Glycogen Synthase Kinase-3 Signaling in Acute Kidney Injury

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Keywords

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Abstract

Acute kidney injury (AKI) is a common clinical syndrome that involves renal tubular epithelial cell death and leads to acute decline in renal function. Improper tubular regeneration following AKI often leads to CKD. We discuss the role of a serine/threonine protein kinase called glycogen synthase kinase-3 (GSK3) in renal tubular injury and renal fibrosis. We also highlight the importance of GSK3 as a potential drug target in AKI patients and molecular mechanisms promoting tissue regeneration.

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Background

Acute kidney injury (AKI) affects over 13 million people and causes 1.7 million deaths every year worldwide. Long-term consequences of AKI include prolonged renal

dysfunction, cardiovascular effects, progression to CKD, and death [1]. AKI is commonly caused by nephrotoxic agents, infection, drug toxicity, or ischemia-reperfusion (I/R) and is often seen in hospitalized patients. AKI involves apoptotic or necrotic cell death of renal tubular epithelial cells, especially of the proximal tubules. Although the injury to the nephron is often reversible, approximately 15% of AKI patients can advance to CKD within 24 months. AKI to CKD progression is mainly attributed to faulty tubular repair and the development of renal fibrosis after AKI. Hence, it is important to develop therapies to ensure proper repair of tubules and minimize the chances of AKI progressing to CKD. This review examines the progress made in understanding the role of a serine-threonine protein kinase called glycogen synthase kinase-3 (GSK3) in renal tubular injury, repair, and renal fibrosis after AKI.

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GSK3 Regulates Apoptotic Cell Death of Renal Tubular Epithelial Cells in AKI

Mammalian GSK3 consists of α and β isoforms which share 97% sequence homology in their kinase domain and were first cloned by James R. Woodgett. Although initially discovered as a key kinase in the insulin signaling pathway, a flood of studies in the 1990s and 2000s revealed that GSK3 acts as a gatekeeper of many cellular signaling pathways, including Wnt, mTOR, sonic hedgehog, and notch signaling. GSK3 is inhibited by phosphorylation of serine-21 and serine-9, respectively, on GSK3 α and GSK3 β isoforms or by their cytoplasmic sequestration. Many of the above mentioned pathways when active, lead to inhibition of GSK3, and consequently GSK3 is active when these signaling pathways are inactive, as in fully differentiated cells. GSK3, thus, plays an important role during embryonic development and during injury/repair processes in adult tissues [2].

A pro-apoptotic role of GSK3 β in kidney cells was first shown by our study in which overexpression of a constitutively active GSK3 β induced apoptosis, while GSK3 inhibition using small molecule inhibitors SB216763 and SB415286, or LiCl reduced apoptosis following hypertonic stress [3]. The first studies examining the role of GSK3 in AKI [4] came from the hypothesis that GSK3 inhibition could have anti-inflammatory effects. This hypothesis was based on prior findings that global GSK3 β gene deleted mice died post-birth and showed a similar phenotype to mice with suppressed NF- κ B activation, due to p65 or I κ B kinase 2 gene deletion [2]. Pretreatment with GSK3 inhibitor protected rodents from lipopolysaccharide-induced endotoxemic renal failure, and specifically proximal tubular injury [4, 5]. Following this, multiple in vivo studies showed that GSK3 plays an important role in apoptosis in AKI models including I/R [6, 7] or nephrotoxicity induced by diclofenac, cisplatin, mercuric chloride, gentamicin, or paraquat [8–11]. Isoform nonspecific pharmacologic inhibitors of GSK3 such as LiCl, TDZD-8, SB216763, SB415286, or BIO reduced apoptosis of renal tubular epithelial cells in the above mentioned studies. SB216763 acts as an ATP-competitive inhibitor, whereas TDZD is a non-ATP-competitive inhibitor of GSK3. TDZD has also been hypothesized to interact with cysteine 199, an important amino acid residue located in the active site of GSK3. GSK3 is inhibited by BIO which interacts with its ATP-binding pocket and by LiCl which competes with Mg²⁺ [12, 13].

The main mechanism by which GSK3 promotes apoptosis is by the regulation of mitochondrial permeability

transition. Plotnikov et al. [14] observed swollen and fragmented renal proximal tubular mitochondria with reduced membrane potential following renal I/R in rats, which was partially restored by LiCl treatment. Furthermore, Rogung Gong's group demonstrated the presence of GSK3 β in mitochondria of cultured cells. They also showed that elevated reactive oxygen species in AKI increased GSK3 β activity and GSK3-mediated phosphorylation of cyclophilin F and voltage-dependent anion channel in the mitochondria, leading to mitochondrial permeability transition and cell death. Consistently, GSK3 inhibition reduced mitochondrial permeability transition and apoptosis in in vitro and in vivo AKI models [7, 8, 11]. Similarly, Wang et al. [6] found that overexpression of constitutively active GSK3 β led to activated Bax and cleaved caspase-3, and increased apoptosis after ATP depletion in vitro, while GSK3 inhibition by TDZD-8 reduced tubular cell apoptosis in rats following I/R. The role of the GSK3 β isoform in survival was further shown by our study employing a proximal tubule-specific GSK3 β gene-deleted mouse, which showed reduced tubular injury and preserved renal function in a mercuric chloride-induced AKI mouse model. Reduced renal tubular cell apoptosis in the GSK3 β -knockout mice was accompanied by reduced levels of activated Bax6A7 and caspase-3 [9]. In addition, multiple in vitro and in vivo studies not discussed here have observed that increased phospho-GSK3 β Ser9 levels correlate with reduced apoptosis and kidney damage in various AKI and CKD models. Thus, pretreatment with GSK3 inhibitors could confer renal protection during AKI.

