NF-E2-Related Factor 2 and Its Role in Diabetic Nephropathy

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Diabetic nephropathy is the leading cause of chronic kidney disease and end-stage renal disease. Oxidative stress has been recognized as a major contributor to its pathogenesis. Defensive mechanisms have also widely been studied. One of them, the NF-E2-Related Factor 2, is reviewed in this article.

INTRODUCTION

Diabetic nephropathy is the leading cause of chronic kidney disease (CKD) and end-stage renal disease.[1] It is characterized by initial hyperfiltration, albuminuria, thickening of the basement-membranes, expansion of the mesangial matrix, and subsequent loss of kidney function.[2]

Reactive oxygen species (ROS) include superoxide anion, hydrogen peroxide, and hydroxyl radical. Reactive oxygen species are formed continuously as byproducts of aerobic metabolism. Sources of ROS production are different and include the mitochondrial electron transport chain, cytochrome P450 enzymes, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, or nitric oxide synthetases.[3] Reactive oxygen species contribute to killing bacteria, and genetic defects of NADPH oxidase cause chronic granulomatosis.[4] However, ROS may cause chemical damage to DNA, proteins, and unsaturated lipids, and may lead to cell death. Reactive oxygen species contribute to multiple pathologic processes.[5,6] Reactive oxygen species can disturb the homeostasis of cells and tissues, which ultimately threatens the integrity of the organ. One general mechanism that cells employ to protect themselves against this persistent assault is to maintain a reducing intracellular milieu.[7]

The ROS production in response to hyperglycemia, protein kinase C (PKC), advanced glycosylation end products, and transforming growth factor (TGF)-β1 contributes to diabetic nephropathy.[8] Furthermore Inflammation, as reveals with its

markers such as interleukin-4, interleukin-6, and high-sensitivity C-reactive protein, has an important role in pathogenesis of diabetic nephropathy.[9-11]

PATHOGENESIS

Protein Kinase C-dependent Nicotinamide Adenine Dinucleotide Phosphate Oxidase Activation

Increased diacylglycerol levels and PKC activity in the retina, aorta, heart and renal glomeruli, especially the α, β1/2, and d isoforms, have been reported in diabetes mellitus.[12] Activation of NADPH oxidase is abolished in diabetic PKC β -/- mice, suggesting that NADPH oxidase is activated via a PKC-dependent pathway. Lack of PKC β can protect against diabetes-induced kidney dysfunction and fibrosis.[13]

Angiotensin II-mediated Activation of Nicotinamide Adenine Dinucleotide Phosphate Oxidase

Using a type 2 diabetic mouse model, Moon and coworkers found that mice co-infused with angiotensin II and angiotensin (1-7) had a lower increase in urinary albumin-creatinine ratio than mice infused with angiotensin II alone.[14] In this animal model, angiotensin (1-7) attenuated angiotensin II-mediated NADPH oxidase activation and ROS production in diabetic glomeruli and mesangial cells.[14]

NF-E2-Related Factor 2

The vertebrate transcription factor NF-E2-related

factor 2 (NRF2) and its invertebrate homologs, including SKN-1 (in worms) and CncC (in flies) have emerged as master regulators of cellular detoxification responses and redox status.[15-18] They function both in situations of acute challenge and as regulators of baseline antioxidant activity. In mice- and in human-cultured cells, many protective genes are induced in response to oxidative challenges in an NRF2-dependent manner.[19]

Under physiological conditions, ROS are eliminated efficiently by antioxidant defense systems, and oxidative stress occurs when ROS production exceeds the capacity of antioxidant defenses. The NRF2 is a redox-sensitive transcription factor, which binds to the antioxidant response element in the promoter region of phase II detoxifying and antioxidant enzymes (Figure), leading to an upregulation of antioxidant gene expression.[20] Among NRF2-regulated gene products are phase II detoxification enzymes as well as a broad range of redox regulators that includes enzymes for glutathione synthesis, glutathione S-transferases, NADPH quinone oxidase 1, heme oxygenase-1 (HO-1), and many others. Upon exposure of cells to oxidative stress, NRF2 activity is markedly increased.[5]

