NADPH Oxidase 4 at the Nexus of Diabetes, Reactive Oxygen Species, and Renal Metabolism

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NADPH oxidases (NOXs) catalyze the transfer of electrons from NADPH to molecular oxygen to produce superoxide and/or hydrogen peroxide, two major reactive oxygen species (ROS). Clarity on in vivo function and relevance to human disease are best exemplified by NOX2, which is responsible for generating the respiratory burst (i.e., large quantities of microbicidal ROS) in phagocytes. In turn, NOX2 deficiency is a cause of chronic granulomatous disease, an inherited disorder characterized by recurrent and persistent infections. Whereas NOX2 and its function within phagosomes were the first to be discovered and are the most widely recognized, in total, seven NOXs have been identified (NOX1, -2, -3, -4, and -5 and dual oxidase 1 and -2) with differences in tissue distribution, intracellular localization, and regulation. In addition to their potential contribution to oxidant stress, NOX ROS production is now recognized to play a fundamental role in cell signaling, with diverse effects on cell growth, differentiation, motility, and survival. These insights have motivated significant interest in nonphagocytic NOX in human health and disease.

NOX4 is the predominant NOX isoform expressed in the kidney. Unlike other isoforms, it is constitutively expressed with basal ROS production implicated in several normal renal physiologic functions. NOX4 expression is increased in tubular epithelial cells, mesangial cells, and podocytes cultured in high-glucose media and kidney tissue from different rodent models of diabetes. The deleterious effects of NOX4 in these contexts are numerous and extend beyond free radical-oxidant–mediated tissue injury. For example, in addition to reducing ROS levels, transient Nox4 knockdown has been shown to reduce Akt/PKB and extracellular signal–regulated kinase-1/2 phosphorylation in cortical homogenates from diabetic mice and inhibit TGF-β1–induced renal myofibroblast activation. Furthermore, the NOX1/NOX4 inhibitors GKT136901 and GKT137831 have been shown to attenuate ROS production, albuminuria, renal fibronectin and TGF-β content, and glomerular hypertrophy in db/db and OVE25 mice, respectively. Finally, global deletion of Nox4 attenuated albuminuria and glomerular injury in streptozotocin–treated ApoE−/− mice. Collectively, these data outline a critical role for NOX4 in the pathogenesis of diabetic nephropathy, albeit one that is complex and incompletely understood.

In this issue of JASN, You et al. make a valuable contribution to the literature, expanding the role for NOX4 in diabetic kidney disease to include the regulation of intermediary metabolism. More specifically, You et al. show that NOX4 modulates levels of the tricarboxylic acid (TCA) cycle intermediate fumarate through its effects on fumarate hydratase (FH) expression (FH catalyzes the hydration of fumarate to malate). Using a metabolomics screen, You et al. found dramatic increases in several TCA cycle intermediates in the urine of F1 Akita mice, a model of type 1 diabetic kidney disease, compared with F1 controls. Only fumarate levels, however, fell in a dose-dependent manner after NOX4 inhibition with GKT137831. Consistent with these findings, renal FH levels in F1 Akita mice were lower than in controls and increased with GKT137831 treatment. Furthermore, You et al. generated a podocyte–specific NOX4 transgenic mouse that recapitulates several pathologic characteristics of diabetic kidney disease and found decreased cortical expression of FH in the knockouts compared with control mice. In vitro, NOX4 overexpression also resulted in decreased FH expression, and treatment with hydrogen peroxide increased fumarate levels. Thus, across a series of elegant experiments, You et al. have established a novel link between increased NOX4 activity, decreased FH expression, and fumarate accumulation.

Is FH deficiency or fumarate excess a causal participant in diabetic nephropathy? Although these studies do not definitively answer this question, a review of the literature suggests both plausibility and potential mechanisms. As You et al. note, biallelic inactivation of FH causes hereditary leiomyomatosis and renal cell cancer, a finding that has stimulated extensive research on the effects of FH deficiency on cellular metabolism and proliferation. One area of focus has been on the aberrant activation of cellular hypoxia response pathways through hypoxia-inducible factor-1α (HIF-1α) stabilization. Excess fumarate has been purported to competitively inhibit HIF-1α proline hydroxylation, thus preventing its recognition and targeting for proteasomal degradation by the Von Hippel–Lindau complex. Consistent with this, You et al. found that HEK293 cells increased HIF-1α expression when exposed to millimolar concentrations of fumarate, and they raise the possibility that NOX4 is the major driver of HIF-1α in diabetic nephropathy. Notably, genetic deletion of Hifs does not prevent cyst formation in Ph1-deficient
mice, indicating that fumarate accumulation activates HIF-independent oncogenic pathways as well. Thus, more recent studies have considered fumarate’s propensity to covalently modify cysteine residues, a reaction known as succination. For example, succination of the cysteine–rich, Kelch–like, ECH–associated protein 1 results in the release and nuclear accumulation of its binding partner nuclear erythroid–related factor 2 (NRF2), a master transcriptional activator of the antioxidant response. Conversely, succination of glutathione has also been observed in HF-deficient cells, contributing to increased oxidative stress. Thus taken together, the current literature supports an important and multifaceted role for fumarate metabolism in redox homeostasis.

Although the results by You et al. motivate particular interest in FH, it will be of interest to know whether NOX4-mediated changes in FH expression are specific to this enzyme or part of a systematic shift in metabolic flux between the TCA cycle and glycolysis. A dramatic increase in glycolytic flux, despite aerobic conditions, is a hallmark of proliferating cancer cells, including FH-deficient cells, and it is possible that the mitogenic effects of NOX ROS production could participate in this shift (e.g., through PI3-Akt signaling). Alternatively, because increased NOX ROS production triggers an antioxidant response through NRF2 activation (perhaps in part because of fumarate accumulation), secondary effects of NOX4 on metabolism must also be considered. In a seminal study, Mitsuishi et al. showed that NRF2 increases glucose flux through the pentose phosphate pathway and diverts malate away from the TCA cycle, changes that promote proliferation by increasing nucleotide biosynthesis and NADPH production. Notably, these effects were mediated directly by NRF2 transcriptional activation at the relevant metabolic gene promoters. Ultimately, the effect of NOX4 on cellular metabolism is certain to be complex and will likely depend on several factors, including the magnitude and duration of activation, secondary responses (e.g., NRF2), and the presence or absence of other proliferative signals.

In addition to revealing new biology, metabolomic screens have the potential to identify novel biomarkers. Whether urinary fumarate reports on renal NOX4 activity certainly warrants additional investigation. However, it is worth noting that the concordance between renal tissue and urinary fumarate levels in F1 Akita mice does not necessarily establish the former as the source of the latter. In general, urine composition would be expected to reflect the net effects of filtration, absorption, and secretion on plasma composition rather than alterations in intracellular renal metabolism (e.g., within podocytes).

In summary, You et al. have leveraged metabolomics in the F1 Akita mouse treated with an NOX1/NOX4 inhibitor and a podocyte-specific NOX4 transgenic mouse to shed new light on the nexus of diabetes, oxidative stress, and cellular metabolism. The exciting results by You et al. raise additional questions (for example, regarding the mechanism and specificity of NOX4-mediated modulation of FH and whether FH overexpression can ameliorate NOX4-mediated renal injury). Beyond these experimental questions, however, the greatest area of interest is whether modulating the ROS system at the source (NOX4 inhibition) will prove to be clinically efficacious in diabetic nephropathy in contrast to the disappointing experience with augmenting the antioxidant response with bardoxolone (NRF2 activation).

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DISCLOSURES

None.

REFERENCES


