Hepatocyte growth factor twenty years on: Much more than a growth factor

doi: 10.1111/j.1440-1746.2010.06549.x

Hepatocyte Growth Factor Twenty Years on: Much More than a Growth Factor

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4,999 words
74 references
3 tables
4 figures

Summary

Liver regeneration depends on the proliferation of mature hepatocytes. In the 1980s, the method for the cultivation of mature hepatocytes provided an opportunity for the discovery of hepatocyte growth factor (HGF) as a protein that is structurally and functionally different from other growth factors. In 1991, the scatter factor, tumor cytotoxic factor, and 3-D epithelial morphogen were identified as HGF, and Met tyrosine kinase was identified as the receptor for HGF. Thus, the connection of apparently unrelated research projects rapidly enriched the research on HGF in different fields. The HGF-Met pathway plays important roles in the embryonic development of the liver and the placenta, in the migration of myogenic precursor cells, and in epithelial morphogenesis. The use of tissue-specific knockout mice demonstrated that in mature tissues the HGF-Met pathway plays a critical role in tissue protection and regeneration, and in providing less susceptibility to chronic inflammation and fibrosis. In various injury and disease models, HGF promotes cell survival, regeneration of tissues, and suppresses and improves chronic inflammation and fibrosis. Drug development using HGF has been challenging, but extensive preclinical studies to address its therapeutic effects have provided significant results sufficient for the development of HGF as a biological drug in the regeneration-based therapy of diseases. Clinical trials using recombinant human HGF protein, or HGF genes, are in progress for the treatment of diseases.

Key words: growth factor, HGF, hepatocyte growth factor, Met, tissue regeneration

Introduction: Before 1991

A mysterious phenomenon, perhaps even now, is the vital ability of the liver to regenerate. 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. The entire process can be completed within only one week. An *in vitro* cell culture technique for rat hepatocytes provided a scientific background to help explain liver regeneration. In 1984, hepatocyte growth factor (HGF) was discovered as a mitogenic protein for rat hepatocytes,[1, 2] and was thereafter purified from rat platelets,[3] human plasma,[4] and rabbit plasma.[5]

In 1989, cDNA for human HGF was cloned and the primary structure of HGF was clarified, by which HGF was identified as a novel growth factor with unique structural characteristics.[6, 7] 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 four kringle domains, while the β-chain contains a serine protease-like structure. A striking fact is that HGF has a structural similarity to plasminogen, which is a heterodimeric serine protease containing five kringle domains.

In 1991, we contributed to this journal by providing our review article entitled "Hepatocyte growth factor: molecular structure and implications for a central role in liver regeneration."[8] In that review we described the structural characteristics of HGF, changes in its expression following liver injury, and newly identified target cell types and the biological action of HGF. Before 1991, studies on HGF were done only by a small number of research groups, and total publications on HGF numbered less than 20 (Table 1). Research on HGF has widely spread and currently over 300 publications appear each year worldwide. After 20 years, recombinant human HGF is currently in clinical trials for treatment of diseases. In this review, we attempt to track 20 years of HGF research and development.

In 1991

In 1991, there was unexpected landmark progress in research on HGF (Table 1). The

scatter factor, originally identified as a fibroblast-derived cell motility factor for epithelial cells,[9] was shown to be an identical molecule to HGF. Similarly, tumor cytotoxic factor, a fibroblast-derived factor that induces cell death for several cancer cell types, was shown to be an identical molecule to HGF.[10] These bioactive molecules were identified and characterized using biological assays from different groups.

The induction of branching tubulogenesis in renal epithelial cells by HGF had a particular impact on cell and developmental biologists, because among growth factors and bioactive molecules HGF was the first to induce 3-dimensional (3-D) morphogenesis.[11] It had long been recognized that morphogenesis of epithelial tissues required an interaction with the adjacent mesenchyme (during embryonic development) or with the adjacent stroma (during postnatal life), which is known as the epithelial-mesenchymal interaction. However, the molecule that mediated the inducing effect of mesenchyme or stroma on epithelial morphogenesis was yet to be identified. HGF was the hitherto unidentified mesenchymal-derived molecule in epithelial-mesenchymal interaction that was responsible for epithelial morphogenesis.

The receptor for HGF was identified as a product of the c-met proto-oncogene in 1991.[12, 13] The Met receptor is composed of structural domains that include the extracellular Sema, PSI and IPT domains, the transmembrane domain, and the intracellular juxtamembrane and tyrosine kinase domains (Fig. 1B). The binding of HGF to the Met receptor induces activation of Met tyrosine kinase and the autophosphorylation of tyrosine residues in Met.

Scientific research sometimes unexpectedly turns in unanticipated directions. The connection and integration of research and molecules previously thought to be unrelated, i.e., HGF, scatter factor, tumor cytotoxic factor, 3-D epithelial morphogen, and Met, rapidly enriched the research on HGF in different fields.

3-D Morphogenesis and organogenesis in development

The essential roles of the HGF-Met pathway in mammalian development have been defined by the targeted disruption of the HGF or Met genes.[14-16] In these studies, the

embryonic liver is reduced in size and shows extensive apoptotic cell death. The knockout is embryonically lethal due to impaired organogenesis of the placenta and liver. In the placenta, the number of labyrinthine trophoblasts is markedly reduced. It is noteworthy that HGF-Met participates in a long-distance migration of cells in development, which indicates a particular role for HGF in cell movement. In the mouse embryo, HGF is strongly expressed in the limb bud mesenchyme and septum transversum (which develops into the diaphragm), and the migration of Met-positive myogenic precursor cells from dermo-myotome in the somite to limb buds and diaphragm is impaired in $Met^{-/-}$ embryos. Consequently, skeletal muscles of the limb and diaphragm are not formed in mutant mice.[16] Thus, HGF provides spatially defined chemoattractant-like motogenic signals for the migration of myogenic precursor cells.[17]

Using a gene knock-out/knock-in approach and sterotaxic injections of HGF or neutralizing antibody into the striatum in mice reveals the critical role of HGF in the development of the nervous systems.[18] HGF functions as an axonal chemoattractant for spinal motor neurons and for the projection of motor neurons to limb muscle.[19] In addition, HGF plays important roles in the development of sensory, sympathetic, parasympathetic, and cortical neurons. HGF regulates the proliferation of oligodendrocyte progenitor cells and their differentiation into oligodendrocytes.[20] Thus, HGF is implicated in neuronal as well as glial development in an orchestrated manner.

HGF regulates epithelial development and morphogenesis in different organs.[17, 21] In organ culture experiments, antibodies against HGF inhibit branching tubulogenesis of developing epithelia in the kidney and mammary glands. In tooth germ culture, antisense oligonucleotide to HGF induces impaired morphogenesis of tooth epithelium, which subsequently differentiates into ameloblasts. In organ cultures of the developing lung, branching morphogenesis of developing lung epithelia is inhibited by neutralization of HGF or use of an antisense strategy. During the development of various tissues, Met is expressed in the epithelia, while the HGF in mesenchymal cells is in close vicinity in various organs. These expression patterns indicate that HGF is a mesenchymal-derived factor that predominantly acts on neighboring developing epithelia.[17] Interactions between epithelium and mesenchyme,

i.e., epithelial-mesenchymal interactions mediate crucial aspects of development, affecting tissue induction and epithelial morphogenesis. Thus, HGF plays important roles as a mesenchymal-derived factor that regulates epithelial growth and morphogenesis.

