Renal tubular epithelial cells response to injury in acute kidney injury

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Summary

Acute kidney injury (AKI) is a clinical syndrome characterized by a rapid and significant decrease in renal function that can arise from various etiologies, and is associated with high morbidity and mortality. The renal tubular epithelial cells (TECs) represent the central cell type affected by AKI, and their notable regenerative capacity is critical for the recovery of renal function in afflicted patients. The adaptive repair process initiated by surviving TECs following mild AKI facilitates full renal recovery. Conversely, when injury is severe or persistent, it allows the TECs to undergo pathological responses, abnormal adaptive repair and phenotypic transformation, which will lead to the development of renal fibrosis. Given the implications of TECs fate after injury in renal outcomes, a deeper understanding of these mechanisms is necessary to identify promising therapeutic targets and biomarkers of the repair process in the human kidney.

Keywords: Acute kidney injury; Renal tubular epithelial cells; Adaptive repair; Phenotypic transformation

Introduction

Acute kidney injury (AKI) is a commonly encountered clinical syndrome associated with various aetiologies, such as ischemia, hypoxia, obstruction, sepsis, metabolism, toxic, and infections, leading to decreased kidney function.[1] Importantly, an episode of AKI is not only associated with short-term adverse outcomes, such as fluid overload, electrolyte or acid-base derangement, and immune dysfunction, but also has a long-term adverse effect on survival, which is associated with considerable morbidity, mortality, and health care burden.[2] In clinical practice, it is now recognized that full recovery of kidney function is uncommon, which leaves these patients at risk of development of chronic kidney disease (CKD) or end-stage renal disease.[3]

Packed with mitochondria and dependent on oxidative phosphorylation, the tubular epithelial cells (TECs) are particularly vulnerable to injury (obstructive, ischemic, hypoxic, oxidative, and metabolic), resulting in decline of kidney function.[4] The principal histopathological features of AKI encompass the depletion of the epithelial phenotype in proximal tubular epithelial cells (PTECs), evident by brush border detachment, flattened and localized desquamation of TECs, inflammatory cell infiltration, and the formation of casts containing an abundance of Tamm-Horsfall protein.[5] The injured TECs display cellular plasticity wherein some cells are damaged and die in the early stage of AKI, and the surviving TECs initiate an adaptive repair process. Through dedifferentiation, migration, proliferation, and redifferentiation, new repaired cells replace the damaged TECs, resulting in complete recovery from mild AKI. However, severe or repeated TEC injury leads to abnormal adaptive repair, irreversible structural damage, and loss of function, eventually resulting in tubular inflammation and fibrosis. However, the exact mechanism of phenotypic transformation of TECs after AKI remains incompletely understood, which is partly due to phenotypic heterogeneity, phenotypic switching, surface marker expression, and functional diversity of TECs that overlap in the kidney during health and injury.

In this review, we provide a comprehensive overview of current research on the intrinsic mechanisms of endogenous repair, pathological responses, and phenotypic transformation of TECs after AKI to facilitate the design of novel treatment strategies.

TECs death in AKI

AKI is not necessarily characterized by loss of renal epithelial cells, but cell death is common in severe episodes of AKI, and these dead cells accumulate in the tubular lumen. The usual modes of regulatory cell death can be classified as apoptosis and regulated necrosis. Renal tubular cells may be susceptible to death by apoptosis, a non-immunogenic process, during development and regeneration. However, after AKI, regulated necrosis, a pathway associated with loss of plasma membrane integrity, kills renal cells. A large body of evidence accumulated in the past suggests that regulated necrosis, such as ferroptosis, necroptosis and

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pyroptosis plays a central pathophysiological role in acute tubular necrosis, glomerular loss, and renal fibrosis. A primary injury may activate different cell death programme in the same cell depending on the severity, and several different modes of death are often interrelated and mutually exclusive, so that inhibition of one will result in the death of another, leading to the possibility that multiple modes of cell death may coexist simultaneously or sequentially in the injured kidney. One such process that simultaneously triggers pyroptosis, apoptosis, and necroptosis in innate immune cells is termed PANoptosis, which generates PANoptosome complexes that incorporate components of individual cell death pathways.[6,7] In contrast to apoptosis, regulated necrosis leads to the release of cellular contents and cytokines, which can subsequently trigger an inflammatory response in neighbouring tissues. This necroinflammatory environment can lead to severe organ dysfunction and lasting tissue damage and subsequently renal dysfunction.

Apoptosis

Apoptosis, a caspase-mediated non-inflammatory cell death programme, reflects the morphological pattern of lethal cell injury and removes unwanted or damaged cells during development and at homeostasis. Lerolle et al.[8] stained renal biopsy samples from patients who died of septic shock and found that apoptotic cells were present in the proximal and distal tubules of all septic samples. Apoptosis has subsequently been observed in renal tubular cells in a number of different animal models of sepsis-AKI.[9,10] However, the contribution of apoptosis to AKI is negligible in most animal models of AKI under other triggers.[11] Recent evidence suggests that reactive oxygen species (ROS) production,[12] mitochondrial dysfunction, and activation of tumour necrosis factor (TNF)-α signalling are involved in the triggering of TECs apoptosis in sepsis. In apoptotic cells, caspases are activated and high levels of phosphatidylserine (PtdSer) are exposed to the outer leaflet of the cytoplasmic membrane.[13] PtdSer can be recognized by macrophages as a feeding signal through PtdSer receptors, such as kidney injury molecule-1 (KIM-1), and then, triggers phagocytosis.[14,15] KIM-1 has been shown to be highly upregulated and functions as a biomarker in AKI associated with tubular necrosis and nephron loss.[16] In addition, KIM-1 was shown to induce fatty acid uptake by TECs.[17] Notably, during apoptosis, the plasma membrane of the cells does not lose its integrity, does not release damage-associated molecular patterns (DAMPs), and elicits only macrophage responses and tissue messenger release in an immunosilenced manner.[18]

Ferroptosis

Ferroptosis, a non-classical cell death pathway, is a non-cell-autonomous mode of death triggered by the inability to control lipid peroxidation.[19] It has been demonstrated that ferroptosis plays an important contributory role in most AKI models,[20,21] occurring in the entire segment of TEC in the proximal renal tubule and the thick ascending branch.[22] Unlike apoptosis and necroptosis, ferroptosis occurs in the course of iron-dependent peroxidation of plasma membrane phospholipidsplasma membrane phospholipids, followed by the formation of lipid peroxides, such as oxidised phosphatidylethanolamine, oxidised PtdSer, and oxidised phosphatidylinositol and disruption of the plasma membrane.[23,24] The sensitivity of tubular cells to ferroptosis will depend on iron availability, the ability of enzymatic or non-enzymatic pathways of lipid peroxidation, and the cellular defences against lipid peroxidation, including the maintenance of adequate glutathione via the system Xc-cystine/glutamate antiporter accessing the stores.[25]

Ferroptosis was found to propagate rapidly from cell to cell in a wave-like manner in renal tubules of IRI and oxalate crystal-induced AKI models.[26] Therefore, synchronized necroptosis or other forms of cell death are triggered by ferroptosis in other TECs and may affect the entire renal tubule. That is, in the earliest stages of insult, the predominant mode of TEC death is likely to be ferroptosis. A single prophylactic administration targeting a specific form of cell death could be administered at this stage to avoid the primordial wave of TEC death caused by ferroptosis and subsequent progression of AKI, which scenario is more difficult to realize in the actual course of the clinic. Whereas apoptosis and necrosis occurred in isolated cells, this may explain the differences in the extent and distribution of renal tubular involvement in the AKI model.[27] Inhibition of ferroptosis has also been shown to be protective in a variety of AKI models, including preclinical models of IRI-AKI, nephrotoxin-induced AKI, cytokine storm-associated AKI, and crystal-induced AKI. Interestingly, ferroptosis may create an anti-inflammatory environment in which the adaptive immune system is suppressed while macrophages and neutrophils function to remove necrotic debris to promote regeneration of necrotic renal tubules.[28]

