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

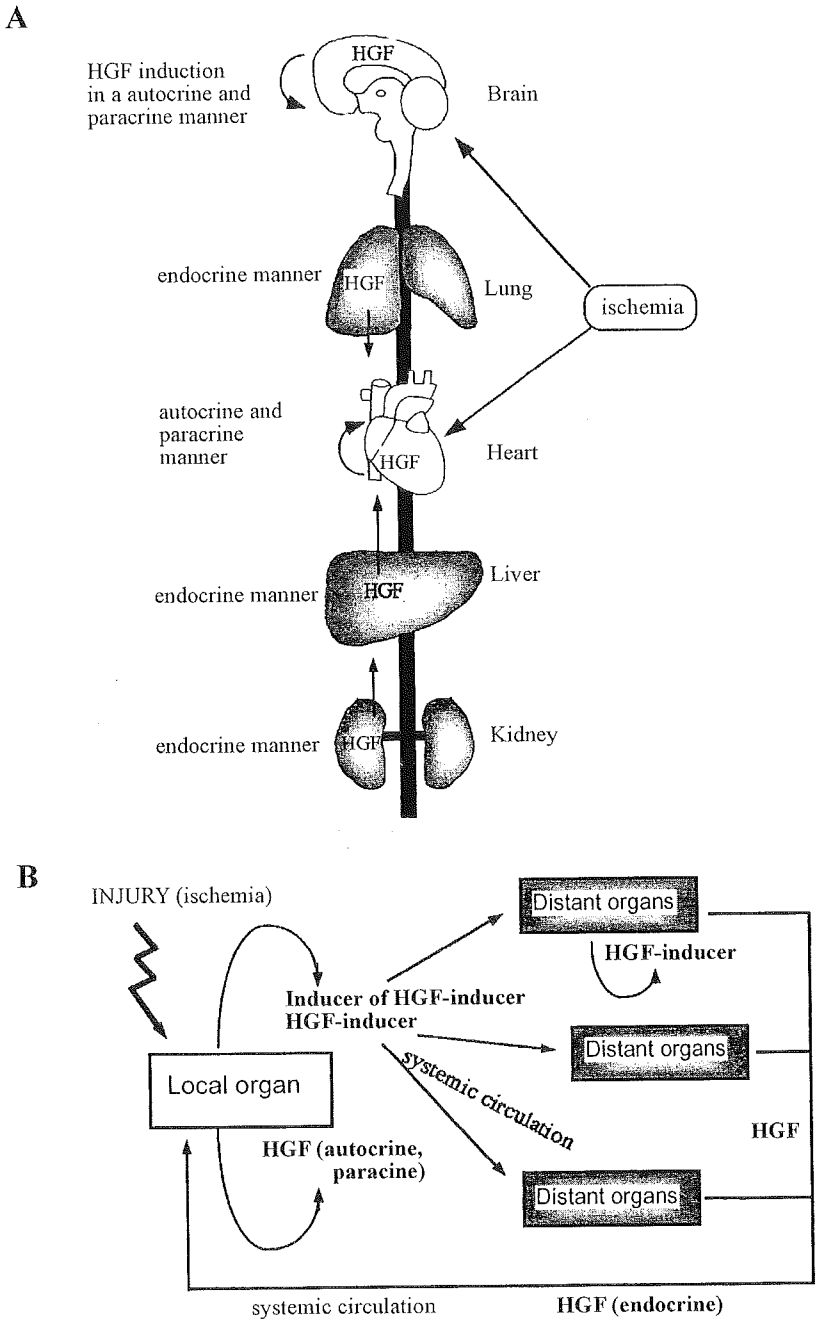

Fig. 2. Example of the regulation of HGF in an autocrine, paracrine, and endocrine manner after heart and brain injury. (A) Schematic representation of HGF regulation after heart and brain injury. HGF is induced in an autocrine and paracrine fashion at the injured region. In addition, HGF can also be 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

