MetMAb, the One-Armed 5D5 Anti-c-Met Antibody, Inhibits Orthotopic Pancreatic Tumor Growth and Improves Survival

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Abstract

The hepatocyte growth factor (HGF) and its receptor, c-Met, have been implicated in driving proliferation, invasion, and poor prognosis in pancreatic cancer. Here, we investigated the expression of HGF and c-Met in primary pancreatic cancers and described in vitro and in vivo models in which MetMAb, a monovalent antibody against c-Met, was evaluated. First, expression of HGF and MET mRNA was analyzed in 59 primary pancreatic cancers and 51 normal samples, showing that both factors are highly expressed in pancreatic cancer. We next examined HGF responsiveness in pancreatic cancer lines to select lines that proliferate in response to HGF. Based on these studies, two lines were selected for further in vivo model development: BxPC-3 (c-Met+, HGF+) and KP4 (c-Met+, HGF−) cells. As BxPC-3 cells are responsive to exogenous HGF, s.c. tumor xenografts were grown in a paracrine manner with purified human HGF provided by osmotic pumps, wherein MetMAb treatment significantly inhibited tumor growth. KP4 cells are autocrine for HGF and c-Met, and MetMAb strongly inhibited s.c. tumor growth. To better model pancreatic cancer and to enable long-term survival studies, an orthotopic model of KP4 was established. MetMAb significantly inhibited orthotopic KP4 tumor growth in 4-week studies monitored by ultrasound and also improved survival in 90-day studies. MetMAb significantly reduced c-Met phosphorylation in orthotopic KP4 tumors with a concomitant decrease in Ki-67 staining. These data suggest that the HGF/c-Met axis plays an important role in the progression of pancreatic cancer and that targeting c-Met therein may have therapeutic value.

Introduction

Pancreatic cancer is a highly lethal disease with a rising incidence (1). Worldwide, over 200,000 people die annually of pancreatic cancer, making it the fourth leading cause of cancer death in the United States (2, 3). Prognosis has not been improved substantially over the past few decades and 5-year survival rates remain <5% (4–6). Therefore, novel treatment strategies for pancreatic cancer are urgently needed.

The hepatocyte growth factor (HGF)/c-Met pathway has been linked to the cancer progression by driving proliferation, motility, invasion, and angiogenesis (7–10) and further has been linked to malignancy in pancreatic cancer (11–20). The c-Met receptor is overexpressed in pancreatic cancer cells, whereas tumor-associated fibroblasts produce HGF in a paracrine manner (11, 13–20). HGF/c-Met signaling not only stimulates growth, locomotion, and invasion of pancreatic cancer cells in vitro but also promotes pancreatic tumor growth and invasion and metastasis in vivo (16–26).

We have developed MetMAb, a monovalent monoclonal antibody (mAb) against c-Met, which blocks HGF binding to c-Met and subsequent pathway activation. MetMAb and other anti-HGF antibodies have shown potent activity in the U-87 MG autocrine glioblastoma tumor model (27–29); however, it had previously not been evaluated in pancreatic cancer models. As prognosis in pancreatic cancer has been linked to ligand-driven c-Met activity, modeling ligand-driven activation in animals would be ideal. Unfortunately, mouse HGF does not activate human c-Met (30, 31), making it essential to either use autocrine models or generate engineered human HGF paracrine models to evaluate the role of ligand in driving tumor progression.

In the present study, we investigated expression of HGF and c-Met by mRNA in primary pancreatic samples and explore functionality of HGF/c-Met signaling in cell-based and animal models to validate this pathway as a therapeutic target in pancreatic cancer. We describe the development of two novel HGF-driven s.c. pancreatic xenograft models: a BxPC-3 paracrine-driven model using osmotic pumps to provide exogenous human HGF and a KP4 autocrine-driven model, both of which showed responses to MetMAb. We further developed KP4 in an orthotopic setting, allowing us to explore long-term survival studies and the efficacy of MetMAb in a HGF-driven model. Together, these studies help further our understanding of the role of HGF/c-Met signaling in pancreatic cancer and help to validate MetMAb as a promising therapeutic against HGF/c-Met–driven cancers.

