Mechanisms of Epithelial Repair and Regeneration After Acute Kidney Injury

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Summary: Acute kidney injury (AKI) is a common clinical problem and is associated with high mortality rates. It is accepted that after AKI cellular regeneration of the proximal tubule occurs from intrinsic tubule cells. Recently, scattered tubular cells (STCs) were discovered as a novel subpopulation of tubule cells involved in regeneration. STCs have a distinct morphology, unique protein expression profile resembling that of parietal epithelial cells, proliferate more than the remaining proximal tubule cells, and are less susceptible to injuries. In response to AKI, STCs become more numerous, independent of the primary insult (ischemic, acute obstruction, and so forth). STCs can be detected with the highest sensitivity and manipulated by the parietal epithelial cell–specific, doxycycline inducible transgenic mouse line PEC-rtTA. In cell fate tracing experiments it was shown that STCs are not a fixed progenitor population. Rather, STCs arise from any surviving proximal tubule cell. Thus, the STC phenotype is a transient, graded, and specific transcriptional program facilitating tubular regeneration. Understanding this program my open new approaches to prevent and/or treat AKI.
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Acute renal failure can be defined as an abrupt decrease in glomerular filtration with resultant azotemia, in most cases caused by acute ischemic and/or toxic insults.[1] In both cases, the proximal tubule is the main site of injury, therefore the term *acute tubular necrosis* often is used synonymously. The mammalian kidney is particularly susceptible to these two kinds of acute kidney injuries (AKIs) for several reasons. First, the mammalian kidney has no portal blood supply (unlike the mesonephros in fish, amphibians, or reptile-like animals). Because all blood first has to pass through the glomeruli in mammals, glomerular vasoconstriction may decrease the blood supply of the entire kidney (eg, in hypovolemia). The proximal tubule is particularly sensitive to ischemia[2] because it relies predominantly on aerobic adenosine triphosphate production (mitochondrial Krebs cycle) and it cannot use the ischemic salvage pathway of glycolysis efficiently.[3]

Furthermore, the proximal tubule reabsorbs most of the filtered substances including toxins, in part by endocytosis. For example, gentamycin is taken up by the cubilin-megalin complex and gentamycin toxicity is increased in a water-retaining kidney (induced by withholding liquids, salt, or volume depletion).[4–6]

The subsequent mechanisms for AKI still are controversial. Besides a reduction in glomerular filtration rate, tubular obstruction likely represents a major factor.[7–9] In brief, after AKI, cellular debris and protein casts obstruct individual nephrons transiently. Depending on the severity of AKI, many or just a few tubules are obstructed, resulting in the transient loss of renal function. Tubular obstruction may last long enough to drive the affected nephron into reversible or irreversible degeneration—similar to tubular degeneration after unilateral ureteral obstruction. In addition, complex interactions with cells of the immune system and release of inflammatory mediators likely play a role during the course of AKI (reviewed by Cantaluppi et al[10]).

Nevertheless, tubules have a remarkable capacity to regenerate lost cells, usually within less than a week. The present article focuses on recent insights into the mechanisms of epithelial repair and regeneration. In particular, the role of a recently discovered subpopulation of tubule cells is discussed: scattered tubular cells (STCs). These cells become abundant in response to AKI and likely play a major role in the regenerative process.

UNIFORM RESPONSE TO AKI: TRANSITION INTO THE STC PHENOTYPE

In 2011, a novel subpopulation of proximal tubular cells was described.[11] Because these cells showed a distinct morphology and were scattered as single cells among fully differentiated inconspicuous tubular cells

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throughout the entire proximal tubule, these cells were termed *scattered tubular cells*.