Necroptosis

Necroptosis, a highly pro-inflammatory form of non-cell-autonomous cell death, is mediated by the ripk3-dependent phosphorylation pathway[29] of pseudokinase mixed-lineage kinase domain like (MLKL).[30,31] Unlike apoptosis, necroptosis is usually triggered by external stimuli, which include TNF, other members of the TNF death ligand family, such as Fas and TNF-related apoptosis-inducing ligand (TRAIL), and interferon.[32] In AKI caused by various forms of IRI, sepsis, and drug toxicity, necroptosis is one of the important factors in TEC death.[33] In addition, necroptosis can lead to rupture of the TEC plasma membrane, contributing to the release of TEC pro-inflammatory mediators, DAMP and

cytokines,[34,35] accompanied by dendritic cell (DC) activation and initiation of T cell cross-population, which drives the adaptive immune system and exacerbates renal injury. Necroptosis also impairs renal tubular regeneration, delaying the recovery of renal function after AKI and leading to fibrosis and irreversible loss of nephron.[36] The two modes of cell death, Necroptosis and Ferroptosis, are referred to as necroinflammation as both are associated with the release of DAMPs, thereby creating an inflammatory response to necrotic debris. Thus in the event of unsuccessful prevention of the original wave of initially regulated necrosis, inflammation and a second wave of inflammatory cytokine-mediated regulated necrosis such as necroptosis may be triggered.[37] In such cases, it is necessary to intervene in inflammation-associated cell death, which is the common clinical practice to intervene with therapy after the diagnosis of AKI.

Pyroptosis

Pyroptosis, a pro-inflammatory form of regulated necrosis, is thought to play a role in the pathophysiology of AKI. The key feature of pyroptosis is the creation of cell membrane pores from N-terminal fragments formed by the cleavage of gasdermins,[38,39] which releases intracellular contents and DAMPs, leading to a pronounced inflammatory response.[40] Caspases and Gasdermins (GSDMs) family of molecules play a crucial role in the activation of focal death.[41] Unlike apoptosis, pyroptosis is dependent on the activation of caspase-1, which in turn is involved in innate and adaptive immune responses. In addition, the potential of caspase 3 to cleave gasdermin E (GSDME) and caspase 8 to cleave gasdermin D (GSDMD) provides a link between apoptosis and Pyroptosis.[42] Available evidence supports the protective effect of inhibiting Ferroptosis and Necroptosis in various preclinical models of AKI, including preclinical models of IRI-inducedAKI, poison-induced AKI, crystal-induced AKI, and cytokine storm-associated AKI, whereas the role of targeting pyroptosis in AKI is still controversial.[43–45]

TECs regeneration in AKI

Under physiological conditions, TECs maintain a quiescent state, refraining from active mitosis. Nonetheless, tubular regeneration and successful renal repair are observed in most patients with mild AKI. Surviving TECs undergo adaptive tubular repair, undergoing dedifferentiation and proliferation in a microenvironment of balanced inflammation and tubular response to replenish lost cells and restore injured renal tubules.[46] Findings from genetic fate mapping studies in mouse models of AKI have elucidated that extrarenal tubular or non-tubular kidney cells do not partake in the regeneration of epithelial cells, highlighting the confinement of tubular regeneration within the tubules.[47,48] However, this observation does not preclude the likelihood of resident intratubular progenitor cells facilitating epithelial repair, thus instigating ongoing debates regarding the precise origin of proliferating intratubular cells following injury.[49,50] The hypotheses below represent the prevailing perspectives in the current scientific discourse (Fig. 1).

Resident intra-tubular progenitor/stem cell population

The first conjecture posits the presence of pre-existing, fixed progenitor cell populations within the renal tubules, governing the process of tubular regeneration following injury. This hypothesis primarily derives support from the identification of $CD133^{+}CD24^{+}$ cells implicated in the repair of proximal tubules in both human and murine systems.[51,52] In vitro experiments have demonstrated that these cells not only express stem cell markers but also display characteristics akin to mesenchymal stem cells, exhibiting self-renewal capability and generating multiple clone cell lines upon induction.[53] Conversely, in vivo studies of pericytes from most organs lacked similar multipotentiality, highlighting the discrepancy with in vitro findings.[54]

Genetic lineage tracing techniques have proven effective in locating and discerning subpopulations of cells promoting tubular repair in the postnatal kidney of rodents. However, translating this approach directly to human studies poses challenges. Rinkevich et al.[55] have verified the existence of specific progenitor cells within the glomeruli and segments of the tubules. In contrast, Corbeil et al.[56] observed abundant expression of CD133 in the proximal tubules of healthy mouse kidneys, casting uncertainty on the precise stem/progenitor cell labeling of this cell population. Owing to the limitations in conducting genetic fate mapping analyses on $CD133^{+}CD24^{+}$ cells in the human body and the contentious expression patterns observed in mouse kidneys, coupled with the lack of well-defined molecular characteristics, conclusive evidence to substantiate the existence of stem/progenitor cell populations within the renal unit is currently elusive. Nonetheless, the potential presence of these specialized TECs, harboring potent in vivo replicative capacity, remains a topic of interest and ongoing investigation.[49]

Scattered tubular cell (STC) phenotype

The second hypothesis posits that all surviving TECs possess the potential to differentiate and the probability to transiently adopt the scattered tubular cell (STC) phenotype during the repair process. This enables them to re-enter the cell cycle, undergo proliferation, and differentiate to effect tubular repair, eventually reverting back to their original TEC state. Some researchers have conducted genetic lineage tracing studies in mice, revealing that during the post-injury repair phase, there was no decline in genetic markers, suggesting that

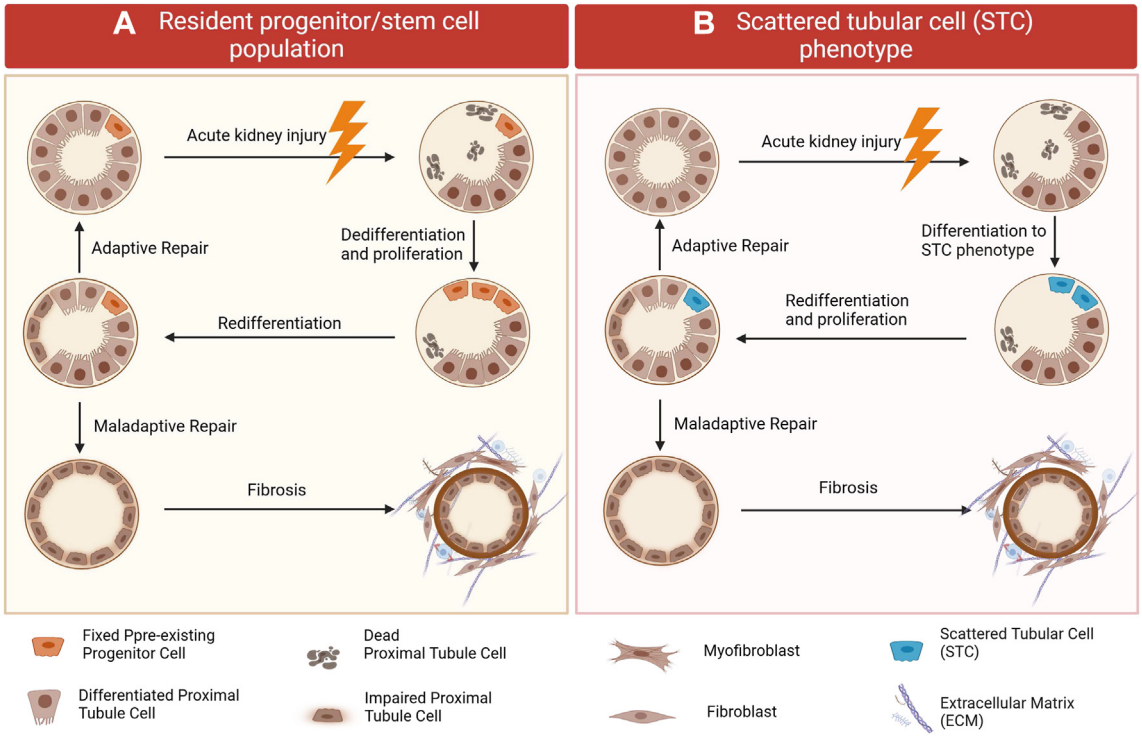

***Fig. 1:* Origin of proliferating tubule cells**. A. The first hypothesis suggests that renal tubular repair originates from a pre-existing population of fixed progenitor cells that exhibit MSC-like characteristics, possess self-renewal capacity, and are induced to give rise to multiple clonal cell lines. B. The second hypothesis suggests that renal tubular repair originates from all surviving TECs, which are all capable of temporarily adopting a scattered tubular cell (STC) phenotype and re-entering the cell cycle, thus having the potential to differentiate.

