Minireview

Copper promotion of myocardial regeneration

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Impact statement

Copper promotes angiogenesis, but the mechanistic insights have not been fully elucidated until recently. In addition, the significance of copper promotion of angiogenesis in myocardial regeneration was increasingly revealed. Copper critically participates in the regulation of hypoxia-inducible factor 1 (HIF-1) of angiogenic gene expression. Interestingly, myocardial ischemia causes copper efflux from the heart, leading to suppression of angiogenesis, although HIF-1α, the critical subunit of HIF-1, remains accumulated in the ischemic myocardium. Strategies targeting copper specific delivery to the ischemic myocardium lead to selective activation of HIF-1-regulated angiogenic gene expression. Vascularization of the ischemic myocardium re-establishes the tissue injury microenvironment, and rebuilds the conduit for communication between the tissue injury signals and the remote regenerative responses including stem cells. This process promotes myocardial regeneration. Thus, a simple and effective copper supplementation to the ischemic myocardium would become a novel therapeutic approach to the treatment of patients with ischemic heart diseases.

Abstract

Myocardial regeneration is the key to the functional recovery of ischemic heart. Angiogenesis plays a pivotal role in myocardial regeneration by resetting a rejuvenation microenvironment under ischemic conditions. Hypoxia-inducible factor 1 (HIF-1) is the predominant transcription factor in the regulation of angiogenesis. In prolonged myocardial infarction, HIF-1α, the critical subunit of HIF-1, is accumulated in the infarcted myocardium, but fails to activate angiogenesis, suggesting a missing of a critical factor in the HIF-1 regulation of angiogenesis. Copper is involved in multiple steps of HIF-1 regulation of target gene expression. However, copper is deprived during myocardial ischemic injury, leading to deactivation of HIF-1-regulated angiogenesis. Multiple approaches are applied to increasing copper availability in the ischemic heart, effectively reactivating transcription of HIF-1 target angiogenic genes. Copper-induced angiogenesis thus reconstructs the conduit for the transduction of tissue injury signaling, recruitment of tissue repair materials such as stem cells, and the homing of stem cells, leading to the promotion of myocardial regeneration. Thus, copper promotes myocardial regeneration through reactivation of HIF-1-regulated angiogenesis. This would constitute an alternative therapeutic approach to ischemic heart disease.

Keywords: Copper, myocardial regeneration, angiogenesis, hypoxia-inducible factor 1, stem cell, tissue injury signaling

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Introduction

Loss of cardiomyocytes in the ischemic heart impairs the contractile function of the heart.[1] Myocardial regeneration is the key to functional recovery of the ischemic heart.[2] This process needs sufficient support from microenvironment, including oxygen and nutrition, growth factors and cytokines, suitable extracellular matrix, and supply of repair materials.[3] Blood provides an appropriate microenvironment that guarantees the survival and normal function of cardiac cells.[4] However, blood flow is dramatically blocked in the heart subjected to ischemic insult.[5,6] As a consequence of the deteriorate hypoxic condition, myocardial regeneration is suppressed due to the disturbance of the regeneration-favorable microenvironment. In contrast, collagen deposition takes place to maintain the structural integrity, further deteriorating myocardial contractile function.[6,7]

Angiogenesis is the most promising approach to myocardial regeneration. The increase in blood supply to the injured tissue leads to the improvement of local supply of oxygen, nutrition, growth factors, and cytokines; reestablishment of the communication between the tissue injury signal and the remote repair system, such as mobilization of bone marrow stem cells[8,9]; and enhancement of stem cell homing to ensure the process of myocardial regeneration.

After ischemic injury takes place, a self-repair mechanism is immediately initiated, including angiogenesis in the acute phase of myocardial ischemia.[10] However, this self-repair mechanism is depressed in the chronic phase of myocardial injury.[11] Angiogenesis is regulated predominately by the expression of hypoxia-inducible factor-1 (HIF-1) target angiogenic genes.[12] HIF-1α, the critical subunit of HIF-1, is accumulated in the hypoxic tissue as the result of myocardial ischemia, and remained at a high level as the progression of the hypoxic condition.[13,14] The expression of angiogenic genes is up-regulated within several days, but depressed in the chronic phase, after the injury.[10,14,15] The consistent accumulation of HIF-1α proteins but depressed angiogenesis indicates the missing link between tissue injury signal and the response of the repair mechanism.

Copper is an essential trace element for humans, and is required for the activation of HIF-1-regulated expression of angiogenic genes.[16] However, copper is deprived in the ischemic myocardium.[14,17,18] Multiple approaches to increasing the availability of copper to the ischemic myocardium indeed reactivate the transactivation of HIF-1-regulated angiogenic genes,[19–21] leading to improved angiogenesis and promotion of myocardial regeneration.

