Hepatocyte growth factor and Met in drug discovery

Review Article

Hepatocyte Growth Factor and Met in Drug Discovery

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Summary

Activation of the HGF-Met pathway evokes dynamic biological responses that support the morphogenesis, regeneration and survival of cells and tissues. A characterization of conditional Met knockout mice indicates that the HGF-Met pathway plays important roles in the regeneration, protection and homeostasis of cells such as hepatocytes, renal tubular cells, and neurons. Preclinical studies in disease models have indicated that recombinant HGF protein and expression plasmid for HGF are biological drug candidates for the treatment of patients with diseases or injuries that involve impaired tissue function. The phase-I and phase-I/II clinical trials of the intrathecal administration of HGF protein for the treatment of patients with amyotrophic lateral sclerosis and spinal cord injury, respectively, are ongoing. Biological actions of HGF that promote the dynamic movement, morphogenesis and survival of cells also closely participate in invasion-metastasis and resistance to the molecular-targeted drugs in tumor cells. Different types of HGF-Met pathway inhibitors are now in clinical trials for treatment of malignant tumors. Basic research on HGF and Met has lead to drug discoveries in regenerative medicine and tumor biology.

Two-Pronged Physiological Roles

Growth factors and their receptors play definitive roles in the development, regeneration and homeostasis of tissues. Aberrant regulation of the growth factor signaling in both positive and negative directions participates in the onset and progression of diseases and pathology. Based on the physiological and pathological significance of growth factors, both they and their receptors have been targets in drug discovery and development. Selective inhibitors against ligand growth factors, and their growth factor receptors, are currently indispensable in the molecular targeted therapy of malignant tumors.

Hepatocyte growth factor (HGF) was molecularly cloned as a mitogenic protein for hepatocytes (1, 2). The promotion of hepatocyte replication by HGF, and the increase in HGF levels following liver injury indicated its role in liver regeneration. Independently, the scatter factor, originally identified as a fibroblast-derived cell motility factor for epithelial cells, was shown to be an identical molecule to HGF (3). In 1991, the receptor for HGF was identified as a c-met proto-oncogene product of transmembrane receptor tyrosine kinase (4, 5). These early findings indicated the biological and physiological roles for HGF and Met in liver regeneration, tumorigenesis, and tumor invasion.

Characteristic biological responses that are driven by the HGF-Met receptor pathway include the dynamic movement, morphogenesis and survival of cells. The induction of epithelial branching tubulogenesis for renal tubular cells in a 3-dimensional collagen matrix by HGF had a particular impact, because HGF was the first of the growth factors and bioactive molecules to induce epithelial tubulogenesis/morphogenesis (6). Impairment in both the survival of hepatic progenitor cells and in the migration of myogenic precursor cells that is seen in the conventional knockout of *Met* gene in mice indicates potent actions of the HGF-Met pathway in dynamic migration and promotion of cell survival. Based on studies in the tissue-specific disruption of *Met* gene and the efficacy of HGF in preclinical disease models, recombinant HGF protein and HGF gene have become biological drug candidates for the treatment of patients with diseases that involve impaired tissue function (7). Conversely, the induction of dynamic 3-dimensional morphogenesis and migration and the promotion of cell survival by HGF also are the biological basis for the invasion and survival, respectively, of tumor cells. Activation of the HGF-Met pathway closely participates not only in tumor development, tumor invasion and metastasis but also in resistance to anticancer therapies (Fig. 1). Therefore, the development of drugs that inhibit the HGF-Met pathway has gained much attention in anticancer drug development (8−10).

Interaction between HGF and Met

HGF is a heterodimeric molecule composed of an α-chain and a β-chain, which are linked by one disulfide bridge (Fig. 2A). The α-chain contains 4 kringle domains, while the β-chain contains a serine protease-like structure (1, 2). HGF is secreted as a single-chain precursor HGF and the extracellular processing into a two-chain mature HGF is coupled to the activation of HGF. Serine proteases such as HGF-activator and matriptase are responsible for the processing of HGF (11). The Met receptor is composed of structural domains that include the extracellular Sema, PSI (a similar structure is found in the plexins, semaphorins and integrins) and IPT (a similar structure is found in the immunoglobulin-like fold shared by plexins and transcriptional factor) domains, the transmembrane domain, the intracellular juxtamembrane, and the tyrosine kinase domains. The Sema domain serves as a key element for ligand binding (12), while the involvement of IPT-3 and IPT-4 in the binding to HGF has been demonstrated by another approach (13).

HGF binds to Met through two different mechanisms: the α-chain binds with high affinity while the β-chain binds with low affinity. In the α-chain, the NK1 (the N-terminal and first kringle domains) 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 (14). When Met is occupied by the α-chain, the low-affinity binding of the β-chain induces the activation of Met and of biological responses. Hence, the α-chain is a high-affinity binding module to Met, while the β-chain is an activation module for Met, as well as a low-affinity binding module to Met. The structure of the extracellular complex between HGF and Met was proposed using structural analyses such as small-angle X-ray scattering (Fig. 2A) (12). In this structure model, two HGF molecules form the dimer, wherein NK1 domains provide a dimerization module for the HGF-Met heterotetramer complex. The structure of the complex between the β-chain of HGF and the Sema-PSI domains of Met was revealed by crystallographic analysis (Fig. 2B) (15, 16).

The Met tyrosine kinase domain follows a conserved bilobal protein kinase architecture with an N-terminal, β-sheet-containing domain linked through a hinge segment to the α-helical C lobe (Fig. 2C) (17, 18). A characteristic feature of Met is the presence of the C-terminal tail that contains tyrosine residues ([1349]YVHVNAT[1356]YVNV). The binding of HGF to the extracellular region of Met results in the receptor phosphorylation of multiple tyrosine residues within the cytoplasmic region. The phosphorylation of Tyr1234 and Tyr1235 within the tyrosine kinase domain positively regulates the catalytic activity of tyrosine kinase. 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). Gab1 is the most crucial substrate and adapter for the HGF-Met pathway, and the direct interaction of Gab1 with tyrosine phosphorylated Met is mediated by the Met-binding sequence in Gab1 (19).

