



# Ischemia-reperfusion injury in renal transplantation: 3 key signaling pathways in tubular epithelial cells

OPEN

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Renal ischemia-reperfusion injury (IRI) is a significant clinical challenge faced by clinicians perioperatively in kidney transplantation. Recent work has demonstrated the key importance of transmembrane receptors in the injured tubular epithelial cell, most notably Toll-like receptors, activated by exogenous and endogenous ligands in response to external and internal stresses. Through sequential protein-protein interactions, the signal is relayed deep into the core physiological machinery of the cell, having numerous effects from upregulation of pro-inflammatory gene products through to modulating mitochondrial respiration. Inter-pathway cross talk facilitates a co-ordinated response at an individual cellular level, as well as modulating the surrounding tissue's microenvironment through close interactions with the endothelium and circulating leukocytes. Defining the underlying cellular cascades involved in IRI will assist the identification of novel interventional targets to attenuate IRI with the potential to improve transplantation outcomes. We present a focused review of 3 key cellular signalling pathways in the injured tubular epithelial cell that have been the focus of much research over the past 2 decades: toll-like receptors, sphingosine-1-phosphate receptors and hypoxia inducible factors. We provide a unique perspective on the potential clinical translations of this recent work in the transplant setting. This is particularly timely with the recent completion of phase I and ongoing phase 2 clinical trials of inhibitors targeting specific components of these signaling cascades.

*Kidney International* (2019) **95**, 50–56; <https://doi.org/10.1016/j.kint.2018.10.009>

KEYWORDS: ischemia-reperfusion injury; tubular epithelial cells

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Received 1 February 2018; revised 25 September 2018; accepted 2 October 2018

Treatment options for end-stage renal disease include kidney transplantation and dialysis. However, a significant gap exists between the demand and the supply of brainstem-dead donor organs for transplantation; at the time of writing, 95,003 patients in the United States<sup>1</sup> and 4803 patients in the United Kingdom<sup>2</sup> are awaiting kidney transplantation. Significant efforts are being made to bridge this gap and reduce waiting lists. In the United Kingdom, a rise in organ donation after circulatory death and use of expanded-criteria organs has contributed to rising rates of organ donation and transplantation.<sup>3</sup>

Ischemia-reperfusion injury (IRI) might be considered the transplant surgeon's enemy and is a particular threat during transplantation of kidneys donated after circulatory death because of their long exposure to warm ischemia.<sup>4</sup> IRI leads to loss of tubular epithelial cell (TEC) function, contributing to the development of acute kidney injury, delayed graft function, and acute and chronic organ rejection.<sup>5</sup> Research during the past decade has vastly improved our understanding of the myriad interconnected molecular pathways that contribute to IRI and has identified potential interventional pharmacologic targets and prognostic biomarkers.<sup>6</sup> Translation of these bench-side advances to novel therapeutics ultimately may lead to improved graft function and clinical outcomes for kidney transplant recipients.

The molecular and cellular events that occur in IRI are complex, involving oxidative damage and the activation of the innate immune system.<sup>7</sup> Signaling components of particular relevance to IRI in TECs are the pathways used by Toll-like receptors (TLRs),<sup>8</sup> sphingosine-1-phosphate (S1P) receptors,<sup>9</sup> and hypoxia inducible factors (HIFs).<sup>10</sup> From a physiological perspective, this inherent pathway complexity *in vivo* has numerous advantages, enabling integration of molecular messaging, specificity of cellular response, and response amplification. In the context of IRI, in addition to influencing the individual injured TEC, these pro-inflammatory cascades have diverse modulating effects on the surrounding microenvironment through upregulation of chemokines and cytokines, promoting recruitment of leukocytes and triggering dilation of the vasculature. As such, the TECs can be considered to be both the victims and the perpetrators of IRI. TEC signaling pathways have potential for significant intra- and intercellular influence, making them powerful targets in ameliorating IRI.

### Toll-like receptors

TLRs, a class of receptors that form part of the innate immune cellular armory, belong to the interleukin (IL)-1 receptor family.<sup>11</sup> They recognize pathogen-associated molecular patterns, conserved structural motifs. The TLR-activated cellular pathways result in production of proinflammatory cytokines and chemokines. The human TLR family comprises 10 members, TLR1 to TLR10.<sup>12</sup> Multiple studies suggest that ischemia upregulates TLR2 and TLR4 expression in TECs,<sup>13–17</sup> as determined by immunohistochemical and reverse transcriptase–polymerase chain reaction techniques across various IRI models *in vitro* and *in vivo*. In a murine genetic knockout model, TLR2 and TLR4 have shown equal importance in initiating apoptosis in renal IRI.<sup>15</sup> Ischemia-driven TLR expression is fine-tuned by the action of cytokines such as interferon- $\gamma$  and tumor necrosis factor- $\alpha$ ,<sup>16</sup> as well as the enzyme spermidine/spermine N1-acetyltransferase that is upregulated in IRI.<sup>17</sup>

TLR2 and TLR4 display multivalent ligand-binding activity, because they are activated by exogenous ligands (e.g., lipopolysaccharide [LPS]) and endogenous ligands such as heat-shock proteins, nonhistone chromatin-binding protein high-mobility group box 1, and extracellular matrix components (hyaluronan, fibronectin, heparan sulfate, and biglycan).<sup>13</sup> For instance, Allam *et al.*<sup>18</sup> demonstrated that direct injection of histones into the renal arteries of mice induced leukocyte recruitment, microvascular vascular leakage, renal inflammation, and structural features of acute kidney injury in a TLR2/TLR4-dependent manner and was suppressed by antihistone IgG.<sup>18</sup>