Physiological roles

Far beyond the initial prediction that HGF plays a particular role in the regulation of liver growth and regeneration, the diverse biological and physiological roles of the HGF-Met pathway have been studied in diverse cell types (Table 2). In addition to the mitogenic, motogenic (enhancement of cell motility), and 3-D morphogenic activities of HGF — those known prior to 1991 — an increasing number of studies done in the early 1990s on apoptosis were connected to the remarkable action of HGF in the suppression of apoptotic cell death (Fig. 2). Activation of the Fas receptor by agonistic antibody in mice induces fulminant hepatitis associated with massive apoptosis of hepatocytes, whereas HGF potently suppresses apoptotic death of hepatocytes, thereby preventing the onset of fulminant hepatitis in mice.[22] The suppression of cell death by HGF participates in the biological, physiological, and therapeutic actions of HGF. The impaired development of the HGF or Met gene in the embryonic livers of systemic knockout mice was explained by submassive apoptosis in the hepatoblasts. The cytoprotective action of HGF explains how it suppresses the onset of tissue damage, including acute liver injury. However, it is noteworthy that HGF bidirectionally regulates cell survival in a cell type-dependent manner. In a model of liver cirrhosis in rats, HGF suppressed apoptosis of hepatocytes but facilitated apoptosis of α-smooth muscle and actin-positive myofibroblasts, the cells responsible for tissue fibrosis.[23]

Induction of proteases involved in breakdown of the extracellular matrix scaffold was also revealed as a particular action of HGF. HGF induces or up-regulates expression of urokinase-type plasminogen activator and matrix metalloproteinases (MMPs) such as membrane-type MMP and MMP-9. Induction of these proteases also participates in the biological, physiological, and therapeutic actions of HGF (Fig. 2). For 3-D epithelial branching tubulogenesis by HGF, activation of urokinase-type plasminogen activator and

membrane type 1-MMP play a crucial role. Enhancement in the expression of MMPs is a mechanism by which the fibrotic change in tissues is suppressed and/or the resolution of fibrosis is facilitated by HGF. Instead, the induction of MMPs, such as membrane types 1-MMP and MMP-9, participates in the 3-D spreading and invasion of cancer cells.

In accord with the initial implication of HGF as a humoral hepatotrophic factor that enhances liver regeneration, expression of HGF is increased in response to liver injuries. Conversely, neutralization of endogenous HGF enahances liver damage, for example by increasing apoptosis/necrosis of hepatocytes and/or suppression of liver regeneration. The hepatotrophic role of HGF was definitively demonstrated using a conditional knockout of the Met gene in mice.[24-26] Mice lacking the Met gene in hepatocytes were hypersensitive even to mild liver injury caused by administration of a low-dose of agonistic anti-Fas antibody, indicating that anti-apoptotic activity of HGF plays a role in protection of the liver. Liver- or hepatocyte-specific $Met^{-/-}$ mice showed delayed liver regeneration associated with persistent inflammatory reaction, and were susceptible to fibrotic change in the liver. Likewise, after bile duct ligation that causes chronic cholestatic liver injury, hepatocyte-specific Met-/- mice showed increases in hepatocyte apoptosis, inflammation, and profibrogenic responses, and were more susceptible to chronic inflammation and fibrotic change compared with control mice.[26] These effects in liver- or hepatocyte-specific $Met^{-/-}$ mice clearly indicate that the physiological roles of the HGF-Met pathway in protection, regeneration, anti-inflammation, and anti-fibrosis of the liver cannot be substituted by other growth factors, cytokines, and bioactive molecules. Thus, the hepatotrophic and hepatoprotective roles of HGF have been well established during the past 20 years.

Similar to the story of HGF-Met in the liver, the involvement of the HGF-Met pathway in tissue protection and/or regeneration has been demonstrated in different tissues, though the dependency on the HGF-Met pathway is different depending on tissue types. The HGF-Met pathway supports the protection and regeneration of kidney, lung, nervous system, cardiovascular, cutaneous, and gastrointestinal tissues. Collectively, the HGF-Met pathway plays definitive roles not only by promoting survival, proliferation, migration, and 3-D

morphogenesis but also in preventing inflammation and fibrotic change in tissues. Studies using tissue-specific Met knockout mice provided clear evidence for the roles of the HGF-Met pathway in protection, regeneration, and anti-fibrosis/inflammation in different cell and tissue types.

Mice with conditional knockout of Met in the collecting duct of the kidney were more susceptible to interstitial fibrosis and tubular necrosis after unilateral ureteral obstruction, while they had reduced capacity in tubular cell regeneration after release of the obstruction, leading to diminished functional recovery.[27] In podocyte Met conditional knockout mice, no pathology was seen, whereas the mice developed more severe podocyte apoptosis and albuminurea in comparison with control mice.[28] Disruption of the Met gene in epidermal keratinocytes demonstrated in an indispensable role for the HGF-Met pathway in skin wound healing.[29] Surprisingly, Met-deficient epidermal keratinocytes were unable to contribute to the re-epithelialization of skin wounds, though other growth factors and bioactive molecules were functional.

Conditional knockout mice with selective disruption of Met in pancreatic β-cells displayed significantly reduced plasma insulin after a glucose challenge. *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 conditional Met knockout mice were not accompanied by changes in total β-cell mass, islet morphology, and β-cell proliferation.[30] Another group using β-cell-Met$^{-/-}$ mice displayed mild hyperglycemia and a complete loss of acute-phase insulin secretion in response to glucose.[31] 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.

Because aberrant activation of growth factor receptors is generally associated with tumor development, a lack of growth factor receptor-mediated signal could be associated with less tumor development. However, a role of the HGF-Met pathway in hepatocarcinogenesis has been debated. Unexpectedly, when compared with control mice, liver-specific Met$^{-/-}$ mice treated with N-nitrosodiethylamine developed significantly more tumors that were larger and

had a shorter latency. N-nitrosodiethylamine induced oxidative stress, whereas administration of antioxidant blocked the hepatocarcinogenesis in liver-specific $Met^{-/-}$ mice.[32] Thus HGF-Met signaling is essential for maintaining normal redox homeostasis in the liver and has tumor suppressor effect(s) in this model. In a model of hepatocarcinogenesis induced by N-nitrosodiethylamine and phenobarbital, liver-specific $Met^{-/-}$ mice showed a higher prevalence of macroscopically visible liver tumors, while there was only minor differences in the number of preneoplastic and neoplastic lesions in response to phenobarbital-induced promotion. These results indicate that a defect in Met-mediated signaling increases chemically induced tumor initiation in liver but does not significantly affect phenobarbital-mediated tumor promotion.[33]

Processing and physiological relevance

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. It has been proposed that several proteases in the serum or cell membranes are involved in the activation of single-chain HGF, including HGF activator (HGF-A), urokinase-type plasminogen activator, plasma kallikrein, coagulation factors XII and XI, matriptase, and hepsin. Among them, HGF-A, matriptase, and hepsin are the most efficient in processing proHGF. HGF-A was purified and cloned as a serum-derived protease that activates HGF.[34, 35] HGF-A is a member of the kringle-containing serine protease superfamily. HGF-A is biosynthesized primarily by hepatocytes and circulates in the plasma as an inactive single-chain proHGF-A. Because significant activation of proHGF occurs in injured tissues and thrombin activates proHGF-A, the conversion of prothrombin to thrombin, which occurs during activation of the coagulation cascade, is involved in the activation of HGF in response to tissue injury. In addition, kallikrein 1-related peptidases participate in the activation of proHGF-A, and this activation is believed to occur in the pericellular microenvironment.

