ACUTE KIDNEY INJURY

Nitric oxide protects against AKI by reprogramming metabolism

Pierre-Yves Martin and Sophie de Seigneux

New findings demonstrate that endothelial nitric oxide synthase regulates major metabolic pathways in the kidney proximal tubule, which confers protection against oxidative stress during acute kidney injury (AKI). These findings give new insights into AKI pathophysiology and nitric oxide biology, and identify new targets for the treatment of AKI.

Refers to Zhou, H.-L. et al. Metabolic reprogramming by the S-nitroso-CoA reductase system protects against kidney injury. *Nature* **565**, 96–100 (2018).

Acute kidney injury (AKI) is a global health problem associated with high morbidity and mortality. AKI is also associated with the development of chronic kidney disease (CKD) and subsequent development of end-stage renal disease[1]. Several biological targets for AKI have been identified in animal models but these have not yet translated into beneficial therapeutic strategies for humans. A new study by Zhou et al. opens new avenues in this field, showing that nitric oxide (NO) produced by endothelial NO synthase (eNOS) protects against AKI through *S*-nitrosylation of pyruvate kinase M2 (PKM2), leading to activation of antioxidant pathways[2].

The signalling molecule NO is synthesized by enzymes called NO synthases, including eNOS[3]. eNOS has previously been shown to be protective against AKI, but the mechanisms underlying this protection are poorly understood. NO signalling is mediated by different mechanisms, including protein *S*-nitrosylation[4], which involves the covalent addition of NO groups to cysteine thiols to generate *S*-nitrosothiols (SNOs). This reversible addition is an important regulatory mechanism that accounts for the majority of the cyclic guanosine monophosphate (cGMP)-independent effects of NO on signal transduction and cellular function. Dysregulation of protein *S*-nitrosylation is associated with several pathophysiological conditions, including haemoglobinopathies, pre-eclampsia, pulmonary hypertension, Parkinson disease, heart failure, septic shock and diabetes mellitus. Available evidence suggests that protein *S*-nitrosylation is regulated by *S*-nitrosylases (which are involved in the transfer of NO groups to cysteine thiols) and denitrosylases (which are involved in the removal of NO groups from cysteine thiols). In 2014, the same research group identified SNO-CoA reductases as a new functional class of enzymes that regulates protein *S*-nitrosylation in yeast and mammals, by acting as a denitrosylase[5]. In particular, they identified the aldo-keto reductase family member AKR1A1 as a SNO-CoA reductase in mammals; however, its physiological role was not known. In their new study, Zhou et al. describe the physiological function of AKR1A1 and link this function to AKI pathophysiology. They first demonstrate that AKR1A1 is abundantly expressed in proximal tubules — the same tubular segment in which eNOS is highly expressed. In detailed analyses using *Akr1a1*$^{-/-}$ and *Akr1a1*$^{-/-}$*Enos*$^{-/-}$ mice subjected to ischaemia–reperfusion-induced AKI, they show that mice deficient in *Akr1a1* are protected from AKI, and that this protection is lost by concurrent deficiency in *Enos*. The protection afforded by *Akr1a1* deficiency is dependent on SNO and is in part mediated by inhibitory *S*-nitrosylation of PKM2, a key metabolic enzyme that mediates the last step of glycolysis (FIG. 1). This inhibitory *S*-nitrosylation of PKM2 leads to an increase in upstream glycolytic intermediates such as glucose-6-phosphate (G6P), dihydroxyacetone phosphate (DHAP) and phosphoenolpyruvate (PEP), suggesting a block at the last step in glycolysis. In support of this hypothesis, deletion of PKM2 specifically in mouse proximal tubules recapitulated the renal protection against AKI observed in *Akr1a1*$^{-/-}$ mice. Using a metabolic profiling approach, the researchers finally demonstrated that inhibition of PKM2 leads to increased flux through the pentose phosphate pathway (PPP). The PPP is a metabolic pathway that generates nicotinamide adenine dinucleotide phosphate (NADPH), which can increase the antioxidant glutathione (GSH) and activate antioxidant enzymes. Inhibition of PKM2 by inhibitory *S*-nitrosylation therefore reprogrammes the metabolism of the proximal tubule from fuel utilization (glycolysis) to redox protection (through PPP flux).

The discovery that NO reprogrammes metabolism identifies a new role for this signalling molecule beyond its vasodilatory, neurotransmission, antimicrobial and cytotoxic roles. This study represents a major step forward in deciphering the complex role of NO in ischaemia–reperfusion injury. Moreover, it opens a new field of investigation in AKI. Although this disease is known to generate toxic oxygen radicals, approaches to counteract this process have so far been unsuccessful. Although confirmatory and further exploratory studies are needed, the link between metabolic reprogramming and antioxidant pathways might represent a new avenue for anti-ischaemic

