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Hepatocyte Growth Factor and Met in Tumor Biology and Therapeutic Approach with NK4

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Summary

Hepatocyte growth factor (HGF) and Met/HGF receptor tyrosine kinase play a role in the progression to invasive and metastatic cancers. A variety of cancer cells secrete molecules that enhance HGF expression in stromal fibroblasts, while fibroblast-derived HGF, in turn, is a potent stimulator of the invasion of cancer cells. In addition to the ligand-dependent activation, Met receptor activation is negatively regulated by cell-cell contact and Ser985 phosphorylation in the juxtamembrane of Met. The loss of intercellular junctions may facilitate an escape from the cell-cell contact-dependent suppression of Met-signaling. Significance of juxtamembrane mutations found in human cancers is assumed to be a loss-of-function in the negative regulation of Met. In attempts to block the malignant behavior of cancers, NK4 was isolated as a competitive antagonist against HGF-Met signaling. Independently on its HGF-antagonist action, NK4 inhibited angiogenesis induced by vascular endothelial cell growth factor and basic fibroblast growth factor, as well as HGF. In experimental models of distinct types of cancers, NK4 inhibited Met activation and this was associated with inhibition of tumor invasion and metastasis. NK4 inhibited tumor angiogenesis, thereby suppressing angiogenesis-dependent tumor growth. Cancer treatment with NK4 suppresses malignant tumors to be ‘static’ in both tumor growth and spreading.

1 Introduction

Among malignant behavior of tumors, tumor metastasis is the most important factor affecting survival of cancer patients. If metastatic tumors can be suppressed to non-metastatic tumors, the rate of cancer cures is considered to show much improvement. The dissociation and migration of tumor cells, adhesive interaction of cancer cells towards the extracellular matrix, and proteolysis of extracellular matrix proteins play a definitive role in tumor invasion and metastasis. Although mutational alterations that occur in oncogenes and tumor suppressor genes are of a genetical background for tumorigenic transformation of cells, invasive and metastatic behavior of tumor cells is largely regulated by extracellularly acting growth factors. Among them, hepatocyte growth factor (HGF) particularly affects metastatic behavior in a wide variety of cancer cells [1-3].

HGF was first identified and cloned as a mitogenic polypeptide for hepatocytes [4-6]. The receptor for HGF is a c-met protooncogene product of transmembrane tyrosine kinase [7, 8]. HGF is involved in development and morphogenesis of embryonic tissues, while in mature tissues HGF plays roles in regeneration and protection of various tissues, including the liver [9, 10]. HGF has angiogenic and lymphangiogenic activities respectively for vascular and lymphatic endothelial cells [11-14]. In tumor tissues, however, tumor cells utilize the biological actions of HGF for their invasive and metastatic behavior. HGF stimulates 1) the dissociation of cancer cells at the primary site, 2) invasion through the basement membrane and host stroma by enhancing cell-matrix interactions, protease networks for the breakdown of extracellular matrix proteins, and motogenic responses [1-3]. Angiogenic and lymphangiogenic activities of HGF may facilitate cancer metastasis. Thus, the abrogation of HGF-Met receptor coupling or Met receptor-mediated signaling events is a strategy toward prevention of tumor metastasis.

NK4 was isolated as a competitive antagonist for HGF and the Met receptor, and subsequent studies showed that NK4 is bifunctional: it is an HGF-antagonist and an angiogenesis inhibitor [15-17]. NK4 can be considered for use in cancer treatment by targeting the malignant behavior of tumor cells, rather than by direct killing of the tumor cells. This review focuses on tumor invasion and metastasis regulated by the HGF-Met system and experimental cancer treatment with NK4.

2 HGF-Met in tumor-stromal interactions

Stromal components associated with carcinoma cells are composed of extracellular matrix and distinct types of cells, including those comprising the vasculature (endothelial cells, pericytes, and smooth muscle cells), inflammatory cells (lymphocytes, neutrophils, and macrophages), fibroblasts, and tumor (or carcinoma) -associated fibroblasts with myofibroblast-like characteristic. Host stromal influence on epithelial neoplasia, tumor angiogenesis and malignant behavior in carcinomas are evident in various types of cancers, including prostate, stomach, skin, oral cavity, mammary gland, and colon cancers [18, 19]. Recombinant grafts in which non-tumorigenic epithelial cells are combined with particular cancer-associated fibroblasts induce tumorigenic progression [20]. Recent studies have demonstrated that experimentally induced genetic alterations, i.e., inactivation of transforming growth factor-β type II receptor gene, in stromal fibroblasts induce epithelial neoplasia and invasive carcinoma [21].

The stromal cells participate in not only neoplastic transformation but also tumor invasion and metastasis. The involvement of stromal fibroblast-derived factor in the invasion of carcinoma cells was first demonstrated using oral squamous cell carcinoma cells cultured on a collagen gel [22]. When the cancer cells were cultured alone, no invasion was observed, whereas they invaded collagen gels in which fibroblasts were incorporated or when fibroblast-derived conditioned medium was added to the culture. These findings indicate that fibroblast-derived soluble factor is responsible for the induction of invasive behavior in oral squamous cell carcinoma. The factor was later shown to be HGF [23]. When cancer cells were cultured in collagen gels, HGF induced dissociation and invasion of cancer cells, whereas epidermal growth factor had marginal effect on invasive behavior of these cancer cells (Fig. 1A).

The enhancement of migration and invasion of cancer cells in the presence of stromal fibroblasts was further demonstrated in a variety of cancer cells, including squamous cell carcinoma cells, breast carcinoma, gallbladder carcinoma, esophageal cancer, and prostate cancer [24-26]. In these studies, the enhancement in cancer cell invasion by co-cultivation with fibroblasts was inhibited by NK4, a specific competitive antagonist against HGF-Met (see below for details) or a neutralizing antibody against HGF. The induction and enhancement of cancer cell invasion by HGF has been demonstrated in a wide variety of

cancer cells [27]. These results indicate that the invasion of various cancer cells is enhanced by fibroblast-derived factors and that HGF is a significant factor responsible for cancer cell invasion mediated by tumor-stromal interactions.

Among various stromal cell types, fibroblasts derived from a variety of tissues express HGF. In addition it has been shown that vascular endothelial cells, vascular smooth muscle cells, neutrophils, and macrophages are cellular sources of HGF [2]. The co-expression of HGF and Met in several different types of carcinoma cells have been noted, however, the collective expression of HGF is restricted not exclusively but predominantly to stromal cells in a variety of carcinoma tissues. For example, in human lung adenocarcinoma tissues, a detailed analysis of the expression of HGF and Met using the laser-beam microdissection of cancer cells and tumor-associated stromal cells indicated that HGF mRNA is expressed exclusively in the stromal cells in different tumor samples [28]. Although the mechanisms by which carcinoma cells acquire the ability to express HGF are unknown, the autocrine activation of Met may possibly be associated with the epithelial-mesenchymal transition of cancer cells, a phenomenon that is occasionally observed in highly aggressive cancer cells.