undifferentiated progenitor cells might not directly participate in kidney repair, raising uncertainties regarding the existence of renal stem/progenitor cells.[47]

Numerous evidence have demonstrated that after IRI in mice, certain stem cell markers such as CD24, CD133, keratin 19, Bcl2, KIM-1, and vimentin are upregulated, implying that $CD133^{+}CD24^{+}$ cells may represent injured TECs.[53,57] Continuous doxycycline labeling experiments revealed that the number of labeled TECs before injury did not increase, while the number of labeled cells significantly rose after injury.[58] Although this labeling does not precisely confirm the precise proliferation mode of surviving TECs, it supports the notion that repair is likely executed by randomly surviving injured epithelial cells, as a portion of these cells express $Kim\text{-}1^{+}Ki67^{+}$. Another study employing cell lineage tracing demonstrated that proximal tubule cells could transiently acquire regenerative properties upon different injuries, displaying robust regenerative potential.[59] The finding further reinforces the hypothesis that there is no distinct population of progenitor cells in the kidney, and remaining tubular cells can temporarily acquire a transient regenerative phenotype and engage in regenerative repair. And therefore these cells were called as STCs, which are smaller than differentiated tubular cells, with a flat cell morphology and epithelial polarity deficit,[50] a state of dedifferentiation of epithelial cells in response to injury.

Taken together, these findings collectively suggest that renal tubular regeneration occurs through the differentiation of tubular cells, which undergo dedifferentiation, proliferation, and subsequent regeneration of renal tubules, accompanied by transient phenotypic changes. Due to the scarcity of human tissue samples from entirely healthy individuals and the prevalent use of healthy and young experimental animals in rodent studies, additional experiments are imperative to further investigate and elucidate these hypotheses.

Maladaptive repair in TECs after AKI

Normally, TEC have a strong self-renewal ability and can be rapidly repaired when the damaging factors are removed. However, severe or repeated TEC injury leads to non-adaptive repair with cell cycle block, mitochondrial dysfunction and metabolic reprogramming, which will lead to the development of renal inflammation and fibrosis (Fig. 2). Thus, it is well recognized that TEC may face adaptive repair and maladaptive repair after injury which will determine the fate of the kidney (Fig. 3).

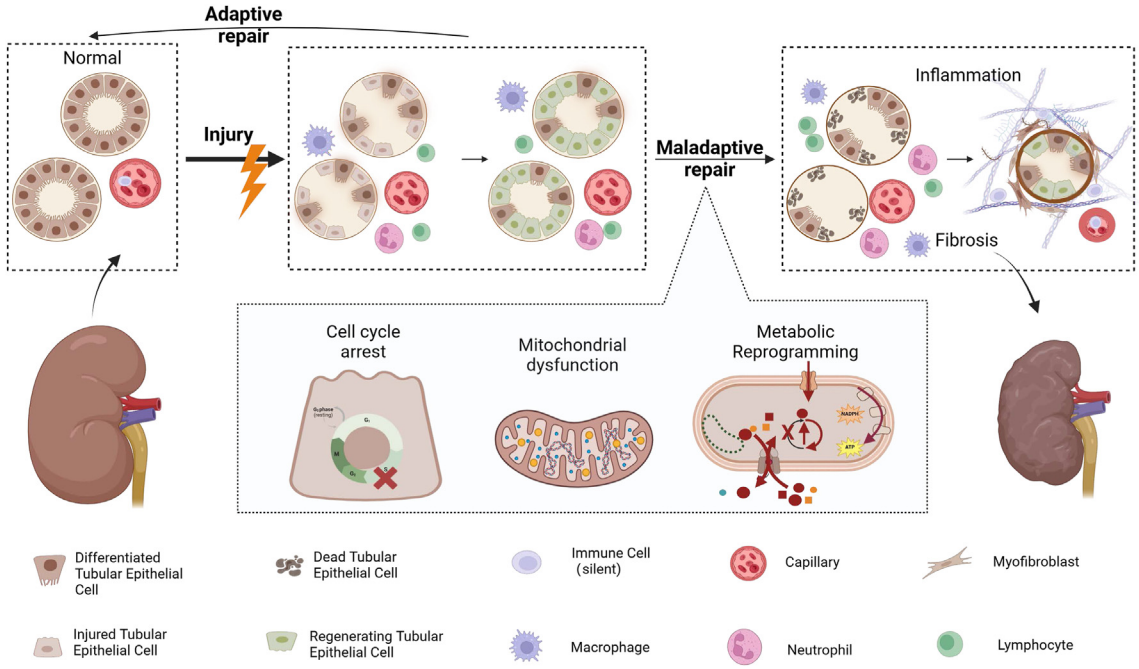

***Fig. 2:* The shematic diagram of major cell state transitions in TEC after AKI.** After AKI, dead TECs release pro-inflammatory and pro-fibrotic factors that chemotaxis immune cells to the site of injury to engulf themselves and repair injured TECs. In less severe injuries, TEC cells undergo adaptive repair and kidney function is restored. However, in the presence of non-adaptive repair such as persistent metabolic reprogramming, mitochondrial dysfunction, and cell cycle blockage, renal fibrosis will eventually result.

Pro-inflammatory phenotype of TECs

Following AKI, damaged dedifferentiated TEC have the ability to transform into a pro-inflammatory phenotype and release a variety of pro-inflammatory mediators that attract immune cells to the site of injury. Damaged TEC exhibiting an inflammatory phenotype can induce the production of a variety of pro-inflammatory cytokines and chemokines, including interleukins (IL), TNF, colony stimulating factor (CSF), growth factors, and the CC chemokine ligand (CCL) subfamily. Notably, activated TECs have been demonstrated to secrete various cytokines, either through autocrine or paracrine mechanisms, such as IL-1β, IL-18, IL-16, IL-34, TNF-α, TWEAK, Fas ligand, connective tissue growth factor (CTGF), and vascular endothelial growth factor (VEGF).[60–62] Additionally, Chi et al,[63] have reported substantial secretion of IL-36α by TECs following injury. Notably, this cytokine promotes IL-1β secretion in macrophages and DC by activating the NLRP3 inflammasome and also induces DC-mediated $CD4^+$ and $CD8^+$ T cell proliferation, thereby promoting interstitial inflammation and fibrosis induced by unilateral ureteral obstruction.

The upregulation of the CCL subfamily, particularly CCL2 (namely monocyte chemoattractant protein-1, MCP-1), is associated with progressive tubulointerstitial inflammation.[64] Furthermore, injured TECs exhibit increased expression of other chemokines, such as CXC chemokine ligand 8 (CXCL8) and CXCL12, which possess chemoattractant properties towards various leukocyte populations.[65] Notably, a previously study also revealed that following the induction of nephrotoxic nephritis, CXCL5 levels escalate in renal tubular cells, thereby playing a crucial role in facilitating neutrophil recruitment during episodes of AKI.[66]

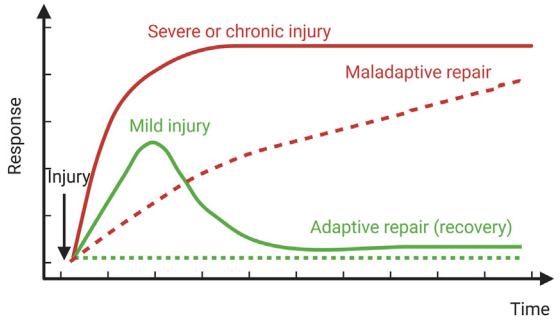

***Fig. 3:* Injury response and prognosis of adaptive versus maladaptive repair.** Two scenarios that may occur in the kidney after AKI with varying degrees of injury are depicted. In the first scenario, mild injury activates the innate immune response in time, leading to adaptive tubular repair and kidney regeneration. In the second scenario, sustained or severe injury leads to phenotypic changes in TEC and maladaptive tubular repair.