Kelch-like ECH-associated protein-1 (KEAP1) negatively regulates NRF2 by targeting it for ubiquitination and proteasomal degradation.[21] Coordinated upregulation of genes coding for detoxification, antioxidant, and anti-inflammatory regulators is seen as a potential therapeutic strategy to protect against oxidative stress that is known to be enhanced by the diabetic milieu.[22] Limited knowledge of the structural biology of NRF2-KEAP1 means that the precise way in which small molecule agents might interact with KEAP1 is still to be fully elucidated. One usual feature appears to be their reactivity with the sulfhydryl groups of the KEAP1 protein.[23]

Cysteine residues of KEAP1 are involved in alterations in the NRF2-KEAP1 complex,
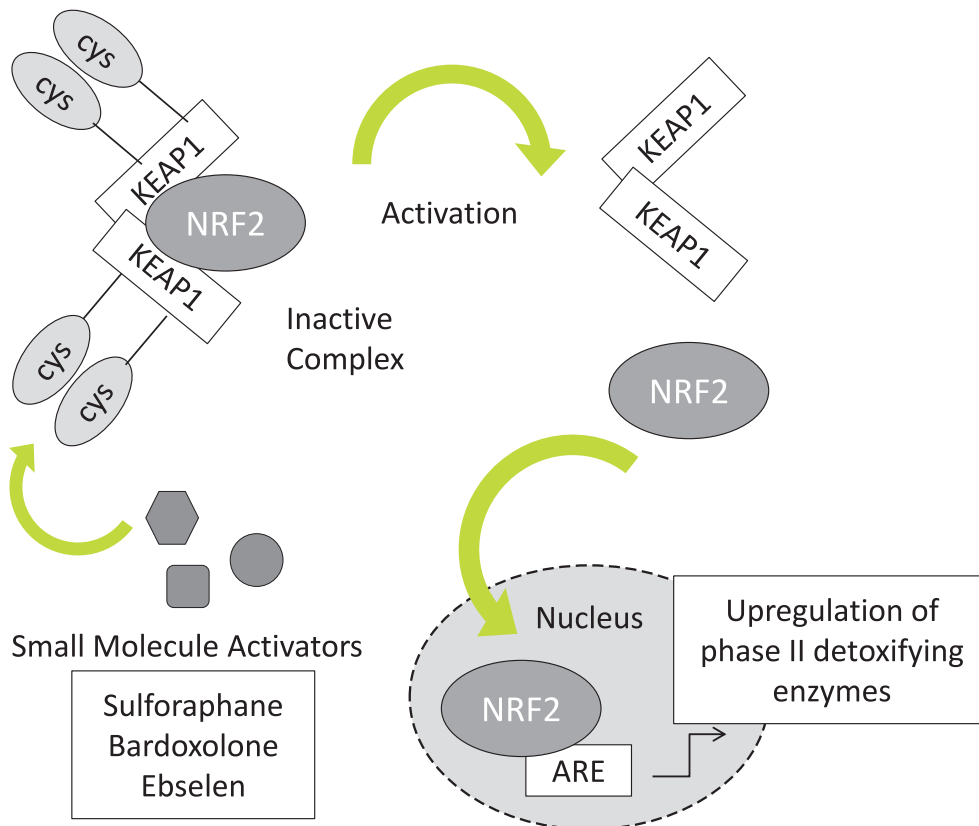
Small molecule activators such as ebselen, sulforaphane, and bardoxolone interact with cysteine residues within kelch-like ECH-associated protein-1 (KEAP1), causing its dissociation from NF-E2-related factor 2 (NRF2). Specificity of a class of activator is thought to reside in the cysteine (cys) residue it interacts with. Unbound NRF2 then translocates into the nucleus where it interacts with antioxidant response elements (AREs) of more than 300 genes. Adapted with permission from an article by de Haan.[22]

preventing proteasomal degradation, enabling newly synthesized NRF2 to accumulate in the nucleus, and activating antioxidant response element-mediated gene expression.[24] Certain classes of NRF2 activators display a greater propensity to modify certain sulfhydryl groups within KEAP1 (for example, sulforaphane modifies Cys151), while the prostaglandin activators require Cys273 for their activation.[25]