Consistent with a lack of autonomous potential to aggressively invade into the scaffold of extracellular matrix in many carcinoma cells, most carcinoma cells do not secrete HGF. However, carcinoma cells not only receive influence of stromal-derived HGF but also facilitate HGF production in stromal cells. Several reports noted that the expression of HGF in fibroblasts in culture is enhanced by the presence of co-cultured carcinoma cells and that many types of carcinoma cells secrete a variety of HGF-inducers, through which HGF production in fibroblasts is up-regulated [24-26, 29, 30]. These carcinoma cell-derived HGF-inducers were identified as interleukin-1β (IL-1β), basic fibroblast growth factor (bFGF), platelet-derived growth factor, transforming growth factor-α and prostaglandin E2. These observations indicate that the presence of crosstalk between carcinoma cells and stromal fibroblasts, mediated by HGF and the HGF-inducers loop: carcinoma cells secrete HGF-inducers for stromal fibroblasts, while stromal fibroblasts secrete HGF, which in turn stimulates cancer cell invasion and metastasis (Fig. 1B). In addition to the role of these mediators that are capable of stimulating HGF expression, mediators that are expressed in cancer tissues are involved in modulating a microenvironment such as angiogenesis, the inflammatory response, and the growth of stromal cells [31-35].

3 Negative Regulation of Met via Cell-Cell Contact and Ser985 Phosphorylation

The loss of contact inhibition, the escape from growth inhibition by cell-cell contact, is associated with cellular transformation, which is a precursor to malignant transformation. Inhibition of cell proliferation by cell-cell contact is a fundamental characteristic of normal cells by which cellular adhesion successfully maintains highly organized tissue architecture. HGF-Met pathway plays roles in reconstruction/regeneration in normal and highly organized tissues, while invasive and metastatic behavior of cancer cells in tumor tissues. In normal cells, Met activation status might be regulated by not only HGF but also tissue organization and cell-cell contact, while in cancer cells Met activation status might be regulated by HGF regardless of cell-cell contact. We recently found the mechanism by which Met activation status is regulated through cell-cell contact, using hepatocytes in primary culture [36].

Proliferation of normal hepatocytes is tightly regulated by cell-cell contact. Hepatocytes do not undergo DNA synthesis even in the presence of excess amount of HGF when the cells are in tight cell-cell contact. Under the sparse condition, HGF induced prolonged Met tyrosine phosphorylation and a marked mitogenic response. Under the confluent condition wherein hepatocytes were in tight cell-cell contact, HGF induced transient Met tyrosine phosphorylation and failed to induce mitogenic response. The activity and expression of the protein tyrosine phosphatase, LAR increased specifically in confluent hepatocytes and not in sparse hepatocytes. LAR and Met were associated, and LAR dephosphorylated tyrosine-phosphorylated Met. Furthermore, specific suppression of the LAR expression prolonged activation of Met and released contact inhibition. Thus functional association of LAR and Met underlies the inhibition of Met-mediated signaling through the dephosphorylation of Met, which specifically occurs under the confluent condition (Fig. 2A).

In addition to the regulation of Met by cell-cell contact, Met activation is regulated by phosphorylation of the 985th Ser (Ser985) in the Met. The juxtamembrane domain of the Met is consisted of highly conserved 47 amino acid residues and Ser985 resides in the juxtamembrane domain. Ser985 is phosphorylated by treatment of cells with 12-O-tetradecanoylphorbol 13-acetate, an activator for protein kinase C [37]. We showed that Ser985 is phosphorylated by protein kinase-C and dephosphorylated by protein

phosphatase-2A [38]. Importantly, HGF-dependent Met tyrosine phosphorylation and subsequent biological responses are suppressed when Ser985 is phosphorylated. These results indicate that the juxtamembrane Ser985 is a negative regulatory mechanism by which Met activation is suppressed even if cells are stimulated by HGF.

Physiological significance of the negative regulatory mechanisms for Met-dependent signal transduction remains to be defined, however, we speculate that the loss-of-function in the Met negative regulation may possibly related to malignant progression of tumor cells. A variety of cancer cells have functional abnormalities in biological machineries involved in cell-cell adhesions [39]. Epithelial-mesenchymal transition, which occurs at boundary between tumor and stromal cells and leads to the loss of intercellular junctions, may facilitate an escape from the cell-cell contact-dependent suppression of Met-signaling. Several mutational alterations in the juxtamembrane domain of Met were noted in different types of human cancers [40-42]. Potential significance of the juxtamembrane mutations as a loss-of-function in the negative regulation of Met-dependent signal transduction is considerable.

4 Structure and Activity of NK4 as an HGF-antagonist

The close involvement of the HGF-Met pathway in tumor-stromal interactions and cancer invasion and metastasis suggests that the HGF-Met pathway represents a potentially important molecular target in cancer treatment. Several lines of distinct approaches for inhibiting the HGF-Met pathway have been demonstrated in experimental models, including small molecules that inhibit the tyrosine kinase activity of the Met [43-45], ribozyme [46-48], small-interfering RNA (siRNA) [49, 50], neutralizing monoclonal antibodies [51], soluble Met receptor [52], and variant molecules of HGF [53]. Among the inhibitory molecules targeting HGF-Met pathway, NK4 has been the most extensively examined for developing a therapeutic approach.

HGF is a heterodimeric molecule composed of a 69 kDa α-chain and a 34 kDa β-chain. The α-chain contains the N-terminal hairpin domain and subsequent four kringle domains while the β-chain contains a serine protease-like domain (Fig. 3A) [5]. NK4 is composed of the N-terminal 447 amino acids of the α-chain, thus it contains the N-terminal hairpin domain and subsequent four kringle domains (thus designated NK4) [15]. NK4 binds to Met receptor, but does not activate the Met receptor, thereby competitively inhibiting the Met

receptor activation induced by HGF (Fig.3B). Because NK1 (N-terminal and the first kringle domains) is the minimum region for high-affinity binding to the Met receptor, NK1, NK2, NK3, as well as NK4, exhibit high-affinity binding to the Met receptor. It is noteworthy that NK1, NK2, and NK3, all variants of the α-chain containing binding domains to the Met receptor retain agonistic activity, not fully but in part [54-56]. Although the mechanism by which high-affinity binding of NK4 to the Met does not activate Met has yet determined, NK4 is the complete competitive antagonist for HGF.

Fig. 3A demonstrates antagonistic activities of NK4 on HGF-induced invasion of cancer cells. When human pancreatic cancer cells were co-cultured with stromal fibroblasts, invasion of the cells was strongly enhanced compared to that seen in the culture of cancer cells alone. In contrast, NK4 almost completely inhibited invasion of cancer cells in the co-culture system [57].