Another layer of complexity to the receptor-ligand interaction is afforded by the presence of a small number of cytosolic adaptor proteins that bridge TLRs and facilitate downstream signal transduction. Adaptor proteins relevant to TLR4 signaling include myeloid differentiation primary response 88 (MyD88), TIR-domain-containing adapter-inducing interferon- $\beta$ , transmembrane adaptor protein, and TRIF-related adaptor molecule; adaptor proteins relevant to TLR2-mediated signaling include transmembrane adaptor protein and MyD88.<sup>19</sup> Of these, MyD88 has proven to be a fruitful target for amelioration of IRI in murine models among pharmacologic and genetic knockout studies to date. Inhibition of MyD88 with small molecular compound TJ-M2010-2 resulted in amelioration of IRI and inhibition of transforming growth factor- $\beta$ 1-induced epithelial-mesenchymal transition of renal TECs.<sup>20</sup> Li *et al.*<sup>21</sup> demonstrated that hypoxia resulted in significant increases in cytokine production and apoptosis/necrosis in wild-type proximal tubular epithelial cells, but these responses were significantly blunted in MyD88(-/-) proximal tubular epithelial cells.<sup>21</sup>

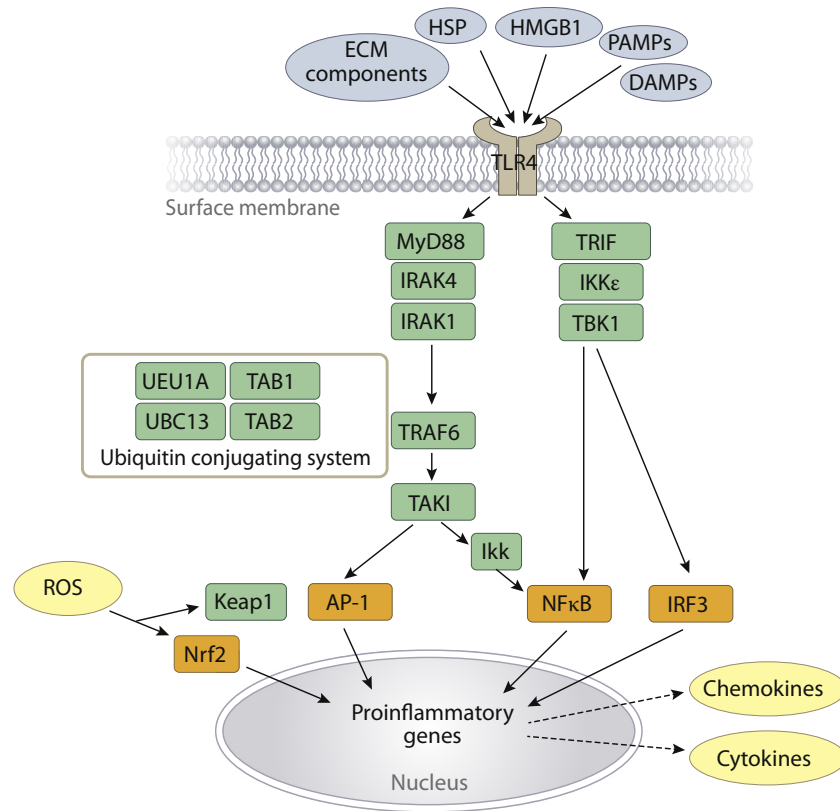
TLR-mediated signal transduction activates proinflammatory transcription factors. The cascade of events is detailed in Figure 1; the MyD88-dependent pathway commences with the recruitment of IL-1 receptor-associated kinase 4, ultimately activating I $\kappa$ B kinase (inhibitor of nuclear factor- $\kappa$ B [NF- $\kappa$ B] kinase complex) and also activation of the

mitogen-activated protein kinase pathway, which causes activation of transcription factors NF- $\kappa$ B and activator protein 1, respectively.<sup>22</sup> It is termed “early phase” NF- $\kappa$ B activation. It is thought that ligand binding can confer specificity in the signaling pathway; for example, biglycan binding will activate p38, whereas heat shock protein 60 activates p38, c-Jun N-terminal kinases 1 and 2, and I $\kappa$ B kinase. The MyD-88 independent pathway promotes TIR-domain-containing adapter-inducing interferon- $\beta$  activation and ultimately results in activation of the transcription factors NF- $\kappa$ B and interferon regulatory factor 3.<sup>12</sup> In both pathways, the transcription factors NF- $\kappa$ B and activator protein 1 contribute to upregulation of proinflammatory products including cytokines and chemokines. Therefore, whereas pharmacologic ligand design may provide the potential to inhibit certain IRI pathways with some degree of specificity at the point of MyD88 divergence, there appears to be pathway convergence at the nuclear level at transcription.

From a more global perspective of cellular signaling, it is important to note the interaction between the TLR and complement pathways in IRI, which occur through at least 2 mechanisms. First, both systems share common activating ligands (e.g., LPS). Second, emerging evidence shows that the TLR-activated pathways interact with the complement system through mitogen-activated protein kinases.<sup>23</sup> However, at present, no studies have been performed specifically in renal TECs to investigate the cross talk between TLR and complement system activation. Zhang *et al.*<sup>24</sup> postulated that the “missing link” between the 2 systems is MyD88. They demonstrated that when decay accelerating factor-deficient mice were treated with the TLR4 agonist LPS, the mice showed increased IL-6, tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , and IL-10 levels. They had lower levels of IL-12 compared with the wild-type control subjects. In dual decay accelerating factor and C3 knockout mice, LPS-induced cytokine release was absent. In dual decay accelerating factor and TLR4 knockout mice, LPS-induced cytokine release also was absent.<sup>24</sup> Therefore one can anticipate that an inhibitor designed to share the multivalency of LPS would possess the ability to dampen IRI through multiple synergistic pathways.

