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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1528160> since 2015-11-12T12:19:47Z

Published version:

DOI:10.1042/CS20150502

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CELLULAR AND MOLECULAR MECHANISMS OF HGF/MET IN THE CARDIOVASCULAR SYSTEM

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Abstract

Met tyrosine kinase receptor, also known as c-Met, is the Hepatocyte Growth Factor (HGF) receptor. The HGF/Met pathway has a prominent role in cardiovascular remodelling after tissue injury. The present review provides a synopsis of the cellular and molecular mechanisms underlying the effects of HGF/Met in the heart and blood vessels. In vivo, HGF/Met function is particularly important for the protection of the heart in response to both acute and chronic insults, including ischaemic injury and doxorubicin-induced cardiotoxicity. Accordingly, conditional deletion of Met in cardiomyocytes results in impaired organ defence against oxidative stress. After ischaemic injury, activation of Met provides strong anti-apoptotic stimuli for cardiomyocytes through PI3K (phosphatidylinositol 3-kinase)-Akt and MAPK (mitogen-activated protein kinase) cascades. Recently, we found that HGF/Met is also important for autophagy regulation in cardiomyocytes via mTOR (mammalian target of rapamycin) pathway. HGF/Met induces proliferation and migration of endothelial cells through Rac1 (RAS-related C3 botulinum toxin substrate 1) activation. In fibroblasts, HGF/Met antagonizes the actions of TGF- β 1 (transforming growth factor- β 1) and ANG (angiotensin) II, thus preventing fibrosis. Moreover, HGF/Met influences the inflammatory response of macrophages and the immune response of dendritic cells, indicating its protective function against atherosclerotic and autoimmune diseases. HGF/Met axis also plays an important role in regulating self-renewal and myocardial regeneration through the enhancement of cardiac progenitor cells. HGF/Met has beneficial effects against myocardial infarction and endothelial dysfunction: the cellular and molecular mechanisms underlying repair function in the heart and blood vessels are common and include proangiogenic, anti-inflammatory and anti-fibrotic actions. Thus, administration of HGF or HGF-mimetics may represent a promising therapeutic agent for the treatment of both coronary and peripheral artery disease.

Short Title

HGF/Met in the Cardiovascular System

Keywords

HGF/Met, Cardioprotection, Angiogenesis, Fibrosis, Immunomodulation, Regeneration

Abbreviations List:

ACE, angiotensin-converting enzyme; Ad, adenovirus; Ang, angiopoietin; ANG, Angiotensin; AP1, activator protein 1; APCs, antigen-presenting cells; Bad, Bcl-2-associated death promoter; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; BM, bone marrow; Bnip3, BCL2/Adenovirus E1B 19kDa Interacting Protein 3; BNP, brain natriuretic peptide; Cbl, casitas b-lineage lymphoma ubiquitin ligase; CBP, CREB binding protein; CPCs, cardiac progenitor cells; CREB, cAMP response element-binding protein; CVD, cardiovascular disease; DCs, dendritic cells; EB1, End binding 1; EMT, epithelial to mesenchymal transformation; EPCs, endothelial progenitor cells; ERK1,2, Extracellular signal-regulated kinase 1,2; Gab1, Grb2-associated binding protein 1; GATA-4, GATA binding protein 4; Grb2, growth factor receptor-bound protein 2; GSK3 β , glycogen synthase kinase-3 β ; HF, heart failure; HGFA, HGF activator; HGF, hepatocyte growth factor; IFN- γ , interferon gamma; IGF1, insulin-like growth factor 1; IgG, Immunoglobulin G; IL, interleukin; iNOS, inducible nitric oxide synthase; IQGAP1, IQ motif containing GTPase activating protein 1; JNK, c-Jun N-terminal protein kinases; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MI, myocardial infarction; MCP-1, monocyte/macrophage chemotactic protein-1; MSCs, mesenchymal stem cells; mTOR, mammalian target of rapamycin; NF- κ B, nuclear factor κ B; NO, nitric oxide; Notch, neurogenic locus notch homolog protein; Nrf2, nuclear factor-erythroid 2-related factor 2; P38MAPK, p38 mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase-C; Rac1, RAS-related C3 botulinum toxin substrate 1; RAS, rat sarcoma; ROS, reactive oxygen species; RTK, tyrosine kinase receptor; SDF-1, stromal cell-derived factor 1; Shp2, SH2 domain-containing

protein tyrosine phosphatase; SHR, spontaneously hypertensive rats; Smad, Sma and Mad related proteins; SMCs, smooth muscle cells; SOS, Son of Sevenless; Sp1, specificity protein 1; TEMs, Tie2 expressing monocytes; TGF- β 1, Transforming growth factor-beta1; Th, helper T cells; TLR, toll-like receptor; TNF- α , tumor necrosis factor-alpha; Tregs, regulatory T cells; VEGF-A, vascular endothelial growth factor A; VEGFR-2, vascular endothelial growth factor receptor 2.

INTRODUCTION

Growth Factors and their Tyrosine Kinase Receptors are becoming important players in the regulation of cellular processes, functions and tissue homeostasis of the cardiovascular system. Among these are the Hepatocyte growth factor (HGF) and its Met receptor, which exert beneficial effects in the setting of cardiovascular injuries. In this review, we will focus on cellular and molecular mechanisms of HGF/Met in the various cells of the cardiovascular system. Cardiomyocytes and endothelial cells are the primary targets of HGF favourable effects. However, also cardiac fibroblasts, leucocytes and dendritic cells have been proposed as potential cell targets, pointing out the anti-fibrotic, anti-inflammatory and immune modulatory properties of HGF.

The HGF/Met pair

The HGF cytokine was originally identified as a potent mitogen for hepatocytes [1] and as a “Scatter Factor” for epithelial cells [2]. *HGF* gene is located on chromosome 7q21.1 and encodes for a protein secreted by cells of mesenchymal origin as a pro-HGF, single-chain inactive precursor, which is bound to heparin-like proteoglycans within the extracellular matrix of most tissues. Pro-HGF is cleaved into the mature $\alpha\beta$ heterodimer (Figure 1) by different extracellular proteases, including HGF activator (HGFA), urokinase-type plasminogen activator, factors XII and XI, matriptase, and hepsin. The receptor for HGF is the tyrosine kinase (RTK) encoded by the *MET* proto-oncogene located on chromosome 7q21-31[3, 4]. The Met receptor is a $\alpha\beta$ - heterodimer, which is expressed at the cell surface of epithelial and endothelial cells (Figure 1). HGF is a pleiotropic factor, which stimulates multiple biological processes, such as proliferation, survival, motility, differentiation and morphogenesis, during embryogenesis, organ regeneration and tumour invasiveness [5, 6]. There is a growing body of evidence indicating that these Met-mediated activities also lead to cardioprotective effects [7, 8].

Major HGF/Met signalling pathways

The multiple effects mediated by the activation of HGF/Met axis on different cell types involve various and complex molecular events. Upon HGF binding, Met undergoes homodimerization and autophosphorylation on Tyr1234 and Tyr1235, which start the intrinsic kinase activity of the receptor. Subsequently, Tyr1349 and Tyr1356 in the C-terminal tail become phosphorylated and form a multisubstrate docking site for intracellular adaptor proteins which recruit the signal transmitter molecules (Figure 1) [5, 6].

Gab1 (Grb2-associated binding protein 1) is the most crucial downstream multi-adaptor protein of Met receptor; it can bind Met directly or indirectly through Grb2 (growth factor receptor-bound protein 2). Once phosphorylated, Gab1 creates extra binding sites for the majority of downstream signalling molecules. This produces the activation of signalling pathways, including PI3K-Akt (phosphatidylinositol 3-kinase-Akt), RAS (rat sarcoma), the MAPK (mitogen-activated protein kinase) cascades, and Rac1 (RAS-related C3 botulinum toxin substrate 1). Activation of PI3K-Akt pathway, which is mediated by direct interaction of p85 subunit with Met, and indirectly by Gab1, has a cell survival role. Moreover, Akt activates mammalian target of rapamycin (mTOR), which stimulates protein synthesis and cell growth. RAS, instead, is activated by Met through binding of guanine nucleotide exchanger Son of Sevenless (SOS) with Shc and Grb2. SH2 domain-containing protein tyrosine phosphatase (Shp2), bound through Grb2 or Gab1, positively reinforces RAS signalling.

RAS stimulation leads to activation of different MAPK cascades: ERK (Extracellular signal-regulated kinase), P38MAPK (p38 mitogen-activated protein kinase) and JNK (c-Jun N-terminal protein kinases) [9]. In addition, Rac1 induction produces P38MAPK and JNK stimulation, resulting in cell migration [6]. The functions played by the different MAPKs subfamilies are quite different: ERK is mainly involved in proliferation, while P38MAPK is implicated in migration and cell survival. Studies using animal models with loss or gain of function of Met signalling pathway

components indicated their involvement in the regulation of embryonic cardiovascular development and in the control of vital cellular processes necessary for normal postnatal growth and maintenance of cardiac function [10-12]. These signalling must be fine-tuned in space and time for correct execution of biological outputs in the cardiovascular system.

In normal tissue, the HGF/Met axis is tightly controlled through a number of mechanisms. Negative regulation of Met receptor kinase activity can be induced through phosphorylation of Ser985 by protein kinase-C (PKC) [13] and through monoubiquitylation, internalization, and degradation of Met by Cbl (casitas b-lineage lymphoma ubiquitin ligase), after recruitment on the phosphorylated Tyr1003 [14]. A variety of protein tyrosine phosphatases can negatively regulate the Met docking site through dephosphorylation of its tyrosine residues [6]. Finally, the activating machinery of HGF ligand is tightly controlled to limit the production of active HGF: for example, two protease inhibitors of HGFA have been identified [15].

HGF PROTECTS CARDIOMYOCYTES AGAINST APOPTOSIS, EXCESSIVE AUTOPHAGY AND OXIDATIVE STRESS

In the heart, a variety of cell types of mesenchymal origin, including cardiomyocytes, produce HGF, which acts in an autocrine/paracrine manner on cells that express Met receptor [16-18].

In different types of cardiac injury, such as ischaemia, chemotherapy cardiotoxicity, cardiomyopathy and heart failure (HF), the main mechanisms of cell damage and death are apoptosis, excessive autophagy and oxidative stress. HGF counteracts these detrimental processes through prosurvival and anti-oxidant activities, thus emerging as a powerful cardioprotective agent.

Apoptosis

After myocardial infarction (MI), HGF levels increase steeply in blood circulation [19] and both HGF and Met receptor expression are enhanced in the heart [20]. HGF protects cardiomyocytes from apoptosis, especially the one caused by ischaemia/reperfusion (reviewed [7]). Cardiomyocytes express Met receptor and respond to HGF by activating PI3K-Akt, P38MAPK and ERK1,2 signalling [21, 22]. All these pathways positively control Met-dependent prosurvival activity and underlie cardioprotective effects. In the context of MI, HGF inhibits Bad (Bcl-2-associated death promoter), a proapoptotic factor, through PI3K-Akt axis [23] and enhances the anti-apoptotic Bcl-xL (B-cell lymphoma-extra large) expression via ERK-dependent phosphorylation of GATA-4 (GATA binding protein 4) [24]. Recently, the presence of a positive signalling feedback loop involving Met and Notch (neurogenic locus notch homolog protein) has been unveiled by analysing the genome response activated by HGF in cardiomyocytes during physiological [18] and pathological [25] conditions. HGF enhances Notch1 and Akt activation in mouse myocardium, thus enforcing Met prosurvival biochemical output (Figure 2). So, during heart injury, HGF receptor recapitulates the activation of molecular and cellular processes which have been implicated in cardiomyogenesis during development [26].

Autophagy

Excessive autophagy produced through generation of reactive oxygen species (ROS) causes uncontrolled 'self-cannibalism' in cardiomyocytes and may contribute to cell death. A cross-talk exists between apoptotic and autophagic pathways: a decrease in Bcl-2 (B-cell lymphoma 2) is accompanied by the concomitant increase in Bnip3 (BCL2/Adenovirus E1B 19kDa Interacting Protein 3) and Beclin-1, these latter being known triggers of autophagy [22]. In fact, Beclin-1 is inhibited by Bcl-2 [27] and, in turn, Bcl-2 is associated with and inhibited by Bnip3 [28]. HGF and Met agonist antibodies protect cardiac cells against hypoxic injury not only via activation of antiapoptotic pathways, but also by inhibiting autophagy. Mechanistically, mTOR is the crucial

protective pathway, downstream to Met, acting against hypoxia-induced autophagic response [22] (Figure 2).

Oxidative stress

Exacerbated ROS production leads to mitochondrial damage, cardiomyocyte apoptosis and impaired cardiac function. Chemotherapy, as exemplified by doxorubicin, causes oxidative stress in cardiomyocytes and generates an important side effect in the heart. Stimulation with HGF is able to counteract doxorubicin-induced apoptosis in cardiomyocytes. The mechanism through which HGF protects from doxorubicin likely involves PI3K-Akt signalling and a novel mechanism of Bcl-xL. However, in cardiomyocytes, cardioprotection against doxorubicin has not been fully investigated. Cardiomyocyte-specific deletion of Met receptor using the Cre- α -MHC mouse line has indicated that Met protects cardiomyocytes against age-related oxidative stress [29]. In fact, α -MHC Met-KO mice show cardiac systolic dysfunction concomitant with reduced expression and activity of catalase and superoxide dismutase, with consequent reactive oxygen species accumulation. The Met-dependent increase of anti-oxidant enzymes is mediated by P38MAPK. In line with this work, it has been shown that loss of Met receptor in hepatocytes creates a pro-oxidant state, which is compensated by constitutive activation of key transcription factors protecting against oxidative and xenobiotic stress, including NF- κ B (nuclear factor κ B) and Nrf2 (nuclear factor-erythroid 2-related factor 2) [30, 31] (Figure 2). This novel function of Met in the antioxidant defence might be exploited to mitigate stress in several cardiac pathological conditions, such as doxorubicin-mediated cardiotoxicity, diabetic cardiomyopathy and HF. On the other side, it must be considered that the heart may be vulnerable to the inhibition of the Met pathway. Met-targeted therapies, which include HGF neutralizing antibodies, Met down-regulating antibodies and Met tyrosine kinase inhibitors, are currently exploited as a novel powerful strategy to treat tumours [32]. When tested by short-term treatments of cellular and pre-clinical models, Met inhibitors, such as Crizotinib or PF-04254644, induce cardiomyocytes death through molecular mechanisms involving ROS production, activation of caspases, alteration of cell metabolism and blockage of ion channels [33, 34]. Given the critical role played by HGF/Met couple in myocardial protection, consideration must be given to the possible cardiac side effects of Met-targeted cancer therapy.

HGF IS A POTENT PROANGIOGENIC FACTOR WHICH ACTS ON ENDOTHELIAL AND SMOOTH MUSCLE CELLS

The formation of new blood vessels from the existing vascular bed is a prime requirement in repair processes of peripheral, coronary and carotid artery diseases. Hence, encouraging therapeutic angiogenesis is an intriguing prospect for modern medicine. Among other growth factors, HGF is a well recognized proangiogenic factor capable to stimulate the formation of new vessels from the pre-existing vascular bed, and increase blood flow [35, 36]. This beneficial function is probably attained through multiple pathways, achieved either by direct or indirect action on endothelial and smooth muscle cells.

Ding et al. [37] reported that Met expression is upregulated in angiogenic growth conditions and remains high during the first steps of endothelial cell migration and proliferation. HGF is also a potent anti-apoptotic factor in endothelial cells [38-40] and can indirectly stimulate angiogenesis by inducing the release of VEGF-A (vascular endothelial growth factor A) from vascular smooth muscle cells (SMCs) [41] (Figure 3). VEGF and HGF cooperate in the induction of proliferation and migration of endothelial cells [41]. Consistently, most of Met downstream signalling pathways are common to VEGF-A signalling [42]. Despite the similarity between HGF-induced and VEGF-A-induced angiogenic responses, only a subset of their target genes is overlapping in endothelial cells [43]. VEGF-A stimulates endothelial permeability [44], leading to oedema, leucocyte adhesion and increased expression of adhesion molecules promoting excessive inflammation. HGF has not these side effects; in contrast, it enhances the endothelial barrier function [45-47], possibly through Rac-specific guanine nucleotide exchange factor Asef [48] and the multifunctional Rac effector

IQGAP1 (IQ motif containing GTPase activating protein 1) [49]. Interestingly, IQGAP1 regulates endothelial barrier function via EB1 (end binding 1)-cortactin cross talk [50], linking the actin cytoskeleton and microtubules (Figure 3). Notably, HGF and VEGF-A display quantitative variations in the stimulation of common downstream signalling pathways, being HGF a more potent stimulator of ERK1,2 and Akt. Moreover, the two factors differentially stimulate the signalling molecules involved in remodelling of the cytoskeleton and cellular adhesion: VEGF selectively increases Rho activity, whereas Rac-1 activity is slightly decreased; in contrast, HGF increases Rac1 and has no effect on Rho activity [51]. The link between VEGF-A/VEGFR-2 (vascular endothelial growth factor receptor 2) and HGF/Met signalling systems could be represented by neuropilins, which bind both VEGF-A and HGF and function as co-receptors, potentiating the proangiogenic activity [52] (Figure 3). In contrast to these findings, it has been demonstrated that HGF inhibits Rac1 activity in endothelial cells and endothelial progenitor cells (EPCs) undergoing Angiotensin (ANG) II oxidative stress [53]. This discrepancy might be explained by different molecular responses of HGF under physiological and pathological conditions such as oxidative stress. Moreover, Sanada et al. [53] have shown that HGF is able to attenuate the ANG II-induced cellular senescence. In the context of MI, stimulation with HGF is associated with increased angiogenesis [54-56], indicating an important role of HGF/Met axis in the neovascularisation, and consequent protection of cardiomyocytes against the ischaemic injury. Such a beneficial effect has been shown for HGF also in hind limb ischaemia [57, 58].

