Apoptosis in Heart Failure – The Role of the β -Adrenergic Receptor-Mediated Signaling Pathway and p53-Mediated Signaling Pathway in the Apoptosis of Cardiomyocytes –

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The heart works as a driving force to deliver oxygen and nutrients to the whole body. Interrupting this function for only several minutes can cause critical and permanent damage to the human body. Thus, heart failure (HF) or attenuated cardiac function is an important factor that affects both patient's the quality of life and longevity. Numerous clinical and basic studies have been performed to clarify the complex pathophysiology of HF and to develop effective therapies. Modulating the β -adrenergic receptor-mediated signaling pathway has been one of the most crucial targets for HF therapy. Impressively, recent reports identified p53, a well-known tumor suppressor, as a major player in the development of HF. The present review highlights the apoptosis of cardiomyocytes, which is one of the important mechanisms that leads to HF and can be induced by both β -adrenergic signaling and p53. Consideration of the cross-talk among these major pathways will be important when developing effective and safe therapies for HF. (*Circ J* 2011; **75**: 1811–1818)

Key Words: Adrenergic signaling; Apoptosis; Heart failure; p53

Because the heart works as a driving force to deliver oxygen and nutrients to the whole body, cardiac function is a critical factor affecting quality of life and longevity. In addition, interrupting heart function for only a few minutes can cause critical and permanent damage to the human body. A report from the United States indicated that the lifetime risk of developing congestive heart failure (HF) is approximately 20%.¹ The most common cause of HF in Western countries is coronary artery disease (CAD). Although controlling the established risk factors for CAD has become more common in general healthcare and treating with several cardioprotective agents, including β blockers, RAS inhibitors, antiplatelet agents, and statins, improves the survival rate, the current prognosis for HF is still not acceptable. Therefore, further developments in HF treatment are one of the greatest issues for extending the healthy life of humans.

Numerous clinical and basic studies have been performed to clarify the complex pathophysiology of HF. These studies have identified several mechanisms that affect cardiac function, and some therapies were developed based on these results. Many years ago, the β -adrenergic receptor (β -AR)-mediated signaling pathway was identified as one of the most important pathways that regulates cardiac function. Modulating this pathway has been one of the most crucial targets for HF therapy.² On the other hand, recent reports identified p53, a well-known tumor suppressor, as a major player in the development of

HF.^{3–5} The present review focuses on these 2 pathways.

Both pathways can induce the apoptosis of cardiomyocytes. It is well known that cardiomyocytes undergo apoptosis in response to harmful stimuli, including ischemia,⁶ reperfusion,⁷ oxidative stress,⁸ stretching,⁹ rapid pacing,¹⁰ etc. Although some signaling mechanisms for inducing apoptosis in cardiac myocytes may be specific, such as those with Bim induction by EPAC,¹¹ others may be shared among different cell types. Since a 1997 report showed that failing hearts were associated with an increased number of apoptotic cardiomyocytes, the importance of apoptosis in the development of HF has been extensively examined and established in many reports.¹² The cell death of terminally differentiated cells, which cannot proliferate, directly affects tissue function. When attempting to develop an effective antiapoptotic therapy for the heart, we reviewed reports that examined the importance of each pathway in cardiomyocyte apoptosis. Based on these findings, we speculate that there is cross-talk among these pathways. Therefore, it will be important to take that into consideration when developing more effective and safe therapies.

