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Review

Novel Molecular Mechanisms and Regeneration Therapy for Heart Failure

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Abstract

Heart failure (HF) is one of the leading causes of mortality in the world. Various molecular mechanisms have been proposed for HF, but its precise mechanisms are still largely unknown. In this review, summarizing the “President’s Distinguished Lecture Award” of XX World Congress of International Society for Heart Research 2010 in Kyoto, Japan, we introduce recent our studies on HF, including 1) p53-induced suppression of Hif-1-induced angiogenesis as a novel mechanism of HF, 2) angiogenesis as a potential therapeutic strategy for HF, and 3) IGFBP-4 as a novel factor for cardiomyogenesis by inhibiting canonical Wnt signaling.

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References

1. Introduction

Heart failure (HF), one of the leading causes of mortality in the world, is a complex disorder that leads to a disturbance of the normal pumping of blood to the peripheral organs to meet the metabolic demands of the body. There are two forms of heart failure, heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). In this review, we focus on systolic heart failure (HFrEF) and discuss about its molecular mechanisms and its novel therapies based on the mechanisms.

Under the conditions of pathological stimuli due to the cardiovascular diseases such as hypertension, valvular diseases and myocardial infarction, the heart initially maintains cardiac function by adaptive mechanisms such as cardiac hypertrophy and dilatation. If the hemodynamic overload is prolonged, however, the ventricular contractility is gradually decreased. Numerous studies have proposed a variety of molecular mechanisms of the transition from cardiac hypertrophy to HF. These mechanisms include, 1) myocyte growth with reemployment of fetal gene programs and decreased adult gene expressions[1], 2) alterations of proteins associated with intracellular Ca^{2+} handling, which play important roles in excitation-contraction coupling [2], 3) myocyte death[3], 4) changes in the extracellular matrix[4, 5], 5) oxidative stress[6], 6) changes of metabolism and energetics[7], 7) over-activation of neurohormones (e.g., norepinephrine and angiotensin II) and their receptors [8], inflammatory cytokines (e.g., tumor necrosis factor- α [TNF- α])[9], other peptides and growth factors (e.g., transforming growth factor- β [TGF- β], endothelin)[10, 11], and 8) gene mutations and epigenetic changes[12]. All of these alterations may cause HF,

however, the precise mechanisms of HF are still largely unknown.

2. Angiogenesis in hypertrophied hearts

In HF patients due to valvular diseases, ²⁰¹Tl myocardial SPECT study has demonstrated that left ventricular systolic function is negatively associated with myocardial perfusion abnormality[13], suggesting that myocardial blood flow is an important regulator of cardiac function. In growing organs or tissues such as hypertrophied heart and cancer, angiogenesis is necessary to maintain their functions. Physiological cardiac hypertrophy induced during development and by exercise is accompanied with angiogenesis[14], while pathological cardiac hypertrophy induced by hemodynamic overload is not accompanied with angiogenesis, leading to myocardial ischemia and cardiac dysfunction known as “hibernation”[14]. In murine model of Akt-induced cardiac hypertrophy, hypertrophic stimuli induced expressions of *vascular endothelial growth factor (VEGF)* and *angiopoietin 2* genes in the adaptive phase of hypertrophy, and inhibition of VEGF signaling with neutralizing antibody reduced capillary density of the myocardium, resulting in an early transition to HF[15]. Furthermore, the transition from adaptive hypertrophy to HF induced by pressure overload was accelerated due to insufficiency of myocardial capillary induction in VEGF deficient mice [16].