5 NK4 as an Angiogenesis Inhibitor

Since vascular endothelial cells express the Met receptor and HGF has angiogenic activity, it was considered that NK4 might inhibit the angiogenic responses induced by HGF. However, when effects of NK4 on human vascular endothelial cells in culture were examined, NK4 unexpectedly inhibited proliferation and migration of endothelial cells enhanced by bFGF and vascular endothelial cell growth factor (VEGF), as well as by HGF [58]. Likewise, when a pellet containing bFGF or VEGF was implanted under the rabbit cornea, bFGF induced extensive angiogenesis, whereas the co-existence of NK4 with bFGF or VEGF in the pellet inhibited angiogenesis induced by bFGF or VEGF [58]. These results suggested that NK4 has an angioinhibitory as well as antagonizing action against HGF and the Met receptor. Because NK4 competitively inhibits biological actions of HGF through high-affinity binding to the Met receptor, we asked if the binding of NK4 to the Met receptor is involved in the antiangiogenic activity of NK4. Because the N-terminal domain in HGF is essential for high-affinity binding of HGF to the Met, the N-terminal domain was deleted from NK4. Deletion of the N-terminal domain in NK4 led to a loss of HGF-antagonist activity, whereas the remaining variant composed of four kringle domains retained antiangiogenic activity [59]. Thus, NK4 is bifunctional, as it is HGF-antagonist and an angiogenesis inhibitor.

The binding of NK4 to the Met receptor is not involved in angioinhibitory action of NK4, then a question how NK4 inhibits signal transduction driven by angiogenic growth factors remained to be addressed. In human endothelial cells in culture, NK4 inhibited tyrosine phosphorylation of the Met receptor induced by HGF, whereas it did not inhibit tyrosine phosphorylation of the VEGF receptor-2 induced by VEGF [58]. Likewise, NK4 did not inhibit activation of ERK-1/2 induced by VEGF and bFGF. These results indicated that NK4 allowed for activation of receptors and subsequent signal transduction, at least, leading to activation of ERK-1/2. Taken together with the finding that the binding of NK4 to the Met receptor is not essential to exert angioinhibitory actions, association of NK4 to a putative binding molecule other than Met receptor may participate in the antiangiogenic signal transduction of NK4.

Merkulova-Rainon et al. [60] reported that the N-terminal hairpin-like domain of HGF did not bind to the Met receptor, whereas it inhibited angiogenic activity of VEGF, bFGF, as well as HGF. Because 1) these angiogenic growth factors have affinity for heparansulfate proteoglycans, 2) the N-terminal domain has affinity for heparansulfate proteoglycans, and 3) the N-terminal domain inhibited binding of these growth factors to vascular endothelial cells, they proposed that the mechanism of anti-angiogenic activity of the N-terminal domain does not depend on binding to the Met receptor and that competitive inhibition of the N-terminal domain for binding of angiogenic growth factors to endothelial glycosaminoglycans may participate in angioinhibitory actions of the N-terminal domain. In addition to the N-terminal domain, involvement of the kringle domain in angiogenesis inhibition was noted. Xin et al. [61] indicated that the first kringle domain of HGF inhibited cell proliferation stimulated by bFGF and enhanced apoptosis in bovine aortic endothelial cells. Taken together, the kringle domains of NK4 are responsible for angioinhibitory activity of NK4, while the N-terminal domain augments anti-angiogenic activities of NK4.

6 Experimental Cancer Treatment with NK4

Colon carcinoma represents one of the most frequently occurring cancers and the majority of deaths from colon cancer are due to metastasis, with the liver being the most frequent site of metastasis. When murine colon carcinoma cells were inoculated into the spleens of mice, the cancer cells metastasized to the liver and formed a number of metastatic

nodules [62]. In this model, the human NK4 gene was expressed predominantly in the liver. The number of intrahepatic metastatic nodules was inhibited by NK4 gene expression (Fig. 4). Likewise, the mean area of each intrahepatic metastases in mice given the NK4 plasmid was much smaller than that in control mice (Fig. 4), indicating that hepatic expression of NK4 suppressed growth of metastases. Because blood vessel density in metastatic nodules was decreased and the number of cancer cells undergoing apoptotic cell death was enhanced by NK4-treatment, expression of NK4 inhibited angiogenesis in intrahepatic metastatic tissues, thereby inhibiting growth of hepatic metastases.

Histological appearances of intrahepatic metastases indicated that expression of NK4 strongly inhibited spreading and invasion of cancer cells to surrounding hepatic tissue. In control mice, tyrosine-phosphorylated/activated Met (pY-Met) was detectable in colon cancer cells in peripheral regions of metastases (Fig. 4), whereas tyrosine phosphorylated Met was mostly undetectable in cancer cells in mice given the NK4 plasmid. Thus NK4 inhibited activation of the Met receptor in colon cancer cells, thereby inhibiting their spreading and invasion as an HGF-antagonist. The life span of the mice was also prolonged by NK4 gene therapy.

Therapeutic effects caused by simultaneous blocking of tumor angiogenesis and Met receptor activation by NK4 have been also demonstrated in metastatic cancer models. When human pancreatic cancer cells were inoculated into the pancreas of nude mice, the pancreatic cancer formed a large mass and spontaneously disseminated into the peritoneal cavity four weeks after tumor inoculation [63]. Control mice started dying with pancreatic cancer from 26 days post-inoculation, and all of the mice were dead within 69 days after the inoculation. In mice treated with NK4, 60% of mice survived for more than 70 days. When moribund control mice were examined, the peritoneum, diaphragm, and mesentery were occupied with numerous metastatic nodules, and the mean number of disseminated metastatic nodules reached 180. In contrast, the number of disseminated metastatic nodules was only 29 in NK4-treated mice. Furthermore, NK4 treatment inhibited ascites accumulation to 24.5% of control levels. Thus, NK4 treatment has unique and static effects on malignant behavior of pancreatic cancer, the result being a prolonged life span of the mice.

Experimental cancer treatment with NK4 protein and NK4 gene therapy have been given for distinct types of cancers (Table 1). NK4-treatment led to anti-invasion/metastasis,

anti-angiogenesis and anti-growth effects in these cancers.

7 Perspectives

HGF has biological activities involved in dynamic tissue remodeling during embryogenesis and tissue regeneration. Breakdown of the extracellular matrix scaffold and concomitant cellular migration, mitogenesis, and morphogenesis driven by the HGF-Met system make way for construction and reconstruction of tissues through epithelial-mesenchymal interactions, fundamental tissue interactions involved in dynamic morphogenesis and regeneration. However, a definitive difference between epithelial-mesenchymal and tumor-stromal interactions is that the expression and functions of mediators and their receptors participating in the former interactions are tightly regulated; once normal tissue architectures are constructed or reconstructed, their expression and functions become quiescent in epithelial-mesenchymal interactions. Elucidation of the negative regulatory mechanisms for HGF-Met pathway and their loss-of-functions in cancers may lead to unique understanding of malignant progression of cancers.

