Cellular and molecular mechanisms of HGF/Met in the cardiovascular system

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-b1 (transforming growth factor-beta1) 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; GSK3b, glycogen synthase kinase-3 beta; 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-kB, nuclear factor κB; 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-b1, 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 αβ 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 αβ- 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 (phosphatidylinositide 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 latters 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-kB (nuclear factor κB) 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 hypertophic 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-b1 secretion [71-73], upregulation of decorin which binds active TGF-b1 and sequester its action [74], promotion of myofibroblasts apoptosis [75], downregulation of TGF-b1 synthesis and interference with TGF-b1-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-b1, 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-kB 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-b1. 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-b1, on the other side. Importantly, the disruption of the autocrine/paracrine local HGF production by TGF-b1 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/mineralcorticoid 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 angiotensine 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 over-stimulation [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 matriptase [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 proliferaton 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.

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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 proteolitic 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

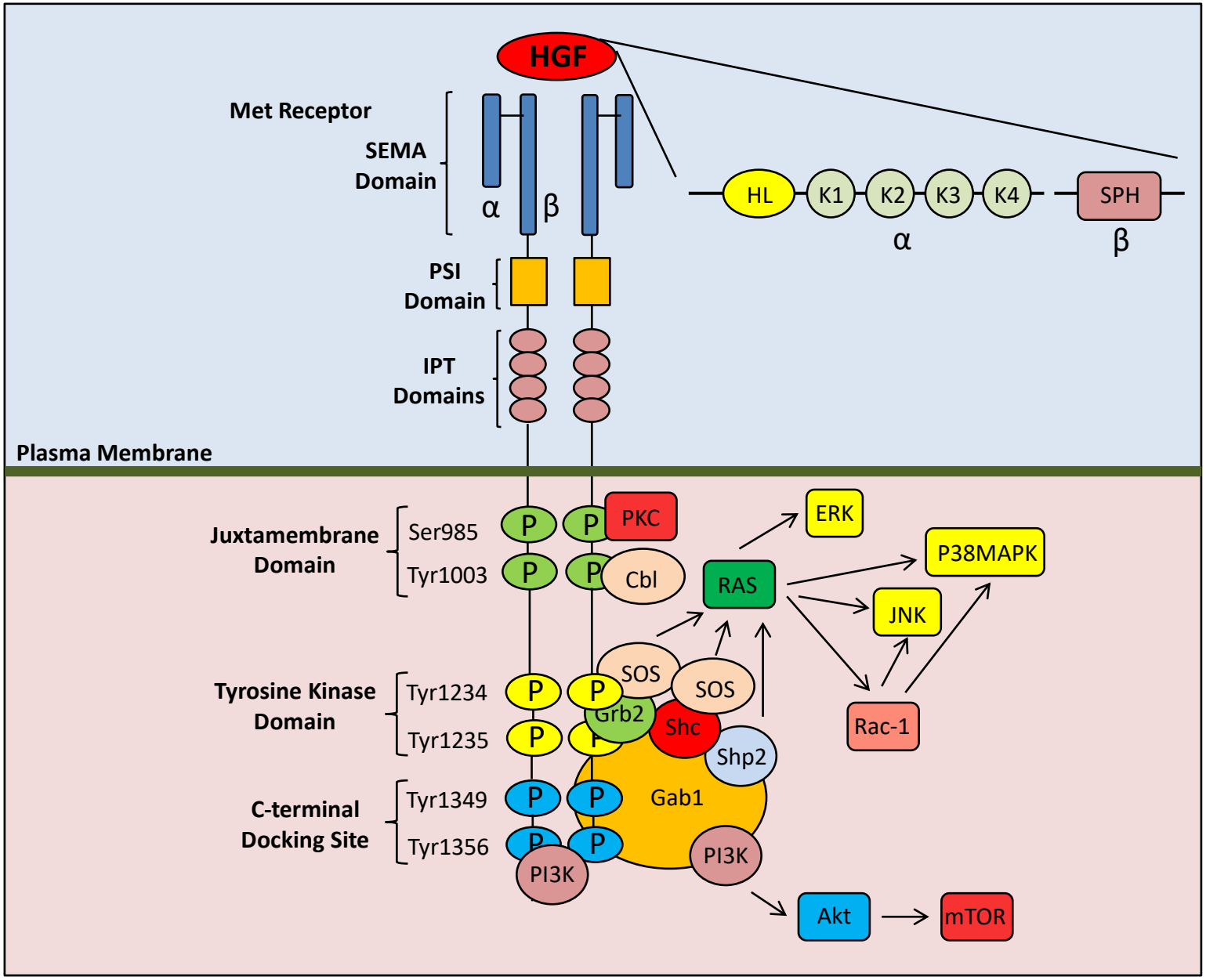