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 the phosphorylated Y1003 of Met, and this Cbl binding results in Met ubiquitination, endocytosis and transport to the endosomal compartment, followed by degradation (20). Cbl-mediated degradation of the activated Met provides a mechanism that either attenuates or terminates Met-mediated signaling. Phosphorylation of Ser985 in the juxtamembrane domain regulates the activation status of Met upon HGF stimulation. Met-Ser985 is phosphorylated by protein kinase-C and is dephosphorylated by protein phosphatase-2A (21). In normal hepatocytes, HGF-dependent Met activation and mitogenesis were suppressed when Met-Ser985 was phosphorylated. In mice, Met-Ser985 phosphorylation was decreased after liver injury and was reciprocally associated with Met activation during liver regeneration. The phosphorylation of Met-Ser985 plays a regulatory role in Met activation in response to quiescence, injury and regeneration. (22).

Tissue Regeneration, Protection and Homeostasis Deduced from Met Disruption

The knockout mice of the *HGF* or *Met* gene in the whole body are lethal in the embryonic stage due to the impairment of organogenesis in the placenta and liver (23, 24). Moreover, HGF provides spatially defined chemoattractant-like motogenic signals for myogenic precursor cells—the migration of myogenic precursor cells from dermo-myotome in the somite to limb buds and the diaphragm is impaired in Met^{-/-} mice, which causes deformed skeletal muscles of the limb and diaphragm in mutant mice (25). These studies in conventional knockout mice have provided the biological roles of HGF and Met during embryonic development. On the other hand, characterizations of conditional Met knockout mice have indicated that the HGF-Met pathway plays important roles in regeneration, protection and homeostasis in various cells and tissues (Table 1).

Hepatocytes subjected to selective loss of the functional Met are highly susceptible to cell death even after mild liver injury, indicating that anti-apoptotic activity of HGF plays a role in protecting the liver (26). Liver- or hepatocyte-specific Met^{-/-} mice show delayed liver

regeneration associated with persistent inflammatory reaction (28). Activation of Met plays a role in the persistence of Erk1/2 activation and in the regulation of the G2/M gene expression program throughout liver regeneration following partial hepatectomy (27). In addition to regenerative responses in mature hepatocytes, HGF-Met signaling supports the *in vitro* sphere formation of hepatic stem cells (oval cells) and *in vivo* hepatic stem cell-mediated regeneration (32). Met-deficient oval cells were more prone to apoptosis when exposed to proapoptotic conditions. After bile duct ligation, hepatocyte-specific Met^{-/-} mice were more susceptible to chronic inflammation and fibrotic change compared with control mice (30). The effects shown by these liver- or hepatocyte-specific Met^{-/-} mice indicate the physiological roles of the HGF-Met pathway in the protection, regeneration, anti-inflammation, and anti-fibrosis of the liver. Thus, HGF seems to be a hepatotrophic factor that plays a major role in liver regeneration.

Loss of functional *Met* in renal tubules led to no appreciable defect in renal function. However, the tubular cell-specific Met^{-/-} mice displayed higher serum creatinine, more severe morphologic lesions, and increased apoptosis, compared to control mice when both were subjected to renal injury (33). In podocyte-specific Met^{-/-} mice, no pathology was seen, whereas, the mice developed more severe podocyte apoptosis and albuminurea in comparison with control mice, when both were subjected to toxic renal injury for podocytes (34). Collecting duct-selective Met dysfunction indicated that there were trends toward increased interstitial fibrosis, infiltration of the interstitium, and acute tubular necrosis following a unilateral obstruction, and there was a reduced regenerative response after the release from the obstruction (35).

Disruption of the *Met* gene in epidermal keratinocytes demonstrated an indispensable role for the HGF-Met pathway in skin wound healing (37). Because the migration of keratinocytes post-wounding was almost completely impaired in Met^{-/-} keratinocytes, re-epithelialization was severely suppressed. Wound closure occurred exclusively in a few keratinocytes that had escaped recombination, indicating that the skin wound process selected and amplified residual cells that express a functional Met. These results indicated a definitive role for the HGF-Met pathway in skin wound healing. In mice with *Met*-deficient dendritic cells, those cells failed to reach skin-draining lymph nodes upon activation while exhibiting an activated phenotype, and the contact hypersensitivity reactions in response to contact allergens were significantly impaired (44). HGF-Met signaling in cutaneous dendritic cells may play a critical role in maintaining normal immune function.

Conditional knockout mice with selective disruption of *Met* gene in pancreatic β-cells displayed significantly diminished glucose tolerance and reduced plasma insulin after a

glucose challenge (39). *In vitro* glucose-stimulated insulin secretion in the islets from β-cell-Met$^{-/-}$ mice was decreased by ~50% compared with control islets. These changes in β-cell function in the conditional Met knockout mice were not accompanied by changes in total β-cell mass, islet morphology, or β-cell proliferation (39). Another group using β-cell-Met$^{-/-}$ mice displayed mild hyperglycemia and a complete loss of acute-phase insulin secretion in response to glucose (38). Therefore, HGF-Met signaling in the β-cell is not essential for β-cell growth, but it is essential for normal glucose-dependent insulin secretion and glucose homeostasis.

A number of allelic or copy variants of the *Met* gene have been associated with autism-spectrum disorders (45). One variant reduced *Met* promoter activity, implicating reduced *Met* gene expression in autism susceptibility (46). Conditional Met deletion in dorsal pallially derived forebrain neurons in mice demonstrated changes in dendrite and dendritic spine morphology in forebrain neurons (41), as well as circuit-specific intracortical hyperconnectivity (42). These studies demonstrated the contribution of Met signaling to the establishment of proper circuit function in the neocortex, which is potentially relevant to autism-spectrum disorders.

