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Review Article

The balance of powers: Redox regulation of fibrogenic pathways in kidney injury

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ABSTRACT

Oxidative stress plays a central role in the pathogenesis of diverse chronic inflammatory disorders including diabetic complications, cardiovascular disease, aging, and chronic kidney disease (CKD). Patients with moderate to advanced CKD have markedly increased levels of oxidative stress and inflammation that likely contribute to the unacceptable high rates of morbidity and mortality in this patient population. Oxidative stress is defined as an imbalance of the generation of reactive oxygen species (ROS) in excess of the capacity of cells/tissues to detoxify or scavenge them. Such a state of oxidative stress may alter the structure/function of cellular macromolecules and tissues that eventually leads to organ dysfunction. The harmful effects of ROS have been largely attributed to its indiscriminate, stochastic effects on the oxidation of protein, lipids, or DNA but in many instances the oxidants target particular amino acid residues or lipid moieties. Oxidant mechanisms are intimately involved in cell signaling and are linked to several key redox-sensitive signaling pathways in fibrogenesis. Dysregulation of antioxidant mechanisms and overproduction of ROS not only promotes a fibrotic milieu but leads to mitochondrial dysfunction and further exacerbates kidney injury. Our studies support the hypothesis that unique reactive intermediates generated in localized microenvironments of vulnerable tissues such as the kidney activate fibrogenic pathways and promote end-organ damage. The ability to quantify these changes and assess response to therapies will be pivotal in understanding disease mechanisms and monitoring efficacy of therapy.

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Abbreviations: AGEs, advanced glycation end-products; ALE, advanced lipoxidation end-products; BH4, tetrahydrobiopterin; CML, N^{ε}-(carboxymethyl)lysine; eNOS, endothelial nitric oxide synthase; ESRD, end stage renal disease; MS, mass spectrometry; $HO^{\bullet}$, hydroxyl radical; HODE, hydroxyoctadecadienoic acid; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; LDL, low density lipoprotein; MPO, myeloperoxidase; NEFA, non esterified fatty acid; NOX, NAD(P)H oxidase; $NO^{\bullet}$, nitric oxide; $NO_2^{\bullet}$, nitrogen dioxide; $ONOO^-$, peroxynitrite; PUFA, polyunsaturated fatty acid; RAGE, Receptor for AGE; RNase, ribonuclease; $O_2^{\bullet}-$, superoxide anion

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1. Background

Oxygen forms the basis of aerobic life but, it is well-recognized that it can be modified by cellular metabolism to form highly reactive free radicals termed as "reactive oxygen species" (ROS) which in turn can form a variety of intermediates including "reactive nitrogen species" (RNS). Oxidative stress may be viewed as an essential consequence related to the fundamental biological need for utilization of molecular oxygen (O_2) for energy production from simple aerobic eukaryotes to more complex mammalian species [1]. While physiological levels of such oxidants have a beneficial role in energy production, cellular signaling and host defense, excess oxidants can lead to pathological consequences.

Acute kidney injury (AKI) is characterized by the sudden deterioration of kidney function and is associated with a high incidence of morbidity and mortality [2]. Chronic kidney disease (CKD) affects approximately 26 million people in the US and premature death from cardiovascular disease and from all causes is higher in adults with CKD compared to adults without CKD [3]. During the past decade these two syndromes were conceptually distinct, however, evidence from experimental models and recent epidemiological studies suggest that AKI and CKD are closely interconnected: AKI can result in end-stage kidney disease (ESKD), AKI is a risk factor for CKD, CKD is a risk factor for AKI, and both are risk factors for cardiovascular disease [2,4]. The exact mechanisms by which AKI initiates or accelerates CKD in humans are unknown and as a result there are currently no therapies to halt or reverse AKI or to address the relentless progression of CKD.

Fibrosis is characterized by maladaptive wound repair following tissue injury that leads to the progressive accumulation of interstitial matrix proteins with gradual destruction of renal tubules and functional nephrons. Increased oxidative stress is a consistent characteristic of both AKI and CKD. Although once conceptualized as a random process, it is now recognized that oxidant-mediated injury occurs along predictable pathways and through specific cell types. One of the ways that oxidant and antioxidants may affect disease pathogenesis is through the modulation of reversible oxidation-reduction (redox) processes. Redox is a dynamic process that involves the ROS/RNS to oxidize a critical protein, such as a kinase, phosphatase or enzyme, and an antioxidant, typically a thiol containing protein such as glutathione (GSH) to reduce the protein back to its original state [5,6]. Redox signaling is advantageous because ROS/RNS are diffusible, permitting action at a distance. In this review, we will discuss the major oxidant and redox sensitive pathways in kidney injury and fibrosis.

