Inhibition of Growth, Invasion, and Metastasis of Human Pancreatic Carcinoma Cells by NK4 in an Orthotopic Mouse Model

Daisaku Tomioka, 2 Naoki Maehara, Keiji Kuba, 2 Kazuhiro Mizumoto, Masao Tanaka, Kunio Matsumoto, and Toshikazu Nakamura

Division of Molecular Regenerative Medicine, Course of Advanced Medicine, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871 [D. T., K. K., K. M., T. N.] and Department of Surgery and Oncology, Kyushu University Graduate School of Medical Science, Maidashi, Fukuoka 812-8582 [N. M., K. M., M. T.], Japan

ABSTRACT

Hepatocyte growth factor (HGF) is involved in malignant behavior of cancers as a mediator in tumor-stromal interactions through enhancing tumor invasion and metastasis. We found recently that NK4, a four-kiringle fragment of HGF, functions as both an HGF-antagonist and an angiogenesis inhibitor. We have now determined whether blockade of the HGF-c-Met/HGF receptor pathway and tumor angiogenesis by administration of recombinant NK4 would inhibit growth, invasion, and metastasis of human pancreatic carcinoma implanted into the pancreas of nude mice. When treatment with NK4 or anti-HGF neutralizing antibody was initiated from the third day after orthotopic injection of SUIT-2 human pancreatic cancer cells, both NK4 and anti-HGF antibody suppressed the conversion of orthotopic pancreatic tumors from carcinoma in situ to abnormally invading cancers during days 3–14. On the other hand, when the treatment was begun on day 10, a time when cancer cells were already invading surrounding tissues, NK4 but not anti-HGF antibody inhibited tumor growth, peritoneal dissemination, and ascites accumulation at 4 weeks after the inoculation. Antitumor effects of NK4 correlated with decreased microvessel density in pancreatic tumors thereby indicating that the antiangiogenic activity of NK4 may have mainly contributed to its antitumor effects. Moreover, although NK4-treatment was initiated from the end stage (day 24 after tumor inoculation), NK4 prolonged survival time of mice, and the suppression of peritoneal dissemination, ascites accumulation, and invasion of metastasized cancer cells into the peritoneal wall were remarkable. We propose that simultaneous targeting of both tumor angiogenesis and the HGF-mediated invasion-metastasis may prove to be a new approach to treating patients with pancreatic cancer.

INTRODUCTION

Pancreatic cancer is one of the major causes of cancer-related deaths in industrialized countries (1, 2). At the time of diagnosis, >80% of patients with this cancer have locally advanced or metastatic disease (3, 4), and only 1–4% of all the patients with pancreatic adenocarcinoma survive 5 years after the diagnosis (2). Even if patients are correctly diagnosed at an early stage, pancreatic cancer frequently exhibits highly malignant phenotypes characterized by extensive invasion into surrounding tissues and metastasis to distant organs, even at an early stage; hence, the prognosis of patients is poor (3, 5, 6). Thus, elucidation of molecular mechanisms related to invasion-metastasis of pancreatic cancers and the novel therapeutic approaches to pancreatic cancer treatment are urgently required.

Materials and Methods

Materials. Polyclonal antitumor HGF and antihuman HGF antibodies were prepared, respectively, as described elsewhere (21, 22, 28, 29). This antitumor HGF antibody cross-reacts with murine HGF but not with human HGF (28). Antitumor HGF IgG (1 μg) neutralizes biological activity of at least 5 ng of murine HGF. Recombinant human NK4 was purified from culture medium of CHO cells, which stably secrete human NK4, using three-step chromatographies. 5 The purity of NK4 was 96.4% as determined by SDS-PAGE and protein staining. The purified NK4 protein and antitumor HGF antibody were analyzed and determined to be negligible for endotoxin levels using a Limulus Amebocyte Lysate kit (BioWhittaker) as described elsewhere (25).
Orthotopic Inoculation of Tumor Cells. Human pancreatic cancer cell lines (SUIT-2, KP-3, and MiaPaCa-2) were donated by Dr. H. Iguchi (National Kyushu Cancer Center, Fukuoka, Japan). SUIT-2 cells were cultured at 37°C in RPMI 1640 supplemented with streptomycin, penicillin, and 10% fetal bovine serum. c-Met receptor expression and effects of HGF and NK4 on growth and invasion of SUIT-2 cells were described elsewhere (20). Cell suspension (50 μl) at 2 × 10^7 cells/ml was injected into the pancreas of 6-week-old male nude mice (BALB/c-nu/nu; Japan SLC, Inc., Hamamatsu, Japan).

