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Significance, Mechanisms, and Progress of Anticancer Drugs Targeting HGF-Met

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1. Introduction

Because growth factors and their receptors play critical roles in cancer development and progression, they are potential target molecules in molecular-targeted cancer therapy. Hepatocyte growth factor (HGF) was discovered and cloned as a mitogenic protein for hepatocytes (Nakamura et al., 1984; Nakamura et al., 1989; Miyazawa et al., 1989). These early studies implicated an important role for HGF in regeneration of the liver. The receptor for HGF was identified as the Met transmembrane receptor tyrosine kinase in 1991 (Bottaro et al., 1991; Naldini et al., 1991). The Met oncogene was first isolated as a fused transforming gene from a human osteosarcoma-derived cell line, wherein sequences from the TPR (translocated promoter region) were fused to the Met sequence (Tpr-Met) (Cooper et al., 1984). In 1991, the scatter factor, originally identified as a fibroblast-derived cell motility factor for epithelial cells (Stoker et al., 1987), was shown to be an identical molecule to HGF (Weidner et al., 1990). These findings implicated further biological and pathophysiological roles for HGF in epithelial wound healing, epithelial-mesenchymal interaction, and cancer development and invasion. Based on its close involvement — not only in tumor development, invasion, and metastasis but also in resistance to anticancer therapies — the HGF-Met pathway has become a hot target in anticancer drug development (Comoglio et al., 2008; Sattler & Salgia, 2009; Hanahan & Weinberg, 2011). In most cases in the relationship between growth factors and their receptor tyrosine kinases, a single growth factor activates multiple receptors that have structural similarities, while a single growth factor receptor has multiple ligands with structural and functional similarities. By contrast, the sole receptor of HGF is Met, while the sole ligand of Met is HGF; the relationship between HGF and Met is a "one-to-one relationship." This unique biochemical characteristic in the HGF-Met pathway promotes drug development by targeting HGF-Met through either the activation or the inhibition of the HGF-Met pathway.

2. Biochemical and biological characteristics

Biologically active HGF, a protein composed of 697 or 692 amino acids, is a heterodimeric molecule composed of an α-chain and a β-chain (Fig. 1A). The α-chain contains 4 kringle

domains, while the β-chain contains a serine protease-like structure (Nakamura et al., 1989; Miyazawa et al., 1989). HGF has a structural similarity to plasminogen, which is a heterodimeric serine protease containing 5 kringle domains. HGF is biosynthesized as a prepro-form of 728 amino acids, including a signal sequence and both α- and β-chains. After cleavage of a signal peptide of the first 31 amino acids, a single-chain HGF is further cleaved between Arg494 and Val495, and this processing is coupled to the conversion of biologically inactive pro-HGF to active HGF (Fig. 1A). Several proteases in the serum or cell membranes are involved in the activation of single-chain HGF, including HGF activator, urokinase-type plasminogen activator, plasma kallikrein, coagulation factors XII and XI, matriptase, and hepsin (Kataoka & Kawaguchi, 2010).

The Met receptor is composed of structural domains that include the extracellular Sema (the domain found in semaphorin receptors), PSI (the domain found in plexins, semaphorins and integrins) and IPT (the domain found in immunoglobulins, plexins, and transcription factors) domains, the transmembrane domain, and the intracellular juxtamembrane and tyrosine kinase domains (Fig. 1B) (Park et al., 1987). The Sema domain serves as a key element for ligand binding (Gherardi et al., 2006), while an involvement of IPT-3 and IPT-4 in the binding to HGF was demonstrated by another approach (Basilico et al., 2008).

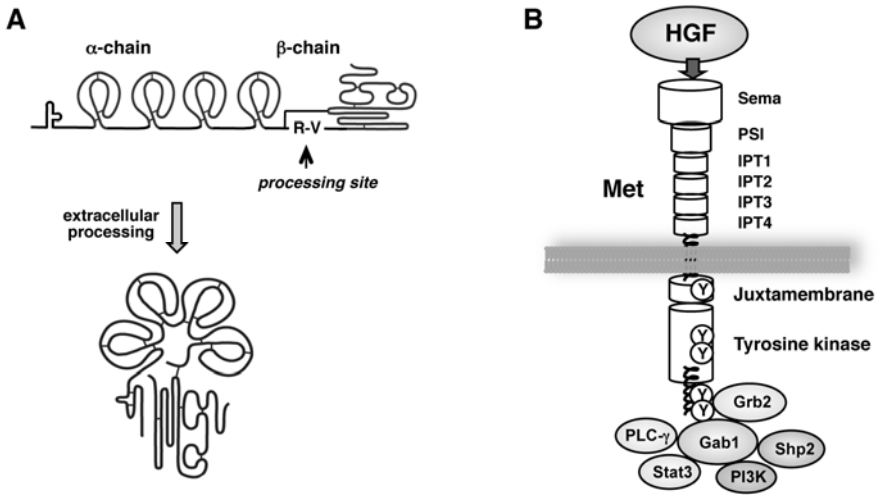

Fig. 1. (**A**) Processing and structure of single-chain proHGF and mature HGF. (**B**) Domain structures of the Met receptor and representative signaling molecules that associate with Met.