In the present review, we summarized the current understanding of HIF-1 regulation of angiogenesis and the critical role of copper in this process, providing a novel insight into the concept of establishing tissue injury signaling for reactivation of self-repair mechanism for myocardial regeneration in ischemic heart disease.[9]

HIF-1 regulation of angiogenesis in ischemic myocardium

Angiogenesis mainly relies on the sprouting of endothelial cells from the existing blood vessels, including the following steps: extracellular matrix modulation, endothelial cells migration, lumen formation, and endothelial cells proliferation and elongation.[22,23] These four steps are regulated precisely by multiple angiogenic factors, including vascular endothelial growth factors (VEGF) and its receptors (VEGFRs),[24] angiopoietin-2 (ANGPT-2),[25,26] placental growth factor (PGF),[27] platelet-derived growth factor B (PDGFB),[28] and stem cell factor (SCF).[12,29] The expression of most angiogenic factors is regulated by a common transcription factor, HIF-1. It was demonstrated that targeting HIF-1 transcription activity to induce angiogenic gene expression was a comprehensive strategy to promote angiogenesis and myocardial regeneration.[30,31]

HIF-1 is a major transcription factor that is activated in response to ischemic injury.[31] HIF-1 is composed of HIF-1α and HIF-1β,[32] in which accumulation of HIF-1α is a key step for the activation of HIF-1 regulation of gene expression.[33,34] HIF-1α contains two different domains in the carboxyl-terminal: the transaction domain[35] and the two oxygen-dependent degradation domains.[36] Under normoxic condition, one or two of the two oxygen-dependent degradation domains (Pro402 and Pro564) on HIF-1α protein are recognized by the prolyl hydroxylase domain-containing proteins (PHDs), catalyzing the hydroxylation reaction.[33,37,38] The hydroxylated HIF-1α is recognized by the von Hippel–Lindau protein (pVHL) of the ubiquitin ligase complex, leading to degradation of the HIF-1α subunit by proteasome.[39,40] Under hypoxic conditions, in contrast, HIF-1α proteins escape from the ubiquitination degradation pathway to accumulate and translocate into the nucleus to form heterodimer with HIF-1β. Interaction with co-factors including CREB binding protein (CBP) and P300 in transaction area is necessary for the formation of HIF-1 transcriptional complex. Then the HIF-1 transcriptional complex binds to hypoxic response elements (HREs), containing the core of HIF-1 binding site sequence 5′-RCGTG-3′ (where R stands for G or A), to initiate expression of target genes. Stabilization of HIF-1α proteins by some transition metals, such as cobalt and nickel, could enhance the transcriptional activity of HIF-1. Factor inhibiting HIF-1 (FIH) is an asparaginyl hydroxylase in the nucleus, catalyzing hydroxylation of asparagine in the C-terminal of HIF-1α, to inhibit the formation of HIF-1 transcriptional complex, leading to suppression of genes expression.

Activation of HIF-1 target gene expression of angiogenic factors is essential for angiogenesis and myocardial regeneration. After ischemic injury, HIF-1α was accumulated immediately in the ischemic myocardium to initiate downstream gene expression, including multiple genes participating in angiogenesis. For instance, the mRNA levels of VEGF, VEGFR1, and ANGPT2 were elevated within a week (the acute or early phase) along with the accumulation of HIF-1α in the ischemic heart.[10] However, after four weeks of ischemic injury (the chronic or prolonged phase), although the accumulation of HIF-1α remained increased, the expression of the angiogenic factors was significantly suppressed, leading to depressed angiogenesis.[14,20] Several studies have focused on enhancing HIF-1 transcriptional activities by using cardiac-specific HIF-1α-overexpressing transgenic mice to restore the expression of downstream genes. The results showed that the elevation of HIF-1α provided a protection for myocardium via preservation of angiogenesis from diabetes-induced impairment of glucose metabolism.[41] It should be noted that this overall increase of HIF-1α proteins would result in a non-specific activation of all HIF-1 target genes, including genes controlling apoptosis and metabolism.[42] It would produce a potential threatening to the ischemic heart.

In the prolonged myocardial ischemic infarction, HIF-1α protein remained accumulated in the infarct area but the expression of HIF-1 target genes was suppressed in human studies[43] and animal models.[13,14] This contradictory phenomenon suggests a missing link between the accumulated HIF-1α proteins and the expression of angiogenic genes (Figure 1). Finding this missing link would provide an

