Activating transcription factor 3 - an endogenous inhibitor of myocardial ischemia-reperfusion injury (Review)

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Abstract. Coronary heart diseases, particularly acute coronary syndrome, have increased in morbidity and mortality in recent decades. Percutaneous coronary intervention, coronary artery bypass grafting and thrombolytic agents are effective strategies to rescue the infarcted myocardium. In addition to acute myocardial infarction, the resulting myocardial ischemia-reperfusion injury (MIRI) leads to serious secondary injury of the heart. Studies have demonstrated that activating transcription factor (ATF)/cyclic adenosine monophosphate response element binding family member ATF3 had a negative regulatory role in IRI, particularly in the kidney, cerebrum and liver. The present review expounded the expression characteristics of ATF3 and its protective effects against MIRI, providing a theoretical basis for the overexpression of ATF3 in the myocardium as a promising gene-therapeutic strategy for MIRI.

Contents

1. Introduction

According to the Heart Disease and Stroke Statistics for 2014, ~620,000 US citizens present with acute myocardial infarction for the first time and ~295,000 experience recurrent acute myocardial infarction per year (1). It was estimated that every 34 sec, one coronary event occurs and every 1 min and 23 sec, one patient succumbs to coronary heart disease (CHD) in the USA (1). Acute coronary syndrome (ACS) is treated using effective methods including percutaneous coronary intervention, coronary artery bypass grafting and thrombolytic agents, which are, however, constrained by a series of pathophysiological changes, including myocardial necrosis, arrhythmia and myocardial stunning following myocardial ischemia-reperfusion injury (MIRI). Hence, identification of endogenous negative regulatory factors of MIRI is urgently required and has become a hot spot of cardiovascular research. In the last decade, activating transcription factor 3 (ATF3) has been found to negatively and directly regulate Toll-like receptor 4 (TLR4) signal transduction (2), which represents the main pathway mediating the MIRI-associated inflammatory response.

ATF3 is a member of the ATF/cyclic adenosine monophosphate response element binding (CREB) family, which is rich in basic-region leucine zipper (bZIP) (3). The ATF3 gene contains four exons encoding a 181 amino-acid protein with a molecular weight of 22 kDa (3). ATF3 forms a homodimer with connection via the bZIP structure and also combines with other ATF/CERB family members or CCAAT/enhancer binding protein (C/EBP) family members to form heterodimers (4). The formation of the ATF3 homodimer and ATF3 heterodimer have numerous functions in the activation of gene-transcription in the cell nucleus (5): The homodimers exert suppressive effects on target gene transcription, while the heterodimers exert suppressive or activating effects based on the status of cell and promoter. Therefore, the transcription of target genes containing a promoter with ability to bind to the homodimer is restrained by ATF3.

2. ATF3 expression in MIRI

Induction factors of ATF3 expression. ATF3 expression is usually ubiquitous at low levels in normal quiescent cells in the absence of cellular stressors. ATF3 is a stress-response gene of the early phase and is induced to activate transcription under several conditions of cellular stress, including oxidative stress, genetic toxicity and inflammatory response, which leads to a large increase in cytokines, growth-stimulating factors (6,7). MIRI is caused by inflammatory responses (8), activation of

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the complement system (9), calcium overload (10), oxidative stress (8), cell apoptosis (11,12) and autophagy (11), which promotes the stimulation of ATF3 expression in the early stage.

Yamamoto *et al* (13) subjected Sprague Dawley rats to IR after lung transplantation and discovered that the expression of ATF3 was markedly elevated at 30 min and even more at 180 min. Furthermore, Wang *et al* (14) reported that in ischemic brains of adult mice, the mRNA expression of ATF3 was 60-fold increased at 6 h, followed by a three-fold reduction at 24 h after ischemic onset. Song *et al* (15) reported that following middle cerebral artery occlusion and reperfusion injury, the expression of ATF3 was markedly increased in the ipsilateral peri-infarct cortex at 1-2 days, but was declined at 3 days. These studies implied that ATF3 expression is transiently and not persistently upregulated after IR injury. When the myocardium is subjected to ischemia or hypoxia, an adaptive upregulation in the expression of ATF3 occurrs. A bioinformatics analysis of gene expression the in left ventricle following acute myocardial infarction performed by Zhang *et al* (16) revealed that a series of genes, including ATF3, were key transcription factors associated with the immune response. Krivoruchko and Storey (17) found that under anoxia, the expression of ATF3 in the heart was increased at the protein and mRNA level.

Signaling pathways stimulating ATF3 expression in MIRI. Upon MIRI, certain endogenous molecules are released, including heat-shock protein 60, fibronectin, hyaluronic acid, defensin 3 and cell debris, which are referred to as danger-associated molecular patterns (DAMPs) (18-20). The recognition of DAMP by TLR4, which is located on the myocardial cell surface, activates the myeloid differentiation factor 88 (MyD88)-dependent pathway as well as the Toll-like receptor adaptor molecule 1 (TRIF)-dependent pathway, the so-called MyD88-independent pathway, to promote the intranuclear translocation of nuclear factor-κB (NF-κB). MIRI induces the production of numerous inflammatory factors and chemotactic factors and activation of immune cells (21,22) as a result of myocardial damage.

