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Role of Forkhead Transcription Factors in Myocardial Ischemic Reperfusion Injury in Diabetes

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Abstract

Diabetes, characterized by hyperglycemia resulting from either insulin deficiency or insulin resistance, is a chronic metabolic disorder. Hearts of subjects with diabetes are more sensitive to myocardial ischemia/reperfusion injury but the underlying mechanism is largely unclear. Recent evidence suggests that alteration in cardiac metabolism is a key contributor to the increased vulnerability of diabetic heart to ischemia/reperfusion injury. The FoxO family of forkhead transcription factors including FoxO1, 3 and 4 play important roles in the regulation of many cellular and biological processes and are critical regulators of cardiac metabolic and cellular oxidative stress in the heart. This brief review focuses on the role of FoxO in regulating cardiac metabolism and its association with myocardial ischemia/reperfusion injury, especially in diabetes.

Keywords: FoxO; Diabetes; Ischemia/reperfusion injury

Introduction

Myocardial infarction is the leading cause of sudden death and long term disability. Unfortunately it is also one of the most common perioperative complication associated with surgery and is particularly prevalent in patients with diabetes [1]. Reperfusion therapies such as thrombolysis and percutaneous coronary intervention are the mainstay of current treatment to restore coronary blood flow in order to salvage the ischemic heart, but paradoxically, reperfusion may itself cause lethal tissue injury, termed “ischemia-reperfusion injury (IRI)” [2].  

Ischemic preconditioning (IPC) achieved by brief episodes of ischemia and reperfusion, given before prolonged ischemia, provides profound protection and has been considered as a powerful maneuver against myocardial ischemic injury in vivo [3,4]. Although effective, IPC requires access to the coronary vessels which is not feasible in the majority of cases. Recently, attention has focused on modifying events occurring at the time of myocardial reperfusion (i.e., ischemic postconditioning, IPostC). This phenomenon, in which the application of transient brief interruptions of reperfusion by ischemic episodes results in reduced myocardial injury, has led to renewed interest in the development of protective maneuvers to combat lethal reperfusion injury [5]. Currently, we and others have found that anesthetic drugs such as isoflurane [6,7], sevoflurane [8] and morphine [9] have similar infarction limitation properties to those of IPC and IPostC, this maneuver is termed pharmacological or anesthetic pre/postconditioning (APC). In addition, antioxidant intervention with respect to reduce oxidative stress and normalize myocardial antioxidant defense, has also been proven to be a cardioprotective way against IRI, which has been demonstrated by Wang et al. and Nie et al. [10,11] and others. Unfortunately, all these effects of current available means of cardioprotection (IPC, IPostC, APC and antioxidant intervention) are abolished or compromised under pathological conditions such as diabetes [12] and yet diabetics are particularly prone to myocardial IRI [13].  

Numerous studies have indicated that the diabetic heart is more sensitive to ischemia/reperfusion injury (IRI). Although the mechanism governing vulnerability of the diabetic heart to IRI is still not fully understood, alterations in cardiac metabolism have been suggested to play an important role in its pathology. At the setting of ischemia, a significant reduction of oxygen supply occurred due to the inadequate blood supply [14]. During prolonged ischemia, there is an increase in anaerobic glycolysis for ATP generation in the myocardium utilized for cardiac contraction, in which results in intracellular acidosis and inhibition of glycolysis as well as mitochondrial dysfunction [15]. In the diabetic heart, the earliest change is altered energy metabolism where, the heart switches from glucose utilization to predominantly using fatty acids (FA) for energy supply, which subsequently increase FA oxidation and triglyceride (TG) accumulation in the heart [16]. When ischemia/reperfusion is imposed in a setting of diabetes, there is a rapid recovery of FA β-oxidation (circulating FA are augmented as a consequence of lipolysis), and glucose oxidation is additionally reduced, this contributes to profound uncoupling of glucose metabolism, provokes mitochondrial uncoupling which increases reactive oxygen species (ROS) generation, aggravates intracellular acidosis, further increasing diabetic heart susceptibility to IRI [14] (Figure 1).

