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Aryl hydrocarbon receptor pathway participates in myocardial ischemia reperfusion injury by regulating mitochondrial apoptosis

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A B S T R A C T

Reducing ischemia reperfusion (I/R) injury has been a major challenge in the treatment of cardiovascular disease. It is widely accepted that mitochondrial apoptosis is an important link in myocardial infarction induced by I/R damage. Studies suggested that aryl hydrocarbon receptor (AhR) pathway plays an important role in the development and function of the cardiovascular system and regulating the mitochondrial homeostasis. AhR played a critical role in regulating mitochondrial homeostasis in response to TCDD-induced stress through translocation of AhR from the cytoplasm into the cell nucleus. And a portion of AhR was found in the mitochondrial inter membrane space. Moreover, there were abundant AhR expression in myocardial cells induced by I/R, and ischaemic post-conditioning reversed apoptotic reperfusion injury involving the AhR signaling pathway. Additionally, AhR is involved in the mechanism of cardiac toxicity of many chemotherapeutic agents. Given the discovery mentioned above, we hypothesize that AhR pathway participates in myocardial ischemia reperfusion injury by regulating mitochondrial apoptosis. Meanwhile, how the AhR pathway is involved in I/R damage and regulating mitochondrial apoptosis is needed to further verified. To evaluate our hypothesis, we will measure the expression of AhR in mitochondria and cytoplasm after myocardial ischemia and reperfusion in vitro and in vivo. And then study the AhR pathway in regulating mitochondrial apoptosis to participate in myocardial I/R injury using mitochondrial protein mass spectrometry analysis and RNA interference technique. If our hypothesis is correct, AhR will be a key target in myocardial I/R injury and myocardial infarction, which could provide important agents.

Introduction

Reducing the ischemia-reperfusion (I/R) injury has been always a key challenge in cardiovascular disease treatment [1–3]. I/R injury main occurs in the early stage of ischemic reperfusion [4,5]. Myocardial apoptosis induced by I/R is believed to be a determinant of the ultimate degree of myocardial infarction and mitochondrial apoptosis is one of the primary pathways [6]. When I/R occurs, a long list of apoptosis signals target to mitochondria lead to the release of relevant substance from mitochondria into cytoplasm by changing the permeability of the mitochondrial membrane. And this progress is believed to mediate the apoptosis of mitochondria and cells. Hence, Mitochondria may play a switch role in cell apoptosis induced by ischemia reperfusion injury [7].

Apoptosis is an active process of various gene regulation. It was shown that Aryl hydrocarbon receptor (AhR) pathway was involved in the regulation of the oxidative stress and the apoptosis of mitochondria [8]. Knocking out AhR gene during mice embryonic development period resulted in the defects of heart structure and function when they were born and eventually developed into heart failure [8]. The results indicated the critical physiological roles of AhR in fetal and neonatal growth and development. Moreover, a portion of AhR was transferred into mitochondrial inter membrane space and involved in oxidative stress and apoptosis of mitochondria in the progress of hepatocytes oxidative stress injury induced by TCDD, a kind of AhR exogenous ligand [9–11]. AhR was also reported to participate in the apoptosis mechanism of cardiac toxicity of various chemotherapy drugs [12]. Furthermore, it was observed that there was not only abundant AhR protein expression in cardiomyocytes but the expression increased after myocardial I/R injury, further supporting a possible interaction between AhR and I/R injury [13]. As a result, we hypothesize that AhR may be a key factor in myocardium preservation by regulating mitochondrial apoptosis. However, the mode of AhR signaling pathway participating in the I/R injury and how it works on regulating mitochondrial apoptosis need further research.

Hypothesis

The hypothesis is that AhR may be involved in myocardial ischemia-reperfusion injury by regulating mitochondrial apoptosis. Studies suggested there were a large number of AhR expressing in myocardial cells, which further induced apoptosis and increased myocardial I/R injury. However, it is unclear how this receptor pathway participates in I/R injury. During the stress reaction induced by exogenous ligands of AhR, some AhR were transported into the mitochondrial membrane space, involves in hepatocellular carcinoma cell mitochondrial oxidative stress injury and apoptosis. Whether this phenomenon occurs in myocardial I/

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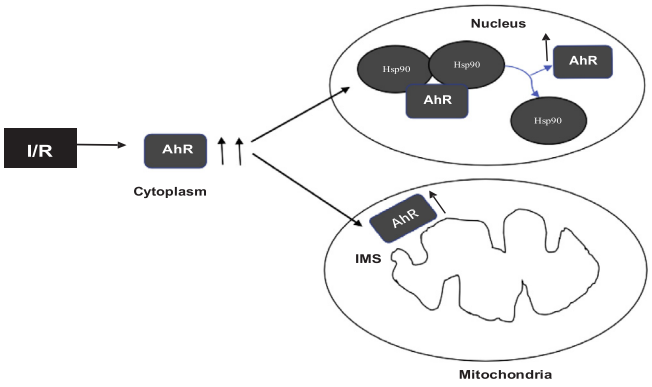

Fig. 1. AhR may be involved in myocardial ischemia-reperfusion injury by regulating mitochondrial apoptosis. I/R: ischemia/reperfusion; AhR: Aryl hydrocarbon receptor; Hsp90: heat shock protein 90; IMS: inter membrane space.

