Regenerative Medicine for Sclerotic Disorders

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Abstract: Hepatocyte growth factor (HGF) was discovered in 1984 as a molecular entity of hepatic regeneration factor, which had long been sought after. In ensuing studies, HGF has been proven to enhance the growth and motility of hepatocytes and other functional cells and to induce morphogenesis represented by tubular structures. Furthermore, HGF is endowed with potent anti-apoptotic activity, thereby preventing various tissue injuries. HGF has been demonstrated in a wide variety of animal disease models to be remarkably effective in the prevention and treatment of acute and chronic organ disorders. It has marked curative/ameliorative effects in chronic diseases, especially such sclerotic disorders as liver cirrhosis, renal failure, nephrosclerosis, lung fibrosis and cardiomyopathy for which there is no definitive treatment to date. The remarkable effectiveness of HGF stems from its ability as a tissue-organizing molecule to provide functional improvement by inhibiting fibrosis and reconstructing normal tissues, as well as from its potent anti-apoptotic activity. These show that HGF is an intrinsic tissue repair factor.

Key words: Hepatocyte growth factor (HGF); Intrinsic regeneration factor; Lung fibrosis; Liver cirrhosis; Chronic renal failure

Introduction

Ingenious tissue regeneration mechanisms in mammals are roughly divided into two distinct systems. One is a system in which undifferentiated, vigorously proliferative stem cells assume the principal role in tissue regeneration. It operates to regenerate and repair tissues comprising differentiated cells that are no longer capable of proliferation, such as the hemopoietic tissue of bone marrow, nerve tissues and muscles. The other, termed the simple duplication system, is the regeneration system for tissues whose cellular components are mature and differentiated, yet vitally capable of proliferation as seen in the regeneration of parenchymal organs such as the liver, kidneys, and lungs. Therefore, for organs with complicated multicellular architecture such as the liver, kidneys, and lungs, treatment of an injury by activation

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of the simple duplication system will be a means of regeneration/repair (therapy) in accordance with nature.

This paper discusses regenerative therapy for sclerotic disorders that arise from failure of the simple duplication system’s self-repair mechanism due to chronic damage.

**HGF as an Intrinsic Regeneration Factor**

Hepatocyte growth factor (HGF) was discovered in 1984 as a long-sought-after hepatotropic factor for vigorous regeneration of the liver, and its structure was clarified by molecular cloning in 1989.1) HGF stimulates proliferation of hepatocytes and practically all types of epithelial cells, as well as some of mesenchymal lineage such as vascular endothelial cells. In 1991, the receptor for HGF was identified to be a tyrosine kinase type c-Met gene product. Ensuing studies have revealed that HGF is endowed not only with the ability to enhance growth of the said target cells (mitogen) but also of diverse biologic activities mediated by HGF receptor. Various organ injuries and C: Inhibition of regeneration and disease exacerbation following neutralization of endogenous HGF.

**Fig. 1 Biologic activities and roles as an intrinsic regeneration factor of HGF**
has other biologic activities such as enhancement of cellular motility (motogen), induction of morphogenesis represented by tubular structures (morphogen), and anti-apoptotic and angiogenetic potential (Fig. 1A).

From the rapid increase in HGF level of damaged tissue or circulating plasma occurring in response to visceral injury, it has even been suggested that HGF acts as an intrinsic regeneration factor upon various organs (Fig. 1B). Blockade of the injury-associated HGF elevation by an anti-HGF neutralizing antibody results in strong suppression of organ regeneration/repair and expansion of tissue damage (i.e., aggravation of disease state) (Fig. 1C). It is obvious also from these events that HGF acts as an intrinsic regeneration factor to promote post-injury regeneration/repair. Conversely, administration of recombinant HGF to mice after onset of induced acute hepatitis, acute renal failure, myocardial infarction, or pneumonia leads to a conspicuous inhibition of tissue damage (anti-apoptotic effect) and enhanced regeneration with consequent marked improvement.

We then investigated the site(s) of origin of HGF, which is promptly generated and supplied in response to tissue injury. Our results showed that, in case of liver damage, for example, HGF mRNA expression occurs immediately in mesenchymal tissues such as vascular endothelial cells, Kupffer cells, and Itoh cells of the damaged liver and that HGF expression is induced also in such distant intact viscera as the lung, spleen, and kidney. Thus, HGF elaborated upon recognition of an injury assumes physiologic roles for regeneration/repair of the damaged organ via both endocrine and paracrine pathways.

**Regenerative Therapy for Chronic Fibrotic Disorders**

In chronic disorders involving persistent tissue damage, destruction of parenchymal cells and loss of vascular endothelial cells that serve organ functions give rise to proliferation of fibroblasts and other interstitial cells and accumulation of interstitial fibrous components which those cells produce, eventually resulting in sclerosis and dysfunction of the organ. These studies have indicated that decrease in expression of the intrinsic regeneration factor HGF occurs with the progression of chronic disease, coupled reciprocally with overexpression of the transforming growth factor (TGF)-β.

