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Hepatocyte Growth Factor and Met: Molecular Dialogue for Tissue Organization and Repair

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Hepatocyte growth factor (HGF), originally discovered and cloned as a powerful mitogen for hepatocytes, is a four kringle-containing growth factor which specifically binds to membrane-spanning tyrosine kinase, c-Met/HGF receptor. HGF has mitogenic, motogenic (enhancement of cell movement), morphogenic (e.g., induction of branching tubulogenesis), and anti-apoptotic activities for a wide variety of cells. During embryogenesis, HGF supports organogenesis and morphogenesis of various tissues, including liver, kidney, lung, gut, mammary gland, and tooth. In adult tissues HGF elicits an organotrophic function which supports regeneration of organs such as liver, kidney, lung, and vascular tissues. HGF is also a novel member of neurotrophic factor in nervous systems. Together with the preferential expression of HGF in mesenchymal or stromal cells, and c-Met/HGF receptor in epithelial or endothelial cells, the HGF-Met coupling seems to orchestrate dynamic morphogenic processes through epithelial-mesenchymal (or-stromal) interactions for organogenesis and organ regeneration. HGF or HGF gene may well become unique therapeutic tools for treatment of patients with various organ failure, through its actions to reconstruct organized tissue architectures. This review focuses on recently characterized biological and physiological functions integrated by HGF-Met coupling during organogenesis and organ regeneration.

Hepatocyte growth factor (HGF) was originally identified as a powerful mitogen for mature hepatocytes in primary culture (Nakamura et al., 1984; Russell et al., 1984), and it was purified from rat platelets (Nakamura et al., 1986; Nakamura et al., 1987) and human plasma (Gohda et al., 1988; Zarnegar and Michalopoulos, 1989). Molecular cloning of HGF revealed that HGF is a heterodimeric molecule composed of the four-kringle-containing α-chain and the serine protease-like β-chain (Nakamura et al., 1989) (Fig. 1). Subsequent purification and molecular cloning of bioactive molecules done in 1990 to 1991 unexpectedly showed that these are identical to HGF: molecular cloning of scatter factor (Weidner et al., 1991; Konishi et al., 1991), tumor cytotoxic factor (Shima et al., 1991), and fibroblast-derived epithelial growth factor (Rubin et al., 1991) were revealed to be identical with HGF. These independent approaches thus joined and further revealed diverse functions of HGF (also see reviews; Zarnegar and Michalopoulos, 1995; Matsumoto and Nakamura, 1996; Matsumoto and Nakamura, 1997).

The tumourigenic *met* oncogene was initially isolated from chemically transformed human osteosarcoma cells (Cooper et al., 1984). Although the primary structure of the *c-met* protooncogene product predicted it to be a receptor-type tyrosine kinase (Park et al., 1987), it remained orphan receptor until two research groups independently identified its ligand to be HGF in 1991 (Bottaro et al., 1991; Naldini et al., 1991). The c-Met/HGF receptor is heterodimeric molecule composed of a 50 kDa chain and a membrane-spanning 145 kDa β-chain which contains the intracellular tyrosine kinase domain (Park et al., 1987) (Fig. 2).

HGF is a family of structurally related factors, including at least one other member, HGF-like protein (HLP) (Han et al., 1991). HLP was later shown to be an molecule identical with macrophage-stimulating protein (MSP) (Yoshimura et al., 1993; Shimamoto et al., 1993). On the other hand, c-Met/HGF receptor has at least two distinct family members; Ron and Sea (Ronsin et al., 1993; Huff et al., 1993). Ron tyrosine kinase was identified as a specific receptor for HLP/MSP (Wang et al., 1994; Gaudino et al., 1994), but a ligand for Sea tyrosine kinase remains to be identified (Fig. 2).

