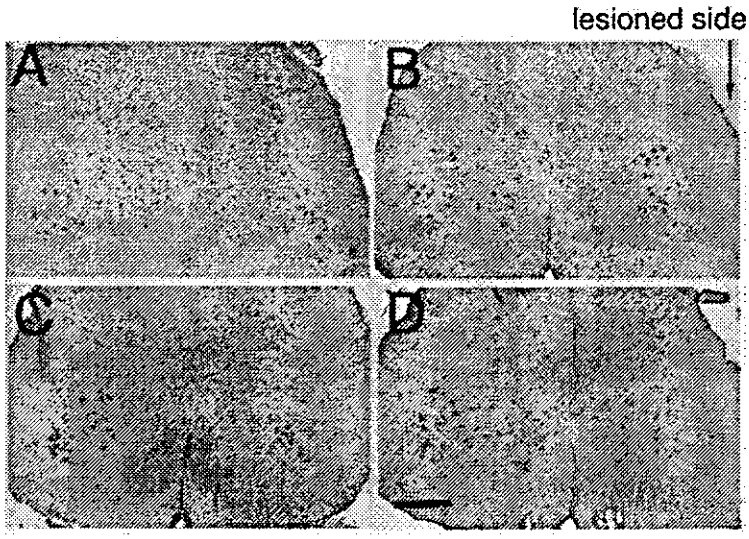

Fig. 3. Photomicrographs showing GluR2 immunoreactivity in the L4–L5 spinal segments at different survival times after avulsion. (A) In control sections, GluR2 immunoreactivity is present in the motor neurons and interneurons of the ventral horn, and is also detectable in the superficial laminae of the dorsal horn. (B) At 24 h after avulsion, GluR2 immunoreactivity starts to decrease in motoneurons on the lesioned side, and this decrease becomes more prominent 3 (C) to 7 (D) days after avulsion. Scale bar in D=400 μm (also applies to A, B and C).

expression patterns of the AMPA receptor subunits that we observed in the spinal cord of uninjured control rats were consistent with previously published observations (Furuyama et al., 1993; Jakowec et al., 1995; Kennis and Holstege, 1997). GluR2 was present in the dendrites as well as the cell bodies of spinal motoneurons, suggesting that the AMPA receptors on the postsynaptic membrane contain GluR2 subunit and are Ca^{2+}-impermeable.

After ventral root avulsion, downregulation of GluR2 subunit was observed in both the dendrites and cell bodies of spinal motoneurons. Although the inability to detect immunoreactivity for a protein does not necessarily imply its absence, the decrease in GluR2 subunit could lead to the formation of GluR2-lacking Ca^{2+}-permeable AMPA receptors in motor neurons. Since AMPA receptor antagonists partially rescued injured motoneurons from avulsion-induced death, AMPA receptor-mediated excitotoxicity in response to endogenous glutamate might contribute to motoneuron death by avulsion injury. It was reported that motoneuron death in neonatal rats induced by sciatic

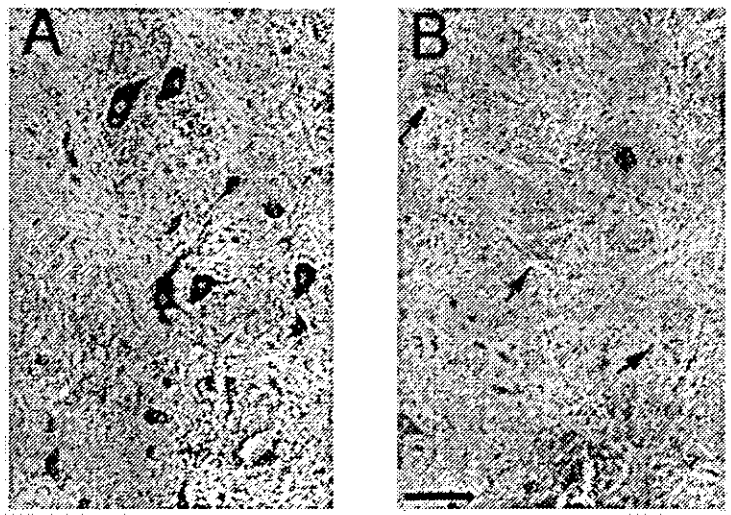

Fig. 4. Higher-magnification photomicrographs of the ventral horn of L4–L5 spinal segments showing GluR2-positive motoneurons at 7 days after avulsion. Whereas many GluR2-positive motoneurons are found on the intact side (A), GluR2-negative motoneurons are observed on the lesioned side (B; arrows). Note that GluR2 is present in both the perikarya and dendrites in (A), while in (B) neither are immunoreactive. Scale bar in B=50 μm (also applies to A).

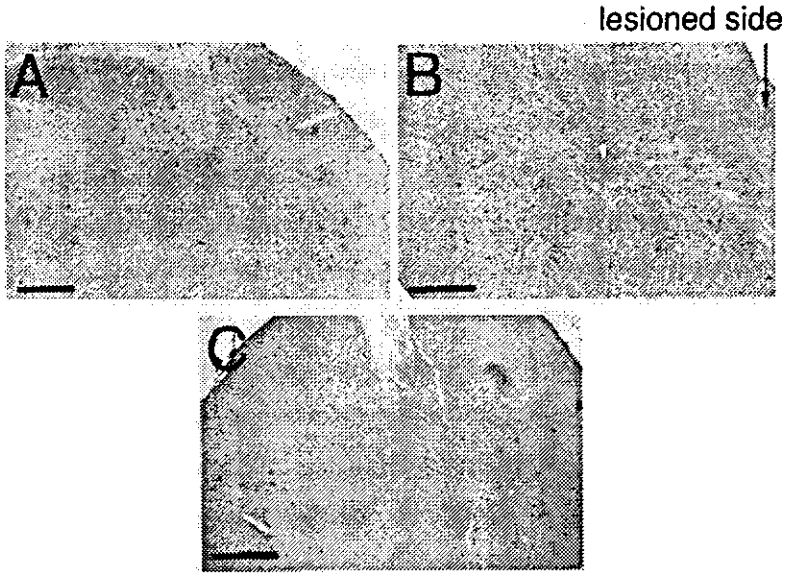

Fig. 5. Immunohistochemical detection of GluR1 (A), GluR4 (B), and NR1 (C) in the L4–L5 spinal segments at 3 days after avulsion. Immunoreactivity for GluR1 (A) and GluR4 (B) is present in motoneurons and interneurons in the ventral horn as well as in cells in the dorsal horn. Staining for NR1 (C) is found in motor neurons and other neurons throughout the gray matter. GluR1, GluR4 and NR1 expression levels in motoneurons on the lesioned side are indistinguishable from the intact side at 3 days after avulsion, in contrast to GluR2 levels. Scale bars=400 μm.

nerve crush was ameliorated by blocking NMDA receptors with MK-801 (Mentis et al., 1993). In contrast, NMDA receptor antagonists showed no effect against avulsion-induced motoneuron death in the present study. The reason for this discrepancy would be due to differences in the methods of neuronal injury and the age of animals. In any case, glutamate toxicity seems to play an important role in these nerve-injury models.

After ischemic brain injury, a specific reduction of the GluR2 subunit has also been observed and this reduction after global (Gorter et al., 1997) or forebrain (Heurteaux et al., 1994) ischemia preceded cell death of the vulnerable hippocampal CA1 neurons, suggesting that the assembly of new, Ca^{2+}-permeable AMPA receptors may contribute to neuronal loss. Aronica et al. (1998) reported that blocking the downregulation of GluR2 with aurintricarboxylic

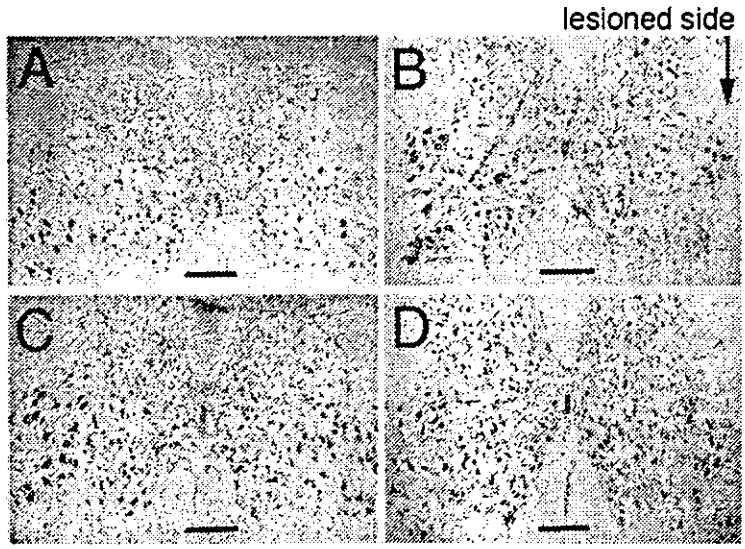

Fig. 6. Photomicrographs of Nissl-stained transverse sections of the L4 spinal cord of adult rats at 2 weeks of treatment with either vehicle (artificial cerebrospinal fluid [aCSF]) (A) or glutamate receptor antagonist following avulsion. Immediately after the avulsion procedures, NMDA-receptor or AMPA-receptor antagonists were continuously infused into the intrathecal lumbar spinal cord region using a miniosmotic pump. The antagonists were dissolved and aliquoted in sterile aCSF to give 2-mM final concentrations, and the nominal infusion rate was 1 nmole/h. Treatment of avulsion-lesioned rats with NBQX (C) or CNQX (D) rescues injured motoneurons from cell death, whereas neither MK-801 (B) nor D-AP5 enhances survival. Scale bars=300 μm.