A number of studies indicated that the TLR4/NF-κB signaling pathway participates in the expression of ATF3 (2,23,24). Whitmore *et al* (23) showed various TLRs that rapidly increased ATF3 expression in macrophages and plasmacytoid dendritic cells in mice and humans. Gilchrist et al (2) performed cDNA microarrays on macrophages following their activation with bacterial lipopolysaccharide (LPS), a TLR4 agonist, revealing that ATF3 mRNA expression peaked at 1 h. These studies suggested that ATF3 expression was induced by TLR4/NF-κB signaling. A study by Suganami *et al* (24) further verified this result *in vitro* and *in vivo* by demonstrating that in RAW264 macrophages, the saturated fatty acids LPS, stearate and palmitate markedly increased the expression of ATF3 at the protein and mRNA level, while palmitate did not affect the mRNA expression of ATF3 in peritoneal macrophages from TLR4-knockdown C3H/HeJ mice. Furthermore, treatment of RAW264 macrophages with the NF-κB inhibitor BAY11-7085 significantly suppressed the palmitate-induced mRNA expression of ATF3, while ATF3 promoter activity was significantly enhanced in HEK293 cells following selective NF-κB activation via transient overexpression of its p65 and p50 sub-units (24). These observations suggested that the expression of ATF3 is induced via the TLR4/NF-κB pathway.

3. Protective effects of ATF3 against MIRI

Role of ATF3 in IRI. While ATF3 expression is induced by activation of the TLR4/NF-κB signaling pathway, ATF3 also negatively regulates the TLR4 signal transduction pathway (2,23), thereby exerting a protective effect against IRI. Of note, the protective effects of ATF3 have been documented with regard to IRI in the kidney (25-27), cerebrum (14) and liver (28). Li *et al* (25) observed that ATF3-deficient mice exhibited increased rates of mortality, kidney dysfunction, inflammation and apoptosis compared with those of wild-type mice following renal IR, while gene transfer-mediated restoration of ATF3 in the kidney protected these ATF3-deficient mice from IR-induced injury. Yoshida *et al* (26) utilized adenovirus-mediated gene transfer to overexpress ATF3 in mice, which had a protective effect against renal IRI. Furthermore, Chen *et al* (27) reported that in ATF3-deficient mice, the induction of adhesion molecules P- and E-selectin, interleukin-6, intercellular adhesion molecule, vascular cellular adhesion molecule and monocyte chemotactic protein-1 was enhanced compared with that in wild-type mice during renal IR-induced inflammation. Their *in vitro* study showed that MCP-1 expression in epithelial cells as well as macrophage migration was inhibited by epithelium-derived exosomal ATF3 RNA and that IR-induced kidney injury was attenuated following administration of exosome containing ATF3 RNA derived from epithelial cells (27). In the research of brain injury after transient focal cerebral ischemia, knockout of ATF3 significantly exacerbated the infarct volume and worsened neurological function and up regulated neural apoptosis, inflammatory gene expression and cellular inflammatory response (14). In mouse models of warm and cold liver IRI, Rao *et al* (28) showed that ATF3 deficiency significantly aggravated IR-induced liver injury and demonstrated that ATF3 mediated local cytoprotection against TLR4-driven inflammation in liver IRI (28).

While only few studies have investigated the role of ATF3 in MIRI, certain protective effects have been indicated (29). Furthermore, Brooks *et al* (30) observed an obvious increase of inflammatory cells and neutrophils in ATF3-null hearts subjected to myocardial IR after ischemic pre-conditioning (30). While genetic deletion of ATF3 did not decrease the inflammatory response and attenuated monocyte and neutrophil infiltration in non-pre-conditioned hearts, it abolished the cardioprotective effects of ischemic pre-conditioning (30). These results suggested that ATF3 may negatively regulate the inflammatory response in MIRI.

Mechanisms of ATF3-mediated attenuation of MIRI

Anti-inflammatory effects of ATF3. The TLR4 downstream signaling cascade of the inflammatory pathway promotes the release of interleukin(IL)-6 and IL-12b, whose promoter regions contain binding sites for CREB/ATF and NF-κB (2). ATF3 binds to the ATF/CREB sites in the promoter regions of the target genes and thereby inhibits the binding of NF-κB, which suppresses the transcription of IL-6 and IL-12b (2). Furthermore, transcription of the inflammatory factor tumor

