miR-21 in ischemia/reperfusion injury: a double-edged sword?

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Xu X, Kriegel AJ, Jiao X, Liu H, Bai X, Olson J, Liang M, Ding X. miR-21 in ischemia/reperfusion injury: a double-edged sword?. Physiol Genomics 46: 789–797, 2014. First published August 26, 2014; doi:10.1152/physiolgenomics.00020.2014.—MicroRNAs (miRNAs or miRs) are endogenous, small RNA molecules that suppress expression of targeted mRNA. miR-21, one of the most extensively studied miRNAs, is importantly involved in divergent pathophysiological processes related to ischemia/reperfusion (I/R) injury, such as inflammation and angiogenesis. The role of miR-21 in renal I/R is complex, with both protective and pathological pathways being regulated by miR-21. Preconditioning-induced up-regulation of miR-21 contributes to the protection against subsequent renal I/R injury through the targeting of genes such as the proapoptotic gene programmed cell death 4 and interactions between miR-21 and hypoxia-inducible factor. Conversely, long-term elevation of miR-21 may be detrimental to the organ by promoting the development of renal interstitial fibrosis following I/R injury. miR-21 is importantly involved in several pathophysiological processes related to I/R injury including inflammation and angiogenesis as well as the biology of stem cells that could be used to treat I/R injury; however, the effect of miR-21 on these processes in renal I/R injury remains to be studied.

miR-21 AND HYPOXIA

Hypoxia plays an important role in I/R injury including renal I/R injury. Under hypoxic conditions, tissues and cells produce a series of hypoxic responses involving complex molecular...
mechanisms. Hypoxia-inducible factor (HIF) is at the center of cellular hypoxia responses (66). HIF is a heterodimeric complex composed of a HIF-α subunit and a HIF-β subunit. While HIF-β remains constitutively high, HIF-α is tightly regulated to control the HIF response. HIF-α has three isoforms (HIF-1α, HIF-2α, and HIF-3α), of which HIF-1α is expressed ubiquitously and has been studied most extensively. Stable expression of HIF-α is an important adaptive response of cells to hypoxia. In the presence of O2, HIF-α is degraded by the ubiquitin-proteasome pathway with the prolyl hydroxylase (PHD). Under hypoxic conditions, the hydroxylation and degradation of HIF-α are inhibited.

The accumulated HIF-α translocates into cell nucleus, and together with HIF-β, binds to the hypoxia-response elements of hypoxia responsive genes, such as vascular endothelial growth factor (VEGF), erythropoietin, glucose transporter 1, and heme oxygenase-1 to promote their transcription. These gene products could increase oxygen supply and capacity for oxygen transport or reduce the oxygen consumption of cells. Reconciliation of the discrepancy between oxygen supply and demand would help to maintain a stable internal environment during ischemia and hypoxia (74).

In recent years many studies have confirmed that miRNAs play an important regulatory role in hypoxic responses as well. A series of hypoxia-related miRNA (HRMs) have been identified in cancer studies, including miRNAs that were upregulated (miR-210, 21, 30, and 192) or downregulated (miR-15b, 20a, and 122a) (70, 71). Fasanaro et al. (34) found that HIF binding sites are present in most HRM promoters, suggesting that HIF may be the key regulator of HRM expression under hypoxic conditions. These findings suggest that HIF regulates alterations in gene expression, not only from direct regulation of hypoxia-responsive genes, but also from indirect regulation of mRNAs targeted by HRMs (52).

miR-21 is one of the HRMs upregulated in tumor tissues, and some evidence suggests that HIF and miR-21 regulation may be interdependent. HIF binding sites are found ~2 kb upstream of the transcription start site of pri-miR-21 (71), which suggests that HIF may act to promote miR-21 expression under hypoxic conditions. We previously found that blockade of HIF abolished miR-21 upregulation in hypoxic human renal epithelial cells, demonstrating a significant role for HIF in the upregulation of miR-21 (120). Interestingly, miR-21 could also regulate HIF-1α expression. miR-21 target gene phosphatase and tensin homology deleted on chromosome 10 (PTEN) has been reported to be an inhibitor of phosphoinositide 3-kinase (PI3K)/Akt signaling pathway (60). The PI3K/Akt signaling pathway plays an important role in the expression and activation of HIF induced by hypoxia, growth factor, and nitric oxide (38, 99, 126). PTEN has been shown to attenuate hypoxia-mediated activation of Akt and HIF-1α stabilization and inhibit HIF-1-regulated gene expression (136). Later Liu et al. (81) demonstrated that miR-21 inhibited PTEN resulting in activation of Akt that can induce expression of HIF-1α and its target gene VEGF. These results indicate that miR-21 can indirectly promote HIF-1 stabilization and activity under hypoxic conditions and act with HIF-1 to form a positive feedback loop.

