

HGF in Tissue Regeneration and Antifibrosis: Mechanisms and Concept

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Liver regeneration is the most dramatic phenomenon in mammalian tissue regeneration. To identify a hepatotrophic factor which stimulates liver regeneration, we utilized rat hepatocytes in primary culture. Hepatocyte growth factor (HGF) was originally identified and cloned as a mitogenic protein for mature hepatocytes (1, 2). HGF is a heterodimeric molecule composed of the α -chain and the β -chain, which are linked by one disulfide bridge. The receptor for HGF is a transmembrane tyrosine kinase, c-Met. The binding of HGF to the c-Met receptor exerts multiple biological actions involved in cell proliferation, migration, morphogenesis, apoptosis, and breakdown of extracellular matrix (3, 4).

Many approaches indicated that HGF is a most potent hepatotrophic factor in liver regeneration. Expression of HGF increases in response to liver injury, while neutralization of HGF results in impairment/retardation of liver regeneration. Recent approach using liver-specific knockout of c-Met function supported critical role of HGF and c-Met in liver regeneration and protection. In addition to the liver, HGF plays a role in regeneration and protection of various tissues from injury and pathology, including the kidney, lung, skin, nervous, and cardiovascular tissues (3, 4). Based on the notion that endogenous HGF supports tissue regeneration, we considered that supplemental administration of HGF would become therapeutic approach for treatment of diseases. Past approaches indicated that HGF has potent therapeutic value in distinct types of acute and chronic disease models. Administration of HGF suppressed cell death and the pathology associated with fulminant hepatitis, cardiac ischemia-reperfusion, and cerebral ischemia (5-7). It is notable that transgenic expression of HGF retarded disease progression and prolonged life span in a mouse model of amyotrophic lateral sclerosis (ALS) (8).

Progressive fibrosis of tissues and organs is a major cause of suffering and death. Liver cirrhosis, renal sclerosis/chronic renal disease, lung fibrosis, arteriosclerosis, and cardiomyopathy are typical progressive fibrotic diseases caused by chronic inflammation and injury. These diseases are characterized by accumulation of extracellular matrix associated

with progressive loss and dysfunction of cells responsible for tissue-specific functions. Past studies established that overexpression of transforming growth factor- β 1 (TGF- β 1) is tightly associated with the pathogenesis of fibrotic disorders. For instance, liver cirrhosis, which usually is as a long-term consequence of chronic hepatic injury caused by alcohol abuse or hepatitis virus infection, is characterized by extensive fibrous scarring of the liver and hepatic dysfunction. Again, TGF- β 1 plays a major role in fibrotic change of the liver.

When the liver was repetitively injured by administration of dimethylnitrosamine in rats, liver cirrhosis developed later than four weeks, which being associated with overexpression of TGF- β 1, expansion of myofibroblasts, extensive accumulation of extracellular matrix, and severe hepatic dysfunction. Administration of recombinant HGF or HGF gene therapy improved pathology associated with liver cirrhosis (9-11). HGF suppressed hepatic gene expression of TGF- β 1, increased collagenase activity, suppressed hepatocyte apoptosis, and stimulated hepatocyte proliferation. Moreover, HGF directly exerted biological activities on liver myofibroblasts, the predominant cells responsible for fibrotic change of the liver. For liver myofibroblasts in culture, HGF counteracted phosphorylation of ERK1/2 and mitogenic stimulus induced by platelet-derived growth factor, while HGF induced JNK-1 phosphorylation and promoted apoptotic cell death (11). In a model of liver cirrhosis, administration of HGF suppressed proliferation while it promoted apoptosis of myofibroblasts. All these events induced by HGF-treatment were associated with reduction in extracellular matrix components, histological resolution from liver cirrhosis, and prevention of mortality. Target cell-dependent distinct biological activities of HGF, which being counteractive against those of TGF- β 1, and reciprocal gene regulation of HGF and TGF- β 1 seem to be responsible for resolution from liver cirrhosis by HGF.

Improvement of tissue fibrosis by HGF was demonstrated in other models such as chronic renal diseases, lung fibrosis, and dilated cardiomyopathy (12-15). In a representative animal model of dilated cardiomyopathy, the late stages hamsters showed severe cardiac dysfunction and fibrosis, accompanied by increases in myocardial expression of TGF- β 1. Conversely, HGF was down-regulated in late-stage myopathic hearts. Treatment with recombinant human HGF for 3 weeks at the late stage suppressed cardiac fibrosis and hypertrophy, accompanied by a decreased expression of TGF- β 1, atrial natriuretic polypeptide and type I collagen (15). Suppression of TGF- β 1 and type I collagen by HGF was also shown in cultured cardiac myofibroblasts. Importantly, down-regulation of the fibrogenic and hypertrophic genes by HGF-treatment was associated with improved cardiac function. Thus, the decrease in endogenous HGF levels may participate in the susceptibility of cardiac tissue to hypertrophy

and fibrosis, and exogenous HGF led to therapeutic benefits in case of dilated cardiomyopathy even at the late stage treatment. Mechanisms by which HGF improves tissue fibrosis have yet fully understood, however, anti-fibrotic action of HGF in distinct models implicates that fundamentally similar mechanisms may participate in anti-fibrotic action of HGF even in distinct tissues.

We recently found that activation of c-Met receptor upon HGF-binding is negatively regulated through a cytoplasmic juxtamembrane domain of the c-Met receptor (16). The juxtamembrane domain of the c-Met receptor is composed of 47 amino acids that are completely conserved between rodent and human. The c-Met receptor deleted with the juxtamembrane domain is naturally expressed as splicing variant. In the juxtamembrane domain, Ser985 was respectively phosphorylated and dephosphorylated by PKC (protein kinase-C) and PP2A (protein phosphatase-2A). When Ser985 was phosphorylated, c-Met tyrosine phosphorylation and c-Met-dependent biological activities upon HGF-binding were suppressed. Following hepatic injury caused by CCl₄ in mice, phosphorylation status of tyrosine and Ser985 of the c-Met receptor was reciprocally regulated, suggesting that dephosphorylation of Ser985 allowed HGF-dependent c-Met receptor activation. When HGF was administered into experimental animals, HGF stimulated tissue regeneration in an injured organ but had no significant effect in non-injured organs. Likewise, when HGF was administered into normal animals, HGF did not mostly exert biological activities. These results suggest that c-Met-mediated signal transduction may be distinctly regulated between injured and non-injured organs. We speculate that regulation of HGF-dependent c-Met receptor activation through the juxtamembrane domain, may possibly be involved in mechanisms by which HGF exerts biological activities in an injured organ-specific manner. Likewise, mechanisms regulating c-Met receptor-mediated signal transduction in response to non-injury/injury may be responsible for a lack of general abnormality in experimental animals administered with pharmacologically effective doses of HGF.

Based on its potent angiogenic activity, HGF gene therapy for treatment of patients with peripheral arterial diseases has been in phase-II/III clinical trial. So far, HGF gene therapy has successfully induced angiogenesis and improved pathology associated with peripheral arterial diseases (17). Clinical trials of HGF for treatment of other diseases are planned. HGF will become regeneration-based therapeutic agent, increasing or compensating an intrinsic ability of tissues to regenerate.

References

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