Materials and Methods

Cell lines. The KP4 pancreatic ductal carcinoma cell line was obtained from the Riken BioResource Center Cell Bank (cell line RCB1005). The Capan-1 cell line was obtained from the German Collection of Microorganisms and Cell Cultures. The BxPC-3, AsPC-1, HPAC, CFPAC-1, and Su86.86 pancreatic adenocarcinoma cell lines and the AS49 non–small cell lung cancer cell line were obtained from the American Type Culture Collection.

Immunoprecipitation and immunoblotting. Cells were cultured overnight in serum-free medium and then pretreated with different concentrations of MetMAb at 37°C for 1 h and then with or without 1 nmol/L HGF (~100 ng/mL) for 15 min. Cells were lysed with lysis buffer (Cell Signaling Technology, Inc.), supplemented with 1 nmol/L phenylmethylsulfonyl fluoride, additional protease inhibitor cocktail, and...
phosphatase inhibitor cocktail 1 and II (Sigma, Inc.). Cell lysates were incubated on ice for 1 h and then centrifuged at 14,000 × g for 5 min and supernatants were collected. Analysis of tumor proteins was done in a similar fashion; however, tumors first were homogenized using a glass Dounce homogenizer. Protein concentrations were determined using the BCA Protein Assay kit (Pierce, Inc.) and samples were immunoblotted. For immunoprecipitations, 6 mg of tumor lysates were incubated with C-28 anti-human c-Met polyclonal antibody (Santa Cruz Biotechnology, Inc.) conjugated to agaroose beads at 4°C overnight with rotation. The beads were washed thrice with lysis buffer at 4°C followed by resuspension in 1× Novex Tris-Glycine SDS Running Buffer (Invitrogen, Inc.) containing 2.5% (w/v) β-mercaptoethanol. Samples were then analyzed by SDS-PAGE and immunoblotting. Antibodies used include the mouse anti-human c-Met DL-21 and mouse anti-phosphotyrosine mAb 4G10 (Upstate Biotechnology/ Millipore, Inc.); rabbit anti-human c-Met C-12 polyclonal antibody (Santa Cruz Biotechnology); and anti-Akt, anti-p44/42 mitogen-activated protein kinase (MAPK), anti-p70 S6 kinase (p70S6K), anti-growth factor receptor binding protein 2-associated binder-1 (Gab-1), rabbit anti-phospho-c-Met (Tyr1234/1235), anti-phospho-Akt (Ser 473), anti-phospho-p44/42 MAPK binding protein 2-associated binder-1 (Gab-1), rabbit anti-phospho-c-Met kinase (MAPK), anti-p70 S6 kinase (p70S6K), anti-growth factor receptor binding protein 2-associated binder-1 (Gab-1), rabbit anti-phospho-p44/42 MAPK (Thr202/Tyr204), anti-phospho-p70S6K (Thr389, 1A5), and anti-phospho-Gab-1 (Tyr307; Cell Signaling Technology), all used according to the manufacturer’s recommendations. Goat anti-mouse IRDye800 (Rockland Immunocincheicals, Inc.) and goat anti-rabbit Alexa Fluor 680 (Molecular Probes, Inc.) were used as secondary antibodies. Immunoblots were imaged and protein levels were quantified and normalized to β-actin levels using an Odyssey imager (LI-COR Biosciences).

**Cell viability assays.** Cancer cells were washed once with PBS, resuspended in serum-free medium, counted, and then added to 96-well plates (2,500 per well). Cell plates were with MetMAb ± human HGF (1 nmol/L). Three-day Alamar Blue assays were performed according to the manufacturer’s recommendations (BioSource International). IC50 values were determined by nonlinear regression analysis with a four-variable model (KaleidaGraph, Synergy Software).

**Antimet Efficacy of MetMAb on Pancreatic Cancer**  
Results are expressed as mean ± SE. To assess differences in tumor size and vascular volume between two groups, Student’s t test was performed. Survival was compared by log-rank (Mantel-Cox) test.