In addition, TECs may interact with immune cells through exosomes that carry signaling molecules, thus facilitating tubulointerstitial inflammation. Our group firstly demonstrated that TECs release exosomes harboring MCP-1 mRNA in various models of kidney injury. This release, in turn, activates and attracts macrophages, thereby promoting tubulointerstitial inflammation.[67] Subsequent gene chip screening investigations have disclosed that these exosomes exhibit a substantial abundance of miR-19b-23a, which triggers activation of the NF-κB pathway by inhibiting the target gene suppressor of cytokine signaling 1, ultimately leading to M1 polarization of macrophages and fostering renal interstitial inflammation.[68] Moreover, we also demonstrated that hypoxic TECs induced the release of exosomes enriched with microRNA-23a to macrophages, thereby facilitating macrophage activation through inhibition of the target gene A20, subsequently culminating in tubulointerstitial inflammation.[69] These findings collectively underscore the notion that TECs employ exosomes as a way of communication with macrophages, thereby contributing significantly to the progression of tubulointerstitial inflammation.

Toll-like receptors (TLRs), a family of transmembrane receptors, are abundantly expressed in TECs and play a crucial role in recognizing signals and activating effector cells via several kinases and NF-κB-dependent mechanisms.[70] Notably, conditions such as IRI-[71] and septic AKI[72,73] lead to the release of various DAMPs from necrotic TECs, acting as danger signals to trigger innate immune responses through TLRs, Nod-like receptors, and NLRP3 inflammasome, subsequently resulting in inflammation.

Pro-fibrotic phenotype of TECs

Following severe or recurrent injury, accompanied by the expression and production of tubulointerstitial fibrosis (TIF)-related tubule-derived factors, TECs can undergo a pro-fibrotic phenotypic shift. Pro-fibrotic cytokines produced by injured TECs, such as transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF), Wnt1, and hedgehog (Hh), can contribute to fibroblast activation.

TGF-β and PDGF have long been recognized as the important pro-fibrotic growth factors.[74] Upon stimulation, TECs could be induced to produce large amounts of TGF-β1, which promotes TIF via paracrine signalling and activates the transformation of neighbouring fibroblasts and pericytes into myofibroblast-type cells.[75] Interestingly, TECs are also one of primary target of TGF-β1 which induces the phenotypic change of myofibroblasts with production of collages.[76] Autocrine TGF-β signalling could also promote the expression of PDGF-β by injured TEC.[77] Its ligand is highly up-regulated only in injured tubules.[78] The expression of PDGF-β by injured TEC may increase the phosphorylation of the PDGF receptor and subsequent signalling on neighbouring fibroblasts, thereby promoting TIF.[79]

The Wnt pathway and Hh signalling are key pathways that regulate cell growth and differentiation and are implicated in the TEC repair process.[80] However, a large body of evidence also supports a role for Wnt/β-catenin signalling in TIF.[81,82] Growing evidence reveal that Wnt proteins and receptors are up-regulated after renal injury, and that β-catenin activity seems to be increased in injured TEC.[83] Injured TECs may produce Wnt ligands, which then activate neighbouring fibroblasts to promote TIF.[84] There are three ligands for Hh, of which Sonic Hh (Shh) has been well studied.[85] Spectral tracing studies have demonstrated up-regulated expression of Shh in the tubules of patients with kidney injury, and that the latter induces fibroblast activation, manifested as α-SMA, fibronectin, collagen and desmin expression.[86]

Cell cycle arrest

The intricate process of cell cycle arrest in TECs following AKI demonstrates both a protective mechanism and a pathological consequence.[87] Initially, during the onset of AKI, cell cycle arrest plays a critical role in preventing the replication of damaged DNA, particularly observed at the G1 phase.[88] This arrest is governed by a complex interplay of cyclin-dependent kinases (CDKs), cyclins, and their inhibitors (CKIs).[89] When induced by AKI-related stress, p53 is stabilized and accumulates within the cell. In addition to halting cell cycle progression, p53 also activates genes responsible for DNA repair and apoptosis.[90] However, this p53-dependent cell cycle arrest serves as a double-edged sword. While aiming to minimize immediate damage by preventing the proliferation of cells with genomic instability, it also results in the accumulation of senescent TECs that are unable to contribute to tissue regeneration.[91,92]

The release of pro-inflammatory cytokines, chemokines, growth factors, and extracellular matrix (ECM) remodeling enzymes by senescent TECs, facilitated by a senescence-associated secretory phenotype, establishes a local pro-inflammatory environment.[93] This conducive environment paves the way for fibrosis, leading to maladaptive repair and eventually contributing to CKD.[94] The transition from a protective to a pathological role of cell cycle arrest emerges as a critical determinant of the long-term outcome following AKI. Recently, therapeutic interventions have targeted the modulation of the cell cycle machinery, with a focus on promoting cell cycle re-entry through selective inhibition of p21.[95] Studies have shown that this approach enhances tissue regeneration in preclinical models. Additionally, exploration of small molecule inhibitors of CDK4/6 aims to bypass G1 arrest and reinitiate the cell cycle in TECs.[96] These strategies aim to transiently and selectively encourage proliferation in the context of injury, with the

objective of optimizing tissue repair while mitigating the risk of adverse outcomes such as fibrosis or oncogenesis.

Furthermore, the understanding of the cell cycle's role in TEC pathology has significantly expanded, presenting new avenues for intervention that could transform the management of AKI and its sequelae. Moreover, the emerging field of regenerative medicine is exploring the use of stem cell-derived exosomes, which carry pro-regenerative microRNAs and proteins, to modulate the cell cycle checkpoints and promote the recovery of TECs after AKI.

Mitochondrial dysfunction in TECs

After AKI, mitochondrial dysfunction in TECs plays a crucial role in the disruption of cellular metabolism, which in turn exacerbates the severity of the initial injury and undermines subsequent repair mechanisms.[97] TECs rely on a dense mitochondrial network to meet their high energy demands.[98] However, following injury, this network undergoes significant structural and functional changes. These changes manifest as mitochondrial fragmentation, reduced mitochondrial biogenesis, and a shift in metabolic substrate preference from fatty acids to glucose.[99] As a consequence, there is an overall decrease in ATP production, which is essential for maintaining cellular homeostasis.

Mitochondrial damage is primarily driven by oxidative stress, which results from an increase in ROS and leads to damage of mitochondrial DNA (mtDNA), lipids, and proteins.[100] This process further exacerbates mitochondrial dysfunction and compromises the activity of the electron transport chain (ETC).[101] Consequently, ATP synthesis is reduced, while ROS production is enhanced, creating a vicious cycle that culminates in energy depletion and cell death.[102] Mitochondrial autophagy, or mitophagy, serves as a cellular quality control mechanism, selectively removing damaged mitochondria. However, dysregulation of mitophagy has been implicated in the pathogenesis of AKI. Excessive or insufficient mitophagy contributes to the accumulation of dysfunctional mitochondria, rendering the cell more susceptible to additional injury and impairing its regenerative capacity.[103]

Following AKI, the insufficient activation of peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) in TECs is a critical factor contributing to maladaptive repair.[104] PGC-1α functions as a transcriptional coactivator, modulating genes involved in mitochondrial replication and repair.[105] Consequently, the suboptimal upregulation of PGC-1α post-AKI hinders the restoration of mitochondrial density and function necessary for recovery in TECs.[105] Therapeutic strategies targeting mitochondrial dysfunction have emerged as crucial in improving AKI outcomes. These strategies encompass pharmacological agents designed to enhance mitochondrial biogenesis, antioxidants to minimize ROS-mediated damage, and modulators of mitophagy that balance the removal of damaged mitochondria with the need for mitochondrial turnover.[106] Additionally, investigations into targeted delivery of mitochondrial protective agents, such as Szeto-Schiller peptides with antioxidant effects targeted at the inner mitochondrial membrane, are underway to prevent TEC damage and promote renal recovery.[107] Moreover, recent advances in mitochondrial transplantation therapy, involving the introduction of healthy mitochondria into injured cells, show promise in restoring cellular bioenergetics and function in experimental AKI models.[108] These innovative approaches highlight the potential of targeting mitochondrial health to boost the intrinsic regenerative capacity of TECs and present promise for novel AKI treatments.

