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REVIEW

Hepatocyte Growth Factor as Cardiovascular Hormone: Role of HGF in the Pathogenesis of Cardiovascular Disease

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Introduction

HEPATOCYTE growth factor (HGF) is a mesenchyme-derived pleiotropic factor which regulates cell growth, cell motility, and morphogenesis of various types of cells, and is thus considered a humoral mediator of epithelial-mesenchymal interactions responsible for morphogenic tissue interactions during embryonic development and organogenesis [1–4]. HGF was originally identified as a potent mitogen stimulating hepatocyte growth [5–7]. In addition, HGF also stimulates various cells such as epithelial cells [8–10]. Interestingly, recent findings that HGF can modulate cardiovascular growth in various types of cardiovascular disease suggest a potential therapeutic strategy using HGF for cardiovascular disease.

I. Role of HGF in cardiovascular disease

1. Signal transduction of HGF in endothelial cells

Numerous recent findings suggest that HGF also stimulates various cells such as endothelial cells [8–10]. Interestingly, the mitogenic activity of HGF in endothelial cells has been reported in many types of cells such as aortic endothelial cells. Initial studies regarding with angiogenic activity of HGF on endothelial cells was reported by Bussolino *et al.* [11]. They also demonstrated the presence of its specific receptor, c-met, in the endothelial cells [11]. Following their report, physiologic quantities of recombinant human HGF have been reported to induce angiogenesis [12]. In contrast, HGF did not stimulate the growth of vascular smooth muscle cells (VSMC), but stimulated the migration of VSMC [13, 14], although VSMC express a specific receptor, c-met [15]. Interestingly, HGF lacks activities related to hemostasis-thrombosis, inflammation and endothelial cells accessory functions [11]. In addition to mitogenic activity in an *in vitro* culture system, HGF has been considered as a novel growth factor for angiogenesis. Initially, activation of the HGF system was reported to promote angiogenesis in a Matrigel system [16], but this system provides far from physiological conditions. Then, *in vivo* evidence of angiogenic activity of HGF was demonstrated in a rabbit ischemia model [17–19]. Administration of recombinant HGF stimulated angiogenesis and improved rabbit hindlimb necrosis, with a significant increase in blood flow [17–19]. Therefore, currently, HGF is considered as potent angiogenic growth factor.

The molecular mechanisms of the angiogenic activity of HGF are still unknown. Our previous study demonstrated that HGF up-regulated ets activity and ets-1 protein in a myocardial infarction model [20]. Members of the ets family play important roles in regulating gene expression in response to multiple developmental and mitogenic signals [21–24]. The ets family of transcription factors has a DNA-binding domain in common that binds to a core GGA(A/T) DNA sequence [25, 26]. *In situ* hybridization studies have revealed that the proto-oncogene c-ets 1 is expressed in endothelial cells at the start of blood vessel formation, under normal and pathological conditions [27, 28]. Thus, the ets family activated the transcription of genes encoding collagenase 1, stro-

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melysine 1 and urokinase plasminogen activator, which are proteases involved in extracellular matrix degradation [29–31]. It is believed that the ets family takes part in regulating angiogenesis by controlling the transcription of these genes whose activity is necessary for the migration of endothelial cells from pre-existing capillaries. Taken together, the molecular mechanisms of the angiogenic activity of HGF may be dependent on the ets pathway. Alternatively, it was reported that angiogenesis induced by HGF was associated with a local synthesis of platelet-activating factor synthesized from macrophages [32].