Collectively, tissue-specific disruption of the functional *Met* in mice indicated that HGF plays a promoting role in the regeneration, protection, and homeostasis of tissues, but an inhibitory role that can progress to chronic inflammation and fibrosis. Thus, enhancement or proper regulation of Met-mediated signaling and biological responses is likely to be therapeutic for the treatment of different types of diseases.

HGF as a Biological Drug Candidate

Therapeutic actions and their mechanisms have been studied in a variety of disease models in experimental animals (7). Here, the clinical studies, and development of recombinant HGF protein and HGF gene as drug candidates are described.

Chronic leg ulcer

Chronic leg ulcer treatment in elderly patients is a widespread healthcare problem. Chronic leg ulcers can result from different causes, such as diabetes and/or inappropriate circulation, and can be very difficult to heal. Involvement of the HGF-Met pathway in the pathophysiology of chronic leg ulcer might be considered by evaluating the status of Met activation in tissues with pathology. In clinical studies focused on cutaneous healing and non-healing wounds, the phosphorylation of Met was most prominent in keratinocytes and dermal cells in normally healing wounds. However, the tyrosine phosphorylation of Met was

barely detectable in non-healing wounds, which suggested reduced Met activation (47).

It is notable that the disruption of functional Met in epidermal keratinocytes indicated that Met-deficient keratinocytes were unable to contribute to the re-epithelialization of skin wounds in mice (37). Therefore, activation of the HGF-Met pathway is essential for a fundamental regenerative process during skin wound healing and may not be substituted for by other bioactive molecules for the generation of hyperproliferative epithelium in skin. In a full-thickness cutaneous excision model in diabetic mice, the topical administration of recombinant HGF protein promoted angiogenesis, extracellular matrix remodeling, re-epithelialization, and wound closure (48, 49).

The first clinical study using recombinant human HGF protein was done to investigate the physiological and therapeutic effects of HGF on chronic leg ulcers. HGF in gel form was locally applied to chronic leg ulcers in 11 patients (50). This pilot study demonstrated excellent (84–100% area reduction) or partial healing (58–59%) in eight out of 11 patients. The microcirculatory perfusion was significantly correlated to the reduction of ulcerous areas in the treated ulcers. This study suggests that the topical application of HGF protein may facilitate the healing of chronic leg ulcers, possibly by improving microcirculation. Because no control group was included in this pilot study, proper control studies must be performed for definitive clinical evaluation.

Critical limb ischemia and HGF gene drugs

Critical limb ischemia is the most severe form of peripheral arterial disease due to atherosclerosis, and is a common clinical problem that has no effective medical therapy. The standard therapy for critical limb ischemia remains to be either lower-extremity revascularization through open bypass surgery or by endovascular techniques, or lower-extremity amputation when revascularization is not an option. Obviously, there is great need for less-invasive therapies to improve limb perfusion in patients with critical limb ischemia.

Met receptor is expressed in different types of vascular endothelial cells, and HGF stimulates the migration and motility of different types of endothelial cells (51−53). Further studies have indicated that HGF supports angiogenesis through multiple mechanisms, targeting not only endothelial cells but also endothelial progenitor cells. In preclinical animal models for limb ischemia, the intramuscle administration of recombinant HGF protein or expression plasmid for HGF facilitated collateral new vessel formation, improved blood flow, and reduced muscle atrophy (55, 56). Thus, HGF is a powerful angiogenic growth factor that is applicable for therapeutic purposes.

The first clinical study of HGF gene therapy by naked expression plasmid was done to investigate its safety for the treatment of patients with arteriosclerosis obliterans or Buerger's disease (57). Subsequently, a multicenter, randomized, double-blind, placebo-controlled clinical trial was performed for the treatment of patients with critical limb ischemia to evaluate the efficacy and safety of HGF gene therapy using naked plasmid (58, 59). This HGF gene therapy was proven safe and effective for critical limb ischemia. Phase-II and Phase-III clinical trials of HGF gene therapy for the treatment of peripheral arterial disease have been completed in the USA and in Japan, respectively.

Hepatitis and acute kidney injury

Conditional *Met* gene disruption in hepatocytes has indicated that hepatocytes were highly susceptible to apoptosis even in mild injury, and the regenerative response in the liver was retarded when mice were subjected to liver injury. In preclinical models for fulminant hepatitis, systemic administration of recombinant HGF suppressed the onset of fulminant hepatitis (60). In a similar manner, conditional Met disruption in mature renal cells indicated mice had no appreciable defect in renal function, whereas they were susceptible to severe renal dysfunction and pathology when they were subjected to renal injury. In preclinical animal models for acute kidney injury, systemic administration of recombinant HGF suppressed tubular cell apoptosis and renal dysfunction, and promoted regenerative cell proliferation (7). HGF protein may be applicable to the treatment of patients with acute hepatitis or acute kidney injury.

The phase-I clinical trial of the systemic administration of recombinant HGF protein was completed in Japan and in the USA. Phase I/II clinical trials indicated that intravenous administration of HGF protein was well tolerated in patients with fulminant hepatitis (61).

Amyotrophic lateral sclerosis (ALS) and spinal cord injury

ALS is a fatal neurodegenerative disease characterized by a progressive loss of motor neurons and a degeneration of motor axons. Approximately 5–10% of patients have familial ALS, and of those ~15–25% carry a mutation(s) in the gene encoding Cu^{2+}/Zn^{2+} superoxide dismutase (SOD1). Neurons with a transgenic expression of mutant SOD1 develop the typical deficits found in both familial and sporadic ALS. Because motoneuronal degeneration is thought to be a primary event in disease progression, treatment approaches have focused on promoting the survival, or at least preventing the death, of motor neurons. In addition to motor neurons, since a reduction in the astrocyte-specific glutamate transporter has been found in ALS patients, astrocytes also seem to be potential targets for ALS therapy.