The complement inhibitor eculizumab, a monoclonal antibody blocking C5, is currently undergoing clinical trials in renal transplantation.<sup>25</sup> Thus far, these trials mostly have been focused on its beneficial effect in ameliorating antibody-mediated allograft injury; however, an ongoing trial in Russia (NCT01756508) is specifically investigating the use of eculizumab for prevention and treatment of reperfusion injury in kidney transplantation. Results are anticipated upon completion of the trial in May 2019.

In summary, potential targets for pharmacologic antagonism in the TLR pathway exist at the level of the transmembrane receptor, cytosolic adaptor proteins, downstream messengers, and transcription factors. Whereas much of our present understanding of the signaling pathway comes from murine models, we are starting to see research making the



**Figure 1 | Toll-like receptor 4 (TLR4) is activated by different ligands (extracellular matrix [ECM] components, heat shock proteins [HSPs], nonhistone chromatin-binding protein high-mobility group box 1 [HMGB1], pathogen-associated molecular pattern molecules [PAMPs], and damage-associated molecular pattern molecules [DAMPs]).** There are 2 main pathways, as illustrated in the diagram. The myeloid differentiation primary response 88 (myD88)-dependent pathway involves recruitment of interleukin-1 receptor-associated kinase 4 (IRAK4) and IRAK1 activation, with myD88 acting as a cytosolic adapter protein. Activation of transforming growth factor  $\beta$ -activated kinase 1 (TAK1) is dependent on tumor necrosis factor receptor-associated factor 6 (TRAF6) interacting with the ubiquitin conjugating (UBC) system. This interaction results in upregulation of activator protein 1 (AP-1) and nuclear factor- $\kappa$ B (NF- $\kappa$ B) and hence upregulation of proinflammatory genes. The alternate pathway involves activation of TANK-binding kinase 1 (TBK1) and I $\kappa$ B kinase epsilon (IKK $\epsilon$ ), with TIR-domain-containing adapter-inducing interferon- $\beta$  (TRIF) acting as an adapter protein. This process results in activation of interferon regulatory factor 3 (IRF3), as well as NF- $\kappa$ B, resulting in upregulation of proinflammatory genes. Of note, there is convergence at this level with pathways activated by reactive oxygen species (ROS), which activate NF-E2 p45-related factor 2 (Nrf2) and Kelch-like ECH-associated protein (Keap1). TAB, TAK1-binding protein.

leap to clinical trials. Of note, anti-TLR2 antibodies have shown some promise in mice and in humans.

Farrar *et al.*<sup>26</sup> demonstrated that antibody-mediated inhibition of TLR2 promoted graft function in an isograft murine model of renal transplantation whereby recipient mice were treated with the antibody prior to reperfusion of the transplanted kidney (which had been exposed to 30 minutes of cold ischemia).

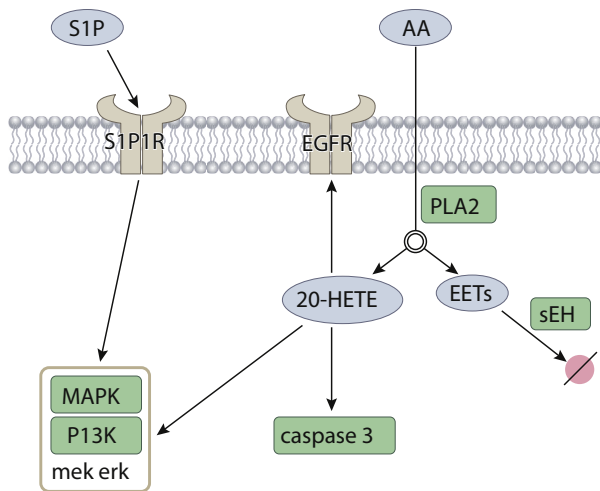
**Clinical trials relating to TLR signaling pathways.** The humanized anti-TLR2 antibody called OPN-305 is currently undergoing clinical trials for delayed graft function in kidney transplantation. An initial phase 1 study performed in 2013 yielded preliminary safety data, and dosing of 0.5 mg/kg given as an infusion over 1 hour fully inhibited the TLR2 and demonstrated an 80% reduction of IL-6 release, with effects for up to 2 weeks.<sup>27</sup>

Arguably, in the context of our review, one limitation of these studies is that OPN-305 does not act specifically on TEC cells; beneficial effects in this context also are likely mediated

by cross-reactivity with circulating leukocytes. Although it is a matter of debate as to whether the antibody indeed passes through the glomerular filtration barrier *in vivo* in a state of physiological normality, in the context of kidney injury, it seems likely that this would occur. After the success of the phase 1 trial, OPN-305 progressed to phase 2 studies in kidney transplant patients; it was administered as an IV infusion for 1 hour at the start of the transplant procedure.<sup>28</sup> The highly anticipated results of this recently completed phase 2 trial (NCT01794663) will arguably provide the most clinically relevant evidence yet on the potential of TLR inhibitors in the field of renal transplantation.