In addition to the mitogenic activity, HGF also has a strong anti-apoptotic action in endothelial cells [33–35]. Of interest, HGF could abrogate the decrease in DNA synthesis and cell death of endothelial cells mediated by serum-free treatment [33]. An additive effect of HGF and bFGF was observed in the prevention of endothelial cell death, equivalent to the effect of serum treatment [13]. HGF should be classed as a new member of growth factors with anti-cell death actions. More importantly, we demonstrated that high D-glucose, but not mannitol and L-glucose, induced aortic endothelial cell death, and L-glucose induced aortic endothelial cell death, probably apoptosis, which was attenuated by addition of recombinant HGF [34]. The mechanisms by which HGF prevented endothelial cell death mediated by these conditions are still unclear. HGF is known to stimulate phosphatidylinositol-3′-kinase (PI3K), protein tyrosine phosphatase 2, phospholipase C-r, pp60$^{c\text{-}src}$ and grb2/hSos1 [36–39]. Moreover, HGF also stimulated the rho- and ras-mediated signal transduction pathways, resulting in an increase in actin fibers [39, 40] (Fig. 1). The activation of these signal transduction pathways suggests that HGF will act to prevent cell death. Especially, phosphorylation of ERK and Akt plays a pivotal role in the mitogenic and anti-apoptosis actions of HGF in endothelial cells [41, 42]. Indeed, we found that HGF up-regulated an anti-apoptotic factor, bcl-2, in human endothelial cells [35]. Up-regulation of bcl-xL was also reported in cardiac myocytes [43]. A unique feature of the HGF signal transduction system is re-phosphorylation of ERK by HGF [41]. Although numerous papers have reported the phosphorylation of ERK induced by various growth factors, this is the first report to describe re-phosphory-

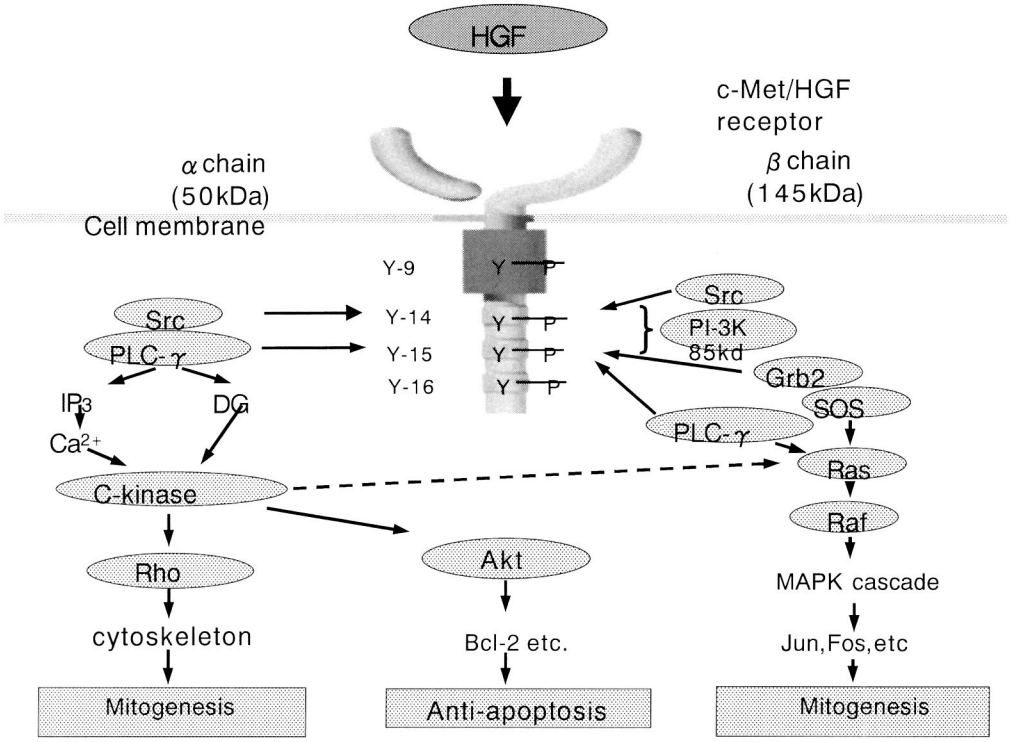

Fig. 1 Signal transduction system of HGF.

lation of ERK. We speculated that re-phosphorylation of ERK was due to auto-induction of endogenous HGF, together with the previous report that endogenously produced HGF induced by transfection of human HGF vector can exert autocrine and paracrine stimulatory effects on endothelial cell growth [44]. Indeed, addition of neutralizing anti-HGF antibody after HGF stimulation attenuated re-phosphorylation of ERK [41]. These data indicate that re-phosphorylation of ERK was due to stimulation of endogenous HGF production. This unique property of the HGF signal transduction system is probably involved in the potent mitogenic activity and anti-apoptotic action of HGF.

