

Chapter Book

Hepatocyte Growth Factor: Cardiotropic Roles and Potential Therapeutics for Cardiovascular Diseases

S. Mizuno¹ and T. Nakamura²

¹Division of Virology, Department of Microbiology and Immunology, Osaka University Graduate School of Medicine, Suita, Japan

²Professor Emeritus, Kringle Pharma Joint Research Division for Regenerative Drug Discovery Center for Advanced Science and Innovation, Osaka University, Japan

INTRODUCTION

Due to prolonged lifespans, cardiovascular disease is on the increase worldwide and is now the leading cause of death, especially in developed countries. Histologically, it is characterized by coronary atherosclerosis, in which the over-proliferation of vascular smooth muscle cells (VSMC) is evident, associated with endothelial injury and foamy cell-like macrophages (i.e., local inflammation) (1,2). Such a sclerotic event triggers narrowed lumens (i.e., stenosis) and a decrease in coronary blood flow leads to local hypoxia, apoptosis, and eventually the onset of myocardial infarction (MI) (2). Thus, the major approaches proposed to prevent or restore MI are: (i) prevention or restoration of coronary atherosclerosis, the primary cause of heart disease; (ii) induction of cardiac angiogenesis with cytokines/growth factors; (iii) inhibition of cardiomyocyte cell death during heart ischemia (i.e., anti-apoptotic therapy); and, (iv) possible reconstitution of cardiomyocytes via recruitment of intrinsic stem cells, or cell transplantation (i.e., regenerative therapy). Many studies have focused on the disease events and identified the molecules involved as a pathogenic regulator(s) (i.e., offensive side) (1,2). However, little is known about the protective mechanisms whereby coronary damage and myocardial cell loss are minimized during heart ischemia (i.e., defensive side). Hepatocyte growth factor (HGF) was identified and cloned, based on the DNA synthesis of adult rat hepatocytes in primary culture (3–5). HGF is now recognized as an organotrophic factor, which is needed for organogenesis in the embryo and tissue repair in almost all organs in adults (3,6-10). Plasma HGF levels increase in response to heart injury under pathological conditions (11,12). We first found that HGF targets cardiomyocytes and elicits anti-apoptotic effects at an acute phase of MI (13), the mechanisms of which will be discussed later. HGF also elicits an angiogenic response in infarcted hearts by enhancing endothelial cell duplication (14) without VSMC hyperplasia. HGF has an anti-inflammatory effect on immune cells (15). These multiple functions are exerted through tyrosine phosphorylation of c-Met, a receptor for HGF(16,17). Of note, the specific inhibition of c-Met signaling by anti-HGF antibody leads to the aggravation of MI-related pathological events, identifying endogenous HGF as a “cardiotrophic factor” (13).

During acute and chronic cardiac disease, HGF production is induced and enhanced, but this response is transient, insufficient and often retarded (10-13,18), leading to the acceleration of cardiac dysfunction. It is, therefore, likely that cardiovascular disease is manifested due to an HGF deficiency or insufficiency. The

present article reviews recent advances in the understanding of the molecular basis underlying the inhibition of heart diseases by endogenous HGF-c-Met signaling. We also discuss a rationale for exogenous HGF supplement therapy as a pathogenesis-based strategy, based on the previous experimental findings.

BIOLOGY AND PHYSIOLOGY OF HGF IN CARDIAC DISEASES

As its name indicates, HGF was originally identified as a mitogen for adult rat hepatocytes in a primary culture (3-5). HGF acts on various types of cells via its receptor, c-Met (16,17), and elicits pleiotropic effects required for embryogenesis and tissue repair (6-10). During heart ischemia, endogenous HGF makes efforts to sustain myocardial morphology and function, and this effect is mediated by a reduction in the apoptosis of myocytes (13) and stimulation of the proliferation of coronary endothelium (14). Prior to discussion on the therapeutic role of HGF, we summarize the physiological value of HGF for heart cells in vitro and in vivo.

In Vitro Effects of HGF on Cardiac Cells

Protective effect of HGF on myocardial cells:

Under a healthy (i.e., non-injury) condition, c-Met expression is much lower in myocardium than in coronary endothelium (19). However, we found that c-Met expression levels dramatically increased in myocardial cells of rats after the heart ischemia (13,20), suggesting a direct therapeutic action of HGF on cardiac muscular cells, as discussed later. In a primary culture, c-Met expression levels increased in the myocardial cells, while recombinant HGF inhibited the apoptotic death caused by hydrogen peroxide (13,20). In this process, the early induction of Bcl-xL, an anti-apoptotic molecule, by recombinant HGF was critical for the inhibition of apoptosis of myocardial cells (13). Recent reports described that the HGF-induced cardio-protection was mediated through c-Met tyrosine phosphorylation and sequential activations of MEK, p44/42 MAPK (ERK1/2) and p90RSK (13,21). Indeed, Kitta et al. demonstrated, using an in vitro assay of myocardial cells, that phosphorylation of GATA-4 via the c-Met-MEK-ERK pathway is critical for HGF-mediated Bcl-xL induction and subsequent anti-apoptotic outcomes (22,23). HGF elicits a mitogenic response in endothelial cells, but not in smooth muscle cells (24). In this process, HGF elicited the ERK phosphorylation in endothelial cells, followed by the STAT3-serine727 phosphorylation and c-fos activation (25). When PD98059, a specific inhibitor of MEK, was added to the culture, STAT3 phosphorylation and c-fos activation were inhibited, resulting in the attenuation of HGF-mediated mitogenesis (25). Thus, it is likely that activations of STAT3 and c-fos by HGF via the c-Met-MAPK-pathways is required for cardiac angiogenesis.

It is critical to inhibit endothelial cell apoptosis for the induction of angiogenesis. In vitro, HGF protected endothelial cells from apoptotic injury caused by TNF- α (25). The effect was restored by pre-treatment with LY294002, an inhibitor of PI3K (25), thus indicating that the anti-apoptotic effect on endothelium is mediated via c-Met-PI3K-AKT pathways. In contrast, HGF prevented serum starvation-induced apoptosis via c-Met-MAPK-pathways (26). Although signaling pathways for HGF-induced anti-apoptosis differ with the type of injury, the mitogenic and protective effects of HGF on endothelium lead to "therapeutic angiogenesis" during cardiovascular disease (14,27), as described later.

