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Kidney Regeneration: Lessons from Development

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Abstract

A number of genes involved in kidney development are reactivated in the adult after acute kidney injury (AKI). This has led to the belief that tissue repair mechanisms recapitulate pathways involved in embryonic development after AKI. We will discuss evidence to support this hypothesis by comparing the mechanisms of development with common pathways known to regulate post-AKI repair, or that we identified as cell-specific candidates based on public datasets from recent AKI translational profiling studies. We will argue that while many of these developmental pathways are reactivated after AKI, this is not associated with general cellular reprogramming to an embryonic state. We will show that reactivation of these developmental genes is often associated with expression in cells that are not normally involved in mediating parallel responses in the embryo, and that depending on the cellular context, these responses can have beneficial or detrimental effects on injury and repair after AKI.

Keywords

Acute kidney injury; ischemia reperfusion; embryonic development; tissue repair; regeneration; fibroblasts; vascular pericytes; endothelial cells; macrophages; tubular epithelial cells; translational profiling

Introduction

Acute kidney injury (AKI) is a common disorder resulting from often multi-factorial ischemic, toxic and septic insults to the kidney^{1, 2}. However, incomplete repair and abnormal tissue remodeling after injury is associated with progressive fibrosis and renal insufficiency such that patients with otherwise apparently reversible AKI are at increased risk of progressive chronic kidney disease (CKD) and eventually end stage renal disease^{3–6}. Despite these sobering clinical data, the mechanisms regulating cellular repair and tissue remodeling after AKI are incompletely understood. Given the lack of progress in developing

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effective clinical therapeutics to enhance tubular repair after AKI, a major focus of AKI research has been to better understand the cell types, responses and signaling pathways mediating these repair mechanisms.

Much of the work studying the cellular mechanisms of repair after AKI has been performed using ischemia-reperfusion models of AKI (IR-AKI) in rodents⁷. These models provide reproducible and defined temporal progression from injury to repair that facilitates analysis. However, there are clearly species differences in responses to AKI. For example, studies using p53 inhibitors in rat and mouse models of IR-AKI suggest that the inflammatory response plays a dominant role in determining the extent of injury in mice⁸, while tubular apoptosis appears to be a more important determinant of renal injury in rats⁹. In addition, there is concern that the severity of injury in rodent models of IR-AKI does not reflect the more focal renal injury seen in patients with AKI¹⁰. This is compounded by the fact that renal biopsies are rarely performed in patients with AKI¹¹, so it is difficult to know whether the pathophysiologic mechanisms of injury and repair are similar or distinct. Having said that, limited post-mortem studies indicate that there are shared elements in rodent and human AKI. These include tubular injury in the cortico-medullary region, including occasional necrosis and apoptosis of tubular epithelial cells, extensive epithelial sloughing, increased tubular proliferation and peri-tubular inflammatory infiltrates^{12–14}. Therefore, while rodent models of IR-AKI have their limitations, we contend that they share the same basic cellular responses to injury as those occurring in human AKI, even though the extent to which these responses contribute to injury and repair after AKI varies between species.

A number of genes involved in embryonic kidney development, including Pax2, Lhx1 and components of the Wnt, Notch, Hedgehog and BMP signaling pathways are reactivated in the adult kidney after IR-AKI in rodents^{15–19}. This has led to the belief that reactivation of pathways promoting cell proliferation and specification during embryonic development drive similar regenerative responses in the adult kidney after injury²⁰. However, with a few exceptions, the functional role of many of these pathways in regenerative repair after AKI has never been tested, or has been shown to promote dysfunctional repair and fibrosis rather than cellular regeneration. In this review we will take a systematic approach to address this problem. We will discuss what is known and what is debated about the cellular mechanisms of repair after AKI. Then, taking advantage of recently published, cell-specific translational profiling studies performed in a mouse model of AKI²¹, we will provide a comprehensive analysis of the expression profiles of genes and pathways involved in embryonic kidney development that are activated after AKI, and discuss what published data is available to explain the functional roles of these genes in regenerative repair and tissue remodeling after AKI. We will also detail that while injury activates many of the same pathways involved in embryonic development, these are often activated in different cell types and lack the coordinated balance required for properly organized tissue regeneration and remodeling after injury. These findings have parallels in cancer biology, where mis-expression and/or subversion of range of different developmentally regulated pathways by cancer cells (or the host micro-environment) have been the focus of therapeutic developments to target initiation and progression of diverse malignancies²².

Cellular mechanisms of repair after AKI

Complete resolution of injury is dependent not only on efficient regeneration of damaged tubular epithelium but also on the restoration of tissue oxygenation by damaged microvasculature^{7, 23}. These responses are determined by the intrinsic ability (or inability) of these cells to regenerate, and by their local microenvironment, in particular the effects of paracrine factors secreted by macrophages and vascular pericytes on tubular repair and vascular stability, respectively^{24, 25}. We will discuss the four dominant cell types involved in tissue repair after IR-AKI: renal tubular epithelial cells, endothelial cells, myofibroblasts and vascular pericytes, and macrophages^{7, 26}. Since each of these cellular compartments has its origins during kidney development, we will discuss the cellular origins and role of these cell types during embryonic development of the kidney, and will discuss how each cell type functions to be part of an integrated cellular response that is required for proper development and for tissue repair after AKI.