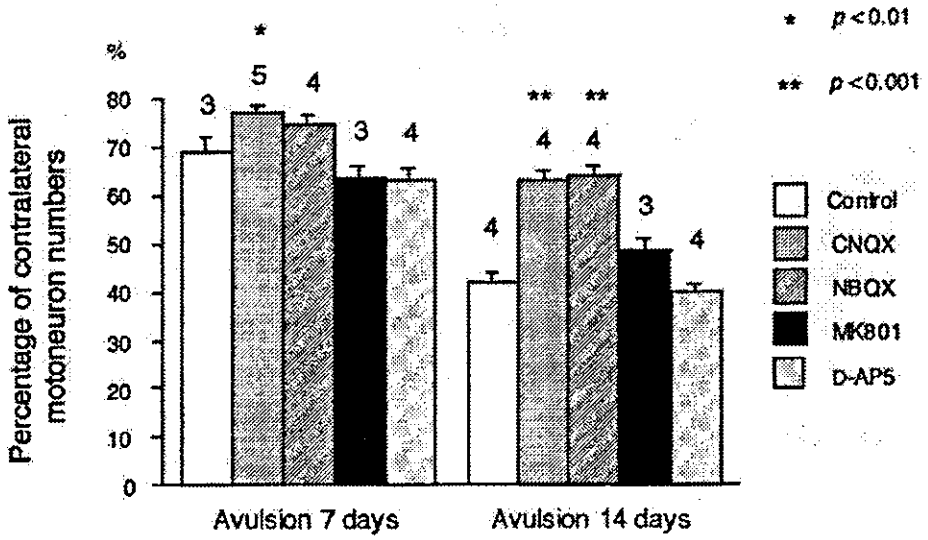

Fig. 7. One week of treatment with CNQX, an AMPA antagonist, produces a significant effect on motoneuron survival after avulsion with 8.2% of the motoneurons being rescued from death compared with the vehicle (aCSF) treatment. Two weeks of treatment with NBQX or CNQX also rescues a significant number (22% or 21%, respectively) of motoneurons from avulsion-induced cell death. In contrast, treatment with MK-801 or D-AP5 has no effect on motoneuron survival. Data are expressed as the percentage (mean±S.E.M.) of the contralateral (control) motoneuron numbers, which represent 100%. Numbers above the bars represent the numbers of animals examined in each group. * $P<0.01$ versus control (aCSF-treated), MK-801- and D-AP5-treated groups; ** $P<0.001$ versus control, MK-801- and D-AP5-treated groups. Statistical comparisons were performed using one-way analysis of variance followed by Newman-Keuls test.

acid after global ischemia prevented delayed neurodegeneration in the hippocampal CA1 neurons of gerbils, further suggesting a role for GluR2-lacking Ca^{2+}-permeable AMPA receptors in neuronal death. In rat spinal cord, sciatic nerve transection (axotomy) has been reported to induce a reduction in GluR2/3 expression in the corresponding motoneurons in the spinal cord, although axotomy itself does not induce motoneuron loss (Popratiloff et al., 1996; Kennis and Holstege, 1997). Furthermore, GluR2 mRNA and, to a lesser extent, GluR4 mRNA in motoneurons are decreased after spinal cord contusion injury in rats (Grossman et al., 1999). In this contusion model, the downregulation of GluR2 was observed in preserved apparently healthy motoneurons, with the reduction in GluR2 being relatively small compared with that seen in brain ischemia (Grossman et al., 1999). These findings suggest that reduced GluR2 expression in motoneurons does not necessarily result in cell death.

It has also been reported that glutamate subunit changes are associated with synaptic plasticity (Garraghty and Muja, 1996; Kaczmarek et al., 1997), and that synaptic strengthening can occur by activation of GluR2-lacking Ca^{2+}-permeable AMPA receptors (Gu et al., 1996). Thus, the downregulation of GluR2 expression in injured motoneurons appears to be related to Ca^{2+}-mediated synaptic plasticity in these neurons.

In the case of avulsion injury, injured motoneurons are completely separated from their peripheral segments, which contain Schwann cells associated with the motor axons, resulting in the complete removal of vital trophic support. Thus, it is possible that injured motor neurons without trophic support may be especially susceptible to Ca^{2+}-permeable AMPA receptor-mediated excitotoxicity. However, the protective effects of AMPA receptor antagonists on avulsed motoneurons were not complete, suggesting that there may also be other mechanisms of avulsion-induced motoneuron death. It has been reported that axotomized neonatal rat facial motoneurons undergo apoptotic cell death (Rossiter et al., 1996) and that motoneuron degeneration after sciatic nerve avulsion in adult rats is also apoptotic (Martin et al., 1999a). Furthermore, the dying motoneurons following avulsion exhibit a sustained accumulation of active mitochondria and oxidative damage as indicated by the formation of nitrotyrosine-modified proteins and hydroxyl radical-modified DNA and RNA in motor neurons (Martin et al., 1999a). AMPA exposure causes mitochondrial depolarization and reactive oxygen species generation in spinal motor neurons *in vitro* (Carriedo et al., 2000), suggesting that the oxidative damage of motor neurons after avulsion injury may be mediated by GluR2-lacking Ca^{2+}-permeable AMPA receptors, eventually leading to apoptotic cell death. Further studies are needed to examine whether anti-apoptotic agents, such as caspase inhibitors, can inhibit avulsion-induced motoneuron death.

In human motoneurons, some studies have found evidence for the presence of GluR2 (Tomiyama et al., 1996; Virgo et al., 1996), while others correlated a low expression or absence of GluR2 with the selective vulnerability of motoneurons (Williams et al., 1996, 1997; Shaw et al., 1999). In rodent motor neurons, most studies, including ours, demonstrated that the GluR2 subunit was present (Furuyama et al., 1993; Jakowec et al., 1995; Kennis and Holstege, 1997). Interestingly, Vandenberghe et al. (2001)

found that GluR2-containing and GluR2-lacking AMPA receptors coexist in single motoneurons *in vitro*. In addition, rat spinal motoneurons expressing GluR2 stained for Co^{2+} after stimulation by kainate, suggesting that they contain Ca^{2+}-permeable AMPA receptors (Van Den Bosch et al., 2000). Thus, both Ca^{2+}-permeable and Ca^{2+}-impermeable AMPA receptors may be coexpressed in spinal motoneurons, suggesting that AMPA receptors with different molecular and functional properties would be expressed on the same motoneurons (Rubio and Wenthold, 1997; Vandenberghe et al., 2001). This hypothesis may help to provide resolution of the apparently conflicting data regarding the presence of GluR2 subunit in human motoneurons. Overall, human motor neurons may coexpress Ca^{2+}-permeable and Ca^{2+}-impermeable AMPA receptors, implying that the presence of Ca^{2+}-permeable receptors renders motor neurons vulnerable to excitotoxicity.

Downregulation of GluR2 relative to the other subunits could increase the Ca^{2+} influx into neurons after stresses such as avulsion or ischemia, possibly contributing to the delayed neurodegeneration (Gorter et al., 1997). GluR2-lacking AMPA receptors apparently constitute only a small fraction of the total AMPA receptor population in motoneurons, but their clustering greatly amplifies the local Ca^{2+} signals that result from their activation (Vandenberghe et al., 2001). Thus, a slight increase in GluR2-lacking AMPA receptors may be sufficient to induce cell death, which would be relevant to the selective motoneuron death in ALS.

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Review

Hepatocyte growth factor: from diagnosis to clinical applications

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Abstract

Hepatocyte growth factor (HGF), initially identified and molecularly cloned as a potent mitogen of primary cultured hepatocytes, has multiple activities in a variety of tissues during the course of development and also in various disease states. HGF plays key roles in the attenuation of disease progression as an intrinsic repair factor. It is also evident that HGF levels are regulated under different conditions, for example, during the course of pregnancy, aging, and disease. This review focuses on the levels of HGF in normal and pathophysiological situations and examines the relationships between HGF levels and disease, disease stage, and disease prognosis. The clinical potential of HGF as a treatment for subjects with various diseases is also given attention.

Keywords: HGF; c-Met; Serum level; Tissue level; Disease; Gene therapy

1. Introduction

Numerous growth factors are regulated in a concerted fashion to maintain homeostasis, not only in healthy individuals responding to their surroundings, but also in subjects with diseases. Therefore, an understanding of the regulation and potential clinical applications of growth factors is of great importance.

Hepatocyte growth factor (HGF) was first identified in 1984 [1,2] and 1985 [3] and purified as a potent mitogen of primary cultured hepatocytes [4–6]. Molecular cloning revealed that it is a heterodimeric molecule composed of a 69-kDa α-chain and a 34-kDa β-chain. The α-chain contains an N-terminal hairpin domain and subsequent four-kringle domains, and the β-chain contains a serine protease-like domain with no enzymatic activity [7–9]. A fibroblast-derived epithelial cell motility factor, termed scatter factor, was identified in 1985 [10] and purified in 1989 [11]. Subsequent characterization revealed scatter factor to be identical to HGF [12–14]. HGF is synthesized and secreted as a biologically inactive

Abbreviations: AD, Alzheimer's disease; ChAT, choline acetyltransferase; CH, chronic hepatitis; CK, creatinine phosphokinase; CRP, C-reactive protein; DMN, dimethylnitrosamine; CsA, Cyclosporin A; CSF, cerebrospinal fluid; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; HGF A, HGF activator; HVJ, hemagglutinating virus of Japan; LC, liver cirrhosis; OA, osteoarthritis; PAOD, peripheral arterial occlusive disease; RA, rheumatoid arthritis; TGF-β, transforming growth factor-β; TIF, tubulointerstitial fibrosis; TIL, tubulointerstitial lesion; uPA, urokinase-type plasminogen activator.