Biological Activities and Target Cells

Although HGF was initially characterized as a mitogenic polypeptide for hepatocytes, as it was named, studies during past decade revealed multipotent characteristics

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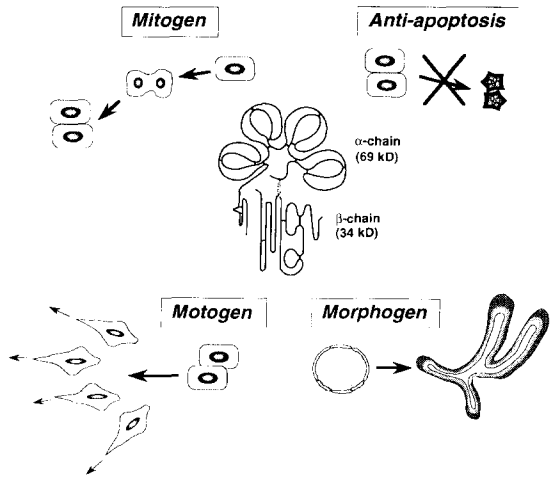

Fig. 1. Schematic structure of HGF and prominent biological activities of HGF in mitogenesis, motogenesis (cell movement), morphogenesis, and anti-apoptosis.

and a wide variety of target cell specificity of HGF (see reviews, Zarnegar and Michalopoulos, Matsumoto and Nakamura, 1996; Matsumoto and Nakamura, 1997). Table 1 summarizes typical biological activities of HGF for cells in embryos and normal tissues. In addition to its mitogenic activity, HGF stimulates motility and migration of various cells as "motogen" (Fig. 1). Among the multipotent characteristics of HGF, the morphogenic activity of HGF is notable and unique. This activity was initially noted in three-dimensional collagen gel cultures using a cell line derived from renal tubular cells wherein HGF induces branching tubulogenesis (Montesano et al., 1991) (Fig. 3). Induction of branching tubular structures in epithelial cells by HGF also occurs in other cells derived mammary gland and

Table 1. Typical biological activities of HGF and target cells

Biological activity	Target cells
Mitogenic	hepatocytes, hepatoblast-like cells, hepatic ductular epithelial cells, renal tubular cells, keratinocytes, hair cells, melanocytes, gastric epithelial cells, corneal epithelial cells, bronchial epithelial cells, alveolar type II epithelial cells, thyroid cells, mammary gland epithelial cells, Schwann cells, placental cytotrophoblasts, prostate epithelial cells, osteoclast-like cells, vascular endothelial cells, articular chondrocytes, muscle satellite cells, hematopoietic progenitor cells, etc
Motogenic	renal epithelial cells, hepatic ductular epithelial cells, keratinocytes, thyroid cell, mammary gland epithelial cells, vascular endothelial cells, articular chondrocytes, myogenic precursor cells, etc
Morphogenic	renal epithelial cells, hepatic epithelial cells, mammary gland epithelial cells, embryonic lung epithelial cells, etc
Anti-apoptosis	hepatocytes, pancreatic islet b cells, vascular endothelial cells, neurons (hippocampal neurons, cerebral cortex neurons, motor neurons) pancreatic β cells, etc

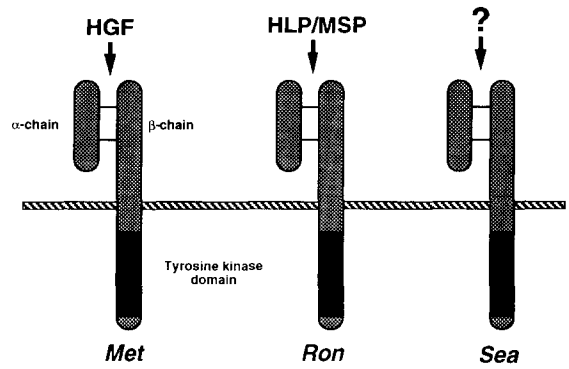

Fig. 2. Members in HGF and c-Met/HGF receptor families and their ligand-receptor relationship.

hepatic duct (Soriano et al., 1995; Niranjan et al., 1995; Yang et al., 1995; Johnson et al., 1993).