HGF functions as a neurotrophic factor for a variety of neurons, including the hippocampal, cerebral cortical, midbrain dopaminergic, motor, sensory, sympathetic, parasympathetic, and cerebellar granule neurons (62). The first implication that HGF might be a therapeutic agent for the treatment of patients with ALS was obtained by transgenic over-expression of HGF in the nervous system in mouse model for ALS with mutant SOD1 (63). Neuronal overexpression of HGF has attenuated motor neuron death and axonal degeneration and prolonged the life span of ALS model mice. HGF expression maintained the levels of astrocyte-specific glutamate transporter in reactive astrocytes. HGF seems to alleviate the symptoms of ALS by reducing glutamatergic neurotoxicity through direct neurotrophic activities on motor neurons and indirect activities on astrocytes.

Human recombinant HGF was continuously administered by intrathecal delivery to transgenic rat models of ALS with mutant SOD1 at 100 days of age (pathologic changes, but animals showed no clinical weakness) and at 115 days (onset of paralysis) for 4 weeks each. Intrathecal administration of HGF attenuated motor neuron degeneration and prolonged the duration of the life span, even with administration from the onset of paralysis (64). The phase-I clinical trial for the intrathecal administration of recombinant HGF protein for treatment of patients with ALS has been conducted at the Tohoku University in Japan.

Spinal cord injury is followed by secondary degeneration, which is characterized by progressive tissue necrosis. Many therapeutic interventions using neurotrophic factors or pharmacological agents have focused on secondary degeneration after spinal cord injury to reduce damaged areas and promote axonal regeneration and functional recovery. The therapeutic action of recombinant HGF protein was tested in a primate (common marmoset) model of contusive cervical spinal cord injury (65). Intrathecal HGF administration preserved the intact spinal cord parenchyma, corticospinal fibers, and myelinated areas, thereby promoting functional recovery. HGF-treatment did not give rise to an abnormal outgrowth of calcitonin gene-related peptide positive fibers compared with that seen in the control group, indicating that this treatment neither induced nor exacerbated allodynia. The phase-I/II clinical trial for the intrathecal administration of recombinant human HGF protein for the treatment of patients with spinal cord injury is ongoing at the Keio University in Japan.

Tumor Microenvironment and Drug Resistance

Invasion and metastasis

The crucial role of stromal fibroblasts in the invasion of cancer cells through 3-D collagen was first demonstrated using human oral squamous cell carcinoma (66), suggesting

the important role of stromal cells in the dynamic invasion of cancer cells in a tumor microenvironment. Independently, as scatter factor, a fibroblast-derived cell motility factor for epithelial cells, is the same molecule to HGF (3), the migration and invasion of different types of cancer cells were markedly enhanced by HGF (67). HGF increases extracellular protease expression coupled with the dissociation of cancer cells and their motility, which promotes invasion in 3-D extracellular matrices and subsequent metastasis. HGF-Met signaling induces the transition of epithelial to mesenchymal cell types (68, 69). The profound action of HGF on cancer invasion has been demonstrated in a variety of cancer cells, and HGF has been established as an important mediator in the tumor-stromal interaction that affects the malignant behavior of cancer (67). Experimental activation of the HGF-Met pathway facilitates metastasis of different types of tumors, and aberrant activation of the HGF-Met pathway has been noted in a variety of human tumors (70, 71). The HGF-Met pathway has become a hot research and development target of molecular targeted therapy for cancer, particularly in attempts to inhibit cancer invasion and metastasis (72).

Drug resistance

The discovery and application of oncoprotein-targeted drugs has improved cancer drug therapy. Mutationally activated protein kinases, particularly growth factor receptor tyrosine kinases and their downstream signaling kinases, define a clinically validated class of targets in the molecular targeted therapy of cancer. However, the efficacy of these inhibitors in patients whose tumors harbor mutated oncogenic protein is invariably limited by innate or acquired drug resistance. Elucidation of the mechanisms by which tumor cells acquire resistance to molecular targeted drugs both before and after treatment is now a very popular focus of research subject to overcome the theme of recurrence.

Gefitinib and erlotinib, selective inhibitors for EGFR tyrosine kinase, have shown favorable responses in non-small cell lung cancer (NSCLC), particularly those expressing activating mutations in EGFR (73, 74). Patients with EGFR mutant NSCLC had superior outcomes with gefitinib treatment, compared with standard first-line cytotoxic chemotherapy (75, 76). However, almost without exception, the patients developed acquired resistance to EGFR tyrosine kinase inhibitors within several years (77). Furthermore, 20–25% of the patients with EGFR-activating mutations showed intrinsic resistance to EGFR tyrosine kinase inhibitors.

Different mechanisms are known to participate in resistance to EGFR tyrosine kinase inhibitors in NSCLC with activating EGFR mutants. The T790M second mutation occurs in about half of all patients with acquired resistance to gefitinib or erlotinib (78). Involvement of

Met activation caused by the *Met* gene amplification (79) and HGF-dependent Met activation (80) have been noted as mechanisms by which NSCLC acquires resistance to EGFR tyrosine kinase inhibitors. *Met* gene amplification was detected in ~20% of patients with acquired resistance to gefitinib or erlotinib (81). Overexpression of HGF 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 (80−82). HGF can be produced by both cancer cells and host stromal cells, such as fibroblasts, within a tumor microenvironment. Collectively, the expression of HGF in cancer cells and/or host stromal cells closely participated in the resistance to EGFR tyrosine kinase inhibitors in NSCLC, even with *Met* gene amplification.