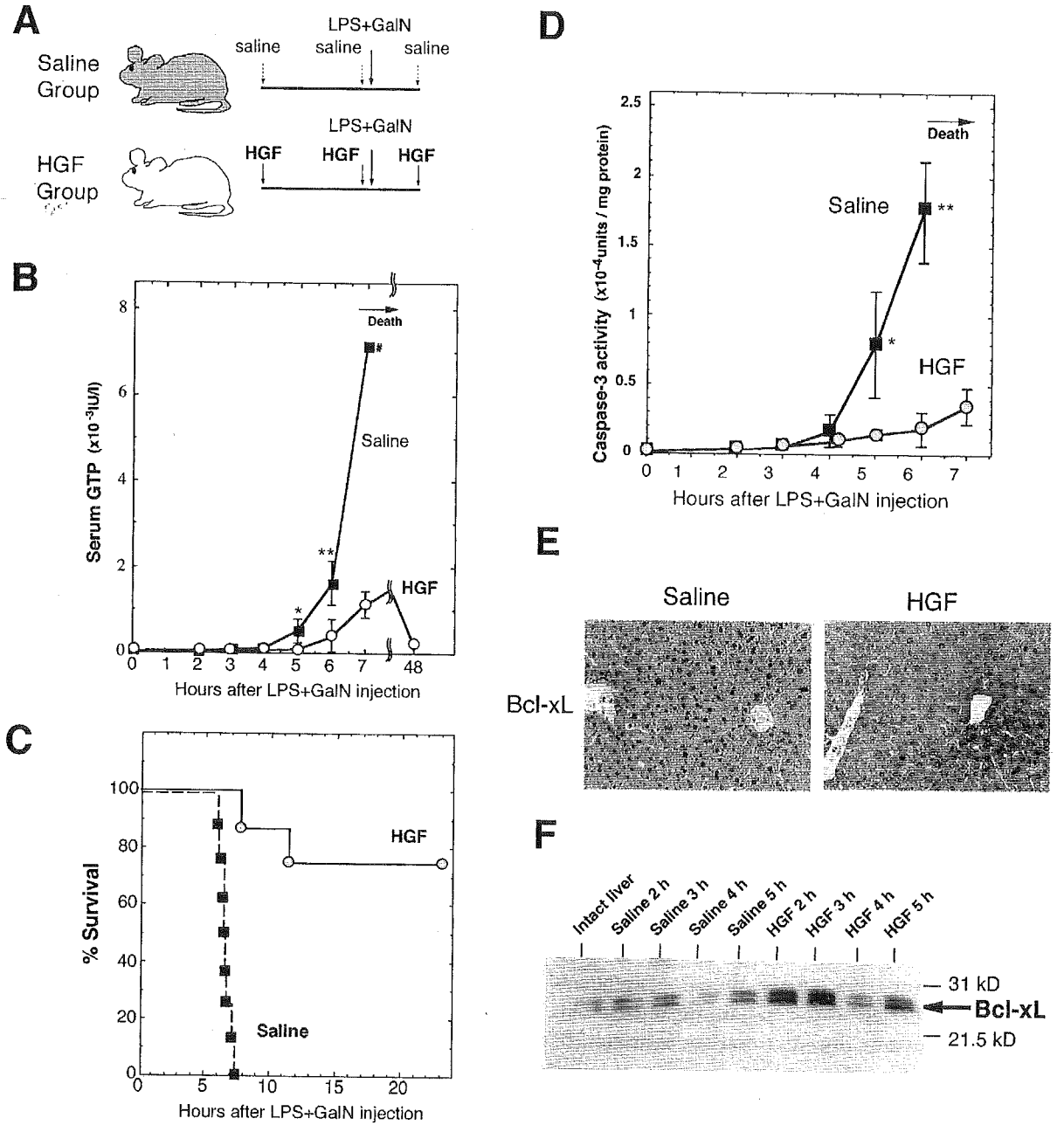

Fig. 3. HGF effectively prevents endotoxin-induced fulminant hepatitis and mortality is decreased, presumably through the suppression of caspase-3 and the induction of Bcl-xL. (A) Schedule of treatment of endotoxin-induced fulminant hepatitis with saline or recombinant HGF. Saline or HGF were injected 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