Apoptosis and anti-apoptosis plays an important roles in homeostasis of multicellular organisms, while a disrupted apoptosis system is involved in many diseases. HGF has a potent anti-apoptotic activity in several types of cells, including hepatocytes, renal epithelial cells, vascular endothelial cells, and neurons (Ishiki et al., 1992; Kawaida et al., 1994; Honda et al., 1995; Hamanoue et al., 1996; Morishita et al., 1997; Morishita et al., 1997a and 1997b; Kosai et al., 1998). Induction of Bcl-xL by HGF seems to be, at least, a molecular mechanism by which HGF prevents apoptotic cell death in hepatocytes (Kosai et al., 1998).

Cell proliferation, cell movement, and morphogenesis of cells are all crucial cellular events, during organogenesis and organ regeneration. Likewise, anti-apoptosis is probably involved in maintenance and homeostasis of functional tissues. As mitogenic, motogenic, morphogenic, and anti-apoptotic activities of HGF in many

Table 2. Possible therapeutic potential of HGF for diseases

Organs	Possible target diseases
Liver	fulminant and acute hepatitis (viral hepatitis, drug-induced hepatitis, etc), liver fibrosis/cirrhosis (viral infection, alcoholism, etc), fatty liver, liver transplantation, liver surgery (liver ischemia), hepatocellular carcinoma
Kidney	acute renal failure (ischemia, drug-induced, etc), chronic renal failure (renal sclerosis), renal transplantation
Lung	acute pneumonia, lung fibrosis (radiation-induced, drug-induced, autoimmune-induced, etc
Gastrointestinal system	gastric ulcer, diabetes millitus
Heart and circular system	myocardial infarction, vascular diseases induced by diabetes millitus, arteriosclerosis obliterans, myocardopathy
Muscular system	muscle atrophy, muscular dystrophy
Brain and neuronal system	brain ischemia, Parkinson's disease

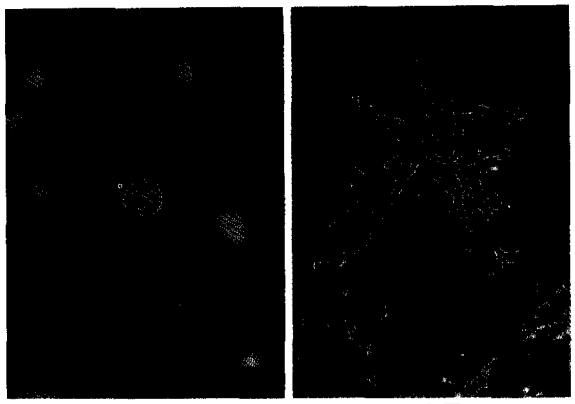

Fig. 3. Induction of branching tubulogenesis by HGF in MDCK renal epithelial cells grown in collagen gel matrix.

types of cells implicate diverse functions of HGF for the construction and reconstruction of multicellular tissue structures, recent studies clearly showed that HGF and Met are specific partner which integrates dynamic biological processes, including embryogenesis, organogenesis, and tissue regeneration.

Roles in Epithelial-Mesenchymal Interactions

Interactions between epithelium and mesenchyme, i.e., epithelial-mesenchymal interactions mediate crucial aspects of normal development, affecting tissue induction, organogenesis, and morphogenesis of multicellular structures. In tissue recombination, growth, differentiation, and morphogenesis of developing epithelia are regulated either inductively or permissively by neighboring mesenchyme, including kidney, lung, liver, pancreas, mammary gland, salivary gland, tooth, etc (Grobstein, 1967; Birchmeier and Birchmeier, 1993). A conceptual framework of epithelial-mesenchymal interactions was established in the 1950s and 1960s, but molecular mechanisms responsible for these interactions have not been elucidated. HGF is now known as a mesenchymal-derived mediator in epithelial-mesenchymal interactions.

