Hepatocyte growth factor: Molecular biomarker and player in cardioprotection and cardiovascular regeneration

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Summary
The liver possesses impressive regenerative capacities. Grafts of embryonic liver explants and liver explant-conditioned media have been shown to enhance the mitotic activity of hepatocytes. Hepatocyte growth factor (HGF), also named scatter factor (SF), has been identified as a primary candidate in promoting and regulating liver regeneration. Although initially thought to be a liver-specific mitogen, HGF was later reported to have mitogenic, motogenic, morphogenic, and anti-apoptotic activities in various cell types. By promoting angiogenesis and inhibiting apoptosis, endogenous HGF may play an important role in cardioprotection as well as in the regeneration of endothelial cells and cardiomyocytes after myocardial infarction. Since serum concentration of HGF increases in the early phase of myocardial infarction and in heart failure, HGF may also play a key role as a prognostic and diagnostic biomarker of cardiovascular disease. Here we discuss the role of HGF as a biomarker and mediator in cardioprotection and cardiovascular regeneration.

Keywords
Hepatocyte growth factor, myocardial infarction, heart failure, biomarker, adult stem cells, cardioprotection

Introduction
The liver possesses impressive regenerative capacities. Grafts of embryonic liver explants and liver explant-conditioned media have been shown to enhance the mitotic activity of hepatocytes (1). Hepatocyte growth factor (HGF), also named scatter factor (SF) (2), has been shown as a primary candidate in the regulation of liver regeneration (3, 4). Increased HGF expression after partial hepatectomy occurs sufficiently early for it to be considered a candidate for initiating the mitogenic signal for liver regeneration (3, 4). HGF is indeed now recognised as a potent mitogenic growth factor for hepatocytes, with half maximal activity of approximately 5 pM in the human body. Although initially thought to be a liver-specific mitogen (5), HGF was later reported to have mitogenic, motogenic, morphogenic, and anti-apoptotic activities in various cell types, including cardiac myocytes (6, 7). Plasma concentrations of HGF increase in response to the damage of several organs. Besides the liver (3), this occurs in the kidney (8) and the heart during the early phases of both acute myocardial infarction (AMI) and heart failure (HF) (9–11). The existence of an apparent contrast between clinical data showing high levels of HGF in patients with AMI and HF, and experimental evidence indicating beneficial effects of HGF administration in animal models of tissue ischaemia (hind limb ischaemia and AMI) poses the question of the significance of increased levels of HGF, as well as the potential role of therapeutic HGF administration in patients with cardiovascular diseases (CVD), and to review the pertinent literature. Here we discuss the role of HGF as a biomarker and player in cardioprotection and cardiovascular regeneration.

The HGF/Met system and cardioprotection: in vivo and in vitro evidence
HGF is a mesenchyme-derived growth factor mainly produced by monocytes and hepatocytes. HGF was originally identified in the plasma of partially hepatectomised rats. Its gene is located in the human chromosome 7, region q22-qter. HGF comprises an amino-terminal domain (N), four kringle domains (K1–K4), and an SPH domain (Fig. 1). HGF is synthesised as a single-chain (82 kDa) precursor, and then converted into the active heterodimer of 120 kDa, consisting of an extracellular α-chain (50 kDa) and a longer β-chain (40 kDa), with a transmembrane helix and a cytoplasmic portion (Fig. 2). In humans, the chromosomal localisation of the

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Met gene is in chromosome 7 q22-q31. HGF binds Met, which subsequently dimerises, undergoes autophosphorylation, and activates the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (ERK) and AKT, determining cell proliferation, differentiation, cell migration and anti-apoptosis (13). The final effects of the HGF/Met signaling in vivo include organ development (14), angiogenesis (15) and tissue regeneration (16, 17).