Since both chemo and radiotherapies are based on the concept to directly kill cancer cells, these therapies have severe side effects and the result is reduction in quality of life and/or in decrease in immune responses. The surgical removal of cancer after early detection can be associated with disease-free survival of patients in case of some malignant cancers, yet surgical treatment often results in incomplete removal of cancer cells, allowing invasion and metastasis of cancer cells. HGF and its partner Met play a definitive role in tumor-stromal interactions, particularly leading to invasive and metastatic cancers. A therapeutic strategy targeting the HGF-Met axis is based on the suppression of the intrinsic characteristics of malignant tumors, i.e., invasion and metastasis. Therapeutic approaches that target the HGF-Met axis warrant further investigation and attention as potential therapy of cancer.

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Table 1. Therapeutic studies on NK4 in experimental models.

Type of tumor (species of tumor)	Inoculation site of tumor cells	Recombinant NK4 protein or NK4 gene expression	Sites of delivery	References
Breast cancer (human)	Subcutaneous	Protein	Subcutaneous near tumor	[64]
Breast cancer (murine)	Subcutaneous	Protein	Subcutaneous, near tumor	[58]
Colon cancer (murine)	Spleen	Gene expression by plasmid	Intravenous	[62]
	Cervical vein	Gene expression by adenovirus	Intraperitoneal	[65]
	Subcutaneous	Gene expression by adenovirus	Intratumoral, combination with dendritic cells	[66]
	Subcutaneous	Gene expression by plasmid	Intravenous	[67]
	Subcutaneous	Stable gene expression	Expression by cancer cells	[68, 69]
	Intravenous	Gene expression by adenovirus	Expression in mesenchymal stem cells	[70]
Colon cancer (human)	Subcutaneous	Gene expression by adenovirus	Intratumoral	[71]
Gallbladder cancer (human)	Subcutaneous	Protein	Subcutaneous, near tumor	[72]
	Intraperitoneal	Gene expression by adenovirus	Intraperitoneal	[73]
Gastric cancer (human)	Subcutaneous	Stable gene expression or adenovirus	Expression by cancer cells or intratumoral	[74]
	Subcutaneous	Gene expression by adenovirus	Intratumoral	[75]
	Intraperitoneal	Gene expression by adenovirus	Intraperitoneal, combination with cisplatin	[76]
	Intraperitoneal	Gene expression by adenovirus	Intraperitoneal	[77]
	Intraperitoneal	Gene expression by liposome	Intraperitoneal	[78]
Glioblastoma (human)	Orthotopic	Protein	Intratumoral	[79]
Hepatoma (human)	Subcutaneous	Gene expression by adenovirus	Intratumoral	[80]
	Subcutaneous or orthotopic	Gene expression by adenovirus	Intratumoral or intravenous	[81]
Lung cancer (murine)	Subcutaneous	Protein	Subcutaneous, near tumor	[58]
	Subcutaneous	Gene expression by adenovirus	Intratumoral, combination with dendritic cells	[66]
	Subcutaneous	Gene expression by plasmid in cationized gelatin	Subcutaneous, around tumor	[82]
Lung cancer (human)	Subcutaneous	Gene expression by adenovirus	Intratumoral or intraperitoneal	[65]
	Subcutaneous	Cancer cell-specific gene expression by adenovirus	Intratumoral	[83]
Lymphoma (murine)	Subcutaneous	Gene expression by adenovirus	Intratumoral, combination with dendritic cells	[66]

Melanoma (murine)	Subcutaneous	Gene expression by adenovirus	Intratumoral, combination with dendritic cells	[66]
Ovarian cancer (human)	Intraperitoneal	Stable gene expression	Expression by cancer cells	[85]
Pancreatic cancer (human)	Orthotopic	Protein	Intraperitoneal	[63]
	Intraperitoneal	Gene expression by adenovirus	Intraperitoneal	[86, 87]
	Orthotopic	Stable gene expression	Expression by cancer cells	[88]
	Subcutaneous	Protein released by cell sheets	Implantation of cell sheet	[89]
	Orthotopic	Gene expression by adenovirus	Peritumoral, combination with gemcitabine	[90]
Prostate cancer (human)	Subcutaneous	Protein	Subcutaneous, near tumor	[91]

Figure Legends

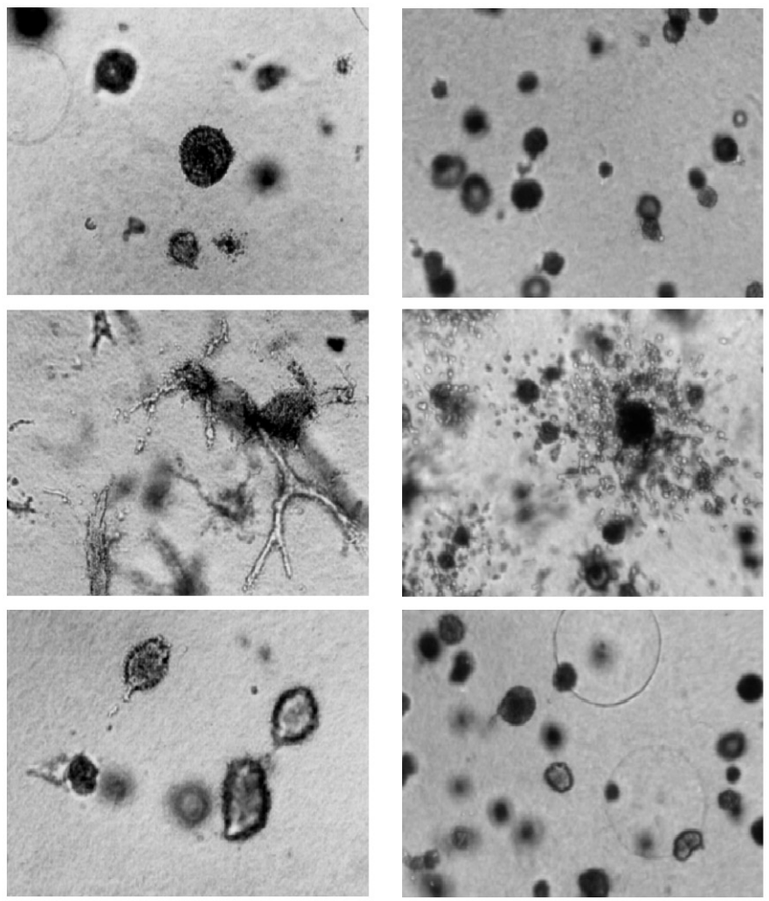
Fig. 1. Cancer cell invasion induced by HGF (A) and the crosstalk between tumor cells and stromal cells mediated by HGF-inducers and HGF (B). In (A), human cholangiocarcinoma cells and mouse lung carcinoma cells were cultured in collagen gel in the absence or presence of HGF or epidermal growth factor (EGF).

Fig. 2. Negative regulation of the Met receptor activation through cell-cell contact (A) and the juxtamembrane Ser985 phosphorylation (B). In hepatocytes in tight cell-cell contact, expression of LAR is up-regulated and LAR protein tyrosine phosphatase inactivates/dephosphorylates Met [36]. Ser985 in the juxtamembrane domain of Met is phosphorylated by protein kinase-C (PKC) and dephosphorylated by protein phosphatase-2A (PP-2A). When Ser985 is phosphorylated, tyrosine phosphorylation of Met is suppressed [38]. Loss-of-function in these negative regulatory mechanisms in the Met is potentially considerable in cancer cells.