The role of HGF in SMCs is less studied than that in endothelial cells. In addition to angiogenesis, HGF participates in the stabilization of newly formed blood vessels. In fact, it is induced upon angiopoietin (Ang)/Tie2 ligand receptor activation and promotes the recruitment of SMCs to endothelial cells [59]. Interestingly, HGF attenuates ANG II-induced oxidative stress in vascular SMCs, inhibiting SMCs growth and inflammation [60] (Figure 3). A significant reduction of vascular HGF was observed in diabetic compared to non-diabetic rats [61, 62]. This phenomenon gives rise to the hypothesis that disruption of the autocrine-paracrine local HGF system in the blood vessels by ANG II may result in endothelial dysfunction [63]. ANG II blockade by imidapril treatment resulted in improvement of endothelial dysfunction in diabetic spontaneously hypertensive rats (SHR), accompanied by an increase in vascular HGF. This latter might thus contribute to the protection against organ damage, especially against the endothelial dysfunction induced, for example, by diabetes and hypertension. HGF is produced and Met is up-regulated by SMCs of injured carotid arteries, making cells responsive to HGF with high motility [64, 65]. Strong Met expression was also observed in the SMCs of the atherosclerotic lesions of apolipoprotein E ^{-/-} mice, whereas HGF was expressed by macrophage-derived foam cells, suggesting that HGF and Met are key mediators of the SMC response in atherogenesis. Whether HGF has beneficial effects on atherosclerosis is questionable, as a recent study suggested that HGF may participate in pathological atherosclerosis through vascular calcification of SMCs [66].

HGF IS AN ANTI-FIBROTIC FACTOR FOR THE HEART

Upon tissue injury, the remodelling of connective tissue contributes to restoration of tissue integrity. However, the abnormal formation of scar tissue may lead to fibrotic diseases. Indeed, fibrotic disease is a major contributor to the degraded function of the heart, secondary to many pathological states including MI. The fibroblast/ myofibroblast cell populations are involved in normal healing and pathological fibrosis. Transforming growth factor-beta1 (TGF-b1) and ANG II both play important roles in regulating the fibrogenic process after cardiac injury. ANG II increases expression of TGF-b1 in cardiac fibroblasts and TGF-b1 increases fibroblast production of extracellular matrix [67-69]. TGF-b1 has a crucial role in promoting an unfavourable myocardial remodelling in hypertrophic growth induced by ANG II [68, 70].

HGF showed a strong anti-fibrotic effect with remarkable effectiveness in ameliorating tissue fibrosis in a wide range of animal models. Notably, HGF counteracts the action of TGF-b1 by

multiple mechanisms: inhibition of TGF- β 1 secretion [71-73], upregulation of decorin which binds active TGF- β 1 and sequester its action [74], promotion of myofibroblasts apoptosis [75], downregulation of TGF- β 1 synthesis and interference with TGF- β 1-initiated Smad (Sma and Mad related proteins) signalling [76] (Figure 4). Thus, one of these or even more mechanisms may operate in the protection from fibrosis by HGF in models of acute ischaemia [56, 77, 78] and dilated cardiomyopathy [71, 79]. A further proof is the observation of increased expression of pro-fibrotic molecules, such as TGF- β 1, in absence of Met signalling in hepatocytes [80]. The profibrotic effects of Met deficiency is mediated also by increased oxidative stress: loss of Met aggravates fibrosis by disrupting redox homeostasis which is compensated by upregulation of NF- κ B and Nrf2 signaling [30, 31, 80]. Further mechanisms of HGF anti-fibrotic action operate in endothelial cells: block of their transformation into myofibroblasts [81] and upregulation of iNOS (inducible nitric oxide synthase) followed by increased production of NO (nitric oxide) [82]. NO counteracts the upregulation of angiotensin-converting enzyme (ACE), which converts ANG I into ANG II [83] (Figure 4). In a complementary way [84], the inhibition of ANG II with an ACE inhibitor or with ANG II type I receptor increases HGF production and attenuates fibrosis in the heart of cardiomyopathic hamsters [72]. Overproduction of HGF, resulting from the inhibition of ANG II, is associated with degradation of extracellular matrix and inhibition of TGF- β 1. In conclusion, a large body of evidence indicates that there is a counter-regulatory axis between HGF, on one side, and ANG II and TGF- β 1, on the other side. Importantly, the disruption of the autocrine/paracrine local HGF production by TGF- β 1 and ANG II may result in an imbalance in the regulation of ECM (extracellular matrix), which in turn leads to pathological processes in the heart and vasculature. The final outcome of pro- and anti-fibrosis signalling may depend on transcription factors which regulate matrix genes and ECM remodelling, including members of the Ets family, Sp1 (specificity protein 1) and AP1 (activator protein 1).

HGF HAS ANTI-INFLAMMATORY PROPERTIES

During organ injury, the inflammatory response is an important process leading to recruitment and activation of cells which play a role in tissue destruction and repair. However, the inflammatory response may be also detrimental and is involved in atherosclerosis and coronary heart disease. A large body of evidence indicates the importance of HGF/Met cascade in the regulation of inflammatory events and immune cell functions that are common to many diseases and organ systems. In this and the next chapter we describe some of the most consolidated results which are relevant for the cardiovascular system.

Met receptor is expressed in hematopoietic progenitor cells and the HGF/Met pathway has a role in hematopoiesis and immunity (reviewed in [85, 86]). Met is expressed in peripheral blood monocytes [87] and their tissue-differentiated forms, macrophages [87, 88] and monocyte-derived dendritic cells (DCs) [89-91]. Resident macrophages participate in immunosurveillance and may support host defence against pathogens in the heart. They release pro-inflammatory cytokines, induce vasodilation and increase vascular permeability, permitting the migration and extravasation of leucocytes, mainly neutrophils, out of the bloodstream into the tissue. Leucocytes (such as neutrophils, eosinophils and macrophages) are key players in accelerating inflammation. Recently, Finisguerra et al. [92] have reported an unprecedented role of Met in neutrophils and inflammation. In fact, Met genetic deletion studies showed that Met is required for neutrophil chemoattraction and cytotoxicity in response to HGF. Met deletion in mouse neutrophils leads to reduced neutrophil infiltration to primary tumours and metastatic sites. Similarly, Met was shown to be necessary for neutrophil transudation during colitis, skin rash or peritonitis [92], linking Met to inflammation.

In the ischaemic heart, blood monocytes are mobilized and recruited to the heart, replenishing the resident macrophages. Although some reports have suggested that HGF may modulate monocyte/macrophage activation and migration during inflammation [93, 94], a large body of evidence indicates a protective action of HGF against inflammation. In monocytes/macrophages,

HGF upregulates IL (interleukin) - 10 [95, 96], a cytokine that plays an important role in limiting inflammation [97], indicating that HGF may play an anti-inflammatory role in the acute inflammatory response. Indeed, it has been shown that in macrophages exposed to microbial LPS (lipopolysaccharide) stimuli the production of IL-6 following LPS-TLR (toll-like receptor) signalling leads to the production of HGF which, in turn, activates Met in an autocrine/paracrine manner, then stimulating phosphorylation and inactivation of GSK3b (glycogen synthase kinase-3 beta). Inactive GSK3b can then promote the association of phospho-CREB (cAMP response element-binding protein, Ser 133) with CBP (CREB binding protein) and sequester CBP away from p65 NF-Kb [96]. Thus, mechanistically, HGF may prevent NF-kB from exerting its inflammatory action. These signalling changes are associated with a switch from pro- to anti-inflammatory pathways with a resultant increase in IL-10 production (Figure 5). Interestingly, administration of HGF increases serum levels of IL-10 in patients with coronary heart disease [98]. IL-10 has protective actions against atherosclerotic disease: IL-10 knockout mice show enhanced formation of atherosclerotic vascular lesions [99]. Indeed, IL-10 inhibits the adhesion of monocytes to endothelial cells by downregulation of the adhesion molecules CD18 and CD62-L on immune competent cells [100] (Figure 5). Higher IL-10 plasma levels have been found in patients with stable coronary artery disease, compared with those with unstable coronary syndromes [101], indicating that IL-10 may function as a plaque-stabilizing cytokine. Moreover, the administration of exogenous HGF to patients with coronary heart disease decreases serum IL-8 concentrations [98] (Figure 5). IL-8 is a proinflammatory cytokine with chemoattractant and mitogenic effects on vascular SMCs [102], monocytes [103] and neutrophils, directing them to the site of tissue injury [104, 105]. HGF may thus exert protective effects against atherosclerotic disease not only by increasing anti-inflammatory IL-10, but also by decreasing pro-inflammatory IL-8 levels. These findings suggest that HGF-based therapies could be effective in the treatment of atherosclerosis. In abdominal aortic aneurism tissue, exogenously added HGF in the presence of tumor necrosis factor-alpha (TNF- α) enhanced the secretion of anti-inflammatory IL-10 and suppressed the secretion of proinflammatory monocyte/macrophage chemotactic protein-1 (MCP-1) [106]. Thus, HGF may influence the inflammatory macrophages also in the aortic tissue (Figure 5). Two subsets of macrophages have been described: the inflammatory M1 subset, with pro-inflammatory activity, is believed to have potent antimicrobial activity, while the tissue repairing M2 subset is involved in immunomodulatory function, remodelling of the ECM and promotion of angiogenesis. Activated M2 macrophages, which arise from exposure to the Th (helper T cells) 2 cytokines, IL-4 and IL-13, and increase the expression of anti-inflammatory factor and mannose receptor 1 (CD206), produce HGF [107]. M2 macrophages are commonly associated with the second wave of inflammation and proficient tissue repair after cardiac injury, when the number of neutrophils decreases, and endothelial cells and fibroblasts proliferate [108]. In this respect, another population of monocytes must be recalled, Tie2 expressing monocytes (TEMs), which support angiogenesis in tumours and remodelling tissues [109]. HGF is also produced by TEMs, but its role in this macrophage subpopulation remains unclear.

THE ROLE OF HGF IN DENDRITIC CELLS AND IMMUNE TOLERANCE

As described above, HGF is critical for tissue protection during inflammatory diseases by influencing the macrophage population. What is more, HGF protects organs from the immunogenic challenge by means of its potential to induce “immune tolerance”. Such a capacity might reveal a powerful tool against vasculopathies, and significantly improve the success of heart transplantation procedures.

HGF has immunosuppressive effects, as indicated by the immunologic tolerance induced by HGF treatment in a model of autoimmune myocarditis [110] and in ectopic heart transplantation [111]. This protective effect appears to be due to reduction of IFN- γ (interferon gamma) and increased production of IL-4 and IL-10. The precise mechanisms of HGF immunomodulatory potency in

myocarditis and cardiac allografts leading to T cell-mediated immunosuppression remain to be determined. However, recent studies suggest a fundamental role for DCs. Interestingly, HGF promotes the differentiation of immature DCs into tolerogenic DCs, which promote the shift from Th1 to Th2 response and favour the expansion of IL-10-producing regulatory T cells (Tregs), thus maintaining T cells in a low state of activation [89, 95, 112]. Although DCs are the most proficient professional antigen-presenting cells (APCs), in the appropriate environment they can promote self-tolerance by inducing immunosuppression of T cell response. On the other hand, Tregs have been shown to inhibit the maturation and alter the cytokine production of DCs. Thus, a reciprocal circuit exists from DCs to Tregs and viceversa, which determines the induction and maintenance of “immune tolerance”.

IFN- γ plays a key role in acute rejection [113, 114]. In the study by Yamaura et al. [111], the administration of HGF resulted in a significant reduction in IFN- γ and in an increase in IL-10 expression in cardiac allografts, which were associated with the prolongation of graft survival. Besides the latter effect, HGF treatment protected from cardiac allograft vasculopathy, which is the major cause of late graft loss in heart transplant recipients. Suppression of IFN- γ may be crucial, as mice deficient in IFN- γ do not develop cardiac allograft vasculopathy [115]. In addition, HGF may have a protective effect on vascular endothelium and attenuate the severity of intimal hyperplasia. Thus, HGF may represent a therapeutic target in vasculopathic disease states.

HGF AND CARDIOVASCULAR REGENERATION

Over the last decade, it has been shown that the heart contains a pool of stem cells with regenerative capacity, albeit at very low extent. Based on this new paradigm, stem cell-based therapies have been initiated to cure the damaged heart with the goal of eliciting cardiac repair and regeneration. Stem cell engraftment forming new myocytes has seldom proved effective. Nevertheless, multipotent bone marrow (BM) cells, mesenchymal stem cells (MSCs), EPCs, myoblasts and other cells have been injected into the damaged heart or into coronary circulation, in both pre- and clinical trials, showing some beneficial effects. Nowadays, it is believed that the therapeutic benefit of stem cell-transplantation is mostly due to the release of paracrine factors able to stimulate migration, proliferation and survival of endogenous cardiac progenitor cells (CPCs) [116-118]. Besides important cardioprotective mechanisms, including cell survival, contractility, remodelling, and neovascularization, paracrine factors may also control endogenous stem/progenitor cell generation and proliferation.

HGF is released by different types of stem cells, such as MSCs [119, 120], multipotent BM-derived cells [121], EPCs [122-124], and adipose stem cells [125]. Among paracrine cytokines, HGF has been identified as an important factor secreted by implanted MSCs, which can promote migration, proliferation, and differentiation of CPCs [126, 127]. Various adult CPCs have been independently described on the basis of specific surface markers and different isolation approaches (reviewed in [128-130]). The nature and origin of CPCs are still controversial: they may reside in the heart from fetal life or derive from the bone marrow and colonize the myocardium following injury. CPCs have self-renewal properties and the ability to differentiate into the three main cardiac populations: endothelial cells, vascular SMCs and cardiomyocytes [121, 131, 132]. Among other growth factor receptors, CPCs express Met receptor [121]. Upon stimulation with HGF, they respond with mobilization, expansion and differentiation into cardiomyocytes and vascular cells [121, 127] (Figure 6). HGF/IGF1 (insulin-like growth factor 1) therapy attenuates pathologic cardiac remodelling and increases the formation of small, newly formed cardiomyocytes and vascular cells. HGF is also secreted by cardiosphere-derived stem cells and, together with VEGF and IGF1, mediates beneficial effects on cardiomyocytes and endothelial cells [117, 133]. Thus, it seems promising to boost the paracrine potency of HGF as a cardiac therapeutic tool per se, especially by means of gene therapy, and as an enhancer of cell engraftment for efficacious cell therapy.

Recent evidence suggests that stem cell niches exist in the heart and provide the support for self-renewal and stemness state of the cardiac precursor pool (reviewed in [134, 135]. Interestingly, Notch1 receptor has been identified as one of the critical components which sustain cell fate decision of CPCs [136]. Notch activation has a major role in regulating the fate of immature precursors in many different tissues, including the heart [26]. Notably, the integrated and coordinated activity of Notch and Met has a cardioprotective role in MI [25] (Figure 6). Genetic studies have revealed that Met is essential for organ/tissue regeneration after injury, through reactivation of stem/progenitor compartment [137-139]. The importance of Met in maintaining stemness is confirmed by studies in cancer stem cells, in which Met is often expressed at high levels and sustains a stem-like status [140-143].

The epicardium is another potential source of progenitor cells and paracrine signals for cardiac repair. Epicardial cells undergo the process of epithelial to mesenchymal transformation (EMT), giving rise to epicardial progenitor cells, which, upon injury, are very sensitive to thymosin b4 and regain the potential to form vascular cells and new cardiomyocytes [144]. Thus, EMT, which is essential for heart development, is also important for the generation of cells with properties of stem cells. At present, no data are available on the expression of Met on epicardial progenitor cells. However, the epicardium secretes - among other factors - HGF [145], which has been demonstrated to control EMT in the developing myocardium [17]. A recent study shows that HGF/IgG (Immunoglobulin G) protein complexes are the key epicardial factor in the conditioned medium of human epicardial-derived cells, which is able to reduce vascular injury in vivo after cardiac ischaemia with reperfusion. This function is likely linked to Wnt signalling, as HGF/IgG complexes were able to induce concomitant phosphorylation of Met and RYK, a receptor tyrosine kinase, which, along with Frizzled and Ror, functions as a Wnt receptor [146] (Figure 6).

HGF/MET COOPERATES WITH CXC CHEMOKINES TO MEDIATE CELL HOMING

Various different sources of circulating stem and progenitor cells with pro-angiogenic function have been shown to augment heart function after MI. They include haematopoietic progenitor cells, EPCs, pro-angiogenic cells of myeloid origin, MSCs isolated from the BM or adipose tissue and mesoangioblasts. The recruitment of these cells, including those administered exogenously, to the site of injury depends on their ability to migrate and to be retained in situ. Among the paracrine factors, which are able to mobilize transplanted and endogenous stem cells, HGF shows a main role (reviewed in [7]). However, the mechanism of progenitor cell homing to the site of injury is poorly understood. The SDF-1 (stromal cell-derived factor 1) chemokine, also known as CXCL12, is one of the factors involved in homing of circulating BM-derived cells [147]. In this respect, it is interesting that a subpopulation of BM-derived cells expresses Met and CXCR4 (the SDF-1 receptor) and responds to HGF/SDF-1 chemotaxis [148, 149] (Figure 6). Met receptor is a powerful driver of the invasive growth program for stem cells [150, 151], which involves migration and invasion of extracellular matrix. CXCR4/ SDF-1 axis has been shown to be important for homing. Indeed, the HGF/SDF-1 interplay has emerged for mobilization and activation of healthy progenitors [152, 153] and cancer stem cells [154-156], thus reinforcing the concept that Met/HGF is involved in the migration, homing and activation of progenitors and stem cells for regeneration. Last but not least, a recent paper has described HGF as a heart-derived factor which primes naïve T cells and enhances their CXC chemokines secretion and responsiveness which support homing during heart inflammation [157].