2. *Regulation of vascular HGF*

Many growth factors have been postulated to work as local vascular modulators in an autocrine-paracrine manner. It is well known that HGF is synthesized in large amounts in the liver and secreted into the blood. However, HGF transcripts and immunoreactive peptide can also be found in numerous locations including the kidney and lung [7, 8]. Moreover, its specific receptor, c-met, has been shown to be expressed in many target organs including brain and kidney [7, 8]. This has led to the speculation that locally synthesized HGF may influence local functions. Of importance, the presence of HGF mRNA was detected in human and rat aortic endothelial cells, VSMC and cardiac myocytes by RT-PCR [15]. The presence of c-met RNA was also detected in human and rat endothelial cells and VSMC. The existence of a local HGF system (HGF and c-met) was also confirmed in the aorta of rat and human *in vivo* [15]. The secretion of HGF was also readily detected by ELISA using specific human anti-HGF antibody in human endothelial cells and VSMC.

In addition to the *in vitro* evidence, HGF mRNA was readily detected in heart, kidney and blood vessels *in vivo* of WKY (Wistar-Kyoto rats) and SHR (spontaneously hypertensive rats) [45–48]. Interestingly, decreased renal, cardiac and vascular HGF concentrations were observed at the time (25 weeks old) that these organs revealed hypertrophic changes due to hypertension, in SHR as compared to WKY [45]. Cardiac HGF concentration also showed a significant negative correlation with left ventricular weight, accompanied by decreased cardiac HGF mRNA. These findings are confirmed by human vessels, since the segment from the injured vessels of patients with ASO demonstrated lower vascular HGF concentration as compared to uninjured vessels [17].

The promoter region of the HGF gene contains a number of putative regulatory elements, such as a B cell- and a macrophage-specific transcription factor binding site (PU.1/ETS), besides interleukin-6 response elements (IL-6 RE), a transforming growth factor-β (TGF-β) inhibitory element (TIE), and a cAMP response element (CRE) [49]. These putative sites seem to affect HGF gene expression. A marked reduction of local HGF production by TGF-β and Ang II treatment was observed in endothelial cells and VSMC [46]. The down-regulation of HGF might be important in the development of cardiovascular disease, as TGF-β and Ang II have been reported to be increased in atherosclerotic lesions and restenotic lesions after angioplasty. Indeed, blockade of the angiotensin system by administration of an ACE inhibitor and Ang II receptor antagonists increased cardiac and vascular HGF in several animal models [46–48]. In addition, we also reported that hypoxia down-regulated local HGF production through a significant decrease in cAMP in vascular cells [19]. The suppression of local HGF production also occurred in more physiological conditions. Local HGF production in endothelial cells and VSMC was markedly suppressed by high D-glucose, probably due to increased TGF-β concentration [34]. Prostaglandin (PG) E, PGI_2 analogue and cilostazol, which are well known to improve peripheral arterial disease in patients with DM, attenuated endothelial cell death induced by high D-glucose through the stimulation of local HGF production via cAMP accumulation [50]. These results suggest that decreased local HGF production may promote the progression of arteriosclerotic vascular changes in diabetes mellitus. On the other hand, HGF itself stimulated local HGF production both in endothelial cells and human VSMC [20, 44]. This phenomenon provides the interesting hypothesis that HGF itself regulates local HGF production by autoloop-positive feedback, and works in an autocrine-paracrine manner (Fig. 2).

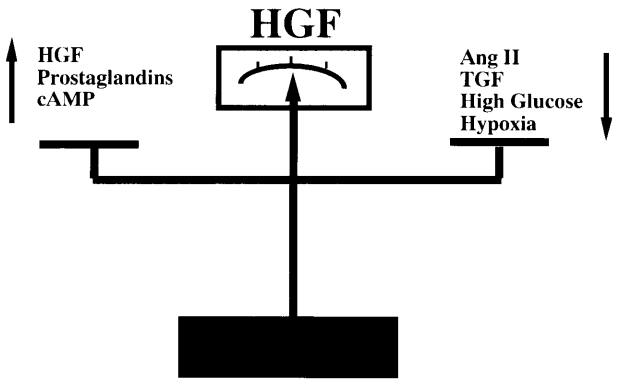

Fig. 2. Regulation of HGF in vascular system.