During organogenesis of the lung, lung bud sprout out from foregut of endodermal tissue and the bronchial epithelia thereafter undergo branching tubulogenesis. Classical experiments using tissue recombination showed that morphogenesis of developing lung epithelium depends on a diffusible factor(s) derived from developing lung mesenchyme. *In situ* analysis of HGF and Met receptor mRNA in developing lung of rat embryo indicates that Met receptor mRNA is expressed in lung epithelia, whereas HGF mRNA is localized in the mesenchymal cells of developing lung (Ohmichi et al., 1998), indicating that HGF is produced in lung mesenchyme, while targets developing lung epithelium. In organ cultures of the developing lung, branching morphogenesis of developing lung epithelia is inhibited by anti-HGF antibody or antisense strategy. Thus HGF is a mesenchymal-derived paracrine factor which supports branching tubulogenesis during lung morphogenesis.

In addition to the lung development, HGF plays important morphogenic roles for the development of other tissues. During rat and murine development, Met receptor gene is expressed in epithelia, while HGF gene in mesenchymal cells in close vicinity in various organs such as kidney, liver, pancreas, lung, intestine, stomach, salivary gland, limb bud, and tooth (Sonnenberg et al., 1993; Tabata et al., 1996; Ohmichi et al., 1998). The expression patterns indicate that HGF is a mesenchymal-derived factor which predominantly acts on neighboring developing epithelia. In organ culture experiments, antibodies against HGF inhibit branching tubulogenesis of developing epithelia in the kidney and mammary gland (Santos et al., 1994; Niranjan et al., 1995; Woolf et al., 1995). In tooth germ culture, antisense oligonucleotide to HGF induces impaired morphogenesis of tooth epithelium which subsequently differentiate into ameloblasts (Tabata et al., 1996).

Essential roles of HGF in the development of mammalian fetal tissues were also defined by targeted disruption of HGF or Met/HGF receptor gene (Schmidt et al., 1995; Uehara et al., 1995; Bladt et al., 1995). These knockout mice are embryonic lethal due to impaired organogenesis of the placenta and liver. In the placenta, the number of labyrinthine trophoblasts is markedly reduced. The embryonic liver is reduced in size and shows extensive apoptotic cell death. In addition to this impaired tissue formation, cell movement in a specific tissue is diminished: the migration of Met-positive myogenic precursor cells from dermomyotome in the somite to limb buds and diaphragm is impaired. Consequently, skeletal muscles of the limb and diaphragm are not formed in mutant mice (Bladt et al., 1995). Since HGF is strongly expressed in limb bud mesenchyme and septum transversum (which develops into diaphragm), HGF provides spatially defined chemoattractant-like motogenic signals for migration of myogenic precursor cells. Moreover, essential roles of HGF for organogenesis was demonstrated in amphibian *Xenopus* (Aoki et al., 1997). Dominant expression of tyrosine kinase-minus Met receptor in *Xenopus* embryo results in liver defects and impaired development of pronephrons, gut, and skeletal morphogenesis in tail region.

Following migration of muscle precursor cells into the limbs, HGF is restrictively expressed in future joint regions and muscle tissues. Since HGF stimulates growth and migration of chondrocytes (Takebayashi et al., 1995), HGF seems to be involved in cartilage development. Furthermore, HGF functions as an axonal chemoattractant for spinal motor neurons, for projection of motor neurons to limb muscle (Ebens et al., 1996). Fig. 4 describes multipotent roles of HGF in embryo and adult tissues.