HGF and Met genes are widely expressed, and HGF is expressed in mesenchymal/stromal cells, predominantly rather than exclusively. Deletion of either the HGF or Met gene in mice lethally disrupts embryogenesis, including impairing development of the placenta and liver, and disabling dynamic migration of myogenic precursor cells (Schmidt et al., 1995; Uehara et al., 1995; Bladt et al., 1995; Birchmeier et al., 2003). In adulthood, HGF and Met play important roles in protection and regeneration of various tissues following injuries and pathology (Nakamura et al., 2011). Tissue-specific deletion of the Met gene revealed that the HGF-Met pathway plays a critical role in regeneration of the liver, kidney, and skin (Huh et

al., 2004; Borowiak et al., 2004; Chmielowiec et al., 2007; Ma et al., 2009). Met-deficient epidermal keratinocytes were unable to contribute to re-epithelialization in skin wound healing, because of a disability in keratinocyte migration (Chmielowiec et al., 2007). HGF induces 3-dimensional (3-D) tubulogenesis in epithelial cells such as renal and mammary grand epithelial cells (Fig. 2) (Montesano et al., 1991). These approaches emphasize a particular role of the HGF-Met pathway in the migration of cells during development, morphogenesis, and regeneration. However, the dynamic actions of HGF in wound healing and tissue reconstruction — even in a 3-D spatial scaffold — remind us of the malignant behavior of tumors, i.e., invasion and metastasis (Fig. 2). Aberrant activation of the Met receptor in tumor cells participates in the malignant progression of tumor cells.

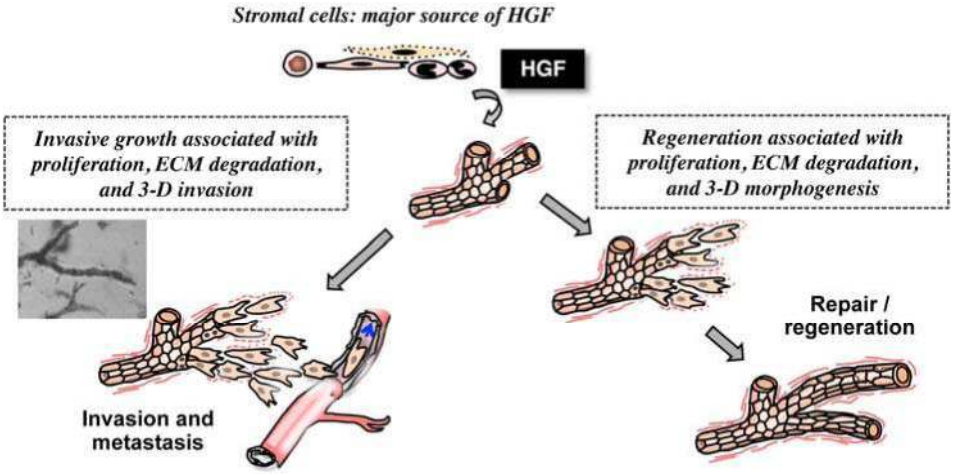

Fig. 2. Outline for biological actions of HGF in tumor invasion-metastasis and tissue regeneration.

3. Met receptor activation

HGF binds to Met through 2 different mechanisms: the α-chain binds with high affinity while the β-chain binds with low affinity. Among the α-chain, NK1 (the N-terminal and first kringle domains) in the α-chain of HGF provides a high-affinity binding site for Met. The α-chain alone exhibits high-affinity binding to Met, whereas the binding of the α-chain does not activate Met (Matsumoto et al., 1998). When Met is occupied by the α-chain, the low-affinity binding of the β-chain induces activation of Met and biological responses. Hence, the α-chain is a high-affinity binding module to Met, while the β-chain is an activation module for Met. The structure of the complex of HGF β-chain and Sema was revealed by crystallographic analysis (Fig. 3A) (Stamos et al., 2004). The HGF β-chain binds to a series of protruding polar side chains from Met, which originate from 3 separate loops: residues 124–128, residues 190–192, and residues 218–223. Although the α-chain of HGF binds to Met with much higher affinity than that of the HGF β-chain, the crystalline structure for the interaction between the HGF α-chain and the extracellular region of Met is yet to be determined.

The Met tyrosine kinase domain follows a conserved bilobal protein kinase architecture mainly with an N-terminal, β-sheet-containing domain linked through a hinge segment mainly to the α-helical C lobe (Fig. 3B) (Schiering et al., 2003; Wang et al., 2006). The characteristic feature of Met is the presence of the C-terminal tail that contains tyrosine residues (^{1349}YVHVNAT^{1356}YVNV). Binding of HGF to the extracellular region of Met results in receptor dimerization and phosphorylation of multiple tyrosine residues within the cytoplasmic region. Phosphorylation of Tyr1234 and Tyr1235 within the tyrosine kinase domain positively regulates the catalytic activity of tyrosine kinase (Fig. 3B). The staurosporine analog K-252a inhibits Met tyrosine kinase through its binding in the ATP pocket (Schiering et al., 2003). The phosphorylation of C-terminal tyrosine residues Tyr1349 and Tyr1356 recruits intracellular signaling molecules, including PI3K (phosphatidylinositol 3-kinase), Grb2 (growth-factor-receptor-bound protein 2), Gab1 (Grb2-associated binder 1), PLCγ (phospholipase Cγ), and Shp2 (SH2-domain-containing protein tyrosine phosphatase 2). Direct interaction of Gab1 with tyrosine phosphorylated Met is mediated by the Met-binding site in Gab1, and Gab1 is the most crucial substrate for the HGF-Met pathway (Ponzetto et al., 1994; Sachs et al., 2000).