II. Therapeutic angiogenesis using HGF

1. Treatment of peripheral arterial disease

Application of angiogenic growth factors has been recently proposed to treat ischemic disease such as peripheral arterial disease. Critical limb ischemia is estimated to develop in 500 to 1000 individuals per million per year [51]. In a large proportion of these patients, the anatomical extent and distribution of arterial occlusive disease make the patients unsuitable for operative or percutaneous revascularization. Thus, the disease frequently follows an inexorable downhill course. Of importance, there is no optimal medical therapy for critical limb ischemia, as the Consensus Document of the European Working Group on Critical Limb Ischemia concluded [51]. Thus, in patients with critical limb ischemia, amputation, despite its associated morbidity, mortality, and functional implications [51, 52], is often recommended as a solution to the disabling symptoms, in particular excruciating ischemic rest pain [53, 54]. Indeed, a second major amputation is required in nearly 10% of such patients. Consequently, the need for alternative treatment strategies in patients with critical limb ischemia is compelling. Therefore, novel therapeutic modalities are needed to treat these patients.

In the pathophysiology of the disease, in the presence of obstruction of a major artery, blood flow to the ischemic tissue is often dependent on collateral vessels. When spontaneous development of collateral vessels is insufficient to allow normal perfusion of the tissue at risk, ischemia occurs. Recently, the efficacy of therapeutic angiogenesis using VEGF (vascular endothelial growth factor) gene transfer has been reported in human patients with critical limb ischemia [55–58]. Most of the studies have used VEGF, also known as vascular permeability factor, as well as being a secreted endothelial-cell mitogen. The endothelial cell specificity of VEGF has been considered to be an important advantage for therapeutic angiogenesis, as endothelial cells represent the critical cellular element responsible for new vessel formation [59, 60]. A clinical trial using VEGF 165 gene demonstrated greater efficacy than expected in treating peripheral arterial disease. Since then, numerous angiogenic growth factors such as VEGF121, VEGF-2 and bFGF have been tested in clinical trials.

As mentioned above, HGF is a member of angiogenic growth factors. Unexpectedly, the mitogenic activity of HGF was more potent than that of VEGF in human aortic endothelial cells *in vitro* as well as in a rabbit hindlimb ischemia model *in vivo* [14, 18]. Moreover, its specific receptor, c-met, has been shown to be up-regulated in ischemic tissue [43, 61]. Thus, it is reasoned that HGF should be a potential therapeutic angiogenic growth factor, in addition to VEGF. Recently, therapeutic angiogenesis using HGF has been reported [17–19]. In addition to recombinant protein, transfection of the human HGF gene by naked plasmid DNA or HVJ-liposome method resulted in a significant increase in blood flow [62]. The angiogenic property of transfection of the HGF gene was also proved in a diabetes model [63]. Based upon these findings, we recently started human clinical trial of gene therapy to treat peripheral arterial disease using the HGF gene. In the case of therapeutic angiogenesis, it may be preferable to deliver a lower dose over a period of several days or more, from an actively expressed transgene in the iliac artery, rather than a single or multiple bolus doses of recombinant protein, to avoid side effects. Regarding economics, which therapy would ultimately cost more to develop, implement, and reimburse, particularly for those indications requiring multiple or even protracted treatment, needs to be considered. In addition, the feasibility of a clinical trial of recombinant protein is currently limited by the lack of approved and sufficient quantities of human quality grade recombinant protein, due in large part to the nearly prohibitive cost of scaling up from research grade to human quality grade recombinant protein.

Thus, the feasibility of gene therapy using HGF to treat peripheral arterial disease seems to be in the realm of possibility in the near future.

2. *Treatment of myocardial infarction*

Another obvious major target of gene therapy based on therapeutic angiogenesis is cardiac disease, including myocardial infarction and cardiomyopathy. Recent reports have described that the administration of bFGF or VEGF into the pericardium salvaged infarcted myocardium via formation of neovasculature in several animal models [64–66]. The feasibility of a novel therapeutic strategy using angiogenic growth factors by expediting and/or augmenting collateral artery development has recently entered the realm of treatment of ischemic cardiac disease [67–71]. Similar to human trials in peripheral arterial disease, transfection of VEGF gene into the myocardium of patients with ischemic cardiac disease resulted in a marked increase in blood flow and improved clinical symptoms, without apparent toxicity. In addition to these angiogenic growth factors, overexpression of HGF was also reported to stimulate angiogenesis and collateral formation in a rat myocardial infarction model [20]. Moreover, it was reported that intramuscular injection of HGF gene into the ischemic myocardium resulted in a significant increase in blood flow and prevention of cardiac dysfunction in a canine model [72]. Interestingly, c-met/HGF receptor expression rapidly increased in the ischemic myocardium [43, 61]. Probably, upregulation of receptor may enhance angiogenic activity of HGF in myocardial infarction. More recently, HGF has been reported to be cardioprotective due to its anti-apoptotic effect on cardiomyocytes following transient myocardial ischemia and reperfusion [43].

