Review

Angiogenesis and Cardiac Hypertrophy
Maintenance of Cardiac Function and Causative Roles in Heart Failure

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Abstract: Cardiac hypertrophy is an adaptive response to physiological and pathological overload. In response to the overload, individual cardiac myocytes become mechanically stretched and activate intracellular hypertrophic signaling pathways to re-use embryonic transcription factors and to increase the synthesis of various proteins, such as structural and contractile proteins. These hypertrophic responses increase oxygen demand and promote myocardial angiogenesis to dissolve the hypoxic situation and to maintain cardiac contractile function; thus, these responses suggest crosstalk between cardiac myocytes and microvasculature. However, sustained pathological overload induces maladaptation and cardiac remodeling, resulting in heart failure. In recent years, specific understanding has increased with regard to the molecular processes and cell–cell interactions that coordinate myocardial growth and angiogenesis. In this review, we summarize recent advances in understanding the regulatory mechanisms of coordinated myocardial growth and angiogenesis in the pathophysiology of cardiac hypertrophy and heart failure. (Circ Res. 2014;114:565-571.)

Key Words: anoxia ■ angiogenesis factor ■ heart failure ■ hypertrophy ■ ischemia

The heart is one of the first organs to develop in the embryo. During embryonic development, the heart grows via proliferation and hypertrophy of cardiac myocytes.1 Heart tube formation is initiated around embryonic day 8 in the mouse, and this primitive avascular structure consists of a few layers of cardiac myocytes, which are located adjacent to endocardial cells and receive nutrients and oxygen through diffusion.2 Because the myocardial wall increases in thickness by myocyte proliferation, the endocardial surface area also increases by progressive trabeculation, allowing maximal diffusion. However, as the myocardium grows further, nutrients and oxygen delivered by diffusion become insufficient, a primitive vascular plexus starts to develop shortly after initiation of heart contraction.3 Angiogenic precursor cells, including angioblasts from the proepicardial organ and from the sinus venosus, differentiate into endothelial cells and assemble into a primitive capillary network in a process known as coronary vasculogenesis. Subsequently, this primitive capillaryplexus expands by endothelial sprouting from preexisting capillaries in a process referred to as coronary angiogenesis.2,3

Because cardiac myocytes discontinue the cell cycle soon after birth, subsequent growth of the heart is achieved predominantly by hypertrophy of individual cardiac myocytes during postnatal development. Individual cardiomyocytes increase in size ≥3- to 4-fold after birth.2 The increased oxygen and metabolic demands of growing cardiac myocytes are accommodated by a significant expansion of the myocardial vasculature. These vessels further differentiate and acquire specific properties of coronary arteries or veins. After birth, the myocardial vascular plexus expands through not only angiogenesis but also physiological neovascularization, in which endothelial progenitor cells are involved.2,3

Cardiac hypertrophy in normal growth or in trained athletes is referred to as physiological hypertrophy and is characterized by normal or enhanced contractile function and normal architecture and organization of cardiac structure.4 In contrast, cardiac hypertrophy in patients with hypertension, myocardial infarction (MI), cardiomyopathy, or structural heart diseases is referred to as pathological hypertrophy and is often associated with contractile dysfunction, interstitial fibrosis, and re-expression of fetal cardiac genes, such as genes coding natriuretic peptides and the β-myosin heavy chain.4,5 Pathological hypertrophy is also associated with cardiac structural remodeling and myocardial fibrosis, and sustained pathological hypertrophy leads to congestive heart failure, arrhythmia, and sudden death.6 During the development of hypertrophy, interstitial cells, such as capillary endothelial cells and cardiac fibroblasts, also dynamically undergo a phenotypic change to support contractile function of the myocardium.5,6 According to morphometry of hypertrophied hearts in animal models, capillary microvasculature and myocytes grow in proportion to the increase of heart mass,7,8 suggesting that disproportional

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growth of capillaries and myocytes could cause myocardial ischemia in pathologically hypertrophied hearts. In this review, we summarize recent advances in our understanding of cardiac hypertrophy and myocardial angiogenesis and discuss molecular mechanisms regulating capillary angiogenesis and cardiomyocyte hypertrophy in pathophysiological conditions.

