

FORUM REVIEW ARTICLE

Angiogenesis in the Infarcted Myocardium

Clement Cochain,¹ Keith M. Channon,² and Jean-Sébastien Silvestre¹

Abstract

Significance: Proangiogenic therapy appeared a promising strategy for the treatment of patients with acute myocardial infarction (MI), as *de novo* formation of microvessels, has the potential to salvage ischemic myocardium at early stages after MI, and is also essential to prevent the transition to heart failure through the control of cardiomyocyte hypertrophy and contractility. **Recent Advances:** Exciting preclinical studies evaluating proangiogenic therapies for MI have prompted the initiation of numerous clinical trials based on protein or gene transfer delivery of growth factors and administration of stem/progenitor cells, mainly from bone marrow origin. Nonetheless, these clinical trials showed mixed results in patients with acute MI. **Critical Issues:** Even though methodological caveats, such as way of delivery for angiogenic growth factors (e.g., protein *vs.* gene transfer) and stem/progenitor cells or isolation/culture procedure for regenerative cells might partially explain the failure of such trials, it appears that delivery of a single growth factor or cell type does not support angiogenesis sufficiently to promote cardiac repair. **Future Directions:** Optimization of proangiogenic therapies might include stimulation of both angiogenesis and vessel maturation and/or the use of additional sources of stem/progenitor cells, such as cardiac progenitor cells. Experimental unraveling of the mechanisms of angiogenesis, vessel maturation, and endothelial cell/cardiomyocyte cross talk in the ischemic heart, analysis of emerging pathways, as well as a better understanding of how cardiovascular risk factors impact endogenous and therapeutically stimulated angiogenesis, would undoubtedly pave the way for the development of novel and hopefully efficient angiogenesis targeting therapeutics for the treatment of acute MI. *Antioxid. Redox Signal.* 18, 1100–1113.

Introduction

HEART FAILURE FOLLOWING MYOCARDIAL INFARCTION (MI) remains one of the major causes of death and disability worldwide, and its treatment is a major challenge of today's cardiovascular medicine (99). Despite a wide therapeutic arsenal, recovery of cardiac function and prevention of the transition to heart failure in MI patients remains unsatisfactory, urging the need for the development of novel therapeutic alternatives (99). In the past two decades, proangiogenic therapy to promote reperfusion and function of the ischemic heart appeared a promising strategy, but so far, clinical trials have failed to meet the expectations raised by exciting preclinical studies (69, 112). These disappointing results highlight the need for a comprehensive understanding of the mechanisms of angiogenesis in the ischemic heart as a prerequisite for the development of novel proangiogenic therapies for the treatment of MI.

Why Should We Target Angiogenesis?

Angiogenesis represents the emergence of newly formed microvessels from pre-existing capillaries. When exposed to

angiogenic signals, such as hypoxia, growth factors, or nitric oxide (NO), quiescent endothelial cells become activated. Junctions between endothelial cells loosen and mural cells (pericytes, smooth muscle cells) detach from the vascular wall, resulting in increased vascular permeability. Extravasation of plasma protein allows formation of a provisional matrix onto which endothelial cells migrate. The microvascular sprout is guided by a specialized endothelial cell termed Tip Cell, while the neighboring endothelial cell (termed stalk cell) proliferates to elongate the sprout. Ultimately, a vessel lumen is formed and mural cells are recruited to ensure neovessel stability (19).

This process is to be opposed to arteriogenesis, the maturation and enlargement of blood vessels, and collateral growth, which represents flow-mediated remodeling and enlargement of pre-existing arteries (90). In this review, we will focus on the microcirculation in the ischemic heart, and mainly address angiogenesis and the maturation of newly formed capillaries. Further information regarding collateral growth after MI can be found in other reviews (96).

Ischemia-induced tissue damages and the cardiomyocyte loss depend on several factors, such as the extent of the

¹Paris Cardiovascular Research Center, INSERM UMR-S 970, Paris Descartes University, Paris, France.

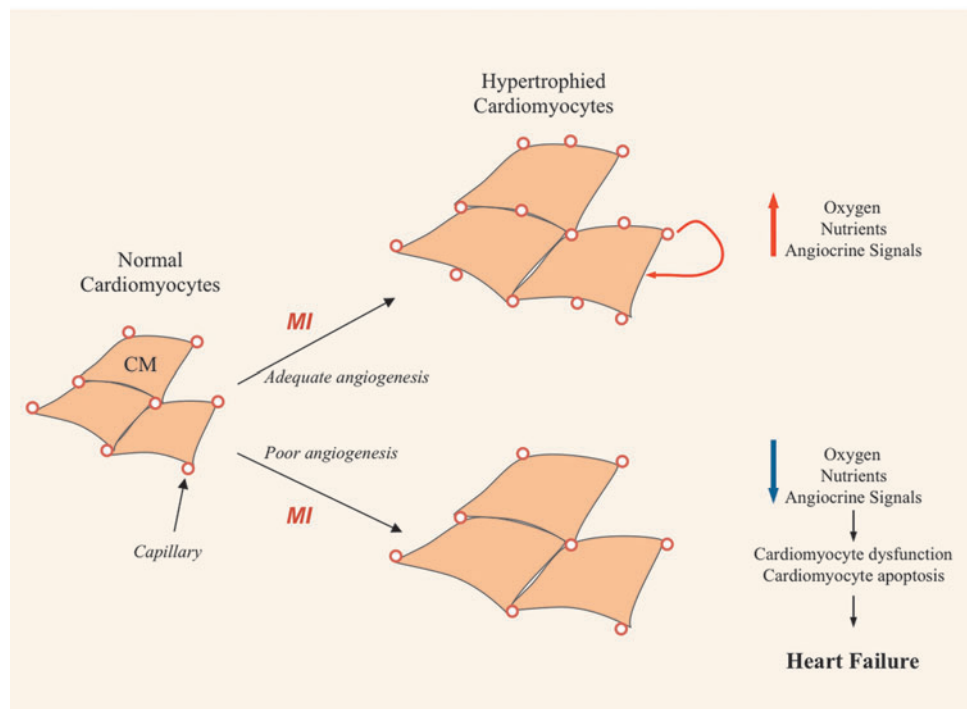
²Department of Cardiovascular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom.