Malignant melanomas harboring a mutant *BRAF* gene encoding BRAF-V600E show marked responses to BRAF inhibitors. However, these responses are usually partial, and the tumors often recur within 6 months of treatment. Among growth factors, the importance of HGF in the tumor microenvironment to the innate or acquired resistance of tumor cells against BRAF inhibitor has been noted (83). That study was based on a hypothesis that the resistance to anticancer drugs might be influenced by the tumor microenvironment, and the growth/survival of a variety of tumor cells in the presence of anticancer drugs was compared for two conditions: when tumor cells were cultured alone or when tumor cells were co-cultured with a variety of stromal cells. A comprehensive analysis demonstrated a significant resistance of tumor cells conferred by the interaction with stromal cells. In particular, HGF was identified as a key factor that is secreted from stromal cells and confers resistance against BRAF inhibitors. Increases in the stromal expression of HGF in malignant melanoma tissues were correlated with a poor response to the BRAF inhibitor vemurafenib (83).

Cancer stem cells

The concept of a cancer stem cell (or tumor initiating cell) proposes that tumors are organized in a cellular hierarchy, and they are maintained by a subpopulation of cells displaying stem cell characteristics such as self-renewal and differentiation that contributes to cellular heterogeneity and tumor bulk. The HGF-Met pathway plays an important role in regulating the self-renewal (stemness) and invasiveness of cancer stem cells (84−86).

Glioblastoma is an aggressive tumor that is associated with high morbidity, mortality, and recurrence. In a glioblastoma model, cancer stem cells aggressively invaded surrounding normal brain tissues, whereas tumor cells hierarchically descendant from tumor-initiating cells were not invasive and were located in the center of the tumor tissue (84). Microarray

analysis was used to analyze the difference in gene expression profiles between brain tumor-initiating cells and non-tumor-initiating cells. The results indicated that Met expression was much higher in tumor-initiating cells. It is important to note that strong *in situ* Met tyrosine phosphorylation was seen in the population of the cancer stem cells, but not in the population of their descendant cells. HGF enhanced invasion of the brain tumor-initiating cells in 3-D collagen gel culture. Extensive gene expression profiling study using the cultures of neurospheres obtained from patients with glioblastoma tissues indicated that in Met-positive neurospheres the subpopulation of cells that express high levels of Met display clonogenic potential and long-term self-renewal ability along with enhanced growth kinetics (85). HGF sustained the proliferation, clonogenicity, expression of self-renewal markers, migration, and invasion of Met-positive neurospheres. Thus, Met is likely to be a functional marker of glioblastoma stem cells.

Wnt/β-catenin signaling is crucial in maintaining stemness in normal colon cells and is a common pathway that is deregulated in most colon cancers. Despite the presence of mutations in APC or β-catenin, which are generally understood to constitutively activate the Wnt signaling cascade, colorectal cancers show cellular heterogeneity. In colon adenocarcinomas, high activity of the Wnt pathway was observed preferentially in tumor cells located close to stromal myofibroblasts, indicating that Wnt activity and cancer stemness might be regulated by extrinsic cues (86). Myofibroblast-derived factors, specifically HGF, activated Wnt/β-catenin signaling and subsequently the clonogenicity of colon cancer stem cells. Therefore, together with Wnt activity, HGF plays a role in maintaining the stemness of colon cancer stem cells through tumor-stromal interaction in the microenvironment.

HGF-Met inhibitors in Clinical 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, monoclonal antibodies, soluble forms of Met, and antagonists composed of selected domains in HGF (e.g., NK4). Among them, only the development of small synthetic Met tyrosine kinase inhibitors and humanized monoclonal antibodies against HGF or Met has progressed to clinical trials.

Based on the wealth of accumulated knowledge gained from the success of the preclinical and clinical development of small synthetic tyrosine kinase inhibitors, several small synthetic Met tyrosine kinase inhibitors have entered clinical trials (Table 2). Crizotinib was originally developed as a Met tyrosine kinase inhibitor. It also strongly inhibits anaplastic lymphoma

kinase (ALK), which makes it a dual inhibitor for both ALK and Met (54). Crizotinib binds to a catalytic pocket in the tyrosine kinase domain of the Met in an ATP-competitive manner (54) (Fig. 3A). Because the fusion oncogene *EML4-ALK* has been effective for a distinct clinicopathologic subset of patients with non-small cell lung cancer, crizotinib has been approved for the treatment of the patients who harbor the *EML4-ALK* fusion gene.

The development of humanized antibody drugs, anti-HGF, and anti-Met antibodies have also entered the clinical trial stage. Onartuzumab is a monovalent mAb that binds to Sema-PSI domains in Met (Fig. 3B). The original bivalent monoclonal antibody was used for the subsequent discovery of onartumab bound to Met and exhibited agonistic action via receptor dimerization (16). Onartuzumab blocks the binding of HGF to Met and binds to Met without dimerization, thereby inhibiting HGF-dependent Met activation. Rilotumumab (AMG102) and ficlatuzumab (AV-229) are humanized monoclonal antibodies against HGF.

Several small synthetic Met tyrosine kinase inhibitors are multi-target inhibitors, rather than monospecific inhibitors, while antibody drug candidates are monospecific. The characteristic of multi-target specificity may have an advantage in certain types of cancers wherein the co-activation of target kinases is an addictive key signal for their growth and survival. From past clinical experiences using small synthetic kinase inhibitors, resistant cancers appear within a certain period of time in most cases. Because mutations in the kinase domain structurally confer resistance to kinase inhibitors, the extracellular inhibition of HGF-Met protein-protein interaction by antibody drug candidates may have a potential advantage over small synthetic kinase inhibitors during the period marked by the emergence of resistant forms of cancer. The characteristics of the therapeutic effects from each of the HGF-Met inhibitors will be defined in further clinical trials.