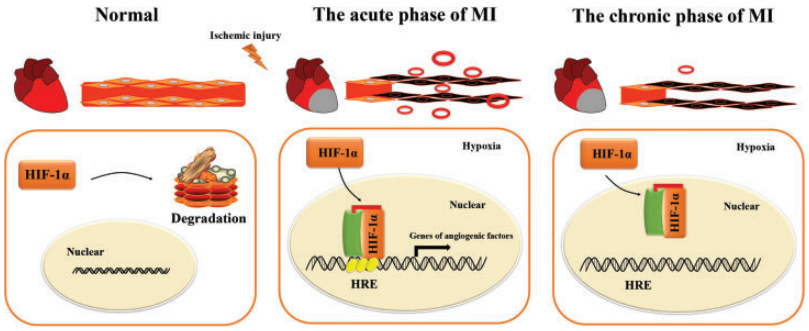

Figure 1. HIF-1 regulation of angiogenesis in myocardial ischemia. HIF-1 is a major transcription factor that is activated in response to ischemic injury. Under normoxic condition, HIF-1α is unstable and prone to degradation. Under hypoxic conditions, HIF-1α is accumulated in the cytosol and translocated to the nucleus, forming the HIF-1 transcriptional complex, and then binding to the HRE sequence to initiate the expression of pro-angiogenic genes. During the acute phase of ischemic injury, angiogenesis is activated in the injured heart under the control of the elevated angiogenic factors. However, in the chronic phase of myocardial ischemia, the sustained accumulation of HIF-1α does not result in an elevation of HIF-1 transcriptional activity. Insufficient signals of angiogenesis thus suppress the process of vascularization, ultimately leading to inadequate blood distribution to the injured myocardium resulting in ischemic infarction. (A color version of this figure is available in the online journal.)

alternative therapeutic target to reactivate angiogenesis and promote myocardial regeneration in ischemic heart disease.

Copper regulation of HIF-1 transcriptional activity

The essential role of copper in angiogenesis has been known for several decades since McAuslan and Gole[44] first discovered that copper induced intraocular vascularization in anterior chamber implants in rats in 1980. Later in 1982, *in vivo* studies conducted in other species by Ziche *et al.*[45] and Raju *et al.*[46] showed that copper stimulated microvessel formation in avascular corneas of rabbits. Further studies continued and supported that copper is an angiogenesis stimulator affecting numerous processes of angiogenesis, including endothelial cell proliferation,[47–49] migration,[49–51] tube formation,[49,52] and vessel maturation.[53] This pro-angiogenic effect of copper was primarily through the regulation of a series of adhesive and growth-promoting factors involved in angiogenesis, such as VEGF.[54] Therefore, it was reasonable to suggest that copper is involved in the regulation of transcription factors for these pro-angiogenic genes.

HIF-1, the key transcription factor in regulation of angiogenesis, was later proved to be a major mediator of copper-induced angiogenesis. In 2007, Jiang *et al.*[20] showed that dietary supplementation of physiologically relevant levels of copper restored VEGF levels and promoted angiogenesis in hypertrophic hearts in mice. Further studies demonstrated that copper-induced angiogenesis was HIF-1 dependent as indicated by the fact that small interfering RNA targeting HIF-1α abolished copper-induced VEGF expression in cultured human cardiomyocytes.[16] Therefore, the effect of copper on angiogenesis is dependent on the regulation of HIF-1 activity. How does copper regulate HIF-1 transcriptional activity?

Mechanisms by which copper regulates HIF-1 angiogenic activity

Multifunctional regulation of HIF-1 by copper

Copper regulates HIF-1 activity at multiple sites, including HIF-1α protein stabilization, transcriptional complex formation, and binding to the HRE sequence of target genes. As previously noted, the stability of HIF-1α protein is censored by two systems, PHDs in the cytosol and FIH-1 in the nucleus. Some transition metals such as cobalt and nickel enhance HIF-1 activity by inhibiting these two systems leading to stabilization of the HIF-1α protein.[55–57] It has been shown that the treatment with high levels (100 μM) of copper in different cell lines was also capable of inhibiting the activity of PHDs, promoting the accumulation of HIF-1α protein and its target genes expression, such as VEGF, ceruloplasmin, and GLUT1, even under normoxic conditions.[58,59] However, copper at physiologically relevant levels do not influence either the production or the stability of HIF-1α protein, but instead, it is required for HIF-1 transcriptional complex formation and binding to the HRE sequence of target genes.[16,60–62] Copper deprivation by tetraethylenepentamine (TEPA), a copper chelator, suppressed HIF-1 activation induced by either IGF-1 treatment in cultured human cardiomyocytes[20] or hypoxia in HepG2 cells.[16] Further analysis using an enzyme-linked immunosorbent assay or an electrophoretic mobility shift assay (EMSA) found that copper deprivation significantly reduced the binding of HIF-1 to the HRE of the target genes.[16,20] Moreover, copper deprivation inhibited the recruitment of cofactors, such as p300, to HIF-1 transcriptional complex, which was likely via affecting FIH-1 activity.[16] Thus, these observations demonstrated that copper is required for HIF-1 activation through regulation of HIF-1 binding to the HRE and the formation of the HIF-1 transcriptional complex.

