The Dual-specificity Phosphatase 3 (DUSP3): A Potential Target Against Renal Ischemia/Reperfusion Injury

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Abstract. Renal ischemia/reperfusion (I/R) injury is a common clinical challenge faced by clinicians in kidney transplantation. I/R is the leading cause of acute kidney injury, and it occurs when blood flow to the kidney is interrupted and subsequently restored. I/R impairs renal function in both short and long terms. Renal ischemic preconditioning refers to all maneuvers intended to prevent or attenuate ischemic damage. In this context, the present review focuses on the dual-specificity phosphatase 3 (DUSP3), also known as vaccinia H1-related phosphatase, an uncommon regulator of mitogen-activated protein kinase (MAPK) phosphorylation. DUSP3 has different biological functions: (1) it acts as a tumor modulator and (2) it is involved in the regulation of immune response, thrombosis, hemostasis, angiogenesis, and genomic stability. These functions occur either through MAPK-dependent or MAPK-independent mechanisms. DUSP3 genetic deletion dampens kidney damage and inflammation caused by I/R in mice, suggesting DUSP3 as a potential target for preventing renal I/R injury. Here, we discuss the putative role of DUSP3 in ischemic preconditioning and the potential mechanisms of such an attenuated inflammatory response via improved kidney perfusion and adequate innate immune response.

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INTRODUCTION

Over the past decade, the clinical application of ischemic preconditioning (IPC) has generated growing enthusiasm as an innovative approach in kidney transplantation (KTx). Indeed, KTx is a clinical paradigm of renal ischemia/reperfusion (I/R).[1] I/R induces sterile inflammation, characterized by a massive production of reactive oxygen species, the recruitment of neutrophils, monocytes, macrophages, and the activation of various pro-inflammatory stimuli.[2-4] Furthermore, I/R is associated with acute rejection due to an increased immunogenicity favoring T-cell–mediated rejection and can cause progressive interstitial fibrosis.[5] Since I/R typically affects the innate immune system,[3] attenuation of I/R damage and preservation of kidney architecture and function is theoretically feasible by modulating this system.

The dual-specificity phosphatase 3 (DUSP3) or vaccinia H1-related phosphatase (VHR) is an uncommon regulator of mitogen-activated protein kinase. Human DUSP3 is a protein with 185 amino acids that has a 93% identity with its murine orthologue Dusp3, therefore, sharing similar functions across these 2 species.[6] DUSP3 is highly expressed in endothelial cells, as well as in platelets and monocytes, where it plays an essential role as a positive regulator of the innate immune response.[7] DUSP3 modulates the cell cycle and various signaling pathways, including extracellular signal-regulated kinase 1/2 (ERK1/2), the c-Jun NH2-terminal kinase (JNK), signal transducer and activator of transcription, and epidermal growth factor receptor.[8] In addition, the production of tumor necrosis factor (TNF)-α during inflammation requires the presence of DUSP3[9] (Figure 1A).