ischemic injury—namely, the size of the initial infarct-, duration of ischemia and efficiency of reperfusion (121). Early reperfusion of the occluded epicardial coronary artery has substantially improved the outcome of MI patients by restoring blood supply to the infarcted area, hence reducing myocardial necrosis (99, 121). However, some patients remain ineligible for such therapy, and microvascular rarefaction and/or dysfunction in the ischemic heart prevent efficient reperfusion of the entire myocardium (114). Hence, *de novo* formation of microvessels, namely angiogenesis, has the potential to salvage ischemic myocardium at early stages after MI, and is also essential for long-term left ventricular remodeling to prevent the transition to heart failure (99, 114). After the initial ischemic event, the infarcted myocardium undergoes a vast process of tissue remodeling, which can be separated in three distinct, but overlapping phases (34). During the early inflammatory phase, macrophages and neutrophils clear the wound of necrotic cardiomyocytes. This is followed by a proliferative phase, where endothelial cells and fibroblasts proliferate to form a vascularized granulation tissue, which then matures into a collagen-rich scar after endothelial cell and fibroblast apoptosis (34). This whole process, in turn, tends to increase the physical load on the neighboring viable myocardium (34). Although angiogenesis occurs in the granulation tissue that will ultimately form the infarct scar, neovascularization of surrounding, viable myocardium in the infarct border zone is also crucial during this process of tissue remodeling. Indeed, hypoperfusion of otherwise viable myocardial areas, consecutive to microvascular dysfunction or increased metabolic demand due to mechanical overload, can promote regional decrease of contractile function, and the extent of microvascular rarefaction or dysfunction is associated with irreversibility of myocardial hibernation. Moreover, efficient perfusion provided by microvessels is required to prevent cardiomyocyte death, which can lead to infarct expansion, left ventricular dilation, and heart failure (101).

Cardiomyocyte growth, survival, and contractile function are dependent upon microvascular function and angiogenesis (Fig. 1), as illustrated by mice deficient in the laminin-alpha4 chain (*Lama4*^{-/-} mice), an extracellular matrix protein abundantly expressed in the basement membrane of blood vessels in the myocardium. In *Lama4*^{-/-} mice, enlargement of the perivascular space around microvessels triggers cardiomyocyte hypoxia and necrosis, and leads to the development of heart failure (116). In pressure overload cardiac hypertrophy, angiogenesis is essential in preventing the transition to heart failure, as inhibition of angiogenesis in hearts undergoing transgenically induced hypertrophy precipitates heart failure (101). On the other hand, angiogenesis itself promotes cardiomyocyte hypertrophy (111). Cardiac-specific overexpression of a secreted proangiogenic growth factor, PR39, produces massive angiogenesis in normal mouse myocardium, spontaneously triggering cardiomyocyte hypertrophy. Most interestingly, angiogenesis-induced cardiomyocyte hypertrophy ameliorates cardiac function after experimental MI (111). In humans, angiogenic imbalance during peripartum cardiomyopathy has recently been described and modeled in mice with cardiac-specific deficiency of PGC-1 α , a strong inducer of Vascular Endothelial Growth Factor (VEGF) expression, and provides a striking example of cardiomyocyte dependence upon the microvasculature, even in the absence of underlying pathology (87).

Endothelial cell/cardiomyocyte cross talk also controls cardiomyocyte contractility (118), and promotes pharmacological protection of cardiomyocytes after ischemia and reperfusion in an NO-dependent manner (64). Similarly, microvascular endothelial cell support organ regeneration of the liver (24) and lung (25) through so called angiocrine signals, which include expression of Hepatocyte Growth Factor (HGF), wnt2, and metalloproteinase (MMP)-14-mediated activation of the Epithelial Growth Factor receptor. This concept

FIG. 1. Model of angiogenesis/cardiomyocyte function coupling after myocardial infarction (MI). After the onset of MI, mechanical overload of cardiomyocyte (CM) in the vicinity of ischemic areas leads to CM hypertrophy. Adequate angiogenesis provides CM necessary perfusion to deliver oxygen and nutrient, and Endothelial Cell/CM cross talk through angiocrine signals could also promote CM function. In contrast, poor angiogenesis leads to CM hypoperfusion, dysfunction, and death, which eventually precipitates heart failure. (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)



might be relevant to heart disease, and angiocrine signals from cardiac microvessels might promote cardiomyocyte survival and function (Fig. 1).

Major Angiogenic Pathways and Their Role in Myocardial Angiogenesis After Infarction

Hypoxia inducible factors

Hypoxia-inducible transcription factors, such as hypoxia-inducible factor 1- α (HIF1- α), mediate cellular adaptation to hypoxia and control both developmental and postnatal angiogenesis (91). HIF1- α is activated by hypoxia and targets a wide array of genes, which include proangiogenic genes, such as VEGF or the progenitor cell mobilizing chemokine Stromal Cell-Derived Factor 1- α (SDF1- α or CXCL12), placing it as an upstream initiator of ischemia-induced angiogenesis (Fig. 2) (91). HIF1- α and the related HIF isoform HIF2- α proteins are expressed in cardiomyocytes, endothelial, and inflammatory cells early after MI, and their expression can persist up to 4 weeks after MI in rats (54). Mice constitutively expressing HIF1- α in cardiomyocytes display improved cardiac function after MI, associated with increased VEGF expression and angiogenesis in the myocardium (55). The prominent role of the HIF pathway in postischemic angiogenesis has further been shown through its indirect activation by Prolyl Hydroxylase Domain proteins (PHDs) and Factor

Inhibiting HIF (FIH) inhibition. Oral administration of a nonisoform-specific PHD inhibitor in rats improved microvascular density in the peri-infarct area, preventing deterioration of cardiac function and left ventricle (LV) dilation after MI (8). Moreover, combined shRNA-induced knockdown of PHD-2 and FIH in mice improved LV function after MI, and also enhanced neovascularization (49). In a model of hindlimb ischemia, shRNA-induced knockdown of PHD-1, -2, or -3 enhanced neovascularization through upregulation of VEGF and endothelial Nitric Oxide Synthase (eNOS) as well as recruitment of proangiogenic myeloid cells (67), further demonstrating the pleiotropic role of the HIF1- α pathway during tissue ischemia angiogenesis. Along this line, HIF1- α controls the extent of endothelial progenitor cell (EPC) recruitment to ischemic areas and angiogenesis through the creation of SDF-1 gradients (20).