Administration of NK4. Experimental schedules for NK4-treatment of mice implanted with pancreatic cancer cells are described in Fig. 1. Mice were i.p. administered twice daily with 1.5 mg/kg/day NK4 or BSA in saline. When the mice were treated with antibody, antirat HGF IgG or normal rabbit IgG (8.7 mg/kg/day) was administered twice daily. At autopsy, size of the tumors in all of the mice was measured using a dial caliper, and the tumor volume was determined using the formula width × (length)^2 × 0.52. The number of macroscopically metastatic nodules >1 mm in diameter in the peritoneal cavity was counted, and the volume of ascites was measured. For histological procedures, tissues were fixed in formalin or EtOH, embedded in paraffin, and tissue sections were stained with H&E unless otherwise mentioned. Survival analysis was computed by the Kaplan-Meier method and compared by the log-rank test.

Immunohistochemistry and Measurement for Tissue HGF Levels. For blood vessel staining, tumor tissues were fixed in EtOH and embedded in paraffin. These tissue sections were then quenched with 3% hydrogen peroxide in PBS for 5 min, washed in PBS, and treated with 0.1% trypsin at room temperature for 20 min. The sections were exposed for 30 min to 10% normal rabbit serum and incubated with the anti-CD31/PECAM-1 antibodies (diluted 1:50; PharMingen) overnight at 4°C. Next, the sections were incubated with biotinylated horseradish peroxidase-conjugated rabbit antirat IgG antibodies (diluted 1:200; DAKO) for 30 min. The reaction was observed by incubating the sections with substrate solution containing diaminobenzidine and hydrogen peroxide. The sections were then washed in PBS and stained with hematoxylin. The number of blood vessels was counted under a light microscope at a 200-fold magnification using ≥10 randomly selected fields per each sample. To detect proliferating and apoptotic cells, tissue sections were respectively analyzed by immunohistochemistry for PCNA and terminal deoxynucleotidyl transferase (Tdt)-mediated nick end labeling assay, as described elsewhere (22, 25, 28).

To analyze the expression of HGF and human c-Met, tissues were fixed in EtOH and formalin, respectively. The tissue sections were incubated overnight with 10 μg/ml antirat HGF IgG, antihuman HGF IgG, or antihuman c-Met (C-12; Santa Cruz Biotechnology) at 4°C then were incubated with the DAKO rabbit Envision plus visualization system for 30 min. The sections were counterstained with hematoxylin.

For measurement of tissue HGF levels, pancreatic primary tumors were excised on day 28, and tissue extracts were prepared as described elsewhere (28, 29). The protein levels of human HGF and murine HGF were determined in ELISAs, respectively using IMMUNIS human HGF enzyme immunoassay and rat HGF EIA (Institute of Immunology, Tokyo, Japan). These ELISA kits have no cross-reactivity between human and rodent HGF.

Invasion Score. To semiquantitate the invasiveness of the implanted pancreatic cancer, we defined the invasion score based on histological observations as follows: score 0, invasion was undetectable, and the tumor was not surrounded by a capsule; score 1, invasion was undetectable, but the tumor was not surrounded by a capsule; score 2, invasion was partial; score 3, invasion was extensive, and normal pancreatic and tumor regions could not be distinguished.

Data Analysis. For statistical analyses, we used unpaired Student’s t test (two-tailed) unless otherwise mentioned. Differences were considered to be statistically significant at P < 0.05.

RESULTS

Possible Involvement of HGF-c-Met Interaction in the Progression of Pancreatic Cancer. On the basis of our previous report (20), we initially implanted two c-Met-positive human pancreatic cancer cell lines (SUIT-2 and KP-3) or c-Met-negative MiaPaCa-2 human pancreatic cancer cells into the pancreas of nude mice. Orthotopically implanted c-Met-negative MiaPaCa-2 cells did not form tiny nodules in the pancreases of nude mice for up to 4 weeks after tumor inoculation. In contrast, two c-Met-positive cells led to invasive tumors in a reproducible manner (100% tumor uptake in both lines). Because SUIT-2 pancreatic tumors showed a more aggressive and malignant phenotype than KP-3 pancreatic tumors, we used SUIT-2 cells in the orthotopic transplantation model of human pancreatic cancer.