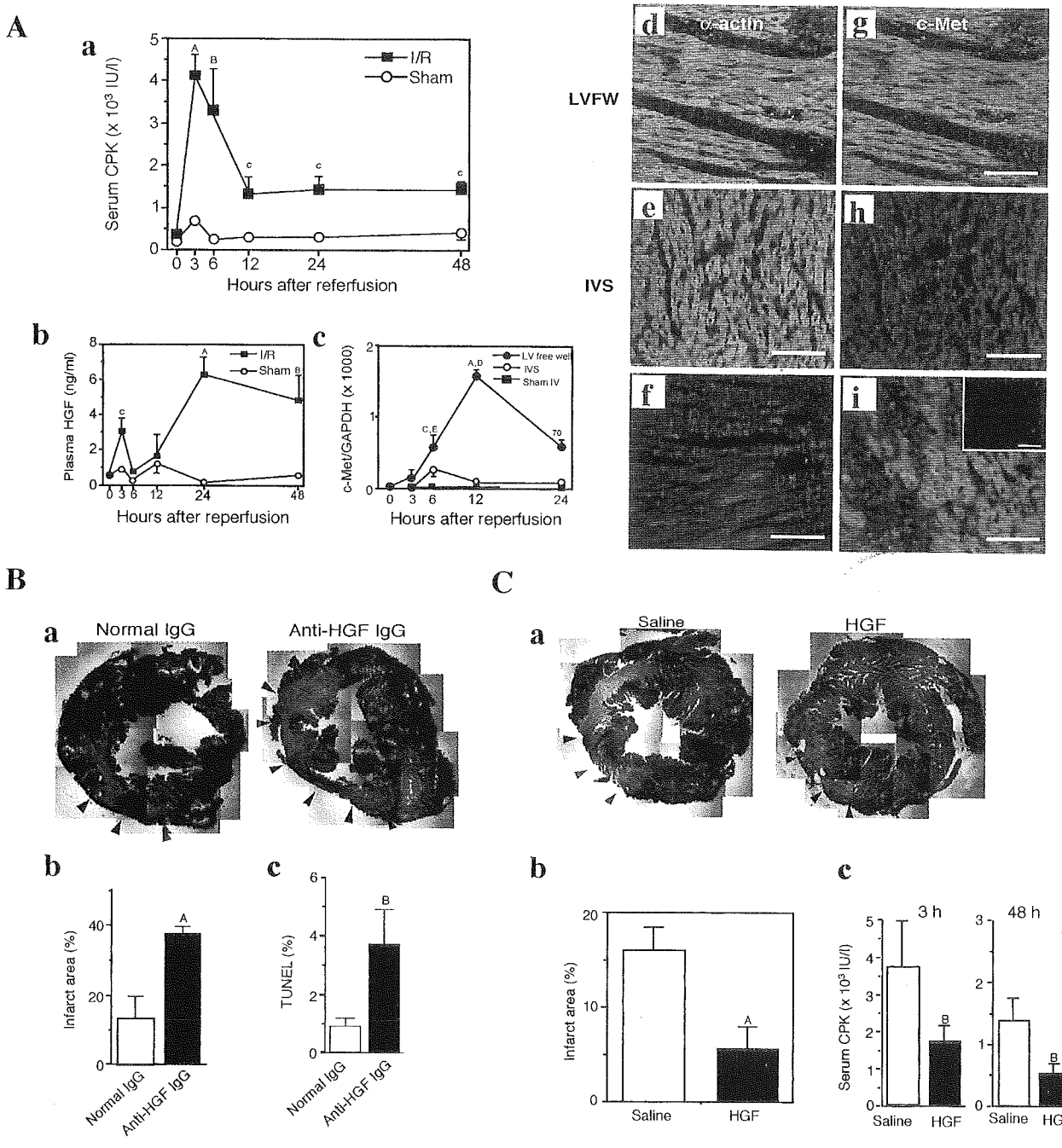

Fig. 4. Myocardial protection from ischemia/reperfusion injury by HGF. (A) Changes in HGF/c-Met expression compared with serum CPK levels in rats with ischemia/reperfusion injury. The images show double immunohistochemistry of α-sarcomeric actin and c-Met in the heart resected 48 h after reperfusion. 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

findings together with neurotrophic and angiogenic activities of HGF indicate the therapeutic potential of HGF for treating a variety of neurological diseases (Table 4A).

5.7. Gastric disease

A local submucosal injection of HGF into rats with gastric ulcers, induced by serosal application of acetic acid, resulted in a significant decrease in gastric acid secretion, acceleration of the rate of ulcer healing, and hyperemia at the ulcer margin. A similar effect was observed when HGF was administered systemically [136].