Myocardial Angiogenesis Induces Cardiac Hypertrophy

Under a spectrum of conditions that trigger both physiological and pathological myocardial hypertrophy, the myocardium secretes angiogenic growth factors, which stimulate coordinated vascular growth to meet demands for blood supply that is sufficient to sustain the increase in myocardial mass and performance. Interestingly, enhanced vascular growth promotes myocardial hypertrophy even in the absence of hypertrophic stimulations. Although transgenic overexpression of vascular endothelial growth factors (VEGFs) in the myocardium induced the formation of vasculature with abnormal structure and connectivity without promoting cardiac hypertrophy, transgenic overexpression of proline-arginine–rich with a size of 39 residues or placental growth factor (PlGF) induced cardiac angiogenesis and cardiac hypertrophy. Importantly, proline-arginine–rich with a size of 39 residues and PlGF had no direct hypertrophic effect on cultured cardiomyocytes, suggesting that enhanced formation of coronary vasculature itself leads to myocardial hypertrophy in mice. The precise mechanisms underlying angiogenesis-induced myocardial hypertrophy remain to be fully elucidated. One possible explanation is that superfluous delivery of nutrients and oxygen, transported because of an increase in capillary mass, might simply promote the hypertrophic growth of cardiomyocytes (Figure 1). Alternatively, an increase in capillary mass might boost the production of endothelium-derived secreted factors, which promote myocardial hypertrophy. Endothelium-derived nitric oxide (NO) is one of the critical factors that mediate angiogenesis-induced myocardial hypertrophy (Figure 1). This mediation is evidenced because an NO synthase inhibitor, NG-nitro-L-arginine methylester, or genetic disruption of endothelial NOS were both able to prevent cardiac hypertrophy induced by proline-arginine–rich with a size of 39 residues or PlGF partially, respectively. Mechanistically, NO promoted proteasomal degradation of regulators of G-protein signaling, and thereby potentiated G-protein–mediated hypertrophic signaling involving the phosphatidylinositol 3-kinase γ-thymoma viral proto-oncogene (Akt)/mammalian target of rapamycin C1 pathway (Figure 1). Either NG-nitro-L-arginine methylester treatment or transgenic rescue of regulators of G-protein signaling 4 were able to attenuate the activation of the Akt/mammalian target of rapamycin C1 pathway and cardiac hypertrophy in PlGF transgenic mice. It will be of particular interest to elucidate the mechanisms further underlying angiogenesis-induced myocardial hypertrophy.

Hypertrophic Responses Induce Angiogenesis

A significant increase in the number of myocardial capillaries was observed in physiological cardiac hypertrophy, whereas capillary density was reduced in pathological hypertrophy, suggesting that myocardial capillary number is controlled by the myocardium, and that rarefaction of capillary density may cause myocardial hypoxia and contractile dysfunction (Figure 2). Myocardial angiogenesis is regulated by secreted angiogenic growth factors, including VEGFs, angiopoietin-1 and -2, fibroblast growth factors, transforming growth factors, and platelet-derived growth factors. Among them, VEGFs and angiopoietins are the prime regulators of myocardial angiogenesis, and their functions and roles have been investigated thoroughly. Akt is a serine-threonine protein kinase that mediates hypertrophic growth of cardiac myocytes. Short-term Akt1 activation induced physiological hypertrophy via coordinated upregulation of VEGF expression, whereas long-term Akt1 activation promoted pathological hypertrophy. Shiojima et al have reported, using cardi-specific inducible Akt1 transgenic mice, that short-term Akt activation in the myocardium increased production of angiogenic growth factors, such as VEGF-A and angiopoietin-2, and maintained myocardial capillary density in the adaptive phase. Conversely, inhibition of VEGF signaling resulted in capillary rarefaction and an early transition to heart failure. This study suggested that cardiomyocytes themselves produce angiogenic factors to maintain capillary density, oxygen supply, and their function. Furthermore, in endothelial cells, brief activation of Akt attenuated damage caused by ischemia, whereas prolonged Akt activation results in unorganized blood vessel formation, similar to tumor vasculature. In the heart failure model of Dahl salt-sensitive rats, exercise training attenuated heart failure through further activation of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway, suggesting that effects of Akt signaling on heart failure depend on the timing and length of Akt activation. Transverse aortic constriction, a model of pathological cardiac hypertrophy caused by pressure overload, induced an increase in myocardial oxygen demand, because of a high workload against an increased afterload. In addition, transverse aortic constriction decreased coronary perfusion pressure and increased extrinsic compressive forces on microvasculature. In transverse aortic constriction–operated hearts, coronary resistance also increased, and the imbalance between myocardial demand and oxygen supply led to relative ischemia/hypoxia in hypertrophied hearts. In VEGF-deficient mice, transverse aortic constriction induced cardiac hypertrophy, which was associated with a rarefaction of myocardial capillary density, and accelerated the transition to decompensated heart failure. In contrast, supplementation of VEGF for a failing heart preserved systolic function of the heart. These results suggest that
Angiogenesis

Angiogenesis as a crucial factor in cardiac hypertrophy.