Cellular compartments during mammalian kidney development

Tubular epithelial cells are derived from nephron progenitor cells within the cap mesenchyme (CM)^{27, 28}. Coordinated signals derived from ureteric bud (UB) epithelium, which gives rise to the collecting duct (CD) system, as well as paracrine signals from the surrounding stroma, regulate survival and differentiation of CM cells into polarized epithelium of the renal vesicle (RV). As a result of coordinated signaling from within the RV, these structures subsequently undergo expansion and patterning to form proximal and distal nephron segments, fusing with ureteric epithelium at an early point to form the complete nephron. Stromal mesenchyme, which along with the CM is essential for patterning the UB, gives rise to fibroblasts, mesangial cells and vascular pericytes in the adult kidney²⁹. Paracrine factors secreted by CD epithelium also induce stromal cells to differentiate into peri-tubular smooth muscle cells³⁰. Endothelial cells in the adult kidney are largely (but probably not entirely) derived from Flk1 positive endothelial progenitor cells within the stromal mesenchyme³¹. Patterning of the renal vasculature is not completely understood, but the formation of glomerular capillaries is regulated by epithelium and mesangial cell-derived factors^{32, 33}, while global patterning of renal vasculature is dependent on the stromal mesenchyme itself³⁴. Conversely, tissue oxygenation from vascular perfusion is required for CM differentiation during the last trimester of pregnancy (after 15.5 days post-fertilization in mice)³⁵. As far as we are aware, there are no published data on the functional role of macrophages in kidney development. However, macrophages are required for normal breast, bone and brain development as well as correct patterning of the retinal vasculature³⁶. Moreover, temporal analysis of macrophages in the developing kidney shows that these cells are intimately associated with tubular epithelium from the earliest stages of kidney development, and that these cells express markers of regenerative or trophic macrophages³⁷. Therefore, while our understanding of the functional role of all four-cell types in kidney development is incomplete, these data establish that there are extensive functional interconnections between epithelial, endothelial, stromal and macrophage lineages during embryonic kidney development.

Cellular compartments involved in tissue repair after AKI

Epithelium—Tubular epithelial cells, particularly cells in proximal tubular segments of the kidney, are the primary targets of injury after IR-AKI⁷. Recovery of this damaged epithelium is essential to restore normal renal function, and incomplete renal tubular repair is thought to be responsible for tubular atrophy and interstitial fibrosis that gives rise to progressive CKD in patients post-AKI³⁸. In IR-AKI, tubular repair, which occurs a short time after the initiating injury, is characterized by robust proliferation and subsequent re-differentiation of injured but surviving tubular epithelial cells^{39–41}. Genetic lineage studies indicate that tubular repair does not depend on the migration of extra-tubular stem cells into the regenerating tubule^{40, 42}. Moreover, clonal analyses and sequential thymidine analogue pulse-chase experiments indicate that regenerating cells are uniformly distributed throughout the regenerating proximal tubular epithelium after IR-AKI^{40, 41, 43, 44}. This supports the hypothesis that this regenerative response is unlikely to involve the proliferation of a subset of intra-tubular stem cells. However, this issue is still vigorously debated since there is evidence that there is a subset of epithelial cells that express the stem cell markers CD133 and CD24 in adult human kidneys, and that these cells have the capacity to regenerate tubular segments *in vitro* and in orthotopic transplant models^{45, 46}. However, regenerating tubular cells express *CD133*, *CD24*, the tubular injury marker *Kim1*, and the de-differentiation marker, *vimentin* mRNAs after IR-AKI in mice⁴⁰, and CD24 positive cells have also been shown to express *Kim1* in human kidneys⁴⁷. This has led to the alternate hypothesis that CD24 and CD133 expression reflects de-differentiation of injured cells rather than a stem cell state^{44, 48, 49}. Having said that, long-term lineage pulse-chase experiments indicate that tubular cells have a remarkable regenerative capacity after IR-AKI⁴³. This raises the possibility that expression of these markers reflects an acquired phenotypic change from an adult, terminally differentiated epithelium to a progenitor-like state with greater proliferative capacity, more closely resembling the embryonic kidney epithelium.

Endothelium—Endothelial injury increases the extent of injury after IR-AKI by further decreasing capillary blood flow and increasing inflammatory cell recruitment²³. Importantly, there is increasing evidence that peri-tubular capillary rarefaction, which occurs after IR-AKI^{50, 51}, promotes long-term tissue hypoxia which impairs tubular repair and resolution of fibrosis⁵². The mechanism of microvascular rarefaction is unclear since there is little evidence of endothelial cell death or proliferative repair after AKI^{51, 53}. However, bi-directional signaling between vascular pericytes and endothelium regulates vascular stability, suggesting that capillary rarefaction might result from loss of normal pericytes-endothelial interactions after AKI⁵⁴. Support for this hypothesis comes from a series of studies demonstrating that interference with pericytes-derived signals that stabilize (TIMP3 and EphrinB2) or destabilize (VEGF and ADAMTS1) the microvasculature exacerbates or attenuates respectively, renal fibrosis after AKI^{55–57}.