STCs show very characteristic morphologic and ultrastructural features[12,13] (Fig. 1A). They generally are smaller than fully differentiated tubular cells and may have different shapes.[12] In the normal kidney, they occur as single cells or, less often, as doublets or triplets. They are surrounded by fully differentiated tubular cells, mostly with an abrupt transition. In this setting, STCs often show a narrow flask-like shape. Importantly, STCs show a dramatic decrease in mitochondria compared with neighboring proximal tubule cells.[12,13]

In the normal human kidney, STCs can be detected, preferentially at the inner turn or along infoldings of the tubule (eg, along the tubular plicae where the tubule makes a hairpin turn).[11–13] The reason for this preferential location is controversial. Increased mechanical forces could push the cells into the STC phenotype, or a hairpin turn could represent a microniche for a fixed progenitor population. However, this would imply that no STCs should be expected along the pars recta of the proximal tubule (ie, the S2 and S3 segment), however, this is not the case.

In contrast to differentiated tubule cells, STCs do not have a pronounced apical brush border. STCs also express only very low levels of the classic multitarget protein endocytic transporter megalin. STCs also lack the basolateral labyrinth of extensive membrane infoldings, which is characteristic for differentiated proximal tubular cells.[13] We have shown previously that the infoldings of the basolateral membrane extend almost up to the apical aspect of proximal tubule cells. Filtered albumin is taken up by differentiated proximal tubular cells from the primary filtrate and released into the apical aspects of the basolateral labyrinth from where it diffuses back into the tubulointerstitial capillaries or lymphatics.[14] Absence of an apical brush border and a basolateral labyrinth strongly suggests that STCs are less active endocytically compared with differentiated proximal tubule cells.

In the regenerative phase after AKI, STCs may become rather abundant and also mostly acquire shapes similar to the surrounding tubular cells.[13,15]

To date, it has not been investigated systematically which stimuli can push tubule cells into the STC transcriptional program. We have shown previously that proteinuria or transient ischemia-reperfusion injury