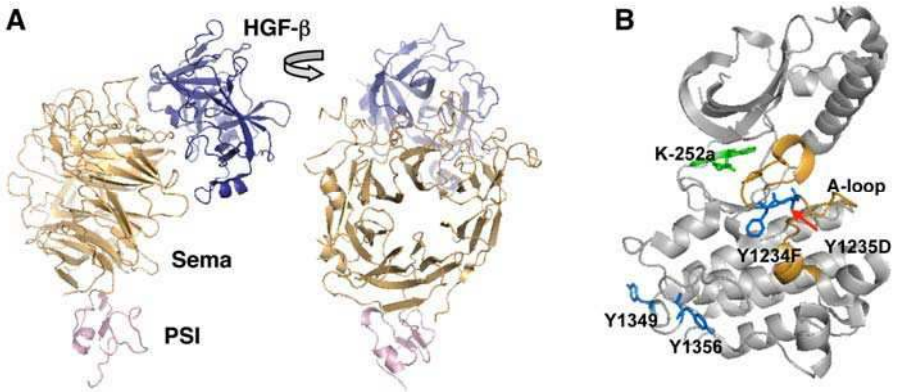

Fig. 3. Crystal structures for the complex of HGF β-chain and the Met Sema domain (**A**) and the Met tyrosine kinase domain (**B**). The crystal structures for the complex of HGF β-chain and the Met Sema domain were reported by Stamos et al. (2003) (PDB number: 1SHY). The crystal structure for Met tyrosine kinase was reported by Schiering et al. (2003) (PDB number 1ROP). In **B**, the activation loop (A-loop) is shown in yellow, K-252a in green, and selected tyrosine residues (Y1234F, Y1235D, Y1349, Y1356) are in blue.

The cytoplasmic juxtamembrane domain, which is composed of 47 highly conserved amino acids, acts as a negative regulator in terms of Met-dependent signal transduction. Cbl, an E3 ubiquitin ligase, binds phosphorylated Y1003 of Met, and this Cbl binding results in Met ubiquitination, endocytosis and transport to the endosomal compartment, then degradation (Peschard et al., 2001). Cbl-mediated degradation of the activated Met provides a mechanism that attenuates or terminates Met-mediated signaling. Phosphorylation of Ser985 in the juxtamembrane domain regulates the activation status of Met upon HGF stimulation. Ser985 is phosphorylated by protein kinase-C and is dephosphorylated by protein phosphatase-2A (Gandino et al., 1994; Hashigasako et al., 2004). In cells in which

Ser985 is phosphorylated, HGF-induced activation of Met is suppressed. Therefore, activation of protein kinase-C, which occurs by different types of extracellular stimuli, regulates HGF-dependent Met inactivation/activation.

4. HGF-Met in cancer development and progression

4.1 Cancer development

In normal tissues the activation of the Met receptor is tightly regulated, perhaps exclusively in a ligand-dependent manner. Aberrant activation of Met is associated with tumor development or progression to a tumor with malignant characteristics (Comoglio et al., 2004; Christensen et al., 2005; Matsumoto & Nakamura, 2006). Overexpression of Met through transcriptional upregulation has been noted in several cancers, including thyroid, ovarian, pancreatic, prostatic, renal, hepatocellular, breast, and colorectal cancers. Overexpression of Met through gene amplification was found in cancers with highly invasive and malignant characteristics, including gastric and esophageal carcinomas, medulloblastoma, and non-small-cell lung carcinomas (NSCLC) with acquired resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (see below). Autocrine and paracrine activation of Met through overexpression of HGF has been noted in breast cancer, glioblastoma, rhabdomyosarcoma, osteosarcoma, and in NSCLC with acquired and intrinsic resistance to EGFR tyrosine kinase inhibitors.

The missense mutations in the Met gene are the causative genetic disorders in inherited, and in some sporadic, papillary renal carcinomas (Schmidt et al., 1997). Mutations found in papillary renal carcinomas are located in the tyrosine kinase domain of the Met receptor, and these Met mutations are likely to be gain-of-function mutations (Jeffers et al., 1997; Michieli et al., 1999). In addition to papillary renal carcinoma, missense mutations in the Met gene have been found in different types of cancers, including lung cancer, hepatocellular carcinoma, and gastric cancer in the Sema, IPT, juxtamembrane, and tyrosine kinase domains (Christensen et al., 2005; Cipriani et al., 2009).

4.2 Cancer invasion and metastasis

The biological programs regulated by the HGF-Met pathway are adopted in cancer tissues, particularly for their invasive and metastatic behavior (Birchmeier et al., 2003; Matsumoto & Nakamura, 2006): 1) the dissociation of cancer cells at the primary site; 2) invasion, i.e., detachment from the primary site and migration through the basement membrane and stroma; and, 3) escape from apoptosis in anchorage-independent conditions during circulation. In a unique 3-D invasion in collagen gel, HGF was identified as a fibroblast-derived factor that definitively induces invasiveness of oral carcinoma cells (Matsumoto et al., 1989; Matsumoto et al., 1994). HGF increases extracellular protease expression coupled with the dissociation of cancer cells and their motility by which HGF promotes invasion in 3-D extracellular matrices and subsequent metastasis. HGF-Met signaling participates in the transition of epithelial to mesenchymal cell types (Birchmeier et al., 2003). Angiogenic and lymphangiogenic activities of HGF may facilitate cancer metastasis (Jiang et al., 2005). Collectively, the HGF-Met pathway has become a hot target in research and development of molecular targeted therapy for cancer, particularly to inhibit cancer invasion and metastasis (Hanahan & Weinberg, 2011).