Fibroblasts and pericytes—Wound healing studies indicate that expansion of collagen producing myofibroblasts plays an important role in tissue remodeling, including epithelial repair and vascularization⁵⁸. However, persistent expansion of myofibroblasts is associated with fibrosis. In the kidney, it is likely that myofibroblasts are derived from different cell

types⁵⁹. However, fate mapping using FOXD1 Cre mice to label stromal lineages during embryonic kidney development, indicates that the bulk of these cells originate from vascular pericytes that have delaminated from the vessel wall post-AKI²⁹. Thus expansion of myofibroblasts is intimately linked with microvascular de-stabilization. Moreover, close proximity between tubular epithelium, interstitial inflammatory cells and myofibroblasts provides the ideal microenvironment for cross talk between these cell types during tissue repair.

Macrophages—Endothelial and tubular injury promotes recruitment of neutrophils, macrophages and lymphocytes in the kidney after AKI^{60, 61}. These events amplify the inflammatory response, extending tissue injury and facilitating phagocytosis of dying cells. However, paracrine signaling from renal macrophages also plays an important role in promoting tubular repair and interstitial remodeling at later time points after AKI^{25, 62}. Signals promoting phenotypic switching from pro-inflammatory to regenerative macrophages include active phagocytosis of dying cells (including infiltrating neutrophils)²⁵, as well as the secretion of macrophage growth factors CSF-1 and CSF-2 by tubular epithelial cells^{63, 64}.

Using knowledge of embryonic kidney development to understand cellular repair after AKI

The coordinated mechanisms of repair involving both cell autonomous (intrinsic) and paracrine interactions between epithelium, endothelium, pericytes, and macrophages after AKI is reminiscent of the cellular interactions between parallel cell types in kidney development. We therefore reasoned that our understanding of the pathways involved in kidney development could be exploited to inform our understanding of the cellular mechanisms of repair after AKI. To do this, we took advantage of a recent study that used genetic, cell-specific labeling techniques to interrogate the genome-wide translational expression profiles in tubular epithelium, endothelium, vascular pericytes and macrophages 24 hours after IR-AKI²¹. These studies used a new technology, translating ribosome affinity purification (TRAP), which allows for the affinity purification of actively translating mRNAs. Using a series of Cre lines to activate TRAP in different cellular compartments (Six2 Cre: epithelium; FoxD1 Cre: fibroblasts; Cdh5 Cre: endothelium; Lyz2 Cre: macrophages), they isolated cell-specific mRNAs in the intact kidney immediately after lysis. This allowed them to perform simultaneous analysis of cell-specific mRNA expression profiles without the need to separate individual cellular compartments by fluorescence activated cell sorting (FACS). Using the deposited gene profile datasets from these TRAP studies (from the NCBI GEO database⁶⁵), we searched for expression of intrinsic and paracrine factors involved in kidney development. The list of developmental genes that we used to interrogate the TRAP datasets is based on information contained in a series of recent, comprehensive reviews^{27, 28, 31, 66–74}. We only included those genes and pathways that have been shown either genetically or pharmacologically to play a functional role in regulating renal development. For each of these genes, we evaluated expression of intrinsic factors in the corresponding cell types in development and injury (CM/RV/PTA and UB: epithelium; EC: endothelium; Stroma: fibroblasts, pericytes and mesangial cells), as well as expression of paracrine factors by neighboring cells (including macrophages), reasoning that different cell types may co-opt the same paracrine responses after injury. Results are only

shown for those genes that showed significantly increased or decreased expression compared with sham-operated controls adjusted for multiple comparisons ($p < 0.05$). We evaluated a total of 120 genes that have been shown to regulate kidney development, of which 51 were significantly regulated in one of the four cellular compartments 24 hours after IR-AKI. Additional genes not included in this analysis include the putative renal progenitor cell markers, CD24 and CD133, which are upregulated in epithelial cells 24 hours post-AKI (CD24: 1.6 fold, $p = 0.0008$; CD133: 1.4 fold, $p = 0.046$). These findings confirm previous studies (discussed above), and validate the approach. Results are summarized in Tables 1 and 2 with information about developmental expression, cellular targets and function of each gene in kidney development.

Developmental pathways known to play a role in cellular repair after AKI

While the TRAP data are limited to a single time point 24 hours after injury and so may fail to capture important gene signatures that are only expressed at later time points, we were able to identify a number of developmental genes and pathways that are already known to play a role in post-AKI repair.