Selective regulation by copper of HIF-1-controlled angiogenesis

Copper is not required for the expression of all HIF-1-regulated genes.[61–63] This was initially demonstrated by an *in vitro* study in which the treatment of HUVECs with TEPA suppressed the expression of a group of HIF-1 target genes such as BNIP3 and VEGF, but did not affect other HIF-1 target genes such as IGF-2.[63] This copper selective regulation of the expression of HIF-1-controlled genes was further defined in studies of monkey model of

myocardial ischemic infarction.[14] It was observed that the accumulation of HIF-1α was accompanied by suppressed expression of HIF-1α-controlled angiogenic factors, including VEGF, tyrosine-protein kinase receptor Tie-2, angiopoietin-1 (Ang-1), and fibroblast growth factor-1 (FGF-1) in the ischemic myocardium. This paradoxical phenomenon, HIF-1α accumulation versus suppression of HIF-1 target angiogenic gene expression, was most likely attributed by the reduced copper concentrations in the ischemic heart, as the expression of other copper-independent HIF-1 target genes such as IGF-2 was activated in the ischemic myocardium.[14] A recent study, by coupling ChIP-sequencing and RNA-sequencing method, comprehensively identified 281 copper-dependent and 10 copper-independent HIF-1 target genes across the genome under hypoxic conditions.[62] Copper, as a cellular modulator, selectively regulates HIF-1α binding sites across the genome to cope with varying environmental conditions such as hypoxia.

The mechanism by which copper selectively regulates the binding site of the HIF-1 target genes was recently revealed by an *in vitro* study in HUVECs.[61] In this study, copper deprivation by TEPA completely suppressed the binding of HIF-1α to HRE site (−412/−404) of BNIP3 along with a complete inhibition of BNIP3 mRNA expression, but the binding of HIF-1α to the HRE site (−354/−347) of IGF-2 or the expression of IGF-2 mRNA was not affected under hypoxic condition. Furthermore, *de novo* motif analysis of all 218 copper-dependent and 10 copper-independent HIF-1 target genes further revealed that the core bases "GGAA" and "TTCC," previously identified as the core motifs for E26-transformation-specific (ETS) family,[64] constituted the critical motifs for the binding sites of copper-dependent genes, while no specific motif found in copper-independent genes except the motif for HIF-1α.[62] The differences in the binding loci and patterns between all the copper-dependent and copper-independent HIF-1 target genes indicated that copper, by affecting the binding of HIF-1α to the critical motifs in the promoter and putative enhancer regions of HIF-1-regulated genes, selectively regulates the expression of HIF-1-controlled angiogenic genes.

Copper-binding proteins involved in the regulation of HIF-1

There is virtually no detectable free copper ion in cells,[65] and copper regulation of HIF-1 transcriptional activity in the nucleus upon hypoxia is most likely through copper-binding proteins (CuBPs). Copper chaperone for superoxide dismutase-1 (CCS) is the first CuBPs that has been proposed to mediate the action of copper on HIF-1 activity. In a study of cultured human cardiomyocytes, CCS gene silencing inhibited IGF-1-induced activation of HIF-1 and VEGF expression, mimicking the effect of copper chelation, but not been reversible upon addition of excess copper.[20] This effect of CCS on regulation of HIF-1 activity was also confirmed by latter studies using different cell models treated with hypoxia[16] or hypoxia mimics, such as cobalt,[60] indicating that copper regulation of HIF-1 is CCS dependent.

In addition to CCS, copper metabolism MURR1 domain containing-1 protein (COMMD1), a critical CuBP involved in intracellular copper transportation and located in both cytosol and nucleus,[66] has been reported to function as a negative regulator of HIF-1 activity. Mouse embryos deficient for COMMD1 showed an increased expression of HIF-1-regulated genes (i.e. VEGF, PGK (Phosphoglycerate kinase 1), and BNIP3), corresponding to increased HIF-1α protein stability.[67] Conversely, overexpression of COMMD1 in human cell lines clearly inhibited HIF-1 activity.[67] Further studies revealed that COMMD1 directly binds to the amino terminus of HIF-1α in the nucleus, preventing its dimerization with HIF-1β and subsequent DNA binding and transcriptional activation.[68] However, COMMD1 is actively exported from the nucleus in a CRM1-dependent manner to cope with low oxygen concentrations.[69] This hypoxia-driven nuclear export of COMMD1 thus become a critical step to maintain the integrity of HIF-1 transcriptional complex in the nucleus and transactivation of HIF-1 under hypoxic conditions.

A recent study using copper immobilized metal affinity chromatography followed by proteomic analysis identified 17 differentially expressed nuclear CuBPs under DMOG-induced hypoxic condition.[70] Among these CuBPs, albumin (ALB), lamini A/C (LMNA), and heat shock protein beta-1 (HSPB1) showed a significant increase in the nucleus after DMOG treatment, which was also confirmed under hypoxic condition. Considering the increased nuclear copper concentrations upon hypoxia,[30,61,62] these newly identified CuBPs could be the targets for further study of copper regulation of HIF-1 activity in the nucleus.