5.8. Pancreatic diseases

Transgenic overexpression of HGF using the rat insulin promoter showed positive effects of HGF on beta-cell mitogenesis, glucose sensing, beta-cell markers of differentiation, and transplant survival. In addition, the mice overexpressing HGF showed a dramatically attenuated response to the diabetogenic effects of streptozotocin [36,137]. HGF also attenuated Caerulein-induced acute edematous pancreatitis, manifested by a 41% decrease in DNA synthesis, a 53% inhibition of pancreatic blood flow, significant increases in plasma amylase and lipase activity, plasma interleukin-1 beta and interleukin-6 concentrations, as well as the development of histological signs of pancreatic damage (edema, leukocyte infiltration, and vacuolization) [138]. These findings indicated the possibility of using HGF to attenuate the immune response, preventing pancreatic beta cell apoptosis, and thus supporting the effective transplantation of functional beta cells.

5.9. Cartilage disease

HGF injection into rabbit knee joints where 4-mm diameter osteochondral defects had been made, revealed that HGF effectively repaired osteochondral defects. The repair process of the articular cartilage defects using HGF was shown to be much more effective than saline injection, as seen on all macroscopic and histological examinations. Although the observation period was short, HGF is one of the most promising candidates for repairing articular cartilage defects seen clinically [139]. In addition to the expression of HGF in the cartilage in patients with osteoarthritis (OA) HGF-stimulated the production of collagenase 3, an enzyme that is possibly involved in OA cartilage remodeling, in human OA chondrocytes, as determined by western and Northern blotting. These findings suggested the implication of HGF in the pathophysiology of OA and the possible therapeutic potential of OA-related cartilage remodeling [140,141].

6. Cancer

Tumor–stromal interaction plays an important role in tumor growth and invasion, and HGF is a critical factor involved in this interaction. In this sense, it may be beneficial to inhibit the HGF-c-Met/HGF receptor loop to suppress tumor progression. Date et al. studied the 447 N-terminal amino acid sequence of the alpha-chain of HGF, which contains the N-terminal hairpin domain and the subsequent four-kringle domains of HGF (HGF/NK4). This act as a strong antagonist to HGF and inhibits invasion of GB-d1 cells with stromal fibroblasts; also the invasion of HuCC-T1 human cholangiocarcinoma cells, and ME-180 human cervical carcinoma cells in collagen gels in vitro [142,143]. In vivo suppressive effects on tumor growth, invasion, and metastasis through antagonistic activity against HGF and anti-angiogenic activity were seen in murine transplantation models of Lewis lung and Jyg-MC(A) mammary carcinoma, SUIT-2,

Table 4B
Potential diseases for the therapeutic application of HGF

Potential cancer (NK4)
Lung cancer
Breast cancer
Pancreatic cancer
Colon cancer
Ovarian cancer
Gallbladder carcinoma
Uterus carcinoma
Melanoma
Others

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

and human pancreatic cancer cells [144,145]. Gene therapy using an adenoviral vector expressing human NK4 (AdCMV.NK4) inhibits tumor growth/invasion of cells of human lung cancer cell lines in mice through competition with HGF for its receptor, c-Met, by inhibition and *demonstrates* potent anti-angiogenic activity [146]. *Thus*, NK4 might be a potential therapeutic agent for patients with a variety of cancers (Table 4B).

7. Summary

In this review, we have summarized data on the circulating levels of HGF under normal conditions and in diseases and have discussed the critical relationships between the levels of HGF and disease, disease stage, and disease prognosis. Increased levels of HGF correlate well with the progression and prognosis of many diseases, including cancer. Therefore, the HGF levels can predict the activity and prognosis of certain diseases. We have also described the injurin system *that might* control HGF levels in serum and tissues in an autocrine, paracrine, and endocrine fashion. On the other hand, although endogenous HGF levels are regulated in response to injury, sometimes HGF levels do not reach sufficient levels to accelerate tissue regeneration. In such cases, an exogenous supplementation of HGF may attenuate disease progression and support the regeneration and remodeling of injured organs. Based on its strong therapeutic potential, HGF gene therapy has begun for treatment of patients in Osaka University Hospital. To date, HGF gene therapy appears to be effective for most ASO patients, without producing side effects. We hope that further investigation may lead us to define diagnostic and clinical roles for HGF for a variety of hitherto incurable diseases.