Wnt signaling—Wnt signaling plays an essential role in regulating cell fate specification at different stages of embryonic development⁶⁸. For example, Wnt9b and Wnt11 are expressed in the UB and regulate CM differentiation and early UB branching patterns, respectively. Wnt4 is expressed in early nephron progenitors (PTA and RV), and is required for growth and specification of CM-derived epithelia. However, Wnt4 is also expressed later in embryonic development in stromal mesenchyme surrounding the UB and plays a role in regulating smooth muscle differentiation⁷⁵. This is a common finding that the same genes may have distinct functional roles in different cell types at different stages of embryonic development. Early nephron patterning events regulated by Wnt9b and Wnt4 are mediated by canonical, β -catenin signaling⁷⁶, while Wnt11 is thought to act through non-canonical pathways to regulate UB branching⁷⁷. In the TRAP datasets, there was a significant increase in expression of Wnt4 and Wnt9b in endothelial cells, while Wnt11 expression is down-regulated in macrophages 24 hours after IR-AKI (Table 2). There was no change in expression of these, or other Wnt ligands involved in renal development (notably Wnt7b) in either epithelial or stromal lineages. Increased expression of Wnt4 has been described in epithelial cells early after IR-AKI¹⁷, and in interstitial myofibroblasts at later time points⁷⁸. However, deletion of Wnt4 in myofibroblasts has no effect on post-injury fibrosis even though conditional activation of canonical Wnt signaling in vascular pericytes promotes spontaneous renal fibrosis⁷⁸. This suggests that an alternative Wnt ligand may play a role in post-injury repair by activating canonical Wnt signaling. The role of Wnt4 expression in injured renal tubular epithelial cells is unknown, and there are no other published data on the regulation of Wnt9b or Wnt11. However, Wnt7b is detectable in renal macrophages 5 days after IRAKI and regulates epithelial repair and remodeling of the extracellular matrix after injury⁷⁹. The TRAP data suggests alternate sites of Wnt ligand expression that may play a role in regulating post-AKI repair. Interestingly, the TRAP dataset indicates that Sfrp1, a secreted Wnt antagonist expressed by stromal mesenchyme that represses UB branching and RV differentiation in cultured embryonic kidney rudiments⁸⁰, is markedly repressed in

epithelia, fibroblasts, endothelial cells and renal macrophages 24 hours after IR-AKI (Tables), suggesting an alternate mechanism to activate Wnt signaling post-AKI.

Notch signaling—Notch signaling plays an essential role in specifying proximal segments of CM-derived renal tubular epithelium (proximal tubules and glomeruli), and in determining CD cell fates⁷⁴. The Notch ligands Jag1 and Dll1, and Notch receptor, Notch1, are expressed in the mid-region of the RV (which is thought to give rise to proximal tubules and glomeruli), while Notch2 is more widely expressed in the RV. More recently it has been shown that Notch1 and 2 also specify mesangial cell fate in the renal stroma⁸¹. In addition, while there are no published data on the role of Notch signaling in specification of the renal vasculature that we are aware of, Notch1 and the Notch ligand, Dll4, play an important role in regulating VEGF-dependent embryonic vascular development⁸². In the TRAP dataset, there is increased expression of Jag1, Notch1 and Notch2 in epithelial cells, as well as increased Jag1 expression in endothelial cells and macrophages 24 hours after IR-AKI (Tables). These findings are consistent with data showing an early increase in renal expression of Jag1, Dll1, Notch1 and Notch2 after IR-AKI^{16, 83}, and increased Notch1 activation and Jag1 protein expression in proximal tubular cells after folic acid-induced kidney injury⁸⁴, but also suggest that Notch ligands may be more widely expressed in different cell types after IR-AKI. Pharmacological inhibition as well as cell-specific loss and gain of function studies indicate that prolonged Notch signaling in tubular epithelial cells increases fibrosis after folic acid-induced AKI⁸⁴, and increases the severity of injury, inflammation and tubular cell apoptosis after IR-AKI^{85, 86}. In contrast to these findings, inhibition of Notch signaling has also been shown to delay both functional and structural recovery from IR-AKI⁸³. One possibility is that persistent activation of Notch signaling in epithelial cells, which occurs after folic acid-induced injury and IR-AKI in aged mice^{84, 86}, increases tubular injury, while more transient activation of Notch signaling in younger mice with IR-AKI may have beneficial effects that recapitulate the developmental program in kidney development. An alternative possibility that has yet to be explored is that activation of Notch signaling plays a more dynamic role in regulating capillary vascular stability after AKI. In this regard, it is interesting to note that the TRAP dataset identified a 1.4fold increase in expression of Dll4 in endothelial cells 24 hours after IR-AKI ($p=0.0014$, data not shown), and that systemic administration of Dll4 accelerates functional recovery after IR-AKI⁸⁷.