The activity of HGF-A is regulated not only by its processing from proHGF-A, but also by a specific inhibitor, HGF-A inhibitor-1 (HAI-1).[36] HAI-1 is a membrane-bound serine protease inhibitor that is mainly expressed on the basolateral surfaces of epithelial cells. HGF-A can be localized to the pericellular microenvironment via its affinity to heparansulfate and/or binding to HAI-1. The activity of HGF-A is suppressed by its binding to the cell surface HAI-1. On the other hand, the HGF-A and HAI-1 complexes on the cell surface can potentially be released by metalloprotease-mediated shedding of the HAI-1 ectodomain, and this process is enhanced by inflammatory cytokines such as interleukin-1β. Perhaps, HAI-1 plays a role not only as an inhibitor but also as a cell-associated reservoir of HGF-A. The regulatory mechanism for the activation of proHGF by HGF-A and HAI-1 can be considerable as the physiological link between inflammation and tissue regeneration. In this context, it is notable that inflammatory mediators such as interleukin-1β, prostaglandin E_2, and prostaglandin I_2 are potent inducers for the expression of HGF, again implicating a physiological link from inflammation to tissue regeneration.

The physiological significance of HGF-A has been studied by loss-of-function approaches.[35, 36] The inhibition of HGF-A by neutralizing antibody resulted in impairment in activation of proHGF.[35] The knockout approach using mice lacking HGF-A indicated that the sera from HGF-$A^{-/-}$ mice were unable to activate proHGF, indicating that HGF-A is the major protease responsible for the activation of HGF in serum.[37] Although HGF-$A^{-/-}$ mice showed normal development, the attenuation of initial regeneration after mucosal injury was associated with impaired restitution of epithelia. Pathophysiological study has indicated that fibroblasts from patients with idiopathic pulmonary fibrosis have a lower capacity to activate pro-HGF compared with control fibroblasts.[38] Therefore, the process for activation of HGF also plays an important role in the regulation of tissue regeneration and susceptibility to pathological conditions.

Therapeutic approaches and clinical development

The highlights in research on HGF during the past 20 years have resulted in extensive

therapeutic approaches using different disease models for different tissues (Table 3). Even in different injury and tissue types, the mechanisms responsible for the therapeutic effects of HGF are likely to overlap (Fig. 2). The protective actions of HGF are explained by prevention of cell death against various types of stresses and injury. Prevention of cell death by HGF seems to be associated with less subsequent inflammation, or an anti-inflammatory effect, whereas mechanisms by which HGF could regulate immunological responses have yet to be addressed. Recent studies have indicated that HGF regulates the function of immune cells such as dendritic cells and a subset of regulatory T cells.[39-41] These biological actions of HGF on immune cells are likely to be the underlying mechanism, at least in part, by which HGF exerts therapeutic effects on diseases associated with allergy, inflammation, and fibrosis.

On the other hand, promotion of cell proliferation, migration, and 3-D morphogenesis by HGF explains the recovery and re-organization of tissues from injury, whereas the dynamic reconstruction of 3-D tissue structure substantially supports functional recovery. Chronic tissue injury is tightly associated with the onset of fibrotic change, and this is particularly relevant to the pathogenesisof liver cirrhosis and chronic kidney disease; there has been no effective therapeutic approach for the treatment of such chronic fibrotic diseases. It should be emphasized that, at least in animal models, HGF-treatment is highly effective for the treatment of chronic fibrosis in various disease models, including liver cirrhosis, chronic kidney disease, dilated cardiomyopathy, lung fibrosis, and vocal fold scarring.

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.[42] The first clinical study of HGF gene therapy by naked expression plasmid was done to investigate its safety for treatment of patients with arteriosclerosis obliterans or Buerger disease.[43] 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.[44] 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 has been completed in both the USA and Japan. The phase-I clinical trial of the systemic administration of recombinant HGF protein and the Phase-I/II clinical trial for the local application of recombinant HGF protein are both in progress (http://www.kringle-pharma.com/en/index.html).

Structural understanding

The C-terminal multifunctional docking site of Met plays a crucial role in the activation of Met-dependent intracellular signal transduction and biological activities.[45] The phosphorylation of C-terminal tyrosine residues in the docking site recruit 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). Among signaling molecules, a scaffolding adaptor protein, Gab1, is the most crucial substrate for the HGF-Met pathway.[46] Direct interaction of Gab1 with tyrosine phosphorylated Met is mediated by the Met-binding site in Gab1, and it allows a direct and robust interaction between Met and Gab1. Knockout mice with the Gab1 gene exhibited phenotypes similar to those seen in HGF and Met knockout mice.[46]

In terms of Met-dependent signal transduction, the cytoplasmic juxtamembrane domain, which is composed of 47 highly conserved amino acids, acts as a negative regulator. Cbl, an E3 ubiquitin ligase, binds phosphorylated Y1003 of Met, and this Cbl binding results in Met ubiquitination, endocytosis, transport to the endosomal compartment, then degradation.[47] 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. In cells in which Ser985 is phosphorylated by treatment with protein kinase-C, HGF-induced activation of Met is suppressed.[48, 49] Therefore, activation of protein kinase-C, which occurs by different types of extracellular stimuli, regulates HGF-dependent Met activation.

HGF binds to Met through two different mechanisms: the α-chain binds with high affinity while the β-chain binds with very low affinity. Among the α-chain binding sites, NK1 (the N-terminal and first kringle domains) in the α-chain of HGF provides high-affinity binding to Met (Fig. 1A). The α-chain alone exhibits high-affinity binding to Met, whereas the binding of the α-chain does not activate Met.[50] 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).[51] The Sema domain of Met forms a seven-bladed β-propeller, which makes the shape of the Sema domain resemble a funnel. Generally, in β-propellers each of the blades is formed by four antiparallel β-strands. The HGF β-chain binds to the bottom face of the propeller, and forms contacts with residues that protrude from blades 2 and 3. The HGF β-chain binds to a series of protruding polar side chains from Met, which originate from three separate loops: residues 124-128, residues 190-192, and residues 218-223 (Fig. 3A). Although the α-chain of HGF binds to Met with much higher affinity than that of the HGF β-chain, the crystal structure for the interaction between the HGF α-chain and the extracellular region of Met has yet to be determined.

The Met tyrosine kinase domain follows the 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).[52, 53] The characteristic feature of Met is the presence of the C-terminal multifunctional docking site that contains tyrosine residues (^{1349}YVHVNAT^{1356}YVNV). In an unphosphorylated Met kinase domain, Met 1229 in the activation loop (A-loop, yellow in Fig. 3B) projects into the ATP-binding pocket, and the direction of the Glu 1127 residue involved in ATP-binding is changed, by which ATP is unable to form an appropriate structure for the kinase-substrate complex.[53] This structure corresponds to the quiescent autoinhibited Met kinase without HGF stimulation. On the other hand, the autoinhibited Met kinase structure changes upon the phosphorylation of Tyr 1234

and Tyr 1235 in the activation loop, which allows a complex formation between the Met kinase and ATP.

The staurosporine analog K-252a inhibits Met tyrosine kinase through its binding in the ATP pocket.[52] Because the structure of the Met kinase domain, complexed with the K-252a as shown in Fig. 3B, was obtained using the recombinant Met kinase domain where Tyr 1234 and Tyr 1235 were replaced by Phe and Asp, respectively, Met kinase complexed with K-252a is expected to represent a structure of Met tyrosine kinase that is activated upon HGF-stimulation.