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Results

**HGF and MET expression in pancreatic cancer.** To explore expression levels of HGF and c-Met in pancreatic cancer, we obtained Affymetrix HG-U133 chip-based microarray data for 59 human pancreatic adenocarcinomas and 51 normal pancreatic tissue samples from Gene Logic. HGF showed higher expression in pancreatic adenocarcinomas than normal controls ($P = 1.44 \times 10^{-8}$) with a higher degree of variation of expression (Fig. 1A). Twenty-seven of 59 (45.8%) tumors had HGF levels greater than twice the mean of normal, suggesting that in nearly half of pancreatic cancers HGF is expressed to a greater extent. Pancreatic adenocarcinomas also showed much higher expression of MET than normal ($P = 1.84 \times 10^{-12}$; Fig. 1B). Forty-five of 59 (76.3%) tumors had MET levels greater than twice the mean of normal. Because primary pancreatic biopsies can vary widely in tumor content, and relative expression levels may vary with respect to tumor content, we further binned samples by tumor content (<10%, 10–25%, 25–50%, and 50–90%; Fig. 1). Again, there was a clear increase in the mean HGF or MET expression levels in all groups versus normal tissues. Although there seemed to be a trend of lower HGF levels in samples with high tumor content, this expression relationship was not statistically significant.

**MetMAb inhibits HGF-dependent signal transduction and proliferation.** To identify potential models of pancreatic cancer, seven pancreatic cancer cell lines were selected by relative MET and HGF expression and tested for responsiveness to HGF in proliferation assays (Supplementary Table S1). All lines responded to HGF with 1.2- to 3.2-fold increase in proliferation; two of which were selected for further analysis (Fig. 2A): BxPC-3 cells, expressing moderate levels of MET and responding to exogenous HGF with a 2.9-fold induction in proliferation, and KP4 cells, which express high levels of both MET and HGF in an autocrine manner. HGF-induced proliferation of BxPC-3 and KP4 was blocked with MetMAb at IC$_{50}$ of 8.73 and 5.0 nmol/L, respectively; however, KP4 cells were also sensitive to MetMAb in the absence of HGF (IC$_{50}$ 2.64 nmol/L), indicating that KP4 cells rely on active HGF/c-Met signaling for growth and survival (Fig. 2A).

Inhibition of KP4 cells by MetMAb was also associated with inhibition of c-Met phosphorylation and activation of several downstream pathway components. In KP4, c-Met was constitutively phosphorylated and was further elevated by adding exogenous HGF (Fig. 2B). MetMAb strongly inhibited c-Met and Gab-1 phosphorylation in a dose-dependent manner, resulting in 93% and 67% inhibition (1 µmol/L MetMAb, normalized to total β-actin), respectively (Fig. 2B). Likewise, MetMAb inhibited the phosphorylation of Akt, extracellular signal-regulated kinase (ERK) 1/2, and p70S6K (at 1 µmol/L) by 48%, 70%, and 32%, respectively (Fig. 2B). MetMAb also inhibited c-Met phosphorylation and downstream pathway activity in unstimulated KP4 cells to a similar extent as HGF-activated KP4 cells (Supplementary Fig. S1). These results show that MetMAb inhibits HGF-induced c-Met activation in paracrine- and autocrine-driven cell lines.

**MetMAb inhibits BxPC-3 tumor xenograft growth in the human HGF osmotic pump model.** Because mouse HGF does not activate human c-Met (30, 31), modeling ligand-driven activation of c-Met in animal tumor models requires either autocrine lines, such as KP4, or engineered paracrine models. We therefore sought to develop a novel *in vivo* model to grow BxPC-3 xenograft tumors in the presence of human HGF provided by implanted osmotic pumps. Human HGF pumps placed in the i.p. space led to high human HGF serum levels (in the 1–10 ng/mL range) with equivalent levels in each group (vehicle group mean = 3.8 ± 1.3 ng/mL; MetMAb group mean = 4.2 ± 1.3 ng/mL; Supplementary Fig. S2). Mice receiving human HGF pumps showed enhanced tumor growth compared with dextran sulfate pump and no pump control arms (Fig. 2C). Treatment of 140 mm$^3$ established BxPC-3 tumors in human HGF pump mice with MetMAb (30 mg/kg in 100 µL, i.p., twice weekly) resulted in significant inhibition of tumor growth (Fig. 2D). MetMAb inhibited BxPC-3 tumor xenograft growth in the human HGF osmotic pump model. Because mouse HGF does not activate human c-Met (30, 31), modeling ligand-driven activation of c-Met in animal tumor models requires either autocrine lines, such as KP4, or engineered paracrine models. We therefore sought to develop a novel *in vivo* model to grow BxPC-3 xenograft tumors in the presence of human HGF provided by implanted osmotic pumps. Human HGF pumps placed in the i.p. space led to high human HGF serum levels (in the 1–10 ng/mL range) with equivalent levels in each group (vehicle group mean = 3.8 ± 1.3 ng/mL; MetMAb group mean = 4.2 ± 1.3 ng/mL; Supplementary Fig. S2). Mice receiving human HGF pumps showed enhanced tumor growth compared with dextran sulfate pump and no pump control arms (Fig. 2C). Treatment of 140 mm$^3$ established BxPC-3 tumors in human HGF pump mice with MetMAb (30 mg/kg in 100 µL, i.p., twice weekly) resulted in significant inhibition of tumor growth.