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single-chain precursor form, and further processing by serine proteases into the two-chain form is coupled to its activation (Fig. 1). Serine proteases responsible for the activation of HGF include HGF activator or HGF-converting enzyme and urokinase-type plasminogen activator (uPA) [15–18]. The receptor for HGF was identified as a c-met proto-oncogene product [19–21]. The c-Met receptor is composed of a 50-kDa α-chain and 145-kDa β-chain. The α-chain is exposed extracellularly, while the β-chain is a transmembrane subunit containing an intracellular tyrosine kinase domain. Binding of HGF to the c-Met receptor induces activation of tyrosine kinase, an event that results in subsequent phosphorylation of C-terminally clustered tyrosine residues (Fig. 1) [22]. Phosphorylation of these tyrosine residues recruits intracellular signaling molecules containing the src homology (SH) domain, including Gab-1, phospholipase c-γ (PLC-γ), Ras-GTPase activating protein (Ras-GAP), phosphatidylinositol 4,5-bisphosphate 3-kinase (PI-3 kinase), c-Src, Shp-2, Crk, and Grb-2. A potential contribution of Bag-1 and STAT3 for HGF signaling was also reported [23,24]. Although HGF was initially identified as a potent mitogen for hepatocytes, considerable evidence indicates that intracellular signaling pathways driven by HGF-c-Met receptor coupling lead to

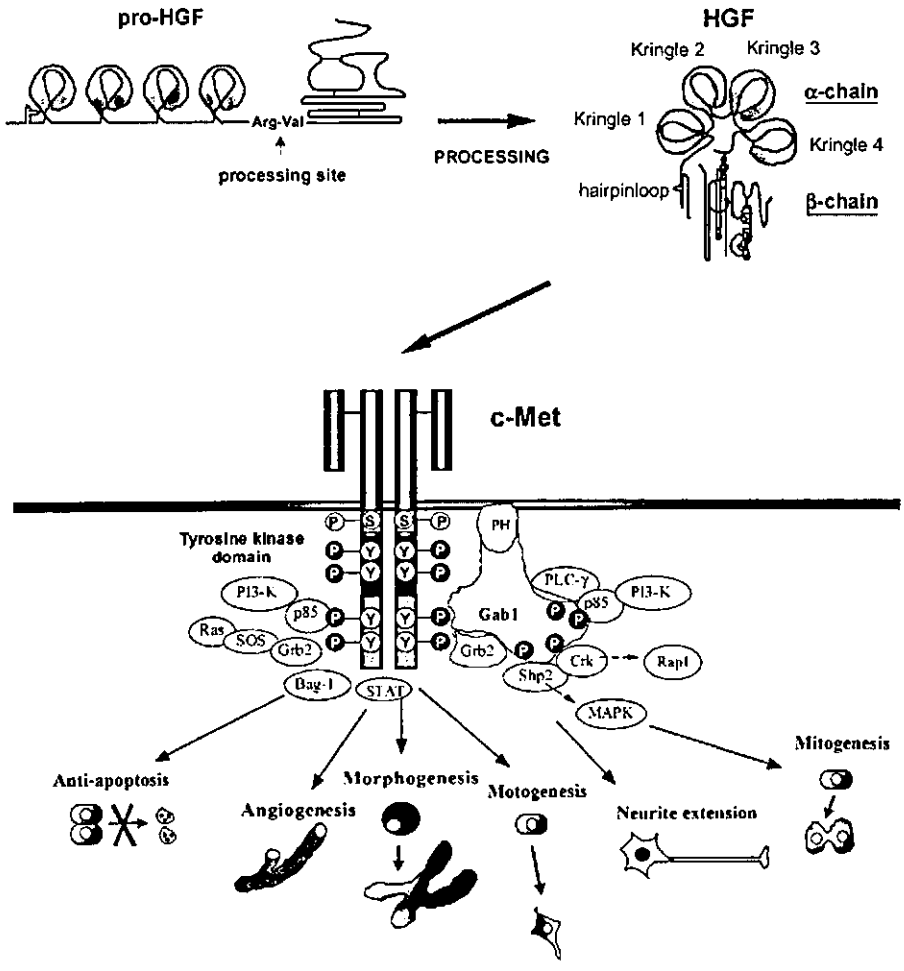

Fig. 1. Schematic structure of mature HGF processed from proHGF, and biological activities of mature HGF mediated by intracellular signals of the c-Met/HGF receptor.

multiple biological responses in a variety of cells, including mitogenic, motogenic (enhancement of cell motility), morphogenic, neurite extension and anti-apoptotic activities.

The essential role for HGF and the c-Met receptor in mammalian development was defined by the disruption of *HGF* or the *c-met* gene in mice; these mice died during development (embryonic days 13–15) as organogenesis of the placenta and liver was impaired. HGF is also involved in the formation of the kidney, lung, mammary gland, teeth, muscle, and neuronal tissues [25–31], and the organotypic role of HGF in the liver has been well defined [25].

In addition, HGF has an organotrophic role in the regeneration and protection of various organs, including the liver, lung, stomach, pancreas, heart, brain, and kidney [32–38]. As correlated with the role of HGF in such diseases, HGF levels are regulated in serum, bronchoalveolar lavage, cerebrospinal and synovial fluids, and/or tissues. In this review, we focus on the relationships between HGF levels and normo-physiological alterations or disease states and the clinical potential of HGF to treat individuals with diseases.

2. Hemodynamics of HGF

An understanding of the hemodynamics of HGF is essential to estimate the clinical relevance of an alteration of serum HGF levels. An injection study revealed that the percentage of ^{125}I-labeled HGF in the peripheral blood was 92.3% at 3 min, 86.5% at 30 min, 80.4% at 60 min, 69.4% at 120 min after the injection, and that ^{125}I-labeled HGF diffuses into the liver, adrenal gland, spleen, kidney, lung, stomach, and intestine within 3 min after intravenous injection of HGF, but does not diffuse into the heart or brain [39–43]. The clearance rate of ^{125}I-labeled HGF is about 70% in the liver and less than 10% in the kidney. Two mechanisms of HGF clearance have been hypothesized: one is internalization of HGF with c-Met and the other is trapping by binding molecules, such as heparan sulfate. When a large amount of recombinant HGF is administered intravenously, c-Met levels in the liver decrease, resulting in the reduction of internalization efficiency. In cases of liver damage, plasma HGF levels are likely to increase, not only because of the up-regulation of HGF, but also because of the lower clearance of HGF in the liver.

3. Alteration of serum HGF levels in normal conditions

HGF levels are altered by various factors, such as aging and pregnancy. For example, serum HGF levels in females are 0.36 ng/ml at 10 years of age, increase to 0.39 ng/ml at 20–29 years of age, and decrease with aging to 0.26 ng/ml at 50 (Table 1) [44–47]. There are small differences in HGF levels between males and females: the levels are 0.33 ng/ml in females and 0.29 ng/ml in males at 40–49 years of age (Table 1). Serum HGF levels increase throughout pregnancy from 0.30 ng/ml in the early pregnancy to 0.41 ng/ml in mid-gestation and to 0.48 ng/ml in the late stage of pregnancy (Table 2) [48]. These data suggest the importance of physiological changes in HGF levels in age, sex, and pregnancy.

4. Relationships between HGF levels and diseases, disease stages, and the prognosis for diseases: clinical relevance of HGF levels

Circulating HGF levels change in the presence of different diseases and the correlation between HGF levels, disease parameters, and disease stages are evident. In diseases such as cancer, a correlation between HGF levels and prognosis has been reported (Tables 3A and B).

Table 1
Alteration of HGF levels in serum during aging and by sex

Age (years)	Serum HGF level in healthy controls (ng/ml)		Reference
	Females	Males	
10–19	0.36 ± 0.16	0.35 ± 0.25	[147]
20–29	0.39 ± 0.25	0.37 ± 0.22	
30–39	0.37 ± 0.19	0.33 ± 0.19	
40–49	0.33 ± 0.17	0.29 ± 0.17	
50–59	0.26 ± 0.17	0.29 ± 0.15	

Table 2
Alteration of HGF levels during pregnancy

A

Stage of pregnancy	Serum HGF level in pregnancy (ng/ml)	Reference
Early	0.3	[44]
Middle	0.41 ± 0.21	
End	0.48 ± 0.25	

B

Stage of pregnancy	Amniotic fluid HGF level in pregnancy (ng/ml)	Reference
Early	15 ± 8	[44] ([45])
Middle	48 ± 23	
End	6 ± 3	

C

Stage of pregnancy	Cord vessel HGF level in pregnancy (ng/ml)	Reference
<37 weeks	0.78 (0.46–1.03)	[48]
>37 weeks	1.11 (0.78–1.45)	

A. HGF level in serum. B. HGF level in amniotic fluid. C. HGF level in Cord vessels.

4.1. Liver disease

4.1.1. Serum HGF levels in various hepatic diseases

Serum HGF levels are significantly higher in patients with acute and chronic hepatitis compared with findings in normal controls [49,50]. The levels increase in patients with acute hepatic failure, and are increased 36-fold compared with findings in patients with acute hepatitis. The data for patients with acute hepatitis correlate significantly with serum bilirubin and γ-GTP levels and with the tissue activation index in patients with chronic hepatitis, thereby reflecting histological inflammation and tissue fibrosis [49]. Serum HGF levels in the presence of chronic hepatitis (CH), liver cirrhosis (LC), or hepatocellular carcinoma (HCC) are 0.40, 1.05, and 1.06 ng/ml, respectively. That is significantly higher than in controls (Table 3A). Serum HGF shows a positive correlation with C-reactive protein (CRP) and a negative correlation with albumin. However, no relationship between HGF and alpha2-microglobulin has been observed [51]. Serum HGF levels in patients with hepatitis C are highest in patients with acute hepatitis (AH) and levels tend to be higher in patients with LC