Angiogenesis and Cardiac Hypertrophy

Figure 1. Angiogenesis-induced myocardial hypertrophy. An increase in capillary mass leads to increased production and release of endothelium-derived NO. NO promotes proteasomal degradation of regulators of G-protein signaling 4 (RGS4), and thereby, relieves G protein-coupled receptor (GPCR)–mediated hypertrophic signaling involving the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin C1 (mTORC1) pathway.

Figure 2. Hypertrophic responses induce myocardial angiogenesis. Hypertrophic stimuli activate intracellular signaling and transcription factors, such as GATA4 and Hif-1α. GATA4 induces the gene expression of structural and contractile proteins, resulting in hypertrophy. Hif-1α is also activated by relative hypoxia/ischemia of the hypertrophied myocardium, and Hif-1α transactivates VEGF gene expression to induce angiogenesis. GSK indicates glycogen synthase kinase; mTOR, mammalian target of rapamycin; and VEGF, vascular endothelial growth factor.

Molecular Mechanisms of Transition From Compensated to Decompensated Hypertrophy

Complex pathophysiological changes have been reported to occur in hypertrophied hearts, including suppressed angiogenesis, vascular rarefaction, endothelial dysfunction, ventricular dilation and remodeling, and fibrosis, which physically hamper oxygen diffusion. These complex changes predispose the myocardium to advanced hypoxia/ischemia. For example, progression of heart failure was related to NO imbalance and endothelial dysfunction, and dysregulation of coronary circulation resulted from altered endothelial function. NO reduction in heart failure influenced endothelial progenitor cells, impairing endothelial repair and regeneration.

The action of VEGF is mainly mediated by 2 receptor tyrosine kinases: VEGF receptor 1 (VEGFR-1, also known as FMS-like tyrosine kinase 1) and VEGFR-2 (also known as Flk-1/kinase insert domain protein receptor). VEGFR-1 and its soluble form VEGFR-1 were upregulated in the heart, and soluble form of VEGFR-1 prevented capillary growth by trapping VEGF in pressure-overloaded hearts of rats. Inhibition of soluble form of VEGFR-1 by PGF, which caused the release of VEGF, was sufficient to induce angiogenesis and to provide cardioprotective effects. These results suggest that soluble form of VEGFR-1 is one of the causative factors inhibiting myocardial angiogenesis, and that VEGF is needed to maintain angiogenesis and cardiac function.

Suppression of capillary density and angiogenesis in the myocardium has been observed in the transition from compensated hypertrophy to decompensated heart failure. In the pressure-overloaded heart, the myocardium becomes ischemic, and the DNA-binding activity of hypoxia inducible factor-1 (Hif-1α) increased significantly. Hif-1α is a transcription factor that is stabilized in hypoxic conditions to transactivate various genes encoding hypoxia- and angiogenesis-associated proteins, such as VEGF (Figure 2) and erythropoietin. However, in the course of prolonged pathological hypertrophy, Hif-1α and VEGF were downregulated despite persistent myocardial hypoxia in the hypertrophied myocardium. This mismatch of Hif-1α downregulation in pathologically hypertrophied hearts is one of the critical mechanisms that underlies exacerbated myocardial hypoxia and accelerated myocardial damage and dysfunction.

Suppression of Hif-1 in the Hypoxic Myocardium in the Failing Heart

We have recently reported that transformation-related protein 53 (p53), a tumor suppressor protein, was critically involved in this paradoxical downregulation of Hif-1 in...
pressure-overloaded hearts. Low levels of intracellular p53 were maintained in cardiac myocytes by degradation through the ubiquitin-proteasomal pathway, involving E3 ubiquitin ligases, such as transformed mouse 3T3 cell double minute 2 (MDM2). However, p53 accumulated in cardiac myocytes in response to adriamycin-induced genotoxic stress and in response to hypoxic stress induced by pressure overload or MI. We also observed p53 accumulation in a mouse model of human dilated cardiomyopathy and in adriamycin-induced cardiomyopathy. These observations suggest that p53 accumulation in the heart is a critical part of the common transition from a functionally healthy heart to heart failure. In general, p53 degradation is mediated by MDM2-dependent ubiquitination, and mRNA expression of MDM2 is positively regulated by p53 protein.