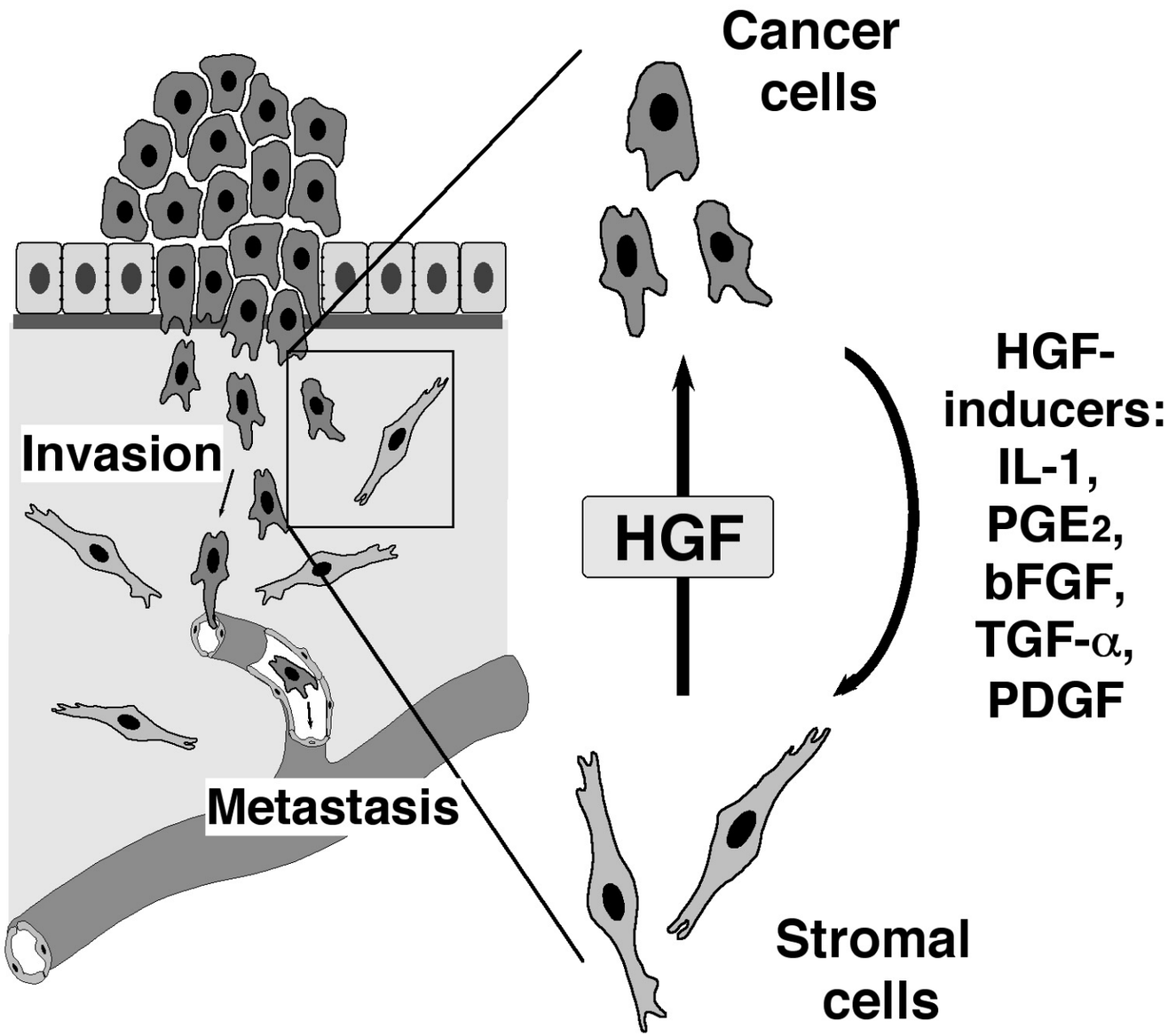
Fig. 3. Structure and biological activity of NK4 as a competitive HGF-antagonist. (A) Schematic structures of HGF and NK4. NK4 was originally isolated from proteolytic fragments of HGF [15]. (B) Outline for inhibitory action of NK4. NK4 binds to Met but does not activate Met, thereby competitively inhibiting activation of Met induced by HGF. (C) Inhibitory effect of NK4 on invasion of pancreatic cancer cells. Cancer cells were cultured on Matrigel basement membrane components in the absence or presence of stromal fibroblasts and the number of invading cells was determined.

Fig. 4. Inhibition of metastasis and invasive growth of colon cancer by NK4 in mice [62]. In the liver metastasis model of colon cancer, NK4 gene therapy inhibited hepatic metastasis (inset photographs) and growth of hepatic metastases (middle graph). Likewise, NK4 gene therapy inhibited tyrosine phosphorylation of the Met in cancer cells in hepatic metastases (lower panels) and this was associated with inhibition of spreading and invasion of cancer cells into hepatic tissue.