BMP signaling—BMP signaling plays an important role at different stages of kidney development⁶⁹. BMP7, which is expressed in the CM and UB, acts in a cooperative manner with the secreted BMP co-activator, Crossveinless-2 (CV-2), to maintain CM expansion without differentiation^{88, 89}. The BMP antagonist, Gremlin, which is also expressed in the CM, suppresses Smad signaling in the UB, facilitating UB branching⁹⁰. BMP4 is expressed in the metanephric mesenchyme (MM) prior to UB invasion where it prevents ectopic budding of the UB⁹¹. Later, BMP4 expression is restricted to the UB stroma where it regulates stromal differentiation into ureteral smooth muscle cells⁹². BMP4 expression in podocytes also regulates Vegfa-dependent glomerular vascularization⁹³. In adult, BMP7 expression in the kidney is reduced after IR-AKI^{18, 94}, and while the function and cellular origin BMP7 expression during post-AKI injury and repair are unknown, systemic treatment

with BMP7, or the BMP7 mimetic THR-123, reduces tubular injury after IR-AKI^{95, 96}. There is added complexity to this pathway since expression of the secreted selective BMP7 antagonist, Chordin-like 1, is also down-regulated in injured proximal tubular epithelial cells after IR-AKI, and this is associated with increased BMP-dependent Smad signaling in regenerating epithelial cells⁹⁷. In the TRAP dataset, BMP4 and 7-expression were both increased in endothelial cells 24 hours after IR-AKI (Table 2), but there was no change in expression of either ligand, or the BMP antagonist Gremlin, in any of the other cell types. Failure to detect changes in BMP7 expression in epithelia post-AKI may reflect differences in the timing of injury, but localized expression in endothelial cells suggests that BMP signaling may be playing a role in regulating vascular injury and/or stability after IR-AKI.

Hedgehog signaling—Hedgehog (HH) signaling has a more limited role than Wnt, Notch and BMP signaling during early kidney development, but plays an important role in regulating smooth muscle differentiation of stromal mesenchyme surrounding the UB after the embryonic kidney has undergone initial dorso-ventral patterning. The Hedgehog ligands Shh and Ihh are expressed in distal CD and CM-derived epithelia, respectively, but only loss of Shh expression in the CD has an effect on renal development. The HH receptor Ptch1, which acts by repressing HH pathway activation by the Smo receptor, is restricted to the stroma surrounding medullary UB⁹⁸. However, deletion of Ptch in the CD results in ectopic activation of HH signaling and reduced UB branching. Moreover, active repression of HH responses by expression of the downstream HH repressor, Gli3, inhibits ectopic HH signaling in nephron progenitors and UB⁷⁰. Gli3 is expressed in UB tips, CM and RV structures, and Gli3 null mice have increased expression of HH activators, Gli1 and Gli2, and increased expression of the Gli targets Pax2, Sal1, Cyclin D1 and N-Myc⁹⁹. In addition, *Gli3* null mice have increased proliferation in the CM indicating that HH activation promotes cell proliferation. Therefore the balance of HH activators and repressors plays an important role in regulating cell proliferation and specification in kidney development. After IRAKI, renal expression of Shh, Ptch, Gli1, Gli2 and Gli3 are increased, reaching maximal levels 7–10 days after injury^{19, 100}. Shh expression is restricted to tubular epithelium, while Gli1 expression, indicative of HH responding cells, is limited to peritubular fibroblasts after IR-AKI¹⁰⁰. In addition, inhibition of HH signaling with the Smo inhibitor, Cyclopamine, decreases post-injury fibrosis and myofibroblast proliferation after severe IR-AKI¹⁰⁰, suggesting that delayed activation of HH signaling after IR-AKI promotes interstitial fibrosis by increasing numbers of interstitial fibroblasts. These findings contrast with the TRAP datasets in which there is reduced expression of Smo and Ptch1 in renal fibroblasts. In addition, Shh expression is decreased in renal macrophages but unchanged in renal epithelial cells 24 hours after IR-AKI (Tables). This suggests that HH signaling is decreased early after IR-AKI, and is only activated later when it plays an active role in promoting post-injury fibrosis.

Signaling pathways regulating angiogenesis and vascular stability—The molecular pathways regulating vascular patterning in the developing kidney are poorly understood. What little is known largely relates to the pathways coordinating the formation of glomerular capillaries³¹. Primitive glomerular epithelial cells (podocytes) secrete the pro-angiogenic factor, Vegfa, which promotes endothelial recruitment and proliferation in the

glomerular tuft¹⁰¹. In addition, endothelial cells secrete Pdgfb, which promotes recruitment and proliferation mesangial cells, which are specialized glomerular vascular pericytes¹⁰². Analysis of the TRAP dataset indicates that epithelial expression of Vegfa is down-regulated 24 hours after IR-AKI, while expression in other cellular compartments, notably endothelial cells, is increased (Tables 1 and 2). In contrast, Pdgfb expression by endothelial cells is markedly decreased after IR-AKI. Unexpectedly, systemic inhibition of both Vegf and Pdgf signaling, using soluble VegfR2 or PdgfRb, reduce delamination and proliferation of pericytes and prevent vascular rarefaction and fibrosis after IR-AKI⁵⁵. This suggests that activation of both pathways promotes vascular instability after IR-AKI. This contrasts with an earlier study in which systemic activation of Vegf signaling by treatment with the Vegfa121 isoform reduced vascular rarefaction after IR-AKI¹⁰³, suggesting that the effects of Vegfa (and possibly Pdgfb) on vascular rarefaction may be sensitive to fine changes in pathway activation post-AKI.