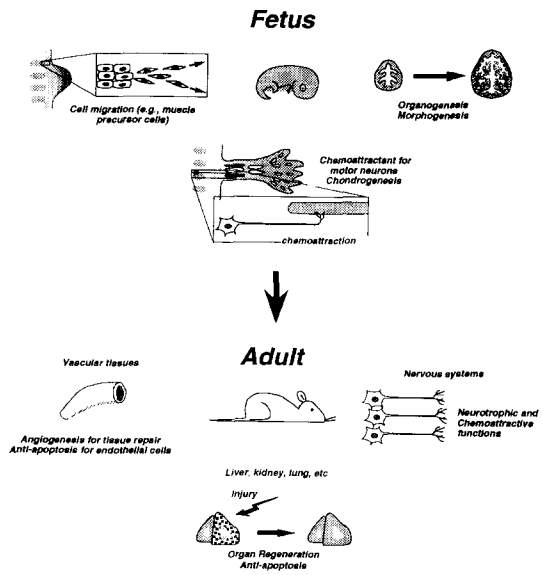

Fig. 4. Pleiotrophic roles of HGF in development, regeneration, and maintenance of various organs and tissues.

Organotrophic Roles

Regeneration of the liver is one of the most dramatic phenomena in higher animals. When 70% of the liver is resected, the cells in the remaining liver rapidly proliferated and the original liver mass is restored within a week. As HGF was initially implicated to be a humoral hepatotrophic factor which enhances liver regeneration, the hepatotrophic roles of HGF have been well-established during the last 10 years (Matsumoto and Nakamura, 1992; Matsumoto and Nakamura, 1996). HGF is now seen to have the role of organotrophic factor for regeneration of other tissues and organs.

In rats and mice, a range of hepatic injuries induce rapid HGF mRNA expression in liver stromal cells such as Kupffer cells (liver macrophages), sinusoidal endothelial cells, stellate (Ito) cells, but not in parenchymal hepatocytes. HGF secreted by these hepatic stromal cells acts on neighboring hepatocytes, i.e., by a paracrine mechanism (Kinoshita et al., 1989; Noji et al., 1990) (Fig. 5). Moreover, HGF functions by similar paracrine mechanisms during regeneration of the kidney (Nagaike et al., 1991; Igawa et al., 1993), lung (Yanagita et al., 1993), and stomach (Takahashi et al., 1995; Schmassmann et al., 1997). In the kidney, mesangial cells and fenestrated endothelial cells express HGF, while HGF elicits a potent mitogenic, morphogenic, and anti-apoptotic activities on renal tubular cells. Similarly, lung stromal cells such as alveolar macrophages, endothelial cells and fibroblasts produce HGF, while HGF acts on bronchial epithelial cells and aleveolar type II epithelial cells in the close vicinity to stromal cells (Mason et al., 1994; Ohmichi et al.,

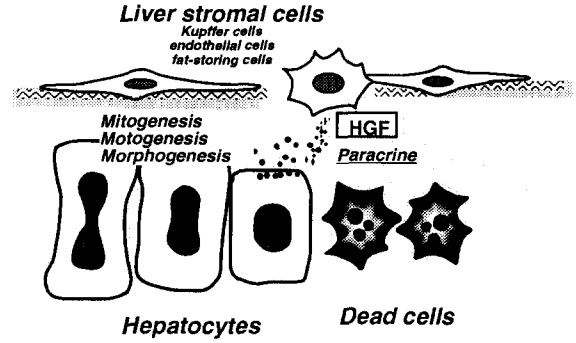

Fig. 5. Possible mechanism of liver regeneration by HGF. HGF is produced in hepatic stromal cells (Kupffer cells, sinusoidal endothelial cells, Ito cells) and predominantly acts on neighboring hepatocytes that express c-Met/HGF receptor in the liver.

1996). In response to the onset of gastric ulcer, HGF expression is up-regulated in stromal cells between regenerative glands and endothelial cells, while Met receptor is expressed in the epithelial cells of the regenerative glands (Schmassmann et al., 1997). Thus, the conceptual framework that HGF mediate epithelial-mesenchymal interactions during organogenesis is likely to be extended to include organ regeneration: HGF is a mediator in epithelial-stromal interactions during organ regeneration.

