ROS Acts as a Double-Edged Sword in the Pathogenesis of Type 2 Diabetes Mellitus: Is Nrf2 a Potential Target for the Treatment?

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Abstract: Although the clear mechanism of T2DM is still to be elucidated, it has been well established that reactive oxygen species (ROS) derived from multiple sources plays a causal role in multiple types of insulin resistance and contributes to β-cell dysfunction thus enhances the development and progression of T2DM. What is incomprehensible is that the detrimental ROS also plays a substantial role in the normal insulin signal transduction and glucose-stimulated insulin secretion (GSIS) in β-cell, which forces us to re-recognize the role of ROS under physiological and pathological conditions in a more broad way. Redox homeostasis is tightly controlled by the transcriptional factor nuclear factor erythroid 2-related factor 2 (Nrf2), whose abnormality is believed to be related with diabetes. Accumulating evidences suggest that there are important cross-talks between Nrf2 and PPARγ, PGC1α, PI3K/Akt on regulating antioxidant enzymes and the development of diabetes. Therefore, these evidences indicate that Nrf2 may be a critical element in taking survival and death decisions when cells are exposed to an oxidant environment. In conclusion, enhancing GSIS and insulin sensitivity through the regulation of Nrf2 levels is a potential avenue for developing new therapeutics. Nrf2 may become a promising target for the treatment of T2DM.

Keywords: Reactive oxygen species, type 2 diabetes mellitus, Nrf2, antioxidant, MAP kinase, PI3K/Akt.

1. THE PREVALENCE OF TYPE 2 DIABETES MELLITUS

Estimates by the American Diabetes Association (ADA) indicate that over the past three decades, there has been an explosive increase in the prevalence of diabetes mellitus (DM) [1, 2], and current estimates suggest that by the year 2030, over 350 million people worldwide will be afflicted with this disease and its debilitating conditions [3], leading to a series of complications such as cardiovascular disease, nephropathy, retinopathy and widespread disease of both the peripheral and central nervous systems. Type 2 diabetes mellitus (T2DM) represents at least 80-85 percent of all DM and is dramatically increasing in incidence as a result of changes in human behavior and increased body mass index [4]. The incidence of undiagnosed diabetes, impaired glucose tolerance and impaired fasting glucose levels raises future concerns in regards to the financial and patient care resources that will be necessary to care for patients with T2DM. Therefore, it is very important to examine the molecular mechanism of T2DM and to explore a therapeutic target for T2DM.

2. PATHOGENESIS OF T2DM: INSULIN RESISTANCE AND β-CELL DYSFUNCTION

T2DM is a heterogeneous disorder characterized by hyperglycemia resulting from defects in insulin sensitivity (insulin resistance) and/or secretion (pancreatic β-cell dysfunction). Under normal condition, insulin lowers the level of blood glucose through suppression of hepatic glucose production and stimulation of peripheral glucose uptake, in which process any dysfunction can lead to insulin resistance. Normal β-cells can compensate for insulin resistance by increasing insulin secretion or β-cell mass, leading to more insulin in circulation, but insufficient compensation leads to the onset of glucose intolerance, resulting in the aggravation of hyperglycemia. Although the clear mechanism of T2DM is still to be elucidated, it has been well established that hyperglycemia-induced generation of reactive oxygen species (ROS) contributes to the development and progression of T2DM and its related contributions [5, 6]. Therapies attributing to lessen oxidative stress could contribute to improve glycemia control and prevent complications of T2DM [7, 8]. Moreover, a number of studies have implicated that excessive ROS plays a pivotal role in both β-cell dysfunction [9] and multiple types of insulin resistance [10].

3. PARADOXICAL ROLES OF ROS IN THE PATHOGENESIS OF T2DM

3.1. Oxidative Stress: Imbalance of the Generation and Elimination of ROS

Systemic oxidative stress is defined as an imbalance between the generation of ROS (also known as free radicals, including superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), peroxynitrite ($\bullet ONOO^-$), and hydroxyl ($\bullet OH^-$)) and the body's ability to eliminate these species *via* endogenous antioxidant defenses [11-14], including phase II detoxifying and antioxidant enzymes, as well as non-enzymatic scavengers of ROS and metal ions [15]. Under physiological conditions, the antioxidant defenses are able to protect against the deleterious effects of ROS, but under conditions when either the ROS

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generation is increased or the antioxidant defenses are decreased, ROS accumulates, leading to cellular and tissue damages [16]. Indeed, ROS acts directly through oxidative damage on macromolecules (proteins, lipids, DNA) or indirectly through activating a number of signal transduction pathways sensible to stress mechanisms, finally resulting in oxidative stress and cell dysfunction.

3.2. Oxidative Stress and Insulin Resistance

Under normal conditions, insulin binds to its receptor (insulin receptor, IR) on cell surface, and then IR and insulin receptor substrate (IRS) will be phosphorylated, which results in activation of various insulin signaling pathways, leading to glucose transportation, glycogen synthesis and protein synthesis (Fig. **1**). Under insulin resistant conditions, main insulin target tissues, including liver, muscle and adipose, become insensible to insulin. A complex network of insulin signaling pathways is involved in the pathophysiology of insulin resistance. Oxidative stress is believed to modify a number of the signaling pathways within a cell that can ultimately lead to insulin resistance. Previous studies had shown that the inhibitory effect of ROS on insulin/insulin-like growth factor 1 (IGF-1) receptor signaling is mediated through activation of serine/threonine kinases [17], which in turn phosphorylate IRS-1 and IRS-2 and cause insulin/IGF-1 resistance, since ser/thr-phosphorylated IRS proteins dissociate from the insulin receptor. As a result, IRS-1 and IRS-2 can no longer activate phosphatidylinositol 3-kinase (PI3K) and thus impair insulin-induced glucose transporter type 4 (GLUT4) translocation and glucose transportation [18]. It is believed that mulitiple so-called stress kinases, such as c-Jun N terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), nuclear factor κB (NFκB) and certain protein kinase C (PKC) isoforms, are involved in the desensitization of insulin (Fig. **1**). The common characteristic of these kinases seems to be their ability to directly or indirectly increase serine/threonine phosphorylation of IRS proteins induced by ROS, thus attenuating normal insulin signaling (Fig. **1**) and resulting in insulin/IGF resistance due to dissociation of IRS from the receptor and PI3K downstream signaling [19]. These results are consistent with the data obtained with adipocytes, which demonstrates a causal role of ROS in insulin resistance [20]. Taken together, these data indicate that excessive generation of ROS has a causal role in multiple forms of insulin resistance [20].