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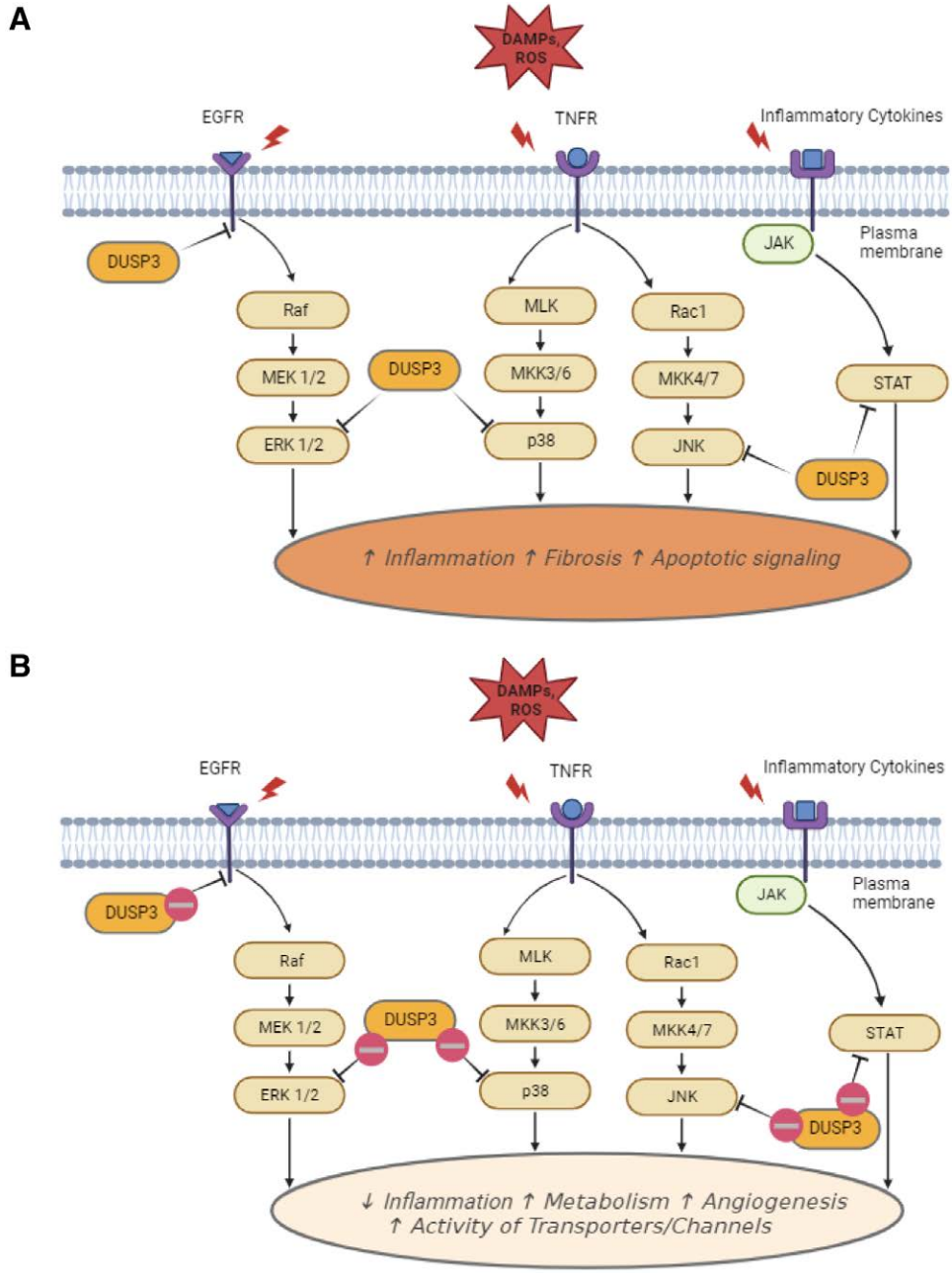

FIGURE 1. DUSP3 in renal I/R cascade. A, Main physiological substrates of DUSP3 (ERK1/2, p38, JNK, STAT, EGFR) and their involvement in different I/R pathways. B, DUSP3 inhibition in I/R is associated with downregulation of inflammatory pathways and increased in cell metabolism, transport and angiogenesis pathways. DAMPs, damage-associated molecular patterns; DUSP3, dual-specificity phosphatase 3; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; I/R, ischemia/reperfusion; JAK, janus kinase; JNK, c-Jun NH2-terminal kinase; MEK, mitogen-activated protein/extracellular signal-regulated kinase kinase; MKK, mitogen-activated protein kinase kinase; MLK, mixed lineage kinase; Rac1, Rac Family Small GTPase 1; Raf, rapidly accelerated fibrosarcoma; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription; TNFR, tumor necrosis factor receptor; Created with BioRender.