Angiogenesis is also an essential event for embryogenesis, tissue regeneration and tumor growth. Vascular endothelial cells from various tissues express Met receptor and HGF stimulates growth and migration of endothelial cells. HGF induces new blood vessel formation *in vivo* (Bussolino et al., 1992; Grant et al., 1993), hence is a member of angiogenic factor. However, since abnormal vascular formation was not seen in knockout mice, probably HGF does not play a major role in neovascularization during embryogenesis. Nevertheless, HGF is an endothelium-specific growth factor in vascular tissue and may play an important role for maintenance and neovascularization in physiological condition and tissue regeneration, respectively. HGF is produced in vascular smooth muscle cells and has potent anti-apoptotic activity on endothelial cells (Morishita et al., 1997a and 1997b).

Therapeutic Potentials

On the basis of its organotrophic roles, the therapeutic potential of HGF has been demonstrated in various disease models. The initial approaches used to define therapeutic efficacy of HGF were done in models for acute organ injuries. Administration of human recombinant HGF into mice stimulates proliferation of hepatocytes after liver insult caused by partial hepatectomy or hepatotoxins (Ishiki et al., 1992). Importantly, HGF also exert potent anti-apoptotic and cytoprotective activity *in vivo*: HGF abrogated the onset of fulminant hepatic failure caused by activation of Fas receptor in

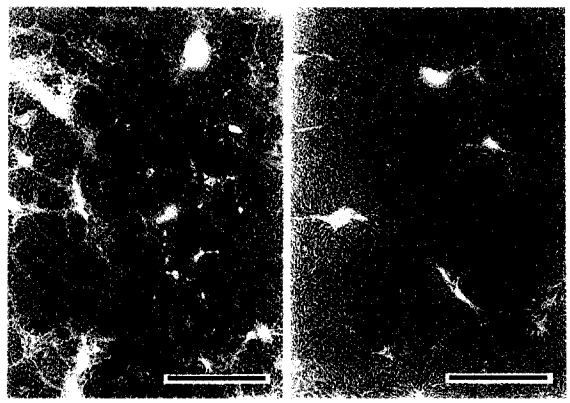

Fig. 6. Prevention of liver cirrhosis by HGF-administration in rats. Dimethylnitrosamine was intraperitoneally administered into rats for 4 weeks and human recombinant HGF (300 mg/kg body weight) was daily injected into these dimethylnitrosamine-treated rats for 4 weeks. HGF clearly prevents the onset of liver cirrhosis (Matsuda et al., 1995). Scale bars=100 μm.

the liver (Kosai et al., 1998). Since excessive activation of the Fas system following hepatitis viral infection in the liver is a critical event for the onset of fulminant hepatic failure whose prognosis is extremely bad with high mortality, the results implicate a potential therapeutic usage of HGF for treatment of fulminant hepatic failure. Similarly, HGF suppresses the onset of acute hepatitis caused by hepatotoxins or endotoxin shock (Ishiki et al., 1992; Kaido et al., 1997). Although acute renal failure often occurs by administration of nephrotoxic drugs such as cisplatin (anti-cancer drug), cyclosporin A, and FK506 (immunosuppressive drugs), or by renal ischemia, HGF potently suppresses the onset of acute renal failure in experimental animals (Kawaida et al., 1994; Amaike et al., 1996; Takada et al., 1996). For acute lung injury, HGF enhances mitogenesis of bronchial and alveolar epithelial cells, implicating therapeutic effect for treatment of patients with pneumonia (Ohmichi et al., 1996).