3.3. Oxidative Stress and Pancreatic β-Cell Dysfunction

Disruption of insulin synthesis and secretion are mainly considered to be due to chronic hyperglycemia in T2DM. Once hyperglycemia becomes apparent, β-cell function gradually deteriorates. Chronic oxidative stress is an important cause of glucose toxicity in β-cells in T2DM [21]. Compared with other tissues, the expression of antioxidant enzymes, such as catalase (CAT) and glutathione peroxidase (Gpx), are very low in β-cells [22, 23], thus β-cells are thought to be a target of oxidative stress-mediated tissue damage [23]. So, it is probable that oxidative stress is an important event involved in β-cell deterioration in T2DM. It has been previously shown that oxidative stress suppresses the insulin gene transcription in β-cells [24]. Pancreatic homeobox factor-1(Pdx-1) and MafA are two important transcription factors for normal insulin gene expression. Anti-

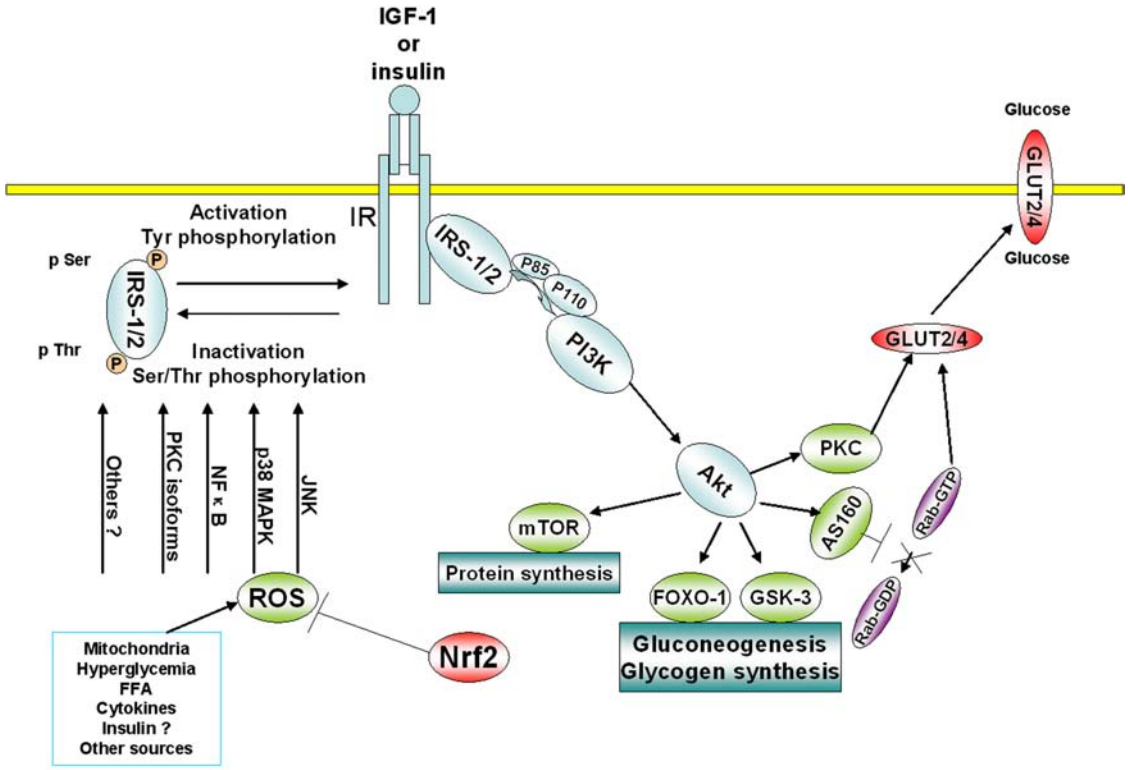

Fig. (1). Schematic representation of insulin signal and the effect of ROS.