GSK3 Regulates Renal Tubular Repair and Fibrosis after AKI

Renal tubules are capable of regenerating after injury, mainly by proliferation of surviving tubular epithelial cells. However, repair is often incomplete, which along with persistent inflammation could lead to the development of fibrosis and CKD. Since pretreatment with GSK3 inhibitors or GSK3 β gene deletion prior to AKI could significantly reduce injury, studies examining the role of GSK3 in repair have used post-AKI GSK3 inhibitor treatment protocols. We found that treatment with TDZD-8 starting 2 days after mercuric chloride induced-nephrotoxic AKI in mice showed significantly better restoration of renal structure and function compared to vehicle-treated AKI group. TDZD-8 treatment post-AKI increased renal levels of pro-proliferative factors such as cyclin D1,

Fig. 1. Pathways regulated by GSK3 β in AKI: GSK3 β induces mitochondrial injury and apoptosis by directly phosphorylating and activating pro-apoptotic proteins Bax and Caspase-3. GSK3 β also phosphorylates and activates the p65 subunit of NF- κ B and enhances its transcriptional response leading to inflammation. Similarly, Nrf2, which plays an important role in antioxidant defense mechanism is suppressed by GSK3 β . Nrf2 is phosphorylated by GSK3 β , which facilitates its nuclear exclusion, resulting in persistent oxidative stress. GSK3 β also regulates cell proliferation by phosphorylating cyclin D1, c-Myc, and HIF-1 α resulting in their nuclear exit and subsequent proteasomal degradation. All these pathways slow down tubular recovery and aids in AKI to CKD transition. AKI, acute kidney injury; GSK3, glycogen synthase kinase-3.

c-myc, and β -catenin (previously known GSK3 β targets for phosphorylation and degradation) and significantly increased cell proliferation of tubular epithelial cells [9]. Increased tubular cell proliferation was also observed in GSK3 β knockout mice subjected to similar nephrotoxic AKI [9]. Consistently, LiCl treatment following cisplatin or I/R-induced AKI was found to significantly improve kidney repair in mice [7]. In vitro studies in murine renal epithelial cell lines exposed to cisplatin showed that GSK3 β interacts with cyclin D1, c-Myc, and hypoxia inducible factor-1 α (HIF-1 α), resulting in their phosphorylation, nuclear exit, and subsequent proteasomal degradation [7]. In a recent study, we have found that GSK3 β suppresses tubular cell proliferation after AKI by inhibiting FoxM1, a crucial regulator of cell cycle progression (revised manuscript under review in FASEB journal).

Progressive renal fibrosis is a major problem associated with AKI and is closely linked to persistent inflam-

mation. GSK3 is a known regulator of NF- κ B activity [2]. In a model of severe acute pancreatitis-induced AKI, GSK3 inhibition reduced NF- κ B activity and pro-inflammatory factors [15]. We demonstrated that inhibition of GSK3 β using TDZD-8 starting 2 days after I/R-induced AKI reduced macrophage infiltration, TNF α , IL6, IL1 β , CCL2, CCL3, and ICAM1 levels [16]. TDZD-8 treatment also reduced renal fibrosis, myofibroblasts, extracellular matrix proteins, such as collagen-1 and fibronectin, and mRNA levels of TGF- β 1, PAI-1, and TGF- β signaling [16]. In vitro, GSK3 inhibitor treatment abolished TGF- β mediated fibroblast to myofibroblast differentiation [16]. Similarly, in a mouse model of chronic alcohol feeding followed by I/R-induced AKI, GSK3 inhibitor pretreatment before I/R reduced renal fibrosis, ECM, myofibroblasts, and TGF- β signaling and improved renal function [17]. These studies suggest that GSK3 could promote renal fibrosis after AKI by activating the TGF- β signaling. In spite of the extensive knowledge that we have gained on the role of GSK3 in AKI, how GSK3 is regulated during renal injury, repair, and fibrosis is still unclear.

Conclusions and Future Directions

GSK3 β plays a critical role in AKI by promoting tubular epithelial cell apoptosis, inflammation and fibrosis, and suppressing repair (Fig. 1). Pharmacologic GSK3 inhibition or GSK3 β gene deletion in animal models of AKI have proven to improve outcomes after various types of AKI. Since AKI is often detected after the injury has occurred, delayed pharmacologic intervention using GSK3 inhibitors after AKI could be a useful strategy to accelerate repair, prevent fibrosis, and minimize the chance of AKI progressing to CKD.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Author Contributions

A.J. and R.R. wrote the manuscript.

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