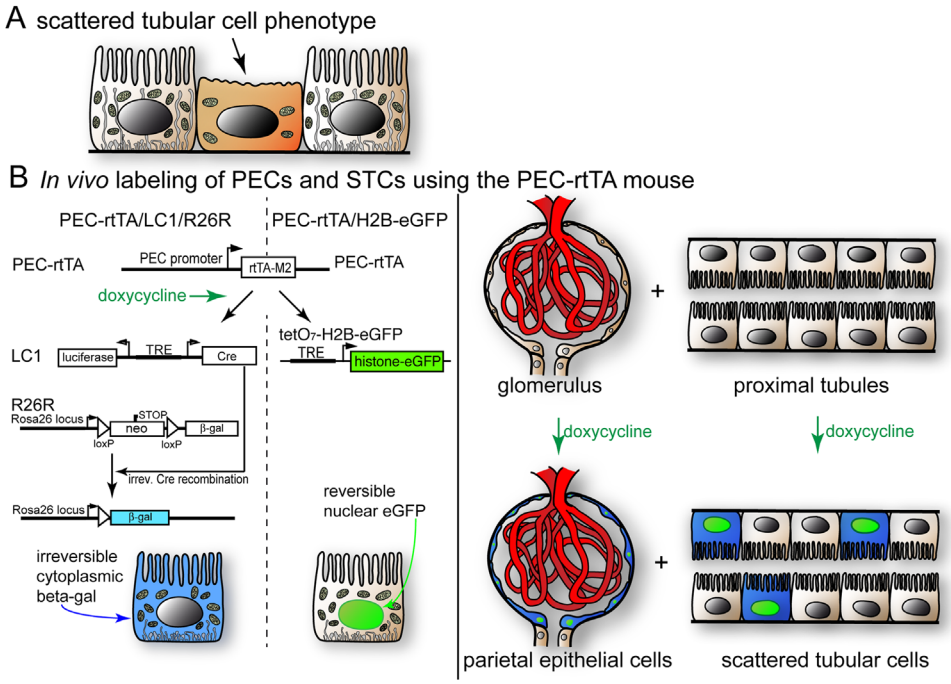

Figure 1. STCs. (A) STCs (arrow) lack a brush border and a basolateral labyrinth and contain fewer mitochondria. STCs express a distinct panel of marker proteins (indicated by the orange color), similar to PECs. (B) The doxycycline-inducible transgenic PEC-rtTA mouse is currently the most sensitive method to mark and/or manipulate proximal tubule cells with the STC phenotype. The transgenic map is shown in the left panel. The PEC-rtTA/LC1/R26R triple transgenic mouse expresses β-galactosidase (β-gal) (blue) irreversibly upon Cre recombination induced by transient administration of doxycycline. The PEC-rtTA/H2B-eGFP mouse loads nuclei with histone-enhanced green fluorescent protein during administration of doxycycline (green). The labeling pattern is shown in the right panel. In the glomerulus, PECs are marked. In the proximal tubule, scattered tubular cells are marked. TRE, tet-responsive element; neo, neomycin resistance cassette; rtTA-M2, improved reverse tetracycline-controlled transactivator. Schematic on the left is modified with permission from Berger et al.[50]

(IRI) induce the STC phenotype in mice and that unilateral ureteral obstruction induces the STC phenotype in mice and rats.[13,15] The multitude of studies reporting tubular cell phenotypes similar to STCs suggests that the STC phenotype can be activated by a multitude of injuries. For example, kidney injury molecule-1 (Kim-1), one of the STC marker proteins, is up-regulated in protein-overload nephropathy, consistent with the notion that nephrotic-range proteinuria alone can increase the frequency of STCs.[16]

STC PROTEIN EXPRESSION PROFILE: DISTINCT FROM DIFFERENTIATED TUBULE CELLS, SIMILAR TO PARIETAL EPITHELIAL CELLS

STCs have a very distinct transcriptional profile compared with fully differentiated proximal tubular cells.[11] In addition, it quickly became obvious that STCs and parietal epithelial cells (PECs) express similar markers. This was first noted by Lindgren et al,[11] who showed that there are higher levels of CD133 and CD24 in isolated STCs from human kidney cortex. In the human kidney, both of these markers also are expressed by PECs.[17] In this context, it should be noted that only a glycosylation isoform of CD133 is expressed specifically on STCs and PECs, termed *glycCD133*.[18] Furthermore, the glycCD133 isoform is specific only for STCs and PECs in human beings, not in rodents. Antibodies directed against total CD133 show a much more widespread expression in the kidney (human beings and rodents).

Currently, there are no antibodies available to stain for CD24 in rodent kidneys. In the rodent kidney, most other described STC markers[13] can be used to identify STCs, in particular Src-suppressed C kinase substrate, annexin A3, or Kim-1.[15] The parietal epithelial cell–specific transgenic PEC–rtTA mouse specifically labels STCs and is the only known mouse model targeting STCs to date[15,19] (Fig. 1B). By using the PEC-rtTA mouse to identify STCs it could be shown that a signal also transmits induction of the STC phenotype to the contralateral kidney after unilateral AKI by an as yet unidentified soluble mediator. This was first reported by Witzgall et al,[20] who showed increased proliferation in the contralateral kidney after ipsilateral IRI. In our recent study, increased induction of the STC phenotype in small but significant numbers of proximal tubule cells in the contralateral kidney was observed during the recovery phase after unilateral ischemic AKI.[15] The STCs in the contralateral kidney showed increased proliferation, validating their identity. They could be identified only by the PEC-rtTA mouse; other STC marker detection remained negative. This shows three major findings. First, the STC transcriptional program is graded. Early indicators for the STC phenotype are increased proliferation and transcriptional activity of the PEC-rtTA transgenic mouse. Kim-1 already is up-regulated in microalbuminuric diabetic patients, suggesting that Kim-1 also may be a relatively early marker for the STC phenotype.[21] Other markers (CD44, SSeCKS, vimentin, and so forth) are up-regulated in tubule cells with significant activation of the STC transcriptional program. Second, the PEC-rtTA mouse is currently the most sensitive method to identify STCs in proximal tubules in mice. Third, these observations also provide evidence that a signal is transmitted to the contralateral kidney, inducing the STC phenotype after unilateral AKI, most likely by a soluble mediator.