Table 3A
HGF levels in various diseases (selected diseases)

Disease	HGF level (ng/ml; ng/mg)	Reference
Serum		
Normal	0.27 ± 0.08	[50]
Alcoholic liver cirrhosis	0.78	[148] ([53])
Acute hepatitis	0.45 ± 0.23	[50] ([52])
Chronic hepatitis	0.40 ± 0.16	
Hepatic cirrhosis	1.05 ± 0.64	
Hepatocarcinoma	1.06 ± 1.45	
Primary bilious cirrhosis	0.44 ± 0.22	
Fulminant hepatitis	16.40 ± 14.67	
Liver transplantation with uneventful postoperative recovery	0.33 ± 0.04	[149]
Liver transplantation with abnormally elevated PT	2.01 ± 0.99	
Angina	0.3 ± 0.1	[60]
Myocardial infarction (6 h)	10.5 ± 9.1	
Myocardial infarction (6–12 h)	6.8 ± 4.6	
Hypertension (WHO stage I)	0.48 ± 0.03	[150]
Hypertension (WHO stage II, III)	0.88 ± 0.1	
Patients with arteriosclerotic lesions	0.35 ± 0.11	[151]
Acute-phase acute renal failure (ARF)	0.55 ± 0.24	[152]
Chronic tubulointerstitial nephritis	0.44 ± 0.37	
Chronic renal failure (non-dialysis case)	0.33 ± 0.1	[79]
Chronic renal failure (less than one year of dialysis)	0.33 ± 0.13	
Chronic renal failure (5–10 years of dialysis)	0.45 ± 0.13	
Acute renal rejection after transplantation	2.17 ± 1.14	[153]
Peak serum HGF in good renal allograft function	2.48 – 5.63	[154]
Interstitial pneumonia	1.16 ± 0.22 ($P<0.01$)	[54]
Bacterial pneumonia	0.96 ± 0.27 ($P<0.01$)	
Pulmonary fibrosis	0.34 ± 0.002 ($P<0.001$)	[57]
Mild and moderate acute pancreatitis	0.63 ± 0.06	[57]

Table 3A (*continued*)

Disease	HGF level (ng/ml; ng/mg)	Reference
Serum		
Severe acute pancreatitis	2.30 ± 0.61	
Clinical presentation before insulin treatment	0.74 ± 0.14	[154] ([156])
Newly diagnosed type 1 diabetes (diabetes duration 1/2–3 years)	0.78 ± 0.40	
Long-standing type 1 diabetes without renal involvement	0.86 ± 0.42	
Long-standing type I diabetes with renal involvement	0.79 ± 0.27	
Polymyositis (PM)	0.63 ± 0.11	[157]
Dermatomyositis (DM)	0.58 ± 0.07	
Inactive SLE	0.788	[158]
Active SLE	1.02	
Ulcerative colitis	1.384 ± 0.107	[159]
Crohn's disease	1.439 ± 0.084	
HELLP syndrome	1.79 ± 0.35	[160]
Bronchoalveolar lavage fluid (BALF)		
Normal (control)	0.23 ± 0.09	([57]) [58]
Idiopathic pulmonary fibrosis (IPF)	0.77 ± 0.88 ($P<0.001$)	
Rheumatoid arthritis (RA)	0.50 ± 0.64 ($P<0.001$)	
Sarcoidosis	0.41 ± 0.61 ($P<0.05$)	
Cerebral cortex		
Normal (average 70 years old)	9.60 ± 4.62	[161]
Alzheimer's disease (average 78.7 years old)	33.7 ± 18.47	
Progressive Parkinson's disease (average 78.5 years old)	20.23 ± 13.55	
Huntington's disease (average 73.8 years old)	36.15 ± 11.98	
Cerebrospinal fluid (CSF)		
Normal	0.346 ± 0.126	[162]
Aseptic meningitis	0.419 ± 0.07	[162]
Bacterial meningitis	6.101 ± 5.20	
Amyotrophic lateral sclerosis	0.58	[91]
Urine		
Normal	19.3 ± 7.1	[78,163]
Acute tubular necrosis (ATN) (non-oliguric)	6.9 ± 0.7 (ng/g)	

Table 3A (*continued*)

Disease	HGF level (ng/ml; ng/mg)	Reference
Urine		
Acute tubular necrosis (ATN) (oliguric)	19.1 ± 4.2 (ng/g)	
Bile		
Normal	0.8 ± 0.1	[164]
One day after hepatic resection (non-diabetic case)	4.0 ± 0.4 ($P<0.05$)	
Control	2.16 ± 1.39	[165]
Rhegmatogenous retinal detachment	2.02 ± 0.84	
Proliferative vitreoretinopathy	3.94 ± 2.29	
Vitreous body		
Non-diabetic case	1.6	[155]
Diabetic retinopathy (rubeosis −)	4.2 ($P<0.05$)	
Diabetic retinopathy (rubeosis+)	7.2 ($P<0.01$)	
Synovial fluid		
Osteoarthritis	0.19	[105] ([166])
Bacterial arthritis	0.18	
Rheumatoid arthritis	1.21	
Placenta		
Normal	6.16 ± 3.32	[46]
Toxemia of pregnancy	4.05 ± 1.44 ($P<0.05$)	

and HCC than in those with chronic hepatitis (CH). Furthermore, serum HGF levels reveal high carcinogenic states in chronic hepatitis and liver cirrhosis type C [52]. Induction is due to the increased production of HGF, not only in the liver, but also in distant organs, such as the lung. With the progression of liver damage, clearance of HGF in the liver diminishes. In addition, although patients with LC show a marked increase in serum HGF levels as the molecule is processed from a biologically inactive single-chain precursor form of HGF into the two-chain active form. Levels may be significantly disturbed in the damaged liver, and a single-chain precursor form can become a major form in the serum. In patients with fulminant hepatitis, extremely high serum levels of HGF (16.40 ng/ml) were detected. In such cases, some of the HGF may exist in the form of

a single-chain precursor. Therefore, exogenous administration of biologically active HGF may be effective in such cases [34,35].

4.1.2. Significance of HGF levels for the prognosis of acute liver diseases

Evidence has accumulated indicating that levels of plasma HGF correlate with the prognoses of liver diseases. In a comparison of HGF levels in patients with acute hepatitis or liver cirrhosis (acute phase) before and after admission to hospital, increased levels of HGF were observed in the patients who died, whereas much lower levels were observed in patients who survived. A correlation between serum HGF levels and prognosis of alcoholic hepatitis was also reported. Serum HGF levels were elevated in all patients (median 0.9 ng/ml; range 0.6–7.7 ng/ml; normal <0.5 ng/ml), and there was a positive correlation between HGF levels and hepatocyte proliferation in liver biopsies [53].

4.2. Lung disease

Serum HGF levels are high in patients with interstitial and bacterial pneumonitis (1.16 and 0.96 ng/ml, respectively: Table 3A). In treatment-responsive patients, the levels of HGF decrease, yet levels are not altered in patients who do not recover, thus demonstrating a correlation between the levels of HGF and the prognosis for individuals with pneumonitis [54,55]. A significant correlation between serum HGF levels and CRP in inflammatory pulmonary diseases has been reported [56]. In addition, the clinical significance of HGF levels in sera and bronchoalveolar lavage fluid (BALF) in patients with pulmonary fibrosis is also evident [57,58] (Table 3A). For example, the HGF concentration in control BALF is 0.23 ± 0.09 ng/ml, whereas that in BALF of patients with idiopathic pulmonary fibrosis is 0.77 ± 0.88 ng/ml. Moreover, HGF levels in serum correlated significantly with those of elastase and CRP in serum, and correlated negatively with pulmonary airway oxygen tension (PaO_2). HGF levels in BALF and the prognosis of patients with acute respiratory distress syndrome (ARDS) have also been reported [59]. These findings indicate a correlation between HGF levels, in serum and/or BALF, and the activity and prognosis of lung diseases.

4.3. Cardiac disease

Plasma HGF levels do not increase greatly in the presence of angina, but they do increase markedly in acute cardiac infarction in parallel with increases in plasma creatine phosphokinase (CK) and the CK isozymes, CK-MB (Table 3A). In particular, the plasma levels of HGF change much earlier than do those of CK and CK-MB: HGF levels markedly increase within 3 h after cardiac infarction, which suggests that plasma HGF levels can serve as an early marker of cardiac infarction [60–62]. HGF is a pertinent prognostic indicator and reflects the clinical course in patients with acute myocardial infarctions. In such conditions HGF levels increase in the heart, liver, and kidney, therefore increased serum HGF levels reflect the combination of autocrine, paracrine, and endocrine deliveries of HGF (Fig. 2A).