A zinc-finger transcription factor GATA4 is reported to be one of the regulators of VEGF[17]. GATA4 binds to transactivation domain of the *Vegf-A* gene and regulates *Vegf-A* expression [17]. GATA4 is expressed in cardiomyocytes from early embryo to

adult[18]. Cardiac-specific deletion of *GATA4* revealed its pivotal roles in cardiac hypertrophy, stress-responses of the heart, myocyte viability as well as regulation of cardiac genes [19]. Endogenous GATA4 protein is kept at low levels in the steady state of the adult heart, but is upregulated in hypertrophied heart in response to pressure overload associating with increased capillary density. In decompensated failing heart, however, GATA4 protein is decreased parallel with capillary density. Taken together, GATA4 directly modifies the angiogenic program regulating *Vegf-A* gene expression in the adult heart [20] (Figure 1). To explore the regulation of myocardial angiogenesis during the transition from compensated cardiac hypertrophy to decompensated HF, we examined murine pressure overload model.

3. Angiogenesis in failing hearts

Transverse aortic constriction (TAC) acutely induces pressure overload on the left ventricle. Initially, TAC induces compensated cardiac hypertrophy (adaptive phase), however, prolonged pressure overload depresses myocardial contractility resulted in decompensated HF (maladaptive phase)[21]. It has been thought that not only insufficient hypertrophy but also a depression of the myocardium's intrinsic contractility cause afterload mismatch and HF[22], however, the precise molecular mechanism by which pressure overload induces cardiac dysfunction remains unclear. After TAC, cardiac hypertrophy indicated by the increases in heart weight-body weight ratio and ventricular wall thickness were gradually enhanced, peaked on day 14 after TAC and not

changed afterward[21]. Noteworthy, cardiac function was preserved until day 14, but significantly reduced at day 28 after TAC. Histological analysis revealed that capillary density was increased during adaptive phase but decreased thereafter[21], resulting in hypoxia in the failing heart, suggesting the important role of angiogenesis in myocardium to maintain systolic function during cardiac hypertrophy. Indeed, an angiogenesis inhibitor, TNP-470, not only suppressed the development of cardiac hypertrophy but also impaired cardiac function even at the adaptive phase. Conversely, promoting angiogenesis by introduction of VEGF and angiopoietin 1 to the TAC heart promoted cardiac hypertrophy with preserved systolic function even at the maladaptive phase. These results further indicate that angiogenesis is enough and necessary for the development of cardiac hypertrophy and maintenance of cardiac function[21]. Consistent with these histological findings of vasculature in hypertrophied myocardium, expressions of VEGF and angiopoietin1 were increased in the adaptive phase and then decreased in the maladaptive phase. A key regulator of angiogenesis under the condition of hypoxia, hypoxia-inducible factor-1 (Hif-1), was markedly upregulated in the adaptive phase and was significantly decreased in the maladaptive phase[21]. Hif-1 is stabilized under hypoxic condition and activates the transcription of various hypoxia-associated genes such as *erythropoietin* and *VEGF* genes[23, 24]. To evaluate specific roles of Hif-1 α in the heart, we analyzed inducible cardiomyocyte-specific deletion of Hif-1 α by using aMHC promoter driven MerCreMer and tamoxifen. In the Hif-1 α -deleted mice, cardiac hypertrophy and systolic function were significantly suppressed even in the adaptive phase, suggesting that Hif-1 is an important mediator for cardiac growth and maintenance

of cardiac function. It has been reported that cardiomyocyte hypertrophy increases diffusion distance and reduces oxygen supply in the myocardium. Indeed, in hypertrophied TAC heart, hypoxia was induced and sustained until day 28[21]. This observation raises the next question why Hif-1 α was downregulated even under the hypoxic condition at 4 weeks after TAC.

4. p53 inhibits Hif-1 in hypoxic myocardium

A tumor suppressor, p53, is kept at very low level in the normal heart but is upregulated when cardiomyocytes are exposed to hypoxic stresses by pressure overload and coronary artery ligation as well as genotoxic stresses with adriamycin treatment[21, 25, 26]. We have also found an increase in p53 expression in the murine heart of dilated cardiomyopathy (DCM) model, in which mutated cardiac α -actin detected in human DCM patients is overexpressed specifically in the cardiac myocytes [27], suggesting that elevation of p53 expression not only reflects the stressed status of the heart, but also plays a key role in the common pathway toward HF. Hif-1 plays important roles in hypoxia-induced p53 accumulation and potentiates p53 regulating gene transcription via direct binding[28], and it has been also reported that severe and/or sustained hypoxia induces p53 expression that inhibits the function or promotes degradation of HIF-1. To elucidate the role of p53 in the cardiac hypertrophy, we imposed pressure overload on p53 knockout mice. Expressions of *Hif-1* and *VEGF* were significantly higher in the heart of p53 knockout mice compared with wild-type mice at maladaptive phase. Consequently,