Other pathways identified from the TRAP datasets that regulate the formation of glomerular capillaries include the CXCL12/CXCR4 axis, which promotes the recruitment and proliferation of mesangial cells through a Pdgfb-independent mechanism³², Angiopoietin 1 (Ang1), which is expressed by podocytes and stabilizes endothelial interactions with mesangial cells¹⁰⁴, and the anti-angiogenic axonal guidance molecule, Sema3a, which inhibits glomerular endothelial proliferation¹⁰⁵. The most striking changes are in the expression of CXCL12, which is markedly down-regulated in epithelial cells, fibroblasts and macrophages, while CXCR4 is the most highly upregulated gene in endothelial cells 24 hours after IR-AKI (Tables 1 and 2). In addition, Ang1 expression is decreased in epithelial cells and fibroblasts, while Sema3a expression is decreased in endothelial cells but markedly increased in macrophages post-AKI. These findings indicate that AKI induces complex changes in a variety of signaling pathways that are known to regulate blood vessel formation in the embryonic kidney. We speculate that these are likely to be important in determining the extent of vascular injury and repair after AKI.

Other genes and pathways that regulate kidney development

In addition to genes that have already been shown to play a role in post-AKI repair, the TRAP datasets include a number of genes that are also regulated after AKI, suggesting additional conserved developmental pathways that may play a role in injury and repair after AKI.

Retinoic acid (RA) signaling—Raldh2-dependent secretion of RA by the renal stroma regulates Ret-dependent UB branching¹⁰⁶, and RA-dependent secretion of ECM1 by the stroma limits the extent of RA-dependent Ret expression in the UB¹⁰⁷. In the TRAP datasets, Raldh2 is the most highly upregulated gene in renal fibroblasts and macrophages, while ECM-1 is one of the most highly upregulated genes in epithelial cells and macrophages 24 hour after IR-AKI (Tables). These findings are reinforced by another recent translational profiling study in which Collagen 1Y1 Cre mice were used to activate TRAP in renal fibroblasts after unilateral ureteral obstruction (UUO)¹⁰⁸. In these studies there was a 52-fold increase in Raldh2 mRNA expression 5 days after UUO. Activation of RA signaling is of particular interest as a candidate for mediating post-AKI regenerative repair of tubular

epithelium since RA signaling plays an important role in amphibian limb regeneration^{109, 110}, and is required for zebrafish fin and heart regeneration after injury^{111–113}.

Cell cycle regulators C-Myc and N-Myc—Myc proteins are a family of proto-oncogene transcription factors that drive cell cycle progression by up-regulating an array of different cell cycle regulators¹¹⁴. C-Myc is expressed in the CM, RV and early tubular epithelium¹¹⁵. Conditional deletion of C-Myc in the CM (but not RV) inhibits CM proliferation¹¹⁶. C-Myc is also the most highly upregulated epithelial gene in the TRAP datasets 24 hours after IR-AKI (Table 1). Similarly, N-Myc, which is expressed in the CM and RV, regulates both CM and UB proliferation^{115, 117}, is also significantly upregulated in the epithelial dataset post-AKI. Since the mechanisms regulating tubular proliferation after AKI are incompletely understood, this suggests a novel mechanism by which reactivation of Myc expression might promote tubular repair.

Other genes—It is notable that very few of the classical CM specific genes (including Six1/2, Cited1, Meox1/2, Sal1/4, Crym, Eya1/4 and Gdnf) were upregulated in injured epithelia in the TRAP datasets 24 hours after IR-AKI. In addition, while some marker of early RV epithelia including components of the Notch pathway and Pax8 (but not interestingly, Pax2), other classical RV markers including Wnt4 and Fgf8 were unchanged, Lhx1 and Bnr1 were actually decreased after IR-AKI. These findings suggest that while injured epithelium takes on some of the markers of embryonic epithelial progenitor cells, this is not a complete reversal to the embryonic state. We would suggest therefore that expression of these developmental markers by epithelial cells reflect their state of cellular dedifferentiation rather their reprogramming to an embryonic cell type. It should also be noted that the functional role of any of the changes in expression of RV markers by epithelial cells after injury is unknown. Other developmentally regulated genes include Cadherin 6 (expressed in the RV) and Sox9 (expressed in stroma), which are highly upregulated in epithelial and fibroblast datasets, respectively (Table 1). During embryonic development, Cadherin-6 regulates RV patterning¹¹⁸, while Sox9 expression indirectly regulates UB branching¹¹⁹. There is also a reduction in components of the renin angiotensin system (RAS), including Angiotensin receptors 1a/b (Atr1a/b) and angiotensinogen (Agt) in epithelial cells, and marked reduction in Agt in macrophages post-AKI (Tables 1 and 2). These components of the RAS pathway regulate UB branching during embryonic development⁷². The functional roles of these changes in developmental gene expression post-AKI are all unknown.