Representation of major molecular mechanisms involved in HGF/Met- mediated anti-fibrotic activity. HGF neutralizes the pro-fibrotic action of TGF-b1 by multiple mechanisms in fibroblasts: inhibition of TGF-b1 secretion (1), upregulation of decorin which binds active TGF-b1 and sequester its action (2), promotion of myofibroblasts apoptosis (3), downregulation of TGF-b1 synthesis (4), interference with TGF-b1-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 GSK3b preventing the association of p65 NFkB 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.


HGF
Met Receptor
SEMA Domain
α
β
HL
K1
K2
K3
K4
SPH
α
β
PSI Domain
IPT Domains
Plasma Membrane
Juxtamembrane Domain
Ser985
Tyr1003
Tyrosine Kinase Domain
Tyr1234
Tyr1235
C-terminal Docking Site
Tyr1349
Tyr1356
P
PKC
Cbl
SOS
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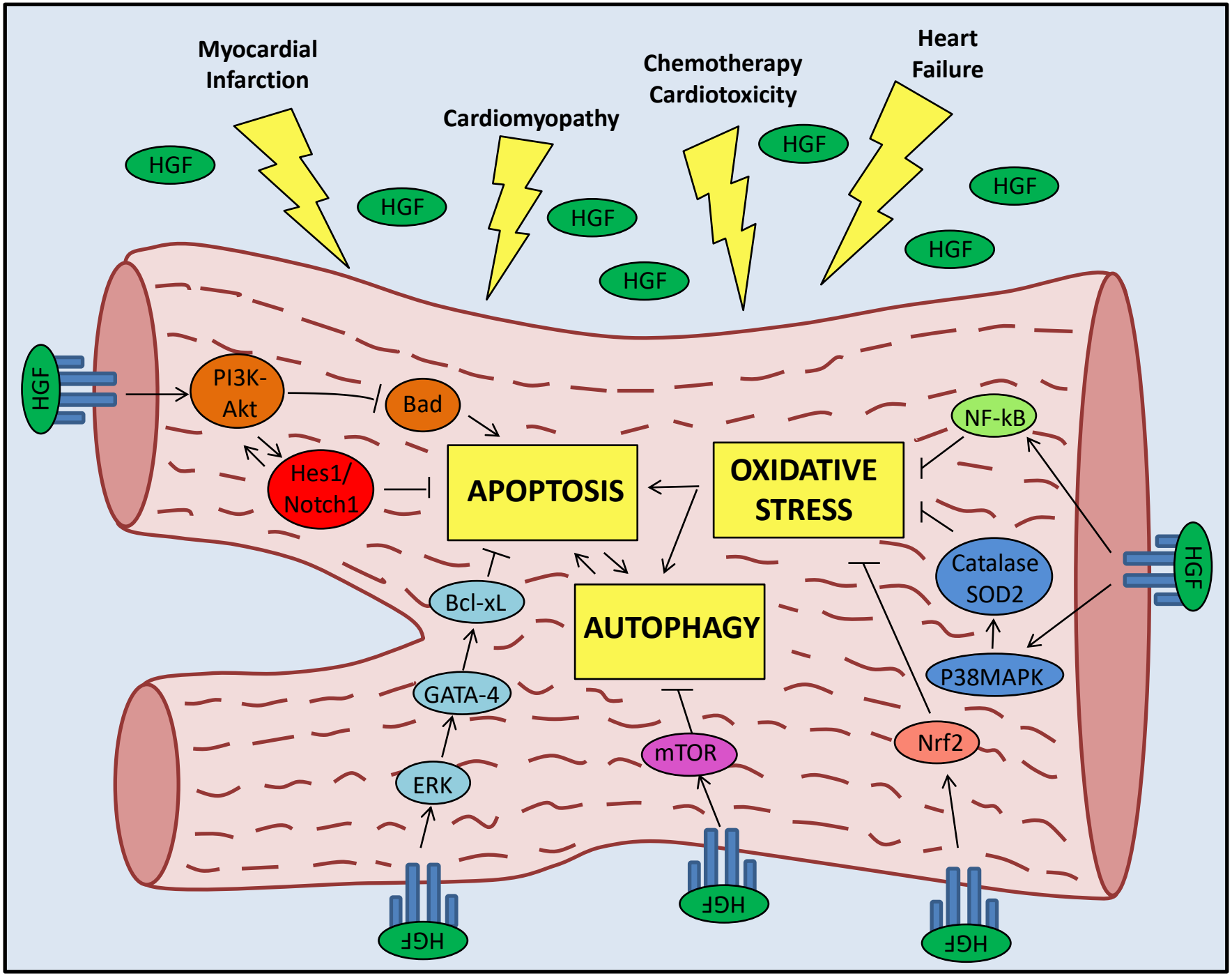
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Cardiomyopathy
Chemotherapy Cardiotoxicity
Heart Failure
HGF
HGF
HGF
HGF
HGF
HGF
HGF
HGF
PI3K-Akt
Bad
Hes1/ Notch1
APOPTOSIS
OXIDATIVE STRESS
NF-kB
Catalase SOD2
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mTOR
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HGF
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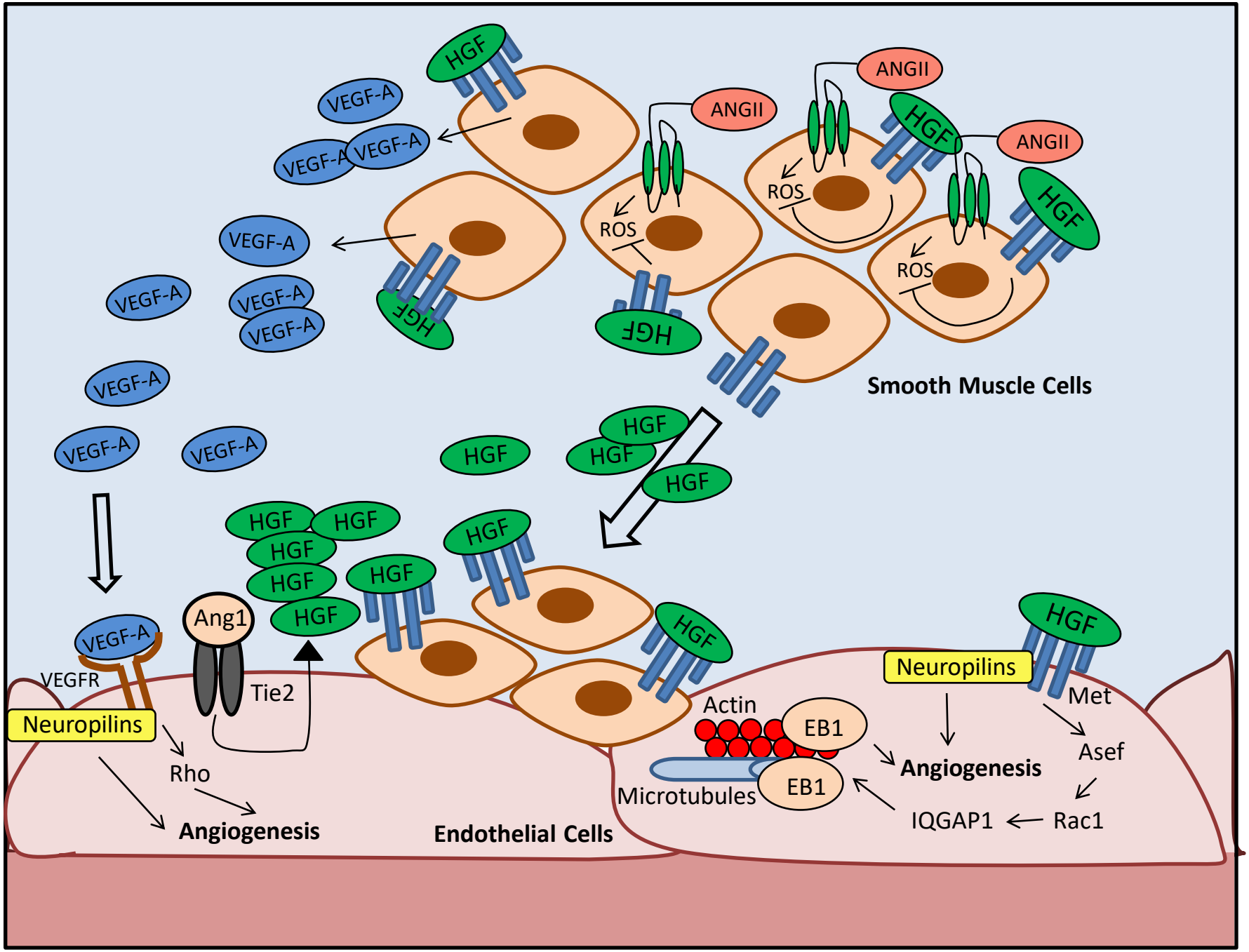
HGF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VEGF-A
ANGII
ROS
Smooth Muscle Cells
Ang1
VEGFR
Tie2
Neuropilins
Rho
Angiogenesis
Endothelial Cells