Metabolic reprogramming of TECs in AKI

Under pathological conditions, the mitochondria of TEC are severely damaged, leading to disruption of oxidative phosphorylation and disturbances in energy metabolism, as well as alterations in the utilization of metabolic substrates (e.g., glucose, amino acids, fatty acids, ketone bodies, citric acid and lactic acid).[109] The metabolic flexibility of TECs is a critical determinant of their survival and function, especially in the context of AKI. Metabolic reprogramming in TECs is a hallmark of their response to injury, characterized by a significant shift from oxidative phosphorylation to glycolysis, along with changes in lipid metabolism.[110,111] This adaptive response, while initially beneficial for cell survival under hypoxic conditions, may become maladaptive if sustained contributing to secondary injury to TECs.

Lipid metabolism reprogramming in TECs

During AKI, TECs undergo a critical adaptive response to cellular stress by reprogramming lipid metabolism. Initially, this response compensates for the cellular energy imbalance; however, dysregulation can create a paradoxical, maladaptive effect.[112] Notably, TECs in AKI undergo a marked decrease in fatty acid oxidation (FAO), a key mitochondrial process for maintaining cellular energy homeostasis.[113] This decrease in FAO leads to the accumulation of lipid droplets, indicating a shift toward lipid storage rather than utilization. Consequently, this metabolic shift contributes to a phenotype of lipid overload, which has been linked to various adverse effects, including cellular lipotoxicity, heightened inflammatory response, and further impairment of mitochondrial function.[114]

The dysregulation of PPARs, a group of nuclear receptor proteins that regulate gene expression in response to lipid ligands, profoundly influences the intricate mechanisms governing lipid metabolism in TECs. Post-AKI, PPARα, a master regulator of genes involved in FAO, has been found to be significantly

downregulated in TECs, leading to reduced FAO capacity and promoting lipid accumulation.[115] This subsequently exacerbates cellular injury through inducing endoplasmic reticulum stress and activating inflammatory signaling pathways, such as NF-κB and JNK, contributing to the pathogenesis of AKI and its progression to CKD.[116] Moreover, ongoing research is elucidating the complex role of sphingolipid metabolism in TECs, particularly the pivotal balance of ceramide and sphingosine-1-phosphate (S1P) levels.[117] In the context of AKI, ceramide accumulation has been associated with the induction of TEC apoptosis, while S1P has been identified as a crucial pro-survival factor.[118] The emerging potential therapeutic target, sphingosine kinase 1 (SphK1), which converts ceramide to S1P, offers an opportunity to modulate the balance of ceramide and S1P.[119] Therefore, the modulation of SphK1 activity and the ceramide/S1P axis represents a novel strategy for reducing TEC apoptosis and promoting cell survival.[120]

The therapeutic implications of these findings are profound. Interventions aimed at restoring PPARα signaling, and thus normalizing FAO, have the potential to mitigate lipid overload and its associated cellular stress responses.[121] Moreover, the pharmacological manipulation of sphingolipid metabolism to favor the generation of S1P over ceramide could provide a double-edged therapeutic benefit by not only reducing pro-apoptotic signals but also enhancing the pro-survival pathways. Compounds such as fenofibrate, a PPARα agonist, and FTY720, a SphK1 activator, are currently under investigation for their efficacy in modulating lipid metabolism in TECs post-AKI.[122]

Furthermore, the interplay between lipid metabolism and autophagy in TECs is gaining attention as a potential therapeutic target. Autophagy, a cellular degradation pathway, has been implicated in the breakdown of lipid droplets, a process termed lipophagy.[123] Enhancing lipophagy could serve as a strategy to clear lipid overload, reduce ER stress, and restore cellular homeostasis. The development of autophagy modulators that can specifically enhance the degradation of lipid droplets without inducing autophagic cell death is a promising area of research.[124]

Glycolytic shift in TECs

During AKI, TECs undergo an adaptive shift to glycolysis as a response to the reduced oxygen availability and mitochondrial compromise characteristic of the condition. This is manifested by the pronounced occurrence of the Warburg effect, in which cells escalate glycolysis despite the presence of oxygen.[125] Hypoxia-inducible factors (HIFs) play a pivotal role in orchestrating this metabolic reprogramming within TECs during AKI by inducing the expression of a wide array of genes that facilitate an increased glycolytic flux.[126,127] Genes coding for glucose transporters and key glycolytic enzymes are transactivated by HIFs, thus augmenting the uptake of glucose and its conversion to lactate.[128] Lactate has long been considered a metabolic waste product. Emerging evidence in recent years suggests that lactate plays an important signaling molecular function in pathophysiological processes such as glucose metabolism, redox homeostasis, post-translational modification of proteins and tumour immune tolerance.[129] Elevated serum lactate levels are considered an indicator of AKI severity, both because this metabolic shift leads to greater lactate accumulation in damaged TECs (whether successfully repaired or not),[130] and because excessive lactate production can in turn mediate TEC damage and thus exacerbate AKI.[131] Mechanistically, lactate can selectively stimulate endoplasmic reticulum Mg^{2+} release, thereby promoting mitochondrial dysfunction aggravating Lipopolysaccharide mediated renal dysfunction.[132]

Recent studies have identified the AMP-activated protein kinase (AMPK) pathway as a critical regulator of the glycolytic switch in TECs, in addition to HIFs.[133] AMPK activation enhances glucose uptake and glycolysis to replenish ATP stores during energy stress. However, chronic activation of AMPK in TECs post-AKI has been linked to the development of TIF, indicating that while this response is initially protective, it may contribute to disease progression if sustained. Another layer of complexity is added by the pentose phosphate pathway (PPP), which branches from glycolysis and serves two major functions: it generates NADPH for reductive biosynthesis and antioxidant defense, and it provides ribose-5-phosphate for nucleotide synthesis.[134] An upregulated PPP during AKI could represent an adaptive response to increase the antioxidant capacity of TECs, thus protecting against oxidative damage. However, the exact role of the PPP in the context of AKI and its long-term implications on TEC function and survival remain to active investigation.[135]

The modulation of the glycolytic pathway in TECs presents potential for therapeutic intervention in AKI. In the early phase of AKI, stabilizing HIFs may promote cell survival under hypoxia.[135] However, prolonged stabilization of HIFs could be detrimental by promoting fibrosis, suggesting a narrow therapeutic window or the necessity for targeted delivery systems. Inhibitors of glycolysis, such as 2-deoxy-D-glucose, have the potential to reduce the acidosis associated with lactate accumulation.[136] However, their use must be carefully timed to avoid exacerbating energy deficits during critical injury phases. Furthermore, the targeting of AMPK signaling provides another therapeutic opportunity. AMPK activators could be beneficial in the acute phase of AKI to promote ATP production, but their long-term use may need to be avoided to prevent fibrotic outcomes. Therefore, developing strategies to temporally modulate AMPK activity could represent a key approach in optimizing TEC energy homeostasis during AKI repair.[137] In

summary, the glycolytic shift in TECs during AKI is a complex, multifaceted response that presents both opportunities and challenges for therapeutic intervention.

TECs crosstalk in AKI

The recovery from AKI is highly dependent on the intricate communication between renal TECs and various resident kidney cells and immune cells (Fig. 4). This intercellular crosstalk is pivotal in coordinating the repair processes, or, conversely, in precipitating maladaptive responses leading to fibrosis. Understanding the nuances of these interactions offers a window into novel therapeutic strategies to mitigate AKI and prevent its progression.