Conclusions

Insights gained from comparative analysis of the cellular and molecular programs involved in regulating normal kidney development with those activated or inhibited after AKI have informed our understanding of the mechanisms of injury and repair after AKI. These changes are not associated with complete reprogramming of cells to an embryonic state, but are associated with often ectopic expression and activation of common pathways that may have both beneficial and detrimental effects of injury and repair after AKI. In addition, functional effects of changes in expression of embryonically regulated genes may be

critically dependent both on the timing in relation to the injury and repair response, and with the balance of signaling events that are being activated at any one time point after injury. Further cell-specific time course analyses as well as cell-specific loss and gain of function studies will be needed to elucidate the functional role of these pathways and gene expression patterns in AKI injury and repair.

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Table 1
Embryonic genes regulated in epithelial and fibroblast compartments post-AKI

Data obtained by interrogating cell-specific TRAP microarray data deposited on the NCBI GEO site (GEO GSE2004) based on published work from Liu et al. JCI 2014²¹. Fold change has been converted from LogFC to a linear scale, and false discovery rate p values after correction for multiple comparisons are indicated. Abbreviations used: IM, intermediate mesoderm; CM: cap mesenchyme; PTA, pre-tubular aggregate; RV, renal vesicle; UB, ureteric bud; S, stroma; PTC, proximal tubular epithelium; GN, glomerular; TF, transcription factor; AF, adhesion factor; ECM, extracellular matrix protein; GF, growth factor; RTP, receptor tyrosine phosphatase; HH, Hedgehog; RTK, receptor tyrosine kinase; RAS, renin angiotensin system; Ang, angiotensin; AGM, axonal guidance molecule

Six2 Cre (CM-derived epithelial cells)						
Gene	Fold-change	adj.P.Val	Expression	Cell targets	Function	Pathway
C-Myc	6.47637943	0.00011547	CM/RV/Tubules	CM	CM growth	TF
Cdh6	3.30745837	0.0001129	RV	RV	RV patterning	AF
ECM-1	3.2546443	0.00019361	UB	UB	UB branching	ECM
PDGFb	2.0151227	0.00694948	EC	Pericyte	Stabilize GN caps	GF
Spry1	1.94634596	0.00015268	UB	UB	UB branching	RTP
Jag1	1.72325331	0.00421987	RV	RV	RV patterning	Notch
Notch2	1.49572507	0.01560838	RV	RV	RV patterning	Notch
Pax8	1.48733756	0.00310193	IM/CM/RTA/RV	IM+RV	IM/RV patterning	TF
Gli3	1.4821436	0.00711689	CM/RV/UB	CM+Stroma	CM/UB differentiation	HH
Notch1	1.34278799	0.01701	RV	RV	RV patterning	Notch
N-Myc	1.30284298	0.01844742	CM/RV	CM+UB	CM/UB growth	TF
Ret	1.23678787	0.02024422	UB	UB	UB branching	RTK
Fgf20	-1.1817532	0.02888695	CM	CM	CM maintenance	FGF
Agtr1b	-1.1879282	0.03005054	UB/S	UB	UB branching	RAS
Fgfr1	-1.206714	0.03634663	RV	RV	RV patterning	FGF
Gli2	-1.2159333	0.03088384	CM/RV/UB/UB S	Stroma	UB S differentiation	HH
Fz4	-1.2808667	0.00939375	UB/RV	UB	UB branching	Wnt
Vegfa	-1.3918525	0.01269938	Podocyte	EC	Patterning GN caps	VEGF
Agt	-1.5161001	0.03699661	PTC/UB/S	UB	UB branching	RAS
Vegfb	-1.553122	0.00400948	S	UB	UB branching	VEGF
Ang1	-1.6218783	0.00362977	Podocyte	EC	Stabilize GN caps	Ang
Ptch1	-1.6721007	0.0014819	UB stroma	Stroma	UB S differentiation	HH
Brn1	-1.7184929	0.00099984	RV	RV	RV patterning	TF
Npnt	-1.7686685	0.02406516	UB	UB	UB branching	ECM
Fgf9	-1.7927826	0.00152379	UB	CM	CM maintenance	FGF
Lhx1	-1.8443451	0.00027397	IM/RV	IM+RV	IM/RV patterning	TF
Agtr1a	-2.2761045	0.00124142	UB/S	UB	UB branching	RAS
CXCL12	-2.6526394	0.00002912	S/podocyte	EC	Patterning (PT/GN caps)	Chemokine

Six2 Cre (CM-derived epithelial cells)						
Gene	Fold-change	adj.P.Val	Expression	Cell targets	Function	Pathway
Gfra1	−3.31917	0.00621682	UB	UB	UB branching	RTK
sfrp1	−4.9082995	0.0000218	S	UB/RV	RV diff/UB branching	Wnt