Several reports have focused on the role of HGF in cardiovascular pathophysiology. Using two distinct approaches, biological neutralization of endogenous HGF and supplementation of recombinant HGF, Nakamura et al. provided evidence of the role of endogenous HGF in cardioprotection after ischaemia/reperfusion injury (18). These authors demonstrated that: (a) the expression of both HGF and its receptor Met in cardiomyocytes, as well as plasma levels of HGF rapidly increase in response to ischaemia/reperfusion injury; (b) the neutralisation of HGF in vivo resulted in the exacerbation of cardiac dysfunction after ischaemia/reperfusion injury; and (c) the administration of exogenous HGF in rats with ischaemia/reperfusion injury resulted in lesser degree of myocardial apoptosis, smaller size of the infarct area and better cardiac function compared with non-treated control rats. These results suggest that increased blood HGF may reflect a defensive reaction and possibly participate in cardioprotection during myocardial infarction. Although the source of plasma HGF following ischaemia/reperfusion injury remains to be defined, Ono et al. showed rapid induction of HGF mRNA expression in distant organs such as the liver, kidney, lung, and spleen, as well as in the injured heart after ischaemia/reperfusion injury (19). These observations indicated that the source of HGF in ischaemia/reperfusion may be far from the heart, thus suggesting postconditioning as a new potential mechanism of HGF-induced cardioprotection. Ueda et al. analysed whether myocyte death after oxidative stress during acute myocardial infarction may be attenuated by HGF administration (20). These authors tested whether increased expression of HGF itself, as well as the activation of its receptor c-Met in cardiomyocytes may confer cardioprotection to cardiac cells at risk. They found that cardiac levels of both the c-Met receptor and HGF are elevated after myocardial infarction in rats, and that the administration of HGF to cardiac myocytes exposed to H2O2 in culture was able to delay or revert cell death (20).

From a mechanistic point of view, HGF exerts anti-apoptotic and angiogenic properties by activating its c-Met receptor and a downstream ERK1/2-mediated signalling pathway in cardiomyocytes and endothelial cells both on the myocardial infarction border zone and in regions of the heart remote from the infarct (21). It seems that cardiomyocytes and endothelial cells are primary targets of endogenous HGF. Nevertheless, other recent work focused on cardiac fibroblasts as potential cell targets, pointing out the anti-fibrotic properties of HGF (22). Whether the effects of cardiac HGF are more apparent in endothelial cells and cardiomyocytes or in cardiac fibroblasts may depend on the type and stage of heart failure. While this question awaits further investigation, the findings of Ueda et al. are of considerable interest: the strong positive correlation of HGF antioxidant effects and its increased expression in tissues bordering the infarcted heart and at sites remote from the infarct site provide a new mechanistic basis for the cardioprotective effects of HGF during myocardial infarction.

**Effects of HGF in cardiovascular regeneration**

Several experimental studies have shown that HGF can stimulate myocardial regeneration by inducing endogenous cardiac stem cells (CSCs) to migrate, differentiate, and proliferate in situ to replace lost cardiomyocytes (23–26) [reviewed by us in (27)]. Rappole et al. demonstrated that HGF and its receptor Met are expressed not only in fully differentiated cardiac cells, but also in myocytes during early cardiogenesis (23). HGF has been shown to be a potent differentiating factor for embryonic stem cells (ESC) (28) and CSCs (26), as well as for the bone marrow (25) and adipose tissue mesenchymal stem cells (MSCs) (24). Overall, treat-
ment of adult and embryonic stem cells with HGF determines cardiac or myogenic commitment through the induction of early and late transcription factors for muscle differentiation, such as Nkx 2.5, guanine/adenine/thymine/adenine (GATA-4), myocyte enhancer factor (MEF)-2C, transcription enhancer factor (TEF)-1 and GATA-4 binding protein, as well as cardiac contractile proteins, such as α- and β-myosin heavy chain (MHC) and troponin I (23–26, 28).