The Forkhead box of class O (FoxO) plays a pivotal role in a number of cellular processes and are critical regulators of cellular oxidative stress response pathways. Abundant evidence now suggests that FoxO is important for cardiac metabolic regulation and maintenance of cardiac function. This review intends to help understand the role of FoxO in regulating the vulnerability of the diabetic heart to IRI and its potential role in regulating the responsiveness of the diabetic hearts to therapeutic interventions like IPC, IPostC, and APC, in order to

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**Figure 1:** Cardiac FoxO regulates metabolism. Diabetes or high glucose/high lipid increases FoxO nuclear retention which leads to metabolic dysfunction with subsequent increase in the production of reactive oxygen species (ROS), making the heart prone to ischemia/reperfusion injury.

**Forkhead Box (Other) Transcriptional Factor: Its Regulation and Biological Function**

Forkhead box (Other) transcriptional factors (FoxO) are members of the forkhead family of transcriptional regulators, which have a DNA-binding forkhead helix domain, a nuclear localization sequence, a nuclear export sequence and a C-terminus containing a transactivation domain. FoxO proteins (including FoxO1, FoxO3 and FoxO4) are expressed in almost every tissue, of which, FoxO1 and FoxO3 are expressed in developing and adult heart, participating in a wide range of cellular processes including oxidative stress resistance, energy metabolism, cell survival and cell death.

FoxO1 and FoxO3 bind to an identical DNA target sequence, thus regulating similar target genes. By binding to DNA through their forkhead domain, these proteins either inhibit or activate target protein gene expression [17]. The fate of FoxO is controlled by three major post-translational modifications, including phosphorylation, acetylation and ubiquitination [18], which determine their cellular compartmentalization, DNA binding, and degradation [19]. Initially FoxO proteins are phosphorylated by the protein kinase Akt, the downstream target of insulin mediated survival signaling pathway, thus insulin is considered as the primary regulator of FoxO [20]. After being phosphorylated by insulin, Akt phosphorylates FoxO1 at site Ser256, making it prone to further phosphorylation at Ser319 and Thr24 by Akt [21]. Following phosphorylation by Akt, FoxO1 binds to 14-3-3, which reduces FoxO DNA binding ability and facilitates its nuclear exclusion and segregation in the cytoplasm, which subsequently undergoes degradation by E3 ubiquitin ligase-mediated ubiquitination [18]. In addition to insulin, stress signals like AMPK and JNK are also known to phosphorylate FoxO at different sites, which disrupt its binding ability to 14-3-3, leading to its nuclear retention [22]. FoxO is also acetylated by CBP/p300, which reduces its DNA binding ability and transcriptional function [23], whereas deacetylation by Sirtuins increase FoxO nuclear retention and transcriptional activity [24].

FoxO plays important roles in many cellular processes. FoxO regulates insulin signaling and glucose and lipid metabolism in the heart [25,26]. Cardiac specific activation of FoxO1 alone is sufficient to adversely alter cardiac metabolism and cause pathological remodeling and dysfunction [27]. Deletion of FoxO1 in cardiomyocytes shifted their metabolic substrate usage, from FA to glucose, and decreased the cardiac lipid content, which reversed high fat diet induced declines in cardiac function [27]. Inhibition or gene knockout of FoxO1 reduces post-ischemic infarction in the brain [28]. Nuclear FoxO activation by Glucocorticoids increase pyruvate dehydrogenase kinase 4 (PDK4) expression and activity, thereby suppressing cardiac glucose oxidation and further lead to cardiac pathologies by upregulating lactate formation and inducing lipotoxicity [29]. Similarly, study in *in vivo* model showed that, administration of intralipid in rats upregulated FoxO1 nuclear presence in cardiomyocytes, contributing to cardiac insulin resistance and cell death via FoxO1-iNOS-CD36 axis [30]. In addition, streptozocin- and diazoxide-induced hypoinsulinemia and hyperglycemia has been shown to increase cardiac nuclear FoxO1 activation which upregulate iNOS expression, and subsequently activates cell death signaling [28]. All these indicate that FoxO1-iNOS may serves as the bridge to connect the changes of cardiac metabolism and cell death pathway stimulations, especially under pathologic conditions like diabetes. Furthermore, these properties of FoxO1 may also make it a potential mediator of the increased sensitivity of the diabetic heart to IRI.