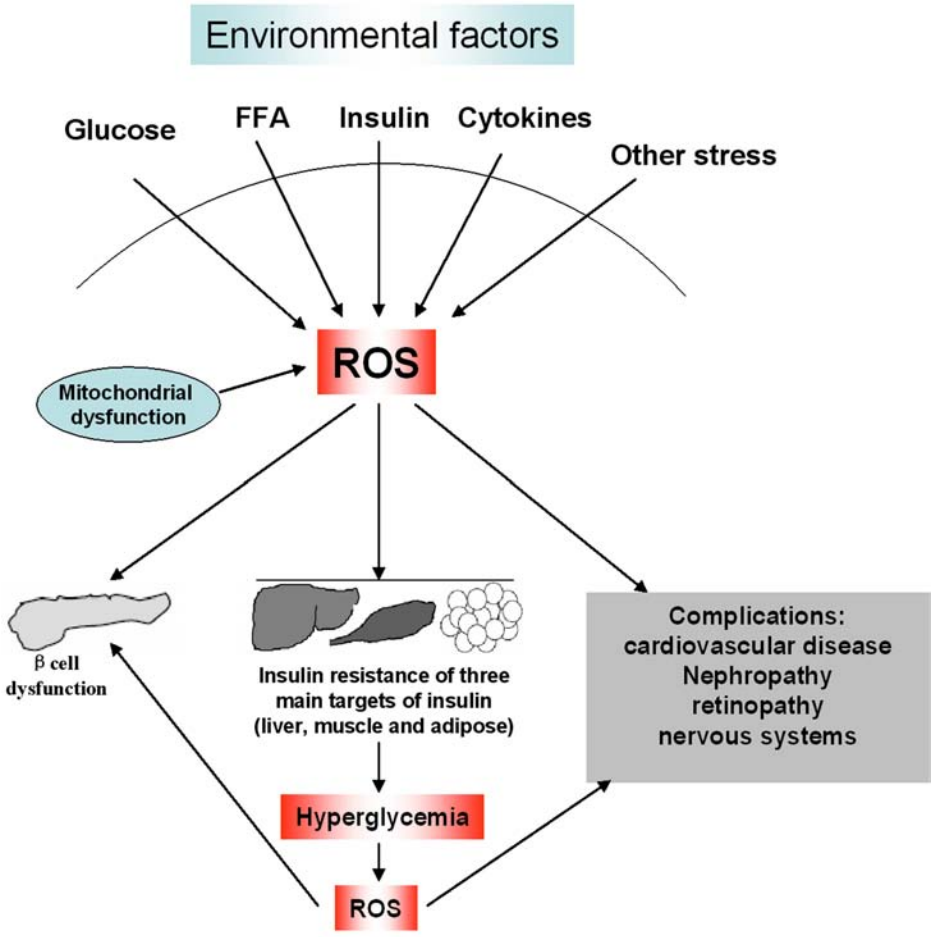

Fig. (2). The role of oxidative stress in the development of T2DM.

oxidant treatment of β-cell lines and models of T2DM were shown to protect against deterioration of insulin gene expression induced by exposure to high glucose, indicating that oxidative stress was responsible for the decrease of Pdx-1 and MafA [25]. Taken together, these results suggest that ROS is a central influencing factor that contributes to insulin resistance and β-cell dysfunction in T2DM and its complications (Fig. **2**).

3.4. ROS also Acts as a Beneficial Signal in the Production of Insulin and Insulin Action

Paradoxically, certain level of ROS may have insulin-mimicking effects, and for example, H_2O_2 can increase glucose transportation and inhibit lipolysis [26-28]. Moreover, Insulin stimulates the rapid production of H_2O_2 and this insulin-dependent H_2O_2 generation is not only involved in the regulation of tyrosine phosphorylation events in the early insulin signaling cascade but also has important effects on the regulation of downstream insulin signaling, including the activation of PI3K, Akt, and ultimately cellular glucose transport in response to insulin [29]. Recently, Loh *et al.* reported that mice lacking one of the key enzymes responsible for the elimination of ROS, glutathione peroxidase 1 (Gpx1), were protected from high-fat-diet-induced insulin resistance [30]. Insulin sensitivity in $Gpx1^{-/-}$ mice was increased, attributing to the enhancement of insulin-induced PI3K/ protein kinase B (Akt) signaling and glucose uptake in muscle, which could be reversed by the antioxidant N-acetylcysteine [30]. Increased insulin signaling correlated with enhanced oxidation of the PTP family member PTEN, which terminates signals generated by PI3K [30]. These studies provide causal evidence for the enhancement of insulin signaling by ROS *in vivo*. Moreover, our recent studies (not shown) have also showed that the effect of ROS on insulin-induced phosphorylation of Akt was concentration-dependent. Relative low level of ROS, including H_2O_2, tBHP and glucose plus glucose oxidase, promoted the phosphorylation of Akt induced by insulin, but high level of ROS inhibited this insulin signaling, which was closely related with glucose metabolism and thus the pathogenesis of insulin resistance.

Defection of glucose stimulated insulin secretion (GSIS) in pancreatic β-cell is instrumental in the progression to hyperglycemia [31]. ROS generation occurs in glucose metabolism and correlates with insulin secretion. In recent years, more and more attention has been paid to the potential role of ROS in glucose signaling and insulin GSIS. The promoting effect of ROS on GSIS has been reviewed in detail by Pi *et al.* [32], which suggests that ROS derived from glucose

