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Selenium deficiency-induced thioredoxin suppression and thioredoxin knock down disbalanced insulin responsiveness in chicken cardiomyocytes through PI3K/Akt pathway inhibition☆

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ABSTRACT

Thioredoxin (Txn) system is the most crucial antioxidant defense mechanism in cell consisting of Txn, thioredoxin reductase (TR) and Nicotinamide Adenine Dinucleotide Phosphate (NADPH). Perturbations in Txn system may compromise cell survival through oxidative stress induction. Metabolic activity of insulin plays important roles in fulfilling the stable and persistent demands of heart through glucose metabolism. However, the roles of Txn and Txn system in insulin modulated cardiac energy metabolism have been less reported. Therefore, to investigate the role of Txn in myocardial metabolism, we developed a Se-deficient chicken model (0.033 mg/kg) for in-vivo and Txn knock down cardiomyocytes culture model (siRNA) for in-vitro studies. Quantitative real time PCR and western blotting was performed. Se deficiency suppressed Txn and TR in cardiac tissues. Significant increases in ROS ($P < 0.05$) levels signify the onset of oxidative stress and in both models. Se deficiency-induced Txn suppression model and Txn knock down cardiomyocytes models significantly decreased ($P < 0.05$), the mRNA and protein levels of insulin-like growth factors (IGF1, IGF2), IGF-binding proteins (IGFBP2, IGFBP4), insulin receptor (IR), insulin receptor substrates (IRS1, IRS2), and glucose transporters (GLUT1, GLUT3, GLUT8), however, IGFBP3 expression increased in Txn knock down cardiomyocytes. In addition, in contrast to their respective controls, Se deficiency-induced Txn depleted tissues and Txn deleted cardiomyocytes showed suppression in mRNA and protein levels of PI3K, AKT, P-PI3K, and repression in FOX, P-FOX JNK genes. Combing the in vitro and in vivo experiments, we demonstrate that Txn gene suppression can cause dysfunction of insulin-modulated cardiac energy metabolism and increase insulin resistance through PI3K-Akt pathway inhibition. Herein, we conclude that inactivation of Txn system can alter cellular insulin response through IRS/PI3K/Akt pathway repression and JNK and FOX expression. These findings point out that Txn system can redox regulate the insulin dependent glucose metabolism in heart and is essential for cell vitality. Moreover, the increased expression of IGFBP3 indicates that it can be a potential negative modulator of metabolic activity of insulin in Txn deficient cells.

1. Introduction

The heart is an energy-consuming organ that demands an uninterrupted and adequate supply of energy. Glucose is the main cardiac energy substrate while, failure to uptake glucose will lead to diabetes. Alterations in the energy metabolism associated with diabetes mellitus contributes the increased oxidative stress which leads to diabetic cardiomyopathy [1]. Insulin promotes the absorption of glucose from the blood which is converted into either glycogen via glycogenesis or fats (triglycerides) via lipogenesis, or into both [2]. Insulin metabolism is closely related to the energy supply [3]. The biological effects of insulin depend on the transduction of intracellular signaling molecules, and the inhibition or degradation of any signal molecule can lead to the occurrence of insulin resistance such as essential hypertension due to obesity, glucose intolerance, dyslipidemia [4], and some angiocardiopathy [5]. Insulin plays a key role in the regulation of various aspects of cardiovascular metabolism through glucose metabolism, protein synthesis and vascular tone. Insulin augment cardiomyocytes contraction, increases ribosomal biogenesis and protein synthesis, stimulates vascular endothelial growth factor (VEGF) and thereby suppresses apoptosis, promotes cell survival and increase blood perfusion of myocardium principally through PKB/AKT signal pathway [6].

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Increased Akt pathway signaling has been shown to be directly correlated with increased rates of glucose metabolism while inhibition of the Akt pathway inhibits glucose consumption and induces glutathione disulfide and thioredoxin reductase (TR) activity in human head and neck cancer cells (HNSCC) [7].Under physiological conditions, almost 90% of energy is produced by a large number of myocardial mitochondria via oxidation of different substrates including glucose [8]. In conjunction with, these reactions also continuously generate a large quantity of reactive oxygen species (ROS) as by-product. Excessive ROS cause cellular dysfunction, protein and lipid peroxidation, and DNA damage and can lead to irreversible cell damage and death, which have been implicated in a wide range of pathological cardiovascular conditions [9]. A study indicate that ROS production precedes insulin resistance in cultured 3T3-L1 adipocytes and ROS scavenging rescues insulin sensitivity, providing evidence toward ROS as a primary cause of insulin resistance [10]. As we all known, the target organs of insulin resistance are liver, muscle and fat, and there are many researches had been done about these. Impaired liver regeneration can cause insulin resistance for ROS excessive produce [11], mitochondria and other sources of ROS can lead skeletal muscle insulin resistance [12]. Nonetheless, cells can defend themselves from oxidative stress injuries through various redox systems consisting of key redox signaling components such as thioredoxin (Txn), glutathione (GSH) and pyridine nucleotide redox couples [13]. Txn, TR and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) constitute the Txn system [14], chiefly responsible for redox homeostasis which is critical for cellular viability and integrity. Txn-TR-NADPH, as an important cellular redox system, regulates oxidative stress [15].