capillary density was significantly increased and cardiac function was conserved in p53-deficient mice even at maladaptive phase. Next, we examined the effects of p53 activation by administering quinacrine, a p53 activator, to mice with TAC. Quinacrine treatment upregulated p53, and decreased expression of *Hif-1* and *VEGF*. Quinacrine treatment significantly decreased capillary density, and suppressed cardiac hypertrophy and cardiac function even in the adaptive phase. Together, these results indicate novel molecular mechanisms underlying transition of cardiac hypertrophy to HF; p53 inhibits angiogenesis by suppressing HIF-1, resulting in myocardial hypoxia and cardiac dysfunction (Figure 1).

5. Angiogenesis as a potential therapeutic strategy for heart failure

If the hypoxia is a cause of HF, induction of angiogenesis might be a novel strategy for HF. In our previous studies of angiogenic therapy for animal models and human patients with limb ischemia, implantation of peripheral blood mononuclear cells (PB-MNC) into skeletal muscle significantly improved limb ischemia by upregulation of various kinds of angiogenic growth factors from the regenerated muscle [29, 30]. Most of the patients of limb ischemia have ischemia also in the heart and some of them showed cardiac dysfunction. Implantation of PB-MNC into skeletal muscle improved cardiac function as well as cardiac ischemia possibly by angiogenic growth factors which are secreted from the skeletal muscles [30]. These findings also support our concept that the development of HF is caused by myocardial ischemia (Figure 2).

Several strategies to enhance myocardial angiogenesis have been investigated, including delivery of proteins or complementary DNAs of angiogenic growth factors such as VEGF-A [31-34], VEGF-B [35-37], fibroblast growth factor-2 (FGF-2) [33, 38, 39], FGF-5 [40, 41], stromal cell-derived factor-1 (SDF-1) [42, 43], hepatocyte growth factor (HGF) [44], and midkine [45]. Although long-term stimulation with VEGF-A promotes immature angiogenesis and increases vascular permeability [46], simultaneous administration of VEGF-A and angiopoietin1 yields coordinated vascular growth and improves the perfusion and function of the diseased hearts [34, 47, 48]. Another potential therapeutic target is Hif-1[49]. Cobalt, which is known to stabilize Hif-1 α , prevented decline in contractile function of perfused rat hearts under hypoxia-reoxygenation [50]. According to recent studies, cobalt-induced stabilization of Hif-1 α is dependent on copper [51, 52], and copper supplementation reversed cardiac contractility and prevented the development of HF in pressure overloaded mice at least in part via induction of myocardial angiogenesis[53].

6. IGFBP-4 is a novel cardiogenic factor by inhibiting Wnt/ β -catenin signaling

Another therapeutic strategy for HF is cardiomyogenesis, and there are two methods to increase cardiomyocytes, 1) cell transplantation of cultured cardiomyocytes and 2) induction of differentiation of cardiac stem cells into cardiomyocytes or of proliferation of cardiac myocytes. Although there are lots of candidate cell types for cell transplantations such as hematopoietic stem cells, mesenchymal stem cells, side population

cells and resident stem cells [54], only embryonic stem (ES) cells or induced pluripotent stem (iPS) cells can efficiently differentiate into cardiomyocytes. There are still many issues even in ES cells and iPS cells to be implanted into human hearts such as immaturity and differentiation efficiency [55], and thus it is important to improve the quality and efficiency of cardiomyocyte differentiation.