HGF in tumor biology and therapeutics

The HGF-Met system drives the breakdown of the extracellular matrix and the concomitant cellular migration, mitogenesis, and morphogenesis, leading to the construction of tissue architecture. In cancer tissues, biological programs regulated by the HGF-Met pathway are adopted particularly for invasion and metastasis, which are the life-threatening events of cancer. Early studies indicating the identity of a fibroblast-derived scatter factor in HGF implicated a role for HGF in cancer spreading and invasion.[54] The crucial role of stromal fibroblasts in invasion of cancer cells through the 3-D collagen was first demonstrated independently using human oral squamous cell carcinoma.[55] The cancer cells invaded aggressively only when they were co-cultured with stromal fibroblasts in collagen gel. The fibroblast-derived factor responsible for the 3-D invasion was thereafter identified as HGF. The profound action of HGF on cancer invasion has been demonstrated in a variety of cancer cells, and it has been established that HGF is a mediator in the tumor-stromal interaction that affects the malignant behavior of cancer.[56]

In addition to the paracrine activation of Met through tumor-stromal interaction, Met activation in cancer often occurs through an autocrine mechanism, or through a mutation in the Met gene. Genetical analysis indicated that missense mutations in the c-met gene are the causative genetical disorder in inherited, and some sporadic papillary, renal carcinomas.[57] Mutations found in papillary renal carcinomas are located in the tyrosine kinase domain of the

c-Met receptor, and these Met receptor mutations are likely to be a gain-of-function mutation.[58] In addition to papillary renal carcinoma, missense mutations in the Met have been found in lung cancer, hepatocellular carcinoma and gastric cancer; sites include the Sema, IPT, juxtamembrane, and tyrosine kinase domains.[59]

The HGF-Met pathway has become a hot target in anticancer drug development.[60, 61] Several distinct lines of approach to the inhibition of the HGF-Met pathway have been demonstrated, including small molecules that inhibit Met tyrosine kinase activity, ribozymes, small-interfering RNA (siRNA), neutralizing monoclonal antibodies, soluble Met receptors, and antagonists composed of selected domains in HGF (Fig. 4A). Among these approaches to the inhibition of the HGF-Met pathway, NK4 was the first-identified specific inhibitor for HGF-Met.[62, 63] NK4 is composed of the N-terminal (N) and four kringle domains (K4) of HGF, and it is a competitive inhibitor of HGF-dependent Met activation (Fig. 4B). Discovery of NK4 as a competitive inhibitor for HGF-Met was soon followed by the experimental treatment of cancer in mice: NK4 inhibited the invasion and growth of gallbladder cancer.[64] It is also noteworthy that NK4 is bifunctional — it is an angiogenesis inhibitor as well as an HGF-Met inhibitor.[63, 65] The inhibition of tumor growth by NK4 treatment has been observed in a variety of tumors, and this inhibitory effect has been associated with a reduction in blood vessels in tumor tissues. NK4 treatment inhibited invasion and metastasis in different types of cancer models, including breast, colon, gastric, lung, ovarian, and pancreatic carcinomas, and malignant melanoma.[63]

If metastatic tumors could be suppressed to a non-metastatic state, there would be a considerable improvement in rate of cancer cures. Recent studies have indicated that cancer stem cells participate in drug-resistance and in the spreading of tumors, indicating that cancer stem cells are therapeutic targets for future advancements in anti-cancer strategy.[66, 67] The activation of the HGF-Met pathway participates not only in the spreading and invasion of cancer stem cells but also in drug resistance to tyrosine kinase inhibitors (e.g., gefitinib and erlotinib) in patients with lung cancer.[67-69] Taken together, it seems likely that HGF-Met inhibitors will provide further advances in the molecular targeting therapy of cancer. Several

inhibitors of the HGF-Met pathway are under preclinical and clinical development.[60, 61]

Perspectives and conclusions

Current attention and progress in stem cell biology has facilitated an understanding of how stemness of stem cells is controlled by genetic programming, and how stem cells participate in tissue regeneration. A small population of hepatic progenitor cells, or stem cells with self-renewal and slow recycling characteristics, participate in long-term liver growth and the renewal of hepatocytes. However, liver regeneration mainly depends on the proliferation of mature hepatocytes. The key to understanding liver regeneration remains to find out how liver tissue architecture and homeostasis of liver mass are precisely regulated, before, during and after the regenerative response.

Growth factors and their receptor tyrosine kinases are divided into families based on structural and functional similarities. Among each family, a single growth factor activates multiple receptors with 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.” Moreover, as demonstrated in systemic and conditional knockout mice with Met, HGF has unique physiological and therapeutic actions that are not substituted by other growth factors, at least not fully. Taken together, HGF and Met have remarkable potential value as targets in drug discovery and development, from aspects that are both agonistic / activating and antagonistic / inhibiting. The one-to-one relationship for HGF-Met should facilitate future drug discovery and design based on the crystal structures of HGF and/or Met for a small molecular activator or inhibitor of HGF-Met. Perhaps small molecular inducers or suppressors for HGF are also potential candidates in drug discovery.

It has been 20 years since we published our first HGF review article (“Hepatocyte growth factor: molecular structure and implications for a central role in liver regeneration”) in the *Journal of Gastroenterology and Hepatology*. The progress and diversity of research on HGF during the past 20 years have been remarkable. It is no longer possible to

comprehensively review this progress in a single article. Our intent was to describe the structural and functional characteristics and the therapeutic significance of HGF. Drug development using growth factors remains a challenge, but extensive studies to address the therapeutic effects of HGF have provided enough significance to develop agonists and antagonists of the HGF/Met system as biological drugs in regeneration-based or anti-cancer therapies. We propose that the further development of HGF as a therapeutic drug target is well worth the challenge.

Acknowledgements

The studies from the authors' laboratories were supported by Grants from the Ministry of Education, Culture, Science, Sports, and Technology of Japan, and by The Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation.

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Table 1. Landmark events in HGF research

Year	Events	References (ref. No.)
1984	Identification and partial characterization of HGF as mitogenic protein for hepatocytes	Nakamura et al., 1984 (1) Russell et al., 1984 (2)
1987	Purification of rat HGF from platelets	Nakamura et al., 1987 (3)
	Identification and partial characterization of scatter factor as fibroblast-derived cell motility factor	Stoker et al., 1987 (9)
1988	Purification of human HGF from plasma	Gohda et al., 1988 (4)
1989	Purification of rabbit HGF from plasma	Zarnegar & Michalopoulos, 1989 (5)
	cDNA cloning for human HGF	Nakamura et al., 1989 (6) Miyazawa et al., 1989 (7)
	Purification of mouse scatter factor from culture supernatant of fibroblasts	Gherardi et al., 1989 (70)
1990	Purification of human scatter factor from culture supernatant of fibroblasts	Weidner et al., 1990 (54)
1991	Establishment of notion that scatter factor is identical molecule to HGF	several reports
	Identification of c-Met protooncogene product as receptor for HGF	Bottaro et al., 1991 (12) Naldini et al., 1991 (13)
	Identification of epithelial 3D-morphogen as HGF	Montesano et al., 1991 (11)
	Identification of tumor cytotoxic factor as HGF	Shima et al., 1991 (10)
1992	In vivo therapeutic actions of HGF using recombinant HGF	Ishiki et al., 1992 (71)
1995	Generation of knockout mice disrupted with HGF or Met gene	Schmidt et al., 1995 (14) Uehara et al., 1995 (15) Bladt et al., 1995 (16)