Txn, a class of small protein (12 kDa), is ubiquitous in prokaryotic and eukaryotic organisms, it exists in the form of heat stable proteins as hydrogen carriers [16], and has two redox-sensitive cysteine residues in a Cys-Xaa-Xaa-Cys (CXXC) motif, which make Txn capable to reduce and to be oxidized by other proteins [17], Txn participates in redox reactions via the reversible oxidation of its active site [18]. Being a key player in redox signaling [19], Txn is induced and secreted in response to oxidative stress to reduce H_2O_2 and scavenge free radicals, thereby protecting cells against oxidative stress [20]. Drechsel's study revealed that Txn system plays a decisive role in inhibiting and eliminating ROS to prevent intracellular oxidative damage [21]. Txn can play protective role in ischemia/reperfusion injury [22]. Txn deglutathionylate proteins in the presence of high levels of Oxidized Glutathione (GSSG) in conditions of oxidative stress, to avoid reperfusion injury in mice [23]. Furthermore, endogenous thioredoxin-1 (Txn-1) in the heart of transgenic mice does not confer cardio-protection in ischemic post-conditioning [24]. Txn can execute its own distinctively antioxidant function, through influencing the expression of some transcription factors [25]. Txn-deficiency will make mitochondrial redox homeostasis imbalanced [26], and Txn systems can regulate hyperoxic-mediated death when pulmonary epithelial cells exposed to hyperoxic [27]. In previous study had revealed that the inhibition of endogenous Txn in the heart can increases oxidative stress and cardiac hypertrophy [28], the overexpression of Txn in transgenic mice can reduce the adriamycin-induced cardiotoxicity [29], and the application of Txn recombinant can treatment the heart disease [30]. These findings indicate that Txn plays a pivotal role in protection against some peroxidating-diseases. Normally, Txn is kept in the reduced state by TR in a NADPH-dependent reaction. Se dependent TR1 in cytosol is the only known reductant of oxidized Txn-1 in vivo so far, while glutathione/glutaredoxin system acts as backup of TR1 for reducing Txn-1 [31–33]. However, Se-deficiency can reduce the expression of TR (TR1, TR2, TR3) [34–36], thereby hindering the antioxidation of Txn-TR-NADPH system and thus compromise the cell survival.

Selenium (Se), a well-known antioxidant [37], mediates its effects mainly through incorporation into different selenoproteins in the form of the 21st amino acid, selenocysteine (Sec), through its own $tRNA^{[Ser]Sec}$ [38]. The incorporation of the Se as selenocysteine at the redox active site is required for the catalytic activity of TRs to avoid ischemia–reperfusion stress and age-related functional cardiac decline [20]. S Mukherjee's study revealed that broccoli is benefit to human's heart for its high amounts of Se, which can produce redox-regulated cardioprotective protein Txn [39]. Conversely, Se deficiency can implicate Keshan disease in humans and a degenerative white muscle disease in animals affecting both skeletal and cardiac muscles [40], which is speculated that energy metabolism plays an important role in myocardial tissue. In a series of studies conducted at our Lab, we observed that Se deficiency implicate oxidative stress damage in chicken myoblasts [41], immune organ [42], liver [43], pancreatic [44], digest system [45], and cardiomyocytes [46] while suppressing various ROS scavenger selenoenzymes including TR and Glutathione peroxidase (GPX). GPX 1 was the first identified selenoprotein, responsible to protect hemoglobin from oxidative damage [47]. Almost all known selenoproteins are oxidoreductases with Sec in the active center which regulate various signaling processes by influencing the redox homeostasis [48]. Se deficiency implicates Keshan disease in humans and a degenerative white muscle disease in animals affecting both skeletal and cardiac muscles [40]. Furthermore, studies indicate that higher levels of plasma Se were found to be associated with upsurge in serum insulin in mice [49]. In vitro studies showed that Se treatment potentially promotes pancreatic islets function and increased insulin content and secretion in mouse [50]. On the contrary, Se deficiency reduced the insulin secretory reserves in rat cardiac and skeletal muscles [51], though oral Se improves glucose-homeostasis of insulin-deficient diabetic rats [52]. AS Reddi et al. stated that Se deficiency can induce renal oxidative stress and diabetic in rats [53]. Moreover, it has been observed that Txn sufficient promotes fetal growth in mice through glucose metabolism [54] and resist energy starvation-induced damage to maintain the energy and redox balance in the heart [55]. Tove and colleagues demonstrated that Txn/Thioredoxin interacting protein (TXNIP) axis may mediate some detrimental effects of glucocorticoid excess on bone tissue in Cushing's syndrome, as alterations in this axis affect glucose metabolism in these patients [56]. H Qin's study revealed that Txn and TR expression significantly decreased in insulin-resistant mouse [57], which revealed that Txn and insulin metabolism had close relationship.

Taking these data into consideration, it is not surprising that Txn and Se share the similar physiological roles in cell as Se-dependent TR act as an integral part of Txn system, and that Se-deficiency can impede the normal function of Txn system. Txn exerts a protective effect on the myocardium by removing excess oxygen free radicals, repairing lipid peroxidation, regulating transcription factor activity, inhibiting cell apoptosis, and regulating intracellular oxidative balance. The expression of Txn and TR in myocardial tissue was inhibited by type 2 diabetes mellitus, and the expression of Txn and TR was compensated and the expression of TXNIP was up-regulated, suggesting that the activity of Txn system in myocardium decreased. The reason may be that the inhibitory protein is upregulated and chemically modified. At present, people pay more and more attention to the Txn system on cell survival of the redox regulatory role, but the Txn system on the regulation of insulin metabolism mechanism is not yet clear, less reported, to be in-depth. The heart is an important organ of energy consumption, however the energy metabolism in the heart get little study, particularly chicken, until now. In-addition, Txn may play role in modulating cellular energy metabolism. However, the mechanism remains unclear. Keeping in view, the present study was designed to understand whether and how Txn can affect the insulin responsiveness in cardiomyocytes. Accordingly, we developed a Se-deficient chicken model to observe the impact of Txn on cardiac energy metabolism through PI3K/Akt pathway. To further explore the role of Txn in energy metabolism, Txn-deficient primary cardiomyocytes culture model was established through gene interference technology.