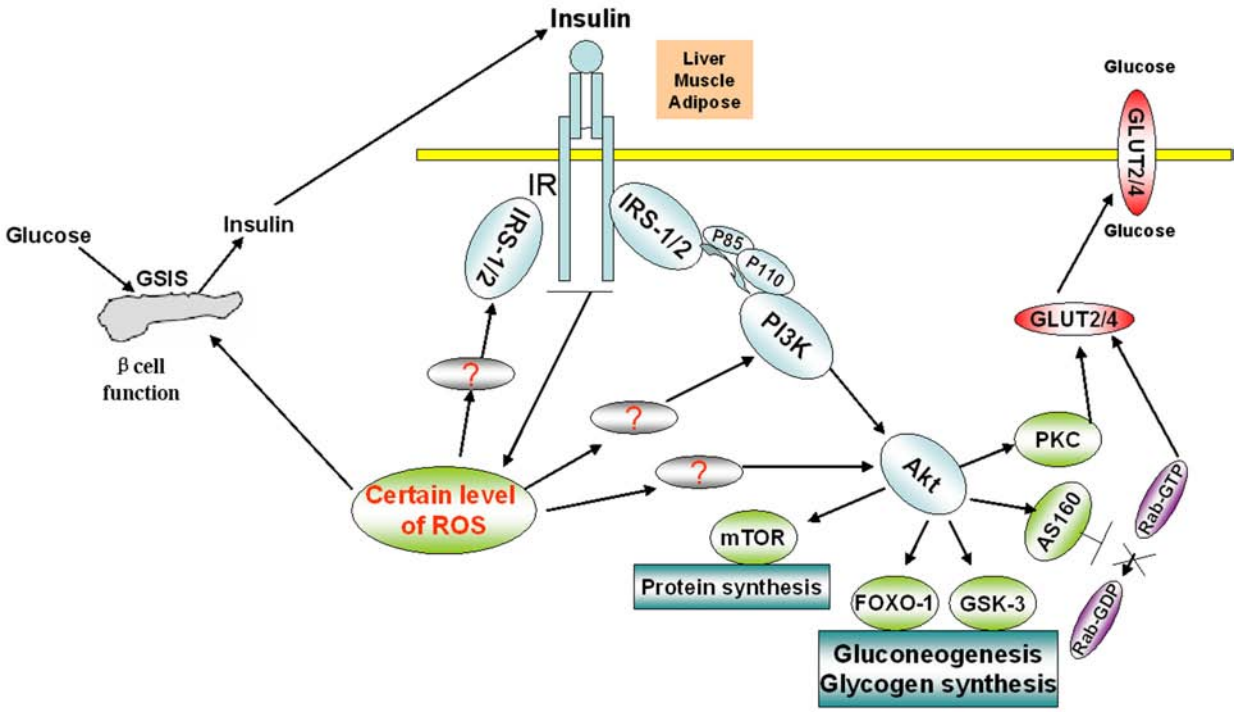

Fig. (3). The role of ROS signaling in the insulin signal and GSIS in β cell.

metabolism are potential metabolic signals that facilitate insulin secretion in β-cell. In conclusion, ROS may be not only the byproduct of metabolism or detrimental agent but also a potential signal molecular in insulin signaling and GSIS in β-cells (Fig. **3**). Indeed, ROS acts as a double-edged sword under normal physiological and pathological conditions.

4. REGULATION OF REDOX BALANCE BY Nrf2

To avoid damage by ROS, a tight regulation of the cellular redox balance is required, which is achieved at least in part by the action of nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 is a member of the "cap 'n' collar" basic leucine zipper family of transcription factors (CNC-bZIP) that can transactivate antioxidant responsive element (ARE) [33-35]. Nrf2 is broadly expressed in tissues, which is kept in the cytosol by kelch-like ECH-associated protein 1 (Keap1) under non-stressful conditions and undergoes proteosomal degradation through a specific ubiquitin–26S proteasome pathway by the Keap1/Cul3-dependent ubiquitin ligase (E3) [36]. Interaction between the inducers and the Keap1 protein through chemical–protein thiol interactions or Nrf2 itself through phosphorylation by kinase initiates the signaling transduction, leading to the stabilization and activation of the Nrf2 protein [37]. Upon recognition of signals imparted by oxidative and electrophilic molecules including ROS, including a number of antioxidants, heavy metals and certain disease processes [38], Nrf2 is released from Keap1, escapes proteosomal degradation and translocates to the nucleus to induce the expression of a series of genes involved in defense and survival [36]. Our and others' experiments [39, 40] have shown that upon activation, Nrf2 mediates the induction of a spectrum of cytoprotective proteins including phase II enzymes and antioxidant proteins (Fig. **4**), including NAD(P)H: quinone oxidoreductase (NQO1), glutamate-cysteine ligase catalytic and modulatory subunits (GCLC and GCLM), peroxiredoxin (PRX) families, thioredoxin (TRX), glutathione S-transferases (GST), cytosolic copper-zinc superoxide dismutase (CuZnSOD), mitochondrial manganese superoxide dismutase (MnSOD) [41], Gpx, glutathione reductase (GR), heme oxygenase-1 (HO-1), through the ARE-dependent pathway [42-49].

5. THE ROLE OF Nrf2 IN THE PATHOGENESIS OF T2DM

For the past few years, accumulative evidence demonstrates that Nrf2 plays a great role in the protection against diabetes. Glucose promotes ROS production in cardiomyocytes and Nrf2 is required for the control of ROS production in the cells under normal conditions and for high glucose-stimulated oxidative stress [50]. Hyperglycemia-induced oxidative stress has been reported to contribute to the dysfunction of renal mesangial or endothelial cells. The glomeruli of human diabetic nephropathy patients are under oxidative stress and Nrf2 levels are elevated [51]. In animal diabetic model, Nrf2 has been demonstrated to be crucial in ameliorating streptozotocin (STZ)-induced renal damage, indicating that Nrf2 plays a protective role in diabetic nephropathy, and that dietary or therapeutic activation of Nrf2 could be used as a strategy to prevent or slow down the progression of diabetic nephropathy [51]. Compared to wild-types, mice lacking Nrf2 (Nrf2-null) have lower basal serum insulin and prolonged hyperglycemia in response to glucose challenge. The absence of Nrf2 worsens hyperglycemia in type 1 diabetic mice, implicating that Nrf2 participates in glucose homeostasis [52]. In diabetic $Nrf2^{-/-}$ mice induced by STZ, hyperglycemia increased oxidative and nitrosative stress and accelerated renal injury [51, 53]. Moreover, Cheng