In the failing heart occurs through various molecular mechanisms. For example, in adriamycin-treated hearts, p53 levels were elevated in response to reactive oxygen species-mediated indirect genomic DNA damage. DNA damage activates the DNA damage response pathway, in which phosphorylated ataxia telangiectasia mutated kinase phosphorylates p53 at its amino terminus, thus, stabilizing p53 by inhibiting MDM2 binding. Another trigger for p53 accumulation was hypoxia in cardiac myocytes and nonmyocytes, the mechanism of which differs from that of p53 accumulation induced by other stresses, such as DNA damage. Hif-1 was required for hypoxia-induced p53 accumulation and induced p53-dependent transcription via direct binding to p53. In the heart, we identified the carboxyl terminus of heat shock cognate protein 70–interacting protein (CHIP) as one of the E3 ubiquitin ligases required for p53 degradation. CHIP, originally isolated as a cochaperone of heat shock cognate protein 70, had ubiquitin ligase activity, which was attributed to its U-box domain. Although expression of CHIP was not induced by p53 to form a negative-feedback loop-like MDM2, CHIP expression was closely related to p53 expression in cardiomyocytes under hypoxic conditions. We also revealed that Hif-1 mediated suppression of mRNA transcription of CHIP under hypoxic conditions, and that transcriptional suppression of CHIP mRNA was responsible for p53 accumulation in cardiac myocytes under hypoxia and in infarcted hearts of mice. However, in pressure-overloaded hearts, transcriptional suppression of CHIP mRNA by Hif-1 was not observed, and further investigations are needed to clarify molecular mechanisms underlying p53 accumulation in the failing heart.

Considering these previous reports, what are the functional roles of accumulated p53 in heart failure? Two major roles of p53 are (1) cell cycle arrest and (2) inhibition of blood vessel formation. In response to severe cellular damage, p53 arrests proliferative cells in the G1 stage of the cell cycle to induce apoptosis or senescence. Although cardiac myocytes do not proliferate after birth, accumulation of p53 induced apoptotic cell death in cardiac myocytes. In hearts, in vivo chemical inhibition of p53 accumulation or transcriptional activity mitigated adriamycin-induced cardiomyopathy and heart failure after MI. However, previous studies using p53 knockout mice revealed that functional significance of p53 in the regulation of myocyte apoptosis differed according to the disease models. For example, genetic deletion of p53 prevented myocyte apoptosis and cardiac dysfunction in mouse model of heart failure induced by adriamycin treatment, mutation of cardiac α-actin gene (Actc1), and pressure overload. However, p53 deletion did not affect apoptosis of cardiac myocytes in mouse model of heart failure induced by coronary artery ligation.

In the pathological hypertrophied heart, p53 elevation in the myocardium attenuated capillary formation by inhibiting the transcriptional activity of Hif-1 (Figure 3). The expression level of Hif-1 mRNA and transcriptional activity of Hif-1 were increased in pressure-overloaded hearts, possibly because of reduced oxygen supply in the hypertrophied myocardium. Activated Hif-1 promoted angiogenesis to prevent progression of hypoxia and maintained functional homeostasis of the myocardium. However, sustained accumulation of p53, by an undefined mechanism, inhibited the transcriptional activity of Hif-1 and, thereby, promoted progression of maladaptive heart failure. These findings indicate that p53 plays pivotal and pathogenic roles in the progression of heart failure via various pathways, including apoptotic cell death and suppression of angiogenesis in the heart.