miR-21 can also be regulated by other mechanisms in hypoxia in addition to HIF. Polyarchou et al. (90) found that binding of nuclear factor kappB (NF-kB), CAM responsive element-binding protein (CREB), and CREB binding protein/p300 to the promoter of miR-21 was induced by the activation of Akt2 in hypoxia.

The studies reviewed here indicate that miR-21 is upregulated in hypoxia and then acts to perpetuate the actions of HIF by indirect activation of Akt via targeting PTEN. Additionally, miR-21 and HIF-1 are in a hypoxia-induced positive feedback loop in which HIF-1α drives miR-21 expression and miR-21 indirectly stabilizes and upregulates HIF-1α. Future directions for research are mutual regulatory mechanism of HIF-1α and miR-21 under hypoxic condition.

miR-21 IN RENAL I/R AND RELATED PROCESSES

The kidney is very sensitive to hypoxia and vulnerable to ischemic or hypoxic injury because of the renal vascular anatomy and the high energy consumption of renal tubular epithelial cells (12). The mechanism of renal I/R injury is extremely complex, however, much of the damage is mediated by reactive oxygen species (ROS) during reperfusion (26). ROS activate inflammatory cells resulting in the release of interleukin (IL), tissue necrosis factor, and other inflammatory factors, promoting cell apoptosis and increasing tissue damage during reperfusion. HIF-1α has also been detected in renal tubular epithelial cells during renal ischemia and postreperfusion (93). ROS could stimulate expression of HIF-1 and upregulate a variety of HRMs under hypoxic conditions through the PI3K/Akt signal pathway (41).

Interest in the relationship between miR-21 and I/R injury has increased in the recent years (25, 44, 56, 96, 120). In studies of renal I/R models by Godwin et al. (44) and Wei et al. (115) several miRNAs including miR-21, miR-7, and miR-192 were shown to be upregulated, while others such as miR-322 were downregulated. In the myocardium, miR-21 is also known to have an important role in cardiac ischemic injury (fibrosis) and myocardial remodeling following I/R (121, 130). The role of miR-21 in brain I/R (stoke) may be related to atherosclerosis (92), possibly involving angiogenesis. A role for miR-21 in lung and liver I/R injury organs has not been reported.

The functional role of miR-21 in renal I/R injury appears to be complex and few studies have explored its role in the many I/R related processes in the kidney. Upregulation of miR-21 by ischemic preconditioning (IPC) contributes to the protection of kidneys from subsequent I/R injury; however, knocking down miR-21 at the time of renal I/R injury has no effect on the I/R injury (120). In ischemia, an I/R-related condition, the overexpression of miR-21 in cultured mouse tubular epithelial cells does not prevent cell death following simulated ischemia (44). In addition, miR-21 may promote renal interstitial fibrosis that occurs after I/R injury (19). The diverse effects of miR-21 on I/R-related processes are likely due to temporal changes in expression of miR-21 target genes and pathway involvement through the different stages of I/R pathology. The relationship of miR-21 to these pathways and pathological processes, which are summarized in Fig. 1 and are discussed in detail in the following sections.

miR-21 and Inflammation

The inflammatory response is initiated quickly after I/R, further exacerbating I/R injury (26). This response involves
NF-κB, inflammatory cytokines, inflammatory cells, and several other factors (26). Studies have shown that suppression of the inflammatory response can reduce renal I/R injury and preserve renal function (15, 32).