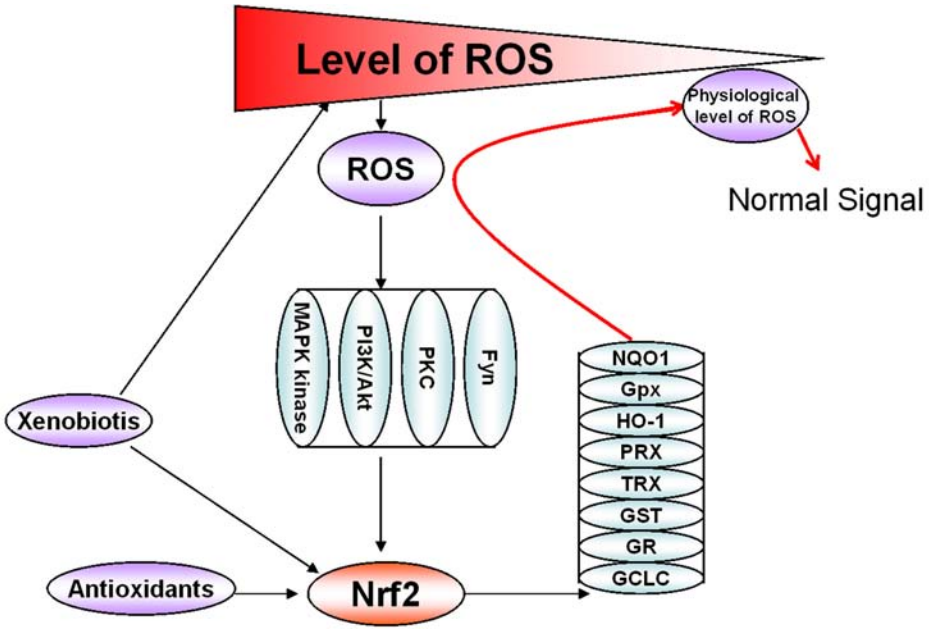

Fig. (4). Regulation of redox balance by Nrf2.

et al. [54] have reviewed the role of Nrf2-Keap1 defense pathway in endothelial cells in diabetes.

High levels of glucose accelerate the formation of advanced glycation end-products (AGEs). In turn, AGEs generate ROS and activate inflammatory signaling cascades through binding with the AGE-specific receptor (RAGE) [55]. AGE-modified bovine albumin (AGE-BSA) has been reported to induce ROS generation, leading to nuclear translocation of Nrf2 and Nrf2-dependent induction of the antioxidant genes, including HO-1 and NQO1, in bovine aortic endothelial cells [56]. And AGE-induced up-regulation of Nrf2-linked antioxidant enzyme activity was considered to play a protective role against sustained oxidative stress in diabetes [56].

Insulin-induced HO-1 mRNA and protein expression were observed in renal adenocarcinoma cells and mouse primary tubular epithelial cells [57]. The transcription factor Nrf2 was found to translocate to the nucleus following insulin treatment in a PI3K-dependent manner, which was responsible for the induction of HO-1 [57]. In addition, insulin-induced activation of PI3K and Nrf2 was also reported to protect against oxidative stress through up-regulation of GCLC in endothelial cells [58, 59]. Decreased GCLC expression due to hyperglycemia and insulin deficiency can lead to decreased GSH levels that impairs activation of Nrf2 and antioxidant defense which indicates an important action of insulin is to increase the expression of GCLC through the activation of Nrf2 [60]. With regard to insulin's ability to induce GCLC expression, PI3K/Akt/mTOR signaling and consequent translocation of Nrf2 to nuclear was required [61]. The result was coinciding with the report which showed that chronic hyperglycemia resulted in enhanced apoptosis in human brain endothelial cells, which was attenuated by insulin [62].

A defect in insulin/IGF-1 signaling in the regenerating liver was identified in the Nrf2-deficient liver [63]. Consistent with data obtained with adipocytes [20], the chronic oxidative stress in hepatocytes of Nrf2-deficient mice resulted in resistance to exogenous insulin or IGF-1 *in vitro*. Alterations in the phosphorylation status of IRS-1 and IRS-2 (enhanced serine/threonine and reduced tyrosine phosphorylation) in Nrf2 knockout mice reduce their association with the receptor [64]. *In vivo*, tyrosine phosphorylation of the IRS-1 and IRS-2 in response to activation of the insulin receptor was strongly reduced in the regenerating liver of Nrf2-deficient mice [65]. Consistent with these data, association of PI3K with IRS-1 in response to insulin was reduced in Nrf2-deficient hepatocytes *in vitro* and reduced association of these proteins was also seen in hepatectomized liver of these mice *in vivo*. One of the inhibitory IRS serine/threonine kinases may be JNK, which is known to be activated by ROS [66] and which showed enhanced and prolonged activation in the injured liver of Nrf2-deficient mice [63]. Moreover, activation of the PI3K/Akt pathway was strongly impaired in the absence of Nrf2 [63]. Nrf2 knockout mice showed strongly reduced activation of Akt and its targets, GSK-3β, p70/S6 kinase and Bad were also reduced [67]. Thus, the strong insulin-mediated activation of Akt was no longer observed in cells from Nrf2-deficient mice [65].

Obesity is believed to be an important risk factor for the pathogenesis of T2DM. Nrf2 inhibits lipid accumulation and oxidative stress in mouse liver after feeding a high fat diet, probably by interfering with lipogenic and cholesterologenic pathways [68]. Moreover, the inhibitory effect of bardoxolone methyl, a synthetic oleanolic triterpenoid and an inducer of Nrf2, on lipogenic gene expression was significantly reduced in Nrf2-disrupted mice [69].

Further studies are needed to define the role of Nrf2 in diabetes and clarify the mechanisms that are responsible for the development of this disease and its complications and even for the insulin resistance and reduced GSIS.