THERAPEUTIC POTENTIAL OF HGF AND HGF MIMETIC AGENTS

A large body of evidence produced in preclinical models of CVDs supports the beneficial role of HGF/Met in cardioprotection and cardiovascular regeneration. In contrast, a few human clinical

trials of HGF for CVD have been performed and the limited number of patients are not sufficient to prove the therapeutic efficacy. Furthermore, there are still severe limitations for the use of HGF in humans, represented by potential side effects such as pathological vessel and tumour formation. These possible side effects have not been reported in preclinical studies and deserve further investigations. This hurdle could be overcome through the development of new tools to effectively deliver the ligand (or other molecules able to stimulate the Met receptor) in target organs.

Biodegradable gelatin hydrogels as a carrier were shown to effectively achieve a sustained and controlled delivery of HGF protein or protein fragments, resulting in improved angiogenesis [127, 158-160] (Table 1). This therapeutic strategy was also successful in improving cardiac function in chronic myocarditis [161], hindlimb ischaemia [162] and spontaneously hypertensive rats [158]. On the other hand, administration of the exogenous HGF protein has been successfully pursued by gene transfer. HGF gene therapy has been shown to improve angiogenesis [55, 56, 163-166] and myocardial remodelling [167], and to reduce apoptosis [77, 167] and fibrosis [56, 81, 168] after MI. Additionally, it has been shown to increase blood flow and capillary density in hindlimb ischaemia [169] (Table 2). This preclinical work has provided encouraging results for human therapy in CVD (cardiovascular disease). Clinical application of HGF gene transfer (AMG0001, AnGes) has been evaluated for safety and improvement of blood perfusion in critical limb ischaemia [170, 171] and a global multicenter Phase III clinical trial (AG-CLI-0206) has begun. Moreover, a clinical safety study of Adenovirus (Ad) HGF to treat ischaemic heart disease has been completed [172, 173] (Table 3), showing safety of the Ad-HGF administration through intramyocardial or intracoronary injection. However, the limited number of patients did not allow to prove consistent signal of efficacy. Viral vectors are considered the best candidates for therapeutic delivery because they transfer genes more efficiently than any of nonviral methods. On the other hand, stem cell-based therapies have shown a higher rate of success when combined with gene transfer of HGF [174-177] and hold even greater promise with the employment of viral vectors which mediate highly efficient cell transduction. This novel ex-vivo approach would overcome the hurdle of the limited duration of the therapy achieved with gene or protein delivery and the high costs associated with protein therapy.

Finally, chemical drugs with agonist activity on Met receptor have been developed. A small molecule mimetic of HGF called BB3 (Angion Biomedical Corp) has been identified and has the advantage of obviating the difficulties associated with gene or protein therapy. BB3 has been shown to possess HGF activities, including protection against cerebral ischaemia [178] and MI. The clinical efficacy of BB3 in patients presenting with acute ST segment elevation myocardial infarction (A-STEMI) who undergo PCI is currently being evaluated (NCT01539590, Table 3). Dihexa, another HGF mimetic compound which belongs to ANG IV-related peptides, enhances HGF/Met signalling in the brain [179]. Dihexa binds to HGF with high affinity and increases the ability of HGF to signal to Met. However, further investigation is required to assess HGF mimetic function of Dihexa in the setting of cardiac repair. Finally, also anti-Met monoclonal antibodies endowed with agonistic activity may act as HGF mimetics [22, 180] and protect cardiomyocytes against ischaemia [22]. Future studies will exploit their use in cardiac repair *in vivo*.

THE DOWNSIDE OF HGF IN CVD

In a variety of cell types, HGF has been shown to exert both positive (tissue repair and regeneration, angiogenesis) and negative effects (invasive growth, tumour vascularization). The double nature of HGF/Met system suggest it to be considered as a Janus, the ancient Roman god depicted to have two faces. Indeed, the effect of HGF/Met axis stimulation in the human organism is a classic example of double-edged sword, whose final outcome varies depending on cell context, dosage, developmental stage, timing and duration. In particular, in the context of CVDs, the biological meaning of HGF upregulation, especially in terms of circulating levels, is still a matter of debate.

HGF has been purported as a diagnostic/prognostic biomarker predicting severity and mortality in a number of CVDs. HGF level raises with age [181, 182] and correlates with increased mortality in hypertension [183, 184], carotid atherosclerosis [39] and HF [181, 185-187].

High plasma HGF levels have been associated with the prevalence and severity of hypertension, systolic blood pressure and carotid artery remodelling, in a number of independent studies [183, 188-192]. In one of these studies, serum HGF concentration was further increased by the occurrence of complications in hypertensive patients [183]. Of importance, hypertensive patients treated with antihypertensive drugs showed the same level of serum HGF as normotensive subjects [183]. Treatment with both Telmisartan or Losartan/Hydrochlorothiazide decreased serum HGF and improved endothelial dysfunction, independently from the antihypertensive effect [193]. Serum HGF concentration was also associated with night-time blood pressure [190] and with the vasodilator response to reactive hyperaemia, an index of endothelial function. However, serum HGF was weakly correlated with the severity of arterial stiffness. Moreover, a relationship between endothelium-dependent vasodilation and serum HGF concentration was observed during treatment with an ACE inhibitor or β -blocker [194]. In parallel, serum HGF concentration in diabetic patients with hypertension was significantly increased compared with normal subjects, and even higher in presence of hypertensive complications. Elevation of serum HGF concentration in hypertensive patients was correlated with the severity of damage in hypertensive target organs [194]. Furthermore, in patients receiving haemodialysis, elevated serum HGF was associated with concentric left ventricular geometry [195]. Finally, the cardiac tissue levels of HGF and Met in 2K-1C and deoxycorticosterone acetate-salt rats were higher compared with normal rats and sham-operated rats [196]. Consistently, HGF was found among the genes upregulated in hearts from aldosterone-treated mice with cardiomyocyte-targeted overexpression of the Mineralocorticoid Receptor [197]. Given the deleterious effects of aldosterone/mineralocorticoid receptor activation in CVD, it is plausible that HGF upregulation may act as a compensatory survival effect.

The studies of Lamblin et al. [181] have, for the first time, suggested that circulating levels of HGF are associated with an increased cardiovascular mortality in patients with HF. These clinical studies demonstrated that HGF is a strong predictor of severity in early HF and mortality in advanced HF. Subsequently, Rychli et al. [198] also found HGF as a strong and independent predictor of mortality in advanced HF, in particular in ischaemic HF. Interestingly, levels of HGF correlated with age, incidence of atrial fibrillation and levels of BNP (brain natriuretic peptide), and inversely correlated with body mass index and dosage of renin angiotensin aldosterone-system inhibitors, including ACE inhibitors and ANG II receptor blockers. Interestingly, HGF and BNP, in combination, had an additive prognostic value for adverse cardiac event. A multi-biomarker risk score which improves prediction of long-term mortality in patients with advanced HF and gives a prognostic value superior to conventional parameters also included, among others, NT-proBNP and HGF [187].

After MI, HGF levels increase in blood circulation [19, 20, 199]. Thus, elevated concentrations of HGF in the serum may be the result of endogenous protective mechanisms for tissue repair. However, compensating effects of HGF may be no longer beneficial in the course of HF. In this line, it should be considered that high levels of continuous HGF secretion desensitize the system by down-regulating Met receptor [21]. Indeed, Met suppression is a recognized adaptation to overstimulation [6]. In addition, HGF is a pleiotropic factor with multiple effects on different cell types and the final biological outcome depends on restoring the balance between opposite functions. Inflammatory cytokines such as IL-1, IL-6 and TNF- α induce transcriptional activation of both HGF [87, 200, 201] and Met [202], indicating that inflammation initiates HGF/Met upregulation. The inflammatory stroma also increases the production of proteases that are involved in pro-HGF activation, such as the plasminogen activation system and matrix metalloproteinase [15]. Thus, HGF is not only overexpressed but also biologically activated. Indeed, HGF has been proposed as a precocious biomarker for the acute phase of the bowel inflammation [203]. On the other hand, HGF/Met plays an important role in resolution of injury through suppression of inflammation, regulation of immune response and tolerance (see above). In this respect, further investigation is required to fully

investigate the role of HGF/Met in hypertension, HF and inflammation, and the putative involvement of the HGF/Met axis in cardiac adverse effects and mortality.

CONCLUDING REMARKS

HGF is known to have many beneficial activities and might constitute a therapeutic option for a number of pathologies, including CVDs. Biological evidence exists which strongly supports the contribution of HGF/Met signalling to heal the ischaemic damage occurring in cardiovascular diseases such as MI and peripheral artery disease. The therapeutic efficacy of HGF and HGF mimetic agents in pre-clinical and clinical trials is being scrutinized. Yet, experimental and clinical facts raise a number of issues which deserve further investigation. For example, the experimental conclusions summarized in the present review (protection from apoptosis and autophagy, anti-oxidant function, proliferation, differentiation, senescence) depend on the context of cell type and/or pathology, and the signalling pathways beneath these cell-specific responses still have to be deeply explored. Our knowledge of positive and negative feedback loops is still rudimentary. Comprehension of the mechanisms that regulate pro-inflammatory versus anti-inflammatory, pro-fibrotic versus anti-fibrotic, pro-angiogenic versus anti-angiogenic responses will have important therapeutic implications. The impact of cellular senescence, tissue fibrosis and oxygenation also influences the migration, survival and proliferation of stem/progenitor cells. We believe that valuable information will be provided by the study of the role of HGF/Met in these cells. Administration of HGF or HGF mimetic compounds can significantly benefit organ regeneration. The biological meaning of elevated HGF concentration in serum, associated with chronic diseases such as hypertension and HF, is still unclear, although it is likely due to activation of compensatory pathways which result from reactive adaptation to chronic stimuli. Strategies targeting HGF/Met in the cardiovascular system may help reach homeostatic balance and resolution of injury.

FUNDING

The work was supported by the Association Francaise contre les Myopathies (AFM) and by CRT.

REFERENCES

- 1 Nakamura, T., Nawa, K. and Ichihara, A. (1984) Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. *Biochem Biophys Res Commun.* **122**, 1450-1459
- 2 Weidner, K. M., Behrens, J., Vandekerckhove, J. and Birchmeier, W. (1990) Scatter factor: molecular characteristics and effect on the invasiveness of epithelial cells. *J Cell Biol.* **111**, 2097-2108
- 3 Bottaro, D. P., Rubin, J. S., Faletto, D. L., Chan, A. M., Kmiecik, T. E., Vande Woude, G. F. and Aaronson, S. A. (1991) Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Science.* **251**, 802-804
- 4 Naldini, L., Weidner, K. M., Vigna, E., Gaudino, G., Bardelli, A., Ponzetto, C., Narsimhan, R. P., Hartmann, G., Zarnegar, R., Michalopoulos, G. K. and et al. (1991) Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. *Embo j.* **10**, 2867-2878
- 5 Birchmeier, C., Birchmeier, W., Gherardi, E. and Vande Woude, G. F. (2003) Met, metastasis, motility and more. *Nat Rev Mol Cell Biol.* **4**, 915-925
- 6 Trusolino, L., Bertotti, A. and Comoglio, P. M. (2010) MET signalling: principles and functions in development, organ regeneration and cancer. *Nat Rev Mol Cell Biol.* **11**, 834-848
- 7 Sala, V. and Crepaldi, T. (2011) Novel therapy for myocardial infarction: can HGF/Met be beneficial? *Cell Mol Life Sci.* **68**, 1703-1717
- 8 Gallo, S., Sala, V., Gatti, S. and Crepaldi, T. (2014) HGF/Met Axis in Heart Function and Cardioprotection. *Biomedicines.* **2**, 247-262
- 9 Graziani, A., Gramaglia, D., dalla Zonca, P. and Comoglio, P. M. (1993) Hepatocyte growth factor/scatter factor stimulates the Ras-guanine nucleotide exchanger. *J Biol Chem.* **268**, 9165-9168
- 10 Nakaoka, Y., Nishida, K., Narimatsu, M., Kamiya, A., Minami, T., Sawa, H., Okawa, K., Fujio, Y., Koyama, T., Maeda, M., Sone, M., Yamasaki, S., Arai, Y., Koh, G. Y., Kodama, T., Hirota, H., Otsu, K., Hirano, T. and Mochizuki, N. (2007) Gab family proteins are essential for postnatal maintenance of cardiac function via neuregulin-1/ErbB signaling. *J Clin Invest.* **117**, 1771-1781
- 11 Sala, V., Gallo, S., Leo, C., Gatti, S., Gelb, B. D. and Crepaldi, T. (2012) Signaling to cardiac hypertrophy: insights from human and mouse RASopathies. *Mol Med.* **18**, 938-947
- 12 Kerr, B. A., Ma, L., West, X. Z., Ding, L., Malinin, N. L., Weber, M. E., Tischenko, M., Goc, A., Somanath, P. R., Penn, M. S., Podrez, E. A. and Byzova, T. V. (2013) Interference with akt signaling protects against myocardial infarction and death by limiting the consequences of oxidative stress. *Sci Signal.* **6**, ra67
- 13 Hashigasako, A., Machide, M., Nakamura, T. and Matsumoto, K. (2004) Bi-directional regulation of Ser-985 phosphorylation of c-met via protein kinase C and protein phosphatase 2A involves c-Met activation and cellular responsiveness to hepatocyte growth factor. *J Biol Chem.* **279**, 26445-26452
- 14 Peschard, P., Fournier, T. M., Lamorte, L., Naujokas, M. A., Band, H., Langdon, W. Y. and Park, M. (2001) Mutation of the c-Cbl TKB domain binding site on the Met receptor tyrosine kinase converts it into a transforming protein. *Mol Cell.* **8**, 995-1004
- 15 Kawaguchi, M. and Kataoka, H. (2014) Mechanisms of hepatocyte growth factor activation in cancer tissues. *Cancers (Basel).* **6**, 1890-1904
- 16 Rappolee, D. A., Iyer, A. and Patel, Y. (1996) Hepatocyte growth factor and its receptor are expressed in cardiac myocytes during early cardiogenesis. *Circ Res.* **78**, 1028-1036
- 17 Song, W., Majka, S. M. and McGuire, P. G. (1999) Hepatocyte growth factor expression in the developing myocardium: evidence for a role in the regulation of the mesenchymal cell phenotype and urokinase expression. *Dev Dyn.* **214**, 92-100
- 18 Gatti, S., Leo, C., Gallo, S., Sala, V., Bucci, E., Natale, M., Cantarella, D., Medico, E. and Crepaldi, T. (2013) Gene expression profiling of HGF/Met activation in neonatal mouse heart. *Transgenic Res.* **22**, 579-593
- 19 Matsumori, A., Furukawa, Y., Hashimoto, T., Ono, K., Shioi, T., Okada, M., Iwasaki, A., Nishio, R. and Sasayama, S. (1996) Increased circulating hepatocyte growth factor in the early stage of acute myocardial infarction. *Biochem Biophys Res Commun.* **221**, 391-395

- 20 Ono, K., Matsumori, A., Shioi, T., Furukawa, Y. and Sasayama, S. (1997) Enhanced expression of hepatocyte growth factor/c-Met by myocardial ischemia and reperfusion in a rat model. *Circulation*. **95**, 2552-2558
- 21 Leo, C., Sala, V., Morello, M., Chiribiri, A., Riess, I., Mancardi, D., Schiaffino, S., Ponzetto, C. and Crepaldi, T. (2011) Activated Met signalling in the developing mouse heart leads to cardiac disease. *PLoS One*. **6**, e14675
- 22 Gallo, S., Gatti, S., Sala, V., Albano, R., Costelli, P., Casanova, E., Comoglio, P. M. and Crepaldi, T. (2014) Agonist antibodies activating the Met receptor protect cardiomyoblasts from cobalt chloride-induced apoptosis and autophagy. *Cell Death Dis*. **5**, e1185
- 23 Liu, Y. (1999) Hepatocyte growth factor promotes renal epithelial cell survival by dual mechanisms. *Am J Physiol*. **277**, F624-633
- 24 Kitta, K., Day, R. M., Kim, Y., Torregroza, I., Evans, T. and Suzuki, Y. J. (2003) Hepatocyte growth factor induces GATA-4 phosphorylation and cell survival in cardiac muscle cells. *J Biol Chem*. **278**, 4705-4712
- 25 Gude, N. A., Emmanuel, G., Wu, W., Cottage, C. T., Fischer, K., Quijada, P., Muraski, J. A., Alvarez, R., Rubio, M., Schaefer, E. and Sussman, M. A. (2008) Activation of Notch-mediated protective signaling in the myocardium. *Circ Res*. **102**, 1025-1035
- 26 de la Pompa, J. L. and Epstein, J. A. (2012) Coordinating tissue interactions: Notch signaling in cardiac development and disease. *Dev Cell*. **22**, 244-254
- 27 Pattingre, S., Tassa, A., Qu, X., Garuti, R., Liang, X. H., Mizushima, N., Packer, M., Schneider, M. D. and Levine, B. (2005) Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell*. **122**, 927-939
- 28 Imazu, T., Shimizu, S., Tagami, S., Matsushima, M., Nakamura, Y., Miki, T., Okuyama, A. and Tsujimoto, Y. (1999) Bcl-2/E1B 19 kDa-interacting protein 3-like protein (Bnip3L) interacts with bcl-2/Bcl-xL and induces apoptosis by altering mitochondrial membrane permeability. *Oncogene*. **18**, 4523-4529
- 29 Arechederra, M., Carmona, R., Gonzalez-Nunez, M., Gutierrez-Uzquiza, A., Bragado, P., Cruz-Gonzalez, I., Cano, E., Guerrero, C., Sanchez, A., Lopez-Novoa, J. M., Schneider, M. D., Maina, F., Munoz-Chapuli, R. and Porras, A. (2013) Met signaling in cardiomyocytes is required for normal cardiac function in adult mice. *Biochim Biophys Acta*. **1832**, 2204-2215
- 30 Gomez-Quiroz, L. E., Factor, V. M., Kaposi-Novak, P., Coulouarn, C., Conner, E. A. and Thorgeirsson, S. S. (2008) Hepatocyte-specific c-Met deletion disrupts redox homeostasis and sensitizes to Fas-mediated apoptosis. *J Biol Chem*. **283**, 14581-14589
- 31 Kaposi-Novak, P., Lee, J. S., Gomez-Quiroz, L., Coulouarn, C., Factor, V. M. and Thorgeirsson, S. S. (2006) Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. *J Clin Invest*. **116**, 1582-1595
- 32 Comoglio, P. M., Giordano, S. and Trusolino, L. (2008) Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat Rev Drug Discov*. **7**, 504-516
- 33 Doherty, K. R., Wappel, R. L., Talbert, D. R., Trusk, P. B., Moran, D. M., Kramer, J. W., Brown, A. M., Shell, S. A. and Bacus, S. (2013) Multi-parameter in vitro toxicity testing of crizotinib, sunitinib, erlotinib, and nilotinib in human cardiomyocytes. *Toxicol Appl Pharmacol*. **272**, 245-255
- 34 Aguirre, S. A., Heyen, J. R., Collette, W., 3rd, Bobrowski, W. and Blasi, E. R. (2010) Cardiovascular effects in rats following exposure to a receptor tyrosine kinase inhibitor. *Toxicol Pathol*. **38**, 416-428
- 35 Bussolino, F., Di Renzo, M. F., Ziche, M., Bocchietto, E., Olivero, M., Naldini, L., Gaudino, G., Tamagnone, L., Coffey, A. and Comoglio, P. M. (1992) Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. *J Cell Biol*. **119**, 629-641
- 36 Grant, D. S., Kleinman, H. K., Goldberg, I. D., Bhargava, M. M., Nickoloff, B. J., Kinsella, J. L., Polverini, P. and Rosen, E. M. (1993) Scatter factor induces blood vessel formation in vivo. *Proc Natl Acad Sci U S A*. **90**, 1937-1941
- 37 Ding, S., Merkulova-Rainon, T., Han, Z. C. and Tobelem, G. (2003) HGF receptor up-regulation contributes to the angiogenic phenotype of human endothelial cells and promotes angiogenesis in vitro. *Blood*. **101**, 4816-4822
- 38 Nakagami, H., Morishita, R., Yamamoto, K., Taniyama, Y., Aoki, M., Kim, S., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T. (2000) Anti-apoptotic action of hepatocyte growth factor through mitogen-activated protein kinase on human aortic endothelial cells. *J Hypertens*. **18**, 1411-1420
- 39 Yamamoto, Y., Kohara, K., Tabara, Y. and Miki, T. (2001) Association between carotid arterial remodeling and plasma concentration of circulating hepatocyte growth factor. *J Hypertens*. **19**, 1975-1979