Although the systemic Dusp3 knock-out (*Dusp3*$^{-/-}$) did not show any spontaneous phenotype,[10] *Dusp3*$^{-/-}$ mice showed resistance to several autoimmune disease models.[9,11] In the context of I/R-induced acute kidney injury (AKI), it has been recently demonstrated that *Dusp3*$^{-/-}$ mice were remarkably resistant to I/R. Glomerular filtration rate was preserved, and albuminuria was reduced by half compared with control mice following I/R. Such an IPC associated with DUSP3 deletion was associated with increased phosphorylation of peptides involved in cell metabolism and vascular endothelial growth factor-related angiogenesis, downregulation of inflammatory pathways, and reduced tissue inflammation[12] (Figure 1B).

Treatment of renal I/R injury remains largely supportive, including fluid maintenance and vasoactive drugs.[13] The recent advances in deciphering the pathophysiology of I/R will lead to novel therapies, including noninvasive approaches of renal IPC. In this review, we discuss the putative role of DUSP3 in IPC against I/R-induced AKI.

KIDNEY TRANSPLANTATION IS A CLINICAL PARADIGM OF RENAL ISCHEMIA/REPERFUSION

The I/R-induced AKI at the time of KTx negatively impacts short- and long-term kidney outcomes.[14,15] The type and duration of ischemia in KTx varies according to the type of kidney donor: living donor (LD) versus deceased donor (DD). Among the DDs, a distinction should be made between (1) heart-beating donors, corresponding to patients with severe and irreversible brain damage, diagnosed as donation after brain death and (2) nonheart-beating donors (donation after circulatory death [DCD]), corresponding to patients whose circulatory and respiratory functions have irreversibly stopped. Furthermore, 2 types of ischemia are defined in KTx: (1) cold ischemia time (CIT), during which the kidneys are immersed in cold preservation solution (4 °C) and stored in ice and (2) warm ischemia time (WIT), which extends from the time of the clamping of the renal pedicle (or the onset of asystole in DCD donors) to the beginning of cold flush. DD kidneys are exposed to more CIT than LD kidneys, which contributes to the shorter graft survival observed in DDs compared with LDs and an increased rate of delayed graft function (DGF). In addition, DCD donors undergo a longer period of WIT than donation after brain death donors. Finally, a second warm ischemia occurs between the removal of the organ from the cold storage until its reperfusion at the time of transplantation itself.[16] As a whole, the I/R process involves a series of lesions linked to hypothermia due to storage conditions, as well as hypoxia and warming associated with reoxygenation/reperfusion.[15] This has an impact on recovery of kidney function, as well as on graft survival. Indeed, a study of over 6400 kidney transplant recipients (KTRs) found that extended CIT is a risk factor for graft failure at 6 y.[17] Another study with 518 KTRs evidenced that DGF and the duration of ischemia are the two most important determinants of long-term graft survival.[18] The pathological link between I/R and the risk of acute rejection in KTRs prompts various strategies aiming to limit the impact of I/R by notably reducing cold and WIT,[19,20] developing novel organ preservation techniques, and identifying innovative IPC approaches to be applied before surgery.[21]

ISCHEMIC PRECONDITIONING AIMS AT ATTENUATING ISCHEMIA/REPERFUSION INJURY

Renal blood flow reaches 1200 mL/min, which equates to 20% of cardiac output, despite the fact that both kidneys account for <1% of total body weight. Due to the tight regulation of the sympathetic nervous system, the complex interplay between the renin-angiotensin system as well as prostaglandin synthesis, renal blood flow remains constant under physiological conditions down to a systolic arterial pressure of 80 mm Hg.[22] When renal blood perfusion is interrupted for an extended period, nutrient delivery ceases and the Po_2 falls. This temporary interruption/reduction of renal blood flow, followed by its restoration and reoxygenation, results in a cascade of cellular and tissue events known as "I/R injury" (Figure 2).