The mechanisms by which HGF is supplied to the systemic circulation from distant organs, when the injury site itself has lost capacity or is insufficient to produce enough HGF, are unclear. To explain this, the injurin system might act as follows. In response to injury, HGF is induced in the lesioned site. In addition, HGF inducers, termed "injurin or injurin-like factors," (see below) are induced in the lesioned site, and then are released into the systemic circulation, reach distant organs and induce the production of HGF in distant organs. The produced HGF is released into the systemic circulation and elevates the levels of HGF in serum in an endocrine fashion [63–65 and our unpublished results] (Fig. 2B). Although such factors are not characterized well in models of cardiac ischemia, several candidates can be considered: one is the proteinaceous factor that induces HGF mRNA in distal non-injured organs, in the intact lung of rats, after partial hepatectomy or unilateral nephrectomy. This factor has been termed "injurin," and it has been partially purified and characterized [66,67]. The others are IL-1β, prostaglandin E2, heparin, bFGF, EGF, and PDGF, which are regulated in response to injury and are known to regulate HGF production [68–74]. The possibility remains that novel injurin-like factors are involved in cardiac ischemia. Therefore, it is of much interest to assess what kinds of injurin-like

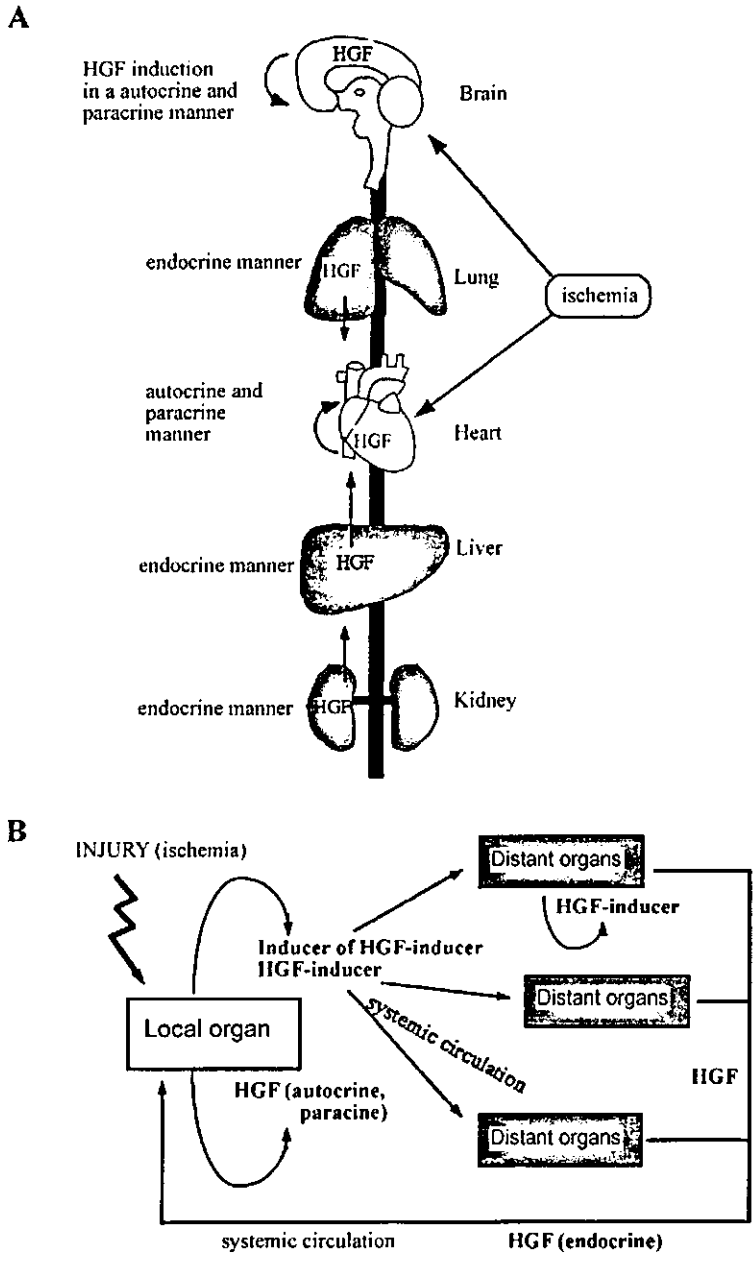

Fig. 2. Example of the regulation of HGF in an autocrine, paracrine, and endocrine manner after heart and brain injury. (A) Schematic representation of HGF regulation after heart and brain injury. HGF is induced in an autocrine and paracrine fashion at the injured region. In addition, HGF can also be supplied to the injured local site from distant regions in an endocrine fashion. (B) Model of HGF and the HGF inducer system after the injury. At the injured site, HGF is induced locally and HGF inducer (possibly an additional inducer of the HGF inducer itself) is induced in response to the injury. HGF inducer is then released into the systemic circulation, reaches the distant organ, and accelerates their production of HGF. HGF produced at distant organs is then released into the systemic circulation and reaches the injured site.

factors play orchestrated roles, spatially and sequentially, to support the maintenance and regeneration of injured organs.

4.4. Vascular diseases

Serum HGF concentrations in patients with peripheral arterial occlusive disease (PAOD) collateral blood vessels tend to be higher than in patients without collaterals (0.43 vs. 0.35 ng/ml; $P=0.06$). Moreover, in patients who underwent bypass surgery or angioplasty, serum HGF concentrations decreased from 0.41 to 0.21 ng/ml after treatment ($P<0.001$) [75]. Therefore, serum HGF may be a useful marker for the diagnosis of PAOD and may play an important role in angiogenesis and collateral vessel growth in patients with PAOD. Vascular HGF concentrations in diseased segments of vessels from patients with arteriosclerosis obliterans (ASO) were found to be significantly decreased when compared with disease-free segments from the same patients ($P<0.05$), and there was a marked reduction in HGF mRNA [76]. Serum HGF concentrations are significantly higher than noted in normal subjects in hypertensive patients with no evidence of complications [77].

4.5. Renal diseases

A marked increase in urine HGF levels was observed in patients with acute renal failure, in contrast to detectable but low levels of HGF in the urine of healthy subjects and in patients with chronic glomerular or polycystic disease [78]. Serum HGF level is elevated in patients with chronic renal failure and may be attributed to the increased production of HGF in response to chronic renal injury [79,80] (Table 3A). Immunohistochemical analysis revealed the positive staining rate for HGF to be 33.3% for IgA nephropathy, 66.7% for membranous glomerulonephritis, and 50% for focal glomerulosclerosis. All patients with drug-induced interstitial nephritis were positive for HGF staining, but no such staining was observed in patients with minimal changes. In patients with renal cystic diseases, the HGF level in the proximal cyst fluid is high (mean 2.45 ng/ml) compared with that in distal cyst fluid (0.42 ng/ml), which suggests an involvement of HGF in mediating the genesis of human cysts [81]. Immunohistochemical staining showed a significant positive correlation between the distribution of HGF and histological damage, the grade of tubulointerstitial lesion (TIL), and several clinical parameters determined at biopsy in patients with IgA nephropathy ($P<0.01$), together with a correlation of HGF levels with the degree of tubular damage in patients with primary glomerulonephritis, as well as acute tubular damage from various drugs [82,83]. In "human rejecting kidneys," transcription of HGF mRNA in the urinal tubular epithelium and in the mesenchymal cells (fibroblasts and smooth muscle cells in chronic vascular rejection and endothelial cells and/or mesangial cells in transplant glomerulopathy) has been observed [84].

4.6. Neurologic diseases

The neurotrophic activity of HGF was first identified in primary cultured hippocampal and midbrain dopaminergic neurons in 1995 and 1996 [85,86]. HGF also shows neurotrophic activities in the cerebral cortical, motor, sensory, sympathetic, and cerebellar granule neurons [85–89]. Therefore, the role for HGF in neurological diseases remains open to speculation. Indeed, increased expression of HGF in senile plaques was identified in the cortex of patients with neurodegenerative diseases, such as Alzheimer's (AD), Parkinson's, and Huntington's diseases. In addition, the HGF activator (HGF A) is present both in normal subjects and in patients with AD. The levels of HGF A inhibitor in the brain decrease in patients with AD [90,161]. Determination of the levels of HGF, HGF A, and HGF A inhibitor may aid in elucidating the role of HGF in other neurological diseases. HGF was present in the cerebrospinal fluid (CSF) of normal subjects (346 ± 126 pg/ml), and represented approximately half of the HGF serum concentrations. HGF levels in the CSF were not significantly changed in patients with chronic CNS disease or with aseptic meningitis (419 ± 71 pg/ml), but were significantly increased in patients with bacterial meningitis (6101 ± 5200 pg/ml: Table 3A). HGF levels in the CSF were not influenced by increased serum concentrations in patients with normal or mildly affected blood–CSF barrier functions [91]. Slightly increased HGF levels in the CSF were observed in patients with amyotrophic lateral sclerosis (ALS: Table 3A, 91).

4.7. Pancreatic diseases

Serum HGF levels in patients with severe acute pancreatitis (2.30 ± 0.61 ng/ml: Table 3A) were significantly higher than in patients with mild and moderate acute pancreatitis (0.63 ± 0.06 ng/ml). Sixteen of seventeen patients in whose serum HGF levels were >1.0 ng/ml were evaluated as having severe acute pancreatitis. Serum HGF levels were significantly elevated in patients with higher Ranson scores, higher APACHE II scores, or higher computed tomography grades [92]. Serum HGF levels are considered pertinent for determination of disease severity, as are plasma CRP levels [93]. Serum HGF levels in patients with chronic pancreatitis are also higher than in disease-free individuals (0.25 vs. 0.37 ng/ml; $P<0.05$) [94].

4.8. Cancer

Plasma HGF levels increase in patients with esophageal, gastric, or colorectal cancer. In addition, HGF levels correlate with disease progression, and the levels increase markedly in recurrent cases [95–99] (Table 3B). In patients with colon cancer, HGF levels also correlate with the pathology in terms of the size of tumor, and the numbers of lymph nodes and liver metastases [99].