6. CANDIDATES OF NATURAL OR SYNTHETIC COMPOUNDS THAT COULD DIRECTLY ACTIVATE Nrf2 AND DECREASE OXIDATIVE STRESS RELATED TO T2DM

Multiple natural or synthetic antioxidants have found to be potent Nrf2 activators, which could decrease oxidative stress related to T2DM, such as oleanolic acid (OA) and bardoxolone methyl.

OA is a natural triterpenoid, which has been used in Chinese medicine for the treatment of liver disorders for many years. OA exists largely in food products (vegetable oils), and is a constituent of the leaves and roots of Olea europaea, Viscum album L., aralia chinensis l. and many others. Our previous study has shown that OA is a potent antioxidant which could activate Nrf2 and protect hepatocytes against tert-Butyl hydroperoxide (tBHP)-induced oxidative stress [39]. The phosphorylation of Akt and extracellular signal-regulated kinase (ERK) were involved in the antioxidant activity of OA [39]. Our previous results have also showed that the administration of OA could significantly reduced STZ-induced increase of blood glucose in rats [70]. It has been also shown that OA could inhibit tBHP-induced insulin resistance in hepatocytes [70], in which process the phosphorylation of ERK and the protective effect on mitochondrial function may be involved. We hypothesize that, through the activation of Akt and ERK, OA activates Nrf2 and plays a potent antioxidant activity, and thus may become a potential pharmacological agent for the treatment of DM.

As mentioned above, bardoxolone methyl is a synthetic oleanolic triterpenoid that is another potent known inducer of the Keap1/Nrf2 pathway and works to suppress both oxidative stress and inflammation [71]. Bardoxolone methyl has entered clinical development for the treatment of moderate to severe chronic kidney disease in T2DM patients. Bardoxolone methyl treatment may reduce stage of chronic kidney disease and improve estimated glomerular filtration rate (eGFR) in kidney function in T2DM patients, which were presented in an oral presentation at the American Society of Nephrology (ASN) Renal Week Conference in Denver [72]. Bardoxolone methyl may be a promising agent for the treatment of chronic kidney disease and other complications in T2DM.

7. CROSS-TALKS BETWEEN Nrf2 AND SEVERAL DIABETES-RELATED FACTORS

Nrf2 and PPARγ

The family of peroxisome proliferator-activated receptors (PPARs) is thought to be involved in the control of fat cell development and glucose homeostasis [73]. PPARγ, which is the target of a new class of insulin-sensitizer agents (thiazolidinediones, TZDs), is expressed predominantly in adipose tissue, resulting in differentiation and triglyceride synthesis [74]. These agents work primarily by binding to nuclear PPARγ [75], thus improving insulin sensitivity, reducing triglyceride levels and decreasing the risk of atherosclerosis in diabetic patients and also exerting direct effects on vascular wall cells [76]. In deed, a number of evident have showed that PPARγ ligands have antioxidant activity and promote the expression of antioxidant enzymes [77]. It has been reported that, in response to the PPARγ agonists, Nrf2 was activated to bind to ARE and stimulate the expression of the GSTA2 gene [78]. TZDs have also been reported to mediate their antioxidant effects through inhibiting nitric oxide synthase, thereby decreasing peroxynitrite and superoxide production [79].

Nrf2 and PGC-1α

Peroxisome proliferator activated receptor (PPAR) γ coactivator 1α (PGC-1α) is a large protein with a number of functional domains that could bind various protein complexes, the N-terminal of which contains an activation domain, which interacts with CBP/p300 [80]. PGC-1α can activate most nuclear receptors and also function as a coactivator to many transcription factors [81]. PGC-1α is a critical regulator of metabolism that links metabolic activity to relevant environmental stimuli in multiple pathways, including those responsible for adipogenesis, gluconeogenesis, myogenesis and mitogenesis [82]. Furthermore, PGC-1α is emerging as a key transcriptional regulator of antioxidant defense system and coordinates the expression of many antioxidant programs in response to oxidative stress [41, 83]. In deed, PGC-1α has been reported to control many aspects of oxidative metabolism, including mitochondrial biogenesis and respiration through the coactivation of many nuclear receptors and factors outside the nuclear receptor family. ERRα, Nrf1 and Nrf2 are key targets of the PGC1s in mitochondrial biogenesis [84]. PGC1α and β are induced when cells are exposed to oxidative stressor, such as H_2O_2. In fact, experiments with RNAi for the PGC1α show that the ability of ROS to induce the antioxidant program depends on the PGC1α [84], indicating that PGC-1α is a key regulator of these protective responses. In the PGC-1α$^{-/-}$ mouse, basal levels of CAT, as well as CuZnSOD and MnSOD (but not Gpx1) appear decreased [85]. Taken together, PGC-1α and Nrf2 are complementary and overlapping regulators of the antioxidant defense system and cooperatively regulate several enzymes in the enzymatic antioxidant defense system. So far, the majority of antioxidant enzymes found to be regulated by PGC-1α locates or is activated in the mitochondria. However, a direct molecular interaction between PGC-1α and Nrf2 has not yet been characterized. PGC-1α promoter contains an ARE [41], although it is not known whether it is functional. Even without a direct interaction between Nrf2 and PGC-1α, it also seems probable that the activation of one gene may regulate the expression of the other *via* redox signaling. Both of the two genes can be induced by ROS and increase downstream antioxidant enzymes, which in turn reduce ROS. Therefore, the diabetes therapeutic strategy that utilizes Nrf2 or PGC-1α must be able to reduce ROS, only to a suitable level that restore redox homeostasis to the cell but do not affect the normal signal in which certain degree of ROS is required [86].