The first cellular victims of ischemia are endothelial cells, which lose their endothelial barrier function. Endothelial cells get activated and injured via the expression of cytokines (eg, TNF-α, interleukin [IL]-6, IL-1β), chemokines (eg, Monocyte Chemoattractant Protein 1, IL-8, Regulated on activation, normal T cell expressed and secreted), and adhesion molecules, which are induced by reactive oxygen intermediates and cause an inflammatory cascade ultimately leading to endothelial cell dysfunction.[23,24] This local inflammatory reaction can be dramatically augmented by the generation of a number of potent mediators produced by ischemic proximal tubules, which overall is thought to represent a "maladaptive tubular response."[23] Ischemia also rapidly induces the inhibition of fatty acid oxidation-associated enzymes in both mitochondria and peroxisomes as well as the reduction of carnitine palmitoyltransferase-1 activity.[24] Changes in kidney metabolomic profiles emphasize the alteration of metabolic pathways affecting lipid metabolism, tricarboxylic acid cycle, and glycolysis.[24,25] The reperfusion of such a damaged tissue is characterized by the activation of the innate immune system within the first hours after I/R, followed by an adaptive immune response contributing to chronic inflammation and rejection in the long term.[26,27] Reperfusion-triggered innate immunity is responsible for the recruitment and activation of monocyte cells,

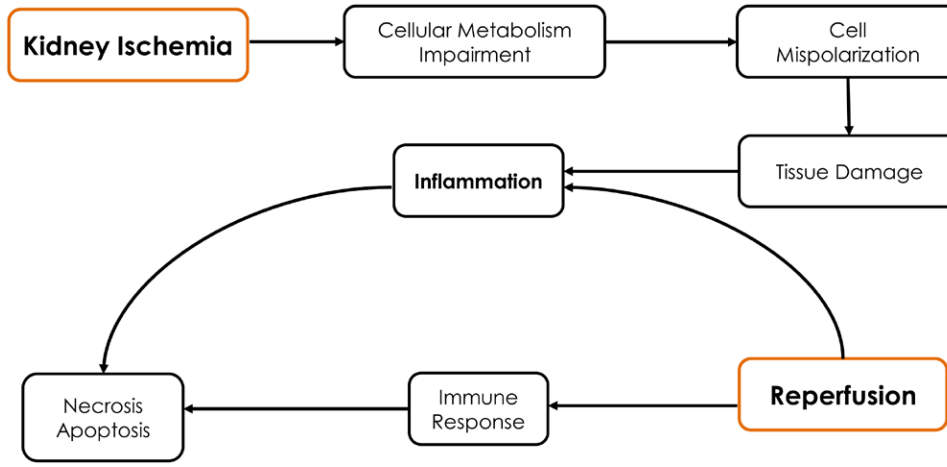

FIGURE 2. Schematic overview of the pathophysiological consequences of I/R. Ischemia: Imbalance in the cell's energy requirements (metabolic alteration/cellular disruption/loss of polarization). Reperfusion: Release of cytotoxic free radicals/pro-inflammatory cytokines. This cascade leads to cell death by apoptosis or necrosis. I/R, ischemia/reperfusion.

neutrophils, and dendritic cells. These different processes are linked to the recruitment of T lymphocytes and the adaptive immune response, the latter occurring few days after reperfusion.[5,28,29]

Renal IPC is a developing concept that encompasses maneuvers aimed at preventing or reducing the severity of renal I/R injury. The classical IPC principle is to mechanically expose the organ to brief waves of ischemia, of the order of 3–5 min in duration, before conservation in extended ischemia takes place to limit the ischemic damage and hasten the functional recovery.[30,31] Such an IPC stimulus is effective when delivered directly to the organ in question (local IPC) or to a tissue placed at distance away from the organ of interest (remote IPC).[32,33] In vivo, the renal IPC makes it possible to maintain the organization of the actin network of the cytoskeleton and the cell polarity, thereby preserving the polarized distribution of ion transporters, such as the basolateral Na^+/K^+-Adenosine triphosphatase. Tissue leukocyte infiltration, as well as the degree of apoptosis and necrosis, decreases substantially when IPC is carried out beforehand.[34]