β -AR-Mediated Apoptosis of Cardiomyocytes

The positive inotropic effect of β -AR stimulation is one of the most effective measures for maintaining cardiac output during urgent care of HF. The β -AR stimulation induces protein kinase A (PKA) activation through G protein, adenylyl

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cyclase (AC) and cyclic adenosine monophosphate (cAMP).² PKA-mediated phosphorylation of many calcium-handling molecules enhances ventricular wall motion.¹³ However, long-term stimulation of these receptors can lead to the deterioration of cardiac function. In addition, the prognosis of HF patients improves with β -AR blocking therapy.¹⁴ One of the mechanisms that contributes to this phenomenon is thought to be the induction of apoptosis upon β -AR stimulation.¹⁵ Failing hearts have been shown to have desensitized β -adrenergic receptor signaling. This response may help maintain cardiac function.¹⁶

Three β -AR subtypes (β 1-AR, β 2-AR, and β 3-AR) are expressed in cardiomyocytes (Figure 1). Norepinephrine or isoproterenol stimulates all β -AR subtypes and induces apoptosis in rat cardiomyocytes. However, not all subtypes of β -AR-mediated signaling induce cardiomyocyte apoptosis. It is thought that the β 1-AR-mediated pathway mainly contributes to apoptosis.¹⁷ Although all 3 subtypes are coupled to Gs, β 2-AR and β 3-AR are also linked to the Gi protein. The β 2-AR exerts antiapoptotic effects through Gi $\beta\gamma$, phosphatidylinositol-3 kinase (PI3K), and AKT activation.¹⁸ In a rat model of myocardial infarction, treating with β 2-AR agonists for 2 weeks preserved cardiac contractility and reduced the number of apoptotic cardiomyocytes.¹⁹ The β 3-AR expression is upregulated in the failing heart.²⁰ It is reported that β 3-AR negatively modulates ventricular contractility by activating endothelial nitric oxide synthase.²¹ Although the role of β 3-AR-mediated signaling in cardiomyocyte apoptosis is

still unknown, it is possible that β 3-AR exerts antiapoptotic effects through nitric oxide.²²

Several mechanisms of β -AR stimulation-induced apoptosis have been reported.

Inducible cAMP Early Repressor (ICER)

ICERs are a group of proteins that are produced from the cAMP responsive element modulator (CREM) gene and known to induce apoptosis. PKA, which is activated by β -AR stimulation, is a key molecule that maintains ICER expression. PKA activates the cAMP-responsive element binding protein (CREB), which transactivates ICER. In addition, PKA stabilizes ICER by reducing ubiquitination.²³ Moreover, ICER attenuates phosphodiesterase (PDE) 3A transcription by interacting with the promoter region of the PDE3A gene. The downregulation of PDE3A results in elevated cAMP levels. Consequently, cAMP–PKA–ICER–PDE forms a positive feedback loop that maintains ICER expression.

ICER promotes apoptosis by downregulating Bcl-2, which is an antiapoptotic protein. Consistent with this function, isoproterenol-treated cardiomyocytes were shown to have induced ICER expression, enhanced apoptosis, and decreased Bcl-2 expression.²⁴ In addition, similar results were obtained in cardiomyocytes that overexpressed ICER.

ICER includes a DNA-binding domain for a cAMP-responsive element (CRE), but lacks the CREM transactivation domain. Therefore, ICER inhibits CRE-mediated transcription by CREM/CREB. Inhibiting CRE-mediated transactiva-

Figure 2. p53-mediated signaling pathway for apoptosis. Stresses that cause heart failure, including hypoxia, tachycardia caused by pacing, oxidative stress, mechanical stress, and anthracyclines, induce the accumulation of p53. The p53 expression level is regulated by MDM2 and MDM4, which promote the ubiquitination and degradation of p53. Accumulated p53 forms tetramers and activates the transcription of various molecules that induce apoptosis and cell growth arrest and inhibit angiogenesis, DNA repair, senescence, etc.

tion of antiapoptotic signaling is thought to be another mechanism of ICER-induced apoptosis.