FoxD1 Cre (vascular pericyte and fibroblasts)						
Gene	Fold-change	adj.P.Val	Expression	Cell targets	Function	Pathway
Raldh2	6.23418194	0.000279	S	UB	UB branching	RA
Sox9	5.47613442	0.000783	UB	UB	UB branching	TF
fgf7	2.13673507	0.03953	CM	UB	UB branching	FGF
PDGFb	0.75471778	0.042824	EC	Pericyte	Stabilize GN caps	GF
Pbx1	−1.3676847	0.012269	CM/stroma	UB/S	UB branching/stroma	TF
fgf9	−1.4431291	0.026046	UB	CM	CM maintenance	FGF
Smo	−1.4610955	0.005545	CM/UB stroma	CM/S	CM diff/UB stroma	HH
Ptch1	−1.6245434	0.006334	UB stroma	Stroma	UB S differentiation	HH
Vegfb	−1.6400474	0.039702	S	UB	UB branching	VEGF
Ang1	−1.9196234	0.016024	Podocyte	EC	Stabilize GN caps	Ang
CXCL12	−3.9407699	0.000787	S/podocyte	EC	Patterning (PT/GN caps)	Chemokine
Sfrp1	−3.9416509	0.000879	S	UB/RV	RV diff/UB branching	Wnt

Table 2
Embryonic genes regulated in endothelial cells and renal macrophages post-AKI

Data obtained by interrogating cell-specific TRAP microarray data deposited on the NCBI GEO site (GEO GSE2004) based on published work from Liu et al. JCI 2014²¹.

Cdh5 Cre (endothelial cells)						
Gene	Fold-change	adj.P.Val	Expression	Cell targets	Function	Pathway
CXCR4	3.38490297	0.0000307	EC	EC	Patterning (PT/GN caps)	Chemokine
Dll1	1.7276432	0.00193236	RV	RV	RV patterning	Notch
Jag1	1.62960485	0.01750718	RV	RV	RV patterning	Notch
wnt11	1.6030653	0.00784358	UB	UB	UB branching	Wnt
ECM-1	1.55669771	0.01539723	S	UB	UB branching	ECM
Vegfa	1.51089858	0.03139186	Podocyte	EC	Patterning GN capillary	VEGF
Raldh2	1.44492489	0.01764699	S	UB	UB branching	RA signaling
wnt4	1.40643807	0.03269299	PTA	PTA/RV	RV patterning	Wnt
CV-2	1.34642046	0.00553672	CM/RV	CM	CM maintenance	BMP
Bmp4	1.30770344	0.00310936	Podocyte	MC	Stabilize GN capillary	BMP
Fat4	1.25212556	0.00998643	S	CM	CM differentiation	Hippo
fgf8	1.24991241	0.04705277	RV	RV	RV patterning	FGF
fgf9	1.18869368	0.00979009	UB	CM	CM maintenance	FGF
Bmp7	1.17549189	0.04114582	CM/UB	CM	CM maintenance	BMP
wnt9b	1.15094784	0.0273861	UB	CM	CM differentiation	Wnt
Sema3a	-1.535023	0.02025937	Podocyte/UB	EC/UB	Stabilize GN cap/UB branch	AGM
Sfrp1	-1.7230822	0.00503227	S	UB/RV	RV diff/UB branching	Wnt
Vegfb	-1.9495597	0.00012436	S	UB	UB branching	VEGF

Lyz2 Cre (macrophages)						
Gene	Fold-change	adj.P.Val	Expression	Cell targets	Function	Pathway
Raldh2	3.57497878	0.00005826	S	UB/S	UB branching/Survival	RA signaling
ECM-1	3.27201955	0.00196424	S	UB	UB branching	ECM
Jag1	2.66705098	0.00069502	RV	RV	RV patterning	Notch
Sema3a	2.19950257	0.043863	Podocyte/UB	EC/UB	Stabilize GN cap/UB branch	AGM
decorin	1.60938392	0.00159439	S	CM	CM maintenance	BMP
CV-2	1.44308259	0.030709	CM	CM	CM maintenance	BMP
Shh	-1.2378484	0.04062207	UB/UB stroma	Stroma	UB stroma diff	HH
wnt11	-1.254778	0.03699451	UB	UB	UB branching	Wnt
fgf9	-1.3903233	0.02143087	UB	CM	CM maintenance	FGF
Vegfb	-1.9296478	0.00049656	S	UB	UB branching	VEGF
PDGFB	-2.0934016	0.00033829	EC	Pericyte	Stabilize GN caps	GF
CXCL12	-2.1777093	0.00069552	S/podocyte	EC	Patterning (PT/GN caps)	Chemokine
Agt	-3.4983139	0.00694161	PTC/UB/S	UB	UB branching	RAS

Lyz2 Cre (macrophages)						
Gene	Fold-change	adj.P.Val	Expression	Cell targets	Function	Pathway
sfrp1	−4.6992125	0.00023495	S	UB/RV	RV diff/UB branching	Wnt

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