A

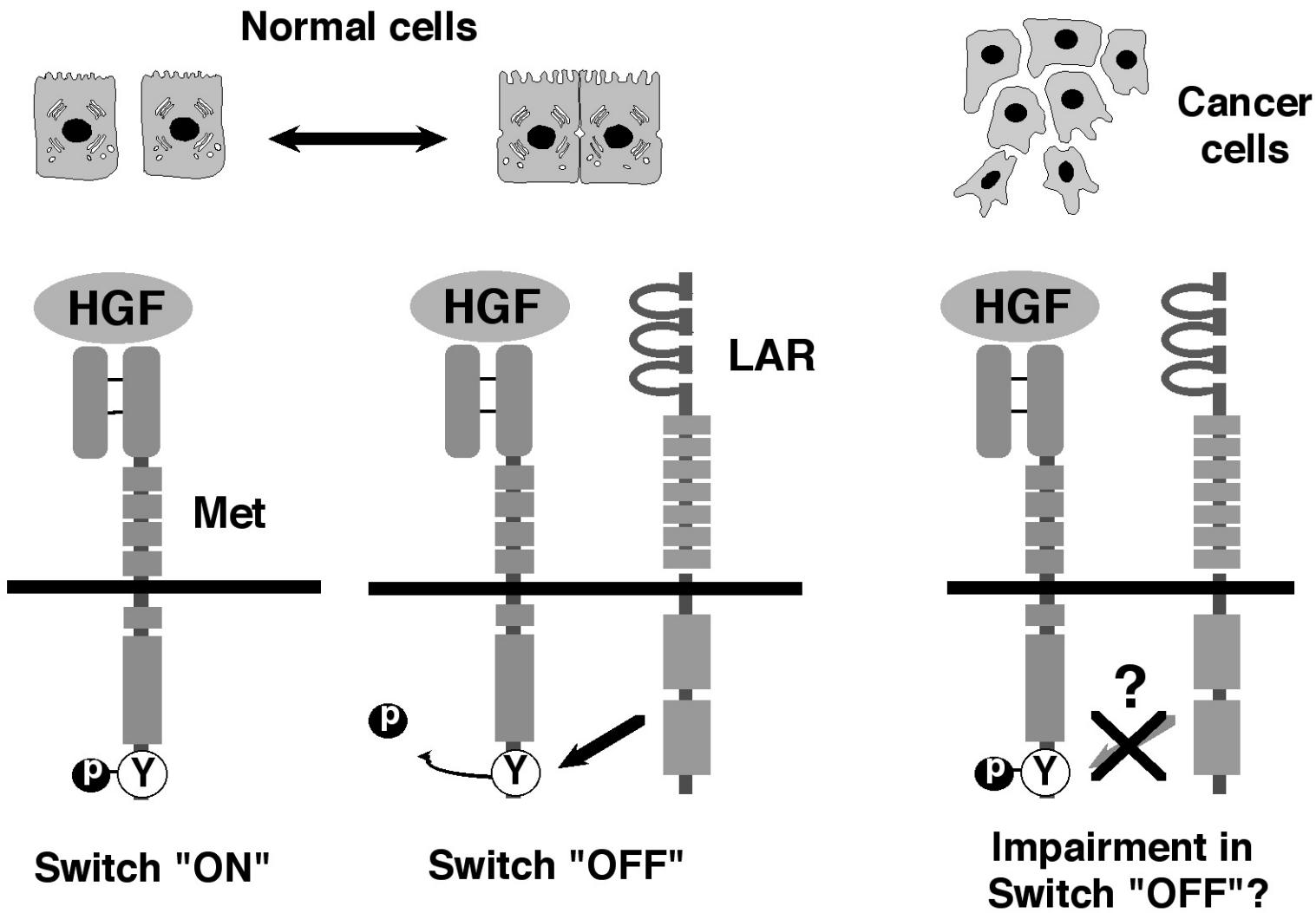

None
HGF
EGF
human cholangio-carcinoma
mouse lung carcinoma

B

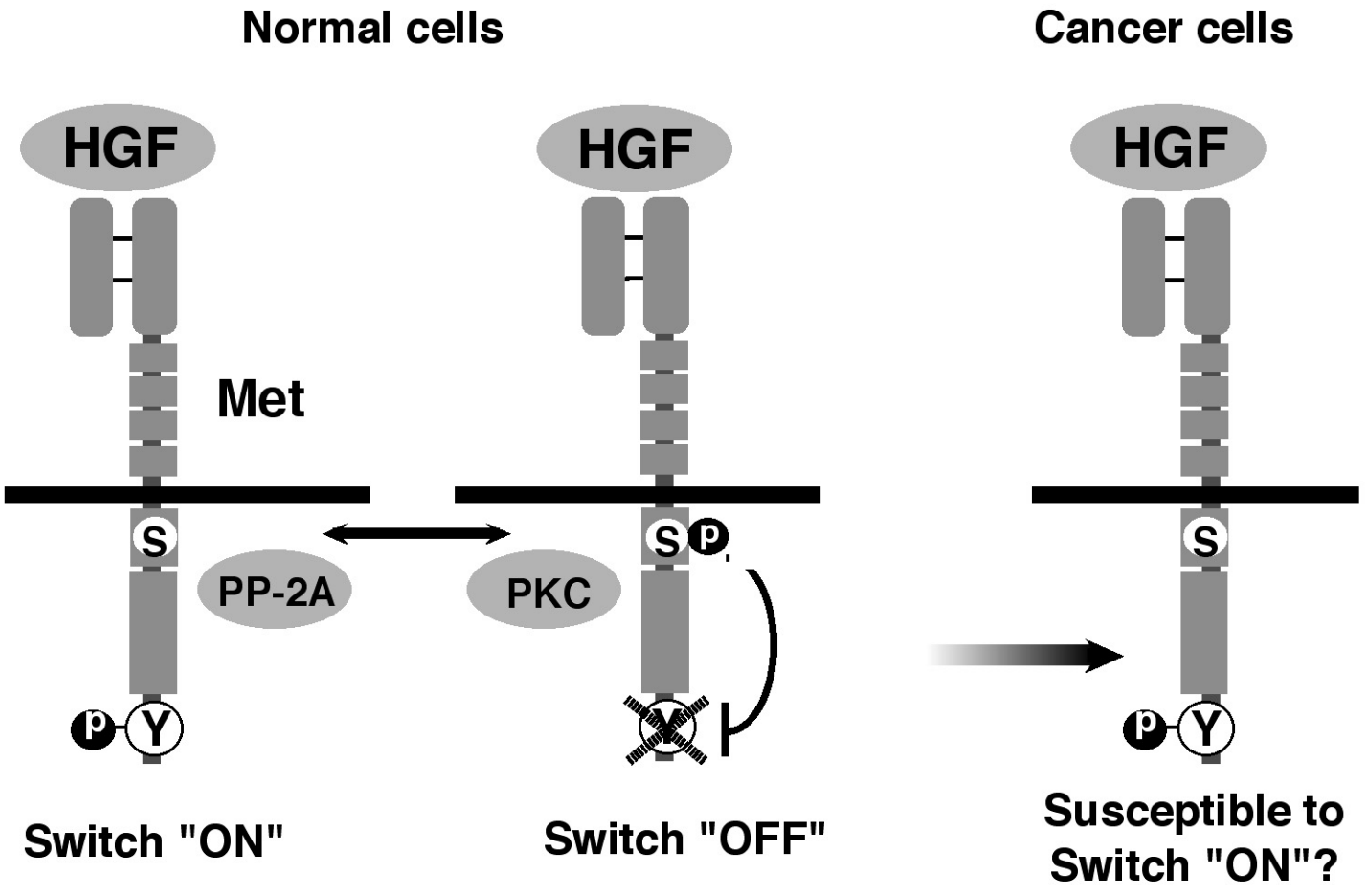

Cancer cells
HGF-inducers: IL-1, PGE2, bFGF, TGF-α, PDGF
HGF
Invasion
Metastasis
Stromal cells