Prevention of fibrosis by HGF was reported by previous studies in which administration of human rHGF or gene transfer of human HGF prevented and/or regressed fibrosis in liver and pulmonary injury models [73, 74]. More recently, we reported that HGF inhibited collagen synthesis through TGF-β, and stimulated collagen degradation through upregulation of MMP-1 and uPA in cardiac fibroblasts [48]. As the mechanisms by which HGF inhibits TGF-β synthesis are not yet clear, further studies are necessary.

3. *Treatment of restenosis after angioplasty*

Another important cardiovascular disease potentially amenable to HGF treatment is restenosis after angioplasty, since the long-term effectiveness of this procedure is limited by the development of restenosis in over 40% of the patients [75]. Balloon angioplasty is one of the major therapeutic approaches to coronary artery stenosis. However, restenosis occurs in 30–40% of patients after angioplasty. Intimal hyperplasia develops in large part as a result of VSMC proliferation and migration induced by a complex interaction of multiple growth factors that are activated by vascular "injury" [75]. The process of VSMC proliferation is dependent on the coordinated activation of a series of cell cycle regulatory genes that results in mitosis. Therefore, inhibition of the cell cycle using genes or oligodeoxynucleotides has been reported in several animal models. Alternatively, it has been hypothesized that rapid regeneration of endothelial cells without replication of VSMC may also modulate vascular growth, because multiple anti-proliferative endothelium-derived substances (PGI_2, NO, CNP) are secreted from endothelial cells. This concept was first tested by overexpression of VEGF 165 gene [76]. Asahara *et al.* reported significant inhibition of neointimal formation by acceleration of endothelial cell proliferation by VEGF [76]. Based upon this finding, a human trial using VEGF 165 gene by hydrogel catheter delivery of naked VEGF 165 plasmid DNA has been started to test its efficacy on restenosis after angioplasty in peripheral arteries [77]. Although the final results have yet to be reported, the preliminary results documented successful inhibition of restenosis after angioplasty [78]. A similar trial using VEGF 165 gene has been started in Finland. In this trial, VEGF gene was transfected by cationic liposomes or adenovirus with a catheter into the coronary artery [79]. A recent report demonstrated the clinical safety of VEGF gene transfer with cationic liposomes or adenovirus [79]. In addition to VEGF, HGF is also considered a potent mitogen that exclusively stimulates the growth of endothelial cells without replication of VSMC [13, 14]. Local delivery of rHGF via a drug delivery catheter attenuated neointimal hyperplasia in response to vascular injury via accelerated re-endothelialization in rabbit model [80]. They also reported that scanning electron microscopy revealed

extensive endothelialization with regular and confluent endothelial cell layer regeneration in the rHGF-treated vessels. In addition, over-expression of HGF gene in balloon-injured arteries could accelerate re-endothelialization, thereby attenuating intimal hyperplasia [81]. Re-endothelialized balloon-injured arteries transfected with HGF gene revealed amelioration of endothelial dysfunction [81]. Further studies are necessary to clarify the utility of HGF to treat restenosis after angioplasty.