Crosstalk between TECs and adjacent epithelial cells in AKI

The regenerative response following AKI relies heavily on the restorative dialogue between TECs and adjacent epithelial cells.[138] This interaction involves a complex interplay of signaling molecules and direct cell-to-cell contact facilitated by junctional complexes.[139] Notably, the Wnt/β-catenin signaling pathway, recognized for its critical role in development and tissue homeostasis, stands out as a crucial mediator in this context. TEC-

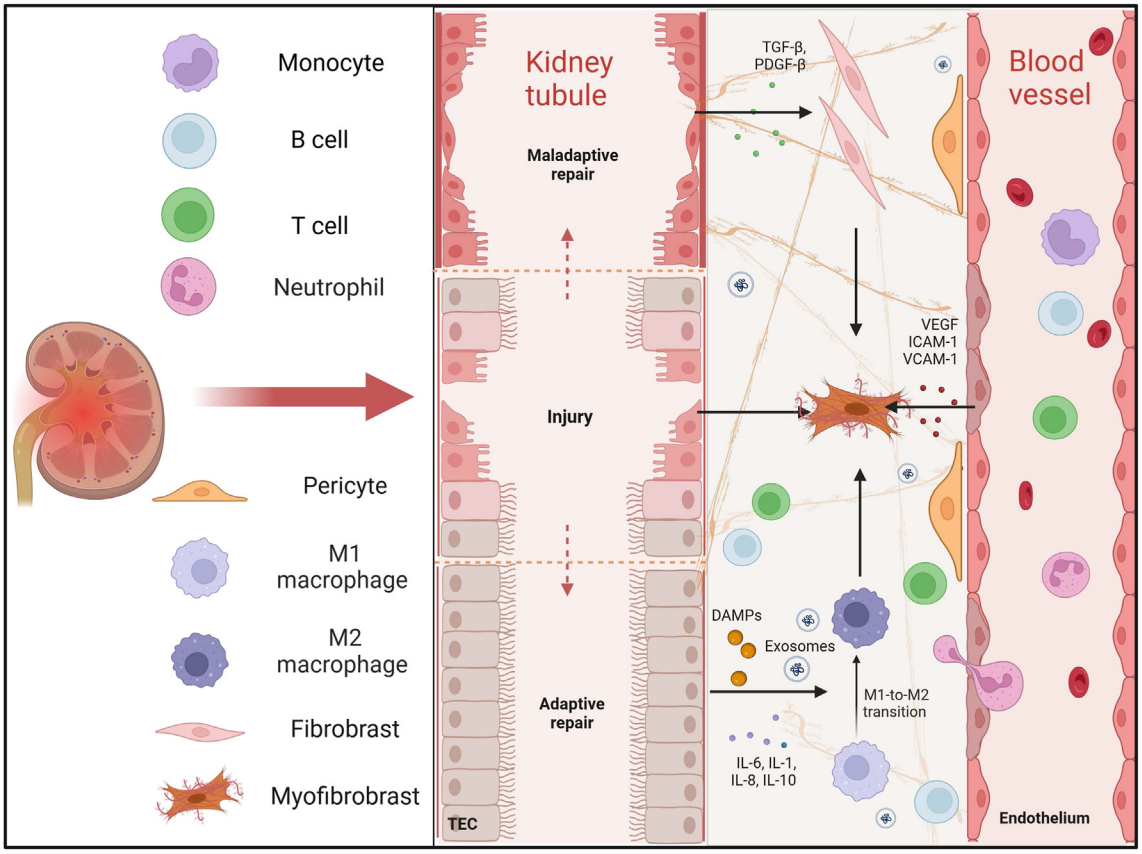

***Fig. 4:* Crosstalk of TEC with other cells in renal repair after AKI.** Interactions between TECs, immune cells and mesenchymal fibroblasts play an important role in renal repair. After AKI, dead TECs trigger inflammation by releasing DAMPs, which activate PRRs on macrophages and recruit immune cells to the site of injury. Surviving TECs undergo dedifferentiation and proliferation and repopulate the tubular basement membrane, where they may also communicate with immune cells via exosomes containing signaling molecules. During renal repair, pro-inflammatory (M1) macrophages change phenotype to anti-inflammatory (M2) macrophages. Pro-fibrotic factors produced by injured TECs also contribute to fibroblast activation into myofibroblasts. In addition to supporting tubular remodeling by providing mechanical support through ECM synthesis, myofibroblasts promote the repair and proliferation of TECs through intercellular contact signaling and paracrine signaling and even the secretion of pro-regenerative factors. The interaction of TECs with endothelial cells is also a key aspect of renal repair, with TECs releasing a wide range of angiogenic factors to promote endothelial cell proliferation and migration, and endothelial cells being able to participate in renal vascular repair and regeneration by regulating chronic inflammatory responses. Disruption of communication between TECs and endothelial cells leads to maladaptive repair and fibrosis. Abbreviations: TEC, tubular epithelial cell; DAMP, damage-associated molecular pattern; PRRs, pattern recognition receptor; ECM, extra cellular matrix; IL, interleukin; TGF, transforming growth factor; PDGF, platelet-derived growth factor; ICAM, Intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

secreted Wnt ligands, such as Wnt9b, initiate a signaling cascade in neighboring epithelial cells, essential for activating regenerative processes, including cellular proliferation, differentiation, and migration crucial for tubular integrity restoration.[140] Besides Wnt signaling, the Notch pathway also plays a pivotal role in the regenerative crosstalk. Notch receptors, upon interaction with their ligands, such as Jagged1, undergo proteolytic cleavage, releasing the Notch intracellular domain, which translocates to the nucleus, influencing the expression of genes involved in cell fate determination.[141] Consequently, the altered expression of Notch pathway components in TECs post-AKI significantly impacts the balance between regenerative repair and maladaptive remodeling. While moderate activation of Notch signalling supports regeneration, dysregulation has been implicated in promoting TEC dedifferentiation and proliferation, contributing to renal fibrosis pathogenesis.[142] Furthermore, Hh signaling, primarily known for its roles in embryonic patterning and organogenesis, has been identified as a key adult TEC plasticity regulator and communication facilitator with neighboring cells. The paracrine activity of Hh ligands, such as Shh, secreted by injured TECs, influences the epithelial response to injury, impacting cell survival, angiogenesis, and the anti-fibrotic response.[80]

Crosstalk between TECs and endothelial cells in AKI

During the reparative phase following AKI, the interaction between TECs and endothelial cells in the renal microenvironment is a critical determinant of recovery and restoration of renal function. This bidirectional communication not only modulates the repair of the injured TECs but also contributes to the regeneration of the renal vasculature, which is vital for re-establishing homeostasis and tissue oxygenation.[143] TECs release various angiogenic factors, including vascular endothelial growth factor (VEGF), angiopoietin-1, and hepatocyte growth factor post-AKI, which play a significant role in promoting endothelial cell proliferation, migration, and tube formation, aiding in the repair and regeneration of the renal vasculature.[144] In particular, VEGF is a key pro-angiogenic factor that enhances endothelial cell survival and vascular permeability, with the VEGF signaling pathway being upregulated in TECs following ischemic injury, indicating its pivotal role in the repair process.[145]

However, disrupted TEC-endothelial cell communication can lead to pathological outcomes, such as capillary rarefaction, persistent hypoxia, and the progression of AKI to CKD. Endothelial dysfunction in the form of reduced nitric oxide production and increased endothelin-1 (ET-1) secretion is a common consequence of AKI, contributing to a pro-inflammatory and pro-fibrotic environment that exacerbates tissue damage.[146] Furthermore, endothelial expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), facilitates the recruitment of inflammatory cells, further promoting a fibrotic response. Recent study has also focused on the role of endothelial-to-mesenchymal transition (EndMT) in AKI, during which endothelial cells lose their characteristic markers and acquire mesenchymal features, contributing to the fibrotic process.[147] TEC-derived factors, including TGF-β and IL-1β, have been implicated in driving EndMT, emphasizing a critical aspect of TEC-endothelial cell interaction that may contribute to maladaptive repair and fibrosis.[148]