The current therapeutic strategies are directed to interact with the major I/R signaling pathways, such as inflammation, vascularization, energy metabolism, or oxygen transport.[4,35] They are based on the supplementation of preservation solutions conventionally used during the KTx process. Based on the identification of cellular targets of major pathways in renal IPC, multiple approaches have been speculated to be capable of replicating these protective processes with the modulation of those circuits (Table 1). Of note, "post-conditioning" are also under development to accelerate tissue regeneration, are among the other transplant-related therapeutic breakthroughs.[53-55]

DUSP3 IS A MODULATOR OF THE INFLAMMATORY RESPONSE

DUSP3 is a small protein of 185 amino acids encoded by *DUSP3* gene located on chromosome 17q21 in humans and on chromosome 11 in mice.[56] It is part of the DUSP family proteins and are so named for their ability to dephosphorylate both threonine/serine and tyrosine residues of their substrates.[7] Unlike the other DUSPs, DUSP3 preferentially dephosphorylates p-Tyrosine over p-Threonine/p-Serine residue.[57] The various biological roles attributed to DUSP3 include its ability to control the cell cycle/cell proliferation and to modulate the immune response. Hence, DUSP3 has been particularly studied in cancers and autoimmune diseases.

Dusp3$^{-/-}$ systemic knockout mice have been generated.[10] Interestingly, no pathologic abnormalities were detected in these animals at baseline.[10] However, when these animals or cells derived from *Dusp3*-Knock-out mice are exposed to various stimuli or stressors, the functional absence of DUSP3 leads to specific phenotypes (Table 2). More particularly, *Dusp3*$^{-/-}$ mice are resistant to lipopolysaccharide-induced endotoxin shock and polymicrobial septic shock. Such a protection has been associated with privileged differentiation of macrophages toward the anti-inflammatory M2 type, as well as to decreased TNF production and impaired ERK1/2 activation.[9] Resistance to sepsis was associated with decreased activity of ERK1/2, phosphoinositide 3-kinase, and protein kinase B.[11] This finding suggests that DUSP3 controls macrophage polarization by regulating TNF production and ERK activity not only in sterile inflammatory models but also in a polymicrobial model of sepsis.

Another study has elucidated the importance of DUSP3 in platelet aggregation.[59] Indeed, this phosphatase seems to be implicated in platelet signaling through collagen receptor glycoprotein VI and C-type lectin-like type II receptors

TABLE 1.

Renal ischemic preconditioning approaches in rodents

Preconditioning approach	Pathways	Cellular modulation	References
Cyclosporine; FK-506	Inflammation	MAPK	4,36-38
Adenosine; MSC		GPCR	
HIF-1/2 genetic invalidation/pharmacological inhibition	Hypoxia	HIF-1/-2	39,40
L-carnitine; N-acetylcysteine	Oxidative stress	Free radicals	41-43
Lithium; SSAT		NOS	
Pharmacological activation	Metabolism	AMPK	44-46
AICAR; metformin; hemin		Hmox1	
X-ray kidney-centered irradiation	Angiogenesis	VEGF, PECAM1	47
	Oxidative stress	Hmox1, HSP70	
Opioids treatment	Hypoxia	HIF-1	48
	Angiogenesis	VEGF, VEGF-R2, IL-6	
Melatonin treatment	Inflammation	iNOS, GPx, SOD	49
	Oxidative stress		
Cilastatin treatment	Hypoxia	HIF-1, AKT/mTOR, PHD/VHL	50
Dexmedetomidine treatment	Inflammation	NF-κB, TNF-α	51
SHP-1 genetic inactivation	Apoptosis	ASK1, MKK4, JNK	52
	Inflammation		