Ca²⁺/Calmodulin Kinase (CaMK), Calcineurin

β -AR stimulation increases intracellular Ca²⁺ through the L-type Ca²⁺ channel, which is essential for the proapoptotic effects of β -adrenergic stimuli. The elevated intracellular Ca²⁺ levels induce the activation of Ca²⁺-dependent kinase, CaMK, and the phosphatase, calcineurin. Both of these proteins reportedly mediate β -adrenergic signaling-induced apoptosis. The increase in the intracellular Ca²⁺ concentration and CaMK activity is induced in a PKA-independent manner in cardiomyocytes.²⁵ However, the detailed mechanisms that lead to the proapoptotic effects of these proteins remain controversial. Calcineurin-independent induction of apoptosis was also observed in isoproterenol-treated cardiomyocytes.^{26,27}

Exchange Protein Directly Activated by cAMP (EPAC)

cAMP, which can be induced by β -AR stimulation, activates EPAC independently of PKA.²⁸ EPAC, a guanine nucleotide exchange factor for the Ras-like GTPase, is involved in several cellular processes, including cell differentiation, cell proliferation, cell survival, etc. EPAC was shown to exert proapoptotic effects by inducing Bim in neuronal cells.¹¹ Bim directly binds to the antiapoptotic protein Bcl-2, thereby inhibiting its function.²⁹ However, EPAC may not play a central role in cardiomyocyte apoptosis. Overexpressing EPAC in cardiomyocytes does not induce significant apoptosis and

1 reason for this finding may be that the heart does not express Bim.

p53-Mediated Apoptosis of Cardiomyocytes

p53 is one of the most famous proteins and a major tumor suppressor, which is a group of proteins that have been well studied in cancer research.³⁰ Mutations in the p53 gene that attenuate p53 function have been found in 50% of human cancers.³¹ This finding indicates the importance of p53 in preventing cancer. p53 mainly functions as a transcription factor and induces a variety of molecules that induce apoptosis (Bax, p53 upregulated modulator of apoptosis (PUMA), NOXA, Death receptor 5 (DR5), Scotin, p53 apoptosis effector related to PMP-22 (PERP) etc.), arrest cell growth, inhibit angiogenesis, function in DNA repair, regulate senescence, etc (Figure 2). Accumulating evidence has elucidated the importance of p53 in various cellular responses. p53 is recognized as a key molecule in the adaptation to a wide variety of harmful stimuli, including hypoxia, oxidative stress, infection, etc. In the cardiovascular system, p53 was recently shown to have a crucial function in the development of HF, arteriosclerosis, cell senescence, metabolism, etc.

We review those reports on the relationship between p53 and HF with particular emphasis on the apoptosis of cardiomyocytes.

p53 Expression During Stresses That Cause HF

A number of reports indicate that p53 expression is upregulated in the heart by the stresses that cause HF. Specifically, reports have shown that p53 is upregulated in the heart by ischemia,^{32,33} oxidative stress,³⁴ mechanical stress,³⁵ and tachycardia caused by pacing.³⁶ Anthracyclines are anti-cancer agents that have been shown to cause cardiomyopathy, which leads to HF. Many reports demonstrated that treating with anthracyclines also induces p53 expression in cardiomyocytes.³⁷ In addition, involvement of telomere dysfunction induced p53 upregulation in the development of HF has been suggested.^{38,39} Although not all reports support these findings,⁴⁰ accumulating evidence indicates that p53 plays an important role in stress-induced apoptosis in the heart.

Roles of p53 in the Development of HF

Several studies have been conducted to clarify the roles of p53 in the development of HF. Many studies indicate that suppressing the function of p53 induces preferable effects on cardiac function. The function of p53 was attenuated by knocking out p53³ or PUMA,⁴¹ which mediates the proapoptotic effects of p53, and overexpressing MDM2,³³ which induces the ubiquitination and downregulation of p53. An examination of these models showed that these direct or indirect changes in p53 function resulted in decreased cardiomyocyte apoptosis, reduced myocardial infarct size, or a better survival rate after myocardial infarction. In addition, p53-deficient mice have decreased susceptibility to anthra-

cycline-induced myocardial apoptosis and HF.⁴² On the other hand, knock-out mice for MDM4, which inhibits the accumulation of p53, develop cardiomyopathy.⁴³ In addition, overexpressing CHIP, which induces the degradation of p53, attenuated the accumulation of p53 and reduced cardiomyocyte apoptosis after myocardial infarction.⁴ These findings indicate that p53 promotes the deterioration of cardiac function.