4.8.1. Hepatocellular carcinoma (HCC) and hepatoblastoma

Plasma HGF levels increase in patients with HCC or hepatoblastoma, while levels decrease in response to treatment in patients with hepatoblastoma (Table 3B). In addition, higher levels of HGF in serum from HCC patients with metastasis were observed compared with findings in those without metastasis, and elevations in serum HGF levels correlated positively with tumor metastasis in human HCC. These findings suggest that HGF may be a useful serological biomarker for clinical diagnosis and follow-up of HCC metastases [100].

4.8.2. Lung cancer

Survival and recurrence rates correlate negatively with HGF levels in lung cancer tissues. Siegfried et al. reported that the HGF content in tumor tissue from 56 patients with non-small-cell lung cancer was associated with recurrence and poor survival: the relative risk was seen to increase with increasing HGF content of the tumor [101]. When HGF exceeded 100 units,

Table 3B
HGF levels in various diseases (cancer patients)

Cancer	HGF level (ng/ml)	Reference
Serum		
Esophageal cancer (stage I/II)	0.47 ± 0.13	[95,96]
Esophageal cancer (stage III/IV)	0.88 ± 1.05	
Esophageal cancer (recurrent)	1.51 ± 1.62	
Gastric cancer (stage I/II)	0.32 ± 0.15	[95,96] ([97,98,167])
Gastric cancer (stage III/IV)	0.49 ± 0.46	
Gastric cancer (recurrent)	0.44 ± 0.29	
Hepatoblastoma (post-chemotherapy)	0.46	[95,96]
Hepatoma (pre-treatment)	0.89	
Hepatocarcinoma	1.06	
Colorectal cancer (stage I/II)	0.35 ± 0.15	[99]
Colorectal cancer (stage III/IV)	0.38 ± 0.19	
Colorectal cancer (stage V)	0.50 ± 0.25	
Colorectal cancer (recurrent)	0.44 ± 0.14	
Breast cancer (primary)	0.38 ± 0.31	[104] ([147,168,169])
Breast cancer (recurrent)	0.59 ± 0.42	
Prostate cancer without metastasis	0.974	[170]
Prostate cancer with metastasis	2.117	
Small cell lung cancer (SCLC) (mean)	0.40 ± 0.17	[103] ([101])
SCLC with limited disease	0.34 ± 0.12	
SCLC with extensive disease	0.47 ± 0.20	
Acute myeloblastic leukaemia (AML)	2.03 (1.055)	[171]
Myeloma		[172] ([173])
Tumor		
Control breast	0.108	[174] ([104,175])
Breast cancer	0.35	

the relative risk was 10, compared with that in patients with a relative risk of 1. Node-negative patients with an elevated tumor content of HGF had significantly poorer outcomes than did node-positive patients with a low tumor content. The same relationship was observed if the patients were stratified according to stage: elevated HGF levels were associated with stage I patients in whom disease recurred and who died of their disease, and stage I patients with elevated HGF levels had poorer survival rates than did higher-stage patients with low levels of HGF. It was also suggested that elevated HGF levels may predict a more aggressive biology in patients with non-small cell lung cancer; thus, the level of HGF may be useful as an indicator of high risk for patients with early-stage lung cancer [101]. A similar up-regulation and/or prognostic role of HGF levels has been reported [102,103].

4.8.3. Breast cancer

The immunoreactive (ir)-HGF concentration in tumor extracts of 82 primary human breast cancers determined using an enzyme-linked immunosorbent assay (ELISA) revealed that such patients with a high concentration of HGF had a significantly shorter relapse-free ($P=0.001$) and overall survival times ($P=0.001$) compared with those with a low ir-HGF concentration at the cutoff point of 21.7 ng/100 mg tissue protein, as determined in another group of 82 patients. In a multivariate analysis, the ir-HGF level was found to be the most important independent factor in predicting relapse-free and overall survival times, such being of greater import than lymph node involvement [104].

4.9. Other diseases

The mean values of HGF in synovial fluid are higher in patients with rheumatoid arthritis (RA) (1.21 ng/ml) than in patients with osteoarthritis (OA) (0.19 ng/ml) ($P<0.01$) and those with septic arthritis (0.18 ng/ml). The levels for patients with RA correlated with serum CRP concentrations ($r=0.626$, $P<0.01$) and IL-6 levels in synovial fluid ($r=0.476$, $P<0.05$) [105]. Higher HGF levels were also observed in patients with diabetes mellitus, polymyositis (PM), dermatomyositis (DM), SLE, acute tubular necrosis, ulcerative colitis, Crohn's disease, and HELLP syndrome (Tables 3A and B).

5. Potential application of HGF in diseases where HGF levels are altered

5.1. Liver disease

Exogenous administration of recombinant HGF or the HGF gene was found to be effective for protection against progression of or regeneration of various liver diseases. Administration of human recombinant HGF following 4 weeks of dimethylnitrosamine (DMN) treatment or during long-term treatment with carbon tetrachloride (CCl_4) suppressed the onset of liver fibrosis induced by stimulated hepatic collagenase activity, prevented the onset and progression of hepatic fibrosis/cirrhosis, accelerated the recovery from liver cirrhosis, and prevented death due to hepatic dysfunction [106,107]. A beneficial effect of HGF in a rat model of lethal liver cirrhosis, as induced by DMN administration was seen, as were repeated transfections of the human HGF gene into skeletal muscles, which induced a high plasma level of human as well as endogenous rat HGF and tyrosine phosphorylation of the c-Met/HGF receptor. Transduction with the HGF gene also suppressed the increase of transforming growth factor-beta (TGF-β), which plays an essential part in the progression of liver cirrhosis, inhibited fibrogenesis and hepatocyte apoptosis, and produced a complete resolution of fibrosis in the cirrhotic liver, thereby improving the survival rate of rats with this severe illness. Thus, HGF gene therapy may be useful for the treatment of patients with liver cirrhosis, which is otherwise fatal and untreatable by conventional therapy [32]. Exogenous administration of HGF has been shown to lead to recovery from alcohol-induced fatty liver in rats [33]. HGF also dramatically improved the survival rate of rats subjected to hepatic warm ischemia/reperfusion injury [108]. Among liver diseases, fulminant hepatitis is thought to be the most severe with an extremely poor prognosis, and the mortality rate is high, with no available effective therapy. Abrogation of Fas or endotoxin can induce a model form of hepatitis, which resembles fulminant hepatic failure. In these models, without HGF, massive hepatocyte apoptosis and severe liver injury occurs, and most of these mice die of hepatic failure. In contrast, recombinant HGF strongly suppressed extensive progress of hepatocyte apoptosis and liver injury, and the mice survived

[33,34]. For example, lipopolysaccharide plus GalN treatment induced fulminant hepatitis in mice and in these mice, serum GTP levels increased, and all the mice died within 7 h of the treatment. Exogenous administration of HGF prevented the induction of serum GTP (induction of liver damage) and improved the survival rate from 0% to 70% (Fig. 3A–C). While the mechanisms of HGF actions in these models are not fully understood, the anti-apoptotic activity of HGF may be explained by the induction of the anti-apoptotic protein Bcl-xL in the liver and the attenuation of caspase-3 induction (Fig. 3D–F) [34,35]. These findings suggest a role for HGF as a critical regenerative factor and the therapeutic potential of HGF for treating patients with acute and chronic hepatitis, fatty liver, hepatic cirrhosis, or fulminant hepatitis (Table 4A).

5.2. Renal diseases

The potential therapeutic roles of HGF in renal diseases are evident and are well summarized in recent reviews [109–111]. Intravenous injection of recombinant human HGF into mice remarkably suppressed increases in blood urea nitrogen and serum creatinine caused by the administration of cisplatin, a widely used antitumor drug, or by $HgCl_2$, thereby indicating that HGF strongly prevents the onset of acute renal dysfunction. Moreover, exogenous HGF stimulated DNA synthesis of renal tubular cells after the renal injury caused by $HgCl_2$ administration and unilateral nephrectomy, and induced reconstruction of renal tissue structures in vivo [112]. Cyclosporin A (CsA) is a potent, widely prescribed immunosuppressant that has serious side effects. When recombinant human HGF (rh-HGF) was co-administrated with CsA to mice, severe digestive and/or neurological symptoms and the degenerative changes in renal tubular cells and hepatocytes seen with cases of CsA administration were remarkably attenuated. Moreover, mortality linked to CsA administration was prevented by rh-HGF treatment [113]. Using mice subjected to unilateral ureter-ligated obstruction, we investigated the roles of HGF in tubulointerstitial fibrosis (TIF), as induced by obstructive nephropathy. Neutralization of endogenous HGF accelerated the progression of TIF, accompanied by increases in TGF-β expression and tubular apoptosis, as well as by decreases in tubular proliferation. In contrast, rhHGF attenuated TIF progression, and there were decreases in TGF-β expression and tubular apoptosis, and an increase in tubular proliferation [114]. We also demonstrated the preventive effect of HGF on the progression of renal dysfunction and fibrosis, in a spontaneous mouse model (ICGN strain) for chronic renal disease (CRD), which is generally thought to be incurable except through renal transplantation. The mice progressively developed glomerular sclerotic injury, tubular atrophy, and renal dysfunction until they were 17 weeks of age. Recombinant HGF was injected into these mice during a 4-week period (from weeks 14 to 17 after birth), DNA synthesis of tubular epithelial cells was found to be 4.4-fold higher than in mice without HGF injection, thereby suggesting that tubular parenchymal expansion is promoted by HGF. Notably, HGF suppressed the expression of transforming growth factor-beta and of platelet-derived growth factor, as well as myofibroblast formation in the affected kidney. Consequently, the onset of tubulointerstitial fibrosis was almost completely inhibited by HGF, while HGF attenuated the progression of glomerulosclerosis, both leading to a prevention of the manifestation of renal dysfunction [38]. In addition, in chronic renal failure/fibrosis in ICGN mice, HGF in the kidney declines in a manner reciprocal to the increase in transforming growth factor-β (TGF-β). Antibody neutralization of HGF leads to acceleration of renal failure/fibrosis, while HGF administration leads to remarkable attenuation, thus indicating the importance of an HGF vs. TGF-β counterbalance in both pathogenesis and therapeutics in cases of chronic renal failure. HGF is being strongly considered for potential treatment of acute and chronic renal failure [115].