Artificial Small HGF

Cyclosporin A, originally isolated from the fungus *Tolypocladim inflatum*, is an orally-bioavailable immunosuppressant bears a highly modified peptide scaffold and its unique chemical characteristics confer drug-like properties, including affinity and specificity to target molecule and serum stability (resistance to proteases and peptidases). Novel technologies to identify peptides or peptide-like molecules have tremendous potential to revolutionize the speed of drug discovery. Random nonstandard Peptides Integrated Discovery (RaPID) system is originally developed as a novel technology to identify macrocyclic peptides that bind to target molecules with very high affinity and selectivity (87).

Very recent study identified macrocyclic peptide capable of activating Met (88).

Macrocyclic Met-binding peptides were identified using RaPID system. These peptides have neither activate Met nor inhibit HGF-induced Met activation. Dimerization of these peptides conferred them Met agonistic activity. Dimerized macrocyclic peptides selectively activate Met and exert Met-mediated typical and unique biological activities mitogenic, motogenic (enhancement of cell motility), and morphogenic (induction of blanching tubulogenesis) activities. The maximal activities of dimerized macrocyclic peptides are indistinguishable to HGF. Extracellular binding of the dimerized macrocyclic peptides to the Met induce dimerization of Met, and such structure in turn seems to give an appropriate length and/or angle for trans-phosphorylation of tyrosine residues between two tyrosine kinase domains (Fig. 4). The method taken in this study is adaptable to generate artificial "non-protein" growth factors structurally unrelated to its original protein. This work suggests alternative ways of developing non-protein regenerative medicines for broad applications in future.

Conclusion

After experimental partial hepatectomy in mice and rats, for which two-thirds of the liver is removed, the residual liver tissue enlarges to make up for the mass of the removed tissue within a week or so. This mysterious phenomenon has attracted much attention from scientists. Impaired regeneration of the liver has been noted in mice subjected to conditional knockout of certain types of genes, including the *Met* gene. By these approaches, our understanding of liver regeneration has definitively deepened. It is reasonable to assume that HGF plays a humoral hepatotrophic role in liver regeneration, which has been suggested by the results from early studies. Gene disruption approaches have defined irreplaceable roles for HGF in the regeneration, protection, and homeostasis of different tissues. As supplemental administration of bioactive peptides or cytokines has become indispensable in the treatment of certain types of diseases, the administration of HGF at the appropriate time and dose is expected to exhibit therapeutic action in patients with impaired tissue function. The dynamic and potent actions of HGF to promote cell movement, morphogenesis, and survival—the original functioning of tissue regeneration and protection—also closely participate in the malignant behaviors of tumors. Inhibition of the HGF-Met pathway has gained much attention in anticancer drug discovery, and HGF-Met inhibitors with different modes of action are either currently under clinical development or are already being marketed. Both basic and applied research on HGF-Met, which is connected to drug discovery, holds great significance.

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Conflict of Interest

K. Matsumoto has acted as a scientific adviser and owns stock in Kringle Pharma, Inc. No other conflicts are declared.

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Table 1. Physiological roles of HGF deduced from conditional knockout mice.

Met$^{-/-}$ tissue/cell types	characteristics	references
Liver		
hepatocytes	- highly susceptible to apoptosis after liver injury - impairment in recovery from liver necrosis after liver injury - impairment in Erk1/2 activation and G2/M transition after liver injury	26 27
hepatocytes	- steatotic change of the liver in aged mice - decrease in mitotic hepatocytes after partial hepatectomy - delayed regeneration after partial hepatectomy	28
hepatocytes	- promoted liver fibrosis after liver injury - extensive necrosis and lower proliferation of hepatocytes after bile-duct ligation - enhanced susceptibility to liver fibrosis	29 30
oval cells	- decrease in oval cell viability - more prone to apoptosis - reduction in oval cell pool - impairment in migration and differentiation into hepatocytes	31 32
Kidney		
tubular cells	- no appreciable defect in kidney morphology and function - aggravated renal injury and inflammation after acute kidney injury	33
podocytes	- neither albuminuria nor overt pathologic lesions - severe podocyte injury and apoptosis, and albuminuria after toxic injury	34
collecting duct	- increased fibrosis and tubular necrosis after unilateral ureteral obstruction - reduced capacity in regeneration after release of the obstruction	35
ureteric bud	- double knockout of Met and EGF receptor in ureteric bud	36

| | - decrease in branching and a reduction in final glomerular number | |

Skin

keratinocytes
- lack of keratinocyte migration after skin wound 37
- severe impairment epidermal wound closure

Pancreas

β-cell
- mild hyperglycemia, and decreased serum insulin levels at 6 months 38
- loss of acute-phase insulin secretion in response to glucose
- impaired glucose tolerance
- diminished glucose tolerance 39
- reduced plasma insulin after a glucose challenge
- normal glucose and β-cell homeostasis 40
- susceptible to streptozotocin-induced diabetes

Nervous

dorsal pallial
forebrain neurons
- increases proximal and reduces distal apical dendritic branching of 41
  neocortical pyramidal neurons in post-pubertal period
- reduced volume of cortical tissue
- increase in spine head volume, but no change in density of spines
- hyperconnectivity in circuit-specific intracortical neurons 42

Heart

cardiomyocytes
- normal heart development 43
- cardiomyocyte hypertrophy and interstitial fibrosis by 6 months
- systolic cardiac dysfunction by 9 months

Immune system

dendritic cells
- impaired emigration toward draining lymph nodes upon 44
  inflammation-induced activation
- impaired contact hypersensitivity reaction to contact allergens