The NF-E2-related factor 2-mediated protection against diabetic nephropathy is, at least partially, through inhibition of TGF-β1 and reduction of extracellular matrix production. Studies show activation or overexpression of NRF2 inhibits the promoter activity of TGF- β1, whereas knockdown of NRF2 by small interfering RNA enhances TGF-β1 transcription.[26]

Yoh and colleagues treated NRF2 knockout mice with streptozotocin. The streptozotocin NRF2 knockout mice did not develop renal hyperfiltration, which was observed in the streptozotocin-treated wild-type mice, but kidney function gradually deteriorated over the 10-week observation period.[27]

Li and coworkers transfected mouse mesangial cells transiently with NRF2-plasmid or the NRF2-specific small interfering RNA. They found that high level of glucose induced ROS and malondialdehyde generation in mouse mesangial cells. Induction of NRF2 overexpression reduced the high-glucose-induced ROS and malondialdehyde production and inhibited cell proliferation and TGF-β1 secretion, accompanied by upregulating the expressions of HO-1 and γ-glutamylcysteine synthetase in mouse mesangial cells. However, knockdown of NRF2 expression displayed reverse effects in mouse mesangial cells. All these results indicated that NRF2 and its downstream antioxidant, HO-1 and γ-glutamylcysteine synthetase, are negative regulators of high-glucose-induced ROS-related mouse mesangial cell dysfunction.[28]

NF-E2-RELATED FACTOR 2 ACTIVATORS

In early experimental diabetes mellitus in hypertensive rats, the administration of tempol, an antioxidant superoxide dismutase (SOD) mimetic, corrected the oxidative imbalance and improved oxidative stress-induced renal injury, decreasing albuminuria and fibrosis.[29] It is not surprising that attention has focused on identifying small molecule activators of the NRF2-KEAP1 pathway.

Many chemically diverse activators have already been identified, including sulforaphane found in cruciferous vegetables,[30] resveratrol in the skin of red grapes and in other fruits,[31] cinnamic aldehyde (found in cinnamon bark), and most recently, bardoxolone methyl.[32,33]

Sulforaphane

Sulforaphane is an organosulfur compound that exhibits anticancer and antidiabetic properties in experimental models, obtained from cruciferous vegetables such as broccoli, brussels sprouts, and cabbages.[34] Studies showed that sulforaphane metabolites were detected in all tissues at 2 and 6 hours after gavage, with the highest concentrations in the small intestine, prostate, lung, and kidney, suggesting that sulforaphane is bioavailable and will be an effective dietary chemoprevention agent for these tissues.[35]

Sulforaphane has garnered particular interest as an indirect antioxidant due to its extraordinary ability to induce expression of several enzymes via the NRF2-KEAP1 pathway.[36] Several studies have shown the preventive effect of sulforaphane via induction of NRF2 on chemical or ischemia-induced renal damage.[37] A recent study has shown that after long-term treatment with sulforaphane, diabetic mice exhibited significant renal prevention from nephropathy via induction of NRF2-mediated antioxidant pathway.[38] Cui and colleagues investigated whether sulforaphane can prevent diabetic nephropathy in type 1 diabetic mouse model induced by multiple low-dose streptozotocin.[39] Diabetic and age-matched control mice were given sulforaphane at 0.5 mg/kg body weight daily for 3 months.[39] At the end of the study, the diabetes-induced damage, shown by kidney dysfunction, oxidative parameters and fibrosis was mostly prevented along with an significant elevation of renal NRF2 expression and transcription in diabetes-sulforaphane group.

In order to figure out effect of blocking of NRF2 expression, human renal tubular human kallikreins 11 cells transfected with NRF2 small interfering RNA. This procedure completely abolished sulforaphane prevention of the profibrotic effect induced by high glucose. These results support that renal NRF2 expression and its transcription play important roles in sulforaphane prevention of diabetes-induced renal damage.[39]

Bardoxolone

Bardoxolone was first introduced as an agent that protected cells from radiation-induced damage (radiation mitigator) through NRF2-dependent and NRF2-independent pathways.[40] In phase 1 trials on cancer patients, bardoxolone unexpectedly improved kidney function, especially in patients with a history of CKD. These results led to evaluation of potential nephroprotective actions in patients with CKD and type 2 diabetes mellitus, in two steps: (1) an exploratory phase II open-label trial, and (2) in a larger randomized clinical trial.

