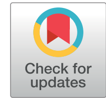

COMMENTARY

New Tricks for Nrf2: Therapeutic Targeting to Restore BK-β1 Expression?

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The transcription factor nuclear factor erythroid-2–related factor 2 (Nrf2) is ubiquitously expressed and a master regulator of antioxidant, phase II, and proteostatic genes induced by oxidative or electrophilic stress (1,2). Basally, Nrf2 is continually synthesized and associates with adaptor protein Kelch-like ECH-associated protein 1 (Keap1), resulting in its ubiquitination and rapid degradation via the 26S proteasome (3–5). Following oxidative challenge, modification of essential cysteine residues on Keap1 prevent Nrf2 degradation, allowing de novo Nrf2 to translocate to the nucleus and initiate transcription of target genes containing an antioxidant or electrophilic response element in their promoter region. In cells, failure to activate Nrf2 defenses increases their susceptibility to oxidative damage and dysfunction, contributing to the pathogenesis of various cardiometabolic disease states including hypertension, cardiomyopathy, gestational diabetes mellitus, and type 2 diabetes (6–8).

In this issue of *Diabetes*, Lu et al. (9) demonstrate a novel role for Nrf2 in the regulation of the large conductance Ca^{2+}-activated K^+ (BK) channel β1 subunit (BK-β1). As with other recently identified Nrf2-regulated solute transporters, this channel has no intrinsic antioxidant activity but is nevertheless important in homeostatic control of cell function. BK channels are expressed on vascular endothelial cells and smooth muscle cells (SMCs) and consist of a tetramer of α-subunits, containing a channel pore domain, voltage sensor, and Ca^{2+}-sensing region. These α-subunits are each associated with a tissue-specific modulatory β-subunit (isoforms β1–4), altering channel activity (10). The β1-subunit is abundantly expressed in vascular SMCs and enhances the BK channels sensitivity to Ca^{2+} (11). Activation of BK channels leads to K^+ efflux, resulting in membrane hyperpolarization and inhibition of Ca^{2+} influx through large conductance L-type Ca^{2+} channels, allowing BK channels to sense and modulate intracellular Ca^{2+} levels.

In the current study, Lu et al. (9) used a mouse model of high-fat diet (HFD) to induce hypertension and insulin resistance. They showed that in HFD mice, diminished Nrf2 expression correlates with a 57% reduction in BK-β1 protein expression. The authors also report that reduced BK-β1 expression coincides with reduced BK channel excitability in patch-clamped single coronary artery SMCs treated with the BK-β1 activator dehydrosoyasaponin-1. Raised mean arterial pressure (MAP) (HFD 105.2 ± 3.7 vs. lean 91.9 ± 4.8 mmHg) and impaired BK-β1–dependent vasodilation of coronary arteries reported by Lu et al. are consistent with previous studies supporting a role for BK-β1 in mediating vasodilation and contributing toward the regulation of myogenic tone and sympathetically mediated MAP.

Previously, deletion of the BK-β1 in mice was shown to uncouple transient Ca^{2+} release from intracellular stores (Ca^{2+} sparks) to BK channel activation, raising MAP (12,13) with primarily nighttime MAP corresponding to peak activity and enhanced sympathetic outflow affected (14). Diminished BK-β1 expression and Ca^{2+} sensitivity are also observed in rat models of genetic hypertension (WKY and SHR) (15) and angiotensin II–induced hypertension (11). In humans, few studies have explored the contribution of BK-β1 in vascular function but do indicate BK-β1 may be a useful therapeutic target for cardiometabolic disease prevention.