Table 2. HGF-Met inhibitors in clinical development*

Classification / name	Target(s)	Stage	Clinical application
Small synthetic			
AMG 208	Met	phase-I	advanced solid tumors
BMS-777607	Met	phase-I/II	advanced/metastatic gastroesophageal cancer, head and neck squamous cell carcinoma, type I papillary renal cell carcinoma
E7050	Met, VEGFR2	phase-II	advanced solid tumors, recurrent glioblastoma, unresectable stage III/IV melanoma
Foretinib (GSK1363089)	Met, Ron, VEGFR1-3, PDGFR, Kit, Flt-3, Tie-2	phase-II	head and neck recurrent/metastatic squamous cell carcinoma, advanced/metastatic gastric cancer
		phase-I/II	HER2-positive metastatic breast cancer
Amuvatinib (MP470)	Met, Ret, Kit, PDGFR, Flt-3	phase-II	small cell lung cancer
MGCD265	Met	phase-I	advanced malignancies, non-small cell lung cancer
MK-2461	Met, Tie-2, Ron	phase-I/II	advanced solid malignancies
Crizotinib (PF-02341066)	Alk, Met	phase-II	stage III non-small cell lung cancer, advanced and/or metastatic type I papillary renal cell carcinoma, advanced and/or metastatic large cell lymphoma, etc.
		phase-I/II	relapsed/refractory solid malignancies
		approved	Alk-mutated non-small cell lung cancer

Cabozantinib (XL184)	Met, VEGFR1-3, Ret, Kit, Flt-3, Tie-2	phase-I	metastatic colorectal cancer (KRAS wild type)
		phase-II	glioblastoma multiforme, plexiform neutofibromatosis, advanced chollangiocarcinoma, carcinoid/pancreatic neuroendocrine tumor, metastatic castrate-resistant prostate cancer, non-small cell lung cancer, metastatic endometrial cancer
		phase-III	hepatocellular carcinoma
		approved	progressive metastatic medullary thyroid cancer
Antibody			
Rilotumumab (AMG102)	HGF	phase-I/II	advanced malignant glioma, prostate cancer, metastatic gastric and esophageal adenocarcinoma
		phase-II	advanced gastroesophageal adenocarcinoma, advanced malignant glioma, advanced renal cell carcinoma
		phase-II/III	non-small cell lung cancer
		phase-III	gastroesophageal junction adenocarcinoma, gastric cancer
Onartuzumab	Met	phase-I	advanced hepatocellular carcinoma
		phase-II	non-small cell lung cancer, metastatic colorectal cancer
		phase-III	advanced and/or metastatic non-small cell lung cancer, metastatic gastroesophageal cancer
Ficlatuzumab (AV-229)	HGF	phase-I	head and neck squamous cell carcinoma
		phase-II	non-small cell lung cancer

*For more current and details: http://clinicaltrials.gov/ct2/home
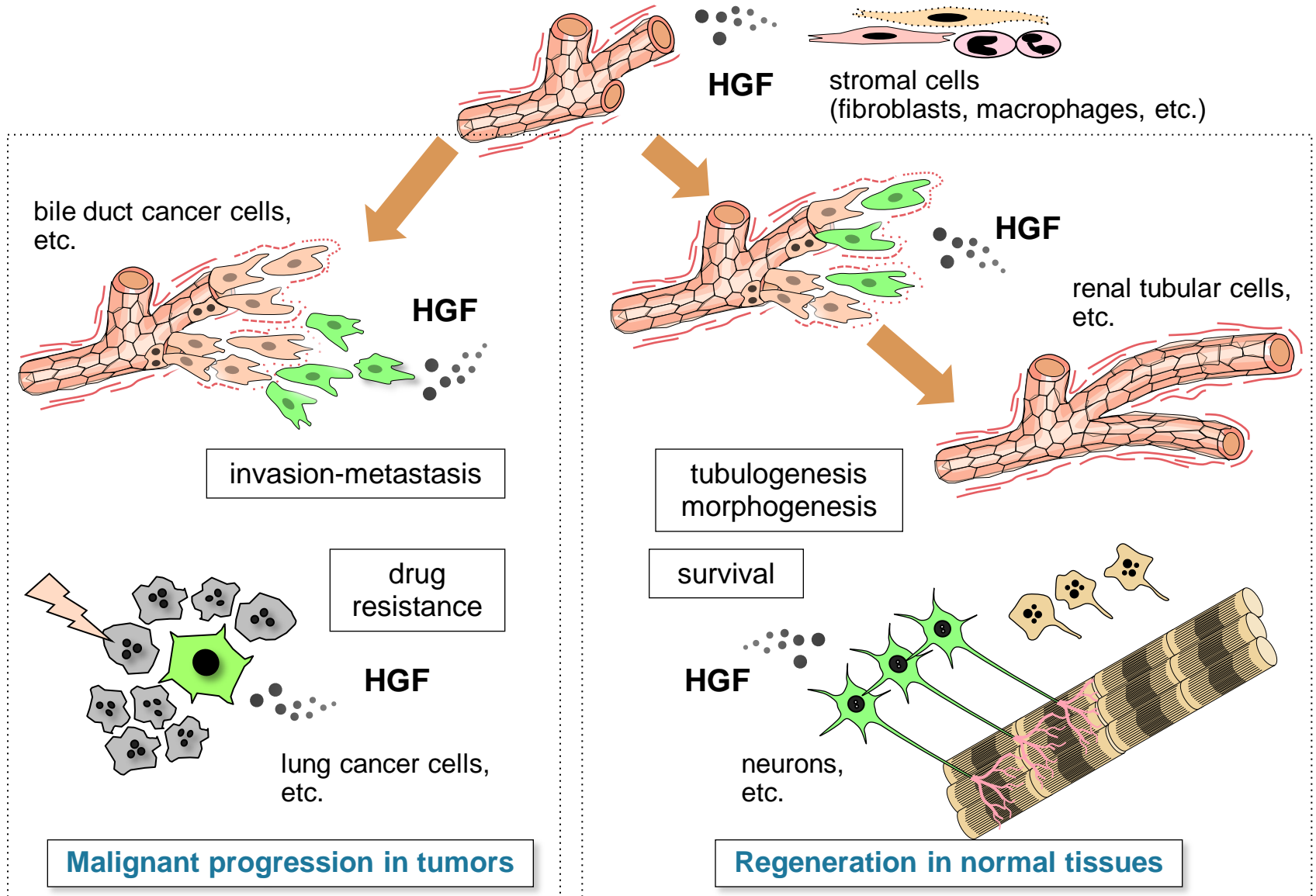
Figure legends