Lindgren et al[11] determined the gene expression profile from total RNA obtained from cortical single-cell suspensions from human tumor nephrectomies based on aldehyde dehydrogenase (ALDH) enzymatic activity, which specifically is up-regulated in STCs. For preparation of single cells, kidney tissues were incubated overnight at 37°C in collagenase IV. Decapsulated glomeruli were removed by sieving. Next, cells were fluorescence-activated cell-sorted according to ALDH enzymatic activity. Thus, the $ALDH_{high}$ cells (putative STC proximal tubule cells) also may have contained PECs. $ALDH_{low}$ cells comprised all remaining cortical cells. Transcriptomes were determined using HumanHT-12 v3.0 Expression Bead Chips (Illumina Inc, San Diego, CA) and deposited under GSE23911 at the National Center for Biotechnology Information Gene Expression Omnibus database. Overall, significant differences in gene expression profiles were noted between $ALDH_{high}$ cells (putative STCs) and the remaining cortical $ALDH_{low}$ cells, emphasizing the profound changes of the STC phenotype.[11] Gene-set enrichment analysis showed that $ALDH_{low}$ cells expressed more genes related to membrane transport and lysosomal proteins consistent with the primary function of tubular cells.[11] $ALDH_{high}$ cells showed down-regulation of some genes involved in apoptosis and up-regulation of hypoxic genes *nuclear factor kappa B* and *interleukin-6* response gene signatures.

Unfortunately, ALDH cannot be used for histologic staining, therefore the exact location of the $ALDH_{high}$ cells could not be determined with certainty (eg, in PECs, in distal tubules or collecting ducts). Furthermore, 7% of all cortical cells were $ALDH_{high}$, which is a relatively high fraction in a 'normal' kidney.[11] These caveats need to be considered when interpreting the presently available transcriptional profiles.

STCs MAY BE MORE SIMILAR TO ACTIVATED PECs THAN TO QUIESCENT PECs

Activation of PECs may occur by as yet unidentified mechanisms in multiple different glomerular pathologies

such as crescentic glomerulonephritis or glomerulosclerosis (reviewed by Shankland et al[18]). Activated PECs show increased proliferation, migration, and matrix production. They can be identified by specific markers, such as de novo expression of CD44.

In immunostainings, we have identified almost 50 additional proteins that are expressed by both PECs and STCs.[13] The most important are vimentin and CD106 (vascular cell adhesion molecule-1). Previously, it was reported that CD106 can be used to differentiate PECs from STCs,[22,23] however, this could not be confirmed in our hands.[13] Kim-1 is expressed at only very low or even negligible levels in quiescent PECs.[13] However, it is significantly up-regulated in activated PECs.[24] The same is true for CD44.[15,25] This implies that the STC phenotype has similarities with the PEC transcriptional program. Activated PECs may resemble the STC transcriptional program more than quiescent PECs.

STCs: CELLULAR DEDIFFERENTIATION OR ACTIVATION OF A SPECIFIC TRANSCRIPTIONAL PROGRAM?

It is an open question whether the STC phenotype reflects cellular dedifferentiation or an alternative cellular program that is activated specifically upon injury. Assuming that cellular dedifferentiation mimics the transcriptional program in development, it is more likely that STCs activate an alternative and specific transcriptional program. Most of the major STC marker proteins are not expressed in proximal tubular cells during renal development (ie, after the S-shaped stage). This has been shown in the human kidney for vimentin,[26] CD24,[27] and CD44,[28] and in the rat for Kim-1.[29] A transgenic mouse line driving Cre expression under control of the Oct-4 promoter does not label tubule cells in development.[30] Oct-4 is very likely a marker for the STC phenotype.[31] In addition, the PEC-rtTA transgenic mouse line showed no transcriptional activity in proximal tubules of juvenile mice.[15]