So far, the in vivo evidence in animals for the HGF-dependency of functional rescue of ischaemic tissues through exogenous stem cell transplantation has relied upon the transplantation of adult stem cells overexpressing HGF by gene transfer (29–31), or the transplantation of stem cells that naturally express and secrete HGF, such as adipose derived stem/stromal cells (ADSC), bone marrow- and adipose tissue-derived stem cells, alone or combined with HGF gene transfer. A summary of the findings of these studies and of the methodology used is reported in Table 1. In particular, Zhu et al. investigated the effects of endogenous transplantation of human adipose tissue-derived stem cells overexpressing human HGF (hHGF) into a rat model of acute myocardial infarction (30). The infarction protocol in this study consisted of a 30-minute coronary artery occlusion by ligation of the left anterior descending coronary artery. Twenty-four hours (h) after the infarction, adipose tissue-derived stem cells (AMI/ADSC group) or adipose tissue-derived stem cells overexpressing HGF (AMI/ADSC/hHGF group, 10^6 cells in 1 ml of phosphate-buffered saline [PBS]), or PBS (AMI/PBS group, as control) were injected into the infarcted rats via the vena caudalis. The authors demonstrated that cardiac function parameters, including ejection fraction (EF), fractional shortening (FS), left ventricular end-diastolic diameters (LVEDd) and end-systolic diameters (LVESd), were improved at seven, 14, and 28 days after cell transplantation in the AMI/ADSC/HGF group, in contrast with the AMI/PBS group. Furthermore, the authors found that transplantation of ADSCs induced angiogenesis by suppressing fibrosis and increasing capillary density in the ischaemic myocardium. More importantly, compared with the AMI/ADSC group, these effects were enhanced in the AMI/ADSC-HGF group (30).

In an effort to deepen our understanding on the translational potential of adult stem cells, such as ADSCs, we have studied the effects of ADSC transplantation, given at multiple doses (10^6–10^7 cells/ml), on cell metabolism and the microcirculation (abundance in capillaries, arterioles and venules) in ischaemic tissues in an animal model of tissue ischaemia (unpublished data). In this rat model of hind limb ischaemia, the intramuscular injection of allogeneic HGF-expressing ADSCs at the time of ischaemia (a) abrogates the ischaemia-associated fall in tissue creatine (tCr), as assessed by proton magnetic resonance (1H-MR) spectroscopy, therefore preventing the bioenergetic deterioration of ischaemic tissues; and (b) resulted in a significant improvement in the microcirculation and neovascularization, as assessed by thermal infrared (IR) imaging and immunohistochemistry. These effects may result from increased blood flow reserve, in turn depending on increased vessel density and decreased fibrosis. Beneficial changes documented with ADSC transplantation were reproduced with conditioned media from ADSC cultures. This indicates that a paracrine effect (i.e. the secretion of soluble products by the stromal cells derived from the adipose tissue) likely play a major role in the functional effects of ADSCs in improving post-ischaemic damage, with an important role of HGF.

**Table 1: In vivo evidence of HGF-dependent functional rescue of ischaemic tissues through exogenous stem cell transplantation.**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Model</th>
<th>Type of injected cells</th>
<th>Administration route</th>
<th>Outcomes</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>(30)</td>
<td>Rat, CA ligation</td>
<td>HGF-overexpressing ADSCs (1x10^6 cells)</td>
<td>i.v. injection</td>
<td>EF, FS, LVEDd, LVESd ↑</td>
<td>angiogenesis ↑, fibrosis ↓</td>
</tr>
<tr>
<td>(29)</td>
<td>Rat, CA ligation</td>
<td>HGF-overexpressing BMSCs (1x10^6 cells)</td>
<td>i.m. injection</td>
<td>EF, FS, LVEDd, LVESd ↑</td>
<td>angiogenesis, apoptosis ↓</td>
</tr>
<tr>
<td>(32)</td>
<td>Mouse, FA ligation</td>
<td>HGF-expressing ADSCs (1 x 10^6 cells)</td>
<td>i.m. injection</td>
<td>LDI ↑</td>
<td>angiogenesis ↑</td>
</tr>
<tr>
<td>(33)</td>
<td>Mouse, FA ligation</td>
<td>HGF-expressing ADSCs (1 x 10^6 cells)</td>
<td>i.m. injection</td>
<td>LDI ↑</td>
<td>angiogenesis ↑</td>
</tr>
<tr>
<td>(34)</td>
<td>Mouse, FA ligation</td>
<td>ADSCs treated with Lenti-siRNA-HGF (1 x 10^6 cells)</td>
<td>i.m. injection</td>
<td>LDI ↓</td>
<td>angiogenesis ↓</td>
</tr>
<tr>
<td>(31)</td>
<td>Rat, CA ligation</td>
<td>HGF-overexpressing skeletal myoblasts seeded in scaffolds (1x10^6 cells)</td>
<td>i.m. injection</td>
<td>dp/dt(max), dp/dt(min) ↑</td>
<td>arteriogenesis ↑</td>
</tr>
</tbody>
</table>