In addition to cardiac myocytes and vascular cells, HGF has pleiotropic biological effects on myofibroblasts (MyoFB), immune cells, and intrinsic stem cells (**Fig. 1**), all of which are critical for HGF to produce therapeutic outcomes in several types of cardiac disease.

Protective Roles of Endogenous HGF-c-Met Axis for Minimization of MI

Several lines of clinical studies demonstrated an apparent increase in blood HGF levels in patients during the acute phase of MI (12,28,29). Similarly, there was an eight-fold increase in the blood HGF levels in a rat model of MI, caused by transient ischemia (13). In this model, c-Met mRNA levels dramatically increased compared with a basal level (i.e., > 1,000-fold) (13), and cardiac immunohistochemistry revealed the predominant expression of c-Met in the non-infarcted area of the myocardium, accompanied by increased levels of plasma HGF. Importantly, when anti-HGF antibody was administered

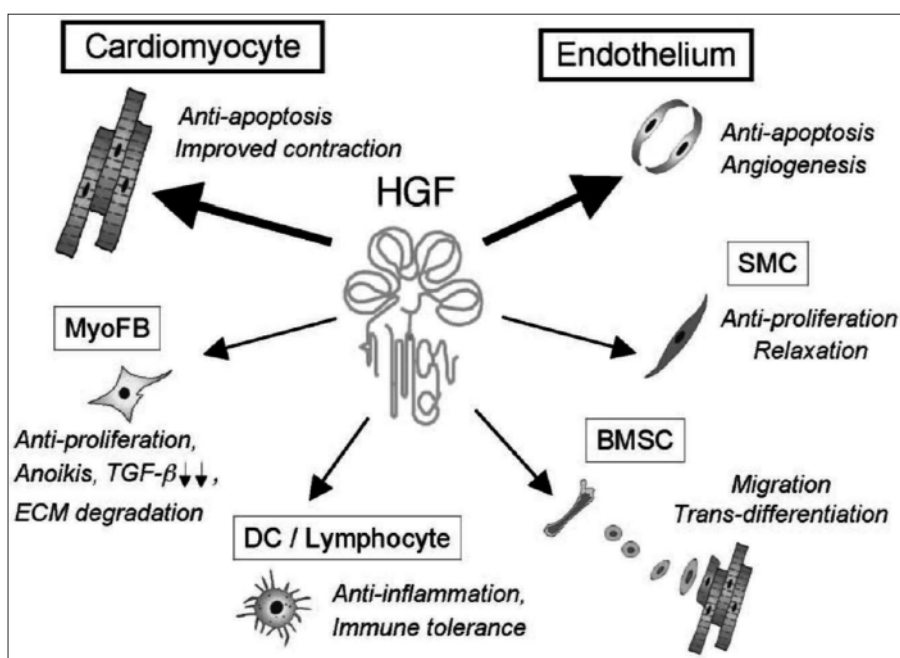


Figure 1. Pleiotropic function of HGF during cardiovascular disease. Multi-faceted biological actions of HGF, mediated via c-Met tyrosine phosphorylation, are outlined. HGF inhibits apoptosis, enhances contraction of cardiomyocytes, and induces angiogenesis via inhibition of apoptosis and stimulation of mitogenesis in endothelial cells. HGF has an anti-proliferative effect on vascular smooth muscle cells (VSMC). Furthermore, HGF elicits anti-fibrotic and anti-inflammatory activities toward myofibroblasts (MyoFB) and dendritic cells (DC), respectively. Recent evidence indicates that HGF stimulates the migration, proliferation and trans-differentiation of bone marrow-derived stem cells (BMSC).

to rats immediately after the release of coronary artery clamp, the infarcted area became wider, along with an increase in cardiac apoptosis (13). As a result, the anti-HGF IgG treatment led to 50%-mortality within 48 hours post-ischemia, while all the rats survived after the normal IgG injections during the acute MI (13). These data demonstrated that the direct action of endogenous HGF on cardiomyocytes is required for minimizing the myocardial apoptosis, infarction and dysfunction post-ichemia.

In the rat model of acute MI, HGF was highly produced in the distant organs (such as the liver, kidneys and spleen) and then delivered to the injured hearts via blood flows (i.e., an endocrine mechanism), as reported in a case of liver injury (10). In addition, HGF is produced in interstitial cells (such as fibroblasts and vascular cells) around the injured heart (i.e., paracrine system). On the other hand, c-Met may be induced in the myocardium through a local hypoxia-related mechanisms such as an increase in HIF-1, a transcriptional factor for the up-regulation of c-Met mRNA (30). Indeed, c-Met expression by myocardium was observed in humans, in accordance with acute cardiac hypoxia (31), as seen in the rat model (13). Thus, signal transduction from blood HGF, or from heart-born HGF, to c-Met-positive myocardium confers an intrinsic defense system to minimize the onset of acute MI (Fig. 2).

THERAPEUTIC EFFECTS OF HGF ADMINISTRATION ON HEART ISCHEMIA

Acute MI is commonly known as a heart attack, due to the interruption of blood supply by atherosclerosis of coronary vessels (1,2). Under hypoxic conditions, the myocardium becomes apoptotic, followed by left ventricular (LV) infarction and dysfunction (32,33). MI is now the leading cause of death in developed countries. Thus, the central interest of the therapeutic strategies is to control myocardial cell death and induce angiogenesis around atherosclerotic areas. Based on the cardioprotective roles of endogenous HGF, the potential application of HGF as a treatment for ischemic heart disease has been tested in animal models.