HGF gene therapy is a feasible option for treating ischemic damage of the kidney, or acute and chronic renal failure, as follows. A single injection of the HGF gene using the hemagglutinating virus of Japan (HVJ) liposomes gave a low but continuous intravenous level of HGF and attenuated ischemic damage in the kidney [116]. Intravenous systemic administration of a naked plasmid containing human HGF cDNA produced substantial levels of human HGF protein in mouse kidneys and significantly ameliorated renal dysfunction and accelerated recovery from the acute injury induced by folic acid [117]. HGF gene delivery

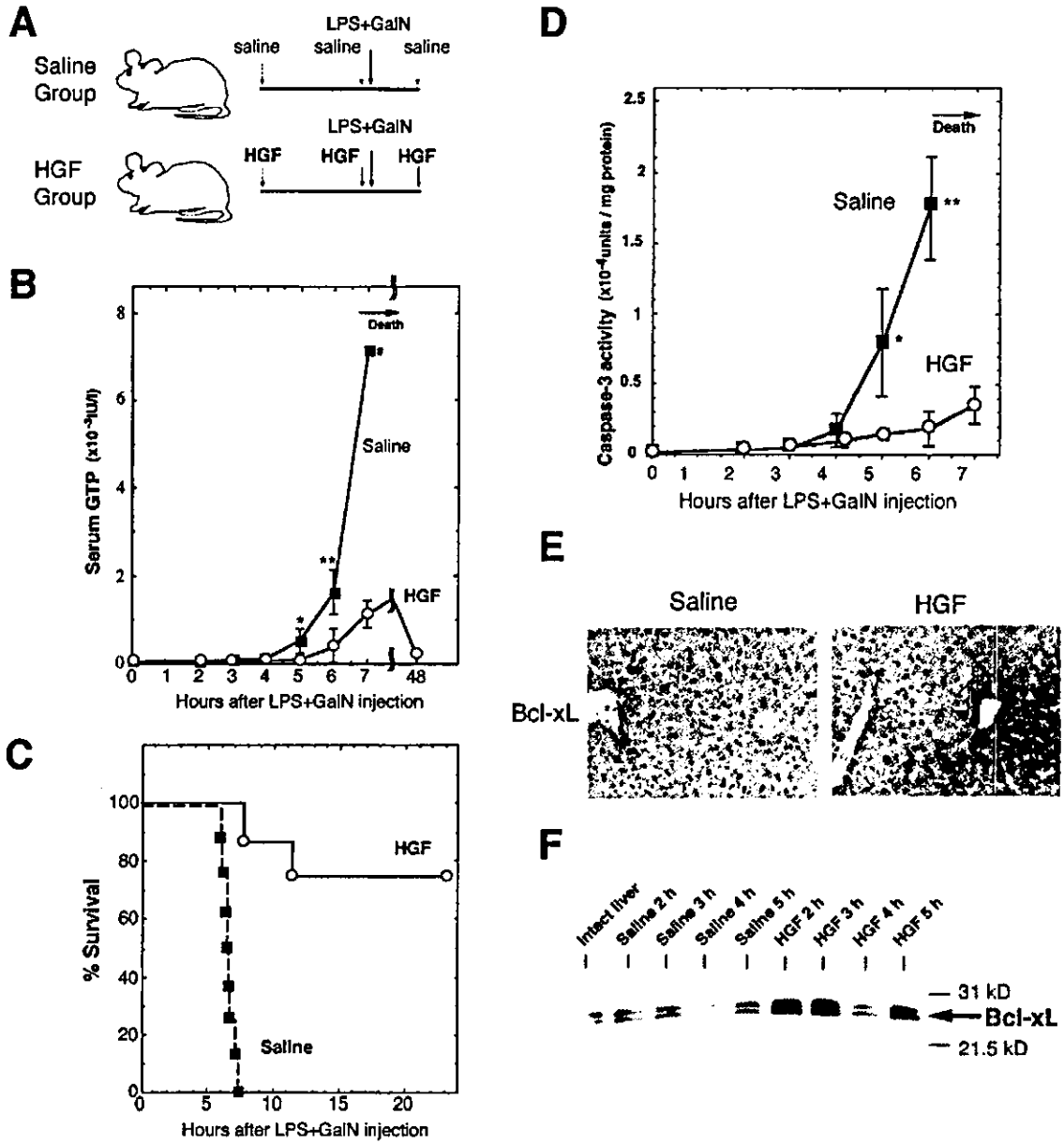

Fig. 3. HGF effectively prevents endotoxin-induced fulminant hepatitis and mortality is decreased, presumably through the suppression of caspase-3 and the induction of Bcl-xL. (A) Schedule of treatment of endotoxin-induced fulminant hepatitis with saline or recombinant HGF. Saline or HGF were injected intraperitoneally at 6 and 0.5 h before, and 3 h after an intraperitoneal injection of lipopolysaccharide (LPS) and D-galactosamine (GalN). (B) HGF effectively prevents massive apoptosis of hepatocytes. Hematoxylin and eosin (H & E) and TUNEL staining views revealed that HGF prevented death of hepatocytes (H & E) and apoptosis caused by endotoxin-induced fulminant hepatitis in mice. (C) Survival of mice after LPS and GalN injection with HGF or saline. $n = 8$ in each group. (D) Changes in CPP32 (caspase-3)-like protease activity in the mouse liver after LPS + GalN injection with HGF or saline. (E) HGF attenuates the activation of caspase-3. (F) HGF induces Bcl-xL in hepatocytes. Upper panel shows immunostaining for Bcl-xL (red). The lower panel shows a western blot analysis of Bcl-xL in endotoxin-induced fulminant hepatitis mice.

Table 4A
Potential diseases for the therapeutic application of HGF

Organ	Potential Disease (HGF)
Liver	Acute hepatitis
	Fulminant hepatitis
	Hepatic cirrhosis
	Fatty liver
	Surgical treatment (liver transplantation, partial resection, ischemia)
Kidney	Acute renal failure (ARF)
	Chronic renal failure (CRF) (nephrotic syndrome, obstructive nephropathy)
	Surgical treatment (renal transplantation, ischemia)
	Diabetic nephropathy
Lung	Acute pneumonia
	Pulmonary fibrosis
	Surgical treatment (lung transplantation, partial resection, ischemia)
Cardiovascular organ	Angina
	Cardiac infarction
	Cardiomyopathy
	Atherosclerosis obliterans (ASO)
Digestive organ	Gastric ulcer
	Diabetes mellitus
Nervous system	Cerebrovascular diseases including a transient ischemic attack (TIA) and stroke
	Neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and Parkinson's disease
	Spinal cord injury
	Diabetic retinopathy
	Peripheral neuropathy
	Spinal canal stenosis
	Deafness
Bone and Joint	Osteoarthritis (OA)
	Rheumatoid arthritis (RA)
Muscle	Muscular dystrophy
	Muscular atrophy
Skin	Skin ulcer
	Burn
	Scleroderma
Whole body	Crush syndrome

Based on the effects of HGF in each organ and in disease models, potential diseases expected for the therapeutic application of HGF are listed.

using a naked plasmid vector, in a similar manner, markedly ameliorated renal fibrosis in an animal model of chronic renal disease induced by unilateral ureteral obstruction [118]. These findings suggest the possibility of treating subjects with renal diseases using a recombinant HGF protein and an HGF gene (Table 4A).

5.3. Lung diseases

HGF markedly and dose-dependently stimulates the proliferation and DNA synthesis of rat tracheal epithelial cells in primary culture. The intravenous injection of human recombinant HGF (10 μg per mouse per day) into mice with acute lung injury induced by intratracheal infusion of 10 mM HCl, stimulated DNA synthesis of airway epithelial cells to levels threefold higher than in mice not given HGF, but it did not stimulate DNA synthesis of alveolar epithelial cells. However, HGF injections at a higher dose (100 μg per mouse per day) stimulated DNA synthesis of alveolar epithelial cells in vivo [119]. Intratracheal administration of rhHGF to C57BL/6 mice with pulmonary fibrosis generated by bleomycin treatment showed that HGF significantly attenuated the induced collagen accumulation, as determined by quantitation of hydroxyproline content and by scoring the extent of fibrosis [120,121]. The protective effect of HGF in hydrogen peroxide-induced acute lung injury in rats was also evident [122]. HGF stimulated proliferation of respiratory epithelial cells during post-pneumonectomy compensatory lung growth in mice, suggesting the potential use of HGF for enhancing compensatory lung growth after partial surgical resection of the lung [123]. These findings indicate that HGF is a potent mitogen for airway epithelial cells and alveolar epithelial cells in vivo as well as in vitro, and may act as a pulmotrophic factor responsible for airway and alveolar regeneration during lung regeneration after acute lung injuries (Table 4A).