- 40 Nakagami, H., Morishita, R., Yamamoto, K., Taniyama, Y., Aoki, M., Matsumoto, K., Nakamura, T., Kaneda, Y., Horiuchi, M. and Ogihara, T. (2001) Mitogenic and antiapoptotic actions of hepatocyte growth factor through ERK, STAT3, and AKT in endothelial cells. *Hypertension*. **37**, 581-586
- 41 Van Belle, E., Witzensbichler, B., Chen, D., Silver, M., Chang, L., Schwall, R. and Isner, J. M. (1998) Potentiated angiogenic effect of scatter factor/hepatocyte growth factor via induction of vascular endothelial growth factor: the case for paracrine amplification of angiogenesis. *Circulation*. **97**, 381-390
- 42 Rosario, M. and Birchmeier, W. (2003) How to make tubes: signaling by the Met receptor tyrosine kinase. *Trends Cell Biol.* **13**, 328-335
- 43 Gerritsen, M. E., Tomlinson, J. E., Zlot, C., Ziman, M. and Hwang, S. (2003) Using gene expression profiling to identify the molecular basis of the synergistic actions of hepatocyte growth factor and vascular endothelial growth factor in human endothelial cells. *Br J Pharmacol.* **140**, 595-610
- 44 Weis, S. M. and Cheresh, D. A. (2005) Pathophysiological consequences of VEGF-induced vascular permeability. *Nature*. **437**, 497-504
- 45 Liu, F., Schaphorst, K. L., Verin, A. D., Jacobs, K., Birukova, A., Day, R. M., Bogatcheva, N., Bottaro, D. P. and Garcia, J. G. (2002) Hepatocyte growth factor enhances endothelial cell barrier function and cortical cytoskeletal rearrangement: potential role of glycogen synthase kinase-3beta. *Faseb j.* **16**, 950-962
- 46 Singleton, P. A., Salgia, R., Moreno-Vinasco, L., Moitra, J., Sammani, S., Mirzapoiazova, T. and Garcia, J. G. (2007) CD44 regulates hepatocyte growth factor-mediated vascular integrity. Role of c-Met, Tiam1/Rac1, dynamin 2, and cortactin. *J Biol Chem.* **282**, 30643-30657
- 47 Birukova, A. A., Cokic, I., Moldobaeva, N. and Birukov, K. G. (2009) Paxillin is involved in the differential regulation of endothelial barrier by HGF and VEGF. *Am J Respir Cell Mol Biol.* **40**, 99-107
- 48 Meng, F., Meliton, A., Moldobaeva, N., Mutlu, G., Kawasaki, Y., Akiyama, T. and Birukova, A. A. (2015) Asef mediates HGF protective effects against LPS-induced lung injury and endothelial barrier dysfunction. *Am J Physiol Lung Cell Mol Physiol.* **308**, L452-463
- 49 Tian, Y., Gawlak, G., Shah, A. S., Higginbotham, K., Tian, X., Kawasaki, Y., Akiyama, T., Sacks, D. B. and Birukova, A. A. (2015) Hepatocyte growth factor-induced Asef-IQGAP1 complex controls cytoskeletal remodeling and endothelial barrier. *J Biol Chem.* **290**, 4097-4109
- 50 Tian, Y., Tian, X., Gawlak, G., O'Donnell, J. J., 3rd, Sacks, D. B. and Birukova, A. A. (2014) IQGAP1 regulates endothelial barrier function via EBI-cortactin cross talk. *Mol Cell Biol.* **34**, 3546-3558
- 51 Sulpice, E., Ding, S., Muscatelli-Groux, B., Berge, M., Han, Z. C., Plouet, J., Tobelem, G. and Merkulova-Rainon, T. (2009) Cross-talk between the VEGF-A and HGF signalling pathways in endothelial cells. *Biol Cell.* **101**, 525-539
- 52 Sulpice, E., Plouet, J., Berge, M., Allanic, D., Tobelem, G. and Merkulova-Rainon, T. (2008) Neuropilin-1 and neuropilin-2 act as coreceptors, potentiating proangiogenic activity. *Blood*. **111**, 2036-2045
- 53 Sanada, F., Taniyama, Y., Azuma, J., Iekushi, K., Dosaka, N., Yokoi, T., Koibuchi, N., Kusunoki, H., Aizawa, Y. and Morishita, R. (2009) Hepatocyte growth factor, but not vascular endothelial growth factor, attenuates angiotensin II-induced endothelial progenitor cell senescence. *Hypertension*. **53**, 77-82
- 54 Wang, Y., Ahmad, N., Wani, M. A. and Ashraf, M. (2004) Hepatocyte growth factor prevents ventricular remodeling and dysfunction in mice via Akt pathway and angiogenesis. *J Mol Cell Cardiol.* **37**, 1041-1052
- 55 Aoki, M., Morishita, R., Taniyama, Y., Kida, I., Moriguchi, A., Matsumoto, K., Nakamura, T., Kaneda, Y., Higaki, J. and Ogihara, T. (2000) Angiogenesis induced by hepatocyte growth factor in non-infarcted myocardium and infarcted myocardium: up-regulation of essential transcription factor for angiogenesis, ets. *Gene Ther.* **7**, 417-427
- 56 Riess, I., Sala, V., Leo, C., Demaria, M., Gatti, S., Gallo, S., Fitou, A., Boero, O., Levi, R., Cuccovillo, I., Molla, F., De Angelis, N., Staszewsky, L., Latini, R. and Crepaldi, T. (2011) A mouse model for spatial and temporal expression of HGF in the heart. *Transgenic Res.* **20**, 1203-1216
- 57 Morishita, R., Nakamura, S., Hayashi, S., Taniyama, Y., Moriguchi, A., Nagano, T., Taiji, M., Noguchi, H., Takeshita, S., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T. (1999) Therapeutic angiogenesis induced by human recombinant hepatocyte growth factor in rabbit hind limb ischemia model as cytokine supplement therapy. *Hypertension*. **33**, 1379-1384
- 58 Hayashi, S., Morishita, R., Nakamura, S., Yamamoto, K., Moriguchi, A., Nagano, T., Taiji, M., Noguchi, H., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T. (1999) Potential role of hepatocyte growth factor, a novel angiogenic growth factor, in peripheral arterial disease: downregulation of HGF in response to hypoxia in vascular cells. *Circulation*. **100**, Ii301-308

- 59 Kobayashi, H., DeBusk, L. M., Babichev, Y. O., Dumont, D. J. and Lin, P. C. (2006) Hepatocyte growth factor mediates angiopoietin-induced smooth muscle cell recruitment. *Blood*. **108**, 1260-1266
- 60 Shimizu, K., Taniyama, Y., Sanada, F., Azuma, J., Iwabayashi, M., Iekushi, K., Rakugi, H. and Morishita, R. (2012) Hepatocyte growth factor inhibits lipopolysaccharide-induced oxidative stress via epithelial growth factor receptor degradation. *Arterioscler Thromb Vasc Biol*. **32**, 2687-2693
- 61 Matsumoto, K., Morishita, R., Tomita, N., Moriguchi, A., Yamasaki, K., Aoki, M., Nakamura, T., Higaki, J. and Ogihara, T. (2002) Impaired endothelial dysfunction in diabetes mellitus rats was restored by oral administration of prostaglandin I₂ analogue. *J Endocrinol*. **175**, 217-223
- 62 Matsumoto, K., Morishita, R., Tomita, N., Moriguchi, A., Komai, N., Aoki, M., Nakamura, T., Higaki, J. and Ogihara, T. (2003) Improvement of endothelial dysfunction by angiotensin II blockade accompanied by induction of vascular hepatocyte growth factor system in diabetic spontaneously hypertensive rats. *Heart Vessels*. **18**, 18-25
- 63 Dzau, V. J. (1993) The role of mechanical and humoral factors in growth regulation of vascular smooth muscle and cardiac myocytes. *Curr Opin Nephrol Hypertens*. **2**, 27-32
- 64 Taher, T. E., Derksen, P. W., de Boer, O. J., Spaargaren, M., Teeling, P., van der Wal, A. C. and Pals, S. T. (2002) Hepatocyte growth factor triggers signaling cascades mediating vascular smooth muscle cell migration. *Biochem Biophys Res Commun*. **298**, 80-86
- 65 McKinnon, H., Gherardi, E., Reidy, M. and Bowyer, D. (2006) Hepatocyte growth factor/scatter factor and MET are involved in arterial repair and atherogenesis. *Am J Pathol*. **168**, 340-348
- 66 Liu, Y., Wang, T., Yan, J., Jiagbogu, N., Heideman, D. A., Canfield, A. E. and Alexander, M. Y. (2011) HGF/c-Met signalling promotes Notch3 activation and human vascular smooth muscle cell osteogenic differentiation in vitro. *Atherosclerosis*. **219**, 440-447
- 67 Campbell, S. E. and Katwa, L. C. (1997) Angiotensin II stimulated expression of transforming growth factor-beta1 in cardiac fibroblasts and myofibroblasts. *J Mol Cell Cardiol*. **29**, 1947-1958
- 68 Lee, A. A., Dillmann, W. H., McCulloch, A. D. and Villarreal, F. J. (1995) Angiotensin II stimulates the autocrine production of transforming growth factor-beta 1 in adult rat cardiac fibroblasts. *J Mol Cell Cardiol*. **27**, 2347-2357
- 69 MacKenna, D., Summerour, S. R. and Villarreal, F. J. (2000) Role of mechanical factors in modulating cardiac fibroblast function and extracellular matrix synthesis. *Cardiovasc Res*. **46**, 257-263
- 70 Kim, N. N., Villarreal, F. J., Printz, M. P., Lee, A. A. and Dillmann, W. H. (1995) Trophic effects of angiotensin II on neonatal rat cardiac myocytes are mediated by cardiac fibroblasts. *Am J Physiol*. **269**, E426-437
- 71 Nakamura, T., Matsumoto, K., Mizuno, S., Sawa, Y. and Matsuda, H. (2005) Hepatocyte growth factor prevents tissue fibrosis, remodeling, and dysfunction in cardiomyopathic hamster hearts. *Am J Physiol Heart Circ Physiol*. **288**, H2131-2139
- 72 Taniyama, Y., Morishita, R., Nakagami, H., Moriguchi, A., Sakonjo, H., Shokei, K., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T. (2000) Potential contribution of a novel antifibrotic factor, hepatocyte growth factor, to prevention of myocardial fibrosis by angiotensin II blockade in cardiomyopathic hamsters. *Circulation*. **102**, 246-252
- 73 Ueki, T., Kaneda, Y., Tsutsui, H., Nakanishi, K., Sawa, Y., Morishita, R., Matsumoto, K., Nakamura, T., Takahashi, H., Okamoto, E. and Fujimoto, J. (1999) Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Nat Med*. **5**, 226-230
- 74 Kobayashi, E., Sasamura, H., Mifune, M., Shimizu-Hirota, R., Kuroda, M., Hayashi, M. and Saruta, T. (2003) Hepatocyte growth factor regulates proteoglycan synthesis in interstitial fibroblasts. *Kidney Int*. **64**, 1179-1188
- 75 Mizuno, S., Matsumoto, K., Li, M. Y. and Nakamura, T. (2005) HGF reduces advancing lung fibrosis in mice: a potential role for MMP-dependent myofibroblast apoptosis. *Faseb j*. **19**, 580-582
- 76 Azuma, J., Taniyama, Y., Takeya, Y., Iekushi, K., Aoki, M., Dosaka, N., Matsumoto, K., Nakamura, T., Ogihara, T. and Morishita, R. (2006) Angiogenic and antifibrotic actions of hepatocyte growth factor improve cardiac dysfunction in porcine ischemic cardiomyopathy. *Gene Ther*. **13**, 1206-1213
- 77 Jayasankar, V., Woo, Y. J., Bish, L. T., Pirolli, T. J., Chatterjee, S., Berry, M. F., Burdick, J., Gardner, T. J. and Sweeney, H. L. (2003) Gene transfer of hepatocyte growth factor attenuates postinfarction heart failure. *Circulation*. **108 Suppl 1**, Ii230-236
- 78 Chen, X. H., Minatoguchi, S., Kosai, K., Yuge, K., Takahashi, T., Arai, M., Wang, N., Misao, Y., Lu, C., Onogi, H., Kobayashi, H., Yasuda, S., Ezaki, M., Ushikoshi, H., Takemura, G., Fujiwara, T. and

Fujiwara, H. (2007) In vivo hepatocyte growth factor gene transfer reduces myocardial ischemia-reperfusion injury through its multiple actions. *J Card Fail.* **13**, 874-883

79 Taniyama, Y., Morishita, R., Aoki, M., Hiraoka, K., Yamasaki, K., Hashiya, N., Matsumoto, K., Nakamura, T., Kaneda, Y. and Ogihara, T. (2002) Angiogenesis and antifibrotic action by hepatocyte growth factor in cardiomyopathy. *Hypertension.* **40**, 47-53

80 Marquardt, J. U., Seo, D., Gomez-Quiroz, L. E., Uchida, K., Gillen, M. C., Kitade, M., Kaposi-Novak, P., Conner, E. A., Factor, V. M. and Thorgeirsson, S. S. (2012) Loss of c-Met accelerates development of liver fibrosis in response to CCl₄ exposure through deregulation of multiple molecular pathways. *Biochim Biophys Acta.* **1822**, 942-951

81 Okayama, K., Azuma, J., Dosaka, N., Iekushi, K., Sanada, F., Kusunoki, H., Iwabayashi, M., Rakugi, H., Taniyama, Y. and Morishita, R. (2012) Hepatocyte growth factor reduces cardiac fibrosis by inhibiting endothelial-mesenchymal transition. *Hypertension.* **59**, 958-965

82 Purdie, K. J., Whitley, G. S., Johnstone, A. P. and Cartwright, J. E. (2002) Hepatocyte growth factor-induced endothelial cell motility is mediated by the upregulation of inducible nitric oxide synthase expression. *Cardiovasc Res.* **54**, 659-668

83 Takemoto, M., Egashira, K., Tomita, H., Usui, M., Okamoto, H., Kitabatake, A., Shimokawa, H., Sueishi, K. and Takeshita, A. (1997) Chronic angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor blockade: effects on cardiovascular remodeling in rats induced by the long-term blockade of nitric oxide synthesis. *Hypertension.* **30**, 1621-1627

84 Tomita, H., Egashira, K., Ohara, Y., Takemoto, M., Koyanagi, M., Katoh, M., Yamamoto, H., Tamaki, K., Shimokawa, H. and Takeshita, A. (1998) Early induction of transforming growth factor-beta via angiotensin II type 1 receptors contributes to cardiac fibrosis induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension.* **32**, 273-279

85 Nishino, T., Hisha, H., Nishino, N., Adachi, M. and Ikehara, S. (1995) Hepatocyte growth factor as a hematopoietic regulator. *Blood.* **85**, 3093-3100

86 Molnarfi, N., Benkhoucha, M., Funakoshi, H., Nakamura, T. and Lalive, P. H. (2015) Hepatocyte growth factor: A regulator of inflammation and autoimmunity. *Autoimmun Rev.* **14**, 293-303

87 Galimi, F., Cottone, E., Vigna, E., Arena, N., Boccaccio, C., Giordano, S., Naldini, L. and Comoglio, P. M. (2001) Hepatocyte growth factor is a regulator of monocyte-macrophage function. *J Immunol.* **166**, 1241-1247