Previous studies demonstrate an autoregulatory role for BK channels, with blockade of BK channels using iberiotoxin reducing vasodilation in response to increased intraluminal pressure in isolated human skeletal muscle arterioles (16). A common polymorphism in the gene encoding the BK-β1 (E65K genotype) results in augmented Ca^{2+} sensitivity of BK channels and is associated with a lower incidence of diastolic hypertension in humans (17). Interestingly, the frequency of this polymorphism and its negative association with diastolic hypertension appear greatest in female participants over the age of 55 years (18). The underlying mechanism(s) responsible for age- and sex-specific differences in BK channel activation is unclear at this stage. Ovariectomy does not alter BK channel expression (19), nor does E65K alter in vitro BK channel activation in response to 17-β estradiol (18), an established activator of BK channels (20). In aged male

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rodents, BK channel α- and β1-subunit expression declines (21) and oxidation of BK α-subunit Cys^{911} reduces the sensitivity of BK channels to Ca^{2+}, reducing channel activation (22). It is therefore conceivable that the pro-oxidative environment found during aging or in the current model of cardiometabolic disease (9) may be needed to fully dissect the contribution of BK-β1 to blood pressure in male and female cohorts.

Highlighting the influence of redox-regulated transcription on BK-β1 expression, the study by Lu et al. (9) demonstrates that Nrf2 attenuates the expression of the muscle-specific E3 ubiquitin ligase muscle ring finger protein 1 (MuRF1) by inhibiting pro-oxidant transcription factor nuclear factor-κB (NF-κB). Using pull-down assays, this group has previously characterized a direct interaction between MuRF1 and BK-β1, with silencing of MuRF1 or inhibition of proteasomal degradation leading to BK-β1 stabilization and enhanced BK channel activity in a type 1 diabetic mouse model (23). Although direct interaction of MuRF1 with BK-β1 strongly suggests enhanced proteolysis does indeed contribute to reduced BK-β1 expression in diabetes, inhibition of global proteasomal activity to prevent BK-β1 degradation, as used in the current study, will produce off-target effects including the stabilization of Nrf2. Future studies genetically ablating the BK-β1–MuRF1 binding site or pulse-chase analysis of BK-β1 protein degradation may provide more definitive evidence for the functional importance of this interaction.

Excitingly, the study by Lu et al. (9) provides strong evidence that induction of Nrf2 can restore BK-β1 expression and activity both in vitro and in vivo. To establish that Nrf2 regulates BK-β1 expression, the authors transfected cultured SMCs with Nrf2 overexpressing and silencing adenoviruses. They showed clearly that enhanced Nrf2 levels result in increased expression of BK-β1 mRNA and protein and that suppression of Nrf2 expression reduces BK-β1 levels. These findings suggest that effect of Nrf2 on BK-β1 levels are at least partly exerted through transcriptional regulation, consistent with the authors' description of an Nrf2 antioxidant response element consensus sequence in the BK-β1 promoter. Further studies are required to resolve the relative contribution of NF-κB–mediated BK-β1 proteolysis versus Nrf2-dependent transcriptional upregulation of BK-β1. Perhaps the most convincing evidence underpinning a potential therapeutic role of Nrf2 in stabilizing BK-β1 is the restoration of BK-β1 expression following the 10-day administration of U.S. Food and Drug Administration–approved Nrf2 activator dimethyl fumarate (DMF) in HFD-treated mice. Acute treatment of human SMCs with DMF confirms upregulation of BK-β1 mRNA and protein, providing further evidence that BK-β1 is a newly identified Nrf2 target gene (Fig. 1).

Although Nrf2 is capable of inducing BK-β1 expression, the observation that basal BK-β1 mRNA expression is stable despite lower Nrf2 protein expression suggests other