**Figure 3. Suppression of hypoxia inducible factor-1 (Hif-1) by transformation-related protein 53 (p53) in the hypoxic failing heart.** In the hypertrophied heart, myocardial angiogenesis is maintained by vascular endothelial growth factor (VEGF), which is induced by Hif-1 in relative hypoxic conditions. However, in the advanced hypoxic condition of the failing heart, Hif-1 is inhibited by p53 accumulation in the myocardium, resulting in the suppression of myocardial angiogenesis and cardiac dysfunction.
Therapeutic Myocardial Angiogenesis as a Potential Therapy for Heart Failure

In hearts manifesting pathological hypertrophy, the capillary density decreased during the transition from cardiac hypertrophy to heart failure. This phenomenon is clinically relevant because an intravascular ultrasound study demonstrated that coronary flow reserve was reduced in patients with hypertension with left ventricular hypertrophy when compared with those without left ventricular hypertrophy. The change of coronary angiogenesis also accompanies cardiac hypertrophy not resulting from pressure overload. When capillary patterns were studied in histological sections, a significant decrease in capillary density was observed in the hearts of patients with dilated cardiomyopathy, ischemic cardiomyopathy, or inflammatory cardiomyopathy. On the basis of these experimental and clinical observations, we found that the therapeutic myocardial angiogenesis is emerging as a promising approach for the prevention and treatment of heart failure.

Several strategies to enhance myocardial angiogenesis have been investigated, including delivery of angiogenic genes or growth factors. The candidate angiogenic factors include VEGF-A, VEGF-B, fibroblast growth factor-2, fibroblast growth factor-5, stromal cell-derived factor-1, hepatocyte growth factor, and midkine. Although long-term stimulation with VEGF-A promoted immature angiogenesis and increased vascular permeability, simultaneous stimulation with VEGF-A and angiopoietin-1 yielded coordinated vascular growth and improved cardiac perfusion and function in rodent models of MI. A combination of fibroblast growth factor-2 and hepatocyte growth factor synergistically stimulated angiogenesis and prevented progression of heart failure in a rat model of MI. To improve the efficacy and safety of the therapeutic interventions for myocardial angiogenesis, further studies are required to determine the optimal combination of angiogenic growth factors and to improve the technology of delivery methods to the myocardium.

One of the potential therapeutic targets is Hif-1, a key transcriptional regulator for the hypoxic induction of angiogenic growth factors. Cobalt is known to stabilize Hif-1α and prevent the decline of contractile function in perfused rat hearts under hypoxia-reoxygenation. According to recent studies, cobalt-induced stabilization of Hif-1α is dependent on copper. Furthermore, copper supplementation reversed contractile dysfunction and prevented transition to heart failure in pressure-overloaded mice, at least in part through promotion of myocardial angiogenesis. In addition to cobalt and copper function, several approved drugs have been reported to affect myocardial angiogenesis. For example, pitavastatin induced myocardial angiogenesis and prevented progression of heart failure in pressure-overloaded mice. The calcium channel blocker, bendipidine, increased capillary density and reduced left ventricular stiffness in Dahl salt-sensitive rats. Although the promotion of myocardial angiogenesis needs much further study before it becomes an established remedy for heart failure patients, a certain amount of preclinical evidence has been accumulated, and the translation of this concept into clinical practice will likely continue in a steady progression in the years to come.

Conclusions

In this review, we summarize the functional association between cardiac hypertrophy and myocardial angiogenesis at molecular and cellular levels. We also discuss dysregulation of capillary angiogenesis in the hypertrophied heart in relation to the transitional process from compensated hypertrophy to decompensated heart failure. Accumulated experimental data provide insights about potential therapeutic strategies for heart diseases. It may be advantageous to stimulate angiogenesis to prevent or reverse heart failure in general or to treat heart failure with a combination of antihypertrophic and proangiogenic agents. However, we still have an array of unanswered questions about an integrative understanding of cardiac hypertrophy and angiogenesis in physiological and pathological conditions. Although it is well established that neurohumoral factors, mechanical and oxidative stresses, metabolic changes, and DNA damage are accompanied by cardiac dysfunction, precise triggers and mechanisms for the disruption of coordinated angiogenesis remain unclear. At the cellular level, it is still unclear how cardiomyocytes, endothelial cells, fibroblasts, and smooth muscle cells coordinate myocardial hypertrophy and angiogenesis in response to environmental changes. Furthermore, experimental studies in this field have been performed mainly using pressure overload and MI, but there is less information about dilated cardiomyopathy or other types of heart failure. Although in theory we consider combination therapy for antihypertrophy and proangiogenesis to be promising, potential molecular targets and mechanisms are still unknown. Further investigations with multidisciplinary approaches would be necessary to resolve these challenging questions and to clarify the whole picture comprised the inextricable link between hypertrophy and angiogenesis in the heart.

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Disclosures

None.

References


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