Recently, both apoptosis and inhibited angiogenesis were suggested to lead to the harmful effects of p53 on cardiac function. In a pressure overloaded mouse model, the cardiac condition transitions from an initial compensatory hypertrophy state to decompensatory HF several weeks after aortic banding. During this transition, p53 is upregulated, hypoxia-inducible factor-1 (HIF-1) expression is attenuated, and microvessels are reduced in the heart. HIF-1 is an established and major inducer of angiogenesis, and p53 was shown to play a pivotal role in downregulating HIF-1 expression during this transition.^{5,44}

Potential Crosstalk Between the β -AR-Mediated Signaling Pathway and the p53-Mediated Signaling Pathway

Although there are only a few reports on the relationship between β -AR stimulation and the p53 expression level, p53 was shown to be upregulated in the presence of isoproterenol

in rat cultured cardiomyocytes.⁴⁵ In addition, p53 mRNA was also upregulated in cardiomyocytes that were isolated from a murine heart after long-term β -AR stimulation.⁴⁶

On the other hand, p53 affects the expression level or activity of several molecules that can be involved in β -AR-mediated proapoptotic signal transduction, such as GSK-3 β and HIF-1.

Accumulated findings obtained from studies of each pathway indicate that there are several possible cross-talk points between the β -AR- and p53-mediated signaling pathways during the induction of apoptosis (Figure 3).

Glycogen Synthase Kinase-3 β (GSK-3 β)

GSK-3 β is a Ser/Thr protein kinase that phosphorylates and regulates many molecules that have a role in cell death, cell proliferation, cell growth, etc.⁴⁷ Several reports indicate that GSK-3 β has proapoptotic effects in cardiomyocytes.⁴⁸ GSK-3 β phosphorylates p53 and Bax, which facilitates proapoptotic signaling. In addition, 1 study reported that GSK-3 β had a proapoptotic role in the isoproterenol-induced apoptosis of cultured adult rat cardiomyocytes.⁴⁹ These findings suggest that GSK-3 β may have an important role in β -adrenergic signaling-induced p53 activation. On the other hand, GSK-3 β can be inactivated via PKA- and AKT-mediated phosphorylation, which can be facilitated by the β -adrenergic signaling pathway.⁵⁰ Therefore, in contrast, β -AR stimulation can mediate antiapoptotic effects through GSK-3 β . Although the mechanism by which β -AR stimulation induces apoptosis through the GSK-3 β pathway is still unclear, studies suggest that a potent GSK-3 β -activating pathway can overcome the effect on PKA- and AKT-mediated GSK-3 β phosphorylation.

Phosphatase and Tensin Homolog (PTEN)–AKT–MDM2–p53 Loop

AKT (also known as protein kinase B) is involved in the development of hypertrophy, contractility, cell survival and inhibition of apoptosis in cardiomyocytes. The role of AKT in the heart was examined by developing a mouse model in which active AKT is specifically overexpressed in the heart. These mice had cardiac hypertrophy, increased contractility,⁵¹ reduced infarct size and apoptosis after ischemia/reperfusion.⁵² AKT is activated upon β -AR stimulation through PI3K and CaMK.⁵⁰ Therefore, β -AR stimuli-induced AKT activation may have a negative role in β -AR-induced apoptosis. Although β -AR signaling induces AKT activation through G $\beta\gamma$, at the same time G α that is also released from β -AR can inactivate AKT by inhibiting the membrane translocation of phosphoinositide-dependent protein kinase 1 (PDK1).⁵³ The expression levels of the molecules that are involved in these β -AR-induced pathways, including AC, are thought to be one of the deciding factors of the consequential effects on the role of these β -AR-induced pathways in AKT activity.⁵⁴