Crosstalk between TECs and interstitial fibroblasts in AKI

The intricate interplay between TECs and interstitial fibroblasts determines the kidney's response following AKI, whether it transitions towards repair or becomes fibrotic. This interaction is governed by a complex network of signaling molecules, ECM components, and direct cell–cell contacts, collectively steering the repair process or driving fibrosis pathogenesis.[149] In response to AKI, TECs experiencing stress or undergoing partial epithelial–mesenchymal transition (EMT) release various fibrogenic cytokines, including the pivotal TGF-β, which drives fibroblast activation and their transformation into myofibroblasts, the principal source of ECM proteins in the injured kidney, leading to tissue scarring and organ dysfunction.[150] Additionally, TEC-derived CTGF and PDGF promote fibroblast proliferation and ECM production, contributing to fibrosis.[151] Recent study has highlighted the role of IL-11 in TEC-fibroblast crosstalk, with injured TECs secreting IL-11 to induce a pro-fibrotic phenotype in fibroblasts, characterized by increased ECM production and resistance to apoptosis.[152] The IL-11 signalling pathway presents a promising target for anti-fibrotic therapy, offering a potential avenue to halt the fibrogenic cascade triggered by TECs.[153] Moreover, physical interaction between TECs and fibroblasts, facilitated by cell adhesion molecules such as integrins and ECM proteins like fibronectin and collagen, can activate signaling pathways, leading to fibroblast activation and ECM deposition.[154] Understanding these molecular interactions is essential for developing strategies to disrupt the profibrotic TEC-fibroblast communication. The modulation of TEC-fibroblast crosstalk offers a promising therapeutic strategy for preventing or treating fibrosis post-AKI.[155]

The intricate and multifaceted crosstalk between TECs and interstitial fibroblasts post-AKI plays a pivotal role in the pathogenesis of kidney fibrosis.[156] A deeper understanding of these interactions will be instrumental in identifying new therapeutic targets and developing effective treatments to halt the progression of AKI to chronic kidney disease. Targeting the TGF-β signalling pathway with specific inhibitors or neutralizing antibodies can potentially prevent fibroblast

activation and myofibroblast transformation.[157] Similarly, antagonists of CTGF or PDGF signaling pathways could inhibit the proliferation of fibroblasts and their ECM-producing activity.[158] Additionally, the development of ECM-modulating agents that can disrupt the adhesive interactions between TECs and fibroblasts, thereby preventing the activation of profibrotic signaling pathways, represents an innovative approach in combating renal fibrosis. Emerging therapies focusing on IL-11 signaling inhibition are also gaining attention as potential anti-fibrotic strategies. Small molecule inhibitors or monoclonal antibodies targeting IL-11 or its receptor could offer a novel means to mitigate the fibrotic response initiated by TECs.

Crosstalk between TECs and immune cells in AKI

Inflammation plays a crucial role following AKI, where intricate bidirectional interactions between renal TECs and immune cells regulate both adaptive repair and inadequate recovery of injured kidneys. During the initial phases of AKI, interactions between TECs and innate immune cells can shift renal injury towards an immune response, pivotal for fostering adaptive tubular repair and renal regeneration. Early AKI, marked by partial TEC death, triggers the release of DAMPs, activating pro-inflammatory innate immune responses to facilitate renal repair.[159] Hence, renal innate immune cells surveil tissue homeostasis by detecting various DAMPs released during tissue injury via TLRs, nod-like receptors (NLRs), etc. These cells can then be recruited to the site of injury by signals emitted from surviving renal cells, releasing inflammatory mediators to aid in restoring injured TECs to a normal cellular state.[160,161]

In addition, inflammation is critical in regulating maladaptive renal repair and for renal inflammation and fibrosis.[162] For a long time, it was thought that the renal tubule was simply a victim of injury. Recently, however, a large body of evidence suggests that TEC plays a key role in renal repair and is involved in the development of chronic kidney disease after severe or prolonged injury.[163] Damaged TEC are activated to produce a wide range of inflammatory factors, chemokines, and growth factors, which interact with immune cells and participate in the repair process after injury. The interaction between injured tubules and immune cells is pivotal in perpetuating chronic inflammation during maladaptive renal repair and the progression to CKD. The exact mechanism, whether stemming from an aberrant inflammatory response or the incapacity of TECs to regenerate due to severe injury, remains incompletely understood. However, recent single-cell studies have revealed that cells undergoing phenotypic alterations fail to revert to their original state, persisting in a reparative phase that fuels inflammation and fibrosis, ultimately resulting in irreversible renal function decline.[164]

Among them, the dual role played by macrophages at different stages of AKI cannot be ignored.[165] It has long been hypothesized by researchers that in the early stages of IRI, macrophages are recruited and thus activated as pro-inflammatory macrophages (M1) to promote injury through the interaction of pattern recognition receptors (PRRs) with DAMPs released from damaged TECs, whereas in the later stages, macrophages change their phenotype to non-inflammatory macrophages (M2) to produce anti-inflammatory cytokines to aid in the repair of TECs.[166] In a follow-up study, Lee et al.[167] provided direct evidence for this theory by tracing fluorescently labelled macrophages. We now know that M2 macrophages have three distinct subtypes, including the M2a subtype that produces and secretes repair-promoting cytokines such as IL-10 and IL-1Rα, the M2b subtype that is readily involved in immunomodulatory processes, and the M2c subtype that can produce anti-inflammatory and fibrotic factors.[168] Furthermore, it has also been shown that macrophage-derived exosomes can transfer secreted miR-195a-5p into TECs, induce mitochondrial dysfunction, and induce TEC death. Although this classification does not truly reflect macrophage subtypes in the in vivo tissue environment, it can be argued, to some extent, that macrophages can influence the prognosis of AKI by interacting with neighboring TECs through their phenotypic heterogeneity and functional diversity.[169]

TECs responses to injury: interrogation by single-cell transcriptomics

Rapidly evolving single-cell technologies in renal research have been shown to play an important role in a dynamic process such as AKI.[170] Previous studies have highlighted the critical role of SOX9 in TEC repair and regeneration.[171,172] However, the mechanisms that trigger and regulate tubular repair are unclear. A large number of single-cell transcriptomics experiments focused on TEC have allowed us to gain a deeper understanding of the regulatory features of adaptive and maladaptive repair after AKI and its long-term consequences (Table 1).

Kirita et al.[173] were the first to characterize injured PTEC by single-cell RNAseq in a mouse model of AKI and identified a distinct pro-inflammatory and pro-fibrotic repair failure of proximal tubular cells (FR-PTC) state. Meanwhile, Rudman-Melnick et al.[174] established the first comprehensive post-AKI renal cell-specific transcriptional profile and validated the AKI characterisation in multiple experiments. Zhao et al.[156] used single-cell RNA sequencing (scRNA-seq) showed that iron death-related genes were mainly expressed in TECs after I/R injury, and thus inhibition of iron death by XJB-5-131 is expected to be an effective therapeutic measure for AKI.

By using single-nuclear transcriptomics, Gerhardt et al.[177] found by single-nuclear transcriptomics that during the injury–repair transition after AKI that

Ref.	Species	Samples	Findings
Kirita et al., 2020[173]	mouse	kidney	First characterisation of injured PTC by single-cell RNAseq in an AKI mouse model, gene expression changes during repair suggest new therapeutic targets.
Rudman-Melnick et al., 2020[174]	mouse	kidney	The first comprehensive renal cell type-specific transcriptional profile, potentially pathological epithelial interstitial crosstalk.
He et al., 2020[175]	human	kidney	Cellular evidence for a potential pathway for SARS-Cov-2 to cause renal injury via angiotensin-converting enzyme 2.
Zhao et al., 2020[156]	mouse	kidney	After IRI, Ferroptosis-related genes are predominantly expressed in TEC, and inhibition of iron mutations by XJB-5-131 is an effective therapeutic strategy for AKI.
Melo Ferreira et al., 2021[176]	human/ mouse	kidney	Spatial transcriptional features were characterized for two mouse AKI models and the approach was mapped to human kidney tissue.
Gerhard et al., 2021[177]	mouse	proximal tubule cells	The diversity of PTC states during the AKI injury–repair transition was revealed, with injury spreading from the cortico-medullary boundary to the cortical regions.
Dixon et al., 2022[178]	mouse	kidney	The new spatial transcriptome sequencing platform was used to generate a spatial map of gene expression in AKI and measure the dynamics of cell–cell interactions.
Cheung et al., 2022[179]	human	urine	Differences in cellular composition and gene expression, several inflammatory immune cell populations and different activation pathways revealed using urinary sediment from COVID-19-associated AKI patients.
De Chiara et al., 2022	mouse	kidney	The distribution of tubular cells that undergo polyploidy during AKI is characterized, revising current pathophysiological concepts of how the kidney responds to acute injury. In addition, targeting YAP1 was found to be a novel drug target to improve the prognosis of AKI survivors.
Hinze et al., 2022[180]	human	kidney	Single-cell transcriptomics reveals common epithelial response patterns in human AKI, highlighting recurrent AKI-associated signatures and inter-individual heterogeneity.
Klocke et al., 2022[181]	human	urine	Non-invasively capturing renal pathological changes in human AKI and reflecting different injury and repair processes including oxidative stress, inflammation and tissue rearrangement.
Gerhardt et al., 2023[182]	mouse	kidney	Lineage tracing and single nucleus multi-omics reveal the regulatory signature of adaptive and maladaptive renal repair after AKI and its long-term consequences.
Chen et al., 2023[128,183]	mouse	kidney	A robust multi-model AKI scRNA atlas was constructed, providing a powerful resource for understanding the homogeneity and heterogeneity of the cytopathological mechanisms of different etiology-induced AKI.
Wang et al., 2023[184]	human/ mouse	kidney/MSCs/ HK2 cells	The transcriptomic diversity of renal TECs and immune cells and the reparative properties of renal progenitor/stem cells were revealed.
Ghag et al., 2023[185]	human	kidney/Urine	Biopsy tissue and urine from COVID-19 AKI patients were also analysed in order to find overlapping COVID-AKI-enriched genes and their corresponding cell types in the kidney from snRNA-seq data.
Hong et al., 2023[186]	mouse	kidney	$WT1^+$ PECs can develop into $WT1^+$ proximal renal tubular epithelial cells through a transient phase of scattered tubular cells, which are essential for proximal tubular regeneration of the kidney after severe AKI.
Lake et al., 2023[164]	human	kidney	For the first time, a high-resolution cellular atlas of the kidney covering 51 major cell types in healthy and diseased states is depicted and 28 cellular states that are altered in kidney injury are defined.