R injury and induce the myocardial mitochondrial apoptosis still need further study. We hypothesize that a small amount of AhR is located in the mitochondrial membrane space and is involved in the maintenance of mitochondrial structure and functional homeostasis under normal conditions. When myocardial cells are damaged by ischemia-reperfusion injury, AhR is translocated to the nucleus from the cytoplasm and induces a series of cascades. At the same time, part of AhR is transported to the mitochondrial inter membrane space, activating its downstream pathway inducing mitochondrial apoptosis ultimately leading to myocardial cell death (see Fig. 1).

Mitochondrial apoptosis and myocardial ischemia reperfusion injury

With the aging of population and the acceleration of the urbanization process, the risk factors of cardiovascular diseases in China are becoming more and more prevalent, and the number of patients with cardiovascular diseases continues to increase. 《2015 China cardiovascular disease report》 revealed that the total number of cardiovascular deaths is still rising rapidly, and mortality of cardiovascular disease accounts for more than 40%, making it the first cause of death among Chinese residents [14]. Myocardial ischemia reperfusion is a potentially fatal event duo to a large area of myocardial infarction. The necrotic myocardial cells are nearly unable to be repaired or regenerated. Therefore, how to reduce the myocardial I/R damage and the death of myocardial cells has always been a hot, tough and meaningful topic in myocardial protection. It has been profoundly multi-facetted that the critical period of I/R injury is early reperfusion stage after ischemia, while the apoptosis caused by reperfusion injury is the main factor which determines the final myocardial infarction [15]. There are three main pathways of apoptosis: mitochondrial pathway, endoplasmic reticulum pathway and death receptor pathway. They are supposed to be interconnected and mediate apoptosis together, in which, mitochondrial pathway is considered as a switch role in apoptosis [7]. In other words, mitochondria are closely related to cell death and recovery, and have positive therapeutic significance [4,16]. It has been discovered that mitochondrial can mediate programmed cell death through autogenic Apoptosis, suggesting that one of the main tasks of mitochondria is to induce cell apoptosis [17,18]. During this progress, as response to apoptotic signals, mitochondria release some proteins such as cytochrome *C*, apoptotic inducing factor (AIF) and endonuclease, etc. through a series of pathways. In addition to mediating cell apoptosis in cytoplasm, which released substance also enter the nucleus and regulate the expression of related apoptotic genes.

Mitochondrion is a closed cystic structure surrounded by two layers of membranes, which is divided into four parts: outer membrane (OMM), inner membrane (IMM), inter membrane space (IMS), and inner matrix (matrix). There are additional structures comprising cytochrome *C*, voltage dependent anion channel (VDAC), ADP/ATP conversion protein, mitochondrial membrane transport channel (MPTP), AIF and other enzymes in the IMS. At the early stage of mitochondrial apoptosis, the permeability of mitochondrial membrane increased [19–21]. AIF is an important bridge between mitochondrial membrane permeability change and nuclear apoptosis [17]. It is supported that upon I/R injury, AIF releases into the cytoplasm from the mitochondrial inner membrane to induce cell apoptosis. Mitochondrial apoptosis is regulated mainly by various apoptotic proteins as well as its complexes in mitochondrial apoptotic pathway. Under normal cell conditions, various pro-apoptotic proteins, along with various apoptosis inhibitors, together regulate cell apoptosis by promoting or inhibiting the caspase cascade.

AhR pathway involves in myocardial ischemia-reperfusion injury

AhR is one of transcription factors belonging to the basic helix-loop-helix (bHLH- PAS) family, which responds rapidly to environmental stimuli through combination of the role of receptor and transcription factors [22]. Inactive AhR is present in the cytoplasm and forms a complex with heat shock protein 90 (HSP90). Upon binding to the ligand, AhR enters the nucleus to form a heterodimeric transcription factor with the aromatic hydrocarbon receptor nuclear translocation protein (ARNT), which in combination with the dioxin-responsive element (DREs) in the target gene promoter region to initiate the transcription of some target gene such as Cytochrome P450 [23]. AhR is a ligand-dependent transcription factor that mediates the metabolism of toxic and chemical substances in the environment, regulation of circadian rhythms, reproduction and the oxidative stress [9,24–27]. Due to the evolutionarily conserved nature of the gene and its widespread expression in the immune and circulatory systems, the environmental adaptation function of the AhR has been used in important physiological processes. At present, lots of evidence points to the critical effects of AhR in the pathogenesis of cardiovascular disease [28–31]. For example, AhR is involved in the regulation of key cardiac development gene expression in the complex regulatory networks of cardiac development and cardiovascular system homeostasis [32,33]. Common cardiovascular events such as cardiac hypertrophy, vascular remodeling and hypertension were all reported in AhR knockout mice [8,29,33]. Besides, Knockout of the AhR gene or AhR agonists during embryonic development can cause structural and functional abnormalities in the heart, leading to changes in fetal cardiac physiology [9,34]. It was found that AhR pathway regulated cardiomyocyte apoptosis and was involved in the cardiotoxic effect of various chemotherapeutic drugs [12,35–37].