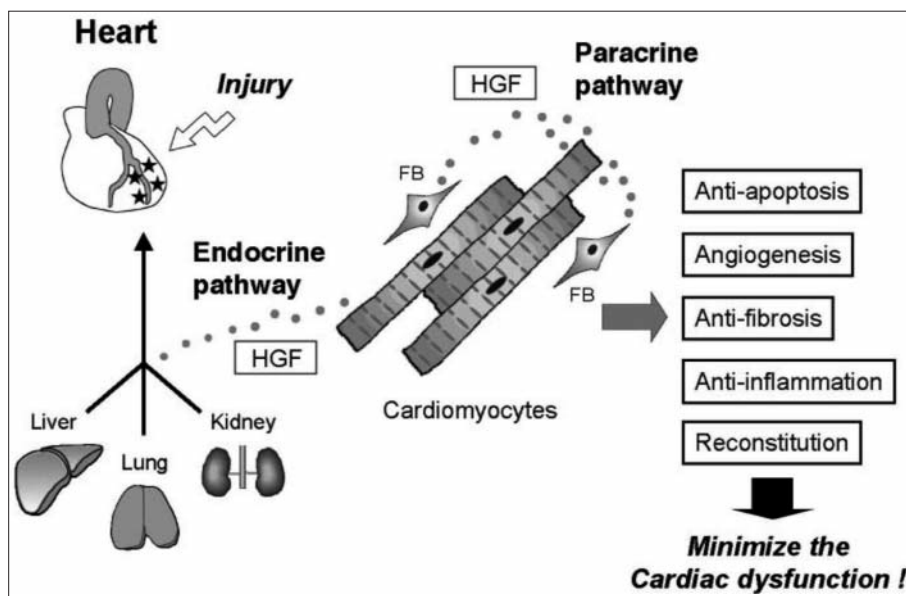


Figure 2. Endocrine and paracrine mechanisms of HGF delivery system in the attenuation of cardiac disease. During cardiovascular injury, HGF is produced in distant organs (i.e., liver, lung and kidney) in response to HGF-inducers, such as norepinephrine, growth hormone, prostaglandins and inflammatory cytokines (i.e., endocrine system). HGF is also produced in intra-cardiac cells, such as fibroblasts (FB) and VSMC (i.e., paracrine system). Both pathways are required for inhibition of cardiac destruction and dysfunction via multifaceted functions.

Anti-apoptotic Therapy with Recombinant HGF During Acute MI

As mentioned above, we found in 2000 that myocardial cells acquired c-Met during heart ischemia in rats (13), accompanied by an increase in blood HGF levels. Of interest, serum obtained from MI-manifesting rats, but not from normal rats, reduced the H₂O₂-mediated apoptosis in a culture of fetal rat myocardial cells (13). The anti-apoptotic effect of serum was diminished by the addition of anti-rat HGF IgG (13), indicating that HGF in serum is responsible for cardio-protection. Thus, we hypothesize that a forced increase in blood HGF, by an injection of exogenous HGF, may be a therapeutic strategy to control acute MI. To test our hypothesis, recombinant HGF was administered to rats after the release of heart ischemia. There was an enhancement of Bcl-xL in the myocardial cells and attenuation of cardiomyocyte apoptosis in the HGF-treated rats post-ischemia. As a result, the infarcted area was reduced, and then the loss of cardiac function was avoided in rats by recombinant HGF therapy (13). Such a beneficial effect of recombinant HGF was reproduced in a rat model of severe MI, in which HGF improved cardiac dysfunction by increasing the stroke volume index (34). Using recombinant HGF in a mouse model of MI, Wang et al. demonstrated that HGF prevented ventricular remodeling, apoptosis and dysfunction, through a PI3K-AKT pathway (35). Given that myocardial apoptosis is the major contributor to the pathogenesis of MI (33), the inhibition of apoptosis by HGF, an endogenous cardioprotective ligand, would be a physiology-based strategy to attenuate the manifestation of acute MI (13).

Angiogenic Therapy Using HGF Gene for Amelioration of Cardiac Hypoxia

Since MI is primarily characterized by a loss of coronary vessel blood flow due to atherosclerosis, numerous investigators have attempted to propose an angiogenic approach, using cytokines or growth factor (36). HGF is a powerful angiogenic factor via eliciting mitogenic and morphogenic effects on endothelial cells, but not VSMC (24-26). Morishita et al. accumulated evidence that supplemental therapy with recombinant HGF (or HGF gene) led to "therapeutic angiogenesis", using animal models of peripheral artery disease (PAD) (14,37). Based on this background, our groups demonstrated, using a rat MI model, that collateral artery development of the coronary vessels by HGF gene leads to an increase in cardiac blood flow (27). In the ischemic myocardium transfected with an HGF cDNA-containing plasmid

vector, there was a significant increase in PCNA-positive endothelial cells, while few PCNA-positive endothelial cells were detected in control-vector-transfected myocardium. Consequently, the number of vessels around the HGF injection sites was increased (27). Such an HGF-mediated angiogenic activity was achieved via the activation of a transcription factor, ets, which is essential for angiogenesis (38). In the rat MI model, the endogenous HGF level was decreased, especially in the infarcted myocardium. Thus, transfer of HGF gene into infarcted myocardium can induce a beneficial response to compensate for the loss of endogenous HGF. The constant production of local HGF resulting from the transgene may be considered as an innovative angiogenic strategy for coronary artery disease (CAD) (14,27).

Anti-fibrotic Therapy with HGF Gene for Amelioration of Advanced MI

As mentioned above, HGF was shown to be effective in controlling the early phase of MI, but its usefulness for post-diagnostic therapy of MI was still unclear. To address this question, Li et al. examined the effect of HGF gene therapy after the onset of MI in mice. An adenoviral vector containing human HGF cDNA was injected into the limb muscles 3 days after heart ischemia, resulting in a persistent increase in plasma human HGF in treated mice (39). At 4 weeks after MI, exogenous HGF reduced ventricular fibrosis, accompanied by an increase in coronary vessels. The systemic HGF gene therapy inhibited the myocardial atrophy, especially near the infarcted areas, such as the papillary muscles and trabeculae (39). Overall, LV remodeling and dysfunction were improved in the HGF-treated mice compared with controls, as indicated by the smaller LV cavity, greater % of fractional shortening and LV+/-dP/dt. The usefulness of "delayed HGF-treatment" was also reported in other models of advanced MI (40,41). Strikingly, HGF supplement therapy was effective in improving chronic MI, even when the adenoviral vector-containing HGF gene was injected 3 weeks after the induction of MI in rats (40), or when intracardial injection of recombinant HGF was started 4 weeks post-MI in dogs (41).

There is now ample evidence demonstrating the efficacy of recombinant HGF (or HGF gene) during acute and chronic MI in animals. These findings hold promise for HGF as a medical drug for the treatment of MI. Induction of coronary blood flow by HGF gene therapy could be an angiogenic strategy to improve CAD (14,27). Furthermore, post-diagnostic treatment of chronic MI with recombinant HGF (or HGF gene) produces anti-fibrotic outcomes (39), and possibly, regenerative effects, as discussed later. Overall, HGF has been shown useful for the inhibition of acute MI (i.e., as a preventive drug) and for the improvement in heart dysfunction of chronic MI (as a regenerative drug) (13,14,42) (**Fig. 3**).