### Sphingosine-1-phosphate receptors

It is believed by some Chinese and Tibetan herbalists that tonic infused with *Isaria sinclairii* is an anti-aging elixir. Although the validity of these claims is beyond the purview of this review, this unusual fungus, native to Asia, produces a substance called myriocin that has been extensively studied



**Figure 2 | During inflammation, sphingosine-1-phosphate (S1P) released by platelets, endothelial cells, and leukocytes results in activation of S1P1 receptors that stimulate the mitogen-activated protein kinase (MAPK) (extracellular signal-regulated kinase [erk]) and P13K (Akt) pathways in tubular epithelial cells.** Convergence occurs at this level with pathways stimulated during inflammation by the production of cytochrome P450-dependent eicosanoids. Arachidonic acid (AA) is converted by phospholipase A2 (PLA2) and cytochrome p450 enzymes to produce 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs). Soluble epoxide hydrolase (sEH) metabolizes EETs to inactive dihydroxyeicosatrienoic acids (DHETs). 20-HETE increases Akt phosphorylation and promotes activation of the epidermal growth factor receptor (EGFR). Overexpression of 20-HETE has been associated with cellular damage mediated by caspase-3 activation. mek, mitogen-activated protein kinase kinase.

and has rejuvenated our understanding of S1P signaling pathways in IRI. S1P is a phospholipid that participates in signaling pathways mediated by G-protein-coupled receptors relevant to IRI, as illustrated in Figure 2. S1P is released during inflammation in ischemia by platelets, endothelial cells, and leukocytes.<sup>29</sup> G-protein-coupled receptors present on renal proximal TECs include S1P<sub>1</sub>R and S1P<sub>2</sub>R; our understanding of downstream signaling from these receptors is still evolving. The study of fungal metabolite sphingosine-like immunosuppressant 1 led to the derivation of a small molecule called fingolimod (FTY720), a nonselective S1PR agonist. Fingolimod has been used in *in vitro* models and also has been featured in clinical trials for a range of different conditions that implicate IRI in their etiology, including acute ischemic stroke and, most recently, in kidney transplant recipients.

Bajwa *et al.*<sup>30</sup> studied S1P in proximal TECs. In a 2010 study, they investigated the effects of S1PR agonists on kidney IRI on lymphopenic mice (Rag1 knockouts). These investigators found that administering FTY720 or selective S1P<sub>1</sub>R agonist SEW2871 reduced IRI in the Rag1 knockout and the wild-type mice. Interestingly, they demonstrated that the S1P<sub>1</sub>R agonist SEW2871 increased activation of the mitogen-activated protein kinase (extracellular signal-regulated kinase) or P13K (Akt) pathways in proximal TECs.<sup>30</sup> In a more recent study in 2015, they again used FTY720, which attenuated IRI. They also found that the agonist reduced the effects of cisplatin-induced AKI.

Using S1P<sub>1</sub> knockout mice, these investigators demonstrated that FTY720 was achieving the effects via the S1P<sub>1</sub> receptor. Cells overexpressing S1P<sub>1</sub> were resistant to the effects of cisplatin. Bajwa *et al.*<sup>30</sup> also presented evidence to suggest that S1P is important in mitochondrial structure and function; they noted that in S1P<sub>1</sub>R overexpressing cells, the mitochondria were longer and thinner, with high rates of basal respiration. However, as they discussed, it is unclear if the protective effect of S1P on mitochondria is mediated through cellular cascades via a cell surface S1P<sub>1</sub> receptor or through a direct effect of S1P on mitochondria.<sup>31</sup> In a rat model of transplantation following 24 hours of cold ischemia, FTY720 has been shown to reduce apoptosis and increase cellular proliferation in the tubular epithelium and can be considered to increase tubular epithelial protection in the presence of severe IRI.<sup>32</sup>

S1P<sub>2</sub>R is thought to mediate proapoptotic, proinflammatory effects.<sup>29</sup> Ishizawa *et al.*<sup>33</sup> demonstrated that in rat TECs, S1P<sub>2</sub>R stimulation activates Rho kinase, resulting in changes to E-cadherin distribution and increased  $\alpha$ -smooth muscle actin expression.<sup>33</sup>

The S1P pathway does not act in isolation; evidence suggests cross talk between S1P and other signaling pathways. For instance, the S1P pathway is influenced by adenosine signaling in IRI. Park *et al.*<sup>34</sup> found that a selective adenosine A1 receptor agonist (2-chloro-N(6)-cyclopentyladenosine) increased S1P synthesis and selectively induced sphingosine kinase 1 in mouse kidney and human kidney-2 cells. Knockout mice deficient in S1P<sub>1</sub>Rs were not protected against renal IRI by 2-chloro-N(6)-cyclopentyladenosine. 2-Chloro-N(6)-cyclopentyladenosine increased the translocation of HIF-1 $\alpha$  in HK-2 cells, and selective HIF-1 $\alpha$  inhibition blocked the A1R-mediated sphingosine kinase 1 induction.<sup>34</sup>

**Clinical trials related to S1P receptors.** Fingolimod (FTY720) reached clinical trials for kidney transplant recipients, with 10 registered complete clinical trials existing on the U.S. database; the last trial was completed in 2006. Unfortunately, fingolimod has not been as successful as anticipated. In clinical trials CFTY720A124 and CFTY720A125, it was associated with decreased graft function, respiratory adverse effects, and increased rates of macular edema compared with mycophenolate mofetil with cyclosporin. As a result, Novartis discontinued its clinical trials for fingolimod in renal transplantation.