4.3 Resistance to EGFR tyrosine kinase inhibitors

Gefitinib and erlotinib, selective inhibitors for EGFR tyrosine kinase, show favorable responses in NSCLC, especially those expressing activating mutations in EGFR (Lynch et al., 2004; Paez et al., 2004). Recent phase III clinical trials demonstrated that patients with EGFR mutant NSCLC had superior outcomes with gefitinib treatment, compared with standard first-line cytotoxic chemotherapy (Maemondo et al., 2010; Mitsudomi et al., 2010). However, almost without exception, the patients developed acquired resistance to EGFR tyrosine kinase inhibitors within several years (Morita et al., 2009). Furthermore, 20–25% of the patients with EGFR-activating mutations showed intrinsic resistance to EGFR tyrosine kinase inhibitors.

Three mechanisms have been noted to induce acquired resistance to EGFR tyrosine kinase inhibitors in NSCLC with activating EGFR mutants. One is the T790M second mutation in *EGFR* (Kobayashi et al., 2005). Second is the amplification of the Met gene (Engelman et al., 2007) (Fig. 4A, left). The T790M second mutation occurs in about half of all patients with acquired resistance to gefitinib or erlotinib. Recent studies showed that Met gene amplification was detected in ~20% of patients with acquired resistance to gefitinib or erlotinib (Bean et al., 2007; Turke et al., 2010). As the third mechanism, HGF-dependent Met activation has been noted (Yano et al., 2008). HGF induces resistance to EGFR tyrosine kinase inhibitors in EGFR mutant lung cancer (Yano et al., 2008) (Fig. 4, right). In clinical specimens, HGF overexpression was detected in a population of specimens from EGFR mutant lung cancer patients who showed intrinsic or acquired resistance to EGFR tyrosine kinase inhibitors indicating the clinical relevance of this resistance mechanism in lung cancer (Yano et al., 2008; Turke et al., 2010; Onitsuka et al., 2010). HGF can be produced by both cancer cells and host stromal cells such as fibroblasts (Matsumoto et al., 1994; Khoury et al., 2005; Matsumoto & Nakamura, 2006) (Fig. 4B). Tumor-associated fibroblasts expressed HGF at high levels in tumors from a population of NSCLC patients, and co-injection of HGF-producing human lung fibroblast cells with gefitinib-sensitive EGFR mutant lung cancer cells caused gefitinib resistance, which could be reversed by anti-HGF antibody and NK4, an antagonist against HGF (Wang et al., 2009). These results indicated that HGF derived from host stromal cells and/or HGF secreted from cancer cells induced resistance to EGFR tyrosine kinase inhibitors through paracrine and/or autocrine actions (Fig. 4B).

In some cases, a small fraction of cells with Met gene amplification pre-exists before exposure to EGFR tyrosine kinases, and HGF accelerates expansion of cells with Met gene amplification in the presence of EGFR tyrosine kinase inhibitors (Turke et al., 2010). HGF expression was higher in the drug-resistant specimens than in the pretreatment specimens (Turke et al., 2010). The results suggested that minor clones with Met gene amplification pre-existed before treatment with EGFR tyrosine kinase inhibitors, and that HGF accelerated expansion of a pre-existing minor population of tumor cells with Met gene amplification, which showed there is a relationship between HGF level and Met gene amplification. In recent studies, the EGFR-T790M second mutation and HGF expression were detected simultaneously in acquired resistant tumors in a considerable number of patients treated with gefitinib or erlotinib. EGFR-T790M second mutation was found in 7 of 10 NSCLC patients who acquired resistance to gefitinib, and 5 of 6 cases with EGFR-T790M second mutation showed high levels of HGF expression (Onitsuka et al., 2010). In 27 patients resistant to EGFR tyrosine kinase inhibitors, EGFR-T790M second mutation was seen in 15 of 27 cases, and 11 of these 15 tumors showed high-level HGF expression (Turke et al., 2010).

Collectively, expression of HGF in cancer cells and/or host stromal cells closely participated in the resistance to EGFR tyrosine kinase inhibitors in NSCLC, even in NSCLC with Met gene amplification.