THE LOSS OF LOCAL HGF PRODUCTION AS A TRIGGER OF CORONARY RESTENOSIS

Coronary restenosis is a frequent adverse effect of medical angioplasty, especially after balloon-dilated catheter therapy (43). The migration and proliferation of VSMC, in response to a loss of endothelial monolayer continuity, play key pathological roles during vascular tissue remodeling (i.e., neointimal hyperplasia), as observed after balloon-dilated catheter therapy. Restenosis is also induced, even when a metal stent(s) is accurately placed in the narrowed lumen of a coronary vessel (44,45). As a result, impairment of coronary blood flow invites reoccurrence of myocardial apoptosis, infarction and LV dysfunction. In a middle stage of endothelial injury, HGF levels in vascular walls are decreased, and then neointimal formation becomes evident. It is, therefore, important to discuss the possible pathogenic role of local HGF-deficiency in the onset and progression of angioplasty-mediated coronary restenosis.

Roles of Endogenous HGF in Sustaining Local Homeostasis in Vessels

In arterial wall tissues, HGF is produced by both endothelium and VSMC, and such an autocrine-paracrine mechanism is required to sustain endothelial monolayer integrity via cell duplication (24,46). *In vitro*, the addition of anti-HGF IgG to co-culture of endothelial cells with VSMC led to a decrease in the number of endothelial cells⁴⁶. As stated before, HGF elicits angiogenesis through MEK-MAPK-dependent (and in part, PI3K-AKT-dependent) mitogenic and anti-apoptotic effects (25,26). Induction of Bcl-2, but not Bcl-xL, by HGF is also involved in the endothelial protection system (47). Moreover, HGF regulates endothelial cell motility via inducing nitric oxide (NO) (48). These regenerative and protective effects of HGF are required to maintain vascular structure and functions.

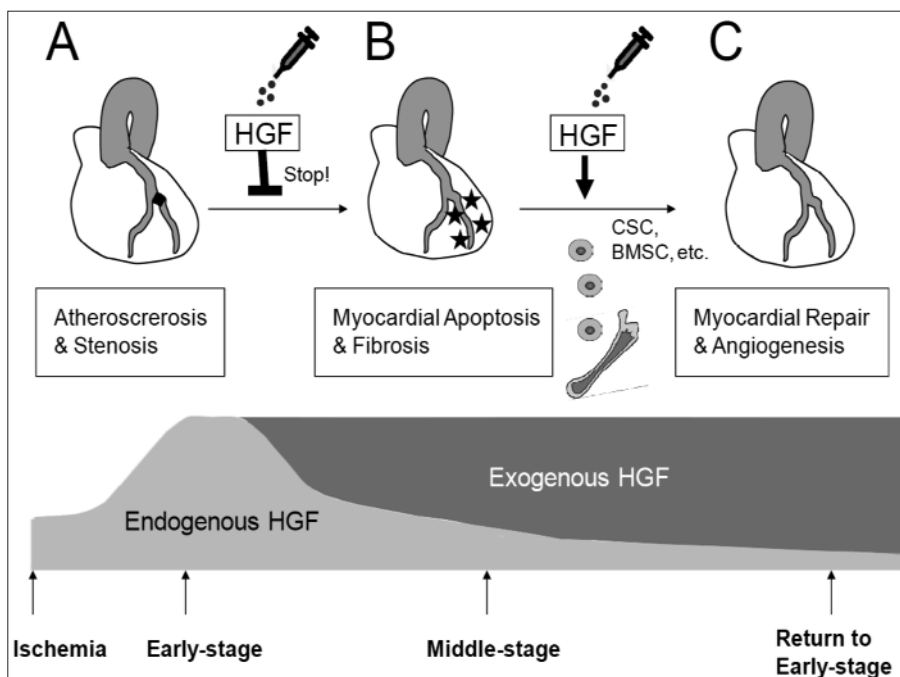


Figure 3. Pathogenesis-based strategy for treatment of acute and chronic MI. **(A)** MI is induced by coronary arterial obstruction, due to atherosclerosis or in-stent restenosis. In the early stage, endogenous HGF production is increased, but this defensive response is transient, followed by the progression of extensive injuries and dysfunction. Early administration of recombinant HGF (or HGF gene) inhibits myocardial apoptosis and fibrosis, accompanied by enhancement of collateral angiogenesis. **(B)** In the middle stage of MI, HGF supplement therapy may enhance the engraftment of BMSC, or of cardiac stemcells (CSC) in the infarcted areas, possibly via stimulating migration and trans-differentiation. **(C)** As a result, infarcted myocardium could be reconstructed after HGF supplement therapy.

It is likely that VSCM-derived HGF stimulates NO in endothelium via induction of NO synthase (48), and in turn, endothelium-derived NO elicits relaxation of VSMC in a healthy state. Such a cross-reaction between endothelium and VSMC functions as a counter-regulatory system to overcome the increase in systolic pressure (24,49). Of note, exogenous HGF can stimulate endogenous HGF production of endothelial cells in an auto-regulatory fashion (50). Under pathological situations, HGF exerts anti-thrombotic and anti-inflammatory effects on endothelial cells (51,52). Based on these data, we predict that vessel-derived HGF is important to sustain vascular homeostasis and regulate the tone of VSMC in normal conditions, and an impairment of the local HGF-c-Met system leads to pathological events related to restenosis.

Mechanisms for HGF Downregulation During Atherosclerosis and Restenosis

Angiotensin-II (Ang-II) is synthesized through a bio-reaction of angiotensin-converting enzymes (ACE) and is involved in stenosis-related pathogenesis as a local mediator through over-growth and constriction of VSMC. It is noteworthy to find that Ang-II suppressed HGF production in endothelial cells *in vitro* (46). *In vivo*, HGF levels were decreased in the carotid artery of rats after the balloon injury (53). Of interest, ACE-inhibitors restored the decrease in HGF production, leading to the attenuation of neointimal formation in rat models (46,53). Thus, from a viewpoint of pathogenesis, a loss of HGF production by up-regulated Ang-II may underlie the mechanisms whereby endothelial injury and neointimal formation are accelerated after balloon-dilated catheter therapy, or during *in-stent* placement therapy.