Because there are multiple sites of copper regulation of HIF-1, there should be multiple proteins involved in the regulation process. The characteristics of these proteins are proposed as follows: a, interaction with copper chaperones (e.g. CCS); b, affecting FIH-1 enzyme activity; c, interaction with HIF-1 subunits or cofactors; d, interaction with ETS family (Figure 2). Most of these proteins have not been identified and further work, especially identification of possible CuBPs, is required to elucidate how copper selectively regulates HIF-1 transactivation of target gene expression.

Copper induced myocardial angiogenesis

The loss of copper content in the ischemic myocardium is accompanied by the depression of angiogenesis in response to ischemic insult.[17,20] Given the essential role of copper in regulating HIF-1 activity,[16,20,60] retuning copper homeostasis in ischemic myocardium would reactivate angiogenesis, thereby promoting myocardial regeneration (in Figure 3).

Cardiac copper efflux after ischemic injury

Myocardial copper concentrations have been found to be abnormally low in persons dying from myocardial infarction since the mid-20th century.[71–73] Further examinations revealed a significant elevation in serum copper concentrations, especially in patients with acute myocardial

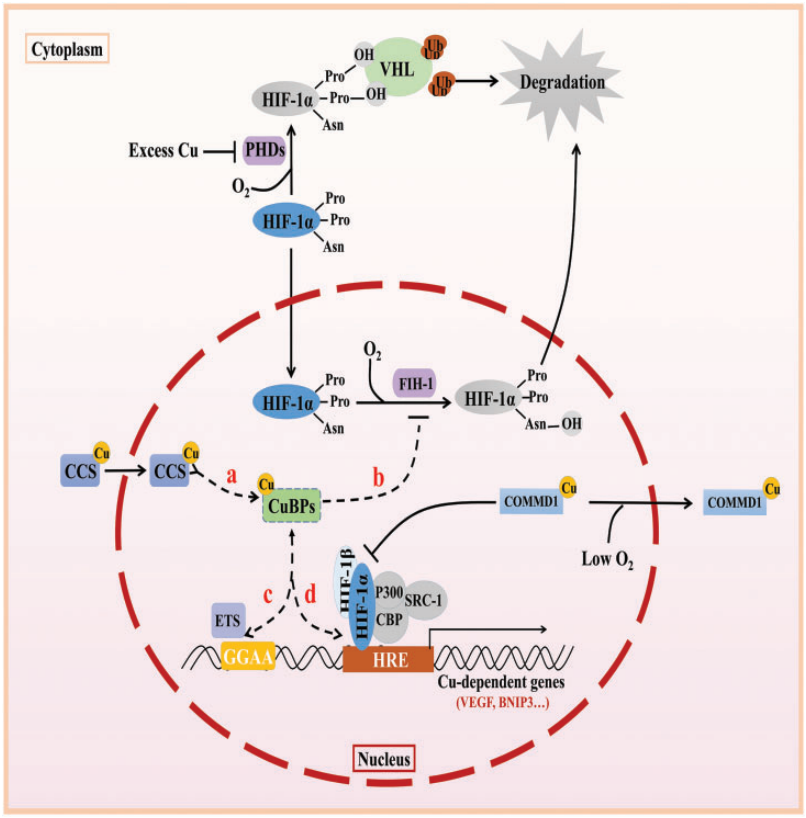

Figure 2. Copper regulation of hypoxia-inducible factor (HIF)-1 activity. HIF-1 is composed of HIF-1α and HIF-1β. Under normoxia, HIF-1α is hydroxylated by PHDs in the cytosol and FIH-1 in the nucleus, and then it undergoes proteasomal degradation. Under hypoxia, HIF-1α escapes from degradation as a result of inhibition of PHDs and FIH-1 and dimerizes with HIF-1β in the nucleus. The heterodimer then recruits cofactors such as p300, CBP and steroid receptor co-activator 1 (SRC1) to form HIF-1 transcriptional complex. Hypoxia-driven nuclear export of COMMD-1 promotes the integrity of HIF-1 transcriptional complex in the nucleus and transactivation of HIF-1. CCS brings copper into the nucleus. Copper is required for the interaction of HIF-1 with the HREs to initiate copper-dependent expression of genes such as VEGF and BNIP3. The core bases "GGAA," as the core motifs for ETS family, constitutes the critical motifs for the binding sites of copper-dependent genes. Copper also acts as an inhibitor of FIH-1. These effects would be mediated by unidentified putative CuBPs. (A color version of this figure is available in the online journal.)