III. HGF as a marker of cardiovascular disease

Elevation of serum HGF level in hypertensive patients

Given the characteristics of HGF as an angiogenic growth factor, the relationship of circulating HGF with blood pressure in normotensive and hypertensive patients was evaluated. As described earlier, TGF-β and Ang II strongly inhibited HGF production, and HGF itself stimulated HGF production in vascular cells. In atherosclerotic and hypertensive models, down-regulation of local HGF production was observed in cardiovascular tissues due to the activation of the vascular renin angiotensin system and TGF-β. HGF is suggested to play an important role in tissue regeneration [15–17], and systemic HGF may act as a humoral mediator in tissue regeneration, in addition to autocrine-paracrine local HGF production. Serum HGF concentration has been reported to be elevated in response to organ damage, such as in hepatitis and nephritis [12–14]. Recent findings show that HGF may play an important role in tissue regeneration. Taken together, we hypothesized that HGF might contribute to the protection or repair of vascular endothelial cells. If so, serum HGF level might be elevated in response to endothelial cell damage induced by hypertension. Our clinical data indicated that serum HGF concentration was significantly correlated with BP [14, 82]. Similar findings were observed in experimental hypertensive rats [45]. Serum HGF concentration was markedly increased in SHR as compared to WKY at any age. Moreover, a significant positive correlation between serum HGF concentration and BP was observed in SHR. Elevation of serum HGF concentration may be considered as a potential index of organ damage induced by hypertension, because there is a significant positive correlation between serum HGF concentration and cardiac hypertrophy. This hypothesis is supported by the present study demonstrating decreased BP and improvement of complications such as cardiac hypertrophy by Ang II blockade, and decreased serum HGF level induced by hypertension. These results are consistent with our clinical data that serum HGF concentration was significantly correlated with BP [14, 82, 83].

In addition, we and others have reported that the circulating level of HGF is elevated in patients with peripheral arterial disease and myocardial infarction [17, 84, 85]. Serum HGF concentration was also evaluated in chronic renal failure patients. Serum HGF concentration in the patients with glomerulonephritis was significantly higher than in the control group ($p < 0.01$), as described in Fig. 3. No significant correlation was found between serum HGF concentration and hemodialysis duration, hematocrit, blood urea nitrogen, serum creatinine or administered erythropoietin dose (data not shown). Of interest, serum HGF concentration in patients who showed calcification was significantly higher than in those without calcification ($p < 0.01$, Fig. 4). There was no significant difference in renal function between the patients with and without calcification. Although serum HGF concentration was significantly higher at the end of dialysis compared with that before dialysis ($p < 0.01$, Fig. 5), serum HGF concentration showed no change compared to that measured after six months (Fig. 6). As serum HGF concentration re-evaluated after 6 months did not show any significant change, it is assumed that, at least in the short term, HGF did not have obvious influences on the kidney. Although systemic HGF may work in tissue-regeneration as a humoral mediator, in addition to autocrine-paracrine local HGF production, systemic HGF may not be sufficient to promote tissue-regeneration due to decrease in local HGF production. Thus, elevation of serum HGF concentration can be considered an index of arteriosclerotic vascular changes. Overall, serum HGF concentration may be considered as a new index of endothelial dysfunction in the patients with cardiovascular disease.

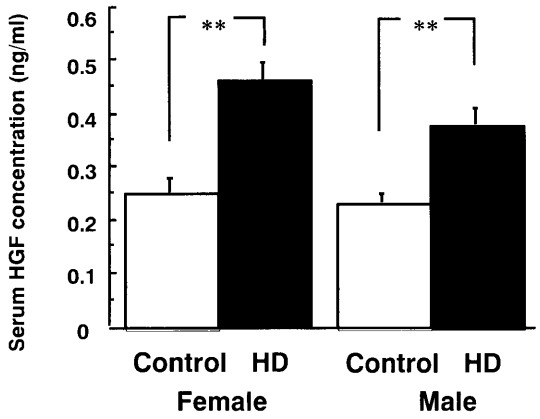

Fig. 3. Serum HGF concentration in chronic hemodialysis patients at the start of hemodialysis as compared with control subjects.
Seventy-one normal control subjects (51.6 ± 6.6 years old; 55 males and 16 females) and age-matched 87 patients with chronic renal failure due to chronic glomerulonephritis, undergoing chronic maintenance hemodialysis (52.9 ± 6.8 years old; 60 males and 27 females) were studied. Subjects were excluded from the study if they showed evidence of hepatic, inflammatory, neoplastic, cardiopulmonary disease or congestive heart failure. Patients underwent 4 hours of hemodialysis 3 times/week. Blood flow in the patients was 150–200 ml/min. Heparin was intravenously administered during hemodialysis as an anticoagulant. *HD: chronic hemodialysis patients, *p< 0.01 compared with control.