We further discuss the mechanisms for the loss of HGF production after the balloon injury. Transforming growth factor- β (TGF- β) is now recognized as a key ligand that elicits fibrotic lesions via stimulating extra-cellular matrix (ECM) proteins (54). TGF- β is up-regulated during neointimal formation in humans, possibly via mechanical stresses (55,56). TGF- β is also a potent suppressor of HGF transcription

in several types of cells, including endothelium (46,57). In other words, it is likely that TGF- β successfully accelerates neointimal hyperplasia and atherosclerosis via the down-regulation of HGF production.

It is noteworthy that endothelial loss and/or dysfunction after angioplasty provokes local hypoxia, and hypoxia also accelerates the endothelial damages (1,2). Such a pathological circuit allows for the accelerated progression of restenosis. Interestingly, hypoxic conditions suppress HGF production at a transcriptional level (37,58). In this process, hypoxia-induced up-regulation of TGF- β may be involved in the loss of HGF production. By contrast, hypoxia stimulates c-Met production via activation of a transcriptional factor, HIF-1 (30), despite the decrease in HGF. Such a reciprocal regulation of ligand and receptor by hypoxia prompted examination of whether HGF supplementation may inhibit the pathological circuit, as follows.

APPROACHES TO HGF SUPPLEMENT THERAPY FOR INHIBITION OF RESTENOSIS

Percutaneous transluminal coronary angioplasty (PTCA), such as balloon-dilated catheter therapy, greatly contributes to improvement in ischemic heart disease. However, restenosis, or reocclusion, after balloon injury remains a serious problem, since restenosis occurs in 30–50% of patients after PTCA (43). The loss of vascular HGF production is one of the key pathogenic causes of restenosis, while HGF has a therapeutic potential for the treatment of restenosis after PTCA, or during *in-stent* therapy in laboratory animals, and possibly in human patients.

Inhibition of Neointimal Formation by HGF Supplement Therapy in Animals

In 2000, two groups independently provided evidence that the administration of HGF led to prevention of neointimal formation, using animal models of balloon injury. Yasuda et al. found that local delivery of recombinant HGF had the potential advantages of allowing a sufficient concentration of HGF, which could be accumulated at the site of injury without systemic adverse effects (59). When recombinant HGF was administered into the balloon-injured iliac artery of rabbits using a drug delivery catheter, vascular endothelial cell regeneration was rapidly induced in the injured arteries, followed by the inhibition of neointimal formation (59). Such a local delivery may have the therapeutic advantage of greater tissue concentrations with a reduced likelihood of systemic effects. In the same year, Hayashi et al. reported similar findings after the use of HGF gene in a rat model of neointima: HGF cDNA-containing HVJ-liposome was injected into the carotid artery immediately after the balloon injury (60). As a result, neointimal hyperplasia was suppressed in the HGF-supplemented rats, accompanied by the enhancement of re-endothelialization (60). A recent report supported the usefulness of HGF *in vivo*. Song et al. demonstrated that intravenous injection of HGF cDNA-transfected endothelial progenitor cells led to reduced neointima and enhanced re-endothelialization in hypercholesterolemic rats (61). These results indicate that a rapid restoration of endothelial damage by HGF supplementation is a reasonable strategy to suppress neointimal formation and re-stenosis after angioplasty.

Molecular Mechanisms of HGF for Reduction of Restenosis

The molecular mechanisms of HGF supplement therapy against restenosis should be discussed. While HGF elicits a rapid re-sheet of the defective endothelial continuity, VSMC in the neointima also expresses c-Met (62), suggesting a direct effect of HGF on VSMC-based pathogenic events. Platelet-derived growth factor (PDGF) is a potent mitogen for stromal cells including VSMC (1,2), while HGF inhibits the PDGF-mediated over-growth of stroma cells (63,64) via the suppression of ERK1/2-phosphorylation. HGF also inhibits Ang-II-induced proliferation of VSMC *in vitro* (65). These anti-mitogenic effects of HGF on activated VSMC may explain the therapeutic outcome after PTCA (59-61), or during pulmonary hypertension (64). Nitric oxide (NO) is important to attenuate intimal formation via relaxation of VSMC, while HGF increases endothelial NO levels during arterial stenosis in rats (60). Moreover, HGF inhibits production of endothelin-1 (ET1), a constrictor of VSMC, in vascular tissues (64). Thus, up-regulation of NO, and/or down-regulation of ET1, by HGF may relax VSMC, making restenosis avoidable during HGF supplement therapy.

Given that the loss of endothelial cells triggers restenosis, other angiogenic growth factors, such as basic fibroblast growth factor (b-FGF) and vascular endothelial growth factor (VEGF) may be applicable for

inhibiting restenosis after PTCA or stent therapy. However, b-FGF is known to stimulate the proliferation of VSMC, and this effect accelerates intimal formation (66). VEGF often elicits inflammatory events and capillary edema in endothelial cells, while HGF inhibits VEGF-mediated inflammation (67,68).

HGF elicits endothelial morphogenesis without edematous changes or VSMC over-growth (14,60). Thus, HGF, rather than VEGF or b-FGF, seems advantageous for clinical use for the inhibition of coronary stenosis.

Enhancement of HGF Production as a Strategy to Prevent Restenosis

Since the loss of HGF production contributes to atherosclerosis, restoration of lowered HGF production via the stimulation of HGF transcription pathways, or via suppression of an HGF-suppressor(s), should be considered. Indeed, several commercially available drugs, used for the treatment of cardiovascular disease, serve to enhance HGF production as follows.

As an example, cilostazol, an anti-platelet agent, inhibits neonatal hyperplasia in diabetic rats after balloon-based carotid injury, accompanied by the increased production of HGF (69). Cilostazol is an inhibitor of type-3 phosphodiesterase, which leads to cyclic AMP (cAMP) accumulation. Notably, the promoter region of HGF gene contains a cAMP-responsive element, and the increased cAMP activates the HGF transcription (69). Indeed, cilostazol enhanced the proliferation of endothelial cells in a co-culture with VSMC, while this effect was blocked by anti-HGF antibody⁶⁹. Of interest, cilostazol-mediated up-regulation of HGF was diminished by Rp-cAMP, an inhibitor of cAMP-dependent protein kinase (69). Therefore, cAMP-dependent up-regulation of HGF may explain the successful effect of cilostazol in a clinical trial (70).