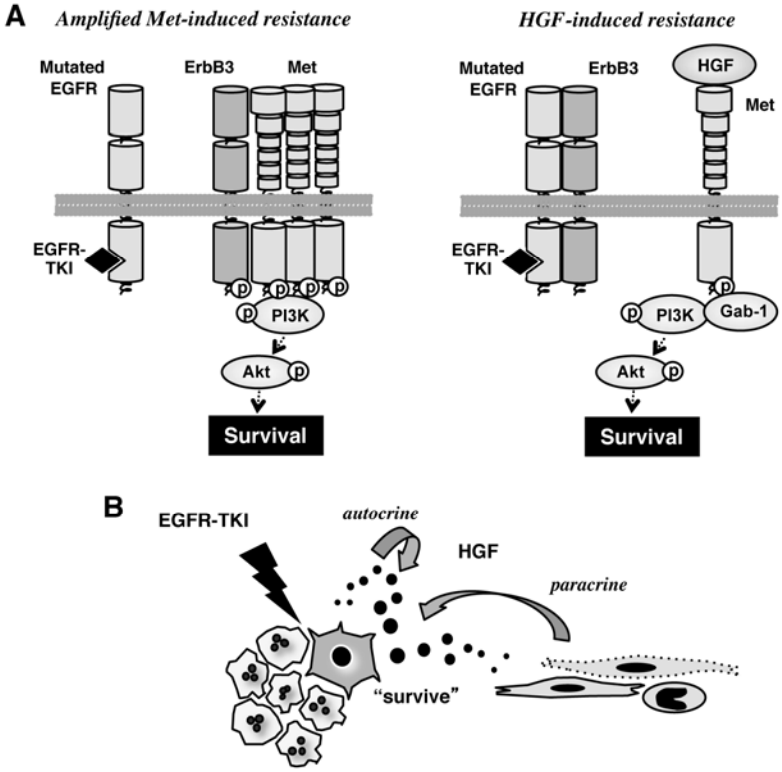

Fig. 4. Drug resistance of non-small-cell lung cancer (NSCLC) against EGFR tyrosine kinase inhibitors (EGFR-TKIs) through HGF-Met pathway. (**A**) Drug resistance through Met gene amplification (left) and HGF-dependent Met activation (right). Amplified Met associates with ErbB3 activates downstream signaling such as the PI3-Akt pathway, leading to the survival of cancer cells. HGF-dependent Met phosphorylation activates the PI3K-Akt pathway, independent of EGFR and ErbB3. (**B**) Outline for the resistance of NSCLC against EGFR-TKI by an HGF-dependent mechanism. HGF acts through an autocrine and/or paracrine manner.

4.4 Resistance to antiangiogenic therapy

Clinical results of antiangiogenic therapy in human patients have not been as promising as expected earlier (Schmidt, 2009). Until recently there had been a question as to why tumors become resistant to antiangiogenic therapy. Experimental studies have suggested that hypoxia generated by angiogenesis inhibitor or the blockage of new blood vessels triggers signaling molecules that make tumors more aggressive and metastatic (Schmidt, 2009).

In a model of pancreatic neuroendocrine cancer, inhibition of vascular endothelial cell growth factor receptor (VEGFR) tyrosine kinase shrank the primary tumor, but it also made the

surviving cancer more aggressive with more metastatic behavior (Casanovas et al., 2005). Pathological and clinical studies indicate that the presence of hypoxic regions within neoplastic lesions correlates with poor prognosis and an increased risk of the development of distant metastases (Höckel & Vaupel, 2001). Importantly, a hypoxic condition induced the transcriptional activation of the Met receptor gene and subsequent amplification of HGF-Met signaling, thereby increasing the invasiveness of cancer cells (Penancchietti et al., 2004). A connection between hypoxia and the Met receptor seems to explain why hypoxia often correlates with invasive and metastatic behavior. Angiogenesis inhibition retards tumor growth by oxygen deprivation, at least in part. However, hypoxia caused by the inhibition of angiogenesis enhances HGF-Met signaling, thereby promoting tumor invasion and metastasis. The involvement of the HGF-Met pathway in the aggressive characteristics in the hypoxic regions of cancers, which includes tumors treated with antiangiogenic drugs, is considerable.

5. Drug discovery and development

Close involvement of aberrant HGF-Met signaling in tumorigenesis and progression to malignant disease has facilitated drug discovery and development. Several distinct lines of approach to the inhibition of the HGF-Met pathway have been demonstrated, including small synthetic inhibitors of Met tyrosine kinase, ribozymes, small-interfering RNA (siRNA), neutralizing monoclonal antibodies (mAbs), soluble forms of Met, antagonists composed of selected domains in HGF, and uncleavable single-chain HGF (Fig. 5). Among recombinant protein-based inhibitors, conventionally called biologics in drug development, mAbs targeting HGF or Met have been in clinical development earlier than the other biological inhibitors, predominantly because of their availability due to established technologies for manufacturing of recombinant mAbs.

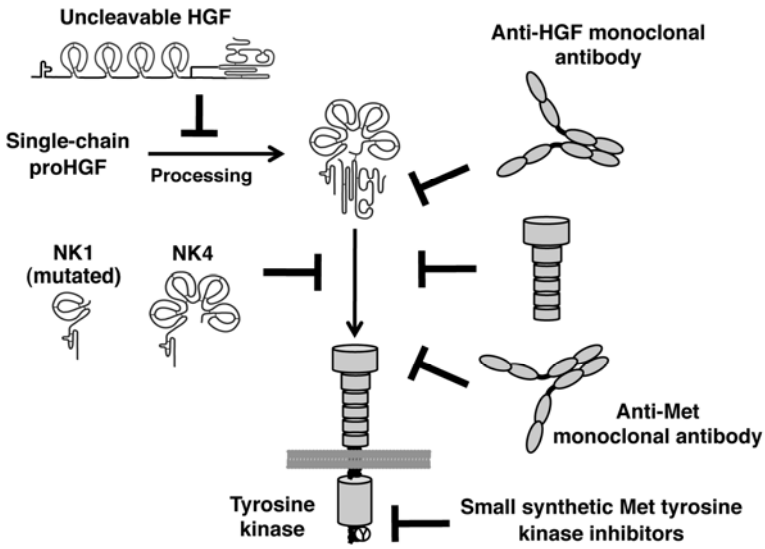

Fig. 5. Outline for different approaches to targeting HGF and Met.