FA, femoral artery; ADSCs, adipose derived stem/stromal cells; BMSCs, bone marrow derived stem/stromal cells; HGF, hepatocyte growth factor; EF, ejection fraction; FS, fractional shortening; LVEDd, left ventricular end-diastolic diameter; LVESd, left ventricular end-systolic diameter; LDI, laser Doppler imaging; IR, infrared imaging; 1H-MRI, proton magnetic resonance imaging; dP/dt, first derivative of ventricular pressure over time.

**Diagnostic and prognostic value of HGF in CVD**

HGF has been proposed as a diagnostic biomarker in AMI in view of its strong increase in expression in the blood after acute myocardial ischaemia (19). Ono et al. (19) indeed first reported that HGF expression in the heart is increased in the ischaemic reperfused region, with plasma HGF peaking at 3 h after reperfusion in a rat model. Matsumori et al. (35) also reported that serum levels of
HGF were increased within 3 h in patients with AMI, and remained elevated for 12 to 24 h. Zhu et al. (36) found raised serum levels of HGF in the acute and subacute stages of a myocardial infarction. In this study, the elevation of HGF in the early stages reflected the extent of the infarct. The authors also found that levels of monocyte HGF were elevated in patients with ventricular enlargement in the course of AMI. They concluded that post-AMI inflammation could contribute to the rise in circulating HGF levels through enhanced HGF production by monocytes.

In addition to AMI, a few studies have found elevated levels of HGF in patients at low mortality risk with early (10), or advanced stages of HF (11). These studies have suggested a prognostic role of HGF in patients with HF. Lamblin et al. showed a moderate—but significant—association of HGF with HF severity and cardiovascular mortality in a cohort of stable, ambulatory patients with HF (10). Of particular importance is the study from Rychli et al., who investigated the role of HGF as a prognostic biomarker in patients with advanced systolic HF (11). Their prospective cohort study enrolled 350 patients (63% with ischaemic HF and 37% with non-ischaemic HF), including patients with NYHA class III or IV at the time of hospital admission; or a cardiothoracic ratio >0.5 and/or left ventricle ejection fraction (LVEF) <40%. The primary end point was all-cause mortality, with cardiovascular mortality as a secondary end point. In this study the median HGF concentration (2.46 ng/ml) was found independently associated with an increased risk for all-cause mortality after adjustment for demographics and other prognostic markers, including brain natriuretic peptide (BNP). The study also found increased cardiovascular mortality in the subgroup of ischaemic HF. Interestingly, levels of HGF were found positively correlated with age, the incidence of atrial fibrillation and levels of BNP, and inversely correlated with the body mass index (BMI) and the dosage of renin angiotensin aldosterone-system (RAAS) inhibitors, including angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers. In the analysis of the combined predictive value of HGF and BNP, patients with both biomarkers above the median had a higher risk of death from all causes or from cardiovascular causes than those with both markers below the median, indicating a potential prognostic value of HGF additive to that of BNP. Overall, the results from Rychli et al. (11), along with those of Lamblin et al. (10) apparently contradicted the in vitro and in vivo experimental evidence, demonstrating beneficial effects of HGF in terms of angiogenesis, anti-fibrosis, cardioprotection and cardiac stem cell recruitment into the ischaemic myocardium. Indeed, these clinical studies demonstrated that HGF is a strong and independent predictor of severity in early HF and mortality in advanced HF, suggesting that HGF exerts an overall deleterious effect in patients with HF. The clinical evidence of unfavorable outcome in HF patients is in agreement with results from additional studies in patients with carotid atherosclerotic disease, showing a role of HGF in worsening plaque formation and even de-stabilising atherosclerotic plaques (37, 38). Possible explanations for these contradictory results are either (a) that increased HGF expression is an endogenous protective mechanism which is no longer effective in the course of HF; or (b) that HGF is a pleiotropic growth factor that may exert both beneficial and detrimental effects on the cardiovascular system. Whatever the underlying mechanism, these results point out the need of caution in considering the HGF administration as a treatment option in patients with CVD, either by direct administration of the recombinant protein or by cell-based gene transfer techniques.