PPAR- γ agonist is now widely used for metabolic diseases and is useful for the attenuation of neointimal formation (71,72). Interestingly, a PPAR- γ agonist increased HGF mRNA levels in a culture of mesangial cells, and this effect was due to a direct binding of the PPAR γ agonist to the PPAR-response element in the promoter region of HGF gene (73). Of note, a PPAR- γ agonist blocked TGF- β -mediated mesangial fibrotic events in vitro, and this effect was abolished by a specific deletion of c-Met through the LoxP-Cre system (73), thus indicating a cascade of the PPAR- γ -HGF-c-Met system in attenuating glomerulosclerosis, an analogue of atherosclerosis.

As mentioned above, Ang-II-blockers decrease the local production of TGF- β 1 and increase the HGF expression levels (14,46). Of note, ACE-inhibitors (such as perindopril and cilazapril) suppressed neointimal changes in the carotid artery-injured rats, accompanied by an increase in HGF production (46,53). At the clinical bedside, ACE-inhibitors and Ang-II receptor antagonists are widely used to reduce restenosis after PTCA. A strategy to block the production, or function, of Ang-II is a practical approach to restore a lowered level of HGF, resulting in the suppressed neointima and reduced stenosis.

In summary, neointimal formation is accelerated, at least in part, by a decrease in local HGF levels, and this is associated with reciprocal increases in Ang-II and TGF- β , or hypoxia. Such an HGF-deficient condition leads to further endothelial damage, VSMC over-growth and restenosis. Low-density lipoprotein (LDL) is known to accelerate restenosis¹, while HGF suppresses the production of LDL in hepatocytes (74). In addition, HGF attenuates LDL-induced endothelial cell injury (75). Thus, HGF supplement therapy (or stimulation of HGF production) is reasonable for the inhibition of pathological circuits after PTCA, or during stent placement (**Fig. 4**).

THERAPEUTIC VALUE OF HGF FOR THE RESCUE OF OTHER CARDIAC DISEASES

As shown in **Fig. 1**, HGF has pleiotropic effects on MyoFB, immune cells and bone marrow-derived stem cells (BMSC), in addition to cardiomyocytes and vascular cells.

Through its direct action toward these cells, HGF inhibits fibrosis and immunological challenge during cardiomyopathy and autoimmune myocarditis, respectively. The possible involvement of BMSC by HGF in myocardial reconstitution will be also discussed.

Use of HGF to Improve Heart Fibrosis and Dysfunction During Cardiomyopathy

Structural remodeling of the myocardium, including myocyte hypertrophy, fibrosis, and dilatation,

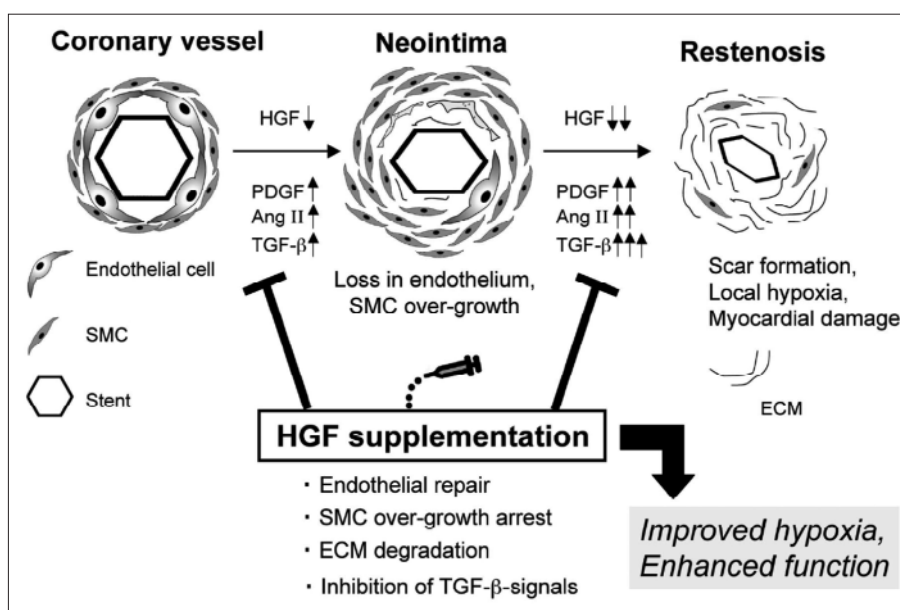


Figure 4. HGF-mediated anti-atherosclerotic effects after in-stent therapy or PTCA. During metal stent placement, vascular endothelial cells are injured by mechanical stress, and local HGF production is impaired by the reciprocal increases in Ang-II and TGF- β , or by local hypoxia. Under the HGF-deficient conditions, VSMC begin to proliferate in response to Ang-II and PDGF, a potent mitogen of stromal cells, resulting in neointimal formation. Furthermore, ECM production is accelerated by TGF- β . These pathological events elicit coronary restenosis and resclerosis, and then myocardial infarction becomes more severe, in accordance with the decrease in HGF production. In this pathological process, HGF supplement therapy suppresses coronary neointimal formation by inhibiting Ang-II- or PDGF-induced over-growth of VSMC at an early stage. Furthermore, exogenous HGF is useful, at an advanced stage, for the attenuation of vascular scarring by inhibiting TGF- β production, or by inducing ECM degradation and MyoFB cell death.

drives functional loss in dilated cardiomyopathy (DCM). Using the TO-2 hamster strain as an animal model of DCM, we examined the possible involvement of HGF in the pathophysiology related to DCM (18). TO-2 hamsters showed severe cardiac dysfunction and fibrosis in the late stage, accompanied by an increase in myocardial TGF- β 1. Interestingly, cardiac HGF levels were lowered in the late-stage heart, suggesting decreased HGF as a risk factor. When recombinant HGF was administered into 27-week-old TO-2 hamsters (i.e., advanced stage) for 3 weeks, cardiac fibrosis was suppressed, accompanied by decreases in TGF- β 1 and type-I collagen (18). In a culture of cardiac MyoFB, HGF inhibited the Ang-II-mediated up-regulations of TGF- β 1 (18). Thus, inhibition of TGF- β production in MyoFB by HGF may be the key mechanisms by which HGF reduces fibrosis during DCM (18). Myocardial apoptosis and remodeling were also suppressed in the hamster by HGF treatment. As a result, the loss of cardiac contractility was improved by HGF treatment (18). Furthermore, local HGF gene therapy enhanced the coronary angiogenesis and improved cardiac fibrosis in the hamster model (76). Overall, these direct actions (i.e., anti-fibrotic effect on MyoFB, anti-apoptotic effect on cardiomyocytes and angiogenic effects on endothelium) participate in HGF-mediated therapeutic outcomes, even in an advanced stage of DCM (Fig. 5).

