Nrf2 Activators as Attractive Therapeutics for Diabetic Nephropathy

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Diabetes is the major cause of chronic kidney disease worldwide (1) with treatment options focused primarily on glucose control, blood pressure, lipid lowering, and the blockade of the renin-angiotensin system (2). However, despite intensive metabolic control and other interventions (3), the unrelenting decline in kidney function means that for many patients the condition progresses to overt kidney failure. This underpins the urgent need for novel approaches to manage the ever-increasing number of patients with diabetes and chronic kidney disease.

One approach that is attracting attention is the use of compounds to bolster the natural cytoprotective responses of the body. The transcription factor NF-E2–related factor 2 (Nrf2), together with its negative regulator, Kelch-like ECH-associated protein 1 (Keap1), is considered one of the most important cellular defense mechanisms to combat oxidative stress (4) with a particular role in the regulation of phase II detoxifying enzymes (Fig. 1). In particular, NADPH quinone oxidoreductase, glutathione *S*-transferase, heme oxygenase-1, and γ-glutamylcysteine synthetase are well-studied targets of Nrf2 that are upregulated through the antioxidant response element found in the promoters of these genes (5). Therefore, the coordinated upregulation of genes coding for detoxification, antioxidant, and anti-inflammatory regulators is seen as a potential therapeutic strategy to protect against insults such as inflammation and oxidative stress that are known to be enhanced by the diabetic milieu.

It is therefore not surprising that attention has focused on identifying small molecule activators of the Nrf2/Keap1 pathway. Many chemically diverse activators have already been identified (6), including the glutathione peroxidase-1 mimetic ebselen (7), sulforaphane found in cruciferous vegetables (8), caffeic acid phenethylester from the bee product propolis (9), cinnamic aldehyde (found in cinnamon bark), and most recently, bardoxolone methyl (10,11). Many have shown promising actions relevant to diabetes complications. For example, activation of Nrf2 by sulforaphane is able to suppress hyperglycemia-induced oxidative stress and metabolic dysfunction in human microvascular endothelial cells (8). A preclinical study by Zheng et al. (12) published in this edition of *Diabetes* also demonstrates the potential utility of the Nrf2 agonists, sulforaphane and cinnamic aldehyde, for improving the metabolic profile and reducing renal injury in mice with streptozotocin-induced diabetes. Specifically, this treatment was associated with reduced oxidative stress and attenuated induction of the profibrotic mediator transforming growth factor-β, the growth inhibitory protein p21, and extracellular matrix proteins in the diabetic kidney. Importantly, sulforaphane and cinnamic aldehyde failed to protect against renal injury in diabetic *Nrf2* knockout mice, suggesting that their renoprotective actions are specifically mediated via activation of Nrf2. Furthermore, specifically silencing Nrf2 also increased matrix synthesis.

Rather than directly targeting Nrf2, many agonists appear to work by suppressing its endogenous inhibitor, Keap1. Indeed, the study by Zheng et al. (12) showed that the specific silencing of Keap1 using small interfering RNA was able to reduce the expression of transforming growth factor-β and matrix proteins in human renal mesangial cells under both normal and high glucose conditions. However, 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 common feature appears to be their reactivity with the sulfhydryl groups of the Keap1 protein. A recent study by Kobayashi et al. (13), using a zebrafish model of a Keap1 mutation, has shown that certain classes of Nrf2 activators display a greater propensity to modify certain sulfhydryl groups within Keap1. In their classification, sulforaphane and several other Nrf2 activators modified Cys151, while the prostaglandin activators required Cys273 for their activation. Hydrogen peroxide, on the other hand, modified multiple cysteine residues of Keap1 (14), suggesting that the specificity of these small molecule activators may reside in the specific modifications of Keap1 cysteine residues. It is likely that more detailed understanding of the molecular interactions between Nrf2-Keap1 and these small molecule activators will pave the way for additional therapeutic interventions.

Beyond actions on renal fibrosis, small molecule activators of Nrf2 may also have direct actions on renal function. In a recently published double-blind, randomized, placebo-controlled trial using the Nrf2 activator, bardoxolone methyl (15), rapid improvements in the estimated glomerular filtration rate have been noted in patients with type 2 diabetes and impaired renal function (estimated glomerular filtration rate 20–45 ml/min/1.73 m^2). That these changes were observed within 4 weeks and were largely reversible when the drug was discontinued suggests a direct hemodynamic effect of this strategy. Given that current intervention appears to slow the decline in renal function by less than 1 ml/min/1.73 m^2 per year at best (16), sustained improvements with bardoxolone methyl of between 5–10 ml/min/1.73 m^2 potentially represent a major advance over standard therapies. Indeed, the result of this clinical trial is encouraging, and it is sure to lead to a flurry

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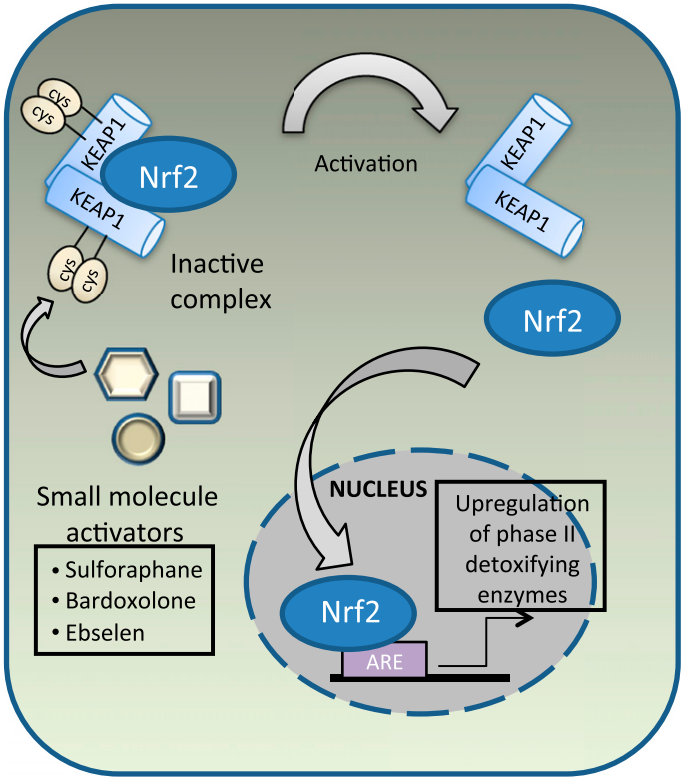

FIG. 1. Upregulation of phase II detoxifying enzymes by small molecule activators involves activation of the Nrf2-Keap1 pathway. The transcription factor Nrf2 is held in an inactive state in the cytoplasm through its interaction with two molecules of its inhibitory partner, Keap1. Small molecule activators such as ebselen, sulforaphane, and bardoxolone interact with cysteine residues within Keap1 causing its dissociation from 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. Upregulation of these phase II detoxification enzymes protects the cell against oxidative stress. Identification of clinically relevant small molecule activators of Nrf2 is offering promise as a new avenue for treatment against diseases such as diabetic nephropathy. (Adapted from Jung and Kwak [17].)

of both preclinical and clinic activity to identify additional Nrf2 activators with possibly even greater efficacy, as well as a deeper understanding of the mode of action of this novel class of compound. If similar improvements in kidney function with other novel activators can be reproduced, the strategy of bolstering antioxidant defenses through modulations of Nrf2 will represent a new class of therapy with potentially major advances over conventional therapy (17).

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