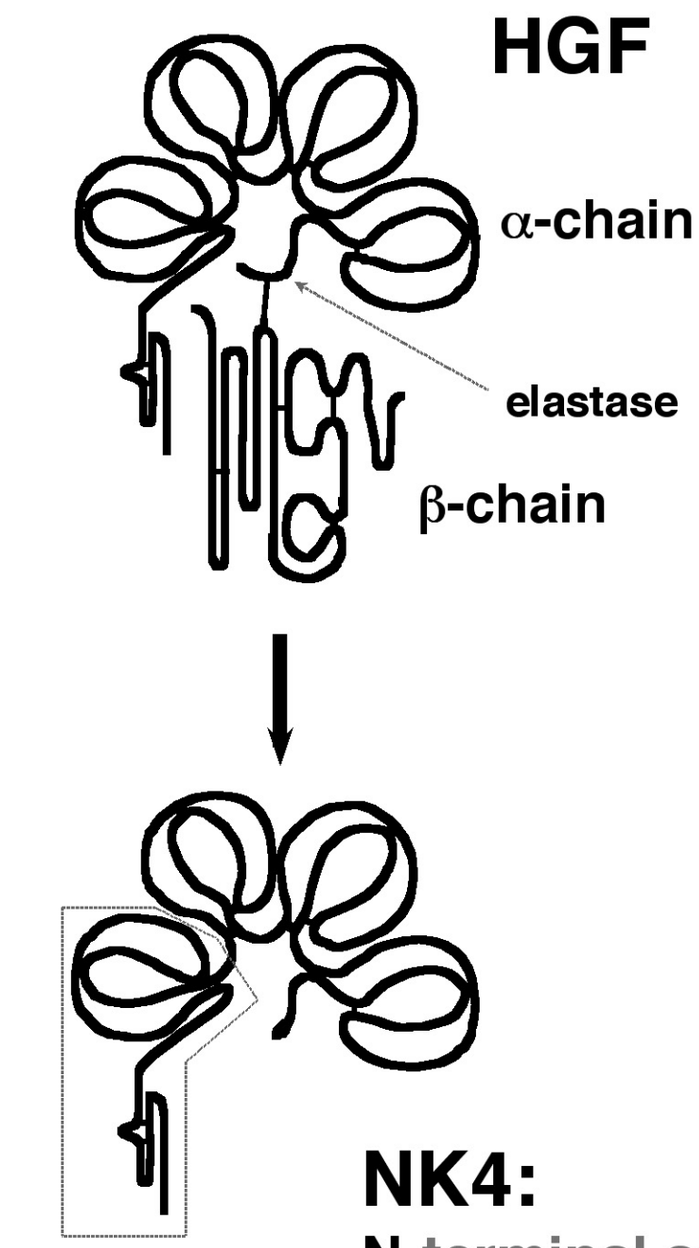
Matsumoto et al. Fig. 2

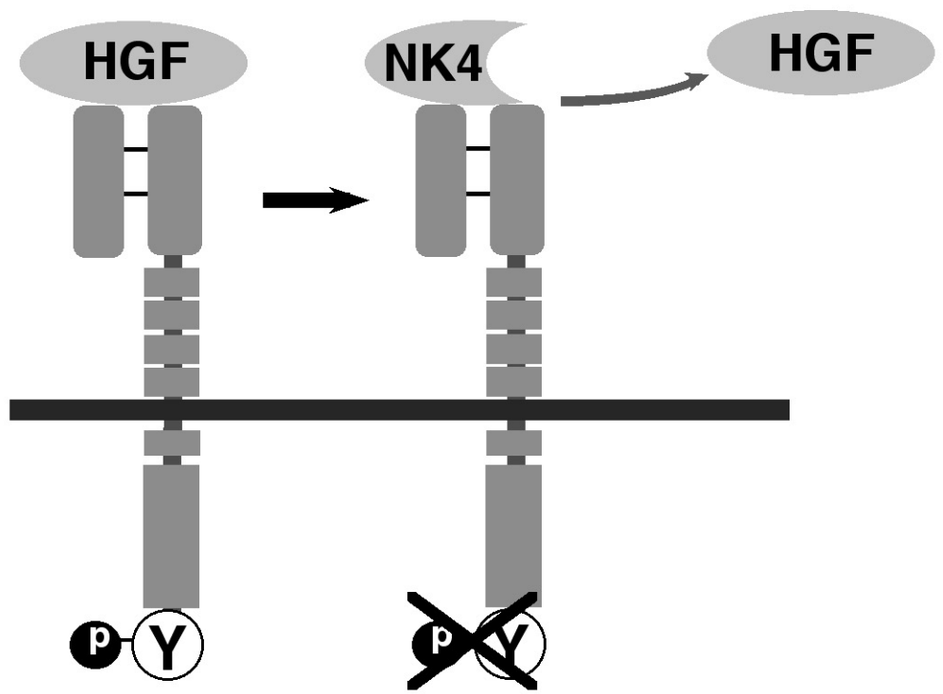
A

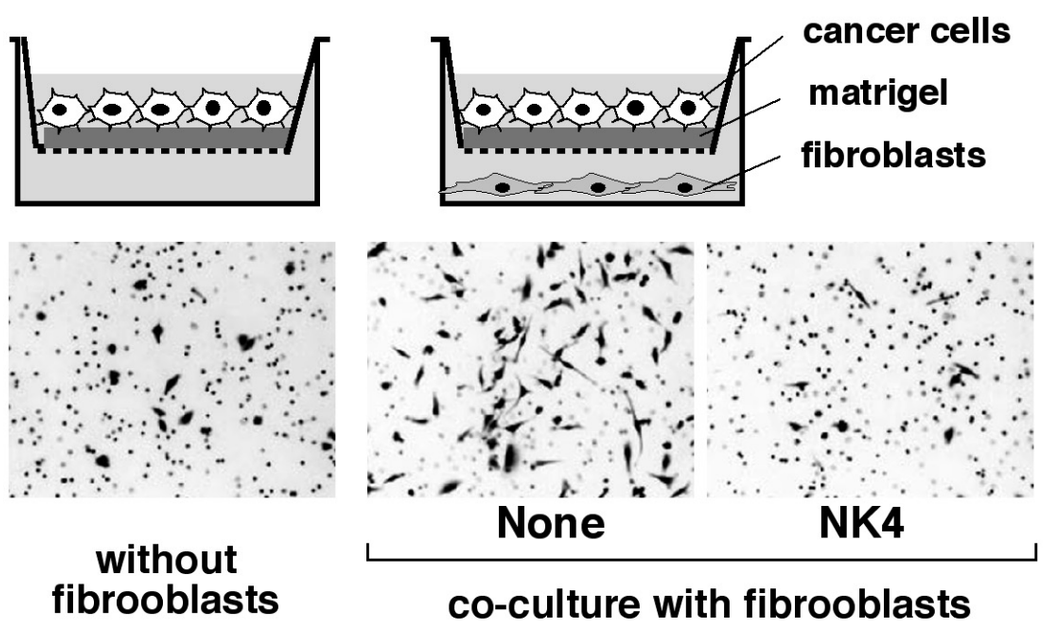


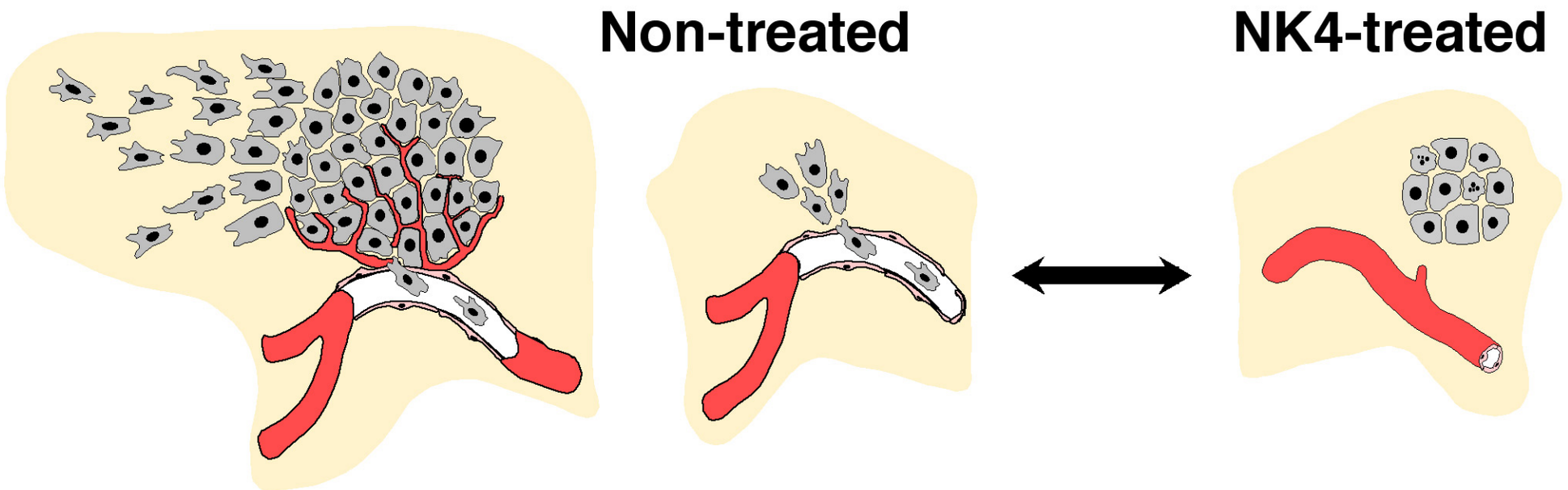
B

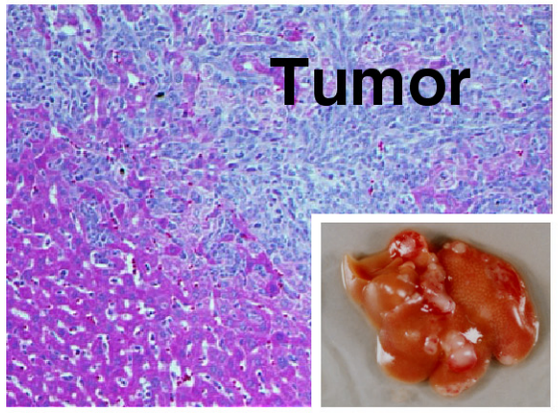


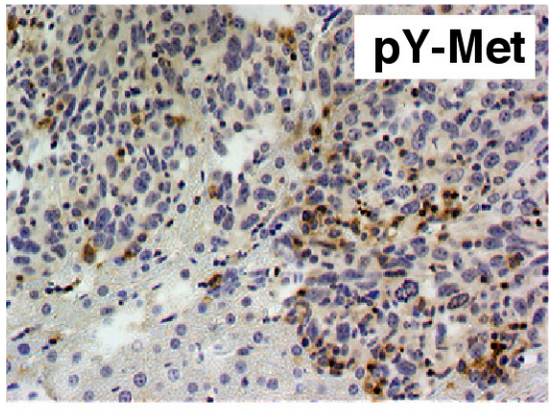

HGF
α-chain
elastase
β-chain
Met-binding domain
NK4:
N-terminal and
4 Kringle domains


HGF