5.4. Cardiac diseases

Using a rat model of ischemia/reperfusion injury, we demonstrated that HGF is endogenously regulated (Fig. 4A) and c-Met is induced in the cardiac area facing the ischemic region. Furthermore, exogenous HGF was cardioprotective, through its anti-apoptotic effect on cardiomyocytes (Fig. 4C). When endogenous HGF was neutralized with a specific antibody, the numbers of myocyte cell deaths increased markedly, the infarct area expanded, and the mortality increased to 50%, as compared with a control group in which there was no mortality (Fig. 4B). Plasma from rats with induced myocardial infarctions showed

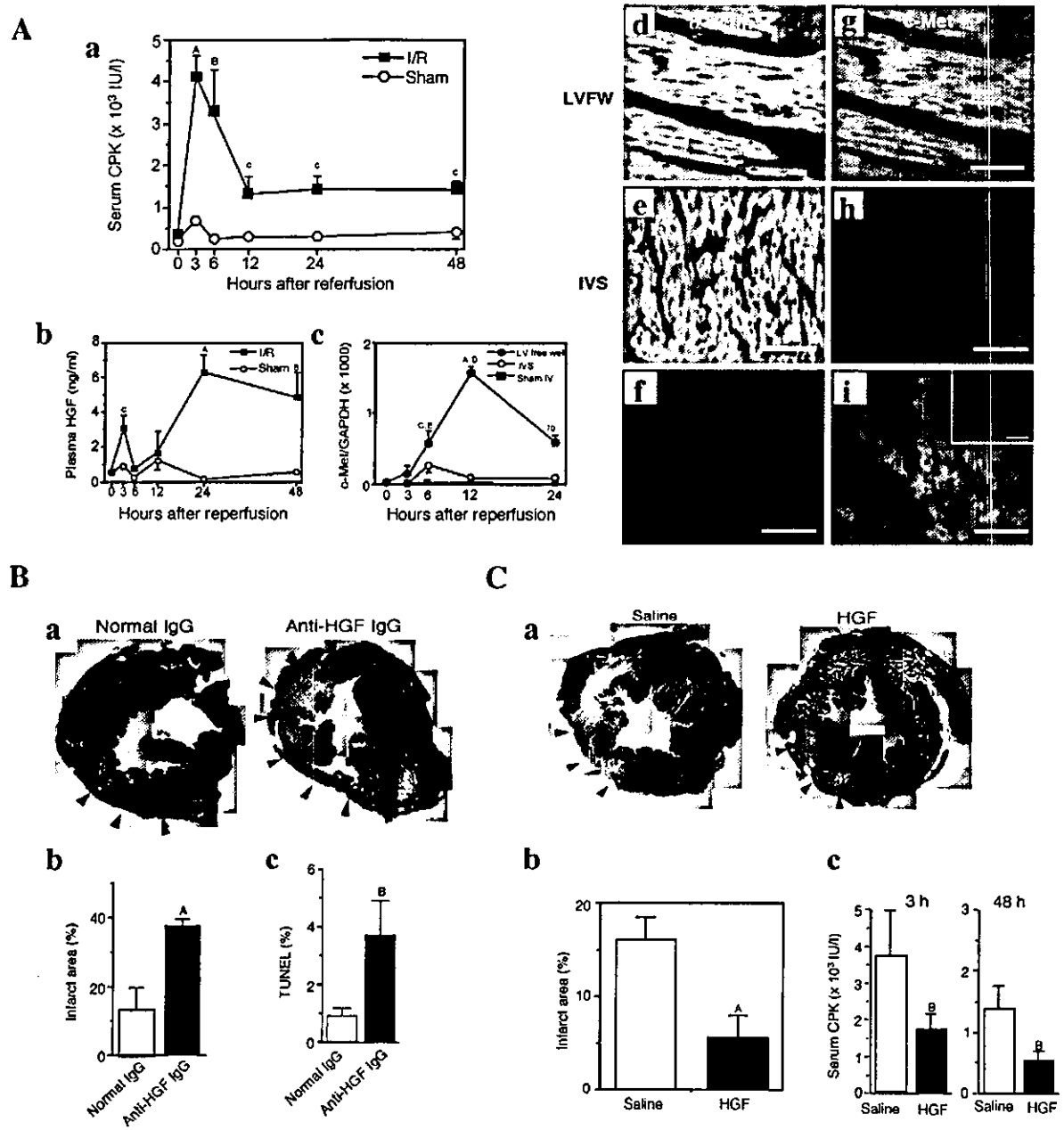

Fig. 4. Myocardial protection from ischemia/reperfusion injury by HGF. (A) Changes in HGF/c-Met expression compared with serum CPK levels in rats with ischemia/reperfusion injury. The images show double immunohistochemistry of α-sarcomeric actin and c-Met in the heart resected 48 h after reperfusion. Photographs of left ventricular free wall (d, g) and interventricular septum (e, h) of a section are shown demonstrating immunostaining of c-Met in the sham-operated myocardium (f) and the border region between infarcted and non-infarcted areas (i). (B) Increase in the number of myocardiocyte cell deaths and expansion of the infarct area by neurtralization of endogenous HGF with a specific antibody. a: Photographs of myocardium treated with normal IgG and anti-HGF IgG. Arrowheads show the infarct area. b: Quantitation of the infarct area treated with normal IgG or anti-HGF. c: Percentages of TUNEL-positive cells in the myocardium treated with normal IgG and anti-HGF IgG. (C) Administration of HGF reduced the infarct area and the induction of CPK. a: Photographs of the myocardium treated with normal IgG and anti-HGF IgG. b: Quantitation of the infarct area. c: Serum CPK levels 3 and 48 h after reperfusion, with or without HGF treatment.

cardioprotective effects on primary cultured cardiomyocytes, but these effects were significantly diminished by neutralizing HGF. By contrast, recombinant HGF administration reduced the size of the infarct area and improved cardiac function by suppressing apoptosis in cardiomyocytes (Fig. 4C). HGF has a high potential to attenuate the death of cardiomyocytes and to promote angiogenesis. Such bifunctional activity suggests the possibility of using HGF administration for patients who have experienced cardiac infarction [37]. HGF gene therapy approach was also feasible for this cardiac infarction model [124,125]. Three days after transfection of the human HGF gene into the normal whole rat heart using HVJ liposomes [125] and subsequent global warm ischemia and reperfusion, a significant increase in human HGF protein levels was noted in the heart. Cardiac function in terms of left ventricular pressure, maximum dp/dt, and the pressure–rate product in hearts transfected with the HGF gene were significantly superior to those of control hearts. In addition, leakage of CK in the coronary artery effluent in hearts transfected with the HGF gene was significantly lower than that in control hearts, suggesting that HGF has a cytoprotective effect on cardiac tissue [126]. Therapeutic angiogenesis was also induced by myocardial injection of a naked plasmid encoding HGF in the ischemic canine heart [127]. The angiogenic activity of HGF may also be beneficial for patients with cardiac infarction. We recently found that when cardiomyopathic hamsters with late-stage pathology were treated with recombinant human HGF, cardiac fibrosis and myocardial hypertrophy were suppressed, and cardiac dysfunction was ameliorated (Nakamura et al., unpublished results). These findings indicate the therapeutic potential of HGF in patients with cardiac infarction and myocardial hypertrophy.

5.5. Vascular diseases

Recombinant HGF administration through the internal iliac artery of rabbits, where the femoral artery had been excised to induce unilateral hind limb ischemia, twice on days 10 and 12 after surgery, produced a significant augmentation of collateral vessel development on day 30 in this model of ischemia ($P<0.01$) [76,128]. In addition to the induction of collateral vascular formation, administration of recombinant HGF improved blood flow and muscular atrophy in rat and rabbit vessel obstruction models of the lower limbs [129]. Furthermore, intramuscular injection of the human HGF plasmid (*HGF* gene) induced therapeutic angiogenesis in a rat diabetic and ischemic hind limb model, as a potential therapy for peripheral arterial disease or in a hind limb ischemic model of lipoprotein (a) transgenic mice [130]. These findings suggested the possibility of clinical application for HGF based on its combined angiogenic and cytoprotective (anti-apoptotic) activities for cardiovascular diseases, such as arteriosclerosis obliterans (ASO), angina, and myocardial infarction [128] (Table 4A). Clinical gene therapy for restenosis and ischemic diseases using the VEGF gene has already been carried out in the United States, and beneficial effects of such strategies were seen. In Japan, gene therapy using the HGF gene for treating ASO began in the spring of 2001 at Osaka University Hospital. In the near future, the therapeutic effects of the HGF gene might be considered for the patients with restenosis, graft failure, cardiomyopathy, renal failure, and possibly cerebral vascular diseases and amyotrophic lateral sclerosis (ALS) (see Section 5.6).

5.6. Neurologic diseases

In adult rats, neurons in the hypoglossal nucleus show a dramatic loss of choline acetyltransferase (ChAT) protein and mRNA after axotomy. This reduction of ChAT was markedly prevented when HGF was administered continuously at the cut end of the nerve, using an osmotic pump [131]. The neuroprotective effect of HGF against transient focal cerebral ischemia in rats was noted in cases of intrastitial administration of rhHGF, which attenuated the death of hippocampal neurons. The intraventricular administration of rhHGF prevented neuronal death after 120 min of occlusion of the right middle cerebral artery and bilateral common carotid arteries [132]. HGF significantly reduced the infarct volume in a dose-dependent manner [133]. In vivo gene transfer of HGF to the subarachnoid space using HVJ liposomes is also effective for the transient occlusion of arteries in gerbils [134]. In addition, we found that HGF gene delivery into amyotrophic lateral sclerosis (ALS) model mice could attenuate motoneuronal death and axonal degeneration, retain motor function, and prolong their life span [135]. These