![Figure 1](cancerres.aacrjournals.org) Analysis of HGF (A) and MET (B) gene expression in pancreatic tissues using microarray data. Multiple probe sets were found to match to each gene, and we chose 209960_at to represent HGF expression and 203510_at for MET because they gave relatively high signal intensities. Y axes, Affymetrix MAS5.0 signal intensity. Open circle, individual normal or adenocarcinoma sample. The box plot denotes 25th percentile, mean, and 75th percentile of each set of expression distribution. Pancreatic adenocarcinomas were binned by their percent tumor content with the number of tumors indicated/bin.
growth ($P = 0.02$ at day 20; Fig. 2C). These data show that MetMAb has antitumor activity in a pancreatic xenograft model driven by exogenous HGF.

**MetMAb inhibits KP4 s.c. tumor xenograft growth.** We next assessed the efficacy of MetMAb in the s.c. KP4 xenograft model. Tumors in vehicle-treated animals grew rapidly, whereas treatment with MetMAb (30 mg/kg in 100 μL, i.p., twice weekly) dramatically reduced tumor growth (Fig. 2D). These results show that KP4 is a HGF-dependent model and that MetMAb treatment can lead to tumor regressions used as a single agent when this tumor is grown s.c.

**Effect of MetMAb on growth of orthotopic tumor xenografts.** To generate models that better reflect pancreatic cancer, we developed an orthotopic model of KP4. The autocrine HGF/c-Met loop here allows for the evaluation efficacy of MetMAb in long-term survival studies a ligand-driven manner. Pilot studies showed that KP4 tumors seemed to grow within the pancreas, showing little local invasion to nearby organ sites. Following inoculation, KP4 tumors were allowed to grow for 12 days and then mice were grouped based on tumor volume measurements taken by micro-ultrasound imaging. Mice were treated with vehicle or MetMAb (30 mg/kg, i.p., twice weekly). The no pump/treatment group (n = 5, gray empty squares, dashed line) received KP4 cells as above, but without pumps and treatment. MetMAb has a significant effect on tumor growth ($P = 0.02$ on day 20). D. MetMAb substantially inhibits growth of established s.c. KP4 tumor xenografts in nude (nu/nu) mice. KP4 pancreatic tumors (n = 10 per group) received vehicle or MetMAb (30 mg/kg, i.p., twice weekly).
Effect of MetMAb treatment on survival in the KP4 orthotopic model. To test whether antitumor activity in the KP4 model translates into a survival benefit, treatment of the animals described above with vehicle or MetMAb (30 mg/kg in 100 μL, i.p., twice weekly) was continued for 11 weeks, and survival was followed for 90 days from study initiation. Continuous treatment with MetMAb for 11 weeks resulted in a substantial improvement in survival (Fig. 3C). Day 90 survival was increased by 424% in MetMAb-treated mice (55.0%) compared with vehicle controls (10.5%). Log-rank (Mantel-Cox) analysis of the plots showed significant survival benefit from treatment with MetMAb ($P = 0.0004$).

To assess whether short-term treatment could also result in a survival benefit in the KP4 orthotopic model, we conducted a survival study in which, 12 days after orthotopic injection of KP4 cells, mice were randomly grouped and treated with vehicle or MetMAb (30 mg/kg, i.p., twice weekly) for 2 weeks, and survival was followed for 90 days. Survival at the end point was 6 of 24 (25.0%) and 12 of 24 (50.0%), respectively, in the vehicle and MetMAb treatment groups (Fig. 3D). Survival benefit from treatment with MetMAb was statistically significant ($P = 0.0299$) as assessed by log-rank (Mantel-Cox) analysis of the plots. These data show that MetMAb significantly improves animal survival even when dosed for a shorter amount of time.