STCs HAVE AN INCREASED CELLULAR PROLIFERATION

Several investigators have reported increased proliferation of STCs. Upon AKI, a subset of tubule cells co-expressed Kim-1 and vimentin and incorporated more bromodeoxyuridine (BrdU), indicating increased proliferation of these cells.[29] Primary cultures of $ALDH_{high}$ cells (STCs) attach better in culture and show an increased colony-forming capacity compared with $ALDH_{low}$ cells (ie, the remaining cells from human kidney cortex).[11] In addition, the $ALDH_{high}$ cells formed spheres in culture on Matrigel (BD Bioscience, San Jose, CA), whereas the $ALDH_{low}$ cells did not.[11] STCs in culture showed a higher proliferative index compared with the remaining tubular cells.[22] In human kidneys with and without acute tubular necrosis, 70% to 100% of proliferating Ki-67–positive proximal tubule cells also expressed CD24.[13] In transgenic mice subjected to AKI, up to 80% of the BrdU-positive cells were STCs.[15]

WHY STCs CAN BE OBSERVED IN NORMAL HUMAN KIDNEY BUT ARE VIRTUALLY ABSENT IN NORMAL MOUSE OR RAT KIDNEY

Given vastly different lifespans in rodents and human beings, it is not surprising that kidneys of adult human beings always contain a small fraction of glomeruli with discrete signs of glomerulosclerosis. Normal human kidneys tend to originate from patients who suffered from some kind of disease (eg, tumor nephrectomies in older patients or autopsy material from patients who recently died as a result of a serious condition). In contrast, normal rodent kidneys usually are derived from healthy young laboratory animals. As outlined in this review, any injury, such as low-grade proteinuria, can induce the STC phenotype. This likely explains why more STCs can be found in the human kidney.

UNINTENTIONAL DESCRIPTIONS OF STCs IN THE LITERATURE

In retrospect, STCs may have been described in several studies, however, their significance could not be determined at that time. Some of them are highlighted here, although more studies likely have made similar observations.

The earliest description of a distinct cell type with similarities to STCs next to fully differentiated tubular cells was reported in regenerating kidneys 2 weeks after acute injury, but their significance was not noted at that time.[32] Houghton et al[33] described two different types of tubular cell morphologies in more detail after AKI using transmission electron microscopy. First, there were 'apparently residual cells'[33] with preserved microvilli, mitochondria, and prominent ER. Cells of the second type were 'apparently residual cells'[33] (putative STCs). The latter cells were wider with a more loosely arranged cytoplasm. There were fewer mitochondria, only rudimentary or absent microvilli, and no basal infoldings in these cells.

With the advent of immunohistology, multiple reports have observed the transition of proximal tubular cells toward a mesenchymal cell (termed *epithelial to mesenchymal transition*), associated with the expression of mesenchymal cell markers

(eg, vimentin, integrin αV, α-smooth muscle actin), which also are STC markers. In 1994, Witzgall et al[20] noted de novo co-expression of the proliferation marker proliferating cell nuclear antigen and vimentin in tubule cells after acute ischemic kidney injury (IRI). As described earlier, vimentin is a marker of the STC phenotype. In the study by Witzgall et al,[20] vimentin was expressed by 0% of all tubule cells at 0 hours and peaked after 24 to 48 hours after IRI in 60% of all cells. Proliferating cell nuclear antigen, on the other hand, was up-regulated in up to 80% of tubule cells after 48 hours.

In 2006, Gupta et al[31] described "multipotent renal progenitor cells." These cells were derived from rat kidney cortex by prolonged culture. The cells co-expressed vimentin, CD90 (thy1.1), Pax-2, and Oct-4, and were negative for other markers of differentiated cells. In vivo, the typical scattered distribution of STCs was observed in transgenic rats driving β-galactosidase expression under control of the Oct-4 promoter.