Anti-immunogenic Effects of HGF on Heart Transplantation and Autoimmune Carditis

Immunosuppressive agents (such as tacrolimus) contribute to overcoming acute rejection and improving the mid-term survival of transplanted hearts. However, the long-term results are still not satisfactory, and cardiac allograft vasculopathy remains the main cause of primary graft loss. Using a mouse model of cardiac allograft, Yamaura et al. found that the inhibition of ischemic injury by the transient use of recombinant HGF resulted in the long-term acceptance of heart allografts, even after withdrawal of tacrolimus (77). During the cardiac allograft, HGF lowered TH1-cytokines, such as IFN- γ , which trigger graft rejections, while the local levels of TH2-cytokines (such as IL-4 and IL-10) were increased by HGF. Such a reciprocal regulation of

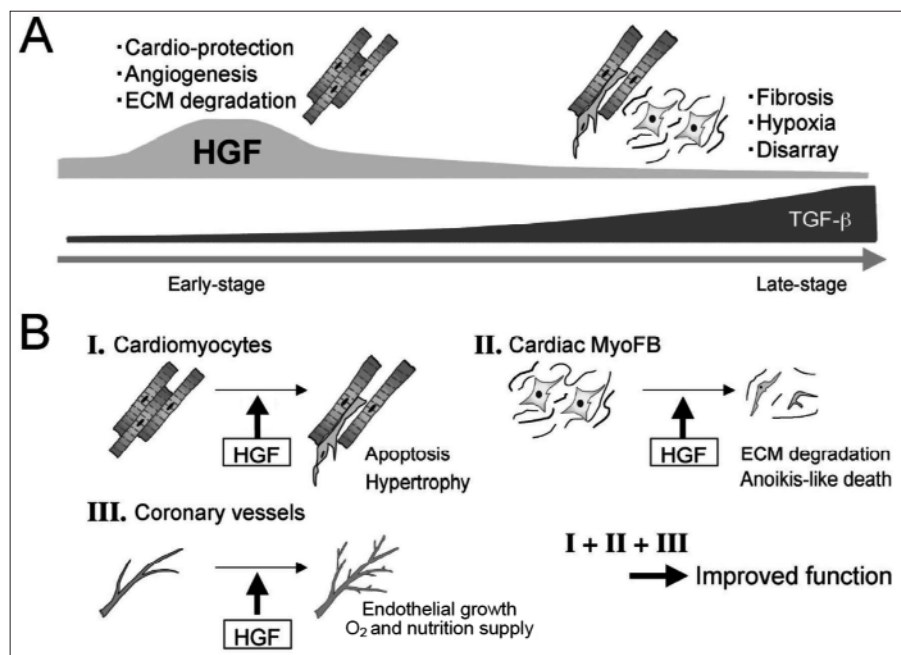


Figure 5. A rationale for HGF supplementation for the treatment of DCM. **(A)** In the early stage of DCM, HGF production is transiently enhanced, in response to fibrotic injury, for inducing ECM degradation and angiogenesis (18). However, HGF production levels are decreased below a basal level, resulting in the manifestation of cardiac fibrosis and remodeling in the late-stage of DCM (18). **(B)** Based on the pathogenic rationale, recombinant HGF injection, or HGF gene therapy, is recommended (18,76). (I) Exogenous HGF directly targets myocytes to inhibit apoptosis and hypertrophy. (II) HGF enhances MMP productions in MyoFB, leading to ECM degradation and anoikis-like cell death. (III) Furthermore, HGF enhances cardiac angiogenesis during DCM. All of these biological effects would contribute to HGF-induced therapeutic outcomes under pathological conditions caused by DCM.

TH1- and Th2-cytokines could lead to HGF-induced immune tolerance. HGF was later shown to directly target dendritic cells (DC) and increase TH2-cytokines in the primary culture system of DC (78). A recent report delineated the potential role of HGF in the induction of regulatory T cells, a key player for the induction of immune tolerance (79).

HGF also has an anti-immune effect on T-lymphocytes under pathological conditions. Using myosin-immunized rats as a model of autoimmune myocarditis (AM), Futamatsu et al. found that HGF targeted activated CD4⁺ cells, and this direct effect of HGF on T-lymphocytes resulted in the improvement of cardiac dysfunction (80). When CD4⁺ cells, isolated from the spleens of AM-manifested rats, were stimulated *in vitro* by the addition of myosin, the CD4⁺ cells began to proliferate, accompanied by an increase in IFN- γ . In the culture, recombinant HGF decreased the IFN- γ levels and attenuated the myosin-induced T-cell proliferation (80). By contrast, HGF increased the levels of IL-4 and IL-10. Such a reciprocal regulation by HGF in activated T-cells led to the attenuation of heart dysfunction during AM in the rats (80).

Possible Involvement of HGF in Heart Repair via Recruitment of Stem Cells

Since the involvement of bone marrow-derived stem cells (BMSC) in infarcted hearts was demonstrated, there is now emerging evidence to show the contributions of BMSC and cardiac stem cells to heart reconstitution (81). Several lines of studies demonstrated the key role of HGF in stem cell biology. *In vitro*, HGF is able to enhance proliferation, migration and trans-differentiation of BMSC (82), or of CSC (83). Recently, it was found that prolonged treatment of MSC, or of embryonic stem cells, with recombinant HGF induced the expression of cardiac-specific markers (Nkx2.5, GATA-4, MEF2C, TEF1, desmin, α -MHC, β -MHC, nestin, etc.) with the concomitant loss of stem cell markers (82,84). The effect of HGF on Nkx2.5 induction was diminished by the addition of LY294002, an inhibitor of PI3-kinase (84), indicating

the involvement of HGF-PI3K pathways in cardiac differentiation. *In vivo*, the local injection of HGF cDNA-transfected MSC ameliorated the functions of impaired myocardium, by diminishing the area of ischemia, increasing the number of capillaries, and reducing collagen contents (85,86). A similar result was also obtained when adipose-derived stem cells, transfected with HGF cDNA, were injected into the ischemic myocardium in a rat model of MI (87). Thus, injection of stem cells, combined with HGF gene transfer, may be a new strategy for the potential regenerations of cardiac muscle and coronary vessels.