Effect of MetMAb on growth of larger orthotopic tumor xenografts. Because tumor size plays a role in determining therapeutic response, we next examined the effects of MetMAb treatment on growth of larger KP4 orthotopic tumors. For these studies, we increased both the number of injected KP4 cells (from 2 to 3 million) and the time for tumor growth before treatment initiation (from 12 to 16 days). These changes succeeded in increasing the mean tumor volume by ~5-fold compared with previous studies as measured by ultrasound and allowed for grouping out similarly sized tumors for MetMAb and vehicle treatment arms ($49.7 \pm 7.1$ versus $49.5 \pm 8.2$ mm$^3$, respectively; $n = 13$ per group). Treatment was begun after 16 days with MetMAb (30 mg/kg, i.p., twice weekly) or vehicle for 2 weeks after tumor cell injection. Log-rank (Mantel-Cox) comparison of survival plots indicated $P = 0.0004$ between the two groups.

**Figure 3.** A to C, MetMAb shows strong antitumor activity in the KP4 orthotopic model of pancreatic cancer by ultrasound. Twelve days after intrapancreatic injection of KP4 cells, nude (nu/nu) mice received ultrasound imaging and were then treated with vehicle or MetMAb (30 mg/kg, i.p., twice weekly) for 2 wk. Ultrasound imaging was repeated 2 wk after treatment initiation. Treatment was continued for 11 wk, and survival was followed for 90 d after cell injection. Animal number was 19 to 20 in each group. There was no difference in tumor volume measured by ultrasound before treatment ($8.34 \pm 3.51$ mm$^3$ and $8.63 \pm 3.61$ mm$^3$ in vehicle- and MetMAb-treated mice, respectively). A, tumor volume measured by ultrasound 2 wk after treatment initiation. B, fold change in tumor volume measured by ultrasound. ***, $P < 0.001$, between groups. C, effect of 11-wk treatment with MetMAb on survival. Log-rank (Mantel-Cox) comparison of survival plots indicated $P = 0.0004$ between the two groups. D, effect of 2-wk treatment with MetMAb on survival in the orthotopic model of pancreatic cancer. Twelve days after intrapancreatic injection of KP4 tumor cells, nude (nu/nu) mice were treated with vehicle or MetMAb (30 mg/kg, i.p., twice weekly) for 2 wk, and survival was followed for 80 d after cell injection. Log-rank (Mantel-Cox) comparison of survival plots indicated $P = 0.029$ between treatment groups.
followed by ultrasound imaging and necropsy. MetMAb or vehicle was dosed over 14 days. Treatment was well tolerated as there was no difference in body weight between the two groups (30.67 ± 0.75 g versus 30.23 ± 0.63 g, respectively), although one animal in the vehicle group died before completing treatment. MetMAb treatment significantly inhibited tumor growth compared with vehicle as measured by ultrasound (817.5 ± 167.1 mm³ versus 1,419.9 ± 205.1 mm³, respectively; \( P < 0.05 \); Fig. 4A). The mean tumor volume in mice receiving vehicle increased by 40-fold, whereas tumor growth was significantly reduced to 18-fold in animals treated with MetMAb (\( P < 0.05 \)). Furthermore, administration of MetMAb significantly diminished tumor weight compared with vehicle by 54% (1.02 ± 0.20 g versus 2.23 ± 0.27 g, respectively; \( P < 0.01 \); Fig. 4B). There was a high correlation between tumor volume measured by ultrasound and tumor weight (\( r = 0.935; P = 0.0001 \); Fig. 4C), indicating that ultrasound measurements are an accurate and reliable means for evaluating tumor responses in this model.

**Representative ultrasound images of one animal per group are shown in Supplementary Fig. S3B.**

**MetMAb inhibits KP4 proliferation in vivo.** To address the potential mechanism of action of MetMAb on KP4 tumors, we determined if MetMAb inhibits tumor cell proliferation, as observed in vitro, by evaluating Ki-67 status (by immunohistochemistry) in KP4 orthotopic tumors treated with MetMAb (30 mg/kg, i.p., twice weekly) or vehicle for 2 weeks. The proliferative index, represented by Ki-67–positive nuclei, was clearly decreased in MetMAb-treated samples relative to those of vehicle-treated samples (Fig. 4D). MetMAb treatment resulted in a reduction in percentage of Ki-67–positive nuclei (52.5 ± 4.79% versus 85.0 ± 2.89%, respectively; \( n = 4 \) per group; \( P < 0.01 \)). Evaluation of apoptosis induction was done in a separate study looking at response of s.c. KP4 tumors to single doses of MetMAb and the results from this showed little change in cleaved caspase-3 immunohistochemical staining (data not shown). This is consistent with data from U-87 MG (29) that showed that overall active caspase-3 is low and is only moderately affected by treatment with MetMAb (one-armed 5D5). These data suggest that the predominant effect of MetMAb in the KP4 autocrine model is antiproliferative.