Langworthy et al[30] showed indirect evidence that target proteins of calcineurin (Nuclear factor of activated T-cells, cytoplasmic (NFATc) proteins) are expressed in presumptive STCs. An increase in intracellular calcium ions triggers the Ca^{2+}-dependent phosphatase calcineurin to dephosphorylate NFATc proteins, which in turn induces their transport into the nucleus. Calcineurin inhibitors inhibit dephosphorylation of NFATc. Langworthy et al[30] used two transgenic mouse lines driving either LacZ or Cre expression under the control of the NFATc promoter locus. By this approach, they showed that the NFATc locus is not significantly transcriptionally active under physiological conditions in developing and adult mice, supporting that STCs are rare under physiological conditions.

Second, Langworthy et al[30] showed that the NFATc locus becomes transcriptionally active after toxic (mercury) AKI in scattered proximal tubule cells within the renal cortex. These cells incorporated BrdU, indicating increased proliferation. Heterozygous $NFATc^{+/-}$ mice showed delayed regeneration after mercury-induced AKI. Similarly, calcineurin inhibitors delayed regeneration after AKI.[34]

STCs: FIXED PROGENITOR CELLS VERSUS A TRANSIENT PHENOTYPE?

Why is this question important? Unraveling the cellular mechanism of proximal tubule regeneration is a prerequisite for developing specific therapies. For this purpose, identification of the actual target cell and/or of the subpopulation of tubule cells mediating cellular regeneration is of prime importance. In a long sequence of studies, and with improving methodology (especially the advent of in vivo cell fate tracking), it has been shown conclusively that cellular regeneration of injured proximal tubule cells occurs from intrinsic kidney tubule cells (see review by Humphreys[35]). However, there is still some controversy about exactly how tubular cell regeneration occurs and specifically what might be the source of the regenerating cells: fixed intratubular progenitor cells or any surviving tubular cell.

In this regard, Fanconi syndrome is of interest. It comprises the clinical consequences of the loss of specific segments of the proximal tubule. It may be acquired after toxic AKI, especially after chemotherapy. Because acquired Fanconi syndrome may persist over a lifetime, it shows that lost segments of the proximal tubule are not repaired from the remaining segments of the proximal tubule.[36,37] Therefore, either committed fixed intratubular progenitors for example in the S1 segment cannot regenerate S2 proximal tubule cells or surviving S1 tubule cells cannot differentiate into S2 tubule cells. Fanconi syndrome teaches us that the origin of regenerating proximal tubule cells already is committed to the affected segment.

STCs, A FIXED PROGENITOR POPULATION?

When STCs were first discovered, it was proposed that they are the most likely candidate cell population to mediate cellular regeneration after AKI.[11,23] Several observations support this notion: STCs become more numerous after AKI, they express similar antigens as hematopoietic stem cells (eg, glyCD133, CD24, and vimentin), and they show a higher proliferation index.[11,22,38] Gupta et al[31] characterized the expression of marker proteins (eg, Oct-4 and Pax-2) on outgrowing cells from the renal cortex and defined these cells as "stem cells." In vitro, the cells showed a capacity for self-renewal for more than 200 population doublings without evidence for cellular senescence (ie, β-galactosidase activity). STCs could be differentiated toward tubule cells but not toward glomerular visceral epithelial cells, indicating some sort of commitment.[22] When injecting STCs into the kidneys of immunocompromised mice after AKI, renal recovery was improved and the transplanted cells engrafted into the kidney. This could not be observed when injecting the remaining tubule cells.[22]

STCs ARISE FROM ANY SURVIVING PROXIMAL TUBULE CELL AFTER AKI

On the other hand, accumulating evidence argues in favor of STCs as a transient regenerative phenotype or transcriptional program as a common reaction to injury. First, STCs are virtually absent in healthy kidneys of laboratory rats or mice.[13,15] If STCs were