### Hypoxia-inducible factors

HIF factors (subtypes HIF-1 and HIF-2) are transcription factors that regulate the expression of numerous transcription targets important in renal function. They are heterodimeric molecules, each composed of an oxygen-sensing  $\alpha$  subunit and a constitutively expressed  $\beta$  subunit.<sup>35</sup> HIFs are stabilized during hypoxia and promote adaptation to a low availability of oxygen. They therefore have an important role in ischemia-reperfusion signaling pathways.

Characterizing HIF-1 and HIF-2 regulatory pathways is relevant to understanding IRI in TECs. Key regulators of HIF include reactive oxygen species (from various sources) and von Hippel-Lindau and prolyl hydroxylase domain (PHD)

proteins. Ubiquitination and degradation plays an important role in regulation of HIF. von Hippel–Lindau proteins and HIF-prolyl hydroxylase domain enzymes 1-3 (PHD1, 2, and 3) coordinate these processes. During normoxia, HIF binds to von Hippel–Lindau proteins and PHD enzymes. This complex then recruits ubiquitin, which results in degradation. During hypoxia, the prolyl hydroxylases are inactivated, resulting in termination of degradation. HIF accumulates and is stabilized.<sup>36</sup>

Several studies suggest an initial protective role of HIF-1 $\alpha$ /HIF-1 during kidney IRI.<sup>37–40</sup> Zhang *et al.*<sup>41</sup> demonstrated that HIF-1 $\alpha$  expression was significantly increased after 60 minutes of hypoxia in a porcine model during simulated partial nephrectomy with warm ischemia. Furthermore, Oda *et al.*<sup>42</sup> demonstrated that upregulation of HIF-1 $\alpha$  after reperfusion may be a predictor of early recovery of graft function after cadaveric kidney transplantation. HIF-2 also has protectant functions. A Japanese group demonstrated the importance of HIF-2 $\alpha$  using a knockout murine model. Kojima *et al.*<sup>43</sup> found that HIF-2 $\alpha$  knockout mice were more susceptible to renal IRI (as assessed by blood urea nitrogen levels and semiquantitative histologic analysis). They demonstrated the importance of endothelial HIF-2 $\alpha$  in the restoration of HIF-2 $\alpha$  in the endothelium by intercrossing with Tie1-Cre mice, which ameliorated renal injury by IRI.<sup>43</sup>

HIF in the injured TEC has been specifically investigated in a number of studies.<sup>44–46</sup> Conde *et al.*<sup>47</sup> demonstrated that HIF-1 $\alpha$  was stabilized in proximal tubule cells during IRI; they observed stabilization of HIF-1 $\alpha$  in ischemia and also during normal oxygen tension in reperfusion. HIF has numerous transcriptional targets including vascular endothelial growth factor, erythropoietin, and heme oxygenase-1<sup>47</sup> and can be considered a gatekeeper to the hypoxia-driven cellular response. In addition to these direct transcriptional effects, evidence is emerging to suggest that HIF activation can modulate other signaling pathways. For instance, He *et al.*<sup>48</sup> demonstrated cross talk between the HIF and NF- $\kappa$ B pathways. In their study, LPS treatment led to HIF-2 $\alpha$  accumulation in endothelial cells in a mouse model of IRI due to NF- $\kappa$ B activation. Inactivation of HIF-2 $\alpha$  resulted in loss of LPS-mediated protection against IRI. LPS-mediated protection is thought to be related to inducible nitric oxide synthase and endothelial nitric oxide synthase.<sup>48</sup>

HIF-1 is implicated in chronic changes as well as the acute IRI response. Luo *et al.*<sup>49</sup> demonstrated that HIF-1 $\alpha$  upregulated  $\alpha$ -smooth muscle actin expression and reduced E-cadherin expression in an *in vitro* model of IRI. The microRNA miR-21 was positively correlated with HIF-1 $\alpha$ , suggesting that miR-21 may be a regulatory factor in the process by which HIF-1 $\alpha$  promotes epithelial to mesenchymal transition in IRI.<sup>49</sup>

PHD inhibitors have been studied therapeutically to protect against ischemia through their mechanism of HIF stabilization.<sup>50</sup> Wang *et al.*<sup>51</sup> demonstrate in a rat model of renal IRI that using PHD-1 inhibitor acetate as a preconditioning agent to stabilize HIF resulted in significantly improved

creatinine levels and renal structure. Bernardt *et al.*<sup>52</sup> used a specific PHD inhibitor called FD-4497 in a rat model of allogenic kidney transplantation. Donors received a single dose prior to nephrectomy, and it was found that donor preconditioning resulted in HIF accumulation. Acute renal injury (as measured by serum creatinine levels) and early mortality were reduced among the recipients after preconditioning with FD-4497.<sup>52</sup>

The role of reactive oxygen species in the regulation of HIF is complex. In addition to having a direct effect on HIF regulation, reactive oxygen species have indirect effects via modulation of intermediates including nitric oxide and microRNAs.<sup>53</sup> Other molecules interact with the HIF pathway, including Mucl and ID1. Pastor-Soler *et al.*<sup>54</sup> studied the role of a cell surface glycoprotein, MUC1, interacting with HIF during IRI in TECs. Mucl was induced during IRI (normally localized on the cell surface but appeared in the cytoplasm and nucleus of tubular epithelia during IRI). Mucl knockout mice exhibited more severe kidney damage compared with control subjects, and Mucl knockouts had reduced HIF-1 $\alpha$  levels.<sup>54</sup> Inhibitor of DNA binding 1 (ID1) is a transcription factor that functions to interact with basic helix-loop-helix transcription factors. Wen *et al.*<sup>55</sup> recently demonstrated that ID1 was upregulated during hypoxia and reoxygenation in an *in vitro* model of renal IRI, with expression localized primarily to the TECs. A relationship exists between ID1 and HIF-1 $\alpha$ , because silencing of the HIF-1 $\alpha$  gene resulted in reduction of ID1 mRNA. The functional significance of ID1 is still being appreciated; it has been previously shown in renal TECs that ID1 can drive cell de-differentiation through suppression of E-cadherin expression.<sup>56</sup>