AKT inhibits the accumulation of p53 by activating MDM2. When MDM2 is phosphorylated by AKT, MDM2 is translocated into the nucleus and promotes the degradation of p53. On the other hand, p53 inactivates AKT by transactivating PTEN. PTEN is a phosphatidylinositol phosphatase and a known antitumor molecule that inhibits AKT. PTEN overexpression causes apoptosis accompanied by AKT inactivation in cardiomyocytes.⁵⁵ Through this positive feedback loop (PTEN–AKT–MDM2–p53 loop),⁵⁶ β -AR-induced antiapoptotic signaling via AKT and p53-mediated proapoptotic signaling may eventually negatively affect each other. Regarding the β -AR-induced and p53-induced proapoptotic effects,

p53 promotes β -AR-induced apoptosis, while β -AR signaling may inhibit p53-induced apoptosis through the signaling loop.

Calcineurin and Nuclear Factor of Activated T Cell (NFAT)

NFAT is a transcription factor that induces a number of molecules that cause apoptosis, cardiac hypertrophy, cell cycle control, etc. NFAT is activated by the Ca²⁺/calmodulin-dependent phosphatase, calcineurin, which dephosphorylates NFAT, causing it to translocate from the cytoplasm to the nucleus. Carcinoma cells were shown to undergo p53-induced apoptosis through the calcineurin-dependent signaling pathway.⁵⁷ In addition, a previous report showed that both p53 and NFAT were involved in angiotensin II-induced apoptosis in vascular smooth muscle cells.^{57,58} On the other hand, other reports have shown that calcineurin has a pivotal role in β -AR stimuli-induced apoptosis in cardiomyocytes.²⁶ Thus, calcineurin and NFAT may be involved in both the β -AR- and p53-mediated proapoptotic pathways.

Cyclic AMP Response Element-Binding Protein (CBP)/p300

CBP/p300 functions as a cofactor for several transcription factors, including p53, and facilitates their function. In addition, CBP/p300 was also shown to have histone acetyltransferase (HAT) activity. p53 is activated by CBP/p300 through acetylation.⁵⁹ When CBP/p300 activates p53, these 2 molecules form a tripartite complex with CREB. The formation of this complex is facilitated by the phosphorylation of CREB by PKA, CaMK, and protein kinase C.^{60,61} PKA and CaMK are activated by β -AR signaling. Taken together, it can be speculated that there may be a situation in which β -adrenergic stimuli affect the p53 induced transactivation through the formation of the CBP/p300–CREB–p53 complex.

HIF-1

HIF-1 expression is induced by hypoxia and it predominantly functions as a transcription factor. HIF-1 transactivates a number of proteins that are involved in angiogenesis, cell proliferation, metabolism, cell survival, apoptosis, etc. HIF-1 can induce not only proapoptotic proteins such as BNIP3 and NIX, but also antiapoptotic proteins such as erythropoietin.⁶² In addition, HIF-1 promotes the accumulation of p53 by directly interacting with MDM2.⁶³ Recent reports indicate that HIF-1 helps preserve cardiac function after hypoxic stress. HIF-1 overexpression attenuated cardiac damage after myocardial ischemia/reperfusion injury in cultured cardiomyocytes and a mouse model.^{64,65} Although the enhancement of angiogenesis by HIF-1 is likely to be the important mechanism, several reports suggest that HIF-1 may modulate the apoptotic signal in cardiomyocytes.^{66–69} Although the role of HIF-1 in the development of apoptosis is not well elucidated, HIF-1 is thought to be a potential factor in the apoptosis of cardiomyocytes.

HIF-1 expression is upregulated by the PI3K–AKT pathway,⁷⁰ which can be activated by G $\beta\gamma$ protein-mediated signaling. In addition, Forskolin, an AC activator that can be activated by Gs protein, also induces HIF-1 expression in cancer cells.⁷¹ On the other hand, some reports indicate that p53 downregulates HIF-1 expression. In addition, this downregulation is inhibited by AKT activation,⁷² which can be induced by β -AR stimulation. Taken together, β -AR signaling upregulates, while p53 downregulates HIF-1 expression. Therefore, HIF-1-mediated regulation of apoptosis may be a type of competitive cross-talk between the β -AR- and p53-mediated signaling pathways.