HIF-2 α is also able to induce expression of various angiogenic genes, such as VEGF or angiopoietins (92), but study of its role in postnatal angiogenesis has been mostly restricted to tumor models so far. Nevertheless, endothelial-specific HIF-2 α has recently been shown to be essential for ischemia-induced angiogenesis (104). Interestingly, HIF-2 α appeared to possess different, but complementary properties with HIF1- α : HIF-2 α deletion in endothelial cells provoked enhanced angiogenesis, but the newly formed vessels failed to mature, thus leading to poor tissue perfusion (104). These properties of

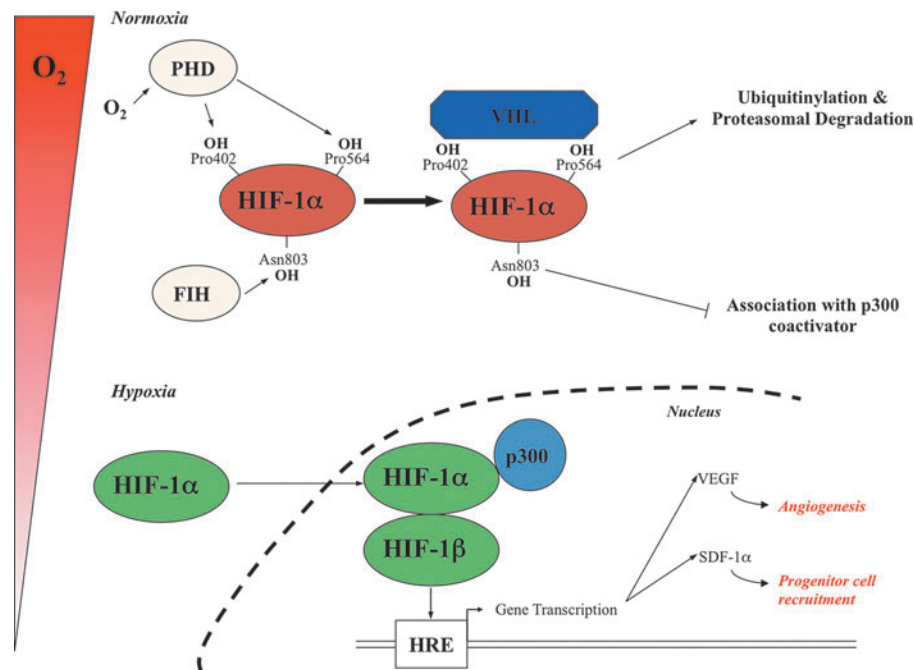


FIG. 2. Oxygen-dependent post-translational modifications of hypoxia inducible factor (HIF)-1 α levels. Under normoxic conditions, HIF-1 α is hydroxylated (OH) on proline residues 402 and 564 (Pro) in the oxygen-dependent degradation domain by specific Prolyl Hydroxylase Domain proteins (PHDs). Once hydroxylated, HIF-1 α is recognised by the von Hippel-Lindau protein (VHL), which is part of an ubiquitin-ligase complex known as E3 ligase complex, which targets HIF-1 α for poly-ubiquitination and subsequent proteasomal degradation. The asparagine 803 (Asn 803) residue of HIF1 α is also hydroxylated by FIH, which impairs the interaction with the transcriptional coactivator p300/CREB binding protein (p300) with the HIF-1 α C-terminal transactivation domain. When oxygen becomes limited, the proline residues are no longer hydroxylated and HIF-1 α μετακινείται στο πυρήνιο, where it dimerises with HIF-1 β and binds to hypoxia-responsive elements (HRE) to the promoter or enhancer sequences of target genes. In addition, interaction with p300/CREB binding protein, due to inhibition of Asn 803 hydroxylation, increases the transcriptional activity of HIF. (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)

endothelial HIF-2 α would be of particular relevance in post-MI angiogenesis, as functionality and persistence of the newly formed microvasculature are required for efficient tissue perfusion.

Growth factors

Numerous growth factors are expressed in the ischemic heart where they promote angiogenesis. For instance, HGF is induced in the myocardium following ischemia (86), and its proangiogenic potential in experimental models of MI is well established (78, 86). Other growth factors, such as Nerve Growth Factor (76) or Insulin-like Growth Factor-1 (26) activate angiogenesis and alleviate cardiac dysfunction after MI, but to date, the most studied growth factors in post-MI angiogenesis are growth factors of the VEGF and Fibroblast Growth Factor (FGF) families.

VEGF family of growth factors

Members of the VEGF family have a prominent role in post-MI angiogenesis (Fig. 3). VEGF acts through binding to its receptor VEGF receptor 2 (VEGFR2), promoting endothelial cell survival, proliferation, and migration (29). The angiogenic properties of VEGF during vascular development or tumor angiogenesis (29) have rapidly led to the evaluation of its role in post-MI angiogenesis. VEGF is rapidly induced in the ischemic heart in humans and rodents (43, 62), not only by hypoxia, but also by mechanical stretch (65). Several experimental reports demonstrated the proangiogenic activity and the associated benefit on cardiac function of VEGF administration in porcine (88) or rodent (97) models of MI. Nevertheless, VEGF alone stimulates the formation of immature, leaky, and disorganized blood vessels (16), and can also inhibit Platelet-Derived growth Factor (PDGF)-BB signaling, an indispensable pathway of vessel maturation. Indeed, VEGFR2 activated by VEGF complexes with the PDGF-receptor β to block signal transduction, hence altering mural cells recruitment and vessel stabilization (40). This phenomenon emphasizes the need of timely regulated angiogenic and ar-

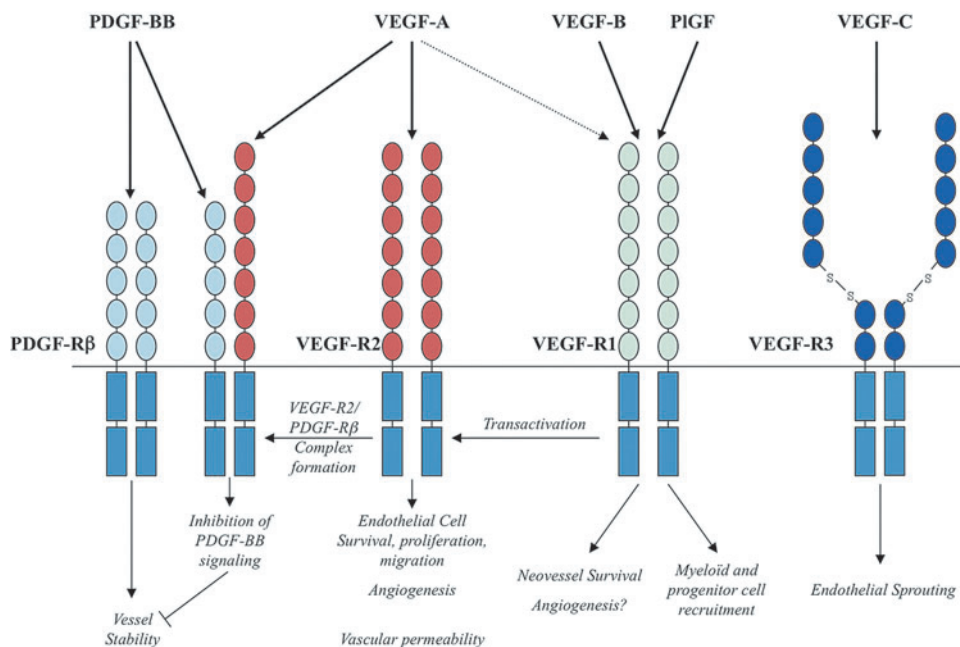
teriogenic signals for the formation of a stable microvascular network.