Chronic inflammatory diseases are generally characterized by fibrotic changes in tissues, including liver cirrhosis, chronic renal failure (or renal sclerosis), lung fibrosis, and cardiomyopathy. These fibrotic diseases are progressive and currently incurable. In terms of the liver, liver cirrhosis often develops due to hepatitis virus infection, hepatotoxic drug administration, and alcohol ingestion. The first therapeutic effect of HGF on fibrotic diseases was shown in a rat model for liver cirrhosis (Matsuda et al., 1995) (Fig. 6). Administration of HGF into rats with liver cirrhosis/fibrosis abrogates the onset of severe liver cirrhosis and has a potent therapeutic effect: HGF decreases accumulation of hepatic extracellular matrix components, enhances liver specific functions, and abrogates mortality due to hepatic dysfunction (Matsuda et al., 1995). Similar potent therapeutic effect of HGF was further demonstrated in distinct models for liver cirrhosis (Yasuda et al., 1996; Matsuda et al., 1997). Moreover, anti-fibrogenic action of HGF was demonstrated in other fibrotic diseases in distinct organs. HGF prevents renal fibrosis/sclerosis and concomitant renal dysfunction in mice model for chronic renal failure (Mizuno et al., 1998). Likewise, HGF-administration prevents lung fibrosis caused by bleomycin (Yaekashiwa et al., 1998). Bleomycin is a widely used anti-cancer drug, but it often causes lung fibrosis in patients. How HGF prevents fibrotic diseases? Currently, transforming growth factor-β (TGF-β) is known as a key growth factor of which over-expression leads to the onset of fibrotic diseases. HGF suppresses TGF-β expression, formation of myofibroblasts (predominant cells responsible for extracellular matrix deposition), and apoptotic cell death in epithelial cells (i.e., hepatocytes, renal tubular cells, alveolar epithelial cells) and endothelial cells, while HGF enhances protease activities responsible for degradation of extracellular matrix components and exerts mitogenic action on epithelial and endothelial cells (Matsuda et al., 1995; Yasuda, et al., 1996; Matsuda, et al., 1997; Yaekashiwa et al., 1998; Mizuno et al., 1998). Table 2 summarizes diseases to which HGF may be used as a therapeutic drug. HGF has an angiogenic activity *in vivo* and prevents high glucose-induced endothelial cell death (Morishita et al., 1997a and 1997b), implicating therapeutic potential for treatment of patients with myocardial infarction and vascular diseases due to diabetes millitus. HGF is a novel member of neurotrophic factor and HGF-infusion into the brain prevents the delayed neuronal death in hippocampal neurons (Miyazawa et al., 1998). In articular tissues, HGF effectively repairs osteochondral defects in rabbit model (Wakitani et al., 1997). HGF is a trophic factor for pancreatic β-cells (Otonski et al., 1994), and a potent mitogen for muscle satellite cells (Allen et al., 1995) and gastric mucosal epithelial cells (Takahashi et al., 1995; Schmassmann et al., 1997). Ongoing studies implicate a possible therapeutic application of HGF for diseases in these tissues.

Perspective

We now know that HGF has various biological activities far over initially implicated function as a potent hepatotrophic factor for liver regeneration. HGF may prove to participate in construction and reconstruction of tissues through mitogenic, motogenic, and morphogenic activities, all these essential to form well-organized multicellular structures. Anti-apoptotic activity of HGF is likely to be involved in the maintenance and homeostasis of multicellular tissue structures. The molecular and cellular mechanism in which HGF-Met signaling integrates endogenous programs (i.e., activation and/or inactivation of specific genes), leading to tissue organization is expected to be understood.

One particular characteristic of HGF is its potential toward therapeutic application for various diseases.

HGF has biological effects on a wide variety of differentiated cells in the liver, lung, kidney, vascular tissue, nervous systems, bone tissue, gastro-intestinal tissues, skin, heart and muscle. It is noteworthy that HGF has strong therapeutic effects on chronic or progressive fibrotic diseases such as liver cirrhosis, chronic renal failure, and lung fibrosis. Progressive fibrotic diseases are characterized by hyperaccumulation of extracellular matrix and apoptosis of differentiated cells, and importantly there has been no cure-oriented therapeutics for these diseases, including liver cirrhosis, chronic renal failure, lung fibrosis, cardiomyopathy, muscular dystrophy, articular sclerosis, and Alzheimer's disease. The therapeutic effects of HGF on fibrotic disease in the liver, kidney, and lung encourage to address efficacy of HGF or HGF gene therapy for other chronic and degenerative tissue diseases of generally incurable.

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