However, only a few reports have examined this pathway in cardiomyocytes, so further studies are required in order to determine the importance of this pathway in the development of HF.

Conclusions

It is crucial to maintain the number of cardiomyocytes in order to maintain the function of a failing heart. Although there have been several attempts to develop therapies that regenerate cardiomyocytes using stem cells or progenitor cells, currently there is not a clinically established method to increase in the number of cardiomyocytes.^{73,74} Therefore, preventing cell death in the failing heart is still a promising approach to manage and prevent HF.

In this review, we focused on apoptosis as one mechanism of cell death in the failing heart. The β -AR- and p53-mediated signaling pathways are 2 major inducers of apoptosis. Many approaches, including gene therapy, have been developed to modulate the signaling of these pathways. Considering their effects on apoptosis, controlling these pathways could be a promising strategy to preserve cardiac function.

When attempting to establish HF therapies that modulate signal transduction, there are several important issues to be considered.

First, it is important to determine when and where signaling should be modulated. As the use of β -AR agonists or antagonists to treat HF depends on the disease state, the timing should be considered when modulating the p53 signaling pathways. p53 is a major tumor suppressor and may exacerbate HF by inducing apoptosis and inhibiting angiogenesis. However, p53 may also cause preferable effects on the heart. For example, p53 may inhibit the development of arteriosclerosis.⁷⁵ In addition, p53 may prevent the proliferation of vascular smooth muscle cells, which is pivotal in coronary restenosis after stent implantation.⁷⁶

The β -antagonists have several side effects, including bronchial asthma, glucose intolerance, and Raynaud's phenomenon, because they affect tissues other than the heart. To avoid these effects, therapies that modulate specific subtypes of AC are near development.⁷⁷ In the same way, we should control p53 function in a tissue-specific manner.

Second, it is important to consider the possibility of cross-talk with other pathways that are involved in the development of HF. Modulating certain signaling pathways may affect others. Understanding the cross-talk among several important pathways would be useful in choosing the time and the method of therapeutic intervention to obtain the maximum effect. In this review, we noted 5 possible cross-talk points between the β -AR- and p53-mediated signaling pathways for apoptosis. Some of these are thought to facilitate another pathway. For example, β -adrenergic signaling may enhance the activity of p53 through several pathways. One of the mechanisms by which β blockers help preserve cardiac function may be by attenuating p53 function. On the other hand, potential points of competitive cross-talk have also been identified. For instance, p53 may downregulate HIF-1, while β -AR signaling may upregulate HIF-1. If there is a situation in which HIF-1 plays a significant role in regulating the apoptosis of cardiomyocytes, understanding these potentially interconnected pathways may lead to the development of more effective therapies that prevent apoptosis. Therefore, it will be important to examine the contribution of each pathway, as well as the cross-talk points under various conditions.

On the whole, to selectively inhibit the cAMP signaling

pathway while preserving the PI3K-AKT pathway seems to be effective for inhibiting the apoptosis that is induced by these 2 pathways. This fact reminds us of the β 1-selective β blockers. However, many pathways and molecules other than these 2 pathways are involved in the apoptosis of cardiomyocytes. Moreover, many mechanisms other than apoptosis are involved in the pathogenesis of HF. Cell death including necrosis, autophagy as well as Ca^{2+} handling, oxidative stress, metabolic state, etc have been identified as important factors that affect the development of HF. This may be one of the reasons why the advantages of β 1-selective β blockers compared with nonselective β blockers for HF therapy seem not to be significant in clinical studies. In the COMET trial, the β 1, β 2, α 1 blocker, carvedilol, extended the longevity of chronic HF patients better than the β 1-selective blocker metoprolol.⁷⁸ Clarifying the relationships and roles of each signaling pathway in the various phases of HF development will lead to the development of more effective and sound treatments.

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