In the first trial, patients with diabetic CKD were evaluated after 8 weeks of bardoxolone at increasing oral doses of 25 mg/d to 75 mg/d. Notably, there was a significant increase in the estimated glomerular filtration rate at the end of study.[41] Cardiovascular death and progression to end-stage renal disease will be studied at 2 years of follow up in an ongoing randomized clinical trial in 1600 patients with advanced CKD (stage 4) and type 2 diabetes mellitus.[42] This study will compare bardoxolone versus placebo in patients receiving standard of care.

Some researchers believe bardoxolone (RTA 405) worsens proteinuria, glomerulosclerosis, and tubular damage.[43]

Resveratrol

During hyperglycemia-mediated oxidative stress, the expression of NRF2 and its downregulatory enzymes such as γ-glutamate-cysteine ligase, μ-glutamylcysteine synthetase, and HO-1 were significantly decreased in the renal tissues of diabetic rats. However, resveratrol treatment significantly modulates the expression of NRF2 in hyperglycemia-mediated oxidative stress by upregulation of γ-glutamate-cysteine ligase, μ-glutamylcysteine synthetase, and HO-1.[44]

Chromium Derivatives

Selcuk and colleagues showed that in kidney tissue, chromium histidinate/chromium picolinate increases NRF2 level, decreases nuclear factor kappa-light-chain-enhancer of activated B cells. It also partially restores nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha levels in high fat diet-streptozotocin group, suggesting that chromium histidinate and chromium picolinate may play a role in antioxidant defense system via the NRF2 pathway by reducing inflammation. This effect is observed through nuclear factor kappa-light-chain-enhancer of activated B cells p65 inhibition.[45]

Angiotensin-Converting Enzyme Inhibitors

In one study, non-obese and hypoinsulinemic C57BL/6-Ins2 Akita (C57BL/6 Akita) diabetic mice were treated with telmisartan, an angiotensin II type 1 receptor blocker, or amlodipine (5 mg/kg/d), a calcium channel blocker, for 4 weeks. The effects of these two antihypertensive drugs were compared in terms of renal NADPH oxidase, SOD, and transcription factor NRF2. The control group exhibited a higher level of renal NADPH oxidase and lower renal SOD activity with increased levels of renal superoxide than the C57BL/6-wild-type nondiabetic mice. Interestingly, telmisartan treatment not only reduced NADPH oxidase activity but also enhanced SOD activity in C57BL/6 Akita mouse kidneys, leading to a reduction of renal superoxide levels. Furthermore, telmisartan-treated C57BL/6 Akita mice increased the renal protein expression of SOD and NRF2.[46]

In parallel with the reduction of renal superoxide levels, a reduction of urinary albumin levels and a normalization of elevated glomerular filtration rate were observed in telmisartan-treated C57BL/6 Akita mice. In contrast, treatment with amlodipine failed to modulate renal NADPH oxidase, SOD and NRF2. In conclusion, this study suggests that NADPH oxidase negatively regulates renal SOD, possibly by downregulation of NRF2, and that telmisartan could upregulate renal SOD by the suppression of NADPH oxidase and subsequent upregulation of NRF2, leading to the amelioration of renal oxidative stress and diabetic renal changes.[46]

CONCLUSIONS

Oxidative stress contributes widely in the pathogenesis of diabetic nephropathy. There are also several defensive mechanisms, including antioxidative enzymes, eg, NADPH quinone oxidase 1 and HO-1. NF-E2-related factor 2 has a major impact on transcription of their genes. Antioxidative agents, sulforafan, bardoxolone, and others achieve their effects via transcription factor NRF2. A couple of studies have focused on ameliorating of renal oxidative stress and diabetic renal changes via prescribing these agents.

CONFLICT OF INTEREST

None declared.

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