AICAR, 5-Aminoimidazole-4-carboxamide ribonucleotide; AKT, protein kinase B; AMPK, AMP-activated protein kinase; ASK1, apoptosis signal-regulating kinase 1; FK-506, Tacrolimus; GPCR, G protein-coupled receptor; GPx, glutathione peroxidase; HIF, hypoxia-inducible factor; Hmox1, heme oxygenase 1; HSP 70, heat shock protein 70; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; JNK, c-Jun NH2-terminal kinase; MAPK, mitogen-activated protein kinase; MKK4, mitogen-activated protein kinase kinase 4; MSCs, mesenchymal stromal cells; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; NOS, nitric oxide synthase; PECAM1, platelet and endothelial cell adhesion molecule 1; PHD, prolyl hydroxylase domain; SHP-1, Tyrosine phosphatase 1; SOD, superoxide dismutase; SSAT, spermidine/spermine N^1-acetyltransferase; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau.

TABLE 2.

Experimental models targeting DUSP3

Experimental model	DUSP3 modulation	Experimental setting	Outcomes and pathways	Phenotype	References
CLP-induced sepsis/LPS-induced endotoxin shock	Genetic deletion	In vivo: *Dusp3*$^{-/-}$ mice In vitro: *Dusp3*$^{-/-}$ peritoneal macrophages	Inflammation (TNF, IL-6) Death rate Adapters: ERK1/2	↓ ↓ ↓	9
CLP-induced sepsis/LPS-induced endotoxin shock	Genetic deletion	In vivo: *Dusp3*$^{-/-}$ mice	ERK1/2, PI3K Protein kinase B activation	↓ ↓	11
LLC-experimental metastasis model	Genetic deletion	In vivo: *Dusp3*$^{-/-}$ mice In vitro: *Dusp3*$^{-/-}$ peritoneal macrophages	Metastasis growth Macrophages (CD11b) Angiogenesis (Ki67)	↑ ↑ ↑	58
Pulmonary thromboembolism	Pharmacological inhibition/deficiency	In vivo: *Dusp3*$^{-/-}$ mice Ex vivo: human platelets	Function Platelet activation (GPVI, CLEC-2) Adapters: Syk, PLCγ2	↑ ↓ ↑	59
Nonalcoholic fatty liver disease	Genetic deletion	In vivo: *Dusp3*$^{-/-}$ mice	Function: ALT, AST Obesity Fibrosis	↓ ↑ ↓	60
AMI-induced LAD	Gene silencing	In vivo: shDUSP3 mice In vitro: shDUSP3 primary neonatal mouse cardiomyocytes	Function (LVEF%, LVFS%, SOD1, TTC) Apoptosis (tunnel) Inflammation (IL-1β, TNF-α, IL-6, NF-κB)	↑ ↓ ↓	61
I/R-induced AKI	Genetic deletion	In vivo: *Dusp3*$^{-/-}$ mice, *Dusp3*$^{-/-}$ kidneys	Function (GFR, BUN, SCr) Inflammation (F4/80, JNK) Metabolism (mTOR ALDO B, PCK1) Angiogenesis (VEGF, BASP1, DLG1)	↑ ↓ ↑ ↑	12

↑, significant functional and morphological improvement; ↓, significant functional and morphological degradation; AKI, acute kidney injury; ALDO B, Aldolase B; ALT, alanine transaminase; AMI, acute myocardial infarction; AST, aspartate transaminase; BASP1, Brain acid soluble protein 1; BUN, blood urea nitrogen; CLEC-2, C-type lectin-like receptor 2; CLP, cecal ligation and puncture; DLG1, Discs large homolog 1; DUSP3, dual-specificity phosphatase 3; ERK, extracellular signal-regulated kinase; GFR, glomerular filtration rate; GPVI, collagen receptor glycoprotein VI; I/R, ischemia/reperfusion; IL, interleukin; JNK, c-Jun NH2-terminal kinase; Ki67, MKI67 cellular marker for proliferation; LAD, left anterior descending coronary artery; LLC, Lewis lung carcinoma; LPS, lipopolysaccharide; LVEF, Left ventricular ejection fraction; LVFS, Left ventricular fractional shortening; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; PCK1, Phosphoenolpyruvate carboxykinase 1; PI3K, phosphoinositide 3-kinase; PLCγ2, phospholipase Cγ2; SCr, serum creatinine; shDUSP3, Short hairpin DUSP3; SOD1, superoxide dismutase 1; Syk, Spleen Associated Tyrosine Kinase; TNF-α, tumor necrosis factor-α; TTC, triphenyl tetrazolium chloride; VEGF, vascular endothelial growth factor.