miR-21 has been reported to be involved in many conditions in which inflammation is central, including lipopolysaccharide (LPS)-stimulated lung inflammation (87), allergic airway inflammation (83), osteoarthritis (104), psoriasis and atopic eczema (137), and many others. In these inflammatory diseases miR-21 may act to regulate immune-related target genes, including confirmed targets IL-12p25 (83) and transforming growth factor (TGF)-β receptor 1 (TGFBR2) (20). miR-21 is also involved in common inflammatory pathways. IL-6, a proinflammatory cytokine activated by NF-κB, can drive miR-21 expression through a signal transducer and activator of transcription 3 (STAT3)-dependent mechanism and STAT3-activated miR-21 directly inhibits PTEN. Additionally, the inhibition of PTEN results in increased Akt activity and subsequent activation of NF-κB, which is required for sustaining the inflammatory positive feedback loop (53). Zhou et al. (129) found another autoregulatory feedback loop between peroxisome proliferator activated receptor-α (PPAR-α) and miR-21.

Despite the previously described studies suggesting that miR-21 is involved with proinflammatory signaling, several studies suggest that miR-21 may be negatively regulating inflammatory processes. Sheedy et al. (101) found that transfection of miR-21 precursor into cells exposed to LPS blocked NF-κB activity and promoted the production of anti-inflammatory IL-10 through miR-21 targeting of programmed cell death 4 (PDCD4). Similarly, Chen et al. (21) found that miR-21 inhibited two important factors in the Toll-like receptor signaling pathway MyD88 and IL-1R-associated kinase 1, thereby reducing NF-κB signaling. miR-21 is also upregulated during the process of T cell differentiation (117). In regulatory T cells (Treg) miR-21 was found to increase expression of forkhead box P3, an important characteristic of natural Tregs (94), that negatively regulate immune cells involved with the inflammatory process.

These miR-21-regulated genes are in pathways that have been well studied in I/R injury through a direct or indirect link to miR-21. Activation of NF-κB is a central part in the inflammation signaling pathway (134). We previously found that prolonged renal ischemia/hypoxia excessively activated NF-κB, increased expression of several proinflammatory factors, and exacerbated the infiltration of monocyte-macrophages, resulting in acute renal failure (58). IL-6 is considered to be an indicator of renal I/R-induced inflammation (113). Tregs have also been found to be protective in renal I/R injury (24). Despite reported interactions between miR-21 and NF-κB, cytokines (IL-6), and Tregs in inflammatory processes, further studies are required to understand how these miR-21 regulation in these pathways would impact the development of inflammation in renal I/R injury.

miR-21 and Angiogenesis

Animal experiments and clinical studies have shown that angiogenesis can be induced following ischemia (myocardium, kidney, and brain) (33, 39, 54). For example, Iruela-Arispe et al. (54) found that endothelial cell proliferation, migration, and new vasculature formation occurred 2–14 days after renal ischemic injury. Ischemia/hypoxia-induced angiogenesis is of great significance in the repair of the ischemic injury because it can help to restore a normoxic environment in the impacted tissue.

It has been reported that knockout of key enzymes in the biogenesis of miRNAs such as Dicer and Drosha could inhibit the generation and migration of capillary endothelial cells and alter factors that regulate angiogenesis, suggesting that dysregulation of miRNAs has a dramatic impact on angiogenesis (69, 104). In addition, miRNAs such as proangiogenic miR-92a and miR-210 and antiangiogenic miR-221 and miR-222 have been reported to regulate ischemia-induced angiogenesis (2, 103).

miR-21 is highly expressed in vascular endothelial cells (45, 55). The endothelial cell responses to shear stress or hypoxia, which are important in vascular remodeling are affected by miR-21 (116). In a recent study by Ji et al. (55), microarray analysis revealed that miR-21 was significantly upregulated in vascular walls following balloon injury. They also found that miR-21 knock-down decreased neointimal hyperplasia following angioplasty, pointing to a proangiogenic function of miR-21. This mechanism may be related to the ability of miR-21 to suppress target PTEN and to indirectly increase B-cell lymphoma 2 expression. Navarro et al. (88) found that miR-21 induced tumor angiogenesis through targeting of PTEN, leading to activation of Akt and extracellular regulated protein kinases 1/2 (ERK1/2) signaling pathways, and thereby enhancing HIF-1α and VEGF expression. A study of miR-21 in a colon cancer model showed that angiogenesis was dependent on the upregulation of miR-21 through regulating VEGF protein levels (127). In addition, miR-21 has been found to induce differentiation of induced pluripotent stem cells into endothelial cells via PTEN/Akt pathway and TGF-β2 activation, which might be involved in angiogenesis (29). Though several studies report a proangiogenic role for miR-21, it is also important to note that miR-21 has also been reported to inhibit angiogenesis.