5.1 Biologicals

Biological inhibitors against HGF-Met include the following: 1) selected domains in HGF (NK4 and engineered NK1); 2) engineered single-chain HGF forms that are resistant to proteolytic processing; 3) truncated soluble forms of the Met extracellular region; and, 4) humanized monoclonal antibodies (mAbs) against HGF or Met.

Among the α-chain of HGF, NK2 (the N-terminal, 1st kringle, and 2nd kringle domains), an alternative splicing variant, was first shown to competitively antagonize the growth stimulation by HGF (Chan et al., 1991). However, NK2 was later shown to stimulate cell motility and enhance HGF-driven metastasis in a mouse model (Stahl et al., 1997; Yu & Merlino, 2002).

NK4 is the first identified HGF-Met inhibitor devoid of biological activity through its Met binding. NK4 is composed of the N terminal and 4 kringle domains (Date et al., 1997; Matsumoto et al., 1998; Matsumoto et al., 2008). NK4 inhibits biological responses triggered by activation of HGF-Met signaling, including the spreading and invasion of cancer cells (Fig. 6). It should be emphasized that NK4 inhibits angiogenesis in addition to its antagonistic action against HGF, and this angioinhibitory action of NK4 is independent of its antagonist action against HGF. NK4 inhibited proliferation, migration, and tube formation of vascular endothelial cells induced by basic fibroblast growth factor and VEGF as well as by HGF (Kuba et al., 2001; Sakai et al., 2009). NK4 binds to perlecan and inhibits the cell-associated assembly of fibronectin, and the impaired fibronectin assembly suppresses integrin-dependent endothelial cell proliferation and migration. Having two different biological activities through completely different mechanisms is unique to NK4. Combination therapy of NK4 with antiangiogenic drugs is expected.

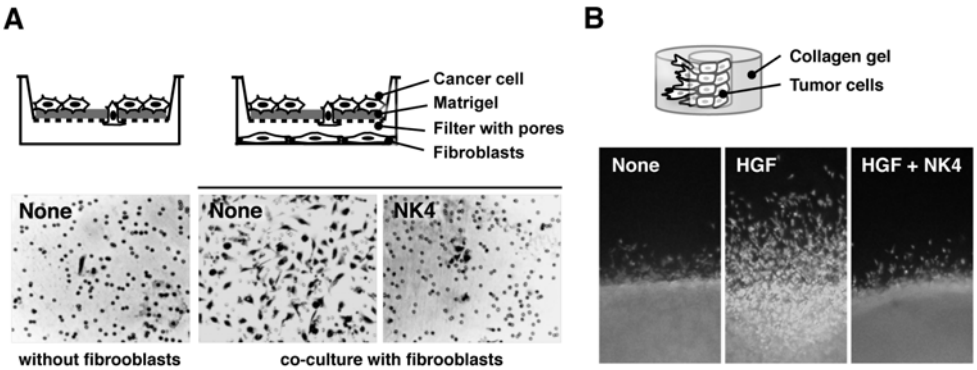

Fig. 6. Inhibition of tumor invasion by NK4. Invasion of human gallbladder cancer cells through the Matrigel basement membrane was induced by co-culture with stromal fibroblasts, and this aggressive invasion was inhibited by NK4 (**A**). 3-D invasion of human pleural malignant mesothelioma in collagen gel was enhanced by HGF, and was inhibited by NK4 (**B**).

The therapeutic effect of NK4 has been demonstrated in a variety of cancer models (Matsumoto et al., 2008). The inhibition of tumor growth by NK4 treatment was observed in a variety of tumors, and this inhibitory effect was associated with a reduction in blood vessels in tumor tissues. NK4 treatment inhibited *in situ* Met tyrosine phosphorylation, and

this was associated with an inhibition of the spread and invasive growth of cancer cells (Matsumoto et al., 2008). NK4 treatment inhibited metastasis in different types of models, including breast, colon, gastric, lung, ovarian, and pancreatic carcinomas, and malignant melanoma. These results provided the base for proof-of-concept; inhibition of the HGF-Met pathway is a way to inhibit the invasive and metastatic growth of cancer. Moreover, the combination therapy of NK4 and gefitinib overcame HGF-induced gefitinib resistance of lung cancer in a mouse model (Wang et al., 2009).