88 Moransard, M., Sawitzky, M., Fontana, A. and Suter, T. (2010) Expression of the HGF receptor c-met by macrophages in experimental autoimmune encephalomyelitis. *Glia.* **58**, 559-571

89 Benkhoucha, M., Santiago-Raber, M. L., Schneider, G., Chofflon, M., Funakoshi, H., Nakamura, T. and Lalive, P. H. (2010) Hepatocyte growth factor inhibits CNS autoimmunity by inducing tolerogenic dendritic cells and CD25⁺Foxp3⁺ regulatory T cells. *Proc Natl Acad Sci U S A.* **107**, 6424-6429

90 Okunishi, K., Dohi, M., Nakagome, K., Tanaka, R., Mizuno, S., Matsumoto, K., Miyazaki, J., Nakamura, T. and Yamamoto, K. (2005) A novel role of hepatocyte growth factor as an immune regulator through suppressing dendritic cell function. *J Immunol.* **175**, 4745-4753

91 Kurz, S. M., Diebold, S. S., Hieronymus, T., Gust, T. C., Bartunek, P., Sachs, M., Birchmeier, W. and Zenke, M. (2002) The impact of c-met/scatter factor receptor on dendritic cell migration. *Eur J Immunol.* **32**, 1832-1838

92 Finisguerra, V., Di Conza, G., Di Matteo, M., Serneels, J., Costa, S., Thompson, A. A., Wauters, E., Walmsley, S., Prenen, H., Granot, Z., Casazza, A. and Mazzone, M. (2015) MET is required for the recruitment of anti-tumoural neutrophils. *Nature.* **522**, 349-353

93 Beilmann, M., Odenthal, M., Jung, W., Vande Woude, G. F., Dienes, H. P. and Schirmacher, P. (1997) Neoexpression of the c-met/hepatocyte growth factor-scatter factor receptor gene in activated monocytes. *Blood.* **90**, 4450-4458

94 Beilmann, M., Vande Woude, G. F., Dienes, H. P. and Schirmacher, P. (2000) Hepatocyte growth factor-stimulated invasiveness of monocytes. *Blood.* **95**, 3964-3969

95 Rutella, S., Bonanno, G., Procoli, A., Mariotti, A., de Ritis, D. G., Curti, A., Danese, S., Pessina, G., Pandolfi, S., Natoni, F., Di Febo, A., Scambia, G., Manfredini, R., Salati, S., Ferrari, S., Pierelli, L., Leone, G. and Lemoli, R. M. (2006) Hepatocyte growth factor favors monocyte differentiation into regulatory interleukin (IL)-10⁺⁺IL-12^{low}/neg accessory cells with dendritic-cell features. *Blood.* **108**, 218-227

96 Coudriet, G. M., He, J., Trucco, M., Mars, W. M. and Piganelli, J. D. (2010) Hepatocyte growth factor modulates interleukin-6 production in bone marrow derived macrophages: implications for inflammatory mediated diseases. *PLoS One.* **5**, e15384

- 97 Yang, Z., Zingarelli, B. and Szabo, C. (2000) Crucial role of endogenous interleukin-10 production in myocardial ischemia/reperfusion injury. *Circulation*. **101**, 1019-1026
- 98 Yang, Z. J., Xu, S. L., Chen, B., Zhang, S. L., Zhang, Y. L., Wei, W., Ma, D. C., Wang, L. S., Zhu, T. B., Li, C. J., Wang, H., Cao, K. J., Gao, W., Huang, J., Ma, W. Z. and Wu, Z. Z. (2009) Hepatocyte growth factor plays a critical role in the regulation of cytokine production and induction of endothelial progenitor cell mobilization: a pilot gene therapy study in patients with coronary heart disease. *Clin Exp Pharmacol Physiol*. **36**, 790-796
- 99 Mallat, Z., Besnard, S., Duriez, M., Deleuze, V., Emmanuel, F., Bureau, M. F., Soubrier, F., Esposito, B., Duez, H., Fievet, C., Staels, B., Duverger, N., Scherman, D. and Tedgui, A. (1999) Protective role of interleukin-10 in atherosclerosis. *Circ Res*. **85**, e17-24
- 100 Mostafa Mtairag, E., Chollet-Martin, S., Oudghiri, M., Laquay, N., Jacob, M. P., Michel, J. B. and Feldman, L. J. (2001) Effects of interleukin-10 on monocyte/endothelial cell adhesion and MMP-9/TIMP-1 secretion. *Cardiovasc Res*. **49**, 882-890
- 101 Smith, D. A., Irving, S. D., Sheldon, J., Cole, D. and Kaski, J. C. (2001) Serum levels of the antiinflammatory cytokine interleukin-10 are decreased in patients with unstable angina. *Circulation*. **104**, 746-749
- 102 Yue, T. L., Wang, X., Sung, C. P., Olson, B., McKenna, P. J., Gu, J. L. and Feuerstein, G. Z. (1994) Interleukin-8. A mitogen and chemoattractant for vascular smooth muscle cells. *Circ Res*. **75**, 1-7
- 103 Gerszten, R. E., Garcia-Zepeda, E. A., Lim, Y. C., Yoshida, M., Ding, H. A., Gimbrone, M. A., Jr., Luster, A. D., Luscinskas, F. W. and Rosenzweig, A. (1999) MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature*. **398**, 718-723
- 104 Molad, Y., Haines, K. A., Anderson, D. C., Buyon, J. P. and Cronstein, B. N. (1994) Immunocomplexes stimulate different signalling events to chemoattractants in the neutrophil and regulate L-selectin and beta 2-integrin expression differently. *Biochem J*. **299 (Pt 3)**, 881-887
- 105 Peveri, P., Walz, A., Dewald, B. and Baggiolini, M. (1988) A novel neutrophil-activating factor produced by human mononuclear phagocytes. *J Exp Med*. **167**, 1547-1559
- 106 Shintani, Y., Aoki, H., Nishihara, M., Ohno, S., Furusho, A., Hiromatsu, S., Akashi, H., Imaizumi, T. and Aoyagi, S. (2011) Hepatocyte growth factor promotes an anti-inflammatory cytokine profile in human abdominal aortic aneurysm tissue. *Atherosclerosis*. **216**, 307-312
- 107 Sakaguchi, S., Shono, J., Suzuki, T., Sawano, S., Anderson, J. E., Do, M. K., Ohtsubo, H., Mizunoya, W., Sato, Y., Nakamura, M., Furuse, M., Yamada, K., Ikeuchi, Y. and Tatsumi, R. (2014) Implication of anti-inflammatory macrophages in regenerative moto-neuritogenesis: promotion of myoblast migration and neural chemorepellent semaphorin 3A expression in injured muscle. *Int J Biochem Cell Biol*. **54**, 272-285
- 108 Dutta, P. and Nahrendorf, M. (2015) Monocytes in myocardial infarction. *Arterioscler Thromb Vasc Biol*. **35**, 1066-1070
- 109 De Palma, M., Venneri, M. A., Galli, R., Sergi Sergi, L., Politi, L. S., Sampaolesi, M. and Naldini, L. (2005) Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell*. **8**, 211-226
- 110 Futamatsu, H., Suzuki, J., Mizuno, S., Koga, N., Adachi, S., Kosuge, H., Maejima, Y., Hirao, K., Nakamura, T. and Isobe, M. (2005) Hepatocyte growth factor ameliorates the progression of experimental autoimmune myocarditis: a potential role for induction of T helper 2 cytokines. *Circ Res*. **96**, 823-830
- 111 Yamaura, K., Ito, K., Tsukioka, K., Wada, Y., Makiuchi, A., Sakaguchi, M., Akashima, T., Fujimori, M., Sawa, Y., Morishita, R., Matsumoto, K., Nakamura, T., Suzuki, J., Amano, J. and Isobe, M. (2004) Suppression of acute and chronic rejection by hepatocyte growth factor in a murine model of cardiac transplantation: induction of tolerance and prevention of cardiac allograft vasculopathy. *Circulation*. **110**, 1650-1657
- 112 Bai, L., Lennon, D. P., Caplan, A. I., DeChant, A., Hecker, J., Kranso, J., Zaremba, A. and Miller, R. H. (2012) Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models. *Nat Neurosci*. **15**, 862-870
- 113 Dallman, M. J., Larsen, C. P. and Morris, P. J. (1991) Cytokine gene transcription in vascularised organ grafts: analysis using semiquantitative polymerase chain reaction. *J Exp Med*. **174**, 493-496
- 114 Saiura, A., Mataka, C., Murakami, T., Umetani, M., Wada, Y., Kohro, T., Aburatani, H., Harihara, Y., Hamakubo, T., Yamaguchi, T., Hasegawa, G., Naito, M., Makuuchi, M. and Kodama, T. (2001) A comparison of gene expression in murine cardiac allografts and isografts by means DNA microarray analysis. *Transplantation*. **72**, 320-329

- 115 Nagano, H., Mitchell, R. N., Taylor, M. K., Hasegawa, S., Tilney, N. L. and Libby, P. (1997) Interferon-gamma deficiency prevents coronary arteriosclerosis but not myocardial rejection in transplanted mouse hearts. *J Clin Invest.* **100**, 550-557
- 116 Mirosou, M., Jayawardena, T. M., Schmeckpeper, J., Gneccchi, M. and Dzau, V. J. (2011) Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. *J Mol Cell Cardiol.* **50**, 280-289
- 117 Chimenti, I., Smith, R. R., Li, T. S., Gerstenblith, G., Messina, E., Giacomello, A. and Marban, E. (2010) Relative roles of direct regeneration versus paracrine effects of human cardiosphere-derived cells transplanted into infarcted mice. *Circ Res.* **106**, 971-980
- 118 Malliaras, K., Zhang, Y., Seinfeld, J., Galang, G., Tseliou, E., Cheng, K., Sun, B., Aminzadeh, M. and Marban, E. (2013) Cardiomyocyte proliferation and progenitor cell recruitment underlie therapeutic regeneration after myocardial infarction in the adult mouse heart. *EMBO Mol Med.* **5**, 191-209
- 119 Neuss, S., Becher, E., Woltje, M., Tietze, L. and Jahnen-Dechent, W. (2004) Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing. *Stem Cells.* **22**, 405-414
- 120 Rosova, I., Dao, M., Capoccia, B., Link, D. and Nolte, J. A. (2008) Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. *Stem Cells.* **26**, 2173-2182
- 121 Urbanek, K., Rota, M., Cascapera, S., Bearzi, C., Nascimbene, A., De Angelis, A., Hosoda, T., Chimenti, S., Baker, M., Limana, F., Nurzynska, D., Torella, D., Rotatori, F., Rastaldo, R., Musso, E., Quaini, F., Leri, A., Kajstura, J. and Anversa, P. (2005) Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. *Circ Res.* **97**, 663-673
- 122 Rehman, J., Li, J., Orschell, C. M. and March, K. L. (2003) Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation.* **107**, 1164-1169
- 123 Cho, H. J., Lee, N., Lee, J. Y., Choi, Y. J., Ii, M., Wecker, A., Jeong, J. O., Curry, C., Qin, G. and Yoon, Y. S. (2007) Role of host tissues for sustained humoral effects after endothelial progenitor cell transplantation into the ischemic heart. *J Exp Med.* **204**, 3257-3269
- 124 Urbich, C., Aicher, A., Heeschen, C., Dernbach, E., Hofmann, W. K., Zeiher, A. M. and Dimmeler, S. (2005) Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. *J Mol Cell Cardiol.* **39**, 733-742
- 125 Cai, L., Johnstone, B. H., Cook, T. G., Liang, Z., Traktuev, D., Cornetta, K., Ingram, D. A., Rosen, E. D. and March, K. L. (2007) Suppression of hepatocyte growth factor production impairs the ability of adipose-derived stem cells to promote ischemic tissue revascularization. *Stem Cells.* **25**, 3234-3243
- 126 Ellison, G. M., Torella, D., Dellegrottaglie, S., Perez-Martinez, C., Perez de Prado, A., Vicinanza, C., Purushothaman, S., Galuppo, V., Iaconetti, C., Waring, C. D., Smith, A., Torella, M., Cuellas Ramon, C., Gonzalo-Orden, J. M., Agosti, V., Indolfi, C., Galinanes, M., Fernandez-Vazquez, F. and Nadal-Ginard, B. (2011) Endogenous cardiac stem cell activation by insulin-like growth factor-1/hepatocyte growth factor intracoronary injection fosters survival and regeneration of the infarcted pig heart. *J Am Coll Cardiol.* **58**, 977-986
- 127 Koudstaal, S., Bastings, M. M., Feyen, D. A., Waring, C. D., van Slochteren, F. J., Dankers, P. Y., Torella, D., Sluijter, J. P., Nadal-Ginard, B., Doevendans, P. A., Ellison, G. M. and Chamuleau, S. A. (2014) Sustained delivery of insulin-like growth factor-1/hepatocyte growth factor stimulates endogenous cardiac repair in the chronic infarcted pig heart. *J Cardiovasc Transl Res.* **7**, 232-241
- 128 Torella, D., Ellison, G. M., Mendez-Ferrer, S., Ibanez, B. and Nadal-Ginard, B. (2006) Resident human cardiac stem cells: role in cardiac cellular homeostasis and potential for myocardial regeneration. *Nat Clin Pract Cardiovasc Med.* **3 Suppl 1**, S8-13
- 129 Barile, L., Messina, E., Giacomello, A. and Marbán, E. (2007) Endogenous cardiac stem cells. *Prog Cardiovasc Dis.* **50**, 31-48
- 130 Leri, A., Rota, M., Pasqualini, F. S., Goichberg, P. and Anversa, P. (2015) Origin of cardiomyocytes in the adult heart. *Circ Res.* **116**, 150-166
- 131 Parmacek, M. S. and Epstein, J. A. (2005) Pursuing cardiac progenitors: regeneration redux. *Cell.* **120**, 295-298
- 132 Leri, A., Kajstura, J. and Anversa, P. (2005) Cardiac stem cells and mechanisms of myocardial regeneration. *Physiol Rev.* **85**, 1373-1416

- 133 Smith, R. R., Barile, L., Cho, H. C., Leppo, M. K., Hare, J. M., Messina, E., Giacomello, A., Abraham, M. R. and Marban, E. (2007) Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation*. **115**, 896-908
- 134 Leri, A., Rota, M., Hosoda, T., Goichberg, P. and Anversa, P. (2014) Cardiac stem cell niches. *Stem Cell Res*. **13**, 631-646
- 135 Pagliari, S., Jelinek, J., Grassi, G. and Forte, G. (2014) Targeting pleiotropic signaling pathways to control adult cardiac stem cell fate and function. *Front Physiol*. **5**, 219
- 136 Boni, A., Urbanek, K., Nascimbene, A., Hosoda, T., Zheng, H., Delucchi, F., Amano, K., Gonzalez, A., Vitale, S., Ojaimi, C., Rizzi, R., Bolli, R., Yutzey, K. E., Rota, M., Kajstura, J., Anversa, P. and Leri, A. (2008) Notch1 regulates the fate of cardiac progenitor cells. *Proc Natl Acad Sci U S A*. **105**, 15529-15534
- 137 Ishikawa, T., Factor, V. M., Marquardt, J. U., Raggi, C., Seo, D., Kitade, M., Conner, E. A. and Thorgeirsson, S. S. (2012) Hepatocyte growth factor/c-met signaling is required for stem-cell-mediated liver regeneration in mice. *Hepatology*. **55**, 1215-1226
- 138 Chmielowiec, J., Borowiak, M., Morkel, M., Stradal, T., Munz, B., Werner, S., Wehland, J., Birchmeier, C. and Birchmeier, W. (2007) c-Met is essential for wound healing in the skin. *J Cell Biol*. **177**, 151-162
- 139 Webster, M. T. and Fan, C. M. (2013) c-MET regulates myoblast motility and myocyte fusion during adult skeletal muscle regeneration. *PLoS One*. **8**, e81757
- 140 Li, Y., Li, A., Glas, M., Lal, B., Ying, M., Sang, Y., Xia, S., Trageser, D., Guerrero-Cázares, H., Eberhart, C. G., Quiñones-Hinojosa, A., Scheffler, B. and Lathera, J. (2011) c-Met signaling induces a reprogramming network and supports the glioblastoma stem-like phenotype. *Proc Natl Acad Sci U S A*. **108**, 9951-9956
- 141 De Bacco, F., Casanova, E., Medico, E., Pellegatta, S., Orzan, F., Albano, R., Luraghi, P., Reato, G., D'Ambrosio, A., Poratti, P., Patane, M., Maderna, E., Pollo, B., Comoglio, P. M., Finocchiaro, G. and Boccaccio, C. (2012) The MET oncogene is a functional marker of a glioblastoma stem cell subtype. *Cancer Res*. **72**, 4537-4550
- 142 Xu, K., Usary, J., Kousis, P. C., Prat, A., Wang, D. Y., Adams, J. R., Wang, W., Loch, A. J., Deng, T., Zhao, W., Cardiff, R. D., Yoon, K., Gaiano, N., Ling, V., Beyene, J., Zacksenhaus, E., Gridley, T., Leong, W. L., Guidos, C. J., Perou, C. M. and Egan, S. E. (2012) Lunatic fringe deficiency cooperates with the Met/Caveolin gene amplicon to induce basal-like breast cancer. *Cancer Cell*. **21**, 626-641
- 143 Gastaldi, S., Sassi, F., Accornero, P., Torti, D., Galimi, F., Migliardi, G., Molyneux, G., Perera, T., Comoglio, P. M., Boccaccio, C., Smalley, M. J., Bertotti, A. and Trusolino, L. (2013) Met signaling regulates growth, repopulating potential and basal cell-fate commitment of mammary luminal progenitors: implications for basal-like breast cancer. *Oncogene*. **32**, 1428-1440
- 144 Smart, N., Bollini, S., Dube, K. N., Vieira, J. M., Zhou, B., Davidson, S., Yellon, D., Riegler, J., Price, A. N., Lythgoe, M. F., Pu, W. T. and Riley, P. R. (2011) De novo cardiomyocytes from within the activated adult heart after injury. *Nature*. **474**, 640-644
- 145 Limana, F., Bertolami, C., Mangoni, A., Di Carlo, A., Avitabile, D., Mocini, D., Iannelli, P., De Mori, R., Marchetti, C., Pozzoli, O., Gentili, C., Zacheo, A., Germani, A. and Capogrossi, M. C. (2010) Myocardial infarction induces embryonic reprogramming of epicardial c-kit(+) cells: role of the pericardial fluid. *J Mol Cell Cardiol*. **48**, 609-618
- 146 Rao, K. S., Aronshtam, A., McElory-Yaggy, K. L., Bakondi, B., VanBuren, P., Sobel, B. E. and Spees, J. L. (2015) Human epicardial cell-conditioned medium contains HGF/IgG complexes that phosphorylate RYK and protect against vascular injury. *Cardiovasc Res*. **107**, 277-286
- 147 Rennert, R. C., Sorkin, M., Garg, R. K. and Gurtner, G. C. (2012) Stem cell recruitment after injury: lessons for regenerative medicine. *Regen Med*. **7**, 833-850
- 148 Kucia, M., Dawn, B., Hunt, G., Guo, Y., Wysoczynski, M., Majka, M., Ratajczak, J., Rezzoug, F., Ildstad, S. T., Bolli, R. and Ratajczak, M. Z. (2004) Cells expressing early cardiac markers reside in the bone marrow and are mobilized into the peripheral blood after myocardial infarction. *Circ Res*. **95**, 1191-1199
- 149 Son, B. R., Marquez-Curtis, L. A., Kucia, M., Wysoczynski, M., Turner, A. R., Ratajczak, J., Ratajczak, M. Z. and Janowska-Wieczorek, A. (2006) Migration of bone marrow and cord blood mesenchymal stem cells in vitro is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases. *Stem Cells*. **24**, 1254-1264
- 150 Boccaccio, C. and Comoglio, P. M. (2014) MET, a driver of invasive growth and cancer clonal evolution under therapeutic pressure. *Curr Opin Cell Biol*. **31**, 98-105