It has been thought that cardiomyocytes are terminally differentiated and withdrawn from the cell cycle after birth. Therefore, myocardial damage due to myocardial infarction causes permanent loss of functional cardiomyocytes and results in cardiac dysfunction. Although many groups including us have identified cardiac stem or progenitor cells such as Sca-1 positive cells [56, 57], c-kit positive cells [58], side population cells [59-61] and islet-1 positive cells [62, 63], these intrinsic stem-like cells do not contribute to the cardiac regeneration with unknown reasons. Strong mediators to induce cardiomyogenesis might be useful for regeneration therapy.

To identify a factor inducing cardiomyogenesis, we screened the cardiomyogenic activity of the media conditioned by many cell types using P19CL6 cells, which can be differentiated into beating cardiomyocytes with dimethylsulphoxide [64]. Within the cell types we tested, media conditioned by murine stromal cell line OP9 effectively differentiated P19CL6 cells into cardiomyocytes without dimethylsulphoxide, indicating that OP9 cells secreted some cardiomyogenic factors [65]. To further identify OP9-derived cardiomyogenic factors, complementary DNA clones isolated by a signal sequence trap method from an OP9 cDNA library were screened for their ability of cardiomyogenesis by transient transfection into P19CL6 cells [66], and one of the genes

which we identified as a cardiomyogenic factor secreted from OP9 cells was insulin-like growth factor-binding protein-4 (IGFBP-4). IGFBP-4 itself also induced cardiomyocyte differentiation of P19CL6 cells and ES cells [65]. IGFBPs directly bind to IGFs and modulate their action, however, cardiogenic activity of IGFBP-4 is independent of IGF[65]. Further investigations revealed that IGFBP-4 regulates cardiomyogenesis by inhibiting canonical Wnt signaling pathway. IGFBP-4 physically interacted with cell surface receptors of Wnt/b-catenin signaling, Frizzled and LRP5/6, and competitively inhibited binding of Wnt to the receptors [65]. In IGFBP-4 knockdown *Xenopus*, the embryonic heart was initially developed but subsequent growth and maturation were interfered, and the embryo resulted in severe heart defect[65], suggesting that IGFBP-4 plays important roles in cardiogenesis by preserving the proliferation and/or the survival of embryonic cardiomyocytes. In vivo, IGFBP-4 was expressed in the anterior liver which exists just next to the heart at late embryonic stage, and it has been reported that cardiac mesoderm secretes FGFs and induces liver development[67], suggesting that there are interactive paracrine signals coordinating the development of each organ between the heart and the liver primordia. In this way, IGFBP-4 is an inhibitory regulator of the canonical Wnt signaling which is required for embryonic cardiogenesis, and is a molecular linkage between IGF and Wnt signaling pathways (Figure 3). On the other hand, IGFBP-4 has been reported to have inhibitory effects on growth factor-induced angiogenesis in chick chorioallantoic membrane[68]. Therefore, further investigations are necessary to elucidate whether administration of IGFBP-4 is beneficial or not for the injured heart.

7. Conclusion

In this review, we have summarized the “President’s Distinguished Lecture Award” of XX World Congress of International Society for Heart Research 2010 in Kyoto, Japan.

The recent advances of experimental techniques have revealed pathophysiology of various diseases including HF, however, we have not identified the key molecules to be targeted by drugs in HF. Many molecular target-based drugs have been recently developed in particular for cancers based on their molecular and cellular mechanisms, and the prognosis of patients with some cancers has become much improved. Although cancers are very different from HF in many aspects, the elucidation of molecular mechanisms and the development of molecular target-based drugs for HF based on its mechanisms might be very important.

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Figure 1. Molecular mechanism of cardiac hypertrophy and angiogenesis.

Muscle ischemic lesion

Figure 2. Molecular mechanisms of angiogenesis induced by PB-MNC.

Figure 3. IGFBP-4 induces cardiogenesis by inhibiting Wnt/β-catenin signaling.