Another member of the VEGF family of growth factor that preferentially binds to the VEGF Receptor 1 (VEGFR1), Placental Growth Factor (PlGF), also activates angiogenesis in ischemic tissues through two main mechanisms. First, PlGF-mediated activation of VEGFR1 leads to transphosphorylation of VEGFR2 and amplification of VEGF-dependent signaling (6). Second, PlGF promotes the recruitment of proangiogenic VEGFR1 expressing myelomonocytic cells (72) and Sca1+ progenitor cells (52) to ischemic tissues.

The role of VEGF-B in post-MI angiogenesis remains controversial. Although an initial report demonstrated a VEGFR1-dependent proangiogenic effect of VEGF-B in a murine model of hindlimb ischemia (103), subsequent studies have suggested that VEGF-B might induce angiogenesis specifically in the murine infarcted heart, but not in other tissues (66), in a VEGFR1- and neuropilin-1-dependent manner (58). Last, using transgenic mice and rats overexpressing VEGF-B in cardiomyocytes, Bry *et al.* finally suggested that VEGF-B only induced coronary artery growth, but not angiogenesis, in transgenic rat hearts, but not in transgenic mouse hearts (14). Besides these confusing results, a study by Zhang *et al.* interestingly demonstrated that VEGF-B was not required for vessel growth in mice, but was essential for their survival through the induction of various antiapoptotic signals in endothelial cells, but also in mural cells, such as pericytes and smooth muscle cells (126). Hence, one might speculate that although VEGF-B might not be necessary for angiogenesis *per se*, its prosurvival actions may allow newly formed vessels to persist and mature in the ischemic myocardium, providing long-term blood supply to cardiomyocytes and delaying adverse remodeling of the LV.

Finally, the VEGF-C isoform, although mainly known to promote lymphangiogenesis *via* its receptor VEGFR-3 (2), has also been shown to activate angiogenesis after ischemia (119) and might participate to post-MI angiogenesis. VEGF-C angiogenic effect might be indirect, as VEGF-C promotes PDGF-B expression and vessel maturation in ischemic tissues (85).

FIG. 3. Vascular endothelial growth factor (VEGF) family of growth factors-dependent angiogenic pathways. Members of the VEGF family have a prominent role in post-MI angiogenesis through direct effect on endothelial cell survival and proliferation, control of new vessel formation and permeability as well as recruitment of inflammatory and regenerative cells. PDGF, platelet-derived growth factor; PlGF, placental growth factor. (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)



Nevertheless, data indicate that VEGF-C binding to VEGFR-3 directly induces vascular sprouting *in vivo* (108) and promotes tumor angiogenesis (57). Still, the role of the VEGF-C/VEGFR-3 pathway in myocardial angiogenesis has yet to be properly evaluated.

FGF family of growth factors

Several members of the FGF family have the ability to promote angiogenesis, but the comprehension of the mechanisms mediating the angiogenic effects of FGF family growth factors is hindered by the substantial amount of FGF ligands (22 in mice and humans), the existence of four tyrosine kinase receptors and relative redundancy in the FGF system (79). First, acidic FGF (aFGF or FGF1) was identified as an endothelial cell mitogen expressed in bovine brain (79). Basic FGF (bFGF or FGF2) was then shown to be expressed by microvascular endothelial cells and to promote their proliferation and survival in a putatively autocrine manner (79). Although FGF2 seems to induce endothelial cell proliferation and angiogenesis *in vivo*, its effect might be at least partially indirect. Indeed, FGF-2 induces VEGF expression in endothelial cells, and VEGF neutralization blocks the proangiogenic effects of FGF-2 (79). FGF-2 can also induce expression of other proangiogenic cytokines, such as HGF or Monocyte Chemoattractant Protein (MCP-1/CCL2) (79). Several reports have demonstrated that FGF-1 (7, 94) or FGF-2 (9, 117) induced angiogenesis in the ischemic heart and promoted cardiac repair after MI.

Interestingly, in the mouse cornea, FGF-2-induced angiogenesis leads to the formation of stable and mature blood vessels, in contrast to VEGF-stimulated neovascularization, which results in disorganized and leaky microvessels (16). This might stem from the synergy between FGF-2 and PDGF-BB signaling, which improves blood vessel stability and maturation, presumably through induction of PDGF-BB receptors expression (15). Coadministration of FGF-2 and PDGF-BB leads to increased angiogenesis and vascular stability in a model of hindlimb ischemia (15) and in a porcine MI model, where it increased angiogenesis, perfusion of the ischemic myocardium, and subsequently improved cardiac function (70).

Other members of the FGF family promote postischemic angiogenesis. In a porcine model of MI, intracoronary gene transfer of FGF-5 promoted recovery of blood flow and cardiac function (39). Recently, a major role of FGF-9 in vessel maturation and stability has been evidenced (36). FGF-9 was shown to be upregulated in vascular smooth muscle cells during neovessel maturation, and to promote neovessel muscularization rather than endothelial proliferation and angiogenesis *per se*. Interestingly, delivery of FGF-9 in a hindlimb ischemia model stimulated the formation of perfused, multilayered, and long-lasting neovessels (36). Although the effect of FGF-9 has yet to be proven in preclinical models of MI, its properties make it an interesting target for proangiogenic therapies, alone or in combination with other factors targeting endothelial cells.