Three days after tumor inoculation, SUIT-2 cells were engrafted in the pancreas in all of the mice, and the tiny mass of cancer cells was encapsulated by multilayers of host stromal cells (Fig. 2B, Day 3). At this stage, these SUIT-2 tumors did not invade surrounding tissues and showed localized tumor growth resembling adenoma or carcinoma in situ. Two weeks after tumor inoculation, SUIT-2 pancreatic tumors...
showed the invasive phenotype. Multilayers of host stromal cells had largely disappeared, and cancer cells were focally invading surrounding pancreatic tissue thereby disorganizing pancreatic acinar structures. In the cancer mass, fibroblast-like stromal cells were integrated within clusters of cancer cells, and angiogenesis in the tumor periphery was apparent. Increase in tumor volume became remarkable >2 weeks after inoculation (Fig. 2A). At 4 weeks after inoculation, the pancreatic tumor was large and occupied the entire area of the pancreas, penetrated the capsule of the pancreas, and spontaneously disseminated into the peritoneal cavity. Visible metastatic nodules in the mesentery and peritoneal wall appeared, and the accumulation of cancerous ascites was apparent (Fig. 2A). Therefore, in this pancreatic cancer model, we designated the 3–14 days as tumorigenic stage, the 2–4 weeks as invasive stage, and 4–10 weeks as end stage (Fig. 1).

To examine the potential involvement of HGF and the c-Met/HGF receptor in malignant behavior of SUIT-2 pancreatic tumors, expression of the human c-Met receptor and murine HGF were immunohistochemically analyzed (Fig. 3). Human c-Met immunoreactivity was observed to be specific in cancer cells but not in host stromal cells and ductal cells (Fig. 3a). Human HGF was not detected in normal regions or in cancerous lesions (Fig. 3b), consistent with the finding that SUIT-2 cells do not produce HGF in vitro (20). Although expression of murine HGF was not detectable in cancer cells or in host ductal cells, it was detected in stromal cells (vascular endothelial cells and fibroblasts), located in border regions between cancer tissue and pancreatic acinar structures (Fig. 3, c and d). It is noteworthy that expression of HGF was scarcely detectable in cells distant from tumor tissue (data not shown), suggesting that HGF expression in these cells might be regulated through interaction with tumor cells. These observations were confirmed by measurement of human and murine HGF levels in tumor tissues. Pancreatic tumors contained significant levels of murine HGF (0.63 ± 0.11 ng/mg protein) but not human HGF (<0.005 ng/mg protein). Together with the finding that HGF potently stimulated invasion of SUIT-2 cells in vitro (20), these results strongly suggest that stroma-derived HGF may play a role in pancreatic cancer cell behavior such as invasion, particularly in cancer cells located in peripheral regions interactive with host cells.

**Inhibition of Pancreatic Cancer Growth and Peritoneal Dissemination by NK4.** We then determined whether the malignant behavior of SUIT-2 pancreatic tumors in the pancreas of nude mice would be suppressed by NK4. Three days after the inoculation of SUIT-2 cells into the pancreas of nude mice, the mice were randomized into two treatment groups (n = 5 in each group), and recombinant NK4 or BSA (1.5 mg/kg/day) in saline was administered i.p. twice daily for 25 days (Fig. 1, Exp. 1), then all of the mice were killed on day 28.

NK4 had no significant effects on body weight or general well-being of the animals (data not shown). NK4 treatment significantly suppressed the growth of SUIT-2 pancreatic tumors by 60.7% (Fig. 4A; P < 0.05). Control tumors aggressively invaded the neighboring spleen and penetrated the pancreatic capsule, whereas NK4-treated tumors neither invaded the spleen nor penetrated the capsule of the pancreas, and macroscopically the tumors had a smooth surface. The effect of NK4 on peritoneal dissemination was assessed by counting the number of metastatic nodules in the mesentery and peritoneal wall. NK4-treatment resulted in 84.1% decrease in the number of metastatic nodules in the mesentry, diaphragm, and peritoneum (Fig. 4B; P < 0.05). These results suggest that HGF plays a role in the malignant behavior of human pancreatic cancers. However, because NK4 functions as an angiogenesis inhibitor independently of its HGF-antagonist activity (25), the antiangiogenic activity of NK4 might have participated in the antitumor effects. To define mechanisms related to the antitumor effects of NK4 on malignant behavior of pancreatic cancers, the potential participation of HGF-antagonist activity and antiangiogenic activity of NK4 should be defined additionally.

