into the prostate. Down-regulation of c-Met expression after ex vivo infection significantly reduced tumor formation or delayed growth in the prostate. Strikingly, in vivo delivery of the ribozyme by intraprostatic injection of Ad-c-Met led to highly similar results. Thus, overexpression of c-Met in prostate tumor cells alone appears to play an important role in the ability of these cells to grow. As c-Met can be activated by HGF/SP in the stromal cells of the prostate (49), these results suggest that inhibition of c-Met in the tumor cells may also inhibit receptor activation induced by HGF in the tumor microenvironment. In vitro studies also demonstrated that down-regulation of c-Met reduced not only proliferation rates but also cellular migration and cellular invasion and VEGF expression, suggesting that c-Met may play multiple roles in prostate tumor progression.

Although a large number of signaling cascades are induced upon c-Met activation [reviewed by Furge et al. (50) and Maulik et al. (51)], we demonstrated that decreased c-Src activity, but not Akt or Erk1/2 phosphorylation, occurred upon c-Met down-regulation. This result is consistent with Src constitutively dock- ing with c-Met when c-Met is overexpressed and therefore activated. Strong biochemical (52) and genetic evidence (53) support a role for Src in several c-Met-mediated functions, including migration and proliferation, and activated c-Met requires functional Src for tumorigenic growth of NIH 3T3 cells in nude mice (54). As Src is involved in a number of survival pathways [reviewed by Schlessinger (55)], it is likely that Src activation also contributes to critical survival pathways in prostate tumor growth and/or progression. However, the multifunctional roles of c-Met in activating signal transduction pathways suggest that additional pathways important to tumor progression may be down-regulated as well and that decreased Src activity may be only one of the factors contributing to decreased VEGF expression. Nevertheless, we have demonstrated previously with this model system that ectopic expression of PTEN inhibited the development of lymph node metastases but not that of primary tumors (56). Furthermore, vascularization of tumors was unchanged (56). Ectopic expression of PTEN does not affect c-Src activity (G. E. Gallick, unpublished observations), suggesting that promotion of angiogenesis by c-Met through Src activation may be early event in prostate tumor progression. Although it is difficult to determine whether the down-regulation of c-Met significantly reduced metastatic potential of the PC3-LN4 cells because of low tumorigenicity and low tumor burden (delayed growth), the in vitro data showing reduced migration and invasion activity suggests that suppression of metastatic activity of the cells by down-regulation of c-Met is highly probable. Thus, altering gene expression in prostate tumor cell lines and examining the effects in orthotopic models is providing insights as to not only what roles specific gene products play in tumor growth and progression but also where in progression of the disease these alterations are important. These results have potential important therapeutic implications as protein tyrosine kinase inhibitors continue to reach the clinic. Understanding which pathways are required for various stage of prostate tumor progression should lead ultimately to understanding which inhibitors and combinations thereof may be of effic- acy in different state tumors.

Acknowledgments

We thank Drs. Roger Abounader and John Laterra (Kennedy Krieger Research Institute) for their generous gift of the control and c-Met ribozyme-expressing adenoviruses, Dr. Isaiah Fidler for his helpful advice in the design of the experiments, and Nila U. Parikh for her excellent technical help.

References


7. Niji, S., Tashiro, K., Koyama, E., Nobno, T., Ohyama, K., Tanugu- chi, S., and Nakamura, T. Expression of hepatocyte growth factor gene in endothelial and Kupffer cells of damaged rat livers, as revealed by in


Reduced c-Met Expression by an Adenovirus Expressing a c-Met Ribozyme Inhibits Tumorigenic Growth and Lymph Node Metastases of PC3-LN4 Prostate Tumor Cells in an Orthotopic Nude Mouse Model

Sun Jin Kim, Marjorie Johnson, Kristen Koterba, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/9/14/5161

Cited articles
This article cites 52 articles, 20 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/9/14/5161.full#ref-list-1

Citing articles
This article has been cited by 15 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/9/14/5161.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.