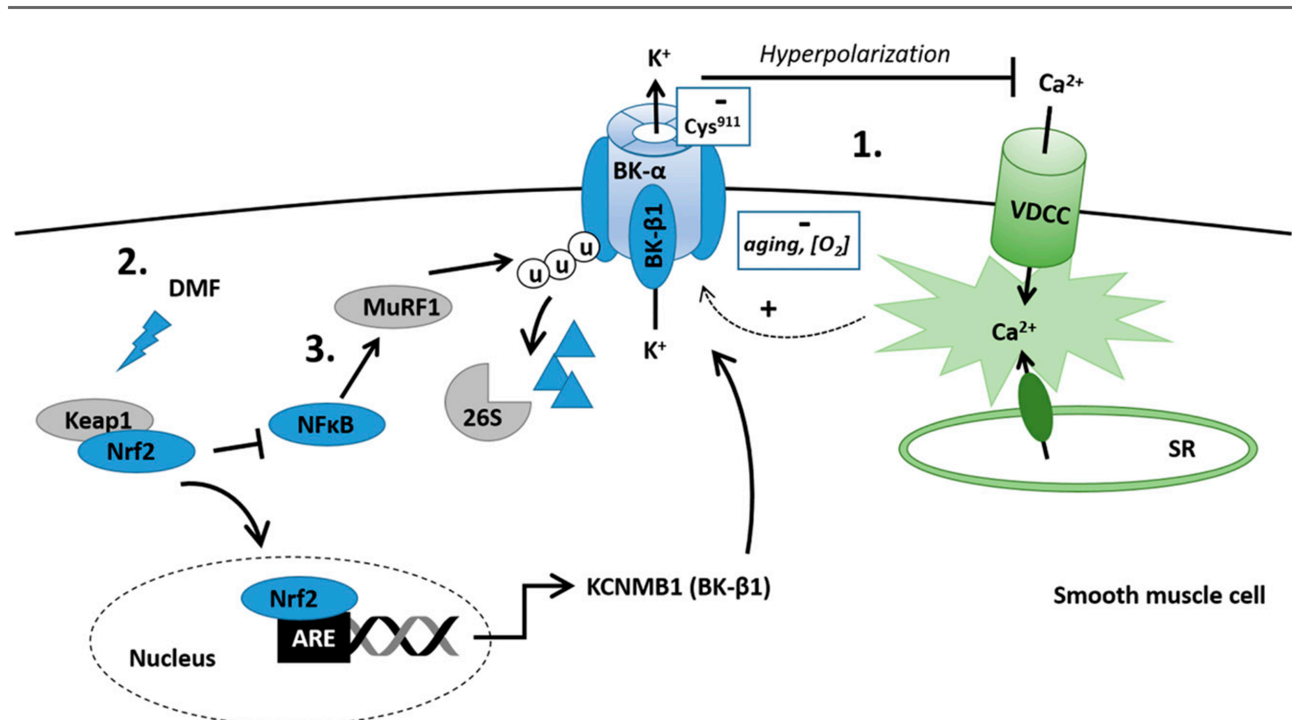

Figure 1—Proposed transcriptional and nontranscriptional regulation of BK-β1 by Nrf2. *1*: BK channels become activated in response to a rise in intracellular Ca^{2+}, with the β1-subunit (expressed in SMCs) increasing channel sensitivity to Ca^{2+}. K^+ extrusion leads to membrane hyperpolarization, promoting inhibition of voltage-dependent Ca^{2+} channels (VDCC), such as large conductance L-type Ca^{2+} channels, thereby preventing contraction. BK α- and β-subunit expression or activity can be further modulated by aging, oxidative stress–induced BK-α Cys^{911} oxidation, or O_2 tension. *2*: DMF relieves Keap1-mediated repression of Nrf2, allowing Nrf2 nuclear accumulation and induction of the newly identified target gene BK-β1. *3*: Nrf2 also attenuates NF-κB stabilization, reducing MuRF1 expression and consequently ubiquitination and degradation of BK-β1 via the 26S proteasome. ARE, antioxidant response element; SR, sarcoplasmic reticulum.

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transcriptional regulators regulate BK-β1 expression and strongly warrants further investigation. Moreover, as both Nrf2 and BK-β1 expression and activity appear to be sensitive to both reactive oxygen species and O_2 tension (22,24,25), conducting further in vitro studies under a physiological O_2 levels may provide valuable insights into BK channel modulation in physiological and pathological settings, allowing age- and sex-associated differences in BK activity to be explored and the efficacy of BK-β1 induction to treat various vasculopathies to be assessed.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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