Nitric Oxide Synthases

The nitric oxide synthase (NOS) enzymes generate NO and are intimately involved in the angiogenic response to ischemia and hypoxia, in part through HIF1- α and VEGF-dependent

pathways described above. Most of the evidence supporting these mechanisms comes from studies of peripheral limb ischemia, but the NOS enzymes also play a role in angiogenesis and ventricular remodeling after MI. In mice with deletion of eNOS (eNOS^{-/-} mice), angiogenesis after MI is reduced, and the ability of statins, known to induce angiogenesis through eNOS-dependent mobilization of EPCs, is reduced in eNOS^{-/-} mice (59). Moreover, treatment of rats with the transcriptional eNOS activator, AVE9488, after MI reduced infarct size, increased eNOS levels, and maintained circulating EPC numbers. The effects of AVE9488 were not seen in eNOS^{-/-} mice compared with wild-type mice after MI (33). More recent evidence suggests that the NOS enzymes and their regulation are more widely involved in myocardial angiogenesis, beyond the well-described role of eNOS. In mouse hearts after MI, inducible NOS and neuronal NOS were both substantially upregulated. Nitrotyrosine formation after MI was reduced in inducible NOS^{-/-} mice, and the ability of the NOS cofactor, tetrahydrobiopterin (BH4), to improve myocardial angiogenesis after MI required the presence of inducible NOS (100), suggesting that inducible NOS coupling is the most important determinant of the effect of BH4 in myocardial angiogenesis and remodeling after MI.

Inflammation

MI induces a rapid and massive influx of inflammatory cells into ischemic areas. These inflammatory cells mainly consist of innate immune cells, such as neutrophils and monocytes (MO) (80). Leukocytes infiltration acts as a double-edged sword, as it can promote beneficial processes, such as dead cell phagocytosis and angiogenesis, as well as induce tissue damage, cardiomyocyte death, and LV dilation (Fig. 4) (35). This is best exemplified by the role of leukocyte-derived proteases urokinase Plasminogen Activator and MMP-9 in the ischemic heart: deletion of urokinase Plasminogen Activator or MMP-9 protects mice subjected to MI from LV dilation and cardiac rupture, but impairs angiogenesis and functional recovery (47).

Historically, MO have first been proposed to promote arteriogenesis and collateral growth in ischemic tissues. Indeed, their presence around collateral vessels is associated with endothelial and mural cells proliferation (3), and the extent of collateral growth in rabbits with hindlimb ischemia is dependent on monocyte circulating levels (46). Nevertheless, MO can also promote angiogenesis through the secretion of various growth factors, such as FGF-2 and VEGF (45) or proteases, such as MMP-9 (53). In mice, monocyte comprises two main subsets, Ly6Chi and Ly6Clo MO, which roughly correspond to human CD14+CD16- and CD14loCD16+ MO, respectively (37). Ly6Chi and Ly6Clo MO infiltrate the ischemic heart sequentially, Ly6Chi MO being predominant in the early phase of myocardial healing, while Ly6Clo monocyte infiltration peaks at later time points (80). The respective role of these subsets in post-MI angiogenesis remains unclear. Although Nahrendorf *et al.* proposed that Ly6Clo MO promoted angiogenesis through VEGF secretion, depletion of either subset in mice hampers neovascularization after MI (80). Moreover, several lines of evidence suggest that Ly6Chi, but not Ly6Clo MO promote angiogenesis in hindlimb ischemia models (17, 22), presumably in a MMP-9 dependent manner (22). Hence, the role of mouse monocyte subsets in

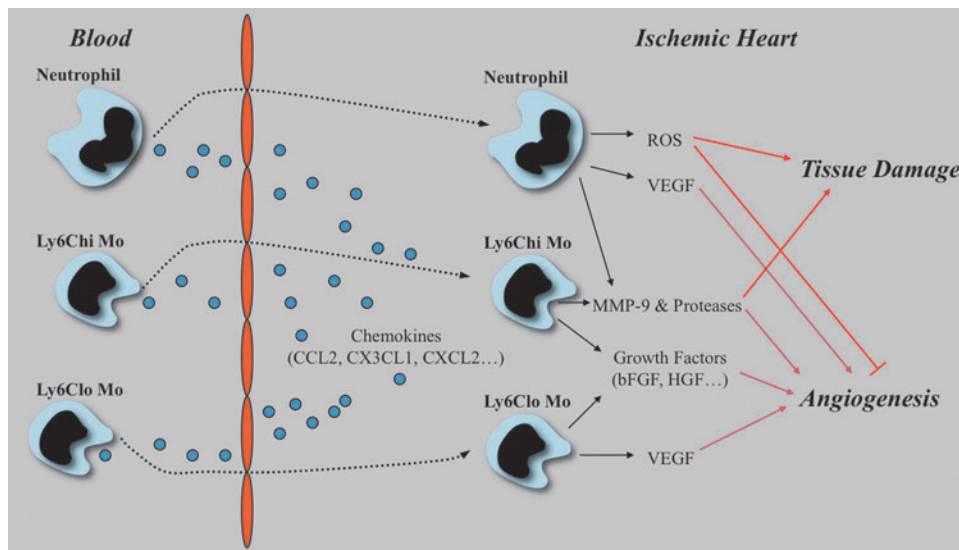


FIG. 4. Multi-faceted role of innate immune cells in the ischemic heart. Ly6Chi and Ly6Clo monocytes (MO) infiltrate the ischemic heart sequentially through the control of chemokines, Ly6Chi monocytes being predominant in the early phase of myocardial healing, while Ly6Clo monocyte infiltration peaks at later time points. Although the respective role of these subsets in post-MI angiogenesis remains unclear, they promoted angiogenesis through growth factor and proteases releases. The role of neutrophils in post-MI angiogenesis is also ill defined. Neutrophils secrete high amounts of elastase, which induces endothelial cell apoptosis and inhibits the proangiogenic effect of progenitor cells. Neutrophils are also a major source of reactive oxygen species (ROS), which, in high quantities, also induce endothelial cell apoptosis. (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)

post-MI angiogenesis, as well as that of their human counterparts, needs to be clarified. Moreover, the impact of monocyte-derived VEGF, initially thought to mediate their proangiogenic effect, appears more complicated than expected. Indeed, monocyte-derived VEGF seems to favor unproductive angiogenesis, as myeloid cell-specific deletion of VEGF produces a stable and mature vascular network in tumors, thus reducing hypoxia in the surrounding tumor cells (106). The relevance of this mechanism has to be evaluated in the setting of post-MI angiogenesis.

The role of neutrophils in post-MI angiogenesis is also ill defined. Even though Granulocyte Colony Stimulating Factor-stimulated neutrophils produce VEGF to stimulate angiogenesis (84), neutrophils secrete high amounts of elastase, which induces endothelial cell apoptosis (120) and inhibits the proangiogenic effect of progenitor cells (51). Neutrophils are also a major source of reactive oxygen species, which, in high quantities, also induce endothelial cell apoptosis (102). Along this line, neutrophils are major mediators of microvascular dysfunction after MI, and notably promote ischemia/reperfusion injury and the no-reflow phenomenon (10, 121).