Table 1: **The studies on AKI using single-cell transcriptomics.**

activated neotranscriptional processes can reactivate diverse gene and pathway activities involved in cell adhesion, migration and proliferation in TECs. In the following study, the team also demonstrated in a follow-up study that AKI triggers cell cycle responses in most renal cells, providing insights into the regulatory features of adaptive and non-adaptive repair of PTCs and their long-term consequences.[182] Dixon et al.[178] used a new spatial transcriptome sequencing platform to compensate for the lack of centralised spatial information from single-cell sequencing technologies, making it possible to define region-specific damage and repair transcriptional responses. Responses possible. Chen et al,[183] constructed a robust multi-model AKI scRNA profile containing 20 cell types and 80,689 high-quality cells, providing a powerful resource for understanding the homogeneity and heterogeneity of the cytopathological mechanisms of AKIs caused by different etiologies, as well as early intervention in AKI progression at the time of single-cell regression. Wilms' tumor 1 (WT1) is expressed in glomerular mural epithelial cells (PECs) in adult kidneys, and Hong et al.[186] found that $WT1^+$ PECs can progress through a transient scattered tubule cell phase to $WT1^+$ PTECs, which have specific stem-cell-like properties and are critical for renal proximal tubule regeneration after severe AKI.

Human kidney tissues and cells have also been extensively studied in recent years. Melo Ferreira et al.[176] characterized the spatial transcriptional profile of two mouse AKI models using single-cell and scRNA-seq datasets and applied them to human kidney tissue, finding that the single-cell and spatial transcriptome datasets are complementary and synergistic. Hinze et al.[180] performed single-nucleus transcriptomics directly on renal tissues from human patients with critical disease-associated AKI and found that in TEC,

Search strategy and selection criteria

Data for this review were identified by searches of PubMed and references from relevant articles using the search terms "tubule", "acute kidney injury", "renal tubular epithelial cells", "cell death", "adaptive repair", "phenotypic transformation", "maladaptive repair", and "metabolic reprogramming". Only articles published in English and up to April 16th, 2024 were included.

four injury-associated cellular states were enriched, which were differentiated and characterized by transcriptomic patterns associated with oxidative stress, hypoxia, interferon response and EMT, respectively. Klocke et al.[181] analysed 42,608 single-cell transcriptomes from 40 urine samples from patients with AKI and demonstrated that the TEC transcriptome reflects renal pathology and different processes of injury and repair. Wang et al.[184] explored the potential mechanisms of MSC treatment of AKI by single-cell RNA sequencing of two groups of IRI mouse models treated with mesenchymal stem cells (MSCs) and controls, revealing transcriptome diversity of renal TECs and immune cells and repair properties of renal progenitor/stem cells. In addition, MSC-derived miR-26a-5p was found to mediate the therapeutic effects of MSCs by inhibiting pro-fibrotic TECs and their subsequently recruited immune cell subsets. The scRNA-seq technique has also been used to obtain cellular evidence of renal injury in patients with Covid-associated AKI. He et al.[175] demonstrated that SARS-Cov-2 invades and is expressed in human renal tissues via the angiotensin-converting enzyme 2 (ACE2)-related pathway. Cheung et al.,[179] on the other hand, assessed differences in cellular composition and gene expression during COVID-19-associated AKI using patients' urinary sediment, and found that transcripts of damage-related pathways were more abundant in renal tubular cells in AKI. Recent studies have analyzed both biopsy tissue and urine from patients with COVID-19 AKI in order to identify overlapping COVID-AKI-enriched genes and their corresponding cell types in the kidney from snRNA-seq data, with a potential role in disease progression.[185]

Taken together, these single-cell transcriptomics revolutions provide an unprecedented approach to understanding adaptive and maladaptive repair responses in TEC after AKI and offer the possibility of identifying new therapeutic strategies for AKI.

Promising therapeutic strategies for AKI

In current clinical practice, the treatment of AKI usually includes supportive therapy and Kidney replacement therapy, which cannot stop the progress of AKI fundamentally, but only relieve the symptoms and wait for the kidney self-repair.[187] Due to the complex pathophysiological mechanism of AKI, there is no specific drug to prevent or cure AKI.[188] A number of promising drugs are in the experimental stage, including new compounds, repurposed drugs, and nanomaterial-modified drugs that can target multiple pathways, including mitochondrial stres, inflammation, antioxidant effects, TEC death and repair mechanisms.[189,190] Exosomes are widely recognized as a focus for developing targeted therapeutic strategies for AKI.[191] Exosomes of many different cellular origins can promote angiogenesis, regulate TEC proliferation and apoptosis, inhibit inflammatory responses and oxidative stress, and prevent fibrosis progression through their contents (e.g., miRNAs, lncRNAs, circRNAs, mRNAs, and proteins) to facilitate AKI repair.[192,193] These targeted therapeutic mechanisms of action all correspond to the course of the TEC response after AKI, offering hope for the development of more effective AKI treatments in the future as a specific therapeutic direction.

Outstanding questions

In synopsis, noteworthy strides have recently been achieved in the delineation of TEC phenotypic transitions and reparative responses subsequent to AKI. The injury-induced dynamic modulation of diverse cellular states accentuates the inherent plasticity of TEC. Accordingly, after kidney injury, the responses of TEC are extremely complex. The TEC could exhibit a diversity of phenotypes, which exert unique functions in the kidney. Meanwhile, the crosstalk between cells and interactions of cell-microenvironment, which create a distinctive adaptive or maladaptive repair niche, play a vital role in the outcomes of kidney disease. To date, the application of omics technologies, especially the multi-omics, provides us a better understanding of the key responses in different stages of kidney disease. Nevertheless, given that each phenotype has its own critical signaling pathway, the key molecules that initiate and determine these responses and the exact molecular mechanisms of cell injury patterns remain poorly understood. Meanwhile, how to comprehensively explicate the entire dynamic spectrum of TEC responses to AKI, along with the intricate mechanisms underpinning their reparative processes is also an outstanding problem that needs to be solved. Thus, more intensive research is required for characterizing the distinctive responses in renal physiology and pathophysiology. Finally, it is also urgent to identify the promising therapeutic targets for treating kidney disease based on the post-injury response of tubules.

Contributors

Z.L.L., X.Y.L., L.L.L, and B.C.L. wrote the manuscript, prepared the figures, and supervised the whole project; Y.Z., and B.W. assisted in

manuscript preparation and editing. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final version of the manuscript.

Declaration of interests

The authors confirm that there are no conflicts of interest.

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