SUMMARY AND FUTURE PERSPECTIVE

During cardiac injury, the HGF-c-Met axis elicits various intracellular signals required for anti-apoptosis, angiogenesis, anti-stenosis, anti-fibrosis, and possibly, myocardial regeneration. Multifaceted activities of HGF are mediated through a conversion from c-Met serine-985 phosphorylation (i.e., switch off) to c-Met tyrosine phosphorylation (i.e., switch on)(10). There is now ample evidence to show the therapeutic potential of HGF in cardiac diseases, including MI, in-stent restenosis, DCM, heart transplantation, and AM (Table 1).

Table 1. Therapeutic potentials of HGF (or HGF cDNA) in acute and chronic cardiovascular diseases

Pathogenic Model (treatment)	HGF therapy	Outcomes	References
I. Myocardial infarction			
Acute MI (Coronary I/R, Rat)	rh-HGF, iv	Bcl-x1 induction, Anti-apoptosis, Improved LV function	Nakamura-T <i>et al.</i> ¹³
Acute MI (Coronary I/R, Rat)	HVJ-HGF cDNA, Intra-myocardium	Increased angiogenesis, Improved hypoxia	Aoko-M <i>et al.</i> ²⁷
Chronic MI (Coronary I/R, Rat)	Adeno-HGF cDNA, im (hind-limb)	Reduced fibrosis, Enhanced angiogenesis, Improved LV function	Li-Y <i>et al.</i> ³⁹
Chronic MI (Coronary ligation, Dog)	rh-HGF,	Enhanced angiogenesis,	Yamaguchi-T <i>et al.</i> ⁴¹
Chronic MI (Coronary I/R, Rat)	Intra-myocardium HGF cDNA in MSC, Intra-myocardium	Improved LV function Increased angiogenesis, Anti-fibrosis, Improved LV function	Duan-HF <i>et al.</i> ⁸⁵
Chronic MI (Coronary I/R, Rat)	rh-HGF hydrogel, Intra-myocardium (DDS)	Increased angiogenesis, Anti-apoptosis, Anti-fibrosis, Improved LV function	Tambara-K <i>et al.</i> ⁸⁸
Chronic MI (Coronary I/R, Rat)	Myoblasts+HVJ-HGF, Intra-myocardium	Improved LV function, Increased angiogenesis, Anti-fibrosis, Myocardial regeneration	Miyagawa-S <i>et al.</i> ⁹⁰
Chronic MI (Human patients)	Adeno-HGF, Intra-myocardium (bypass surgery)	Increased blood flow, Improved hypoxia	Yuan-B <i>et al.</i> ⁹⁷
II. Arterial injury and stenosis			
Neointimal formation (Iliac artery, Ballon Injury, Rabbit)	rh-HGF, Local injection	Re-endothelialization, Reduced neointima	Yasuda-S <i>et al.</i> ⁵⁹
Neointimal formation (Carotid artery, Ballon Injury, Rat)	HVJ-HGF, Local injection	Re-endothelialization, Reduced neointima Increase in NO production	Hayashi-K <i>et al.</i> ⁶⁰
Neointimal formation (Carotid artery, Ballon Injury, Rat)	HGF cDNA in EPC, iv	Re-endothelialization, Reduced neointima	Song-MB <i>et al.</i> ⁶¹
III. Cardiomyopathy			
DCM (Mutant, TO-2 Hamster)	rh-HGF, sc	Anti-apoptosis, TGF- β down-regulation, Reduced fibrosis, Improved LV function	Nakamura-T <i>et al.</i> ¹⁸
DCM (Mutant, TO-2 Hamster)	HVJ-HGF, Intra-myocardium	Angiogenesis, Anti-fibrosis, Reduced hypoxia	Taniyama-Y <i>et al.</i> ⁷⁶
IV. Immunogenic heart diseases			
Cardiac allograft transplantation (B10-to-Balb/c mouse)	rh-HGF, sc (+tacrolimus)	Anti-apoptosis, Anti-fibrosis, Reduced vascular lesions, Increased TH2-cytokines, Immune tolerance	Yamaura-K <i>et al.</i> ⁷⁷
Autoimmune myocarditis (Myosin immunization, Mouse)	HVJ-HGF, Intra-myocardium	T-cell suppression, Increased TH2-cytokines, Anti-apoptosis,	Futamatus-H <i>et al.</i> ⁸⁰

Abbreviations: I/R, ischemia and reperfusion of coronary artery; rh-HGF, recombinant human HGF; LV, left ventricular; HVJ-HGF, HVJ vector-carrying HGF cDNA plasmid; Adeno-HGF, adenoviral vector containing HGF cDNA plasmid; DDS, drug delivery system; EPC, endothelial progenitor cells. See text for other abbreviations.

Recombinant HGF is prepared from stable lines of CHO cells in an active form, and this is useful for the acute phase of MI, because apoptotic events occur within 2 days after coronary artery obstruction (13). The transient use of recombinant HGF (i.e., for approximately 2 weeks) is also practical for inducing immune tolerance after allograft transplantation (77). By contrast, HGF gene therapy may be practical for chronic heart disease, including chronic MI (39) and DCM (76), due to the long-term expression of HGF. Matrix-anchored HGF, such as gelatin hydrogel-bound HGF (88), and HGF-fibronectin chimera that includes a collagen-binding domain (89), are also available as slow-release agents, especially during chronic heart disease. In animal models of MI and DCM, combination therapy with HGF and myoblastic cell transplantation was useful for myocardial reconstruction, which led to the attenuation of LV dysfunction (90,91). In vivo, recombinant HGF injection enhanced the migration, proliferation and differentiation of cardiac stem cells (92), and further studies will elucidate the detailed mechanisms. Co-administration of commercially available drugs, such as ACE-inhibitors and cilostazol (53,69), with recombinant HGF may boost the therapeutic effects.

Based on the known effects of HGF in animal models, clinical studies have been performed to evaluate the safety and effectiveness of HGF as a drug to treat various diseases (93-96). Indeed, a local transfer of HGF gene via an adenoviral vector was performed during the bypass surgery on patients with chronic MI (97-99). Notably, HGF gene therapy improved the cardiac blood flow in patients, without side effects (97), encouraging larger and randomized efficacy trials. We believe that supplemental therapy with HGF would contribute to the reduction of heart disease, and further efforts would shed more light on this conceptual therapy.

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