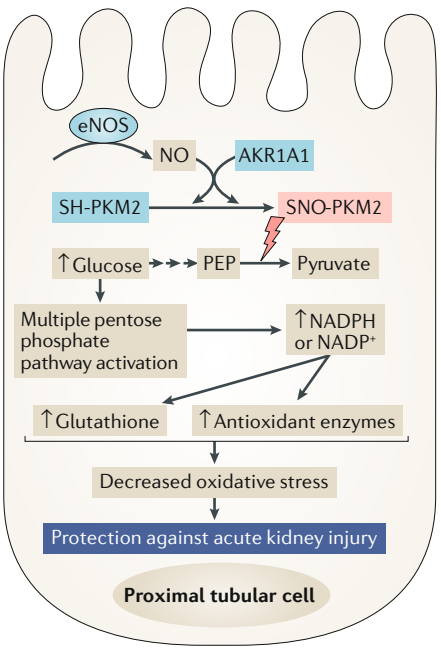

Fig. 1 | **Nitric oxide confers protection against AKI by shifting metabolic flux towards the pentose phosphate pathway.** Nitric oxide (NO) together with the aldo-keto reductase family member AKR1A1 mediate the *S*-nitrosylation of pyruvate kinase M2 (PKM2) by modifying the thiol (SH) groups of cysteine residues, leading to the formation of *S*-nitrosothiol (SNO)-PKM2. This post-translational inhibition of PKM2 leads to inhibition of glycolysis. Glucose subsequently enters the multiple pentose phosphate pathway (PPP), leading to the generation of nicotinamide adenine dinucleotide phosphate (NADPH), a cofactor that increases glutathione and antioxidant enzymes, leading to reduced oxidative stress and protection against ischaemia–reperfusion-induced acute kidney injury (AKI). eNOS, endothelial NO synthase.

therapies, and suggests that AKR1A1 and PKM2 might represent new targets for drug discovery. In addition, these findings suggest that metabolic profiling may be beneficial in characterizing and stratifying AKI in humans and in overcoming some of the limitations of conventional animal models of AKI[6]. It should be noted that activation of PKM2 has been shown to protect against diabetic kidney disease by increasing glucose metabolic flux and inhibiting the production of toxic glucose metabolites[6]. Although this finding seems contradictory, it highlights the differences between acute and chronic injuries and also the need to better understand the role of this enzyme in different cell types and pathologies[7].

Finally, metabolic dysfunction is associated with fibrosis and contributes to the development of AKI-induced CKD. Metabolic reprogramming via PKM2 increases the synthesis of serine, a precursor for lipids, proteins and nucleotides, and enhances the regeneration of injured tissues, which might be an additional advantage of this new mechanism[8]. This study adds further evidence that the regulation of redox status and energy metabolism are of utmost importance in the pathophysiology of AKI and supports previous work showing a protective effect of NAD^+ in AKI by boosting mitochondrial function[9,10]. Together, these studies demonstrate that the field of AKI is exploding in new and promising directions.

Pierre-Yves Martin * *and Sophie de Seigneux*

Service and Laboratory of Nephrology, Department of Medicine, University and University Hospital of Geneva, Geneva, Switzerland.

**e-mail:* *Pierre-yves.martin@hcuge.ch*

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Competing interests
The authors declare no competing interests.

DEVELOPMENT

DNA methylation links intrauterine stress with abnormal nephrogenesis

Samir S. El-Dahr

Scientists have long wondered how maternal diabetes, malnutrition and placental dysfunction impair fetal nephrogenesis. A new study discovered a link between prenatal metabolic stress and nephron deficit via dysregulation of DNA methylation — an epigenetic mechanism that is essential for the renewal and differentiation of nephron progenitors.

Refers to Wanner, N. et al. DNA methyltransferase 1 controls nephron progenitor cell renewal and differentiation. *J. Am. Soc. Nephrol.* **30**, 63–78 (2018).

Human nephrogenesis begins around week 5–8 of gestation and ends by week 34. Interestingly, the final number of nephrons at birth (the so-called nephron endowment) varies widely in humans (by approximately tenfold)[1]. Epidemiological studies have demonstrated that low nephron number at birth is associated with the development of chronic kidney disease and hypertension in later life[2]. Prenatal stressors, such as prematurity, maternal diabetes and exposure to nephrotoxins and infections, have been postulated to rewire the fetal nephrogenesis programme leading to renal hypoplasia, but how such adverse prenatal events reprogramme fetal nephrogenesis is unknown. New findings from Wanner et al.[3] show that DNA methylation is a key mechanism that links the intrauterine environment with fetal nephrogenesis.

Using two rodent models of gestational stress (induced by hyperglycaemia and placental blood flow insufficiency — known causes of intrauterine growth restriction (IUGR)), Wanner et al.[3] show that global DNA hypomethylation is associated with renal hypoplasia. They further show that conditional disruption of *Dnmt1* in nephron progenitor cells (NPCs) impairs their renewal and differentiation. *Dnmt1* encodes the maintenance DNA methyltransferase DNMT1, which faithfully methylates cytosine residues on daughter DNA strands in accordance with the methylation pattern of the parental DNA strands. Surprisingly, however, deletion of the de novo DNA methyltransferases, *Dnmt3a* and *Dnmt3b* — which unlike *Dnmt1* can establish new methylation patterns — had no effect on NPC homeostasis. A strength of this study is the use of sophisticated quantitative 3D imaging reconstruction techniques to characterize the nephron progenitor deficits in the mutant kidneys. Specifically, the investigators used in vivo cell labelling with the nucleoside analogue 5-ethynyl-2′-deoxyuridine (EdU) and co-staining of the whole organ using NPC and other compartment-specific markers followed by optical projection tomography and confocal microscopy. The imaging phase was followed by quantitative analysis with modelling and integration of the morphogenetic events at a cell and tissue level in 3D space and across developmental time. The total number of NPC niches and NPC per niche can be quantified fairly accurately using these techniques. The researchers also used gene expression profiling to reveal that renal hypoplasia occurs in these models as a result of at least three mechanisms: a modest downregulation of a subset of genes that are important for NPC proliferation; downregulation of the WT1–WNT4 signalling axis, which is necessary for NPC differentiation; and perhaps the most novel finding of this study, derepression of the stress interferon–p53–p21 pathway as well as endogenous retroviral elements and non-lineage germline genes. *Dnmt1* seems to uniquely affect self-renewing NPCs, as deletion of this methyltransferase in terminally differentiated podocytes did not affect glomerular development.

> “a simple strategy involving gestational supplementation of methyl donors … might be effective in restoring DNA methylation and gene expression in the NPCs affected by gestational stress”