**Inhibition of Tumorigenesis and Invasive Growth by NK4.** To determine whether NK4 affects tumorigenesis and invasive growth of SUIT-2 pancreatic tumors, NK4 was administered for 11 days from day 3 after inoculation (Fig. 1, Exp. 2). In vehicle-treated mice, cancer cells extensively invaded pancreatic tissue; thus, the border between tumor lesions and intact normal pancreatic tissues was not obvious (Fig. 5A, panels a and d). In contrast, in NK4-treated mice, tumor lesions were still largely encapsulated by host stromal cells, and the tumor lesions were apparently separated from normal pancreatic tissues, thus, indicating that invasion of cancer cells was inhibited by NK4 (Fig. 5A, panels b and e). When tumor invasion seen in histological observations was evaluated semiquantitatively, the tumor-invasive score in control mice reached 2.3 ± 0.1 (Fig. 5B), whereas tumor invasion was significantly inhibited to 1.3 ± 0.2 in NK4-treated
mice ($P < 0.01$). Because HGF potently stimulated invasion of SUIT-2 cells in vitro (20), we speculated that the suppression of invasion of SUIT-2 tumors by NK4-treatment might be attributable to antagonistic actions of NK4 for HGF. To address this possibility, mice were administered neutralizing anti-HGF IgG instead of NK4. Similar to the case of NK4, anti-HGF IgG inhibited tumor invasion (Fig. 5, panels c and f). Administration of normal rabbit IgG had no inhibitory effect on invasive behavior of cancer cells in the control mice (data not shown). Histological estimation of tumor invasion indicated that the invasive score in normal IgG-treated mice reached $2.3 \pm 0.2$, whereas anti-HGF IgG treatment significantly inhibited tumor invasion to $1.3 \pm 0.2$ (Fig. 5B; $P < 0.01$). On the other hand, tumor growth was not significantly affected by either NK4 or anti-HGF IgG, and the poor vasculature at this early stage made it difficult to quantify microvessel density (data not shown). Together with the expression of murine HGF in host stromal cells (Fig. 3, c and d), these results strongly suggest that host stroma-derived HGF promoted tumorigenesis and played a major role in acquisition of the invasive phenotype of SUIT-2 pancreatic tumors. NK4 may have inhibited SUIT-2 cell invasion through its potential to antagonize the biological actions of HGF as an HGF antagonist.

**NK4-induced Inhibition of Tumor Angiogenesis, Growth, and Peritoneal Dissemination.** We next determined whether the HGF-c-Met interaction system plays a role in the malignant behavior of SUIT-2 pancreatic tumors at the invasive stage (advanced cancers already invading or metastasizing during days 14–28 after inoculation). NK4 was administered daily from day 10 after inoculation until day 28. Administration of NK4 significantly decreased (55.6%) tumor volume as compared with findings in control mice (Fig. 6A; $230 \pm 37 \text{ mm}^3$ and $518 \pm 108 \text{ mm}^3$, respectively; $P < 0.05$). In contrast, treatment with anti-HGF IgG had no significant effect on tumor growth. Therefore, we speculated that NK4 inhibited tumor growth through the inhibitory actions of NK4 on tumor angiogenesis.

For analysis of the tumor vasculature, blood vessels in tumor tissues were stained with anti-CD31/PECAM-1 antibodies. The number of CD31/PECAM-1-positive blood vessels in NK4-treated tumors ($9.0 \pm 1.4 \text{ vessels/field}$) was suppressed by 56.5% when compared with findings in control mice ($20.7 \pm 3.5 \text{ vessels/field}$; $P < 0.05$; Fig. 6B). In contrast, treatment with anti-HGF IgG had no significant effect on angiogenesis in tumor tissues. Furthermore, measurement of the number of proliferating and apoptotic cells in tumor tissues, as respectively determined by PCNA staining and terminal deoxynucleo-
Prolonged Survival of Mice by NK4 Treatment.