Nrf2 and PI3K/Akt

Oxidative stress has been implicated in the impairment of PI3K and Akt signaling during T2DM. Also, PI3K/Akt pathways are reported to be involved in HO-1 expression and in Nrf2-dependent transcription [87]. And the PI3K/Akt/Nrf2 signaling has been found to be responsible for insulin-induced HO-1 and GCLC expression [57, 58].

The pathway of PI3K is activated by oxidative stress, leading to rearrangement of actin microfilaments and then depolymerization of actin which causes a complex of Nrf2 bound with actin to translocate into the nucleus [88]. Ginsenoside Rb1 was found to activate PI3K/Akt pathways and the use of specific inhibitors for PI3K/Akt pathways confirmed the involvement of PI3K/Akt in Ginsenoside Rb1-induced HO-1 expression, Nrf2 nuclear translocation, transcriptional activity and cytoprotection [87]. Moreover, Eckol attenuates oxidative stress by activating Nrf2-mediated HO-1 induction *via* ERK and PI3K/Akt signaling [89].

Nrf2 and GSK-3β

In recent years, it has been shown that there is a loss in oxidative stress tolerance with aging which is linked to a parallel increase in glycogen synthase kinase-3β (GSK-3β) activity. GSK-3β is a serine/threonine kinase involved in energy metabolism, neuronal cell development and midline development [90]. In addition to regulating PGC-1α protein stability, GSK3β also seems to negatively regulate Nrf2 in response to oxidative stress, although the precise mechanisms are still to be cleared [41]. H_2O_2 induces tyrosine 216 phosphorylation of GSK-3β resulting in its activation. GSK-3β maintains Nrf2 within the cytosol in mouse N2A neuroblastoma cells [91], excludes Nrf2 from the nucleus and inhibits the transcriptional activity of Nrf2 [91]. Lithium, a GSK-3β inhibitor, promotes Nrf2 transcriptional activity towards an ARE luciferase reporter and cooperated with sulforaphane (SFN) to induce this reporter and to increase the protein expression of HO-1. Conversely, ARE activation by SFN is attenuated by over-expression of active GSK-3β. Prolonged oxidant exposure activates GSK-3β and limits the antioxidant cell response by keeping Nrf2 off the nucleus. Besides, GSK-3β reversed the cytoprotective effect of Nrf2 in the presence of H_2O_2. Previous data reveal that GSK-3β is upstream to Fyn, which phosphorylates Tyr-568 of Nrf2 that regulated nuclear export of Nrf2 [92].

These findings support that there are axises between Nrf2 and PPARγ, PGC-1α, PI3K/Akt and GSK-3β, which could be used as a pharmacological target in prevention of oxidative stress related diseases, especially diabetes. The cross-talks between these important factors implicate that Nrf2 is a pivotal determinative factor which determines the cell fate in response to oxidative stress or certain level of ROS (Fig. **5**).

CONCLUSION

The worldwide prevalence of T2DM makes it the most serious healthcare concern now and in future. Knowledge gained in understanding the complex cellular and systemic processes of T2DM provide essential insight into the pathogenesis of diabetes and its complications. Although the clear mechanism of T2DM is still to be elucidated, it has been well established that ROS derived from multiple sources plays a causal role in multiple types of insulin resistance [10] and contributes to β cell dysfunction thus enhances the development and progression of T2DM and its related contributions [5]. It is incomprehensible that the detrimental ROS also plays a substantial role in the normal insulin signaling

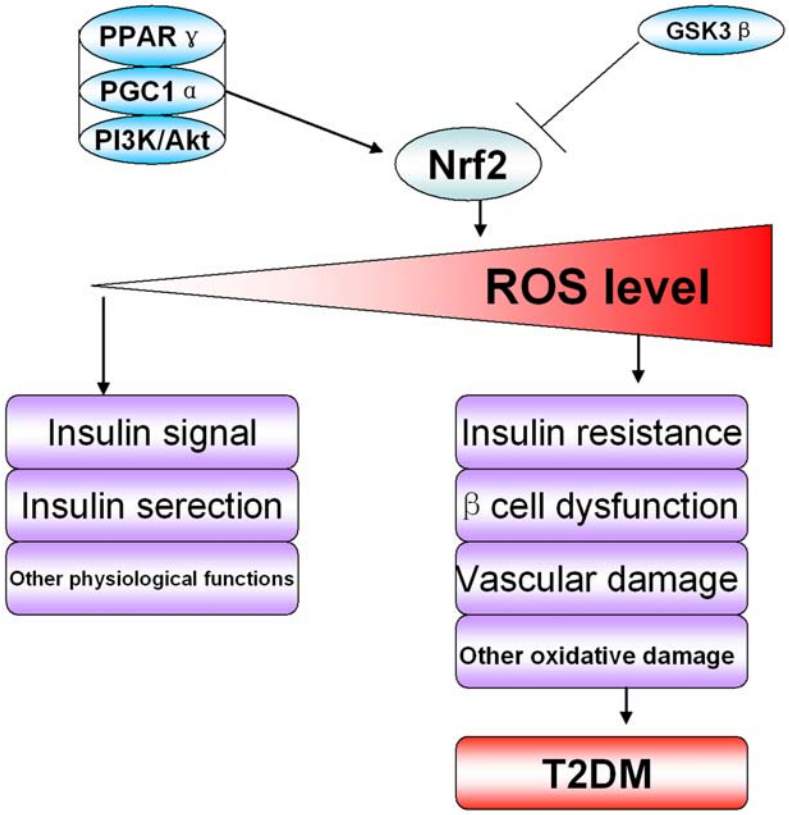

Fig. (5). Regulation of insulin sensitivity and β cell function by Nrf2 through manipulation of ROS level.

transduction and GSIS in β cell. Moreover, the use of antioxidant become more complicated [93], because of the dosage of antioxidant must be appropriate to ensure that ROS level is decreased but not to the level that is not enough for normal physiological function. But it is too difficult because of the nonspecific characteristic of most antioxidants, which has a potential to be "too much of a good thing", producing unexpected effects. These discoveries force us to re-recognize the role of ROS under physiological and pathological conditions in a more broad way.