Adult Progenitor Cells

Endothelial progenitor cells

Discovery of putative circulating EPCs in adults in 1997 (4) has triggered a massive amount of research regarding EPCs biology and their therapeutic potential for ischemic diseases, and in particular MI, in the beginning of the past decade (27). EPCs mainly originate from the bone marrow, but extramedullary EPCs can also be recruited toward ischemic tissues (1). Consequently, whole bone marrow-derived mononuclear cells or medullar cell selected on different markers (CD34+,

CXCR4+, Lin-ckit+...) have often been used as source of EPCs for preclinical studies of therapeutic cell therapy for angiogenesis. Although a substantial number of studies have demonstrated the proangiogenic and therapeutic effect of EPCs in experimental models of MI (27), the mechanisms of EPC-induced neovascularization and the cellular identity of EPCs remain poorly defined (27).

After the seminal discovery of Asahara, the first mechanism of EPC-induced angiogenesis to be proposed has been incorporation of EPCs into a newly formed vascular structure, a process referred to as postnatal vasculogenesis (4). Subsequent studies have shown that EPCs incorporation into neovessels was generally low, and it appears nowadays that the stem cell-like behavior of EPCs and postnatal vasculogenesis cannot solely account for their proangiogenic effects (27). Nevertheless, differentiation of bone marrow progenitors into cells of the vascular lineage is required for their long-term beneficial effect after MI, as elimination of bone marrow progenitors expressing an inducible suicide gene under the control of an endothelial or smooth muscle cells specific gene promoter late after transplantation reverses the original therapeutic benefit (123). The proangiogenic effect of EPCs might at least partially depend on paracrine signals, as EPCs express various proangiogenic cytokines, such as VEGF or Interleukin-8 (50), and injection of a culture medium conditioned by EPCs recapitulates the proangiogenic and therapeutic effects of cell transplantation (23). EPCs can also promote cardiac repair and post-MI angiogenesis by inducing recruitment of endogenous bone marrow-derived cells (BMCs) (21).

One of the major hurdles in EPCs research is the lack of consensus regarding their precise identity. Although efforts have recently been made to standardize the cell surface

markers, isolation procedure and phenotypic properties that define bona-fide EPCs (27, 48), a large number of different EPCs, or EPC-like populations have been used in experimental or clinical studies, and hamper a comprehensive understanding of the existing literature. At least two types of EPCs with divergent properties can be obtained *in vitro* (50). Early EPCs possess a strong paracrine activity, but no vasculogenic potential, while Late EPCs have low paracrine activity, but can incorporate into newly formed vessels (50) (Fig. 5). Interestingly, both cell types can promote postischemic angiogenesis, and act synergistically when cotransplanted (122).

Resident cardiac progenitor cells

Although the myocardium is generally considered to possess poor regenerative ability, the description of multipotent resident cardiac progenitor cells (CPCs) isolated from human and mammalian adult hearts (63) has offered a new alternative for regenerative therapeutics for MI. Similarly to EPCs, consensus is lacking regarding the true identity of CPCs as several markers (Lin-Ckit+, sca1+, Isl+) or isolation procedures (direct isolation followed by expansion or isolation of CPCs arising from cardiospheres during *in vitro* culture of myocardial biopsies) have been used so far (63). Nevertheless,

it is generally recognized that CPCs have the ability to differentiate in several cardiac lineages, including myocytes, vascular endothelial and smooth muscle cells *in vitro* and *in vivo*, and might thus promote neovascularization as well as cardiomyocyte repopulation of the injured myocardium. Although the therapeutic effect of CPCs in animal models of MI has been documented (11, 109), the mechanisms mediating their therapeutic effect, and mainly their ability to differentiate into cardiomyocytes or vascular cells *in vivo* is still controversial as preclinical studies have reported inconsistent results (73). The therapeutic benefit of CPCs transplantation might mostly rely on their paracrine activity, as intracoronary infusion of CPCs in a rat model of MI resulted in very low rates of exogenous cell incorporation, but activated endogenous CPCs to promote functional recovery (109).

Induced pluripotent stem cells

A potential breakthrough in regenerative medicine has emerged from the discovery that adult somatic stem cells, such as skin fibroblasts could be reprogrammed to a pluripotent stem cell state by as few as 4 distinct factors (107). These cells, termed induced Pluripotent Stem cells (iPS), were