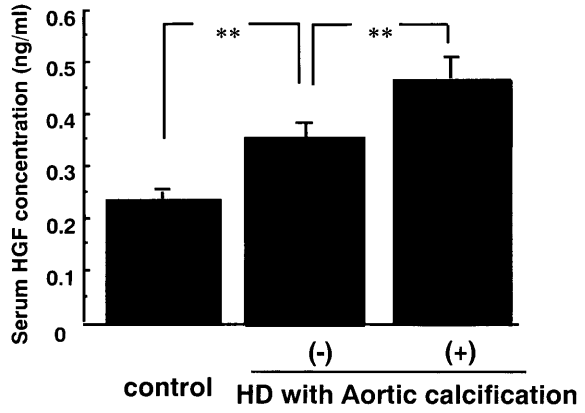

Fig. 4. Serum HGF concentration in patients with and without calcification as compared to control subjects. HD: chronic hemodialysis patients, (−) = without calcification, (+) = with calcification, **p< 0.01 compared with control.

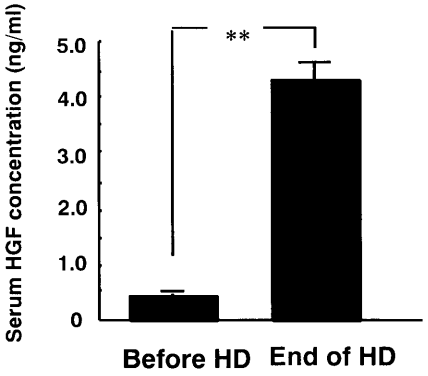

Fig. 5. Serum HGF concentration at the end of hemodialysis as compared with that at the start.
HD: chronic hemodialysis, **p< 0.01 compared with the beginning.

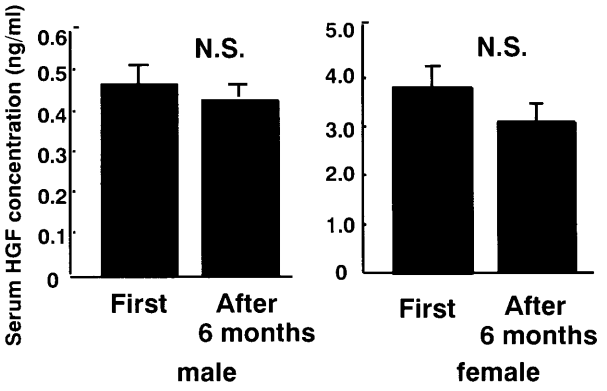

Fig. 6. Change of serum HGF concentration after six months.
HD: chronic hemodialysis

IV. HGF as "Happy Growth Factor"

Overall, HGF has several unique characteristics such as 1) potent endothelial mitogen without replication of VSMC, 2) stimulation of angiogenesis in rat or rabbit ischemic hindlimb, and rat, canine or porcine myocardial infarction model, 3) anti-apoptotic actions in endothelial cells and cardiac myocytes, 4) anti-fibrotic actions in cardiac fibroblasts, 5) acceleration of regeneration of endothelial cells. We speculated that HGF may one day be a therapeutic growth factor for the treatment of cardiovascular diseases, e.g., restenosis after angioplasty, arteriosclerosis oblerance and myocardial infarction. If so, future studies may characterize hepatocyte growth factor as a "happy growth factor".

Summary

Hepatocyte growth factor (HGF) is a mesenchyme-derived pleiotropic factor which regulates cell growth, cell motility, and morphogenesis of various types of cells, and is thus considered a humoral mediator of epithelial-mesenchymal interactions responsible for morphogenic tissue interactions during embryonic development and organogenesis. Although HGF was originally identified as a potent mitogen for hepatocytes, HGF has also been identified as a member of angiogenic growth factors. Interestingly, the presence of its specific receptor, c-met, is observed in vascular cells, endothelial cells and cardiac myocytes. In addition, the mitogenic action of HGF on human endothelial cells was most potent among growth factors. Recent studies have demonstrated the potential application of HGF to treat cardiovascular disease such as peripheral vascular disease, myocardial infarction and restenosis after angioplasty. On the other hand, serum HGF concentration was significantly correlated with blood pressure. These results suggest that HGF secretion might be elevated in response to high blood pressure as a counter-system against endothelial dysfunction, and may be considered as an index of severity of hypertension. In this review, we discussed the potential role of HGF in cardiovascular disease.

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