**MetMAb inhibits c-Met phosphorylation in vivo.** To show that the antitumor effects of MetMAb are tied to inhibition of the c-Met pathway, studies were done to evaluate c-Met phosphorylation in...
KP4 orthotopic tumors following 24 or 48 h after a single dose of vehicle or MetMAb (30 mg/kg). MetMAb reduced the ratio of phospho-c-Met to total c-Met by 81% at 24 h (n = 2 in the vehicle group, and n = 3 in the MetMAb group) and 85% at 48 h (n = 3 in the vehicle group, and n = 2 in the MetMAb group) compared with vehicle-treated tumors (Fig. 5), indicating that MetMAb has a profound effect on KP4 c-Met phosphorylation within the pancreas.

**Effect of MetMAb on tumor phosphorylation in orthotopic KP4 tumors.** KP4 orthotopic tumors were harvested at 24 and 48 h after a single dose of MetMAb (30 mg/kg, i.p.) or control (for each time point, n = 2 in the control group and n = 3 in the MetMAb group). A, tumor lysates were immunoprecipitated by anti-c-Met antibody (C-28) and blotted by anti-phosphoryrosine (4G10) and an anti-c-Met antibody (C-12). Cell lysates from A549 cells treated with or without 100 ng/mL HGF were loaded on the SDS gel as a positive control. B, the intensity of bands of phospho-c-Met (top) and total c-Met (middle) was quantified on a LI-COR Odyssey IR imaging system and the ratio of phospho-c-Met to total c-Met (bottom) from each tumor sample was used to evaluate c-Met phosphorylation. Tumor c-Met phosphorylation level in each treatment group was averaged. MetMAb treatment resulted in an 81% and 85% decrease in c-Met phosphorylation in KP4 orthotopic tumors at 24 and 48 h, respectively.

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**Discussion**

Expression of c-Met and HGF is correlated with tumor proliferation, invasive growth, and poor prognosis in multiple cancer types. In pancreatic cancer, we have shown that expression of HGF and MET is significantly up-regulated. These data are consistent with recent reports that show that HGF and c-Met also show higher protein expression by immunohistochemistry, which seems to be correlated with expression of hypoxia-inducible factor-1α and poor prognosis (37). Therefore, there is a need to generate models for this tumor type and to evaluate potential HGF/c-Met inhibitors. MetMAb, a potent anti-c-Met monovalent antibody, blocks HGF binding to c-Met and downstream activity. Although monoclonal anti-HGF/c-Met antibodies and MetMAb have shown efficacy in animal models of glioblastoma (27–29), we have shown here that MetMAb also has activity against both paracrine-driven (BxPC-3) and autocrine-driven (KP4) pancreatic tumor xenografts and that MetMAb treatment can significantly improve survival in the KP4 orthotopic pancreatic model.

As mouse HGF does not activate human c-Met (30, 31), the role of c-Met is not captured in typical mouse xenografts studies. Therefore, we developed the human HGF osmotic pump model that allowed us to grow BxPC-3 xenografts in a HGF-driven manner (Fig. 2C). Therein, MetMAb showed a statistically significant effect on the ability of BxPC-3 tumors to grow, indicating that MetMAb is capable of blocking HGF-enhanced paracrine signaling in vivo (Fig. 2C).

Identification of the KP4 autocrine pancreatic line was of great utility as it allows for the evaluation of HGF-driven tumor growth without the need for engineered paracrine models, making long-term survival studies more feasible. MetMAb showed great potency against s.c. grown KP4 tumors, indicating that this tumor is heavily dependent on HGF. To better model pancreatic cancer, we
developed the KP4 orthotopic pancreatic tumor xenograft model. Treatment of established tumors (~8.5 mm³) with MetMAb completely abolished orthotopic tumor growth (Fig. 3A and B), consistent with the effects of MetMAb in s.c. models. However, because most patients with pancreatic cancer cannot be diagnosed at an early stage, we increased the pretreatment tumor size by ~5-fold to ~50 mm³. Therein, MetMAb significantly inhibited tumor growth (Fig. 4A and B), although here we did not observe tumor regressions, rather only inhibition of tumor growth.