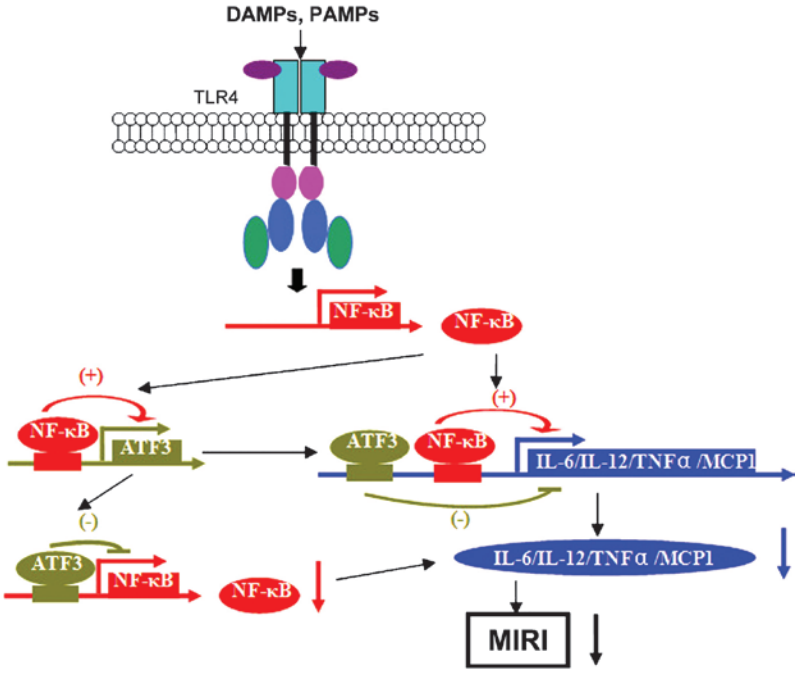

Figure 1. Signal transduction pathway of ATF3 protecting MIRI via TLR-4/NF-κB-mediated inflammation. MIRI, myocardial ischemia reperfusion injury; ATF3, activating transcription factor 3, TLR4, Toll-like receptor 4; NF, nuclear factor; IL, interleukin; TNF, tumor necrosis factor; MCP1, monocyte chemotactic protein 1; DAMP, danger-associated molecular pattern.

necrosis factor (TNF)-α was shown to be restrained by ATF3 by blocking the AP-1 binding site in its promoter to interfere with the transcriptional activation by NF-κB (24,31). The effect of ATF3 binding to the CREB/ATF or AP-1 recognition site of target genes is linked with ATF3-associated histone deacetylase (HDAC), which performs chromosomal remodeling, which has an important role in the regulation of transcriptional activity (32,33). While histone acetyltransferases mediate the acetylation of histones to unwind the chromatin structure, which provides access by positive transcriptional regulators and thereby promotes transcriptional activation, HDACs have the opposite effect and mediate chromatin condensation, which represses transcriptional activation due to the resulting inaccessibility of gene promoters. Numerous studies have confirmed that ATF3-associated HDAC caused condensation of the chromatin structure and blocked the binding of NF-κB to the promoter regions of IL-6, IL-12, TNF-α and MCP-1, which inhibited the transcription of these inflammatory factors and attained the suppression of TLR4-associated inflammatory signaling pathways (23,25,34). Furthermore, it has been evidenced that HDAC in had protective effects against MIRI (35,36). In conclusion, ATF3 inhibits TLR4/NF-κB inflammatory signaling to reduce MIRI (Fig. 1).

Anti-apoptotic effects of ATF3. Yoshida *et al* (26) revealed that adenovirus-mediated overexpression of ATF3 in HK2 cells reduced H_2O_2-induced cell death and this protective effect was associated with a decrease of p53 mRNA and an increase of p21 mRNA. ATF3 was also shown to prevent reactive oxygen species-induced renal injury via upregulation of p21 and downregulation of p53 (33). It is well known that protein kinase inhibitor p21 has a vital role in cell apoptosis by regulating cell-cycle progression, while p53 is able to induce apoptosis and suppress cell proliferation; therefore, enhancement of p21 and reduction of p53 expression following overexpression of ATF3 leads to cell-cycle arrest and prevents apoptosis. Krivoruchko and Storey (17) demonstrated that ATF3 prevented adriamycin-induced myocardial-cell apoptosis by blocking the transcription of p53 through binding to the PF-1 site of its gene promoter, thereby exerting protective effects on the myocardium. Thus, the prevention of apoptosis is another mechanism of the protective effects of ATF3 against MIRI.

4. Conclusion

ATF/CERB family member ATF3 is considered to be a regulatory factor of gene transcription. While ATF3 expression is maintained at low levels in normal quiescent cells, it is induced by several cellular stressors, including IRI, which triggers the activation of the TLR4/NF-κB signaling pathway as an early stage of the molecular stress response. A large number of studies have demonstrated that ATF3 homodimer binds to the promoter regions of its target genes and recruits HDAC, which in turn inhibits the transcription of these genes to interrupt the synthesis of the associated inflammatory and chemotactic factors involved in the TLR4/NF-κB pathway, which ultimately exerts protective effects against MIRI. Although few studies have reported the protective effects of endogenous regulatory factor ATF3 in MIRI, the anti-inflammatory and anti-apoptotic effects of ATF3 are well-documented; therefore overexpression of ATF3 may represent a promising therapeutic strategy for the prevention of MIRI.

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