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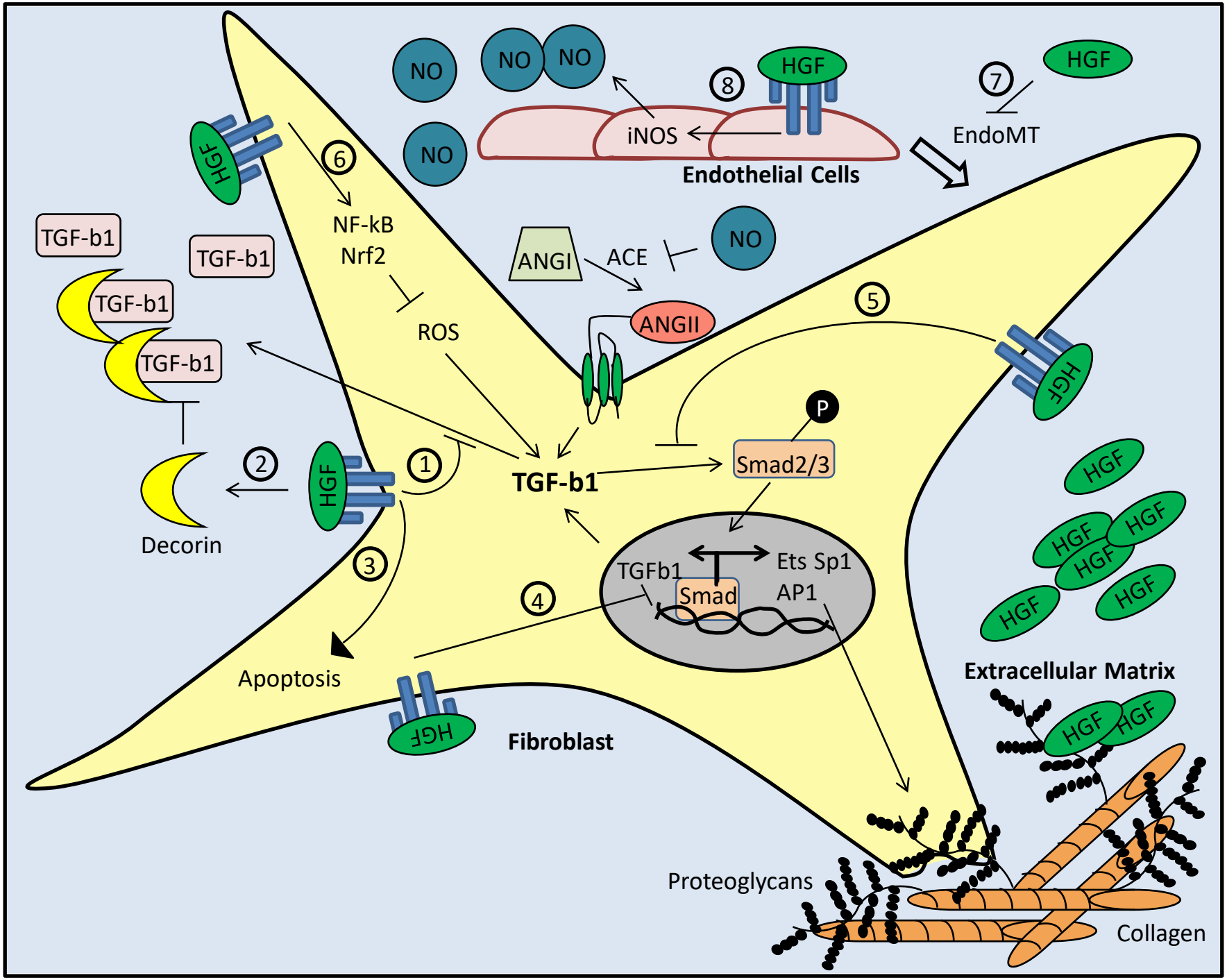
EB1
Microtubules
Met
Asef
IQGAP1
Rac1


NO
NO
NO
NO
HGF
8
iNOS
Endothelial Cells
HGF
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EndoMT
HGF
6
NF-kB
Nrf2
ROS
ANGI
ACE
NO
ANGII
TGF-b1
TGF-b1
TGF-b1
TGF-b1
Decorin
2
HGF
1
TGF-b1
P
Smad2/3
5
HGF
3
4
TGFb1
Smad
Ets Sp1
AP1
Apoptosis
HGF
Fibroblast
HGF
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HGF
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Extracellular Matrix
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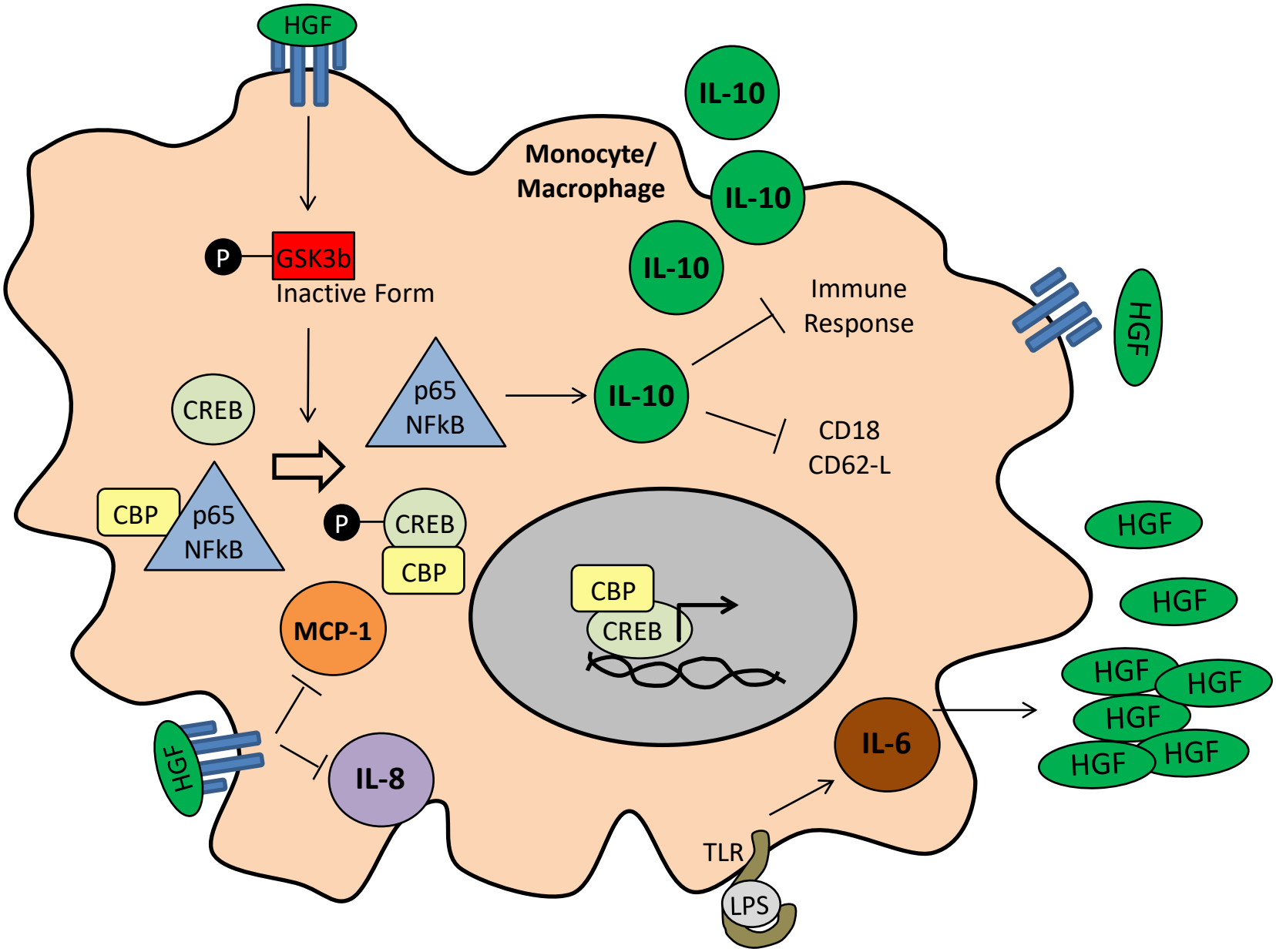
HGF
Proteoglycans
Collagen


HGF
IL-10
IL-10
IL-10
Monocyte/