Interestingly, some evidence shows that hyperoxia can have detrimental effects on the developing kidney. This effect is thought to be mediated through hyperoxia-induced degradation of HIF. Popescu *et al.*<sup>57</sup> demonstrated in a rodent model that HIF-1 $\alpha$  expression was reduced in developing kidneys after exposure to hyperoxia. Administration of the HIF-1 $\alpha$  stabilizer dimethylxalylglycine (a PHD inhibitor) resulted in increased HIF-1 $\alpha$  expression and improved nephrogenesis.<sup>57</sup>

**Clinical trials related to HIFs.** As yet, no HIF-1 $\alpha$  antagonists have entered clinical trials for renal transplantation. However, a locked nucleic acid antagonist called EZN-2968 is being investigated for its role in oncogenesis and potential as an antitumor drug, because HIF-1 is often overexpressed in malignant cells.<sup>58</sup> A number of PHD inhibitors also have been developed and are undergoing clinical trials for the treatment of anemia in persons with chronic kidney disease; it is possible these PHD inhibitors also have relevance and potential applications as ameliorators of IRI.<sup>59</sup>

## CONCLUSION

Signaling pathways are necessary for the detection and cellular reaction to IRI in TECs. Transmembrane receptors, notably TLRs and S1PRs, are of key importance; they are activated by

exogenous and endogenous ligands in response to external and internal stresses. Through sequential protein-protein interactions, the signal is relayed deep into the core physiological machinery of the cell and have numerous effects from upregulation of proinflammatory gene products to modulation of mitochondrial respiration. Interpathway cross talk facilitates a coordinated response at an individual cellular level and modulates the microenvironment of surrounding tissue through close interactions with the endothelium and circulating leukocytes.

Most of the evidence base surrounding renal IRI pathways to date has come from rodent models in which mRNA or protein expression has been examined in isolation. Few investigators have studied the impact of these interventions on transplantation, and therefore further research in these models is required to determine whether targeting theoretical IRI cascades leads to improved graft function. Future research will provide further insight into the potential of microRNA as a specific tool for cell signaling intervention. The work on TLR inhibitors has shown great promise, particularly the TLR2 inhibitors currently undergoing clinical trials in renal transplantation. However, as demonstrated by fingolimod, translating from scientific studies to clinical studies is not always straightforward, with unforeseen adverse effects occasionally emerging at a late stage in trials.

#### DISCLOSURE

All the authors declared no competing interests.

#### ACKNOWLEDGMENTS

The research was funded by the National Institute for Health Research Blood and Transplant Research Unit (NIHR BTRU) in Organ Donation and Transplantation at the University of Cambridge in collaboration with Newcastle University and in partnership with NHS Blood and Transplant (NHSBT). The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, the Department of Health, or NHSBT.