	Therapeutic action of HGF for liver cirrhosis as fibrotic and chronic inflammatory disorder	Matsuda et al., 1995 (72)
1997	Identification of germline mutations in Met gene in patients with hereditary and sporadic papillary renal cancer	Schmidt et al., 1997 (57)
	Isolation of NK4 as specific inhibitory molecule for HGF-Met pathway	Date et al., 1997 (12)
1998	Determination of crystal structure of NK1 (N-terminal and the first kringle domains)	Ultsch et al., 1998 (73)
	In vivo therapeutic action of a specific inhibitor for HGF-Met in experimental cancer model	Date et al., 1998 (64)
1999	Determination of crystal structure of NK1-dimer structure	Chirgadze et al., 1999 (74)
2002	Clinical study for treatment of patients with chronic leg ulcer with recombinant HGF protein	Nayeri et al., 2002 (42)
2003	Determination of crystal structure of Met tyrosine kinase domain	Schiering et al., 2003 (52)
2004	Determination of crystal structure of the HGF β-chain in complex with the Sema-PSI domains of Met	Stamos et al., 2004 (51)
	Clinical study for treatment of patients with critical limb ischemia by HGF gene drug	Morishita et al., 2004 (43)
2009	Clinical trial for safety evaluation using recombinant HGF protein drug	http://www.kringle-pharma.com/en/
2010	Double-blind clinical trial for treatment of patients with critical limb ischemia by HGF gene drug	Shigematsu et al., 2010 (44)

Table 2. Target cell types of HGF.

Tissue type	Target cell type
Hepato-biliary and pancreas	hepatocyte hepatoblast bile duct epithelial cell pancreatic β cell
Gastrointestinal	gastric epithelial cell intestinal mucosal epithelial cell
Kidney	renal tubular cell podocyte
Lung	bronchial epithelial cell alveolar type II epithelial cell
Nervous	neuron (hippocampal neuron, cerebral cortex neuron, midbrain dopaminergic neuron, cerebellar granular neuron, motor neuron, thalamic neuron, sensory neuron, sympathetic neuron, parasympathetic neuron, subventiricular zone neural stem-like cell) Schwann cell astrocyte oligodendrocyte progenitor cell
Cardiovascular and lymphatic	cardiomyocyte (in hypoxic condition) vascular endothelial cell lymphatic vessel cell
Hematopoietic and immune	dendritic cell hematopoietic stem/progenitor cell monocyte-osteoclast macrophage (conditionally)
Skin and eye	keratinocyte melanocyte hair bulb keratinocyte corneal epithelial cell
Muscle, bone, and joint	muscle satellite cell myogenic precursor cell articular chondrocyte
Glands	mammary gland epithelial cell submandibular gland epithelial cell salivary gland epithelial cell prostate epithelial cell thyroid cell
Placenta	cytotrophoblast

Table 3. Therapeutic approaches with recombinant HGF in various disease models in various tissues.

Tissues and disease models	Model for injury or diseases	Observed therapeutic effects	References
Liver			
Acute hepatitis	α-Naphthylisothiocyanate or CCl_4	Enhancement of hepatocyte proliferation Suppression of serum ALT	Ishiki et al., Hepatology, 1992; 16: 1227
	α-Naphthylisothiocyanate	Decreases in serum ALT, AST, bilirubin Suppression of parenchymal lesions	Roos et al., Endocrinology, 1992; 131: 2540
	Dimethylnitrosamine Acetaminophen	Suppression of serum ALT, AST	Masunaga et al., Eur J Pharmacol, 1998; 342: 267
	Ischemia-reperfusion	Suppression of mortality, serum ALT, and necrotic hepatocytes Inhibition of neutrophil infiltration Enhancement of hepatocyte growth	Sakakura et al., J Surg Res, 2000; 92: 261
	Ischemia-reperfusion	Suppression of necrotic hepatocytes, serum ALT, AST Suppression of oxidative stress	Oe et al., J Hepatol, 2001; 34: 832
	α-Naphthylisothiocyanate + partial hepatectomy	Enhancement hepatocyte proliferation Decrease in serum bilirubin	Yoshikawa et al., J Surg Res, 1998; 78: 54
Cholestasis	Bile duct ligation	Reduction in necrotic and apoptotic hepatocytes Decrease in serum AST, ALT	Li et al., Am J Physiol, 2007; 292: G639
Fulminant hepatitis	Agonistic anti-FAS antibody	Suppression of hepatocyte apoptosis, serum ALT, and mortality	Kosai et al., Biochem Biophys Res Commun, 1998; 244: 638
	LPS + D-galactosamine	Suppression of hepatocyte apoptosis, serum ALT, and mortality	Kosai et al., Hepatol, 1999; 30: 151
Liver cirrhosis	Dimethylnitrosamine	Improvement of survival and liver function Reduction in extracellular matrix accumulation Decrease in serum AST, ALT	Matsuda et al., J Biochem, 1995; 118: 643
	CCl_4, dimethylnitrosamine, or porcine serum	Improvement of survival and liver function Reduction in extracellular matrix accumulation and serum AST level	Matsuda et al., Hepatol, 1997; 26: 81
	Thioacetamide	Reduction in extracellular matrix accumulation	Oe et al., J Control Release, 2003; 88: 193
	Dimethylnitrosamine	Reduction in extracellular matrix accumulation and myofibroblasts Increase in apoptosis in myofibroblasts	Kim et al., Am J Pathol, 2005; 166: 1017
	Dimethylnitrosamine	Reduction in extracellular matrix accumulation Decrease in serum ALT and TGF-β levels Increase in serum albumin and liver weight	Kusumoto et al., Int J Mol Med, 2006; 17: 503
Liver cirrhosis + hepatic surgery	Dimethylnitrosamine + portal branch ligation + partial hepatectomy	Promotion of survival Decrease in serum AST, ALT, bilirubin Increase in liver weight	Kaido et al., Hepatol, 1998; 28: 756
Alcoholic steatohepatitis	Ethanol-containing diet	Decrease in hepatic lipids	Tahara et al., J Clin Invest, 1999; 103:

		Increase in serum lipids and lipoproteins	313
Gastrointestinal			
Ulcerative colitis	Dextran sulfate sodium	Suppression of histological damage and loss of weight Enhancement of epithelial cell proliferation	Tahara et al., J Pharmacol Exp Therapeutics, 2003; 307: 146
	2,4,6-trinitrobenzene sulfonic acid	Suppression of colonic ulcer coverage and large intestinal shortening Reduction in inflammatory cells and enhancement of epithelial cell growth	Numata et al., Inflamm Bowel Dis, 2005; 11: 551
Gastric ulcer	Cryoinjury	Enhancement of epithelial cell proliferation	Schmassmann et al., Gastroenterol, 1997; 113: 1858
Gastric injury	Cisplatin		Nakahira et al., Biochem Biophys Res Commun, 2006; 341: 897
Kidney			
Acute kidney injury	$HgCl_2$ or cisplatin	Suppression of renal injury Enhancement of tubular proliferation	Kawaida et al., Proc Natl Acad Sci USA, 1994; 91: 4357
	Ischemia	Enhancement of tubular proliferation	Miller et a., Am J Physiol, 1994; 266: F129
	Cyclosporin A	Enhancement of tubular proliferation Suppression of tubular pathology (vacuolization)	Amaike et al., Cytokine, 1996; 8: 387
	$HgCl_2$	Suppression of renal dysfunction Suppression of tubular apoptosis	Yamasaki et al., Nephron, 2002; 90: 195
	Glycerol	Suppression of tubular necrosis and improvement of renal function Prevention of mortality	Nagano et al., Nephron, 2002; 91: 730
	Tacrolims/FK506	Suppression of injury and decrease in serum creatinine Enhancement of renal cell proliferation	Takada et al., Transpl Int, 1999; 12: 27
Acute renal inflammation	Tumor necrosis factor-α	Decrease in sequestration of circulating macrophages in the kidney Suppression of acute renal inflammation	Gong et al., Kidney Int, 2006; 69: 1166
Septic acute renal failure	Lipopolysaccharide	Suppression of mortality Suppression in blood urea nitrogen and AST	Kamimoto et al., Biochem Biophys Res Commun, 2009; 380: 333
Diabetic nephropathy	Streptozotocin	Decrease in albuminurea Suppression of glomerular and tubulointerstitial fibrosis Improvement of renal function	Mizuno & Nakamura, Am J Physiol, 2004; 286: F134
Chronic kidney disease	Spontaneous due to tensin2 mutation	Improvement of glomerular and tubulointerstitial fibrosis Enhancement of tubular proliferation Improvement of renal function and decrease in albuminurea	Mizuno et al., J Clin Invest, 1998; 101: 1827
	5/6 nephrectomy	Suppression of renal inflammation Decrease in sequestration of circulating macrophages in the kidney	Gong et al., J Am Soc Nephrol, 2006; 17: 2464
	Unilateral ureteral obstruction	Improvement of tubulointerstitial fibrosis Increase in tubular proliferation and decrease in tubular apoptosis	Mizuno et al., Kid Int, 2001; 59: 1304

	Unilateral ureteral obstruction	Improvement of tubulointerstitial fibrosis	Yang & Liu, Am J Physiol, 2003; 284: F349
Glomerulonephritis	Anti-Thy 1.1 antibody	Suppression of mesangial cell proliferation	Bessho et al., 2003; Am J Physiol, 284: F1171
Chronic allograft nephropathy	Ischemia and transplantation	Prevention of tubular cell death after ischemia and transplantaion Suppression of proteinurea and fibrotic change of the kidney Prevention of mortality	Azuma et al., J Am Soc Nephrol, 2001; 12: 1280
Cardiovascular			
Critical limb ischemia	Hindimb ischemia	Enhancement of collateral blood vessel formation Increase in blood vessel density and blood flow	Van Belle et al., Circulation, 1998; 97: 381
	Hindimb ischemia	Enhancement of collateral blood vessel formation	Morishita et al., Hypertension, 1999; 33: 1379
	Hindimb ischemia	Improvement in the recovery of blood flow by slow release delivery	Marui et al., J Vasc Surg, 2005; 41: 82
Neointimal hyperplasia	Balloon injury	Reduction in intimal area Enhancement of regeneration of endothelial cell layer	Yasuda et al., Circulation, 2000; 101: 2546
Coronary artery disease	Chronic ischemia	Improvement in regional myocardial blood flow Improvement in myocardial function	Yamaguchi et al., Surg Today, 2005; 35: 855
Myocardial infarction	Ischemia-reperfusion	Reduction in infract area, apoptosis in cardiomyocytes, and mortality Improvement of cardiac function	Nakamura et al., J Clin Invest, 2000; 106: 1511
	Ischemia-reperfusion	Promotion of improvement in cardiac function Suppression of myocardial apoptosis and hypertrophy	Jin et al., J Pharmacol Exp Therapeutics, 2003; 304: 654
Cardiac allograft vasculopathy	Ischemia and transplantation	Promotion of survival of allografts Suppression of myocardial inflammation apoptosis Prevention of cardiac allograft vasculopathy and interstitial fibrosis	Yamaura et al., Circulation, 2004; 110: 1650
Dilated cardiomyopathy	Mutation in δ-sarcoglycan	Improvement of cardiac fibrosis and echocardiographic function Suppression of myocardial apoptosis and hypertrophy	Nakamura et al., Am J Physiol, 2005; 288: H2131
Respiratory			
Acute lung injury	Intratracheal HCl infusion	Enhancement of proliferation of airway and alveolar epithelial cells	Ohmichi et al., Am J Physiol, 1996; 270: L1031
	Ischemia-reperfusion	Suppression of histological damage Decrease in apoptosis	Makiuchi et al., J Heart Lung Transplant, 2007; 26: 935
Lung fibrosis	Bleomycin	Suppression and improvement of fibrosis	Yaekashiwa et al., Am J Respir Crit Care Med, 1997; 156: 1937
	Bleomycin	Decrease in extracellular matrix deposition Enhancement in proliferation of epithelial cells	Dohi et al., Am J Respir Crit Care Med, 2000; 162: 2302
	Bleomycin	Suppression and improvement of fibrosis	Mizuno et al., FASEB J, 2005; 19: 580
Pulmonary emphysema	Elastase	Regeneration of alveolar structure Promotion of recruitment of bone marrow-derived progenitor cells into	Ishizawa et al., Biochem Biophys Res Commun, 2004; 324: 276

		alveolar epithelial and endothelial cells	
Resection	Left peumonectomy	Enhancement of alveolar and airway epithelial cells	Sakamaki et al., Am J Resp Cell Mol Biol, 2002; 26: 525
Allergic airway inflammation / asthema	Sensitization and challenge with ovalbumin	Suppression of airway inflammation, collagen deposition, smooth muscle hyperplasia, and remodeling Reduction in Th2 cytokines and fibrogenic growth factors	Ito et al., Am J Respir Cell Mol Biol, 2005; 32: 268
	Sensitization and challenge with ovalbumin	Suppression of eosinophylic airway inflammation Suppression of antigen-induced allergic immune responses	Okunishi et al., Int Arch Allergy Immunol, 2009; 149 Suppl. 1: 14
Vocal fold scarring	Removal of the lamina propria	Better vibration interms of mucosal wave amplitude and glottal closure Reduction in collagen deposition and restoration of hyaluronic acid and elastin	Ohno et al., Ann Otol Rhinol Laryngol, 2007; 116: 762
	Removal of the lamina propria	Better function (vibration) of larynge Reduction in collagen deposition	Kishimoto et al., Laryngoscope, 2010; 120: 108
Skin			
Wound healing	Full-thickness cutaneous excision in diabetic mice	Promotion of wound closure, re-epithelialization, angiogenesis, granulation tissue formation	Yoshida et al., Growth Factors, 2004; 22: 111
		Promotion of recruitment of neutrophils, monocytes, macrophages, endothelial cells, and re-epithelialization, granulation tissue formation and angiogenesis	Bevan et al., J Pathol, 2004; 203: 831
Nervous and sensory			
Cerebral ischemia	Occlusion of carotid arteries	Decrease in delayed neuronal death in the hippocanpus	Miyazawa et al., J Cereb Blood Flow Metab, 1998; 18: 345
	Transient occlusion of arteries	Decrease in the infarct size Increase in blood vessel density	Tsuzuki et al., Neurol Res, 2001; 23: 417
	Microsphere embolism	Prevention of learning and memory dysfunction Suppression of endothelial cell apoptosis and necrotic tissue damage	Date et al., J Neurosci Res, 2004; 78: 442
	Transient forebrain ischemia (the four-vessel occlusion)	Suppression of neuronal cell death in hippocanpal neurons	Niimura et al., Neurosci Lett, 2006; 407: 136
	Transient middle cerebral artery occlusion	Decrease in the infarct size Suppression of apoptotic neurons and increase in autophagic response	Shang et al., J Neurosci Res, 2010; 88: 2197
Peripheral nerve injury	Hypoglossal nerve axotomy	Suppression of the loss in choline acetyltransferase	Okura et al., Eur J Neurosci, 1999; 11: 4139
Amyotrophic lateral sclerosis	Mutation in superoxide dismutase	Suppression of degeneration of motor neurons and disease progression Promotion of survival	Ishigaki et al., J Neuropathol Exp Neurol, 2007; 66: 1037
Hydrocephalus	Transforming growth factor-β	Suppression of fibrosis	Tada et al., Neurobiol Dis, 2006; 21: 576-586
Retinal injury	Ischemia-reperfusion	Decrease in apoptosis in ganglion cell layer and inner nuclear layer Increase in the inner retinal thickness and neuronal function	Shibuki et al., Invest Ophthalmol Vis Sci, 2002; 43: 528