NK1 was identified as a product of an alternative spliced variant of HGF, similar to NK2, that consists of the N-terminal and first kringle (K1) domains (Cioce et al., 1996). NK1 binds Met and acts as a partial agonist in cell-based assays and transgenic mice (Lokker et al., 1994; Schwall et al., 1996). Biochemical and structural analysis indicated the following two points: 1) NK1 is responsible for the high affinity binding of HGF to the Met-Sema domain; and, 2) Met dimerization may be mediated by the NK1-NK1 dimer interface (Watanabe et al., 2002; Gherardi et al., 2006). Based on structural analysis, mutations designed to alter the NK1 dimer interface (Y124A, K85A, K85A/D123A, and K85A/N127A) abolish its ability to promote Met dimerization, but these mutated NK1s retain Met-binding activity (Rubin et al., 2001; Tolbert et al., 2007). These NK1 mutants act as Met antagonists by inhibiting HGF-mediated cell scattering, proliferation, and invasion (Gherardi et al., 2006; Rubin et al., 2001; Tolbert et al., 2007). Although it is yet to be determined if NK1 acts as an angiogenesis inhibitor, NK1 can be expected to exert anti-cancer action by inhibiting the HGF-Met pathway.

Single-chain HGF variants that are resistant to proteolytic activation exploit the requirement for processing machinery that converts pro-HGF to mature HGF. Indeed, uncleavable forms of HGF have been engineered by substituting single amino acids in the proteolytic site, and these engineered uncleavable HGFs suppress Met-driven tumor growth, metastasis, and angiogenesis in murine tumor models (Mazzone et al., 2004). Related antagonists consisting of two-chain HGF mutants exploit the mechanism by which proteolytic conversion allosterically stabilizes HGF–Met binding to promote kinase activation (Kirchhofer et al., 2007). Insertion of the newly formed N-terminus of the HGF β chain into the activation pocket stabilizes the interaction between the HGF β chain and Met. Full-length 2-chain HGF mutants engineered to interrupt these interactions efficiently inhibited HGF-mediated Met activation. Studies on the structure-function relationship of Met extracellular domains provided the development of Met-based biological HGF-Met inhibitor. A soluble Met-Sema domain is not only necessary for Met receptor association but is also essential for HGF binding, whereby the Sema domain inhibits HGF-dependent and -independent receptor phosphorylation and functional receptor activation (Kong-Beltran et al., 2004). In a mouse model, soluble Met Sema domain suppressed tumor growth and metastasis (Michieli et al., 2004).

Among different types of mAbs against HGF or Met, one anti-Met mAb decreases Met activation by inducing ectodomain shedding and degradation (Petrelli et al., 2006), while the others inhibit the binding of HGF to Met. Neutralizing mAb against human HGF, such as L2G7, AMG102 and SCH900105 (formerly AV299) inhibited HGF-dependent Met activation and the growth of tumor xenografts in mice (Petrelli et al., 2006; Kim et al., 2006; Jun et al., 2007). AMG102 is currently in phase I and II clinical trials (HYPERLINK "http://www.clinicaltrials.gov" www.clinicaltrials.gov) (Table 1). AMG 102 was well tolerated in humans and adverse events were predominantly low grade (Cecchi et al., 2011). SCH900105 was also well tolerated by patients in phase I trials. In its first completed trial, SCH900105 treatment was associated with a stabilizing of disease in half of the patients (Cecchi et al., 2011).

Classification and name	Target(s)	Stage	Clinical application
Small synthetic			
AMG 208	Met	Phase-I	advanced solid tumors
ARQ197	Met	Phase-I	advanced or metastatic solid tumors, healthy volunteers
		Phase-II	advanced pancreatic cancer, non-small cell lung cancer, unresectable hepatocellular carcinoma, advanced pancreatic cancer
BMS-777607	Met	Phase-I/II	advanced or metastatic solid tumors
E7050	Met, VEGFR2	Phase I	advanced solid tumors
GSK089 (GSK363089/XL880)	Met, Ron, VEGFR1-3, PDGFR, Kit, Flt-3, Tie-2	Phase-I	solid tumors, papillary renal carcinoma, liver cancer
		Phase-II	advanced or metastatic gastric cancer, papillary renal cancer, esophageal adenocarcinoma
JNJ38877605	Met	Phase-I	advanced or refractory solid tumors
MGCD265		Phase-I/II	advanced malignancies, non-small cell lung cancer
MK-2461	Met, Tie-2, Ron	Phase-I/II	advanced solid tumors
MK-8033	Met	Phase-I	advanced solid tumors
MP470	Met, Ret, Kit, PDGFR, Flt-3	Phase-I	solid tumors, metastatic solid tumors, lymphoma
PF-02341066	Met, Alk	Phase-I	advanced cancer, healthy volunteers
		Phase-II	carcinoma, non-small cell lung cancer
		Phase-III	carcinoma, non-small cell lung cancer
PF-04217903	Met	Phase-I	advanced cancer, neoplasms
SGX 523	Met	Phase-I	solid tumors, advanced cancer
XL184	Met, VEGFR1-3, Ret, Kit, Flt-3, Tie-2	Phase I	glioblastoma, advanced malignancies, solid tumors, lymphoma, thyroid cancer
		Phase-II	glioblastoma multiforme, non-small cell lung cancer
		Phase-III	medullary thyroid cancer
Antibody			
AMG102 (Anti-HGF)	HGF	Phase-I/II	advanced malignant glioma, prostate cancer, metastatic gastric and esophageal adenocarcinoma
		Phase-II	renal cell cancer, advanced malignant glioma
MetMab (Anti-Met)	Met	Phase-II	non-small cell lung cancer
SCH900105	HGF	Phase-I	advanced solid tumors, lymphomas, multiple myeloma

Table 1. HGF-Met inhibitors in clinical development.