**Cardiac Metabolic Dysfunction may Increase Diabetic Heart Vulnerability to IRI**

Diabetic heart is more susceptible to IRI [31], but the mechanism is unclear. Alterations in cardiac metabolism may exaggerate IRI. In the healthy heart, approximately two-thirds of the energy required for cardiac contractility derives from fatty acid (FA) oxidation, with the remainder from glucose and lactate metabolism. During cardiac ischemia, glucose becomes the preferred substrate, as it requires less oxygen for oxidation than an equimolar amount of carbon derived from FA [32,33]. This is particularly beneficial in the stressed heart with limited oxygen supply [16]. However, in diabetes, cardiac glucose utilization is depressed, which causes a switch to predominantly using FA for energy supply [14]. When diabetic hearts are subjected to ischemia, high levels of FA uptake suppress glucose oxidation, reduces ATP supply, thereby increasing lactate accumulation and electromechanical uncoupling in cardiomyocytes, resulting in cardiac acidosis and dysfunction [34,35]. In diabetic heart, high levels of FA delivery exceeds the oxidative capacity of the cell, resulting in excessive cardiac FA accumulation, elevated oxygen demand, mitochondrial uncoupling, reactive oxygen species (ROS) overproduction, and mitochondrial dysfunction, which provoke cardiac cell death and exacerbate myocardial IRI [36]. Thus, metabolic and the subsequent mitochondrial dysfunction may be the fundamental mechanism through which diabetic hearts are more susceptible to IRI. Elucidating the mechanism governing metabolic alterations and identifying a potential central controller that may reverse this metabolic switch in diabetes is of importance in preventing IRI in diabetic heart.

FoxO is a recently identified regulator of cardiac metabolism. Activation of FoxO1 upgrades cardiac FA uptake and oxidation [27], and inhibits glucose utilization, whereas FoxO3 deletion rendered mice prone to cardiac hypertrophy [37]. In the heart, both FoxO1
and 3 regulate metabolism [38,39]. This indicates that enhanced FoxO activation may serve as an early key trigger of metabolic dysfunction that renders diabetic heart more susceptible to IRI.

Changes of FoxO1 in Diabetic Hearts and its Potential Impact on Myocardial Vulnerability to IRI

FoxO plays important roles in many cellular processes. FoxO regulates insulin signaling and glucose and lipid metabolism in the heart [25,26]. Three FoxO isoforms, namely, FoxO1, FoxO3, and FoxO4 (with FoxO1 being the dominant member in the adult heart), are critical for the maintenance of cardiac functions, including response to oxidative stress [20] and regulation of metabolism in the heart [38].

Activated FoxOs upregulate PDK4 that inactivate pyruvate dehydrogenase (PDH), a controlling enzyme that favors glucose oxidation, and shifts the cell from glucose metabolism toward FA utilization [40]. FoxOs also increase mitochondrial CD36 (the predominant cardiac FA transporter) and enhance FA uptake [41]. To perform these metabolic functions, nuclear presence of FoxO is mandatory [42]. In vivo study shows that FoxO1 nuclear presence is increased in cardiomyocytes of rats treated with intralipid, which upregulates cardiac inducible nitric oxide synthase (iNOS) and CD36 expression, contributing to activation of nitrosative stress and triglyceride (TG) accumulation in myocardium, which also triggers cardiac Protein phosphatase 2A (PP2A) expression, and may subsequently contributed to cardiac insulin resistance and cell death [30]. In diabetes (streptozotocin- and diazoxide- induced hypeoinsulinemia and hyperglycemia), FoxO activation (in the presence of increased FA supply in diabetes) enhances FA uptake in excess of oxidation, upregulating iNOS expression, which subsequently nitrosylate GAPDH, leading an increased nitrosative stress in the nucleus, and eventually activate Poly [ADP-ribose] polymerase 1 (PARP-1) mediated cell death signaling [28,30] (Figure 1). Indeed, in normal condition, nitric oxide (NO) produced from iNOS may enhance defensive response against reperfusion injury at the early stage, however, after prolonged reperfusion or hyperglycemic condition (e.g. diabetes) that is associated increased in superoxide anion production, NO produced from iNOS is significantly increased, which enhances peroxynitrite formation, resulting in depression of myocardial contractility [43]. This suggests that upregulation of iNOS by FoxO under diabetic condition is detrimental to the heart, especially when the heart is subjected to ischemia and reperfusion. FoxO is regulated by insulin [44] via Akt-dependent phosphorylation at site S253 which inactivate FoxO, making it prone to 14-3-3 binding [44] and subsequent nuclear exclusion to cytosol, reducing its transcriptional activity [23]. In the cytosol, FoxO undergoes ubiquitination and degradation [18]. In diabetes, Akt activation is reduced [10] and cardiac 14-3-3 decreased [45], increasing FoxO nuclear retention. FoxO is also regulated by acetylation, which reduces its activity [46]. Deacetylation of FoxO needs silent information regulator1 (Sirt1) [47], leads to activation of FoxO [48].