#### REFERENCES

- Organ Procurement and Transplantation Network, U.S. Department of Health and Human Services. National data. <https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#>. Accessed November 6, 2018.
- Blood and Transport, National Health Service. Statistics about organ donation. <https://www.organdonation.nhs.uk/supporting-my-decision/statistics-about-organ-donation/>. Accessed November 6, 2018.
- Nath J, Field M, Ebbs SR, et al. Evolution of renal transplant practice over the past decade: a U.K. Center experience. *Transplant Proc.* 2015;47(6):1700–1704.
- Salvadori M, Rosso G, Bertoni E. Update on ischemia-reperfusion injury in kidney transplantation: pathogenesis and treatment. *World J Transplant.* 2015;5(2):52–67.
- Saat TC, van den Akker EK, Uzermans JNM, et al. Improving the outcome of kidney transplantation by ameliorating renal ischemia reperfusion injury: lost in translation? *J Transl Med.* 2016;14:20.
- Ponticelli C. Ischaemia-reperfusion injury: a major protagonist in kidney transplantation. *Nephrol Dial Transplant.* 2014;29(6):1134–1140.
- Chouchani ET, Pell VR, James AM, et al. A unifying mechanism for mitochondrial superoxide production during ischemia-reperfusion injury. *Cell Metab.* 2016;23(2):254–263.
- Wu H, Chadban SJ. Roles of Toll-like receptors in transplantation. *Curr Opin Organ Transplant.* 2014;19(1):1–7.
- Koch A, Pfeilschifter J, Huwiler A. Sphingosine 1-phosphate in renal diseases. *Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol.* 2013;31(6):745–760.
- Akhtar MZ, Sutherland AI, Huang H, et al. The role of hypoxia-inducible factors in organ donation and transplantation: the current perspective and future opportunities. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg.* 2014;14(7):1481–1487.
- Zhao H, Perez JS, Lu K, et al. Role of Toll-like receptor-4 in renal graft ischemia-reperfusion injury. *Am J Physiol Renal Physiol.* 2014;306(8):F801–F811.
- Kawasaki T, Kawai T. Toll-Like receptor signaling pathways. *Front Immunol.* 2014;5:461.
- Wu H, Chen G, Wyburn KR, et al. TLR4 activation mediates kidney ischemia/reperfusion injury. *J Clin Invest.* 2007;117(10):2847–2859.
- Zhao H, Watts HR, Chong M, et al. Xenon treatment protects against cold ischemia associated delayed graft function and prolongs graft survival in rats. *Am J Transplant.* 2013;13(8):2006–2018.
- Rusai K, Sollinger D, Baumann M, et al. Toll-like receptors 2 and 4 in renal ischemia/reperfusion injury. *Pediatr Nephrol.* 2010;25(5):853–860.
- Wolfs TGAM, Buurman WA, van Schadewijk A, et al. In vivo expression of Toll-like receptor 2 and 4 by renal epithelial cells: IFN-gamma and TNF-alpha mediated up-regulation during inflammation. *J Immunol.* 2002;168(3):1286–1293.
- Zahedi K, Barone S, Wang Y, et al. Proximal tubule epithelial cell specific ablation of the spermidine/spermine N1-acetyltransferase gene reduces the severity of renal ischemia/reperfusion injury. *PLoS One.* 2014;9(11):e110161.
- Allam R, Scherbaum CR, Darisipudi MN, et al. Histones from dying renal cells aggravate kidney injury via TLR2 and TLR4. *J Am Soc Nephrol.* 2012;23(8):1375–1388.
- Kawai T, Akira S. Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med.* 2007;13(11):460–469.
- Zhang L-M, Liu J-H, Xue C-B, et al. Pharmacological inhibition of MyD88 homodimerization counteracts renal ischemia reperfusion-induced progressive renal injury in vivo and in vitro. *Sci Rep.* 2016;6:26954.
- Li M, Khan A-M, Maderdrut JL, et al. The effect of PACAP38 on MyD88-mediated signal transduction in ischemia/hypoxia-induced acute kidney injury. *Am J Nephrol.* 2010;32(6):522–532.
- Fujioka S, Niu J, Schmidt C, et al. NF-kB and AP-1 connection: mechanism of NF-kB-dependent regulation of AP-1 activity. *Mol Cell Biol.* 2004;24(17):7806–7819.
- Damman J, Daha MR, van Son WJ, et al. Crosstalk between complement and Toll-like receptor activation in relation to donor brain death and renal ischemia-reperfusion injury. *Am J Transplant.* 2011;11(4):660–669.
- Zhang X, Kimura Y, Fang C, et al. Regulation of Toll-like receptor-mediated inflammatory response by complement in vivo. *Blood.* 2007;110(1):228–236.
- Frémeaux-Bacchi V, Legendre CM. The emerging role of complement inhibitors in transplantation. *Kidney Int.* 2015;88(5):967–973.
- Farrar CA, Keogh B, McCormack W, et al. Inhibition of TLR2 promotes graft function in a murine model of renal transplant ischemia-reperfusion injury. *FASEB J.* 2012;26(2):799–807.
- Reilly M, Miller RM, Thomson MH, et al. Randomized, double-blind, placebo-controlled, dose-escalating phase I, healthy subjects study of intravenous OPN-305, a humanized Anti-TLR2 antibody. *Clin Pharmacol Ther.* 2013;94(5):593–600.
- Opsona Therapeutics. Placebo-controlled study to evaluate the safety and efficacy of OPN-305 in preventing delayed renal graft function. <https://www.clinicaltrials.gov/ct2/show/NCT01794663?term=opsona&rank=1>. Accessed November 6, 2018.
- Kusch A, Hoff U, Bubalo G, et al. Novel signalling mechanisms and targets in renal ischaemia and reperfusion injury. *Acta Physiol.* 2013;208(1):25–40.
- Bajwa A, Jo S-K, Ye H, et al. Activation of sphingosine-1-phosphate 1 receptor in the proximal tubule protects against ischemia-reperfusion injury. *J Am Soc Nephrol.* 2010;21(6):955–965.
- Bajwa A, Rosin DL, Chrosicki P, et al. Sphingosine 1-phosphate receptor-1 enhances mitochondrial function and reduces cisplatin-induced tubule injury. *J Am Soc Nephrol.* 2015;26(4):908–925.
- Fuller TF, Hoff U, Kong L, et al. Cytoprotective Actions of FTY720 modulate severe preservation reperfusion injury in rat renal transplants. *Transplantation.* 2010;89(4):402–408.
- Ishizawa S, Takahashi-Fujigasaki J, Kanazawa Y, et al. Sphingosine-1-phosphate induces differentiation of cultured renal tubular epithelial