mAbs against Met with different characteristics have been developed (Martens et al., 2006; Jin et al., 2008; van der Horst et al., 2009; Pacchiana et al., 2010). Anti-Met mAb, MetMab (formerly OA5D5), is a monovalent mAb that blocked binding of HGF to the Met (Martens

et al., 2006). Anti-Met mAb R13 and R28 synergistically inhibited HGF binding to MET and elicited antibody-dependent cellular cytotoxicity (van der Horst et al., 2009). The combination of R13/28 inhibited tumor growth in various colon tumor xenograft models. MetMab reduced Met phosphorylation, and this was associated with inhibition of orthotopic tumor growth and improvement of survival in a pancreatic xenograft model (Jin et al., 2008). MetMab is currently in phase I/II human clinical trials in comparison with erlotinib for patients with NSCLC (HYPERLINK "http://www.clinicaltrials.gov" www.clinicaltrials.gov).

5.2 Small synthetic kinase inhibitors

As small synthetic Met tyrosine kinase inhibitors, SU11274 and PHA665752 provided the basic notion that small synthetic Met tyrosine kinase inhibitors selectively inhibit Met activation and suppress tumor growth (Christensen ; 2003; Sattler et al., 2003; Berthou et al; 2004; Ma et al., 2005; Smolen et al., 2006). Subsequent research and development led to the discovery of various types of synthetic tyrosine kinase inhibitors with different structures, chemical properties, and target specificity. Based on the wealth of accumulated knowledge gained from the success of preclinical and clinical development of small synthetic tyrosine kinase inhibitors, more than 10 small synthetic Met tyrosine kinase inhibitors have been entered into clinical trials (Table 1).

PF-02341066 targets Met as well as anaplastic lymphoma kinase (ALK) (Sattler & Salgia, 2009). MP470 inhibits PDGFR, Kit, and Met tyrosine kinases. In combination with erlotinib, MP470 inhibited prostate cancer cell proliferation and tumor xenograft growth (Qi et al., 2009). E7050 targets both Met and VEGFR2 (Nakagawa et al., 2009). JNJ-38877605 shows a >1,000-fold selectivity for the Met kinase, compared to a >200-fold selectivity for related receptor tyrosine kinases (Eder et al., 2009). AMG 208 selectively inhibits both ligand-dependent and ligand-independent Met activation. BMS777607 has completed a phase I/II study in metastatic cancer patients (Schroeder et al., 2009). Phase I clinical trials were discontinued for SGX523 after renal toxicity was observed in patients receiving relatively low doses (HYPERLINK "http://www.sgxpharma.com" www.sgxpharma.com).

PF02341066 and XL184 have progressed the furthest of all Met inhibitors in clinical development. PF-02341066 has greater Met selectivity compared with PF-04217903 (Timofeevski et al., 2009). Preclinical studies indicate PF-02341066 is highly effective against the product of the EML4-ALK translocation found in a subset of NSCLC patients (Shaw et al., 2009). PF-02341066t is currently in phase I, II, and III clinical trials (HYPERLINK "http://www.clinicaltrials.gov" www.clinicaltrials.gov). XL184 targets Met, VEGFR2, and Ret. A current phase III trial is investigating the efficacy of XL184 as a first-line treatment, compared to a placebo, in patients with medullary thyroid cancer (HYPERLINK "http://www.clinicaltrials.gov" www.clinicaltrials.gov).

6. Conclusion and perspective

Breakdown of the extracellular matrix scaffold and the concomitant cellular migration, mitogenesis, and morphogenesis that is driven by the HGF-Met system makes way for the construction and reconstruction of tissues during development and wound healing. Perhaps because of this, tumor cells use the HGF-Met pathway as a machine particularly for their spreading, metastasis, and evasion of microenvironmental predicaments. Therefore, activation and inhibition of the HGF-Met pathway are likely to be therapeutic approaches

for the treatment of non-neoplastic diseases with tissue damage and for malignant diseases, respectively. HGF exhibits therapeutic effects for the protection and healing of tissues against tissue damage and pathology. Clinical trials using recombinant HGF or HGF gene drugs have been approved for the treatment of diseases with unmet needs.

Based on the basic knowledge of the significance of the HGF-Met pathway in tumor biology and pathology, during the last several years the one-to-one relationship between HGF and Met has facilitated the discovery and development of drug candidates that selectively inhibit HGF-Met in different ways. Preclinical and clinical development of drugs targeting HGF-Met will move into practice in the near future as new anticancer drugs. However, although drug discoveries in molecular-targeted cancer therapy have been beneficial for patients with malignancies, the appearance of persistent characteristics of malignant tumors in regard to resistance to anticancer therapies and drugs remains an obstacle to disease-free survival. The choice of the better, or best, way to inhibit HGF-Met signaling, i.e., ligand inhibition, receptor inhibition, biologics, mAb, or small synthetic, would gradually become clearer following clinical experiences.

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The book "Advances in Cancer Therapy" is a new addition to the Intech collection of books and aims at providing scientists and clinicians with a comprehensive overview of the state of current knowledge and latest research findings in the area of cancer therapy. For this purpose research articles, clinical investigations and review papers that are thought to improve the readers' understanding of cancer therapy developments and/or to keep them up to date with the most recent advances in this field have been included in this book. With cancer being one of the most serious diseases of our times, I am confident that this book will meet the patients', physicians' and researchers' needs.

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