Photoreceotr degeneration / Retinitis pigmentosa	Sodium iodate	Better structural preservation of the outer retina Better functional preservation of retinal pigment epithelium and photoreceptor cells	Ohtaka et al., Current Eye Res, 2006; 31: 347
	Phototoxicity (strong fluorescent light)	Better the morphological and functional preservation of photoreceptor cells Suppression of apoptosis in photoreceptor cells	Machida et al., Invest Ophthalmol Vis Sci, 2004; 45: 4174
Difficulty in hearing	Noise-induced hearing loss	Protective in the auditory function Reduction in the loss of outer hair cell loss	Inaoka et al., Acta Oto-Laryngologica, 2009; 129: 453
Muscloskeletal			
Articular cartilage injury	Osteochondral defects	Promotion of repair of osteochondral defects	Wakitani et al., Acta Orthop Scand, 1997; 68: 474-480
Skeletal muscle injury	Freeze damage	Inhibition of muscle differentiation and retardation of muscle regeneration	Miller et al., Am J Physiol, 2000; 278: C174
Rheumatoid arthritis	Type II Collagen-induced arthritis	Enhancement of Th2-type immune response Inhibition of development of collagen-induced arthritis	Okunishi et al., J Immunol, 2007, 179: 5504
Ligament injury	Tendon graft into bone	Promotion of histological and biomechanical regeneration and improvement	Nakase et al., Arthroscopy, 2010, 26: 84

Figure legends

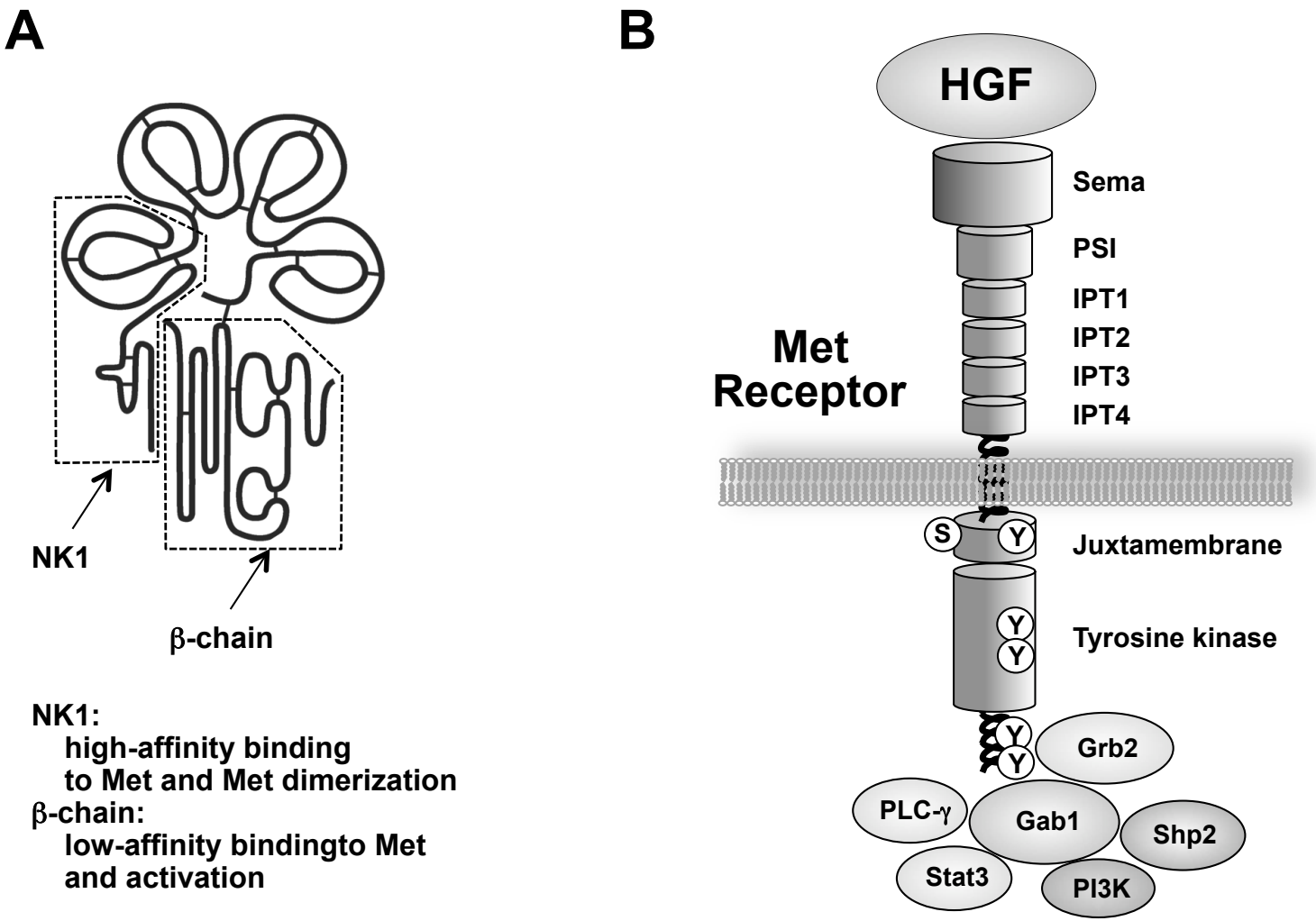
Fig. 1. Structural characteristic of HGF (**A**) and Met (**B**). Sema, the domain found in semaphorin receptors; PSI, the domain found in plexins, semaphorins and integrins; IPT, the domain found in immunoglobulins, plexins and transcription factors.

Fig. 2. Outline for biological and physiological actions of HGF, which participates in tissue protection and regeneration by HGF, and therapeutic actions of HGF.

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.

Fig. 4. Outline for distinct approaches targeting HGF and Met for potential cancer treatment (**A**), outline for biological activity of NK4 as a competitive HGF-antagonist (**B**), and anti-cancer action of NK4 (**C**, **D**). Inhibitory effect of NK4 on tumor invasion (**C**) and cancer metastasis (**D**). Photographs in **C** show invasion of human malignant mesothelioma cells in a 3-D collagen gel. Photographs in **D** show appearance of mesentery with disseminative metastasis of pancreatic cancer in mice. NK4 inhibited 3-D tumor invasion (**C**) and cancer metastasis (**D**).