- cells under Rho kinase activation via the S1P2 receptor. *Clin Exp Nephrol*. 2014;18(6):844–852.
34. Park SW, Kim M, Kim JY, et al. Proximal tubule sphingosine kinase-1 has a critical role in A1 adenosine receptor-mediated renal protection from ischemia. *Kidney Int*. 2012;82(8):878–891.
  35. Rabinowitz MH. Inhibition of hypoxia-inducible factor prolyl hydroxylase domain oxygen sensors: tricking the body into mounting orchestrated survival and repair responses. *J Med Chem*. 2013;56:9369–9402.
  36. Andringa KK, Agarwal A. Role of hypoxia-inducible factors in acute kidney injury. *Nephron Clin Pract*. 2014;127(1–4):70–74.
  37. Hill P, Shukla D, Tran MG, et al. Inhibition of hypoxia inducible factor hydroxylases protects against renal ischemia-reperfusion injury. *J Am Soc Nephrol*. 2008;19(1):39–46.
  38. Bernhardt WM, Campean V, Kany S, et al. Preconditional activation of hypoxia-inducible factors ameliorates ischemic acute renal failure. *J Am Soc Nephrol*. 2006;17(7):1970–1978.
  39. Ma D, Lim T, Xu J, et al. Xenon preconditioning protects against renal ischemic-reperfusion injury via HIF-1 $\alpha$  activation. *J Am Soc Nephrol*. 2009;20(4):713–720.
  40. Matsumoto M, Makino Y, Tanaka T, et al. Induction of renoprotective gene expression by cobalt ameliorates ischemic injury of the kidney in rats. *J Am Soc Nephrol*. 2003;14(7):1825–1832.
  41. Zhang Z, Haimovich B, Kwon YS, et al. Unilateral partial nephrectomy with warm ischemia results in acute hypoxia inducible factor 1-alpha (HIF-1 $\alpha$ ) and Toll-like receptor 4 (TLR4) overexpression in a porcine model. *PLoS One*. 2016;11(5):e0154708.
  42. Oda T, Ishimura T, Yokoyama N, et al. Hypoxia-inducible factor-1 $\alpha$  expression in kidney transplant biopsy specimens after reperfusion is associated with early recovery of graft function after cadaveric kidney transplantation. *Transplant Proc*. 2017;49(1):68–72.
  43. Kojima I, Tanaka T, Inagi R, et al. Protective role of hypoxia-inducible factor-2 $\alpha$  against ischemic damage and oxidative stress in the kidney. *J Am Soc Nephrol*. 2007;18(4):1218–1226.
  44. Rosenberger C, Rosen S, Shina A, et al. Activation of hypoxia-inducible factors ameliorates hypoxic distal tubular injury in the isolated perfused rat kidney. *Nephrol Dial Transplant*. 2008;23(11):3472–3478.
  45. Sutton TA, Wilkinson J, Mang HE, et al. p53 regulates renal expression of HIF-1 $\alpha$  and pVHL under physiological conditions and after ischemia-reperfusion injury. *Am J Physiol Renal Physiol*. 2008;295(6):F1666–F1677.
  46. Tanaka T, Kojima I, Ohse T, et al. Hypoxia-inducible factor modulates tubular cell survival in cisplatin nephrotoxicity. *Am J Physiol Renal Physiol*. 2005;289(5):F1123–F1133.
  47. Conde E, Alegre L, Blanco-Sánchez I, et al. Hypoxia inducible factor 1-alpha (HIF-1  $\alpha$ ) is induced during reperfusion after renal ischemia and is critical for proximal tubule cell survival. *PLoS One*. 2012;7(3):e33258.
  48. He K, Chen X, Han C, et al. Lipopolysaccharide-induced cross-tolerance against renal ischemia-reperfusion injury is mediated by hypoxia-inducible factor-2 $\alpha$ -regulated nitric oxide production. *Kidney Int*. 2014;85(2):276–288.
  49. Luo L, Luo G, Fang Q, Sun Z. Stable expression of hypoxia-inducible factor-1 $\alpha$  in human renal proximal tubular epithelial cells promotes epithelial to mesenchymal transition. *Transplant Proc*. 2014;46(1):130–134.
  50. Rabinowitz MH. Inhibition of hypoxia-inducible factor prolyl hydroxylase domain oxygen sensors: tricking the body into mounting orchestrated survival and repair responses. *J Med Chem*. 2013;56:9369–9402.
  51. Wang Z, Schley G, Türkoglu G, et al. The protective effect of prolyl-hydroxylase inhibition against renal ischaemia requires application prior to ischaemia but is superior to EPO treatment. *Nephrol Dial Transplant*. 2012;27(3):929–936.
  52. Bernhardt WM, Gottmann U, Doyon F, et al. Donor treatment with a PHD-inhibitor activating HIFs prevents graft injury and prolongs survival in an allogenic kidney transplant model. *Proc Natl Acad Sci U S A*. 2009;106(50):21276–21281.
  53. Movafagh S, Crook S, Vo K. Regulation of hypoxia-inducible factor-1 $\alpha$  by reactive oxygen species: new developments in an old debate. *J Cell Biochem*. 2015;116(5):696–703.
  54. Pastor-Soler NM, Sutton TA, Mang HE, et al. Muc1 is protective during kidney ischemia-reperfusion injury. *Am J Physiol Renal Physiol*. 2015;308(12):F1452–F1462.
  55. Wen D, Zou Y-F, Gao Y-H, et al. Inhibitor of DNA binding 1 is induced during kidney ischemia-reperfusion and is critical for the induction of hypoxia-inducible factor-1 $\alpha$ . *BioMed Res Int*. 2016;2016:4634386.
  56. Li Y, Yang J, Luo J-H, et al. Tubular epithelial cell dedifferentiation is driven by the helix-loop-helix transcriptional inhibitor Id1. *J Am Soc Nephrol*. 2007;18(2):449–460.
  57. Popescu CR, Sutherland MR, Cloutier A, et al. Hyperoxia exposure impairs nephrogenesis in the neonatal rat: role of HIF-1 $\alpha$ . *PLoS One*. 2013;8(12):e82421.
  58. Jeong W, Rapisarda A, Park SR, et al. Pilot trial of EZN-2968, an antisense oligonucleotide inhibitor of hypoxia-inducible factor-1  $\alpha$  (HIF-1 $\alpha$ ), in patients with refractory solid tumors. *Cancer Chemother Pharmacol*. 2014;73(2):343–348.
  59. Maxwell PH, Eckardt KU. HIF prolyl hydroxylase inhibitors for the treatment of renal anaemia and beyond. *Nat Rev Nephrol*. 2016;12(3):157–168.