Survival is one of most important end points for preclinical and clinical studies on new anticancer therapies. Long-term (11 weeks) treatment with MetMAb substantially improved survival (P = 0.0004) as assessed by log-rank (Mantel-Cox) analysis (Fig. 3C), as did treatment with MetMAb for 2 weeks (P = 0.0299; Fig. 3D). The 90-day survival rate was increased by 100% after short-term treatment with MetMAb (12 of 24, 50.0%) versus vehicle (6 of 24, 25.0%), whereas long-term treatment enhanced 90-day survival rate by 424% (55.0% for MetMAb versus 10.5% for vehicle). Taken together, these results show the therapeutic potential of MetMAb in a ligand-driven pancreatic tumor model.

Promotion of invasive growth induced by HGF requires activation of multiple downstream signaling transduction pathways (38). HGF binding to the c-Met receptor induces dimerization and c-Met phosphorylation, activating various adaptor proteins, particularly Gab-1, leading to activity in multiple signaling pathways, including phosphatidylinositol 3-kinase (PI3K)/Akt, and the Ras/ERK and MAPK pathway, involved in inducing cell proliferation (39, 40). Activation of both PI3K/Akt and Ras/ERK signaling pathways stimulates p70S6K, contributing to HGF-induced invasion and migration (41). MetMAb markedly inhibited c-Met phosphorylation of KP4 tumor cells in vitro (Fig. 2) as well as in orthotopic KP4 pancreatic tumors (Fig. 5). Our in vitro studies also showed that inhibition of c-Met phosphorylation led to inhibition of Gab-1 and downstream PI3K/Akt, ERK1/2, and p70S6 phosphorylation. Furthermore, MetMAb treatment resulted in a dose-dependent inhibition of KP4 cell viability in vitro. Antiproliferative effects were recapitulated in vivo where MetMAb strongly decreased the Ki-67 proliferative index in orthotopic KP4 tumors. HGF and c-Met have been characterized as angiogenesis-promoting factors (36). HGF has been previously shown to act alone and in synergy with VEGF to induce growth of vascular growth (36, 42). Activation of tumor-expressed c-Met leads to expression of a wide variety of proteases and growth factors known to be involved in tumor angiogenesis, including uPA and VEGF. Treatment with MetMAb was associated with dramatic tumor regressions in the dorsal skin window model; however, this response was not accompanied by a

![Figure 6. MetMAb shows antiproliferative but not antiangiogenic effects in the KP4 dorsal skin window model. A piece (~1 mm in diameter) of KP4 pancreatic tumor was implanted into the center of a dorsal skin window. When the window tumor reached ~4 mm in diameter, animals were treated with vehicle or MetMAb (30 mg/kg, i.p., twice weekly; n = 4 per group). Tumor size was monitored under light microscope, and tumor vessels were imaged by confocal microscopy after i.v. injection of FITC-dextran. *, P < 0.05, between the MetMAb-treated and vehicle groups at the same time point. A, representative light microscopic images of window tumor from one animal per group. B, effect of MetMAb on window tumor size under light microscope. C, representative confocal microscopic images of window tumor vessels labeled with i.v. infusion of FITC-dextran from one animal per group. D, effect of MetMAb on window tumor vascular density under confocal microscope. White columns, vehicle-treated; shaded columns, MetMAb-treated.](image-url)
significant change in tumor vascular density (Fig. 6), suggesting that c-Met–dependent angiogenesis does not play a major role in KP4 tumor growth in mice. These observations show that MetMAb prevents HGF-induced activation of c-Met phosphorylation, thereby inhibiting downstream signaling activity and cellular responses, but in this setting does not seem to affect tumor angiogenesis. In conclusion, we have shown that, in HGF-driven models of pancreatic cancer, MetMAb shows potent antitumor efficacy and these data suggest that targeting HGF/c-Met in pancreatic cancer with MetMAb may have therapeutic potential for this deadly disease.

Disclosure of Potential Conflicts of Interest

All authors are employees of, and have ownership interest in, Genentech, Inc.

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References

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