- 151 Boccaccio, C. and Comoglio, P. M. (2006) Invasive growth: a MET-driven genetic programme for cancer and stem cells. *Nat Rev Cancer*. **6**, 637-645
- 152 Cohen, M. E., Fainstein, N., Lavon, I. and Ben-Hur, T. (2014) Signaling through three chemokine receptors triggers the migration of transplanted neural precursor cells in a model of multiple sclerosis. *Stem Cell Res*. **13**, 227-239
- 153 Konoplyannikov, M., Haider, K. H., Lai, V. K., Ahmed, R. P., Jiang, S. and Ashraf, M. (2013) Activation of diverse signaling pathways by ex-vivo delivery of multiple cytokines for myocardial repair. *Stem Cells Dev*. **22**, 204-215
- 154 Todaro, M., Gaggianesi, M., Catalano, V., Benfante, A., Iovino, F., Biffoni, M., Apuzzo, T., Sperduti, I., Volpe, S., Cocorullo, G., Gulotta, G., Dieli, F., De Maria, R. and Stassi, G. (2014) CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Cell Stem Cell*. **14**, 342-356
- 155 Holland, J. D., Gyorffy, B., Vogel, R., Eckert, K., Valenti, G., Fang, L., Lohneis, P., Elezkurtaj, S., Ziebold, U. and Birchmeier, W. (2013) Combined Wnt/beta-catenin, Met, and CXCL12/CXCR4 signals characterize basal breast cancer and predict disease outcome. *Cell Rep*. **5**, 1214-1227
- 156 Miekus, K., Lukasiewicz, E., Jarocha, D., Sekula, M., Drabik, G. and Majka, M. (2013) The decreased metastatic potential of rhabdomyosarcoma cells obtained through MET receptor downregulation and the induction of differentiation. *Cell Death Dis*. **4**, e459
- 157 Komarowska, I., Coe, D., Wang, G., Haas, R., Mauro, C., Kishore, M., Cooper, D., Nadkarni, S., Fu, H., Steinbruchel, D. A., Pitzalis, C., Anderson, G., Bucy, P., Lombardi, G., Breckenridge, R. and Marelli-Berg, F. M. (2015) Hepatocyte Growth Factor Receptor c-Met Instructs T Cell Cardiotropism and Promotes T Cell Migration to the Heart via Autocrine Chemokine Release. *Immunity*. **42**, 1087-1099
- 158 Sakaguchi, G., Tambara, K., Sakakibara, Y., Ozeki, M., Yamamoto, M., Premaratne, G., Lin, X., Hasegawa, K., Tabata, Y., Nishimura, K. and Komeda, M. (2005) Control-released hepatocyte growth factor prevents the progression of heart failure in stroke-prone spontaneously hypertensive rats. *Ann Thorac Surg*. **79**, 1627-1634
- 159 Sonnenberg, S. B., Rane, A. A., Liu, C. J., Rao, N., Agmon, G., Suarez, S., Wang, R., Munoz, A., Bajaj, V., Zhang, S., Braden, R., Schup-Magoffin, P. J., Kwan, O. L., DeMaria, A. N., Cochran, J. R. and Christman, K. L. (2015) Delivery of an engineered HGF fragment in an extracellular matrix-derived hydrogel prevents negative LV remodeling post-myocardial infarction. *Biomaterials*. **45**, 56-63
- 160 Salimath, A. S., Phelps, E. A., Boopathy, A. V., Che, P. L., Brown, M., Garcia, A. J. and Davis, M. E. (2012) Dual delivery of hepatocyte and vascular endothelial growth factors via a protease-degradable hydrogel improves cardiac function in rats. *PLoS One*. **7**, e50980
- 161 Nakano, J., Marui, A., Muranaka, H., Masumoto, H., Noma, H., Tabata, Y., Ido, A., Tsubouchi, H., Ikeda, T. and Sakata, R. (2014) Effects of hepatocyte growth factor in myocarditis rats induced by immunization with porcine cardiac myosin. *Interact Cardiovasc Thorac Surg*. **18**, 300-307
- 162 Ruvinov, E., Leor, J. and Cohen, S. (2010) The effects of controlled HGF delivery from an affinity-binding alginate biomaterial on angiogenesis and blood perfusion in a hindlimb ischemia model. *Biomaterials*. **31**, 4573-4582
- 163 Funatsu, T., Sawa, Y., Ohtake, S., Takahashi, T., Matsumiya, G., Matsuura, N., Nakamura, T. and Matsuda, H. (2002) Therapeutic angiogenesis in the ischemic canine heart induced by myocardial injection of naked complementary DNA plasmid encoding hepatocyte growth factor. *J Thorac Cardiovasc Surg*. **124**, 1099-1105
- 164 Ahmet, I., Sawa, Y., Yamaguchi, T. and Matsuda, H. (2003) Gene transfer of hepatocyte growth factor improves angiogenesis and function of chronic ischemic myocardium in canine heart. *Ann Thorac Surg*. **75**, 1283-1287
- 165 Wang, W., Yang, Z. J., Ma, D. C., Wang, L. S., Xu, S. L., Zhang, Y. R., Cao, K. J., Zhang, F. M. and Ma, W. Z. (2006) Induction of collateral artery growth and improvement of post-infarct heart function by hepatocyte growth factor gene transfer. *Acta Pharmacol Sin*. **27**, 555-560
- 166 Jin, Y. N., Inubushi, M., Masamoto, K., Odaka, K., Aoki, I., Tsuji, A. B., Sagara, M., Koizumi, M. and Saga, T. (2012) Long-term effects of hepatocyte growth factor gene therapy in rat myocardial infarct model. *Gene Ther*. **19**, 836-843
- 167 Li, Y., Takemura, G., Kosai, K., Yuge, K., Nagano, S., Esaki, M., Goto, K., Takahashi, T., Hayakawa, K., Koda, M., Kawase, Y., Maruyama, R., Okada, H., Minatoguchi, S., Mizuguchi, H., Fujiwara, T. and Fujiwara, H. (2003) Postinfarction treatment with an adenoviral vector expressing hepatocyte growth factor relieves chronic left ventricular remodeling and dysfunction in mice. *Circulation*. **107**, 2499-2506

- 168 Ahmet, I., Sawa, Y., Iwata, K. and Matsuda, H. (2002) Gene transfection of hepatocyte growth factor attenuates cardiac remodeling in the canine heart: A novel gene therapy for cardiomyopathy. *J Thorac Cardiovasc Surg.* **124**, 957-963
- 169 Taniyama, Y., Morishita, R., Hiraoka, K., Aoki, M., Nakagami, H., Yamasaki, K., Matsumoto, K., Nakamura, T., Kaneda, Y. and Ogihara, T. (2001) Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat diabetic hind limb ischemia model: molecular mechanisms of delayed angiogenesis in diabetes. *Circulation.* **104**, 2344-2350
- 170 Morishita, R., Makino, H., Aoki, M., Hashiya, N., Yamasaki, K., Azuma, J., Taniyama, Y., Sawa, Y., Kaneda, Y. and Ogihara, T. (2011) Phase I/IIa clinical trial of therapeutic angiogenesis using hepatocyte growth factor gene transfer to treat critical limb ischemia. *Arterioscler Thromb Vasc Biol.* **31**, 713-720
- 171 Makino, H., Aoki, M., Hashiya, N., Yamasaki, K., Azuma, J., Sawa, Y., Kaneda, Y., Ogihara, T. and Morishita, R. (2012) Long-term follow-up evaluation of results from clinical trial using hepatocyte growth factor gene to treat severe peripheral arterial disease. *Arterioscler Thromb Vasc Biol.* **32**, 2503-2509
- 172 Yuan, B., Zhao, Z., Zhang, Y. R., Wu, C. T., Jin, W. G., Zhao, S., Wang, L. S., Zhang, Y. Y., Zhu, X. L., Wang, L. S. and Huang, J. (2008) Short-term safety and curative effect of recombinant adenovirus carrying hepatocyte growth factor gene on ischemic cardiac disease. *In Vivo.* **22**, 629-632
- 173 Yang, Z. J., Zhang, Y. R., Chen, B., Zhang, S. L., Jia, E. Z., Wang, L. S., Zhu, T. B., Li, C. J., Wang, H., Huang, J., Cao, K. J., Ma, W. Z., Wu, B. and Wu, C. T. (2009) Phase I clinical trial on intracoronary administration of Ad-hHGF treating severe coronary artery disease. *Mol Biol Rep.* **36**, 1323-1329
- 174 Lu, F., Zhao, X., Wu, J., Cui, Y., Mao, Y., Chen, K., Yuan, Y., Gong, D., Xu, Z. and Huang, S. (2013) MSCs transfected with hepatocyte growth factor or vascular endothelial growth factor improve cardiac function in the infarcted porcine heart by increasing angiogenesis and reducing fibrosis. *Int J Cardiol.* **167**, 2524-2532
- 175 Wang, S., Qin, X., Sun, D., Wang, Y., Xie, X., Fan, W., Liang, D., Pei, X. and Cao, F. (2012) Effects of hepatocyte growth factor overexpressed bone marrow-derived mesenchymal stem cells on prevention from left ventricular remodelling and functional improvement in infarcted rat hearts. *Cell Biochem Funct.* **30**, 574-581
- 176 Zhang, J., Wang, L. L., Du, W., Yu, Y. C., Ju, W. Z., Man, Y. L., Li, X. R., Chen, Y., Wang, Z. D., Gu, W. J., Zhang, F. X., Wang, H., Wu, C. T. and Cao, K. J. (2014) Hepatocyte growth factor modification enhances the anti-arrhythmic properties of human bone marrow-derived mesenchymal stem cells. *PLoS One.* **9**, e111246
- 177 Li, N., Pasha, Z. and Ashraf, M. (2014) Reversal of ischemic cardiomyopathy with Sca-1+ stem cells modified with multiple growth factors. *PLoS One.* **9**, e93645
- 178 Chaparro, R. E., Izutsu, M., Sasaki, T., Sheng, H., Zheng, Y., Sadeghian, H., Qin, T., von Bornstadt, D., Herisson, F., Duan, B., Li, J. S., Jiang, K., Pearlstein, M., Pearlstein, R. D., Smith, D. E., Goldberg, I. D., Ayata, C. and Warner, D. S. (2015) Sustained functional improvement by hepatocyte growth factor-like small molecule BB3 after focal cerebral ischemia in rats and mice. *J Cereb Blood Flow Metab.* **35**, 1044-1053
- 179 Benoist, C. C., Kawas, L. H., Zhu, M., Tyson, K. A., Stillmaker, L., Appleyard, S. M., Wright, J. W., Wayman, G. A. and Harding, J. W. (2014) The procognitive and synaptogenic effects of angiotensin IV-derived peptides are dependent on activation of the hepatocyte growth factor/c-met system. *J Pharmacol Exp Ther.* **351**, 390-402
- 180 Prat, M., Crepaldi, T., Pennacchiotti, S., Bussolino, F. and Comoglio, P. M. (1998) Agonistic monoclonal antibodies against the Met receptor dissect the biological responses to HGF. *J Cell Sci.* **111 (Pt 2)**, 237-247
- 181 Lamblin, N., Susen, S., Dagorn, J., Mouquet, F., Jude, B., Van Belle, E., Bauters, C. and de Groote, P. (2005) Prognostic significance of circulating levels of angiogenic cytokines in patients with congestive heart failure. *Am Heart J.* **150**, 137-143
- 182 Kamppa, N., Makela, K. M., Lyytikainen, L. P., Peltonen, N., Hautamaki, J., Seppala, I., Mononen, N., Goebeler, S., Karhunen, P. J., Hervonen, A., Hurme, M., Jylha, M. and Lehtimaki, T. (2013) Vascular cell adhesion molecule 1, soluble Fas and hepatocyte growth factor as predictors of mortality in nonagenarians: the Vitality 90+ study. *Exp Gerontol.* **48**, 1167-1172
- 183 Morishita, R., Moriguchi, A., Higaki, J. and Ogihara, T. (1999) Hepatocyte growth factor (HGF) as a potential index of severity of hypertension. *Hypertens Res.* **22**, 161-167

- 184 Nakamura, S., Morishita, R., Moriguchi, A., Yo, Y., Nakamura, Y., Hayashi, S., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T. (1998) Hepatocyte growth factor as a potential index of complication in diabetes mellitus. *J Hypertens.* **16**, 2019-2026
- 185 Ueno, S., Ikeda, U., Hojo, Y., Arakawa, H., Nonaka, M., Yamamoto, K. and Shimada, K. (2001) Serum hepatocyte growth factor levels are increased in patients with congestive heart failure. *J Card Fail.* **7**, 329-334
- 186 Rychli, K., Richter, B., Hohensinner, P. J., Kariem Mahdy, A., Neuhold, S., Zorn, G., Berger, R., Mörtl, D., Huber, K., Pacher, R., Wojta, J., Niessner, A. and Hülsmann, M. (2011) Hepatocyte growth factor is a strong predictor of mortality in patients with advanced heart failure. *Heart.* **97**, 1158-1163
- 187 Richter, B., Koller, L., Hohensinner, P. J., Zorn, G., Brekalo, M., Berger, R., Mörtl, D., Maurer, G., Pacher, R., Huber, K., Wojta, J., Hülsmann, M. and Niessner, A. (2013) A multi-biomarker risk score improves prediction of long-term mortality in patients with advanced heart failure. *Int J Cardiol.* **168**, 1251-1257
- 188 Nakamura, S., Moriguchi, A., Morishita, R., Aoki, M., Yo, Y., Hayashi, S., Nakano, N., Katsuya, T., Nakata, S., Takami, S., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T. (1998) A novel vascular modulator, hepatocyte growth factor (HGF), as a potential index of the severity of hypertension. *Biochem Biophys Res Commun.* **242**, 238-243
- 189 Nakamura, Y., Morishita, R., Nakamura, S., Aoki, M., Moriguchi, A., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T. (1996) A vascular modulator, hepatocyte growth factor, is associated with systolic pressure. *Hypertension.* **28**, 409-413
- 190 Hayashi, Y., Saitoh, S., Takagi, S., Tuchihashi, K., Miura, T. and Shimamoto, K. (2002) Hepatocyte growth factor and 24-hour ambulatory blood pressure monitoring. *Hypertens Res.* **25**, 655-660
- 191 Vistoropsky, Y., Trofimov, S., Malkin, I., Kobylansky, E. and Livshits, G. (2008) Genetic and environmental determinants of hepatocyte growth factor levels and their association with obesity and blood pressure. *Ann Hum Biol.* **35**, 93-103
- 192 Yamamoto, Y., Kohara, K., Tabara, Y., Igase, M., Nakura, J. and Miki, T. (2002) Plasma hepatocyte growth factor and the relationship between risk factors and carotid atherosclerosis. *Hypertens Res.* **25**, 661-667
- 193 Hu, Z. P., Wang, B. N., Qian, H. Y., Zhou, Q., Wei, W. and Wang, Y. (2010) Fixed-dose telmisartan/hydrochlorothiazide in comparison with losartan/hydrochlorothiazide in decreasing serum hepatocyte growth factor and improving endothelial dysfunction in hypertensive patients. *Int Heart J.* **51**, 252-258
- 194 Komai, N., Ohishi, M., Morishita, R., Moriguchi, A., Kaibe, M., Matsumoto, K., Rakugi, H., Higaki, J. and Ogihara, T. (2002) Serum hepatocyte growth factor concentration is correlated with the forearm vasodilator response in hypertensive patients. *Am J Hypertens.* **15**, 499-506
- 195 Malatino, L. S., Cataliotti, A., Benedetto, F. A., Stancanelli, B., Bellanuova, I., Belluardo, P., Bonaiuto, L., Tripepi, G., Mallamaci, F., Castellino, P. and Zoccali, C. (2003) Hepatocyte growth factor and left ventricular geometry in end-stage renal disease. *Hypertension.* **41**, 88-92
- 196 Akiyama, Y., Ashizawa, N., Seto, S., Ohtsuru, A., Kuroda, H., Ito, M., Yamashita, S. and Yano, K. (1999) Involvement of receptor-type tyrosine kinase gene families in cardiac hypertrophy. *J Hypertens.* **17**, 1329-1337
- 197 Messaoudi, S., Gravez, B., Tarjus, A., Pelloux, V., Ouvrard-Pascaud, A., Delcayre, C., Samuel, J., Launay, J. M., Sierra-Ramos, C., Alvarez de la Rosa, D., Clement, K., Farman, N. and Jaisser, F. (2013) Aldosterone-specific activation of cardiomyocyte mineralocorticoid receptor in vivo. *Hypertension.* **61**, 361-367
- 198 Rychli, K., Richter, B., Hohensinner, P. J., Kariem Mahdy, A., Neuhold, S., Zorn, G., Berger, R., Mörtl, D., Huber, K., Pacher, R., Wojta, J., Niessner, A. and Hülsmann, M. (2011) Hepatocyte growth factor is a strong predictor of mortality in patients with advanced heart failure. *Heart.* **97**, 1158-1163
- 199 Zhu, Y., Hojo, Y., Ikeda, U. and Shimada, K. (2000) Production of hepatocyte growth factor during acute myocardial infarction. *Heart.* **83**, 450-455
- 200 Matsumoto, K., Okazaki, H. and Nakamura, T. (1992) Up-regulation of hepatocyte growth factor gene expression by interleukin-1 in human skin fibroblasts. *Biochem Biophys Res Commun.* **188**, 235-243
- 201 Nakamura, T. and Mizuno, S. (2010) The discovery of hepatocyte growth factor (HGF) and its significance for cell biology, life sciences and clinical medicine. *Proc Jpn Acad Ser B Phys Biol Sci.* **86**, 588-610