We next investigated the therapeutic potential of NK4 for treatment of end-stage pancreatic cancers. We initiated NK4-treatment from day 24 after tumor inoculation (Fig. 1, Exp. 4), a time when peritoneal dissemination and accumulation of cancerous ascites became apparent, yet all of the mice survived. Control mice died of pancreatic cancer only after 26 days, and all of the mice \( (n = 15) \) died within 69 days after the inoculation (Fig. 7). In mice treated with NK4 \( (n = 10) \), 4 mice died within 65 days, whereas 6 mice survived for \( \geq 70 \) days after tumor inoculation \( (P < 0.01) \). Even on day 70 after tumor inoculation, 6 surviving mice appeared active without anorexia, cachexia, and accumulation of ascites, although pancreatic tumors were palpable. Consistent with this observation, NK4-treated mice maintained their weight significantly better than control mice (data not shown). When moribund control mice were examined, peritoneum, diaphragm, and mesentery were occupied with numerous metastatic nodules (Fig. 8C, panels a and b). In contrast, metastatic nodules in NK4-treated mice were apparent but much less numerous than in control mice (Fig. 8C, panels c and d). The mean number of disseminated metastatic nodules reached 180 \( \pm 12 \) in control mice but remained at 29 \( \pm 14 \) in NK4-treated mice (Fig. 8A; \( P < 0.05 \)). Surface of the peritoneum was covered by a relatively thick layer of cancer cells, and cancer cells had extensively invaded muscular tunics (Fig. 8C, panels e and g). A similar histological appearance was noted in large areas of the peritoneum and the diaphragm. In contrast, in NK4-treated mice, large areas of peritoneum were free of cancer cells (data not shown). Even in the region with metastatic cancer cells, the cancer cells were more tightly associated than in the control, and invasion of cells into muscular tissues was remarkably inhibited by NK4-treatment (Fig. 8C, panels f and h). In control mice, ascites accumulation reached 4.0 \( \pm 0.3 \) ml, but NK4-treatment inhibited ascites accumulation to 24.5\% of the control levels (Fig. 8B; 1.0 \( \pm 0.8 \) ml; \( P < 0.05 \)).

**DISCUSSION**

In the orthotopic pancreatic cancer model, blockade of the HGF-c-Met receptor coupling by NK4 or anti-HGF antibody led to inhibition of invasion of pancreatic primary tumors at the tumorigenesis stage, a time when pancreatic tumors underwent phenotypic changes from carcinoma *in situ* to advanced invasive carcinomas. Because the antirat HGF antibody used here does not cross-react with human HGF and SUIT-2 cells do not express human HGF both *in vitro* (20) and *in vivo* (Fig. 3, b), the phenotypic changes in this pancreatic cancer model were not mediated by HGF-c-Met signaling.

Twenty-eight days after inoculation, SUIT-2 pancreatic tumors spontaneously metastasized to the peritoneum. The number of metastatic nodules \( \geq 1 \) mm in diameter reached 22.6 \( \pm 7.9 \) (Fig. 6C). NK4-treatment strongly inhibited disseminated metastasis by 58.4\% of the control value \( (9.4 \pm 5.2; P < 0.05) \). Treatment with anti-HGF IgG had no significant effect on disseminated metastasis. Therefore, the inhibitory effect of NK4 on peritoneal dissemination might be predominantly achieved by antiangiogenic activity and not by HGF-antagonist activity.

Prolonged Survival of Mice by NK4 Treatment. We next investigated the therapeutic potential of NK4 for treatment of end-stage pancreatic cancer by NK4. A, inhibition of primary tumor growth by NK4. B, immunohistological analysis of vascularization of tumors from vehicle-, NK4- and anti-HGF IgG-treated mice. Mice were treated with vehicle, NK4, or anti-HGF IgG from day 10 for 18 days (Fig. 1, Exp. 3). On day 28 after tumor inoculation, the mice were killed, and tissue sections were stained with anti-CD31/PECAM-1 antibodies. Microvessels in a 10 randomly selected field \( (\times 200) \) were counted in tumors in each group. **C**, suppression of peritoneal dissemination by NK4. Six mice were used in each group. *, \( P < 0.05 \). Each value represents the mean; bars, \( \pm \) SE.

Fig. 6. Inhibition of tumor angiogenesis and peritoneal dissemination in pancreatic cancer by NK4. A, inhibition of primary tumor growth by NK4. B, immunohistological analysis of vascularization of tumors from vehicle-, NK4- and anti-HGF IgG-treated mice. Mice were treated with vehicle, NK4, or anti-HGF IgG from day 10 for 18 days (Fig. 1, Exp. 3). On day 28 after tumor inoculation, the mice were killed, and tissue sections were stained with anti-CD31/PECAM-1 antibodies. Microvessels in a 10 randomly selected field \( (\times 200) \) were counted in tumors in each group. **C**, suppression of peritoneal dissemination by NK4. Six mice were used in each group. *, \( P < 0.05 \). Each value represents the mean; bars, \( \pm \) SE.