miR-21 and Protection/Repair

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miR-21 and Cell Survival

miR-21 is a strong prosurvival and antiapoptotic miRNA especially in cancer. miR-21 has been found to be elevated in several types of solid tumors, including breast tumors, colon tumors, and gliomas (11, 18, 112). The antiapoptotic function of miR-21 is mediated, at least in part, by targeting a series of pro-apoptotic genes including PDCD4 (37), PTEN (86), tropomyosin 1 (132).

The antiapoptotic protection mediated by miR-21 has been studied in ischemic heart and cerebrovascular diseases. For example, miR-21 expression has been shown to be inversely correlated with PDCD4 expression and cellular apoptosis in the heart after I/R (23). Additionally, overexpression of miR-21 in the left ventricle reduced cardiomyocyte apoptosis following I/R (91). It has also been reported that miR-21 attenuates the death of ischemic cortical neurons by reducing expression of cell death inducing Fas ligand (FasL) gene (13). Overexpression of miR-21 in the mouse heart inhibited ischemia-induced upregulation of PTEN and FasL, reduced infarct size, and attenuated apoptosis (100). It is interesting to note that suppression of PDCD4 and PTEN could lead to activation of activator protein 1 or Akt signaling, respectively, which could in turn upregulate miR-21, forming positive feedback loops (30, 100, 105).

In renal I/R injury apoptosis contributes to tubular epithelial cell death (79, 97). It has been suggested that miR-21 may also play an important protective role in the renal I/R injury through antiapoptotic regulatory mechanisms (44). We found that in mouse kidneys exposed to 15 min of ischemia miR-21 was significantly upregulated after 4 h of reperfusion and remained significantly higher 4 days later. The preconditioning-induced upregulation of miR-21 protected mouse kidneys from subsequent I/R injury, which might in part be mediated by a decrease in cell apoptosis through miR-21-induced suppression of PDCD4 (120). The functional significance of miR-21 regulation of apoptosis in long-term I/R injury response is not known.

miR-21 in Fibrosis

I/R response is associated with chronic (days to weeks) molecular mechanisms of repair including pathways associated with fibrosis. I/R is known to incite fibrosis in the heart (110), kidney (46), and liver (22) through expression of a series of profibrositic genes, some of which may be regulated by miRNA. As discussed above, miR-21 is upregulated in response to I/R, and several studies have shown that miR-21 is involved with the regulation of fibrosis in lung (80), heart (1, 78, 106), liver (114), and kidney (43, 111, 128).

Renal TGF-β, a key fibrogenic protein, is upregulated in response to I/R. miR-21 is rapidly induced in the kidney after TGF-β treatment (44). Findings from studies in other disease processes suggests that miR-21 may be involved in TGF-β-induced profibrotic signaling in renal I/R injury through several signaling pathways. Dey et al. (27) found that suppression of miR-21 attenuated phosphorylation of endogenous inhibitors of mammalian target of rapamycin complex 1 by inhibiting TGF-β-stimulated phosphorylation of Akt kinase. TGF-β signaling is also amplified by miR-21 through inhibition of Smad7 expression and reduced Smad2 phosphorylation, promoting fibrotic lung disease (80). Other studies have shown that miR-21 contributes to fibrosis by the PTEN/Akt pathway (7, 114). A positive feedback loop with miR-21 targeting of transforming growth factor beta receptor III (TGFBR3) may also be involved with cardiac fibrosis (78). In addition to the PTEN/Akt pathway, Sprouty homolog 1 (Spry1), a miR-21 target gene, in the ERK-MAPK signaling pathway is also involved in cardiac fibrosis. Overexpression of miR-21 promoted proliferation of cardiac fibroblasts through activation of MAPK which was inhibited by Spry1 (106). Roy et al. (95) showed that increased miR-21 was localized to cardiac fibroblasts in the infarct zone of mouse hearts subjected to I/R. They found that targeting of PTEN by miR-21 increased matrix metalloprotease-2 expression via alterations in the PTEN signaling pathway, contributing to fibrosis. The mode of action for miR-21 in renal fibrosis could be different from that in the heart or lung. Recently, Chau et al. (19) found that the ERK/MAPK activation by miR-21 in the renal fibrosis after I/R through target genes Ppar-α and Mpv171, respectively. In the unilateral ureteral obstruction model miR-21 is localized primarily to the tubular epithelial cells, In this model the tubulointerstitium remains ischemic/hypoxic following renal reperfusion, and this has been shown to promote renal fibrosis (73). Serum miR-21 level was increased and associated with the pathological renal fibrosis in renal transplant patients (43). Since miR-21 is hypoxia responsive, and elevations in circulating miR-21 are associated with renal fibrosis, the role that miR-21 plays in the occurrence and development of tubulointerstitial fibrosis and fibrotic repair processes following renal I/R is an important area for future research.