- 202 Bigatto, V., De Bacco, F., Casanova, E., Reato, G., Lanzetti, L., Isella, C., Sarotto, I., Comoglio, P. M. and Boccaccio, C. (2015) TNF-alpha promotes invasive growth through the MET signaling pathway. *Mol Oncol.* **9**, 377-388
- 203 Sorour, A. E., Lonn, J., Nakka, S. S., Nayeri, T. and Nayeri, F. (2015) Evaluation of hepatocyte growth factor as a local acute phase response marker in the bowel: the clinical impact of a rapid diagnostic test for immediate identification of acute bowel inflammation. *Cytokine.* **71**, 8-15

Figure 1 Structure of HGF/Met and molecular signalling components

The HGF α chain is composed by an N-terminal hairpin loop (HL) followed by four kringle domains (K1-K4), while the β chain contains a serine protease homology domain (SPH) devoid of proteolytic activity.

Met receptor β chain, together with extracellular α chain, forms a large semaphorin domain (SEMA), which contains the binding site of HGF. After the SEMA domain, which includes the disulphide bond, the β chain contains a plexin, semaphorin and integrin-rich domain (PSI). These portions of the receptor are linked to the transmembrane segment through four immunoglobulin-plexin-transcriptional factors (IPT) domains. Intracellularly, the β chain of Met receptor includes: i) two phosphorylation sites (Ser985 and Tyr1003) for negative regulation in the juxtamembrane portion; ii) two tyrosines (Tyr1234 and Tyr1235) in the tyrosine kinase domain, which become catalytic upon phosphorylation; iii) a multisubstrate docking site (Tyr1349 and Tyr1356) in the carboxy-terminal tail, whose phosphorylation is required for recruitment of several adaptors and signal transducers.

Figure 2 HGF/Met protects cardiomyocytes from cell death

Representation of signalling mechanisms through which HGF/Met prevents apoptosis, autophagy and oxidative stress in cardiomyocytes after injury.

Figure 3 HGF/Met promotes formation of new vessels through stimulation of endothelial and vascular smooth muscle cells

HGF is a powerful stimulator of proliferation and migration of endothelial cells and is released, together with VEGF-A, by endothelial and smooth muscle cells (SMCs). The link between HGF/Met and VEGF receptors may be represented by neuropilin co-receptors. HGF enhances the endothelial barrier function through Rac1-mediated regulation of actin cytoskeleton and microtubules. HGF is upregulated by Ang1 in endothelial cells and stimulates migration and recruitment of SMCs. HGF counteracts ANG II- dependent oxidative stress in SMCs.

Figure 4 HGF/Met has anti-fibrotic activity

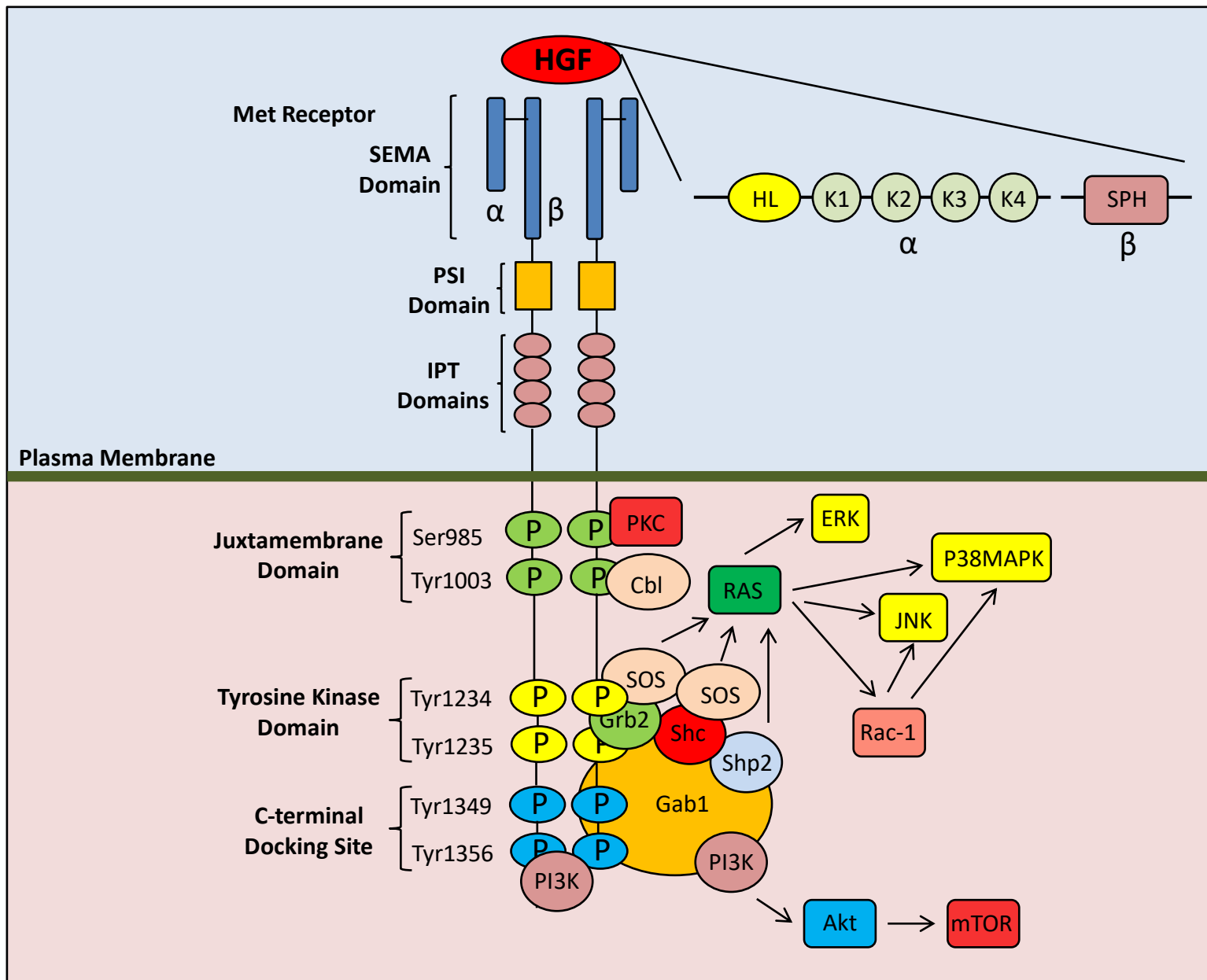
Representation of major molecular mechanisms involved in HGF/Met- mediated anti-fibrotic activity. HGF neutralizes the pro-fibrotic action of TGF- β 1 by multiple mechanisms in fibroblasts: inhibition of TGF- β 1 secretion (1), upregulation of decorin which binds active TGF- β 1 and sequester its action (2), promotion of myofibroblasts apoptosis (3), downregulation of TGF- β 1 synthesis (4), interference with TGF- β 1-initiated Smad signalling (5), protection from oxidative stress (6). In endothelial cells HGF blocks their transformation into myofibroblasts (7) and induces upregulation of iNOS followed by increased production of NO (8).

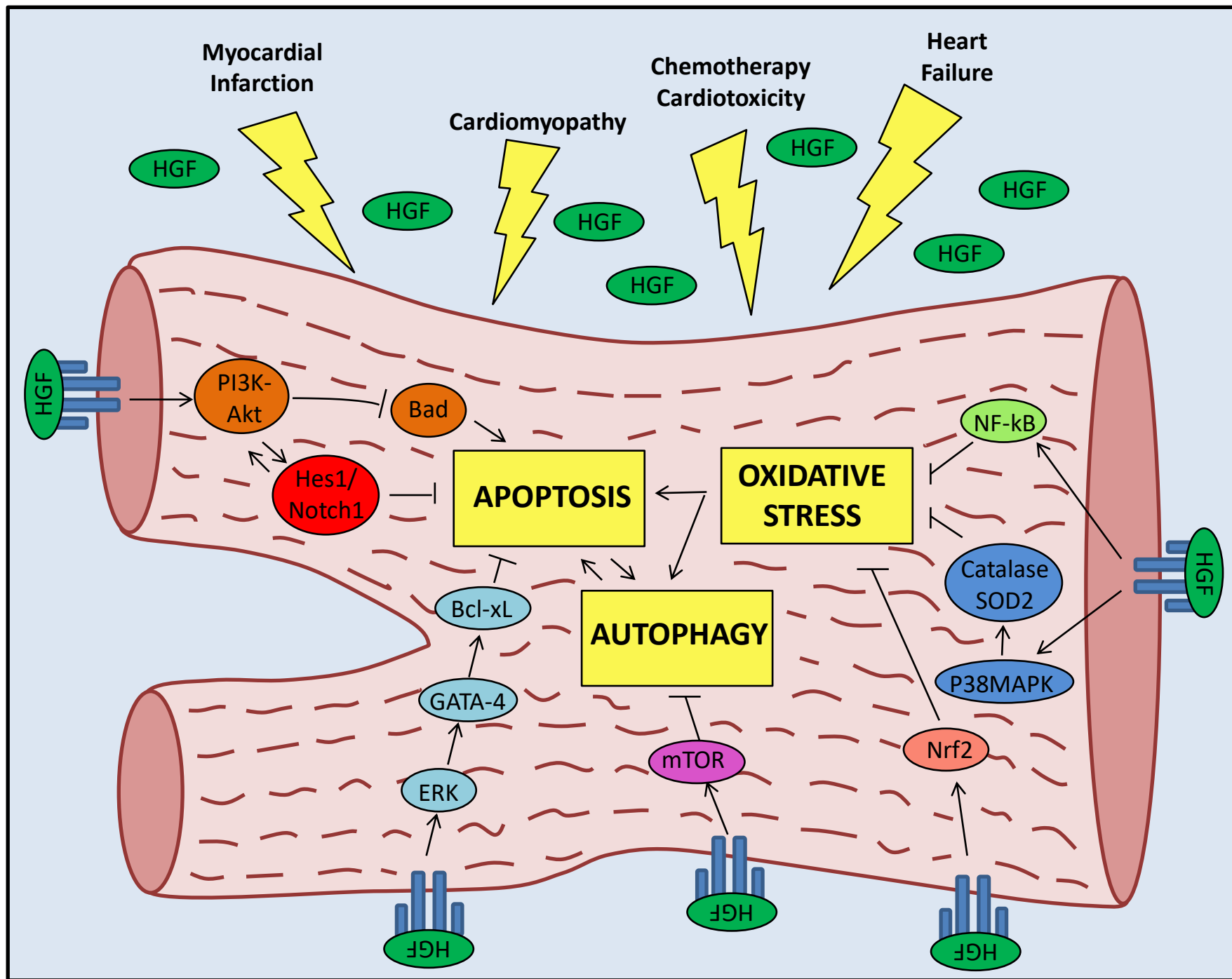
Figure 5 HGF/Met has anti-inflammatory properties in monocytes/macrophages

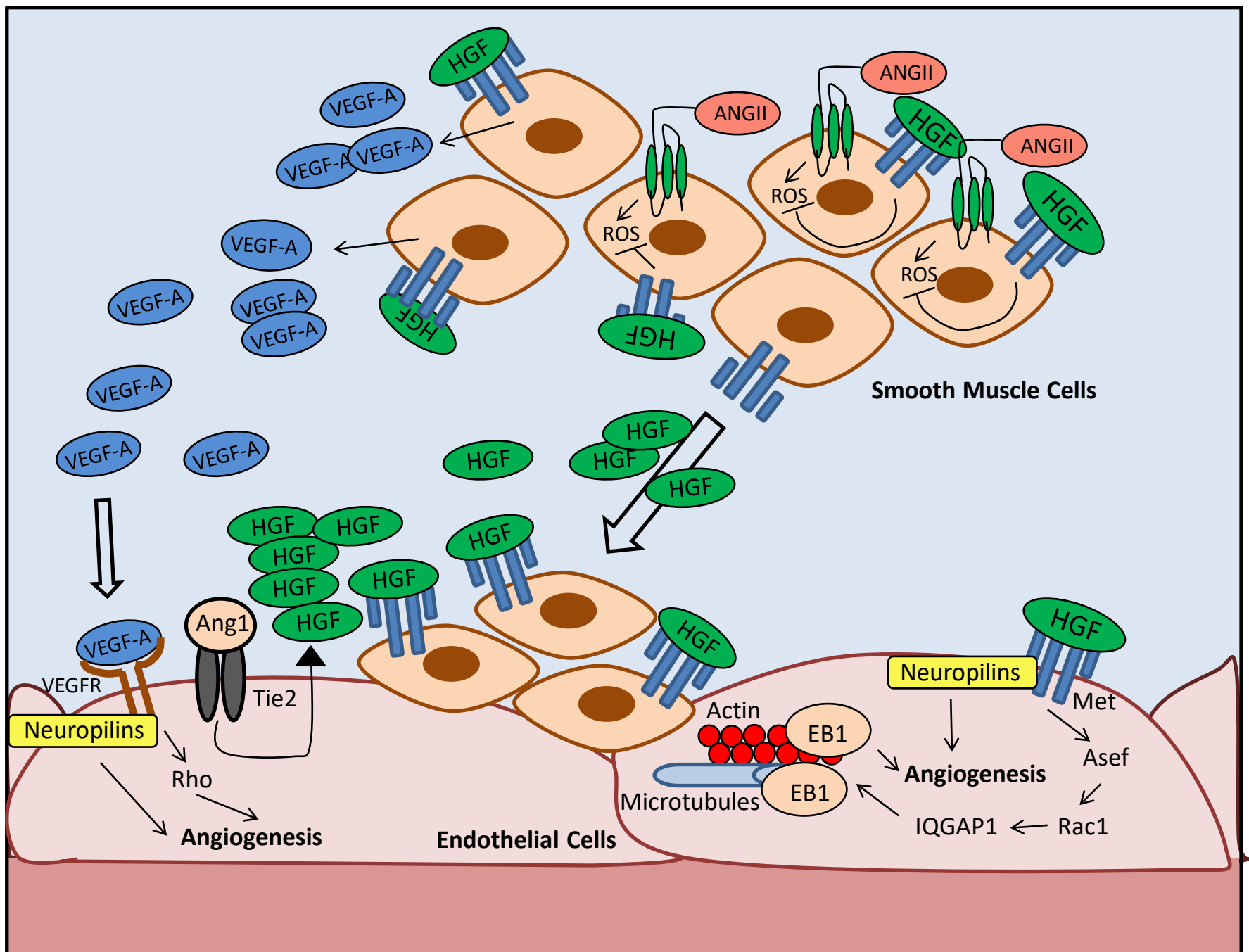
HGF is upregulated in macrophages by LPS-TLR signalling. Stimulation of Met induces phosphorylation and inactivation of GSK3 β preventing the association of p65 NF κ B subunit with CBP. These molecular events result in induction of IL-10 production. IL-10 has anti-inflammatory functions, such as downregulation of adhesion molecules CD18 and CD62-L. In addition, HGF/Met axis exerts its anti-inflammatory activity through decreases in IL-8 and MCP-1 levels.