TGF-β acts as an inhibitor of growth of epithelial cells and vascular endothelial cells and, at the same time, as a promoter for apoptosis. Toward interstitial cells, on the other hand, TGF-β functions as a growth-promoting factor and stimulates production of interstitial fibrous components such as collagen. Overexpression of TGF-β is thus a major cause of dysregeneration and parallel stimulation of tissue fibrosis.

First, we would like to introduce findings demonstrating HGF’s ability to inhibit the development of liver cirrhosis and treat cirrhosis. In Japan alone, more than 20,000 patients develop liver cirrhosis due to chronic hepatitis or congenital biliary atresia annually. Liver transplantation is the only life-saving procedure currently available, however, registered donors are extremely few and most cirrhosis patients on the waiting list lose their lives.

Long-term administration of very low doses of dimethylnitrosamine (DMN), a hepatotoxic substance, to rats led to overt development of hepatic fibrosis and in due course to cirrhosis. Deaths began to occur among the animals in Week 4 of DMN dosing and practically all animals succumbed to hepatic failure by Week 6. When rats were started on daily recombinant HGF dosing at Week 3 of DMN administration, at which period hepatic fibrosis had developed, subsequent development of cirrhosis was almost completely inhibited along with amelioration in preexisting hepatic fibrosis and reconstruction of the normal lobular architecture (Fig. 2A). Thus, the survival rate of rats increased with increasing dosages of HGF;
all rats receiving HGF at 200μg/kg b.w. survived in normal health without incurring hepatic failure. In rats of the HGF-treated groups, there was
evidence of enhanced growth (regeneration) of hepatocytes, increased collagenolysis, inhibition of TGF-β expression as well as hepatocytic apoptosis, along with suppressed extracellular matrix production; the treated animals showed nearly normal histological features of liver tissue architecture.\(^4\) Gene therapy with HGF-expressed plasmid produced similar therapeutic effects in animals with liver cirrhosis.\(^5\)

Next, we would like to discuss the therapeutic effects of HGF in chronic renal failure/nephrosclerosis. ICGN mice, being animal models for chronic nephropathies in humans, show gradual impairment of renal function beginning just after 10 weeks of age, develop marked glomerular and tubulointerstitial fibrosis at 18 weeks of age and after, and eventually develop end-stage renal failure. The renal tissue TGF-β level increases remarkably at 10 weeks of age and thereafter, and the TGF-β elevation is accompanied by accumulation of fibrous components in renal tissues and increased apoptosis of renal tubule epithelial cells and glomerular vascular endothelial cells.

The renal tissue HGF level is decreased, in contrast to the TGF-β elevation, so that regenerative capacity of renal tubule epithelial cells is noticeably depressed. Depressed expression of HGF, which is to act as an intrinsic regeneration factor and elevated expression of TGF-β, is considered to be etiologically largely responsible for the development and progression of chronic nephropathies. In ICGN mice receiving daily doses of recombinant HGF for 4 weeks starting at 14 weeks of age, the renal function indicator BUN level, which had been continuously increasing, was largely restored to normal 7 days after the start of dosing and remained at normal levels thereafter (Fig. 2B).\(^6\)

In the HGF-treated groups of mice, a pronounced suppression of TGF-β expression in kidney tissues, inhibited accumulation of collagen and other fibrous components, suppressed apoptosis of renal tubule epithelial cells, and a marked enhancement of renal tubule epithelial cells were noted. Consequently, their kidneys showed histological features close to normal renal tissues, indicating that HGF effected glomerular and renal tubule tissue reconstruction, as seen in Fig. 2B.\(^6\) Similar results were reproduced in such other chronic renal failure models as chronic kidney graft rejection, hydropnephrosis, and 5/6-nephrectomized rats.\(^7\) If reconstruction of renal tissues could be induced by administration of HGF, thereby liberating chronic renal failure patients from hemodialysis therapy, it would be of great medical and economic significance.

The antifibrotic effect has been demonstrated following treatment with HGF in a mouse model for pulmonary fibrosis induced with bleomycin (BLM) injections (Fig. 2C).\(^8\) We would like to omit the details of the data here on account of limited space. The conspicuous efficacy of HGF lies in regenerating normal tissue architectures as well as in inhibiting apoptosis of parenchymal cells accountable for functions of tissues and organs and in stimulating growth of those cells.

HGF is also an angiogenetic factor with potent ability to promote angiogenesis essential to tissue regeneration/repair. In chronic disorders in particular, depressed HGF expression occurs and eventually leads to elevated expression of fibrosis-promoting factors such as TGF-β. This, in turn, accelerates fibrosis via loss and inhibited regeneration of parenchymal tissues. Administration of HGF to subjects in such conditions enhances regeneration of parenchymal cells and vascular endothelial cells and suppresses TGF-β expression, with consequent inhibition of tissue destruction and fibrosis.

**Conclusion**

This article has briefly described the regenerative therapy of chronic fibrotic disorders for which there has been no definitive treatment by vigorous regeneration/repair *in vivo* using the intrinsic regeneration factor HGF, which is considered to play a crucial role in the simple
duplication system for regeneration/repair inherent in mammals. We feel that there is a fair prospect that regenerative medical care using a recombinant HGF protein or HGF gene will be a concrete therapy that will remove the suffering and promote the welfare of countless diseased individuals.

REFERENCES