**Potential applications of HGF in the treatment of CVD**

The growing body of experimental evidence on the role for HGF in cardioprotection and cardiovascular regeneration has suggested the in vivo application of HGF gene transfer or the administration of HGF as recombinant protein, aimed at enhancing the regeneration of cardiovascular tissues. An early evaluation of the feasibility and efficacy of in vivo HGF gene delivery for therapeutic angiogenesis involved the direct intramuscular injection of HGF DNA using plasmid vectors in patients with otherwise inoperable critical limb ischaemia (39). This open-labelled phase I/IIa study documented the safety and feasibility of this approach, but a major limitation was that the study was not randomised, placebo-controlled, or double-blind. To overcome such limitations, a multicentre randomised, placebo-controlled, double-blind phase II clinical trial was performed in the US to confirm the effectiveness of HGF gene therapy (40). This trial of HGF gene therapy demonstrated a significant increase in transcutaneous (Tc) PO_{2} in the high-dose HGF group compared with the placebo group. One must point out, however, that such findings are preliminary, and do not establish the long-term safety of HGF administration, which may include potential adverse effects such as haemangiomas, cancer, or a worsening of diabetic retinopathy. Because of such potential adverse effects related to the administration of high doses of growth factors, the use of recombinant HGF in in vivo experiments has so far encountered several limitations. In general, because of the short half-lives of growth factors in the body and the need of delivering therapies to specific target sites, growth factor injection does not often yield the anticipated therapeutic effect. The short half-life of growth factors necessitates massive doses and multiple injections to achieve a therapeutic effect, especially for the intravenous administration. The administration of supra-physiological concentrations of growth factors may lead to severe side effects. For example, the use of large doses of vascular endothelial growth factor (VEGF) may lead to pathological vessel formation at non-target sites, with the formation of tumours. This justifies the need for searching new delivery systems of growth factors in the clinical application of regenerative therapies. For HGF, in addition to its high instability, there is an additional limitation represented by the fact that this growth factor is difficult to purify in its heterodimeric biologically active form. In the study by Banquet et al. (41), the authors evaluated a new delivery system for growth factors, such as the targeted intramyocardial albumin-alginate microcapsule delivery, capable of providing spatio-temporally controlled released of fibroblast growth factor (FGF)-2 and HGF. The authors used a
The finding that HGF is not simply related to the regulation of liver regeneration, but plays a role in mitogenic, motogenic, morphogenic, and anti-apoptotic activities in various cell types, opens up many avenues worth being explored on the role of this growth factor in CVD. The following conclusions can be drawn at this time:

- HGF seems to have diagnostic as well as prognostic value in AMI and HF. The clinical evidence of unfavourable outcome in HF patients, along with the worsening of plaque formation and destabilisation in patients with ischaemic heart disease and high levels of HGF however demands caution in considering HGF administration as a possible treatment option in such patients.
- HGF has a role in cardiovascular repair and regeneration. The HGF/Met axis is an important biological system for enhancing the efficacy of cell-based cardiovascular regenerative therapies. The high probability that this therapy may be long-lasting represents a substantial theoretical advantage over treatments with recombinant proteins.
- Combining HGF with other growth factors such as FGF-2 may improve clinical outcomes by stimulating angiogenesis and decreasing fibrosis.

Further studies, particularly more bench-to-bedside translational work, are needed to validate these results and to clarify whether therapeutic modulation of HGF levels (by RNA interference or genome editing) or the administration of HGF (by gene transfer of direct injection of recombinant protein) would be helpful in patients with HF and myocardial ischaemia.

Acknowledgements

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Conflicts of interest

None declared.

References

12. Chmielowic J, Borowiak M, Morkel M, et al. c-Met is essential for wound healing and also associated with protein therapy because of the need for large doses, the transient effects of treatment with generation of unstable blood vessels that regress over time, and safety issues related to the stimulation of unwanted angiogenesis in distant micro-metastases or in the setting of diabetic retinopathy (42).

Conclusions