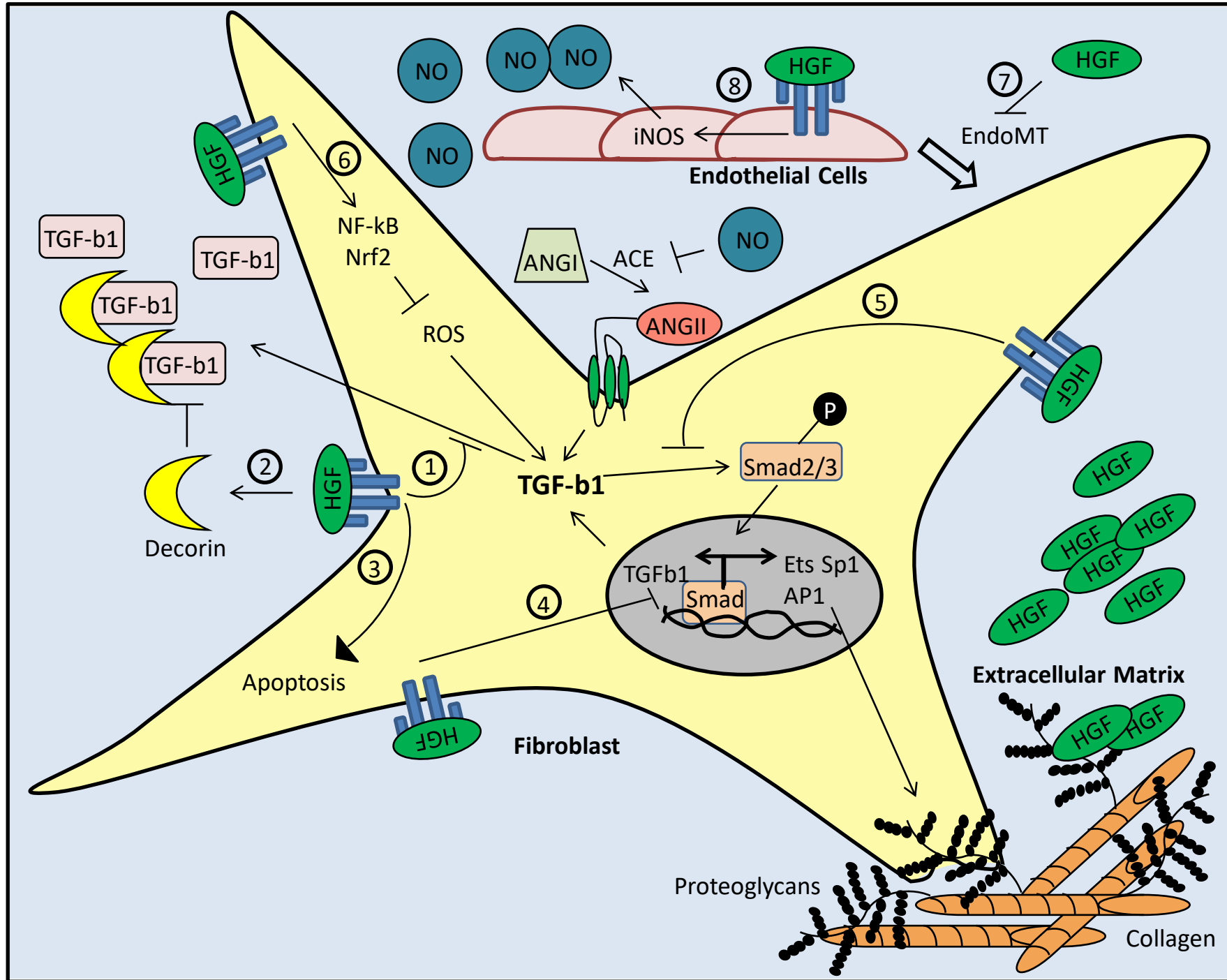
Figure 6 HGF/Met drives cardiac repair and regeneration

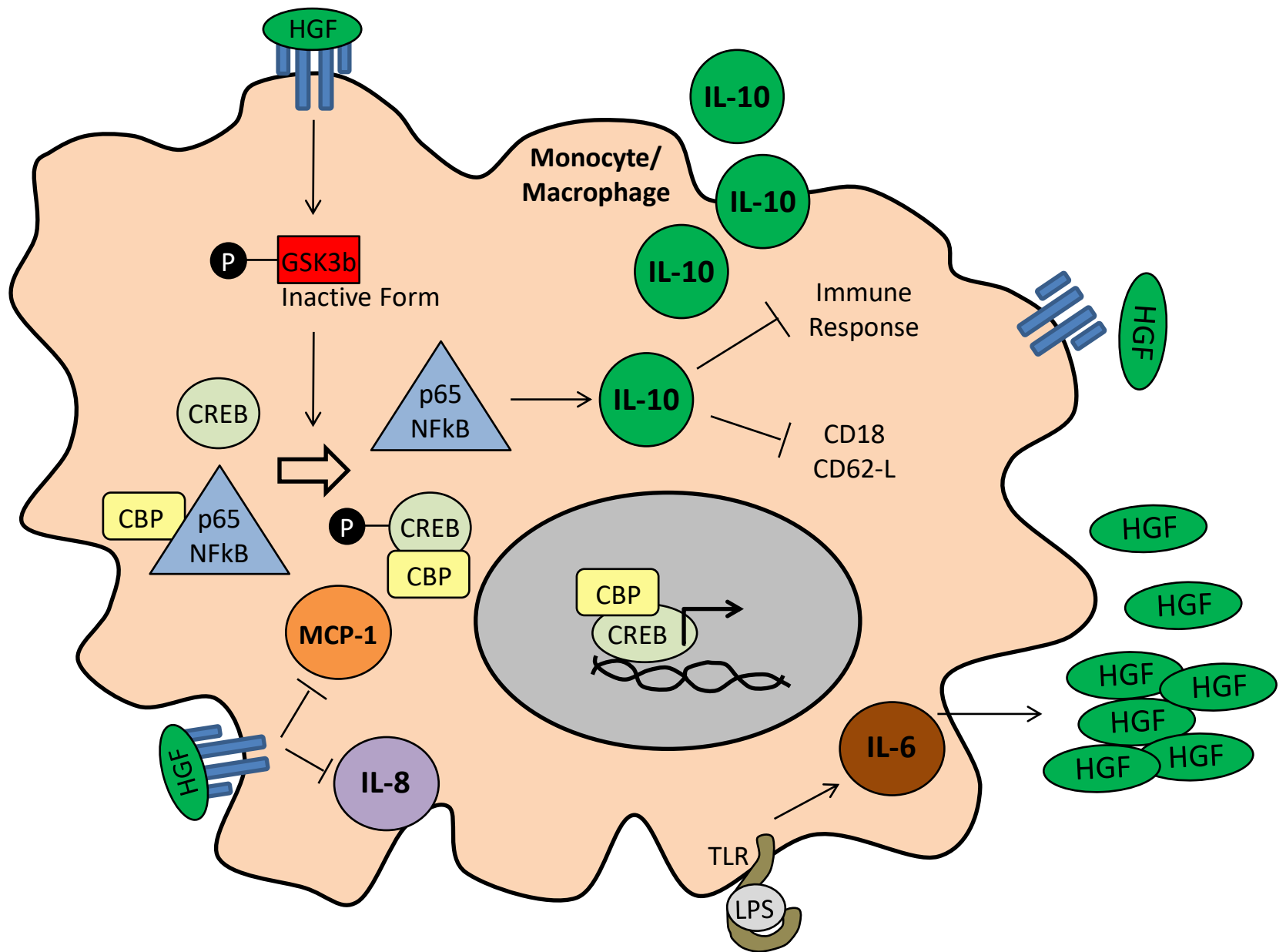
Different pools of progenitor cells contribute to cardiac regeneration: resident cardiac progenitor cells (CPCs) and circulating progenitor cells of various origins. HGF is secreted by mesenchymal stem cells (MSCs), multipotent bone marrow (BM)-derived cells, endothelial progenitor cells (EPCs) and adipose stem cells. HGF stimulates mobilization, expansion and differentiation of CPCs into the three main cardiac populations: cardiomyocytes, endothelial cells and vascular smooth muscle cells. HGF/Met cooperates with Notch1 receptor, which regulates cell fate of CPCs. Another source of progenitor cells is the epicardium. Epicardial cells secrete HGF and undergo the process of epithelial to mesenchymal transformation (EMT), producing epicardial progenitor cells, which differentiate into cardiomyocytes, endothelial cells and vascular smooth muscle cells. HGF/IgG protein complexes and the subsequent Wnt receptor activation are likely the molecular mechanism involved in EMT induction after injury. Finally, HGF mobilizes various different sources of circulating progenitor cells, such as MSCs, hematopoietic progenitor cells, multipotent BM-derived cells, EPCs and adipose stem cells. The mechanism of circulating multipotent BM-derived cells homing to the site of injury involves HGF/Met and SDF-1/CXCR4 chemotaxis.











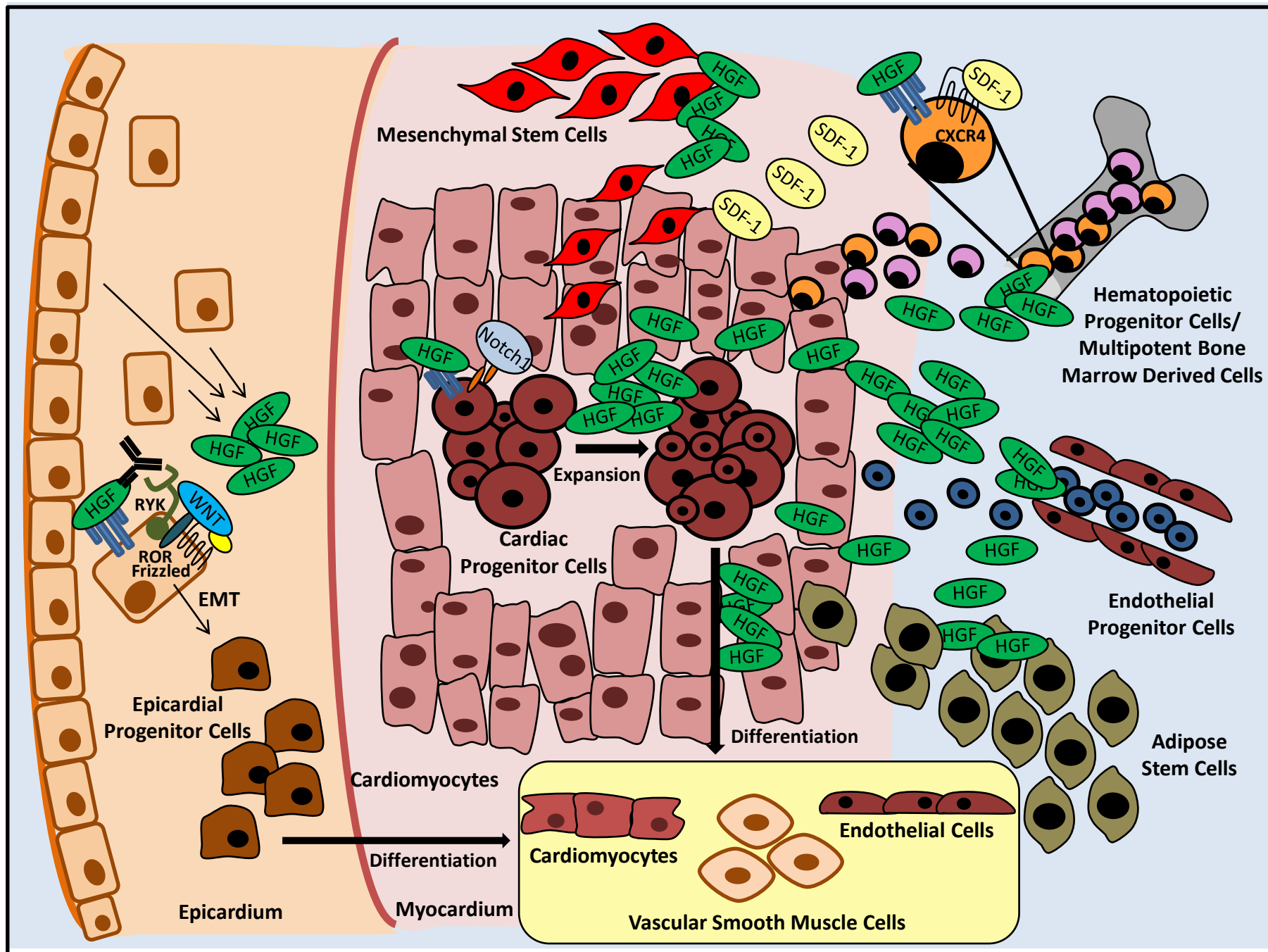


Table 1 **Preclinical Studies of HGF protein carried by hydrogels as therapy for Cardiovascular Diseases**

Reference	Methods	Disease Application	Year	Animal Model	Outcomes
Sakaguchi G. et al. [158]	Placement of HGF-incorporating gelatin hydrogel sheet on the left ventricular free wall of stroke-prone spontaneously hypertensive rats	Heart Failure	2005	Rat	No cardiac adverse effects; Increase of fractional shortening, capillary density and animal survival rate; Reduction of left ventricular diastolic dimension and myocardial fibrosis; Improvement of diastolic functions
Salimath A.S. et al. [160]	Delivered to the infarcted myocardium of a bioactive hydrogels with a protease-degradable crosslinker loaded with hepatocyte and vascular endothelial growth factors	Acute Myocardial Infarction	2012	Rat	No cardiac adverse effects; Increase in angiogenesis and stem cell recruitment; Decrease in fibrosis; Improvement in chronic function
Koudstaal S. et al. [127]	HGF-incorporating pH-switchable and self-healing hydrogel injected in the heart by transendocardial delivery using the NOGA catheter system	Chronic Myocardial Infarction	2014	Pig	Increase of left ventricular ejection fraction, formation of new cardiomyocytes, capillarization and endogenous cardiac stem/progenitor cells population; Reduction of pathological hypertrophy
Sonnenberg S.B. et al. [159]	HGF delivery through an extracellular matrix (ECM)-derived hydrogel	Myocardial Infarction	2015	Mouse	Improvement of tissue blood perfusion; Induction of mature blood vessel network formation
Nakano J. et al. [161]	HGF-incorporated gelatin hydrogel sheets applied to the epicardium	Chronic Myocarditis	2014	Rat	Improvement of fractional shortening and end-systolic elastance; Decrease of fibrotic area and Bax-to-Bcl-2 ratios
Ruvinov E. et al. [162]	HGF in affinity-binding alginate solution injected into the adductor muscle	Hindlimb Ischemia	2010	Mouse	Improvement of tissue blood perfusion; Induction of mature blood vessel network formation

Table 2 Preclinical Studies of HGF gene transfer for Cardiovascular Diseases

Reference	Methods	Disease Application	Year	Animal Model	Outcomes
Aoki M. et al. [55]	HGF gene driven by SRalpha promoter transfected into myocardium by the HVJ-liposome method	Myocardial Infarction	2000	Rat	Increase in PCNA-positive endothelial cells, number of vessels and blood flow
Funatsu T. et al. [163]	Myocardial injection of naked complementary DNA plasmid encoding hepatocyte growth factor	Myocardial Infarction	2002	Dog	Increase in number of myocardial capillaries; Improvement of regional thickening fraction and blood flow
Ahmet I. et al. [168]	Injection of HGF gene directly into the LV myocardium through Japan liposome method	Heart Failure	2002	Dog	LV function improvement; Increase of myocardial perfusion flow and capillary density; Fibrosis and apoptosis reduction
Ahmet I. et al. [164]	HVJ-liposome containing human HGF gene directly injected into ischemic myocardium	Myocardial Infarction	2003	Dog	Increase of percent of nonischemic myocardium, perfusion flow and capillary density
Li Y.W. et al. [167]	Injection into the hindlimb muscle of adenovirus encoding human HGF	Myocardial Infarction	2003	Mouse	Improvement of LV remodeling and dysfunction; Increase of noncardiomyocyte cells (vessels) density; Fibrosis Reduction
Jayasankar V. et al. [77]	Intramyocardial injection of replication-deficient recombinant adenovirus encoding HGF	Myocardial Infarction	2003	Rat	Contractile function and LV geometry preservation; Apoptosis Reduction
Wang W. et al. [165]	Adenovirus5-mediated human HGF transfer into the myocardium via the right coronary artery	Myocardial Infarction	2006	Pig	Improvement of LV ejection, end systolic/diastolic volume and perfusion; Increase collateral arteries and α -SMA+ vessels
Riess I. et al. [56]	Mouse transgenic model with spatial and temporal expression of HGF in the heart	Myocardial Infarction	2011	Mouse	Improvement of cardiac functionality; Enhancement of cardiomyocyte proliferation; Decrease of scarred area
Jin Y.N. et al. [166]	Injection into the infarct border zone of an adenovirus expressing the HGF therapeutic gene	Myocardial Infarction	2012	Rat	Increase of thin capillary density
Okayama K. et al. [81]	Pressure-overloaded in transgenic mice with cardiac-specific overexpression of HGF	Heart Failure	2012	Mouse	Fibrosis decrease; Cardiac function preservation; Inhibition of endothelial cells differentiation into myofibroblasts and conversion of cardiac fibroblasts into myofibroblasts
Taniyama Y. et al. [169]	Human HGF gene intramuscular injection by virus of Japan (HVJ)-liposome method	Peripheral Arterial Disease	2001	Rat	Increase in blood flow and capillary density; Stimulation of MMP-1 production; Activation of Ets-1

Table 3 Human Clinical Trials of HGF or HGF-mimics therapy in Cardiovascular Diseases

Reference	Methods	Phase	Year	Population	Outcomes
Morishita R. et al. [170]	Intramuscular Injection of HGF Plasmid	I/IIa	2011	Critical Limb Ischemia	No serious adverse event; Increase in ankle-brachial index and reduction in ulcer size and in visual analog scale score
Makino H. et al. [171]	Intramuscular Injection of Naked Plasmid DNA encoding HGF	I/IIa	2012	Peripheral Arterial Disease or Buerger disease	No severe adverse effectsIncrease in ankle-brachial pressure index; Reduction in rest pain and in number and size of ischemic ulcers
Clinical Trial: AG-CLI-0206/ NCT02144610	Intramuscular Injections of HGF Plasmid	III	2014	Critical Limb Ischemia	Not Available
Yuan B. et al. [172]	Direct intramyocardial injection of recombinant adenovirus with HGF	Preliminary Clinical Data	2008	Coronary Heart Disease	No adverse effects; Improvement of myocardial perfusion of the Ad-HGF-injected area
Yang Z.J. et al. [173]	Intracoronary administration of adenovirus-mediated human HGF	I	2009	Severe and diffused triple vessel disease	No serious adverse events
Clinical Trial: NCT01539590	BB3 Adjunct to Percutaneous Coronary Intervention	II	2012	Acute ST Segment Elevation Myocardial Infarction	Not Available