Prolonged Survival of Mice by NK4 Treatment. We next investigated the therapeutic potential of NK4 for treatment of end-stage pancreatic cancer by NK4. A, inhibition of primary tumor growth by NK4. B, immunohistological analysis of vascularization of tumors from vehicle-, NK4- and anti-HGF IgG-treated mice. Mice were treated with vehicle, NK4, or anti-HGF IgG from day 10 for 18 days (Fig. 1, Exp. 3). On day 28 after tumor inoculation, the mice were killed, and tissue sections were stained with anti-CD31/PECAM-1 antibodies. Microvessels in a 10 randomly selected field \( (\times 200) \) were counted in tumors in each group. **C**, suppression of peritoneal dissemination by NK4. Six mice were used in each group. *, \( P < 0.05 \). Each value represents the mean; bars, \( \pm \) SE.

Fig. 7. Prolonged survival of tumor-bearing mice treated with NK4. Mice were treated with vehicle or NK4 from day 24 after tumor inoculation. When mice were moribund or NK4-treated mice survived until day 70, they were killed for analysis. A statistically significant difference was evident in the long-rank analysis of a Kaplan-Meier survival curve \( (P < 0.01) \).
In NK4-treated mice, tumor cells on peritoneal wall had a much less invasive phenotype, and muscular tissue was mostly free of invasive cancer cells (\(H_11003\), arrowheads) compared with findings in control mice. Peritoneal walls from control mice had invasive tumor cells in muscular bundles and a thickened layer of mesothelial cells (\(e\) and \(g\)). Peritoneal walls from NK4-treated mice were largely free from metastatic tumors but were partially occupied with tumor cells (\(f\)). In metastatic nodules in NK4-treated mice, tumor cells on peritoneal wall had a much less invasive phenotype, and muscular tissue was mostly free of invasive cancer cells (\(h\)). Original magnifications, \(\times\)40 (\(e\) and \(f\)) and \(\times\)100 (\(g\) and \(h\)).

The formation of metastatic nodules in the peritoneum seems to depend on the invasiveness, growth, and the breakdown of pancreatic-capsule of primary tumor, and subsequent growth of colonized micrometastases. Bifunctional properties of NK4 (HGF antagonist/
angiogenesis inhibitor) make it difficult to clearly address the precise mechanism for the antimitastatic effect of NK4 in this model. However, the failure of anti-HGF antibody to suppress peritoneal dissemination indicates that antiangiogenic activity of NK4 contributed mainly to its antimitastatic action. We therefore speculate that: (a) NK4 suppressed the local invasion and angiogenesis-dependent primary tumor growth, thereby inhibiting metastatic spreading, and/or; (b) NK4 inhibited angiogenesis-dependent growth of newly colonized metastases in the peritoneal cavity. On the other hand, accumulation of ascites is attributable to enhanced vascular permeability and plasma leakage influenced by cancer cells and/or impaired reabsorption of ascitic fluid attributable to massive invasion of cancer cells into lymphatic vessels (38). Because NK4 remarkably suppressed the penetration of cancer cells into muscle layers of the peritoneal wall (Fig. 8C), antagonistic activities of NK4 for HGF may have participated to some extent in suppressing the accumulation of ascites.

Because effective systemic therapy for pancreatic cancer is currently not available, and diagnosing pancreatic cancer in its early stages is difficult, the highly invasive and metastatic behavior of pancreatic cancer lead to difficulty in attaining a long-term survival and a recurrence-free status. Targeting tumor angiogenesis and blockade of HGF-mediated invasion of cancer cells may prove to be potential therapy for patients with pancreatic cancer.

ACKNOWLEDGMENTS
We thank Dr. S. Mizuno (Department of Molecular Regenerative Medicine, Osaka University) for technical advice on the immunohistochemistry and M. Ohara for helpful comments.

REFERENCES
5. Ohara for helpful comments.
7. Nakamura, T., Matsumoto, K., Kiritoshi, A., Tano, Y., and Nakamura, T. Induction of tumor angiogenesis/metastasis and a recurrence-free status. Targeting tumor angiogenesis and block-
Inhibition of Growth, Invasion, and Metastasis of Human Pancreatic Carcinoma Cells by NK4 in an Orthotopic Mouse Model

Daisaku Tomioka, Naoki Maehara, Keiji Kuba, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/20/7518

Cited articles
This article cites 36 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/20/7518.full#ref-list-1

Citing articles
This article has been cited by 29 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/20/7518.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.