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REGULAR PAPER

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Expression of hepatocyte growth factor and c-Met in the anterior horn cells of the spinal cord in the patients with amyotrophic lateral sclerosis (ALS): immunohistochemical studies on sporadic ALS and familial ALS with superoxide dismutase 1 gene mutation

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Abstract To clarify the trophic mechanism of residual anterior horn cells affected by sporadic amyotrophic lateral sclerosis (SALS) and familial ALS (FALS) with superoxide dismutase 1 (SOD1) mutations, we investigated the immunohistochemical expression of hepatocyte growth factor (HGF), a novel neurotrophic factor, and its receptor, c-Met. In normal subjects, immunoreactivity to both anti-HGF and anti-c-Met antibodies was observed in almost all anterior horn cells, whereas no significant immunoreactivity was observed in astrocytes and oligodendrocytes. Histologically, the number of spinal anterior horn cells in ALS patients decreased along with disease progression. Immunohistochemically, the number of neurons negative for HGF and c-Met increased with ALS disease progression. However, throughout the course of the disease, certain residual anterior horn cells co-expressed both HGF and c-Met with the same, or even stronger intensity in comparison with those of normal subjects, irrespective of the reduction in the number of immunopositive cells. Western blot analysis revealed that c-Met was induced in the spinal cord of a patient with SALS after a clinical course of 2.5 years, whereas the level decreased in a SALS patient after a clinical course of 11 years 5 months. These results suggest that the autocrine and/or paracrine trophic support of the HGF-c-Met system contributes to the attenuation of the degeneration of residual anterior horn cells in ALS, while disruption of the neuronal HGF-c-Met system at an advanced disease stage accelerates cellular degeneration and/or the process of cell death. In SOD1-mutated FALS patients, Lewy body-like hyaline inclusions (LBHIs) in some residual anterior horn cells exhibited co-aggregation of both HGF and c-Met, although the cytoplasmic staining intensity for HGF and c-Met in the LBHI-bearing neurons was either weak or negative. Such sequestration of HGF and c-Met in LBHIs may suggest partial disruption of the HGF-c-Met system, thereby contributing to the acceleration of neuronal degeneration in FALS patients.

Keywords Amyotrophic lateral sclerosis · Hepatocyte growth factor · c-Met · Neurotrophic factor · Lewy body-like hyaline inclusion

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Introduction

Amyotrophic lateral sclerosis (ALS), which was first described by Charcot and Joffroy in 1869 [3], is a fatal and age-associated neurodegenerative disorder that primarily involves both the upper and lower motor neurons [11]. This disease has been recognized as a distinct clinicopathological entity of unknown etiology for over 130 years.

Hepatocyte growth factor (HGF) was first identified as a potent mitogen for mature hepatocytes [24] and was cloned in 1989 by Nakamura et al. [25]. Although HGF was discovered as a hepatotrophic factor, recent expression and functional analyses have revealed that HGF is also a neurotrophic factor [8, 21, 23]. HGF exerts neurotrophic effects on the hippocampal, cerebral cortical, midbrain dopaminergic, cerebellar granular, sensory, and motor neurons, as well as on the sympathetic neuroblasts [8, 12, 23]. HGF is one of the most potent *in vitro* survival-promoting

factors for motor neurons and is comparable to glial cell line-derived neurotrophic factor (GDNF) [6]. Neurotrophic effects have been demonstrated *in vivo* on embryonic spinal motor neurons during development and on adult motor neurons after axotomy of the hypoglossal nerve [27, 28, 40]. In addition, overexpression of neuronal HGF has been shown to result in the attenuation of neuronal cell death and progression of disease in a familial ALS (FALS) transgenic mouse model [35]. Therefore, HGF and its receptor, c-Met [9], might be beneficial for motor neuron survival.

An essential histopathological feature of ALS is loss of the large anterior horn cells throughout the spinal cord, and the surviving motor neurons of the spinal cord often exhibit shrinkage. Among these residual large anterior horn cells, some appear to be normal. These surviving neurons in ALS patients are thought to possess some form of self-preservation mechanism. To gain new insight into the survival/trophic mechanism of these residual neurons, we focused on the HGF-c-Met system. To date, there have been no reports demonstrating the immunohistochemical expression of HGF and c-Met in motor neurons of the human ALS spinal cord. In the study presented here, we performed immunohistochemical analyses of the human spinal cord, not only from FALS patients with superoxide dismutase 1 (SOD1) gene mutations, but also from patients with sporadic ALS (SALS), and analyzed the expression of HGF and c-Met.