2. Oxidative regulation of the fibrotic response

Oxidative stress is commonly viewed as a disturbance in the balance between oxidant production and antioxidant defense mechanisms within the tissue. Both acute and chronic kidney injury are characterized by an over-production of oxidants in the presence of a diminished antioxidant reserve. This imbalance of pro-oxidants or free radicals can oxidize macromolecules such as proteins, lipids, and nucleic acids altering redox sensitive pathways resulting in subsequent cell and tissue injury (Fig. 1). Reactive oxygen species (ROS) and reactive nitrogen (RNS) are collective terms that include not only highly reactive oxygen and nitrogen radicals ($O_2^{\bullet -}$, $OH^{\bullet}$, and $NO^{\bullet}$ derived $NO_2^{\bullet}$) but also non-radical derivatives ($ONOO^-$). Several enzyme complexes generate ROS and RNS such as NADPH oxidase, myeloperoxidase, nitric oxide synthase, and superoxide dismutase. Although many of these oxidant complexes are important in kidney injury [7], we will focus on oxidant pathways most thoroughly investigated in regards to redox regulation in kidney disease.

2.1. NADPH oxidase

Superoxide anion ($O_2^{\bullet -}$) is the major free radical generated *in-vivo* through the action of the enzyme complex, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The phagocyte NADPH oxidase enzyme complex is composed of the membrane bound flavocytochrome subunits Nox (formerly known as $gp91^{phox}$) and $p22^{phox}$ and cytosolic regulatory proteins $p47^{phox}$, $p67^{phox}$, $p40^{phox}$, and the GTPase Rac1/2. All Nox proteins contain a heme binding site on a membrane spanning region of the N-terminal half and NADPH- and FAD-binding domains in the C-terminal half. Membrane bound Nox along with $p22^{phox}$ form the catalytic core that can transfer electrons across biological membranes using NADPH as the electron donor to molecular O_2 to generate superoxide anion ($O_2^{\bullet -}$). At neutral pH, $O_2^{\bullet -}$ is a reducing agent rather than an oxidant. However, $O_2^{\bullet -}$ dismutates enzymatically or nonenzymatically into hydrogen peroxide (H_2O_2), which can then oxidize thiol residues, a mechanism for cellular signaling via the inactivation of cysteine-containing phosphatases [8]. It can also function as an oxidizing substrate for heme proteins such as myeloperoxidase (MPO). $O_2^{\bullet -}$ also reacts at a diffusion-controlled rate with nitric oxide (NO) to form peroxynitrite ($ONOO^-$), a powerful reactive nitrogen species that nitrates tyrosine residues on proteins and may induce oxidative damage to other macromolecular substrates.

Nox and $p22^{phox}$ are activated after recruitment and phosphorylation of the three regulatory proteins ($p47^{phox}$, $p67^{phox}$, $p40^{phox}$) and the GTPase Rac1/2, which assemble with the membrane-bound proteins to form a functional NADPH oxidase [9,10]. This regulatory pathway allows Nox to remain inactive in resting cells and rapidly activated to provide the respiratory burst in leukocytes or ROS in nonphagocytic cells.

There are currently seven Nox isoforms (Nox1-5, Duox1, Duox2). Several Nox isoforms (Nox1-4) share a number of critical structural and functional domains with Nox2. Excessive production of ROS by the Nox complex is commonly thought to be responsible for tissue injury associated with a range of chronic inflammatory diseases and has long been considered a unique property of phagocytic cells. Deficiency of Nox2 in humans results in chronic granulomatous disease characterized by multiple abscess formation due to inability to remove bacterial pathogens. However, in animal studies of chronic injury, Nox2 deficiency was associated with enhanced inflammation with subsequent tissue damage [11–15], implying that Nox2 also has beneficial functions in immune responses and cell signaling. Recent studies have broadened our understanding of Nox's function to include cellular processes as diverse as cell proliferation, migration, differentiation, signal transduction, and oxygen sensing [9,16,17].