Role of miR-21 in Preconditioning

The response of a cell to hypoxia/ischemia is bimodal. Initially there is an adaptive protective conditioning reaction that transitions to cell death upon persistence of the insult. Different pretreatments including brief exposures to ischemia, mild heat shock, and certain drugs activate endogenous defense mechanisms, to protect cells or organs against subsequent, sustained ischemic insult. This is known as ischemic tolerance (10). Ischemic tolerance, is, by far, the most powerful endogenous mechanism in the prevention of ischemic organ damage (42). Among studies on various pretreatments to induce ischemic tolerance, IPC was evaluated most frequently. Organ protection phenomenon of IPC has been confirmed in the heart, brain, liver, lung, and kidney (14, 49, 61). Our previous study...
found that IPC could effectively attenuate acute ischemic renal injury by reducing apoptosis and inflammation. We also found that the window of renal protection could be significantly prolonged by adjusting the form of ischemic pretreatment. IPC also inhibited transdifferentiation of glomerular mesangial cells and tubular epithelial cell and significantly reduced tubulointerstitial fibrosis during late renal I/R injury (57).

The protective mechanism of IPC appears to involve a series of protective mediators and/or effectors such as ROS, protein kinase C, HIF, inducible nitric oxide synthase, and heat shock protein (HSP) (42, 49). Recently miRNAs have been identified to be involved with the regulation of IPC. Several miRNAs such as miR-133, miR-200, and miR-23a have been reported to be involved in cardiac, brain, and hepatic IPC (72, 98, 119). A protective effect of miR-21 in cardiac IPC was reported by both Dong et al. (30) and Yin et al. (123). In these studies IPC inhibited the downregulation of miR-21 in the infarcted areas. Overexpression of miR-21 reduced cell apoptosis in the border and the infarcted areas, decreased myocardial infarct size, and improved left ventricle remodeling 2 wk following myocardial infarction. The protective role of miR-21 in cardiac IPC was further supported in a study by Cheng et al. (23), which found that miR-21 was upregulated early after cardiac IPC and protected against cardiac I/R injury. Consistent with these findings, exogenous miR-21 reduced infarct size following I/R by 64% and inhibition of miR-21 abolished the protective effect (124). In addition, Dharap et al. (28) analyzed the spectrum of miRNAs in brain after IPC and found that expression of miR-21 increased most significantly in the rat cerebral cortex 24 h after IPC.

In our study, we utilized a mouse model of delayed renal IPC and found that expression levels of miR-21 were significantly induced in kidneys with 15 min of IPC. The in vivo knockdown of miR-21 during IPC led to worsen renal functional and histological damage resulting from I/R injury 4 days later, indicating that miR-21 contributes to the protection conferred by delayed IPC (120).