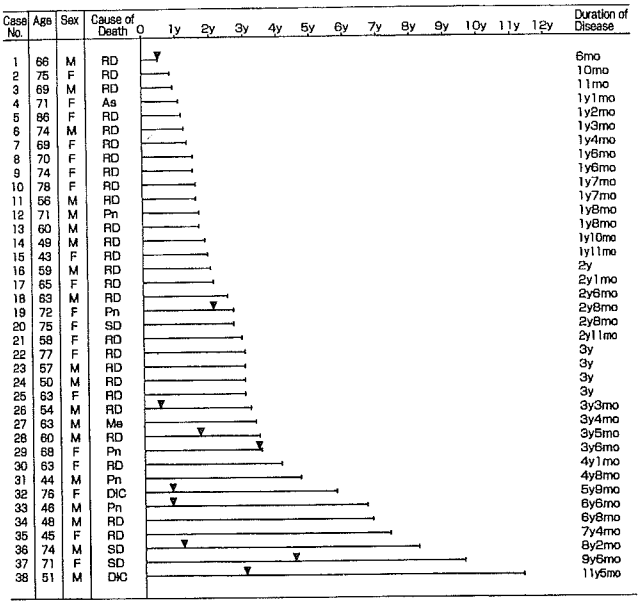

Fig. 1 Characteristics of 38 sporadic ALS cases. This figure includes each patient's age, sex, cause of death, and disease duration. The horizontal lines each show the duration of disease. Arrowheads indicate the time point at which the patients were placed on respirators. (*ALS* amyotrophic lateral sclerosis, *RD* respiratory distress, *As* asphyxia, *Pn* pneumonia, *SD* sudden death, *Me* melena, *DIC* disseminated intravascular coagulation, *y* years, *mo* months)

Materials and methods

Autopsy specimens

Immunohistochemical studies were performed on archival, buffered 10% formalin-fixed, paraffin-embedded spinal cord tissues obtained at autopsy from 38 SALS patients and 5 FALS patients who were members of two different families. The main clinical characteristics of the SALS patients are summarized in Fig. 1. The clinicopathological characteristics of the FALS patients are summarized in Table 1 and have been reported previously [14, 15, 18, 19, 26, 33, 36]. SOD1 analysis revealed that the members of the Japanese Oki family had a two-base pair deletion at codon 126 (frameshift 126 mutation) [14] and that the members of the American C family had an Ala to Val substitution at codon 4 (A4V) [33]. We also examined autopsy specimens of the spinal cord from 20 neurologically and neuropathologically normal individuals (11 males, 9 females; aged 37–75 years). This study was approved by the Ethics Committee of Tottori University (Permission No. 2001-150).

Histopathology and immunohistochemistry

After fixation in buffered 10% formalin, the specimens were paraffin-embedded, cut into 6-μm-thick sections, and examined by light microscopy. Spinal cord sections were subjected to routine staining with hematoxylin and eosin (H-E), Klüver-Barrera, Holzer, and Bielschowsky stains. Representative paraffin sections were used for immunohistochemical staining with the following primary antibodies: an affinity-purified rabbit antibody against human recombinant HGF purified from the culture medium of a Chinese hamster ovary cell that had been transfected with the human HGF expression vector (concentration: 5 μg/ml), and an affinity-purified rabbit antibody to human c-Met (C-12) [diluted 1:500 in 1% bovine serum albumin-containing phosphate-buffered saline (BSA-PBS), pH 7.4] (Santa Cruz Biotechnology, Santa Cruz, CA). Sections were deparaffinized, and endogenous peroxidase activity was quenched by incubation for 30 min with 0.3% H_2O_2. The sections were then washed in PBS. Normal serum homologous with the secondary antibody was used as a blocking reagent. Tissue sections were incubated with the primary antibodies for 18 h at 4°C.

Table 1 Characteristics of five FALS cases (*FALS* familial amyotrophic lateral sclerosis, *SOD* superoxide dismutase, *LBHI* Lewy body-like hyaline inclusion, *mo* months, *y* years, *2-bp* two-base pair, *PCI* posterior column involvement type, + detected, *ND* not determined, *As* asphyxia, *IH* intraperitoneal hemorrhage, *RD* respiratory distress, *Pn* pneumonia)

Case	Age	Sex	Cause of death	FALS duration	SOD1 mutation	Subtype	Neuronal LBHI
Japanese Oki family							
1	46	F	As	18 mo	2-bp deletion (126)	PCI	+
2	65	M	IH	11 y	2-bp deletion (126)	PCI and degeneration of other systems	+
American C family							
3	39	M	RD	7 mo	A4V	PCI	+
4	46	M	Pn	8 mo	A4V	PCI	+
5	66	M	Pn	1 y	ND	PCI	+