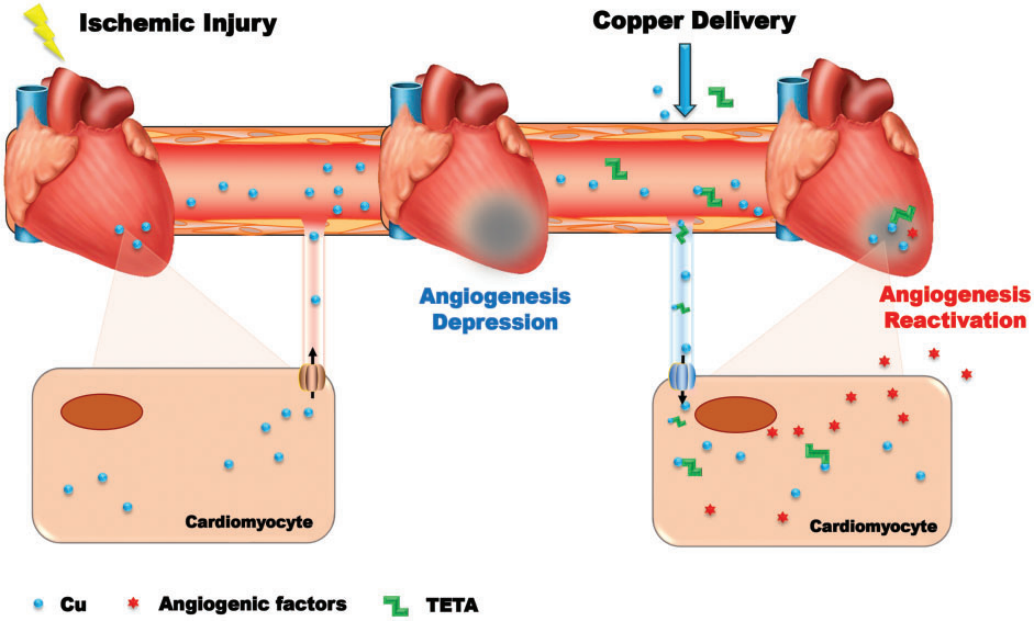

Figure 3. Copper induced myocardial angiogenesis. Myocardial ischemia leads to copper excretion from heart to blood along with angiogenesis depression. Increasing bioavailability of copper via multiple approaches including (1) dietary supplementation and (2) TETA administration, results in copper restoration in the heart. The reutilization of copper improves the transactivation of HIF-1-regulated expression of angiogenic genes as described in Figure 2, leading to improved angiogenesis and promotion of myocardial regeneration. (A color version of this figure is available in the online journal.)

infarction.[74–76] The hypothesis that cardiac copper is mobilized into the blood after ischemic insult was then proposed by an *in vitro* study conducting Langendorff perfusion to isolated rat hearts. This study showed an 8- to 9-fold increment of copper concentrations in the perfusate compared to the pre-ischemic value.[77] However, until recently, the cause-effect relationship between copper loss from the heart and copper elevation in the blood was addressed in a mouse study. In this study, mice were subjected to left anterior descending (LAD) artery ligation leading to myocardial ischemia and copper levels in the ischemic heart were significantly decreased as a function of elapsed time, accompanied by a gradual increase in serum copper concentrations.[18]

Furthermore, the time course of copper loss from the ischemic myocardium was in parallel with the suppression of angiogenesis during the process of myocardial ischemia. A significant angiogenesis was observed prior to the extensive loss of copper in ischemic myocardium in the acute phase of myocardial ischemia (less than four days after myocardial infarction).[78] However, with the prolongation of ischemia, copper content in the ischemic heart is progressively reduced (five days after myocardial infarction), accompanied by significant inhibition of angiogenesis.[78] Therefore, considering the critical role of copper in promoting angiogenesis, effectively increasing copper concentrations in the heart would be conductive to reactivate angiogenesis in the ischemic myocardium.

Myocardial copper restoration and angiogenesis reactivation

Dietary copper supplementation has long been used as the most direct and effective way to increase the copper content of tissues, especially for copper-deficient myocardium.[20,79,80] It was reported that dietary supplementation of physiologically relevant levels of copper restored myocardial copper levels from its loss caused by prolonged pressure overload, thus promoting angiogenesis in the hypertrophic myocardium and eventually recovering myocardial function.[20] Moreover, many other studies using various types of copper formulations to treat cardiomyopathy caused by different pathogeneses have also shown that increasing the bioavailability of copper to the injured myocardium is beneficial for the recovery of cardiac structure and function.[81,82]

In addition to copper supplementation, a copper-selective chelator, trientine (TETA), has also been shown to effectively promote angiogenesis and reverse the hypertrophic cardiomyopathy in diabetic rats.[83–85] The idea of using TETA for the treatment of cardiomyopathy was initially proposed based on the observation of the elevated serum copper levels in the diabetic rats and the fact that oral TETA treatment removes copper from the body under disease conditions such as Wilson's disease.[86] However, a recent study showed that TETA at a lower dosage (21.9 mg/kg day) actually selectively delivered copper to the copper-deficient hypertrophic hearts in rats.[21] Further studies revealed the possible mechanism by which TETA facilitates copper accumulation selectively in cardiomyocytes through the formation of Cu(II)-TETA complex that is transported to cardiomyocytes via an energy-dependent, but CTR1-independent, cross membrane transportation process in cardiomyocytes.[87]

Furthermore, it was shown that diacetyl-bis(N-methylthiosemicarbazone) (ATSM) and elesclomol (ES) specifically delivered copper into hypoxic tissues such as tumors and ischemic heart.[88–90] These synthetic ionophores have a high binding affinity for copper, forming lipophilic copper complexes that enable copper across cell membrane under hypoxic conditions.[91] Thus, copper ionophores are currently used for hypoxic imaging of ischemic diseases and potent chemotherapeutic agents for cancer treatment.[88] In addition, a recent study showed that ES treatment in genetically copper-deficient cells restored intracellular copper homeostasis and recovered mitochondrial function of the cells.[92] This provides a potential application of copper ionophores to increase the bioavailability of copper to the ischemic myocardium.