to promote reduction of Spleen Associated Tyrosine Kinase and phospholipase Cγ2 tyrosine phosphorylation, independently of the overall tyrosine phosphorylation (such as ERK1/2 and JNK). In the absence of DUSP3, thrombus formation was significantly impaired without significant bleeding, suggesting its role in arterial thrombosis and being dispensable for primary hemostasis. Ex vivo experiments further demonstrated that DUSP3 deficiency resulted in defective platelet aggregation, granule secretion, and αIIbβ3 integrin activation in response to collagen receptor glycoprotein VI and CLEC-2 receptor stimulation without affecting G protein-coupled receptor-mediated platelet activation.[59] Finally, a recent study using in vitro and in vivo approaches, suggested that the downregulation of DUSP3 alleviates the extent of acute myocardial infarction damage, namely via the pathways of apoptosis and inflammation. These beneficial effects were most likely mediated through the nuclear factor-kappa B signaling pathway.[61]

DUSP3 INHIBITION MAY REPRESENT A NOVEL STRATEGY IN RENAL ISCHEMIC PRECONDITIONING

As previously stated, there is a body of evidence suggesting that IPC helps to prime organs, including the kidney, to better support an ischemic insult. Because I/R is a type of sterile inflammation, we speculated that the benefits of DUSP3 deletion in reducing an abnormal inflammatory response in several disease models might also apply to I/R. First, we showed that DUSP3 distributes in podocytes and endothelial cells in mouse kidney. Following I/R, the messenger ribonucleic acid and protein levels of renal DUSP3 increased, and immunohistochemical examination revealed that DUSP3 colocalized with markers of proximal tubule cells in addition to podocytes and endothelial cells. *Dusp3*$^{-/-}$ mice and their C57BL/6 male wild-type controls were then subjected to an ischemic episode, with right nephrectomy followed by left 30-min ischemia with 48 min of reperfusion. *Dusp3*$^{-/-}$ mice were significantly more resistant to I/R than controls. Glomerular filtration rate was preserved, and albuminuria was reduced by half compared with wild-type mice exposed to a similar I/R episode. The renal injury in *Dusp3*$^{-/-}$ mice was attenuated, with (1) urinary alpha-1-microglobulin levels comparable to pre-I/R levels, (2) preserved serum levels of blood urea nitrogen, (3) less severe histological Jablonski scores of I/R, and (4) renal abundance of *Kim-1* Messenger ribonucleic acid levels 18-fold lower compared with the levels in wild-type kidneys post-I/R.[12]

There is evidence that, similar to septic shock, DUSP3 deletion in I/R resulted in the modulation of the innate

immune system, with diminished expression of inflammatory markers (IL-1ß, CD11b, IL-6, and TNF) and reduced renal infiltration of F4/80-positive macrophages. Previous work using the same *Dusp3*$^{-/-}$ mouse model reported that DUSP3 plays an important role in macrophage activation and in the innate immune response[58,60,62] by mechanisms involving the control of ERK1/2 activation as well as TNF secretion. The functional absence of DUSP3 seems to be protective by a macrophage-dependent pathway, as suggested by a higher percentage of M2-like macrophages in a sterile inflammation in *Dusp3*$^{-/-}$ mice.[10,11] Several articles show that the induction of M2-like macrophages is associated with preserved renal function.[63,64] In addition, as widely reported, the innate immune system is an evolutionary conserved first line of defence and is capable of acting rapidly upon infection or deleterious environment with a rapid increase in inflammation mediators, like IL-6 and TNF-α, leading inevitably to a worsened renal function after I/R.[65,66] Nevertheless, the identification of yet unknown substrates of this atypical DUSP would greatly facilitate the elucidation of the biological responses and signaling pathways involved in I/R and in DUSP-3-associated IPC.[67]