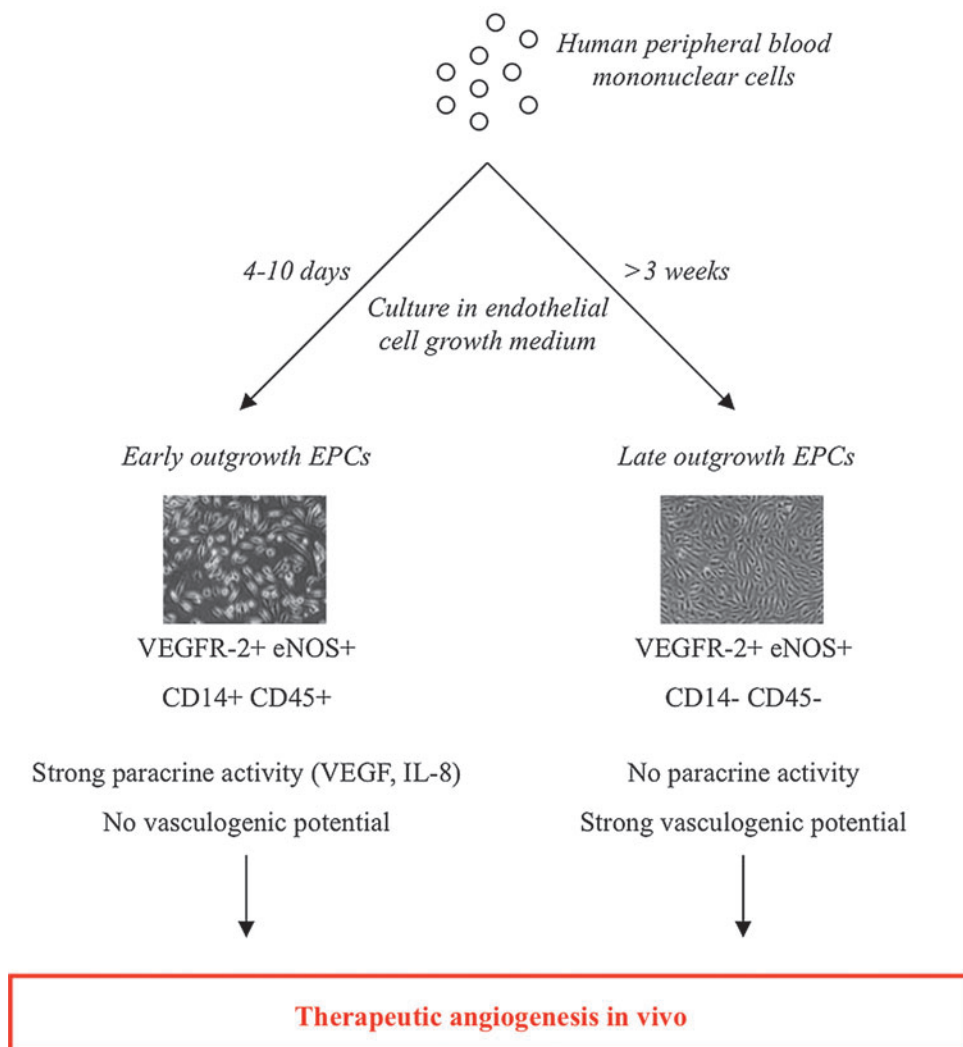


FIG. 5. Two types of endothelial progenitor cells (EPCs) promote therapeutic angiogenesis with different mechanisms. From human peripheral blood mononuclear cells cultured in endothelial cell growth medium, spindle-shaped Early EPCs appear as soon as 4 days of culture, while cobblestone-shaped Late EPCs appear around 3 weeks. Early EPCs express hematopoietic markers and possess strong proangiogenic paracrine, but not vasculogenic properties, while Late EPCs are devoid of hematopoietic markers, have a weak paracrine potential, but strong proliferative and vasculogenic ability. When transplanted in models of tissue ischemia, both cell types can promote therapeutic angiogenesis *in vivo*. Images adapted from Hur *et al.*, (50). (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)

subsequently shown to possess the ability to differentiate in cardiac lineage cells *in vitro*, including cardiomyocytes, vascular endothelial, and mural cells (81). iPS were notably able to promote repair of ischemic myocardium after infarction, where they differentiated into cardiomyocytes, endothelial cells, and smooth muscle cells (82). Endothelial cells differentiated *in vitro* from iPS were also shown to promote angiogenesis in a hindlimb ischemia model (93). Although iPS obviously represent a potentially valuable tool for cell therapy after MI, as they would provide an autologous source of cells with multilineage differentiation capacity, further research is warranted before therapeutic clinical use can be considered. One important concern is the ability of iPS to form teratomas *in vivo*. Moreover, patient-specific generation of iPS is a timely process, which might not compute with the requirement for acute therapy after an ischemic event (83).

Other endogenous adult progenitor cell types, and in particular Mesenchymal Stem Cells and Adipose Tissue-Derived Stem Cells (89) have been endowed with vasculogenic or at least proangiogenic potential and have been successfully used to promote post-MI angiogenesis in experimental studies (56).

miRNA

Micro RNAs (miR) are short RNA molecules that regulate gene expression at the post-transcriptional level. In the past few years, they have emerged as prominent actors in cardiovascular biology, and particularly in post-MI angiogenesis (61). miR-92 thus regulates post-MI angiogenesis through modulation of integrin- α 5 expression in endothelial cells (13). Human miR-424 and its murine homologue miR-322 are induced by hypoxia and indirectly favor HIF-1 α stabilization, hence promoting postischemic angiogenesis (38), and miR-

100 can inhibit angiogenesis by repressing mammalian Target of Rapamycin expression in ischemic tissues (41). Several other miR, such as miR-126 (115), miR-503 (18), or miR-24 (30) regulate postischemic angiogenesis, highlighting the importance of these molecules in the neovascularization process after MI. Targeting miRs for therapeutic angiogenesis after MI could represent a relevant alternative, as miR supplementation or inhibition is technically feasible.

Clinical applications of therapeutic angiogenesis for MI

Exciting preclinical studies evaluating proangiogenic therapies for MI have prompted the initiation of numerous clinical trials (42). Several phase I and a few phase II studies using protein or gene transfer delivery of FGF or VEGF family growth factors have, so far, failed to demonstrate any substantial benefit on LV function in MI patients (42). After the discovery of EPCs and a tremendous amount of experimental evidence pointing toward EPCs or BMCs therapeutic potential for the treatment of MI (68), clinical trials evaluating the benefit of intracoronary administration of BMCs for therapeutic angiogenesis have been conducted in patients with acute MI (5, 71, 77, 95). Although Schachinger *et al.* demonstrated that intracoronary BMCs delivery early after MI (3 to 7 days) improved cardiac function at 4 months and reduced death, recurrence of MI and need for a revascularization procedure at 1 year (95), a simultaneously published trial did not show any benefit of early BMCs transplantation at 6 months of follow-up (71). Moreover, the BOOST trial evidenced a benefit of BMCs transplantation after 6 months that was lost at 18 months follow-up (77). Recently, intracoronary transplantation of BMCs 2 to 3 weeks after MI did not produce any functional benefits (113). Nevertheless, a 2008 meta-