Recent studies have shown that AhR signaling pathway might play a key role in myocardial I/R injury mechanism [12,13,37]. In myocardial ischemia model induced by left anterior descending branch ligation, AhR abundantly expressed in necrotic myocardium, indicating that acute myocardial ischemia can activate AhR and induce inflammation, as well as hsCRP, IL-1 and IL-6 expression. AhR ligands such as baicalin could reduce myocardial necrosis and inflammation by inhibiting cardiac AhR expression [37]. It was reported that AhR expression was significantly increased after ischemia-reperfusion in I/R model of pig myocardium, while ischemic post- conditioning (IPost-Co) could significantly reduce the expression of AhR, suggesting the significance of AhR pathway in ischemic post-conditioning myocardial protection mechanism [13]. However, the specific mechanism of action of this receptor and the pathway during I/R injury is not yet clear. We hypothesize that apoptosis is initiated by activation of the downstream pathway of this signaling pathway in myocardial ischemia-reperfusion injury duo to the overexpression of AhR.

AhR pathway in mitochondrial apoptosis

Based on previous research results, AhR pathway is in great possibility involved in the regulation of apoptosis and its mechanism is still not clear. It has been previously found that AhR affected apoptosis by regulating the expression of apoptotic genes and many AhR ligands could induce apoptosis [11]. Paradoxically, there is also evidence that endogenous AhR and its ligands promote cell proliferation and maintain cell numbers by inhibiting apoptosis [38]. It has been discovered that the AhR pathway is involved in regulation mitochondrial oxidative stress and apoptosis through transmission of stress signals between mitochondrial and nuclear [39]. Mitochondrial homeostasis in adult animal hearts would be severely affected by damaged AhR during development. AhR-dependent mitochondrial ATP synthesis decreased by the AhR exogenous ligand TCDD, and which increased production of superoxide and hydrogen peroxide, then trigger mitochondrial oxidative stress and eventually induce apoptosis [40]. Besides, studies also showed that AhR pathway was highly relevant to cardiomyocyte apoptosis process caused by cardiotoxicity of chemotherapeutic drugs [12].

Gel electrophoresis and protein mass spectrometry analysis revealed that AhR interacted with mitochondrial ATP synthase subunit ATP5α1 and mitochondrial ribosomal protein MRPL40 [39]. AhR could co-purified with mitochondria-specific cytochrome *c* oxidase IV, suggesting that some AhR exist in the mitochondria. Further research in line of mitochondrial protein mass spectrometry analysis found that part of AhR is located in the mitochondrial inter membrane space (IMS) in the mouse hepatoma cell line hepa1c1c7, and mitochondrial AhR plays an important role in the regulation of mitochondrial homeostasis and TCDD-induced stress response [10]. AhR might be transported from the cytoplasm into the mitochondrial membrane space through its cytosolic partners AIP and HSP90. HSP90 may bind to the mitochondrial outer membrane translocation complex and promote the import of mitochondrial proteins which lack the classical targeting sequence [39]. TOMM20 recognizes the N- terminal sequence of the mitochondrial target protein and transports it into the

mitochondrial inner membrane [41], then activate its downstream cascade and inducing mitochondrial apoptosis.

Conclusion

AhR pathway plays an important role in the development and function of the cardiovascular system and regulation of mitochondrial homeostasis. The AhR gene knockout mice develop cardiac hypertrophy, eventually lead to heart failure. some Ah receptors are found in the mitochondrial IMS. There is a large number of AhR expression in myocardial cells induced by I/R, and Post-Co may reduce apoptotic reperfusion injury involves the AhR pathway. AhR is also involved in the mechanism of cardiac toxicity of many chemotherapeutic agents. Hence, the AhR pathway is supposed to be involved in I/R damage and it plays an important role in regulating mitochondrial apoptosis. At first, we will confirm that the expression of AhR in mitochondria and how it participates in regulating mitochondrial apoptosis in isolated myocardial cells; then we establish animal model of myocardial ischemia reperfusion in vitro and in vivo, to observe the regulation of AhR pathway on mitochondrial apoptosis and the role during I/R injury. And providing important scientific basis and theoretical basis for the research and development of new myocardial protective drugs.

Conflicts of interest statement

Authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2018.12.004.

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