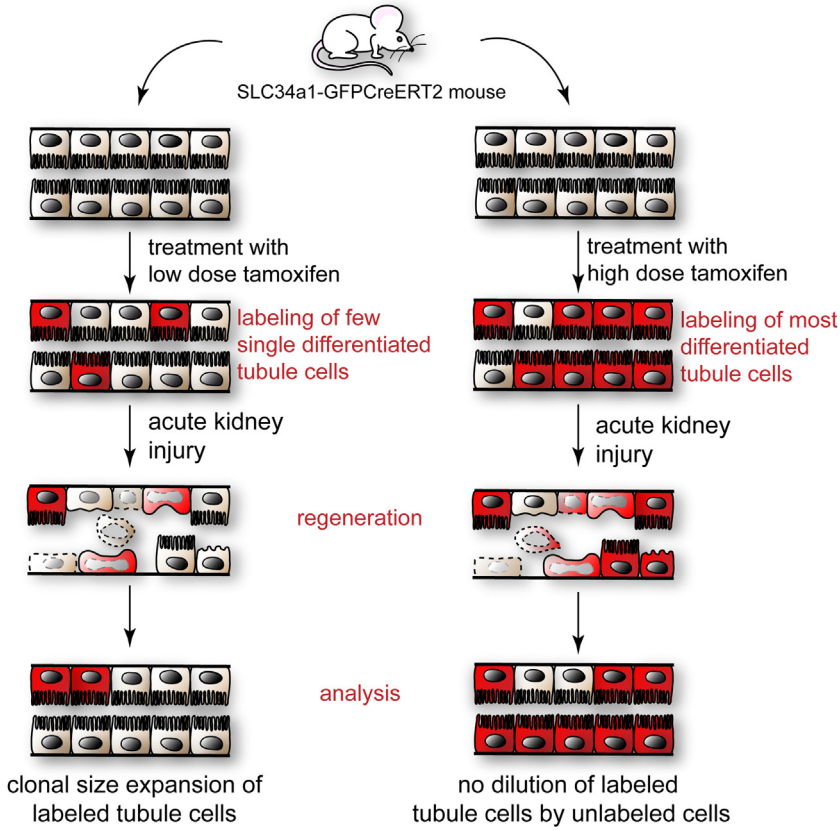

Figure 2. Cell fate tracking of differentiated tubule cells.[39] Left column: When administering low-dose tamoxifen, only a few single tubules are marked genetically (red). After AKI and regeneration, marked cells tend to occur in clusters as a result of clonal expansion of single differentiated tubule cells. Right column: When administering high-dose tamoxifen, the majority of differentiated tubule cells are marked. If regeneration occurred from a presumptive unmarked fixed progenitor population, significantly fewer tubule cells should be unmarked after AKI and regeneration. However, this was not observed in the study by Kusaba et al.[39]

a fixed progenitor population, they always should be detectable in minimal numbers in healthy kidneys.

Retrospectively, similar observations were made by Langworthy et al,[30] who showed that the NFATc locus (as a putative marker for STCs) is not transcriptionally active under physiological conditions in developing and adult mice (see earlier).

Recently, two major studies were published back-to-back investigating the potential existence of a fixed progenitor cell population in proximal tubules.[15,39] Both studies used the current gold standard methodology of irreversible genetic tagging and cell fate tracking, but taking very different approaches. Kusaba et al[39] traced the fate of differentiated tubule cells and Berger et al[15] traced the fate of STCs. In combination, both studies provided extensive experimental evidence against a fixed progenitor population in proximal tubules.

Kusaba et al[39] drove the expression of a tamoxifen-inducible Cre mutant CreER^{T2} within the endogenous gene locus of the sodium-dependent inorganic phosphate transporter SLC34a1. Previously, it was shown that this gene is expressed specifically in the kidney and within the kidney in differentiated proximal tubule cells.[40] Kusaba et al[39] showed that the novel knock-in mouse was transcriptionally active exclusively in the differentiated proximal tubule cells of the S1/2 segment and to a lesser extent also in the proximal part of the S3 segment, recapitulating the expression pattern of the endogenous SLC34a1 gene.