To explore the pathways putatively involved in the IPC observed in *Dusp3*$^{-/-}$ mice, comparative phosphoproteomics between *Dusp3*$^{-/-}$ and Wild type (WT) ischemic kidneys was performed. DUSP3 deletion was significantly associated with an increased phosphorylation of various transporters (Sodium glucose linked transporter 2, Glucose transporters 2), kinases (The mammalian target of rapamycin, AMP-activated protein kinase, Protein Kinase C), and enzymes (Phosphoenolpyruvate carboxykinase 1, Phosphoenolpyruvate carboxykinase, Fructose-1,6-bisphosphatase 1) classically involved in gluconeogenesis, together with a preserved kidney function, suggesting that increased tubular functions in *Dusp3*$^{-/-}$ versus WT mice post-I/R may occur upon inhibition of DUSP3-induced dephosphorylation. In addition, a significant downregulation of signal transducer and activator of transcription 3 and mitogen-activated protein kinase kinase kinase kinase 4 post-I/R, which have been reported as inducers of the c-Jun signaling pathway through activating the mitogen-activated protein kinase kinase kinase-mitogen-activated protein kinase kinase cascade, was highlighted in the phosphoproteomics assay. The phosphorylation status of c-Jun was confirmed to be lower in *Dusp3*$^{-/-}$ versus WT ischemic kidneys.[12] c-Jun has been previously involved in AKI induction post-I/R.[68] JNK inhibition has also been shown to provide significant protection against aristolochic acid-induced AKI.[69] The role of DUSP3 in AKI post-I/R could also be related to its role in platelets. Platelet activation is important in AKI[70] and anti-platelet drugs have been recently proposed as preventive treatment against AKI.[71] In-depth investigations are required to explore the role of platelet-located DUSP3 in renal IPC.

Interestingly enough, a recent study about healthy human kidneys showed by single nuclei-sequencing that DUSP3 is present in multiple cell types, including podocytes.[72] Single-cell RNA-sequencing experiments in a patient with acute kidney rejection showed high DUSP3 expression levels in proximal tubule and monocytes.[73] These preliminary translational observations mimic the data collected in murine kidney disease models.

CONCLUSIONS AND PERSPECTIVES

Prevention of I/R at the time of KTx is an important clinical challenge, with no satisfactory strategies thus far. Pharmaceutical approaches and cell-based therapies have been tested.[13,47,74,75] The present review includes a summary of the general properties of DUSP3, with a particular emphasis on the DUSP3-mediated impact on the immune system and the ischemic conditioning strategy. How can we use DUSP3's involvement in the context of KTx to reduce I/R-induced damage? Would there be a benefit in priming donor kidneys with DUSP3 inhibitors before transplant? Machine perfusion is increasingly used at the time of organ preservation, including marginal kidney grafts, to minimize I/R injury and accelerate organ repair.[76,77] Such a technique may facilitate the continuous administration of DUSP3 inhibitors to the kidney. Are there any protective effects of DUSP3 blockade in the early aftermath of I/R? Would DUSP3 deletion also protect against hepatic or cardiac damage after I/R? Further studies are needed to explore these questions. Based on our recent observations that the loss of DUSP3 activity protects against kidney injury post-I/R, one may expect that the use of a small molecule inhibitor of DUSP3 would be able to recapitulate the nephroprotective phenotype observed in *Dusp3*$^{-/-}$ mice. Several groups have reported the development and use of DUSP3 inhibitors[59,78,79] ex vivo in murine models and on human platelets, showing the specificity of these compounds. Therefore, a specific DUSP3 inhibitory strategy seems technically feasible and could be promising in the quest against I/R-induced AKI.

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