Of note, Sirt1 mediated cardioprotection against IRI needs the participation of FoxO1, which is essential for Sirt1 to upregulate antioxidant manganese superoxide dismutase (Mn-SOD) [49]. Moderate increase of cardiac FoxO1 reduced while its cardiac deletion exacerbated cardiac IRI in mice [20]. However, these studies were performed in non-diabetic conditions, where slight increase in ROS (as occurs during ischemia) may activate FoxO1, which in the presence of Sirt1 promotes vascular resistance to oxidative stress by transcriptional activation of catalase and Mn-SOD that remove ROS [47]. In addition, in vitro studies show that Sirt1 increases the ability of FoxO3 to resistance to oxidative, while, Sirt1 also inhibits FoxO3 to induce cell death [50]. In diabetes, excessive increase in myocardial ROS, not only stimulates stress signals [51] which disrupts the binding of 14-3-3 to FoxO [22] but most importantly it impairs Akt signaling [10,28]. These changes jointly lead to increased myocardial nuclear FoxO1 (especially FoxO1) activation. In type 1 diabetic rats, cardiac Sirt1 initially increased at 4 weeks, but it was soon significantly decreased with the progression of diabetes [52]. Thus, an increase of cardiac FoxO1 especially FoxO1 activation, in the presence of decreased Sirt1, in the late phase of diabetes diminishes FoxO1 function in enhancing antioxidant enzymes while promotes its metabolic regulatory function. Our preliminary study performed in isolated cardiomyocytes showed that incubating cardiomyocytes with high glucose and high lipid increased nuclear presence of FoxO1 with concomitant increase in cardiomyocytes CD36 over-expression which is associated with increased cellular injury when the cells were subjected to hypoxic reoxygenation (Figure 2). Taken together, studies by other and us provide evidence to suggest that under diabetic condition, increase in cardiac FoxO under the stimulation of high glucose and high lipid, may play a central role in rendering diabetic heart more sensitive to IRI (Figures 1 and 2), although further in depth studies are needed to confirm this hypothesis.

Myocardial Conditioning: FoxO as Convergence of Cardioprotective Pathways

Cardioprotective effects of IPC, IPostC and APC (myocardial conditioning) have been extensively studied [6,53,54]. IPC, IPostC and APC confer their cardioprotection via activation of the Reperfusion-Injury-Salvage-Kinase (RISK) pathway (phosphatidylinositol3-kinase (PI3K)-Akt signaling [55]) and the Survivor-Activating-Factor-Enhancement (SAFE) pathway (Jak/STAT3 signaling) [56] which can be activated independent of the RISK pathway [57,58]. However, these pathways are impaired in the diabetic myocardium, making it difficult to pre- or post-condition the diabetic hearts [58]. The mechanism whereby impairment in STAT3 and/or Akt activation compromises myocardial conditioning, cardioprotection in diabetes is unknown.

Akt phosphorylates/inactivates FoxO and confers cellular protection [59]. Inactivation of FoxO1 also needs STAT3, and FoxO1 was persistently activated in STAT-3 deficient mice which led to the loss of cardioprotection induced by sphenogine-1-phosphate (this confers cardioprotection via both the RISK and SAFE pathways) [60]. This suggests that FoxO is the downstream target of both Akt and 3 regulate metabolism [38,39]. This indicates that enhanced FoxO activation may serve as an early key trigger of metabolic dysfunction that renders diabetic heart more susceptible to IRI.
and STAT-3 and serves as critical convergence of the RISK and SAFE pathways. Thus, inhibition of FoxO may not only enhance diabetic heart tolerance to IRI but also restore diabetic heart responsiveness to myocardial conditioning.

Conclusions

Myocardial infarction is the major cause of death in patients with diabetes and there is repaid prevalence of diabetes worldwide. To date, effective therapeutic strategies to protect the diabetic heart from IRI remain limited. FoxO is a major transcription factors that regulate cardiac metabolism, however, its role in the heart during pathological conditions like diabetes has not been fully elucidated. Gaining more insight into the mechanism(s) by which cardiac FoxO is regulated may assist in developing new therapeutic strategies to improve cardiac function during diabetes and to restore effectiveness of cardioprotective interventions (ischemic preconditioning, IPC or ischemic postconditioning, IPostC) in the setting of diabetes.

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