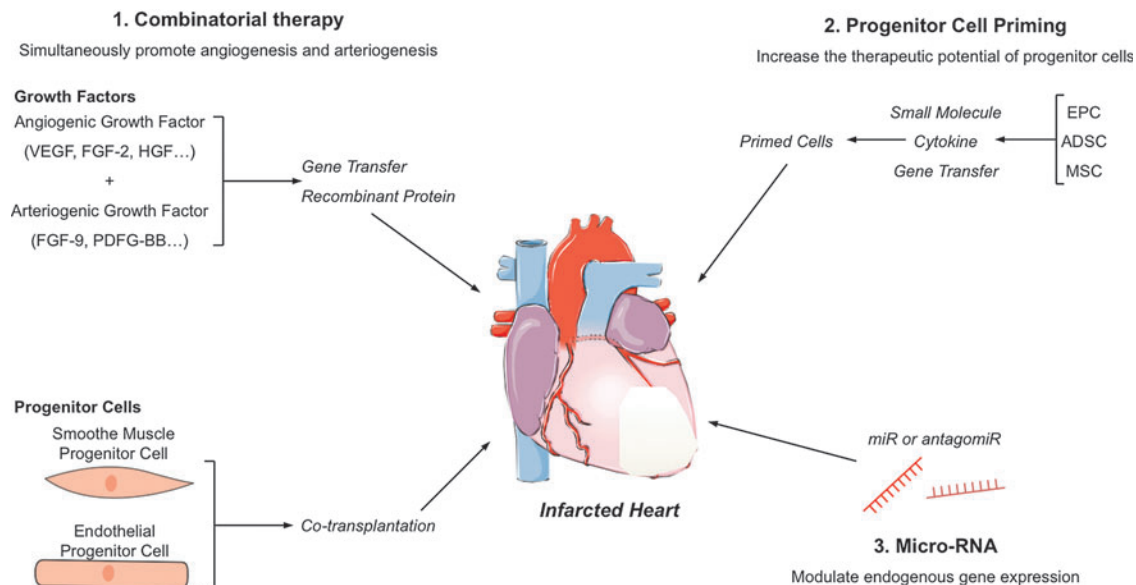


FIG. 6. Future avenues for therapeutic angiogenesis. [1]. Combinatorial therapy with a proangiogenic and a proarteriogenic factor, or cotransplantation of smooth muscle cell and endothelial cell progenitors could promote the formation of a functional and sustainable microvascular network. [2]. Enhancing the endogenous proangiogenic potential of adult progenitor cell through small molecule or cytokine priming, or *in vitro* gene transfer, could increase their therapeutic potential. [3]. Specific microRNA supplementation or inhibition through antagomiR could modulate gene expression to activate endogenous pathways of angiogenesis. ADSC, adipose tissue-derived stem cell; MSC, mesenchymal stem cell. (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)

analysis of 13 trials with a total of more than 800 patients has shown that BMCs transplantation led to a modest (+2.99%), but significant increase in LV ejection fraction in patients receiving BMCs when compared to placebo (75).

Nonetheless, clinical results of proangiogenic therapies remain globally disappointing. Even though methodological caveats, such as way of delivery for angiogenic growth factors (e.g., protein *vs.* gene transfer) (42) or isolation procedure for BMCs (98) might partially explain the failure of such trials, it appears that delivery of a single growth factor or cell type does not support angiogenesis sufficiently to promote cardiac repair. Along this line, several experimental reports have proposed alternatives to optimize proangiogenic therapies, and strategies aiming at simultaneously promoting angiogenesis and vessel maturation appear promising (Fig. 6). As previously mentioned in this review, combined administration of an angiogenic and an arteriogenic growth factor, namely FGF-2 and PDGF-BB, synergistically enhances angiogenesis in the ischemic heart (70), but this concept is also relevant to cell-based therapy. Hence, coadministration of EPCs and smooth muscle progenitor cells is superior to EPCs alone for therapeutic angiogenesis, and produces a mature vascular network in ischemic tissues (31). *Ex vivo* progenitor cell priming with various agents could also increase their proangiogenic and therapeutic potential (32, 125) (Fig. 6).

Recently, the therapeutic use of CPCs in MI patients has been evaluated in two phase 1 trials (12, 74). Although the primary end point of these studies was to assess the safety of CPCs transplantation, both reported encouraging results. Indeed, the SCIPIO trial reported increased LV ejection fraction in the CPCs treated patients 1 year after cell infusion, while in the CADUCEUS study, CPCs delivery led to reduced scar mass, increased viable heart mass, regional contractility and regional systolic wall thickening without significantly improving ejection fraction.

Cardiovascular Risk Factors Limit Endogenous and Therapeutic Angiogenesis

More than being just causes for coronary artery disease and the onset of MI, cardiovascular risk factors, such as hypertension (28), diabetes (105), or hyperlipidemia profoundly impair endogenous and therapeutically induced post-MI angiogenesis, and represent a major difficulty to circumvent in proangiogenic therapy (60). Along this line, several reports have highlighted bone marrow progenitor cell deficiency in diabetic (110), hypertensive (124), or dyslipidemic (44) patients, and might partially explain the disappointing results of proangiogenic BMC therapy for patients with acute MI.

Innovation

Proangiogenic therapy appeared a promising strategy for the treatment of patients with acute myocardial infarction (MI). Exciting preclinical studies have prompted the initiation of numerous clinical trials based on growth factors delivery and administration of stem/progenitor cells. Nonetheless, these clinical trials showed mixed results paving the way for the optimization of proangiogenic therapies, including stimulation of both angiogenesis and vessel maturation and/or the use of additional sources of stem/progenitor cells. Experimental unraveling of the mechanisms of angiogenesis, vessel maturation,

and endothelial cell/cardiomyocyte cross talk in the ischemic heart would undoubtedly increase the efficiency of angiogenesis targeting therapeutics for the treatment of acute MI.

Concluding Remarks

Despite the relative failure of proangiogenic therapies for MI patients so far, targeting angiogenesis to prevent heart failure after MI still appears as a promising strategy, but further mechanistic insight are required to optimize such therapy. Experimental unraveling of the mechanisms of angiogenesis, vessel maturation, and endothelial cell/cardiomyocyte cross talk in the ischemic heart, analysis of emerging pathways, such as miRNA, as well as a better understanding of how cardiovascular risk factors impact endogenous and therapeutically stimulated angiogenesis, would undoubtedly pave the way for the development of novel and hopefully efficient angiogenesis targeting therapeutics for the treatment of acute MI.

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Address correspondence to:
 Dr. Jean-Sebastien Silvestre
 Paris Cardiovascular Research Center
 INSERM U970
 Université Paris Descartes
 56 rue Leblanc
 Paris 75015
 France

E-mail: jean-sebastien.silvestre@inserm.fr

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Abbreviations Used

- ADSC = adipose tissue-derived stem cell
- Asn = asparagine
- BMC = bone marrow-derived cells
- CM = cardiomyocyte
- CPCs = cardiac progenitor cells
- eNOS = endothelial nitric oxide synthase
- EPC = endothelial progenitor cells
- FGF = fibroblast growth factor
- FIH = factor inhibiting HIF
- HGF = hepatocyte growth factor
- HIF = hypoxia inducible factor
- HRE = hypoxia-responsive elements
- iPS = induced pluripotent stem cells
- Lama4 = laminin-alpha4 chain
- LV = left ventricle
- MCP-1 = monocyte chemoattractant protein-1
- MI = myocardial infarction
- miR = micro RNAs
- MMP = metalloproteinase
- MSC = mesenchymal stem cell
- MO = monocytes
- NO = nitric oxide
- NOS = nitric oxide synthase
- OH = hydroxylated
- p300 = p300/CREB binding protein
- PDGF = platelet-derived growth factor
- PHD = prolyl hydroxylase domain proteins
- PIGF = placental growth factor
- Pro = proline
- ROS = reactive oxygen species
- SDF-1 = stromal cell-derived factor 1
- VEGF = vascular endothelial growth factor
- VEGFR = VEGF receptor
- VHL = von Hippel-Lindau protein