Therefore, the following hypotheses may help us understand the biological effect of ROS: (a) Concentration-dependent biological effect of ROS. Relatively low (physiological) level of ROS is a required signal molecule, which is essential in the insulin action and β cell function and many other physiological functions, but under oxidative stress, when the body's antioxidant defense is not able to counteract excessive ROS, ROS become a detrimental agent, which will disturb the insulin signal and GSIS, leading to the occurrence of oxidative stress-related disease, including T2DM. Under physiological conditions, organisms have the ability to control the ROS under a physiological level. When this ability is missed, disease will come to exist; (b) The paradoxical regulation of Nrf2. Nrf2 is the central transcription factor for regulating redox balance. ROS not only can activate Nrf2 through regulating Keap1 and Nrf2 itself, but also can inactivate Nrf2 through the activation of GSK-3β or other potential kinases. And this may contribute to the paradoxical biological effect of ROS. The ability of Nrf2 to respond to the changes of ROS concentration and to correlate with a series of diabetes-related regulators determines Nrf2 as a pivotal factor in the development of T2DM and a novel potent target for the treatment of T2DM; (c) ROS acts as both a signal and a stressor in a broad way. The role of ROS in signal transduction is not very specific. ROS could oxidize a number of signals and/or kinases and thus affect many pathways that could not clear easily; (d) Interactions of different kinds of ROS. For example, H_2O_2 could react with NO, which is benefit for the health, generating $\bullet ONOO^-$ which is detrimental to cells. Therefore, the interactions of different ROS may contribute to the bilateral effect of ROS; (e) Compartment of cells. Cells have compartment to limit different ROS in a local region. ROS only could play as a signal locally because of its high reactivity. But when ROS is excessive, it can extend without being cleared, leading to oxidative stress.

Taken together, these hypotheses may have a common center-Nrf2, which is in the point of decision about what role ROS will play. ROS activate multiple signaling pathways in cells that determine whether they will ultimately tolerate or succumb to this aggression. Nrf2 appears to be one such pathway that will determine the destiny of ROS [94, 95] due to the tightly regulation of redox homeostasis by this transcription factor. The fact that oxidative stress activates contradictory signaling pathways either to survive or die implies that there must be a complex cross-talk between these opposite signals that determine the destiny of cells. Consistent with these hypotheses, PPARγ, PGC1α, PI3K/Akt and GSK-3β are critical regulators of cell fate, survival or death, in normal and pathological physiology [96]. Accumulating evidence suggest that there are important cross-talks between Nrf2 and these factors, on regulating antioxidant enzymes and the development of diabetes. Therefore, these evidence suggest that Nrf2 may be a critical element in taking survival and death decisions when cells are exposed to an oxidant environment [91]. We propose that Nrf2-mediated antioxidant response plays a paradoxical role in insulin action and insulin secretion (Fig. **5**). On the one hand, it protects insulin signal and β-cells from oxidative damage and possible cell death, thus minimizing oxidative damage-related impairment of insulin action and insulin secretion. On the other hand, situations leading to chronic induction of endogenous antioxidants mediated by Nrf2 due to oxidative stress may blunt endogenous ROS signaling, resulting in reduced insulin signal and GSIS. In conclusion, enhancing GSIS and insulin sensitivity through the regulation of Nrf2 levels is a potential avenue for developing new therapeutics (Fig. **5**). Nrf2 may become a promising target for the treatment of T2DM, compared with nonspecific antioxidants. The effectiveness of bardoxolone methyl is a potent example. By inhibiting Keap1, it induces the activity of Nrf2 to suppress ROS formation and ROS-driven inflammation. The future agents could take similar strategy to directly to Nrf2, which could help provide a perspective for the Nrf2 as a potential target. However, further studies are needed to provide more specific answers in this important area of research.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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ABBREVIATIONS

ROS	=	Reactive oxygen species
GSH	=	glutathione
GSSG	=	oxidized glutathione form
CAT	=	catalase
GPx	=	glutathione peroxidase
GCL	=	glutamate cysteine ligase
GCLC	=	catalytic subunit of GCL
γ-GCS	=	γ-glytamyl cysteine synthetase
Nrf2	=	NF-E2-related factor-2
T2DM	=	Type 2 diabetes mellitus
CuZnSOD	=	copper-zinc superoxide dismutase
MnSOD	=	manganese superoxide dismutase
Prxs	=	Peroxiredoxins
NQO1	=	NAD(P)H:quinone oxidoreductase 1
GR	=	glutathione reductase
GST	=	glutathione-S-transferases
TR	=	thioredoxin reductases

HO = heme oxygenases

IRS-1/2 = insulin receptor substrate-1/2

PI3K = Phosphatidylinositol 3-kinase

PPAR γ = Peroxisome proliferator activated receptor γ

PGC-1α = Peroxisome proliferator activated receptor γ coactivator 1α

GSK3 = Glycogen synthase kinase-3

OA = oleanolic acid

tBHP = tert-Butyl hydroperoxide

Akt = protein kinase B

ERK = extracellular signal-regulated kinase

Keap1 = kelch-like ECH-associated protein 1

STZ = streptozotocin

IGF-1 = insulin-like growth factor 1

GLUT4 = glucose transporter type 4

JNK = c-Jun N terminal kinase

NFκB = nuclear factor κB

MAPK = mitogen-activated protein kinase

ARE = antioxidant responsive element

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