Macrophage
P
GSK3b
Inactive Form
Immune
Response
HGF
CREB
p65
NFkB
IL-10
CD18
CD62-L
CBP
p65
NFkB
P
CREB
CBP
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IL-6
IL-8
TLR
LPS

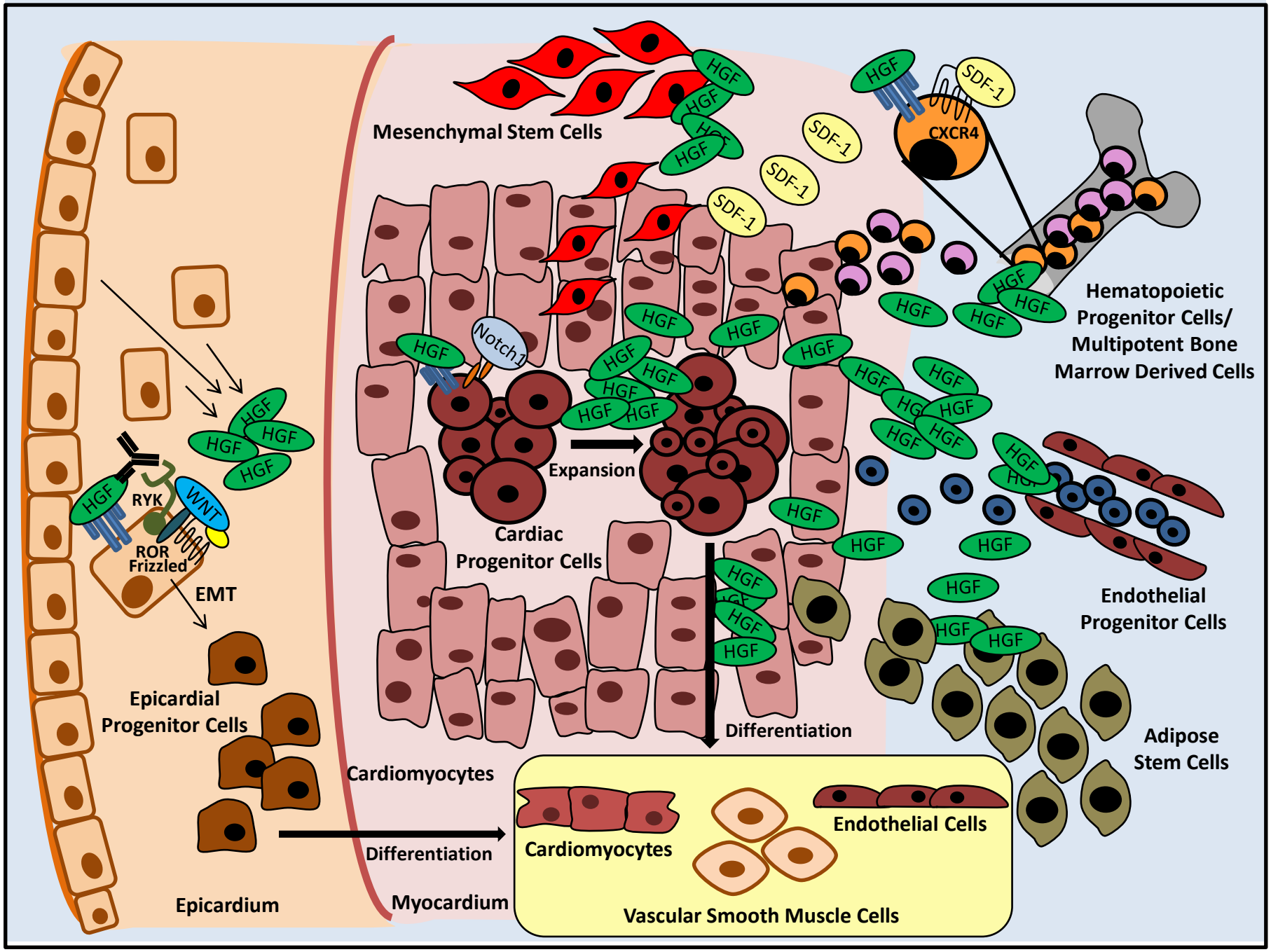
Mesenchymal Stem Cells
HGF
SDF-1
CXCR4
Hematopoietic Progenitor Cells/ Multipotent Bone Marrow Derived Cells
Notch1
Expansion
Cardiac Progenitor Cells
RYK
WNT
ROR Frizzled
EMT
Epicardial Progenitor Cells
Endothelial Progenitor Cells
Adipose Stem Cells
Differentiation
Cardiomyocytes
Endothelial Cells
Vascular Smooth Muscle Cells
Epicardium
Myocardium

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-branchial 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