Fig. 1. Two-pronged roles of HGF. Dynamic blanching morphogenesis (e.g., in renal tubular cells) and promotion of cell survival (e.g., in neurons) mediated by the HGF–Met pathway play roles in tissue regeneration and protection after injury (right part). In tumor tissues, however, similar biological activities, i.e., dynamic cell movement and survival, promoted by Met activation also participate in invasion-metastasis and drug resistance (left part). Cells responding to HGF are conceptually shown in green.
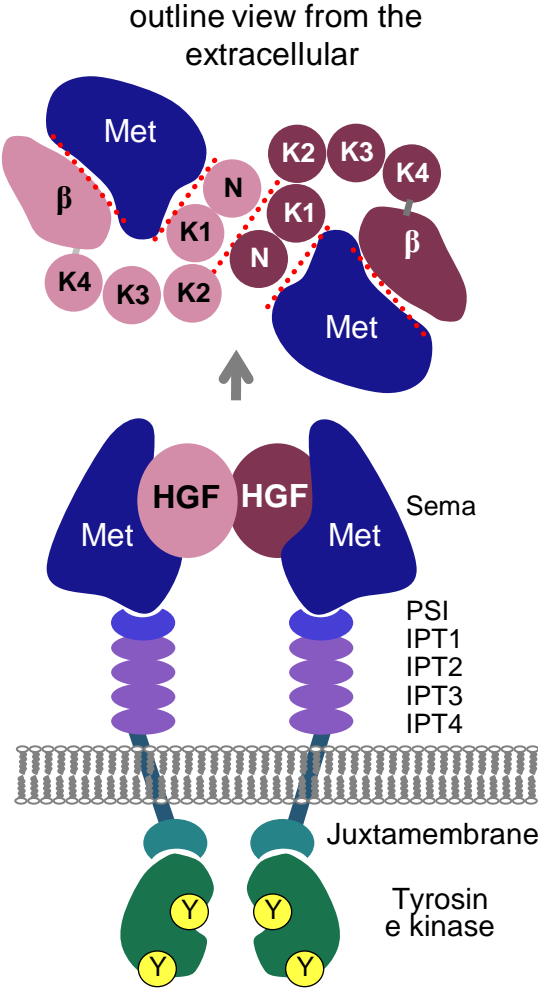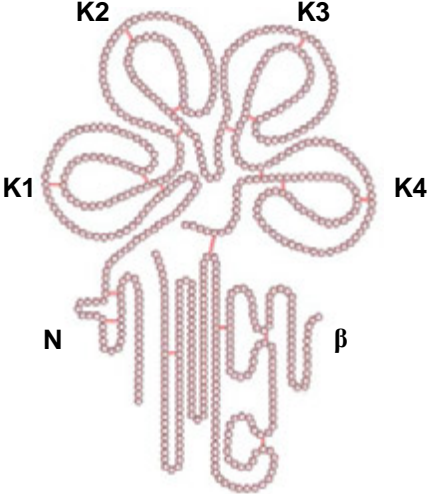
Fig. 2. Structural basis for HGF-Met interactions and Met activation. (**A**) Outline structures of HGF (left) and extracellular interaction between HGF and Met (right). The outline structure for extracellular interaction between HGF and Met was proposed by analysis using small-angle x-ray scattering and cryoelectron microscopy (12). (**B**) Crystal structure for association between the β-chain of HGF and Sema-PSI domains in the Met (16) (PDB: 4K3J). (**C**) Crystal structure for Met tyrosine kinase and a part of the juxtamembrane domain (17, 18) (PDB: 1R0P and 2G15). In the ribbon drawing (upper), structural change of the activation loop occurs between the active status and inactive status shown in red and blue, respectively. Structural change in the catalytic hollow for tyrosine kinase is shown in the surface drawing (lower).

Fig. 3. Structural basis for HGF-Met inhibitors in clinical development. (**A**) Crystal structure for binding of crizotinib (PF-02341066), a dual tyrosine kinase inhibitor for Alk and Met, in the Met tyrosine kinase domain (54) (PDB: 2WGJ). (**B**) Crystal structure for association between Sema-PSI domains of Met and onartumab, a monovalent monoclonal antibody against Met (16) (PDB: 4K3J). Comparison of structures for association between Sema-PSI domains and the β-chain of HGF (Fig. 2B) or onartumab indicate onartumab and the β-chain of HGF bind to opposite positions in Met.

Fig. 4. Conceptual structure for Met receptor activation by the dimerized macrocyclic peptide.

Fig. 1

HGF

stromal cells (fibroblasts, macrophages, etc.)

bile duct cancer cells, etc.

HGF

invasion-metastasis

drug resistance

HGF

lung cancer cells, etc.

Malignant progression in tumors

HGF

renal tubular cells, etc.

tubulogenesis morphogenesis

survival

HGF

neurons, etc.

Regeneration in normal tissues

A

outline view from the extracellular

K2   K3

K1   K4

N   β

Met

β

N

K1   K2   K3   K4

K4   K3   K2   N   K1   β

Met

HGF   HGF   Sema

Met   Met

PSI
IPT1
IPT2
IPT3
IPT4

Juxtamembrane

Y   Y   Tyrosine kinase

Y   Y

B

C

Fig. 3

A

B

Fig. 4

Met

Artificial Met-agonist
by cyclic peptide