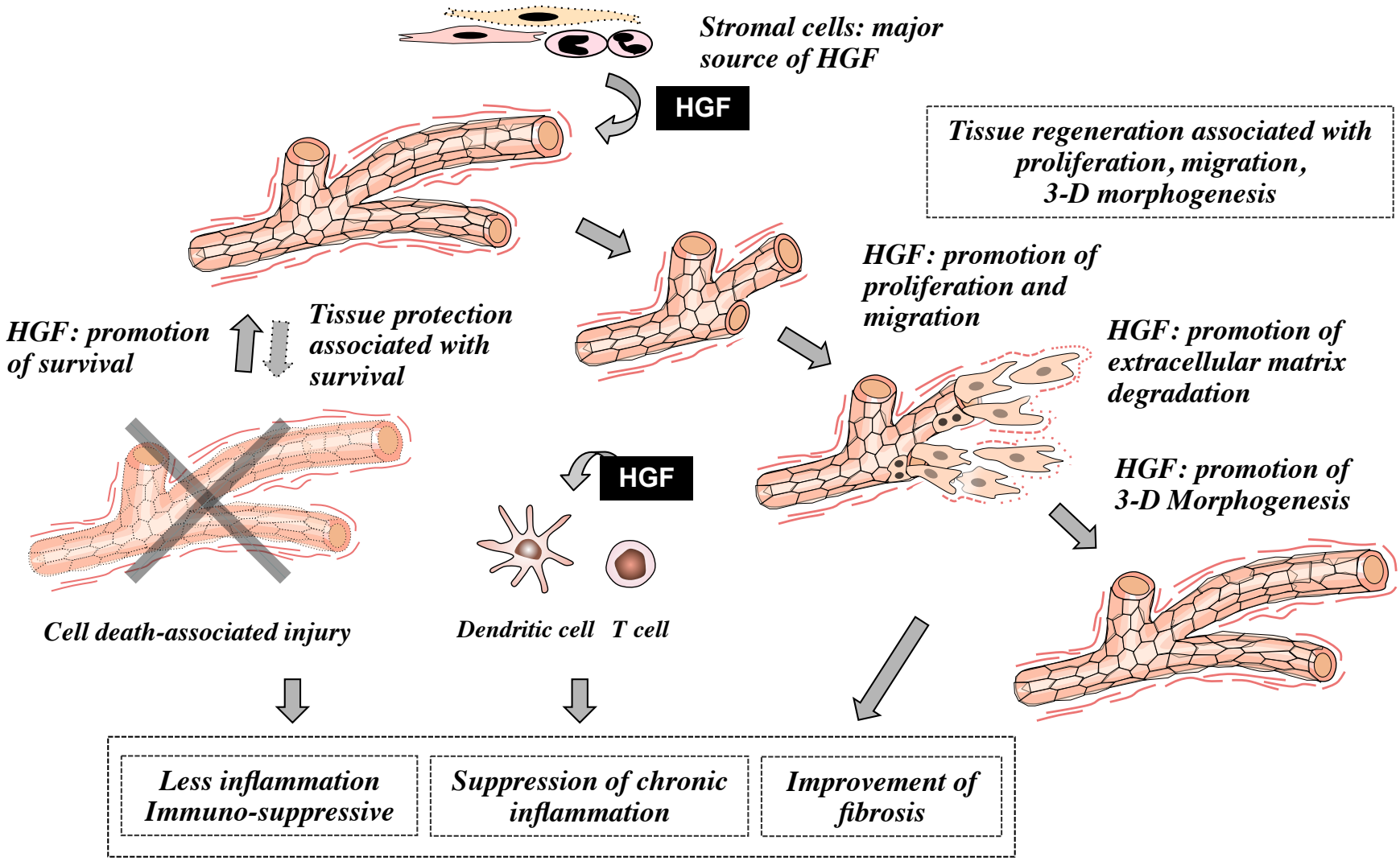
Nakamura et al. Fig. 1



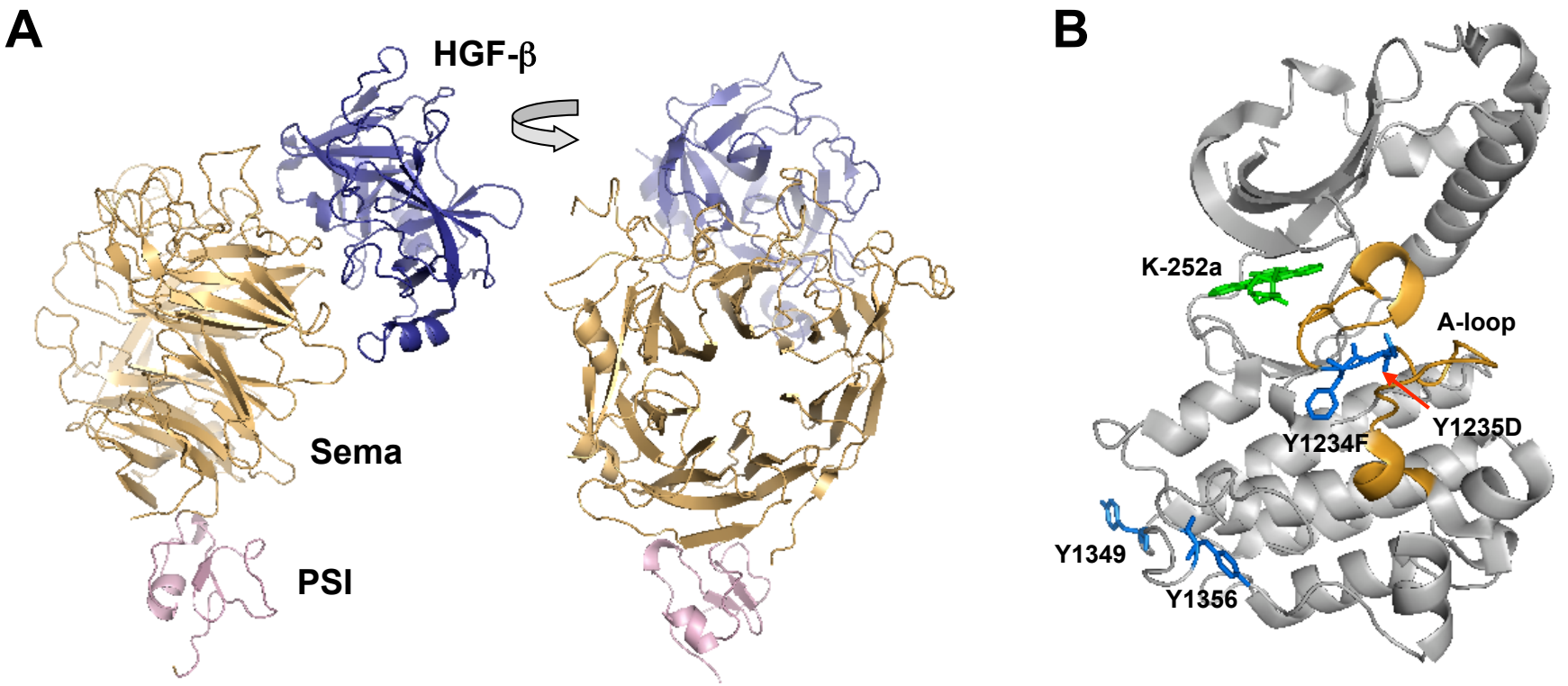
NK1:
high-affinity binding
to Met and Met dimerization

β-chain:
low-affinity bindingto Met
and activation

Nakamura et al. Fig. 2



Nakamura et al. Fig. 3



Nakamura et al. Fig. 4