Furthermore, numerous studies have shown that miR-21 regulates IPC through several signaling pathways. The role of PI3K/Akt signaling pathway has been well established in the protection against I/R injury conferred by early or delayed IPC, mainly through attenuation of apoptosis (64, 109, 118, 122). This suggests that the protective role of IPC-induced miR-21 may be related to its regulatory effects on the PTEN-PI3K/Akt signaling pathway. The miR-21 conferred protection with IPC is also conferred by the regulation of target gene PDCD4 in renal (120) or cardiac (23) tissues. In another study, miR-21 was shown to be involved in the resveratrol-induced cardioprotection against I/R injury through ERK/MAPK pathway (3). In addition to the established IPC-involved pathways, HIF is increasingly being considered as a mediator of ischemic tolerance (9, 31). It has been confirmed that high expression of HIF plays an important role in IPC in the heart, brain, liver, and kidney (14, 65, 82, 85). Our study showed that knock-down of miR-21 did not affect I/R injury in the absence of IPC, suggesting that the protective effect of miR-21 might depend on HIF-1 induction by IPC (120). In addition, xenon (84) or PHD inhibitor (50) pretreatment, which induces overexpression of HIF, also has a protective effect on renal ischemic injury. A study by Jia et al. (56), in our lab, showed miR-21 contributes to renal protection conferred by xenon preconditioning probably through the upregulation of HIF. miR-21 expression induced by cardiac IPC was also found to positively correlate with protective proteins including endothelial nitric oxide synthase, heat shock transcription factor 1, and HSP70 by Yin et al. (123), suggesting the involvement of other signaling pathways and molecules in this process.

**miR-21 and Stem Cell Therapy in I/R**

Unfractionated bone marrow stem cells (59), hematopoietic stem cells (76), and mesenchymal stem cells (MSC) (107, 133) have been studied as sources for stem cell-based repair after I/R injury. Currently, one of the major challenges to successful stem cell therapy in I/R injury is insuring cell survival and differentiation in the harsh microenvironment of damaged postischemic tissues or organs (4–6).

miR-21 was found to function in a lineage-specific manner during differentiation of stem cells into specialized cell types via PTEN/Akt or TGF-β signaling pathway (62, 63). Furthermore, Zou et al. (135) found that upregulation of miR-21 induced by TGF-β treatment in MSCs promoted skin wound healing by increasing proliferation and differentiation of these cells. These results indicate that induced miR-21 in renal I/R injury may regulate the differentiation of stem cell into renal tubule epithelial cell.

Transplanted donor stem cells are sensitive to serum and O2 deprivation in damaged tissues (47). Hypoxic preconditioning (HPC) of stem cells prior to transplantation is an effective protection strategy. In the heart stem cell therapy, HPC has been found to be able to enhance the stability of HIF-1 via the PI3K/Akt signaling pathway, enhancing the antiapoptotic state of MSCs (17, 51), which suggests that miR-21 may play a role in HPC for stem cell therapy by the HIF/miR-21/PTEN/Akt pathway. In addition, Haider et al. (48) found that HPC enhanced the proliferation and survival of injected stem cells in the infarcted hearts, which was associated with preconditioning-induced upregulation of miR-21 via activation of ERK1/2 and STAT3 signaling. Nie et al. (89) also reported that miR-21 was involved in the survival of MSCs under hypoxic condition with serum deprivation. Therefore, miR-21 could be used to improve feasibility and success of stem cell therapy in I/R injury.

**SUMMARY**

In summary, miR-21 clearly plays an important role in I/R injury (Fig. 1). Preconditioning-induced upregulation of miR-21 contributes to the protection against subsequent renal I/R injury. This protective effect of miR-21 may involve targeting of PDCD4 and the interaction between miR-21 and HIF. Long-term elevation of miR-21, however, may lead to the development of renal interstitial fibrosis following I/R injury. miR-21 is importantly involved in several pathophysiological processes related to I/R injury, including inflammation and angiogenesis as well as in the proliferation, differentiation, and survival of stem cells. Despite the reported role for miR-21 in those processes, it remains to be further investigated if and how miR-21 affects inflammation, angiogenesis, and stem cell biology in relationship to I/R injury and its treatment. In addition, it would be important to understand the role of miR-21 in different cell types relevant to I/R injury, including tubular...
epithelial cells, interstitial cells, endothelial cells, and infiltrating immune cells in the kidney.

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DISCLOSURES
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AUTHOR CONTRIBUTIONS

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