NK4
HGF
P
Y
P
Y

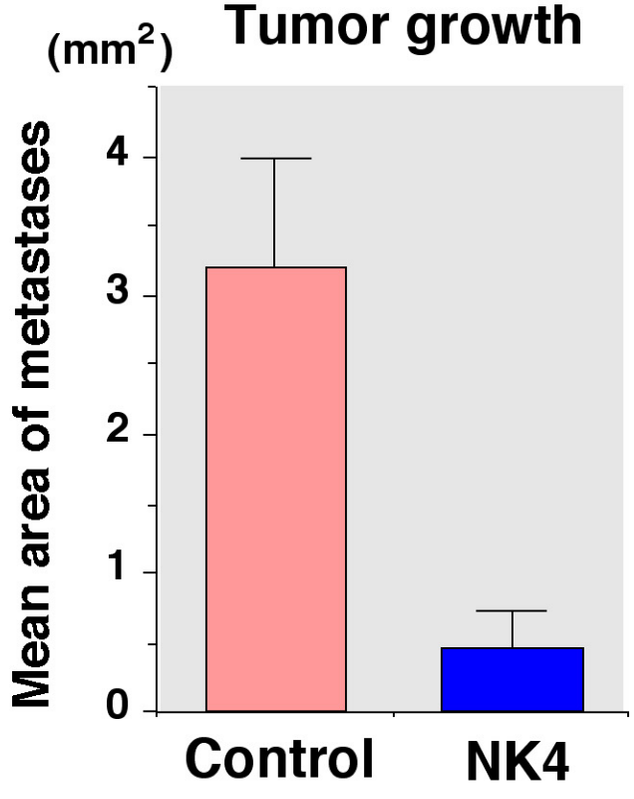

cancer cells
matrigel
fibroblasts
None
NK4
without fibrooblasts
co-culture with fibrooblasts

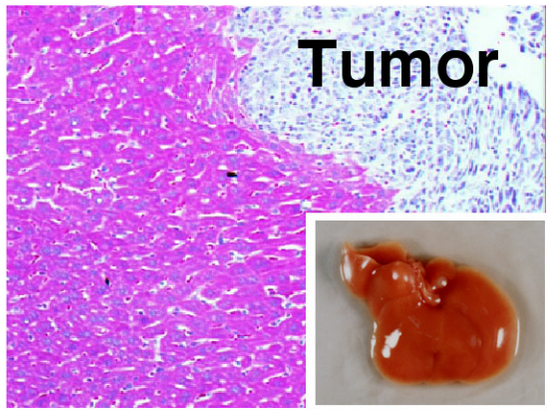

Non-treated
NK4-treated

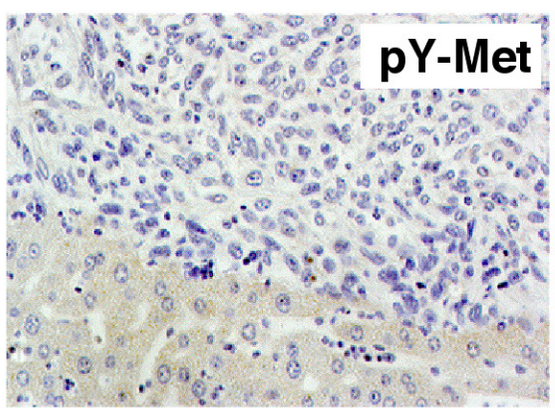

Tumor


pY-Met

Control


Tumor growth
(mm2)
Mean area of metastases
4
3
2
1
0
Control
NK4


Tumor


pY-Met

NK4