These findings thus indicate that multiple approaches to increasing the availability of copper to the ischemic myocardium indeed can be developed. In addition, in consideration for the role of copper in reactivation of the expression of HIF-1-regulated angiogenic genes, this approach would be greatly beneficial to patients with ischemic damage to other organs than just the heart.[93–95]

Angiogenesis in myocardial regeneration

Angiogenesis is the critical step to initiate the process of myocardial regeneration due to the fact that vascularization creates a rejuvenation microenvironment and ensures sufficient supply of regenerative materials to rescue the failing heart.

Rejuvenation of myocardial regeneration microenvironment

Angiogenesis ameliorates the hypoxic environment and improves the vitality and function of cardiomyocytes in the ischemic myocardium. The survival and contraction of cardiomyocytes require abundant energy supply from mitochondrial-based oxidative respiration. In the acute phase of myocardial ischemia, the activation of angiogenesis rescues some parts of the ischemia-injured myocardium.[78] But in the chronic phase, the depressed angiogenesis produces a persistent hypoxic condition, thus damaging the vitality and contractile function of cardiomyocytes.[14] Reactivation of angiogenesis improves the hypoxic condition, restoring the function of mitochondria in the infarcted myocardium,[20] rescuing the contractibility of the heart.

Angiogenesis is also involved in the modulation of extracellular matrix for myocardial regeneration. Formation of scar tissue is closely associated with depressed angiogenesis, constituting a natural barrier for myocardial regeneration.[6] Promotion of angiogenesis activates multiple cytokines including matrix metalloproteinases (MMPs),[96,97] which are responsible for degradation of collagens. In the process of copper-induced angiogenesis, the fibrotic scar is also ameliorated, due to an increase of MMP-2 protein level and activity in the heart.[21] The coordination between

angiogenesis and destruction of fibrotic scar guarantees a suitable microenvironment for myocardial regeneration.

Importantly, angiogenesis re-establishes the communication and transportation system between the injured site and the remote reservoir of repair materials (Figure 4). A burst of cytokines (such as IL-1β, IL-6, TNF-α)[98] and chemokines (such as SDF-1)[99] were accumulated in the injured site, forming a new tissue injury microenvironment. These molecules serving as tissue injury signals, are released to blood activating self-repair mechanism including recruitment of inflammatory cells to eliminate the debris of dead cells, and mobilization of stem cells for myocardial regeneration.[9] However, depressed angiogenesis in the chronic phase of myocardial ischemia blocks the transduction of tissue injury signaling, inhibiting the recruitment of effective self-repair mechanisms for the injured heart. Therefore, the reconstituted conduit resulting from angiogenesis is necessary to allow the tissue injury signal molecules released and transported to remote areas, recruiting regenerative materials, such as bone marrow stem cells, to the injured site for repairing.[9] Moreover, angiogenesis makes a critical contribution to the re-establishment of the communication between cardiomyocytes and surroundings.[100] These enhanced crosstalk among cells and systems actively participate in the process of myocardial regeneration. Taking together, it is concluded that angiogenesis is the key to myocardial regeneration.

Involvement of stem cells in myocardial regeneration

Renewal of injured or dead cardiomyocytes is the fundamental process for myocardial structural and functional recovery from ischemic injury. However, due to the weak proliferative capacity of adult cardiomyocytes, it has been a difficult undertaking to promote myocardial regeneration for a long time.[101,102] Stem cell therapy for ischemic heart disease has been a major focus for a better approach to promote myocardial regeneration. These approaches include intravenous injection of multipotent stem cells[103] and in situ injection of induced pluripotent stem cells (iPSCs).[104] However, these approaches have not achieved the expected results and remain at the animal experimental stage.

Mesenchymal stem cells (MSCs) intrinsically possess unique features that migrate towards the injured area and differentiate into multiple cell types.[105] The homing of these cells to the injured site of the heart is limited by the depression of angiogenesis in the heart.[6] In the process of angiogenesis, recruitment of bone marrow stem cells to the injured site is well documented.[106] Both $CD34^+$ bone marrow stem cells and $CD34^-$ mesenchymal stem cells have been observed to be mobilized from peripheral blood and bone marrow, respectively.[106,107] Furthermore, pluripotent cells, such as $CD117^+$ cells and $CXCR4^+$ cells in the peripheral blood also show a migration potential after the formation of blood vessels.[106] The mobilization and homing of these stem cells are all angiogenesis-dependent, relying on the reconstitution of blood vessels for action.

It is prospected that copper-induced angiogenesis triggers the natural regenerative capacity of the heart (Figure 4). The improvement of myocardial microenvironment through angiogenesis is greatly beneficial to stem cell homing. SDF-1/CXCR4 axis is considered as an important pathway in stem cell homing.[99] SDF-1, a target gene of HIF-1, is markedly upregulated in the myocardium under ischemia, which partially contribute to $CXCR4^+$ cells mobilization and their colonization in the infarcted area after angiogenesis.[99,106]

The mobilized stem cells are involved in myocardial regeneration. Stem cells administered by intravenous injection were found to differentiate into vascular endothelial cells and cardiomyocytes in the ischemic hearts.[108,109]

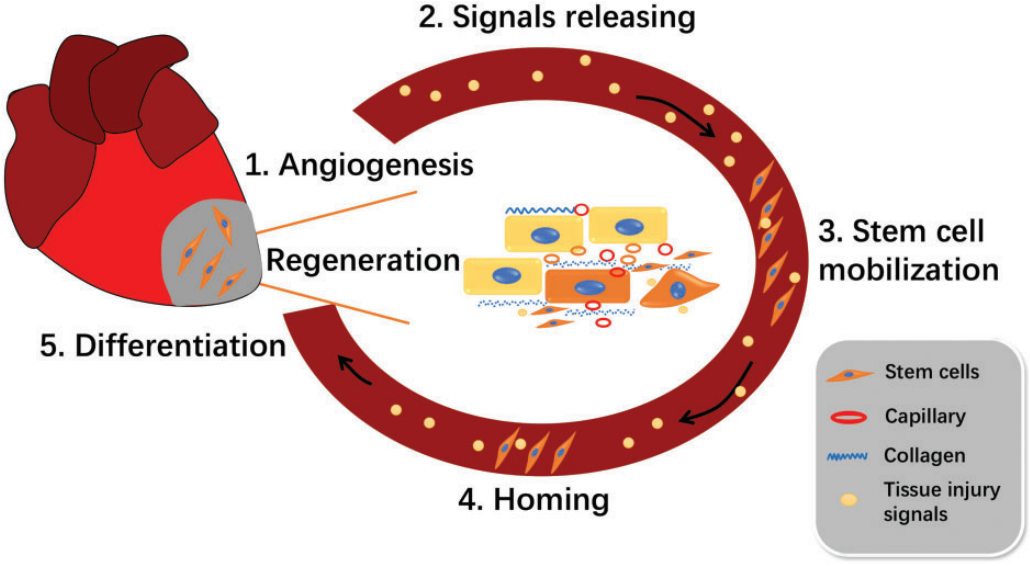

Figure 4. Angiogenesis in myocardial regeneration. The diagrammatic sketch summarizes the process of myocardial regeneration triggered by angiogenesis. As the progress of angiogenesis reactivation, the microenvironment of the injured myocardium improves. Tissue injury signals are released and transported between the injury site and the remote area. Stem cell mobilization takes place in response to the tissue injury signals and stem cell homing is guided by the signal molecules concentration gradient. Situated in the rejuvenation microenvironment, stem cells homing to the injured myocardium differentiate as well as function as a paracrine secretion to promote myocardial regeneration. (A color version of this figure is available in the online journal.)

Another pro-regenerative effect of stem cells appears to be derived from their paracrine activity. Many efforts focused on exosomes, which act as paracrine mediators between MSCs and target cells.[110] Isolation and injection of exosomes extracted from MSCs to LAD artery ligated mice have shown a therapeutic efficacy, improving cardiac function after myocardial infarction.[111] A recent study demonstrated another mechanism for stem cell-mediated myocardial regeneration through acute immune response-induced rejuvenation of the mechanical properties of the infarcted heart, further revealing the beneficial effects of stem cells in myocardial regeneration.[112]

Perspectives

Copper promotion of angiogenesis has been extensively studied for almost 40 years since the first observation that copper was involved in intraocular vascularization in anterior chamber implants in rats in 1980.[44] Copper selectively regulates HIF-1 binding to its target angiogenic genes leading to the expression of copper-dependent angiogenic factors. This understanding highlights the importance of CuBPs in the process of angiogenesis, which would be an important undertaking in future studies. Angiogenesis is the key to myocardial regeneration from ischemic injury to the heart. Therefore, a simple approach to increase the availability of copper to the ischemic myocardium would become an attractive focus for clinical translation studies. This simple and straightforward approach would be greatly beneficial to human patients with ischemic heart disease.

AUTHORS' CONTRIBUTIONS

YX, TW, XS, DY and QC drafted the manuscript and figures. YJK edited, revised and approved the final version of this manuscript.

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DECLARATION OF CONFLICTING INTERESTS

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