Next, the fate of differentiated proximal tubule cells was traced in two alternative experimental approaches (Fig. 2). The first approach was a 'clone size expansion' analysis, in which only a small percentage of differentiated tubule cells were irreversibly genetically labeled by administration of only low amounts of tamoxifen before induction of ischemic AKI. After the regeneration phase, there were fewer solitary proximal tubule cells (a decrease by approximately 50%) and instead more clusters of labeled proximal tubule cells. The clusters typically consisted of 2 to 5 labeled cells, indicating that the parent tubule cell had undergone on average 1 to 3 cellular divisions during the regeneration phase. The average number in clusters correlated with the extent of ischemic injury. Taken together, the results of these experiments show

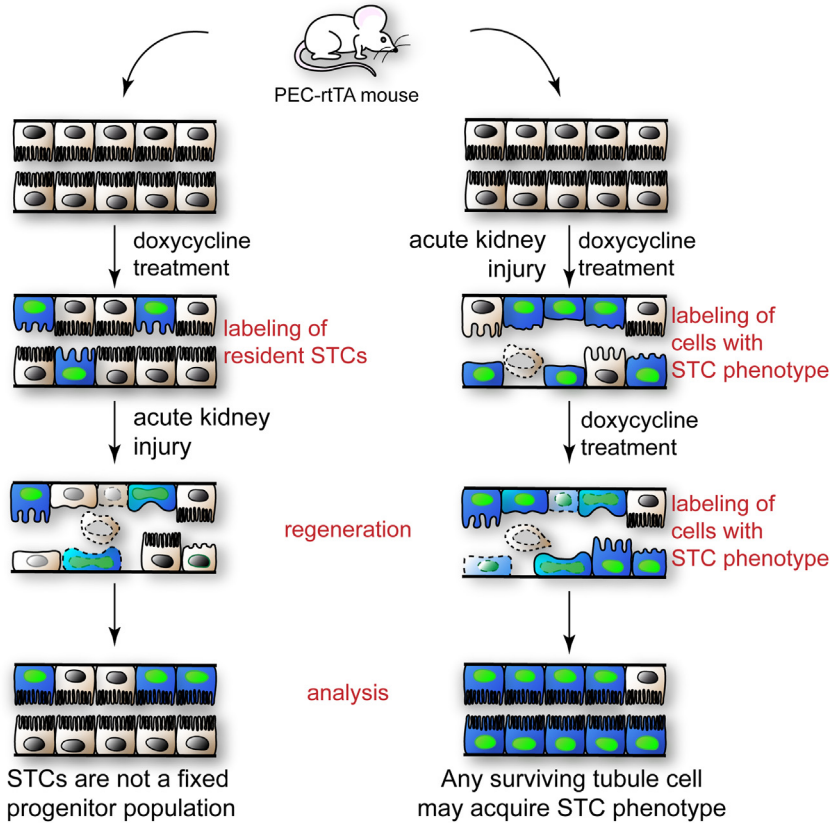

Figure 3. Cell fate tracing STCs after AKI.[15] Left column: The rare STCs in the normal mouse kidney were labeled by transient administration of doxycycline. After AKI and regeneration, the frequency of labeled proximal tubule cells did not increase insignificantly, arguing against the notion that STCs are a fixed progenitor population. Right column: When administering doxycycline after AKI and the subsequent regeneration phase, significantly more tubule cells were marked, arguing that any surviving proximal tubule cell may acquire the STC phenotype after AKI.

that cellular regeneration may occur from differentiated tubule cells.

Next, the investigators performed a dilution experiment, in which as many differentiated proximal tubule cells as possible first were labeled irreversibly by the administration of high doses of tamoxifen (Fig. 2). It is virtually impossible for 100% labeling to be achieved using transgenic mice. The percentage of labeled cells before induction of ischemic AKI then was compared with the percentage after the regeneration phase. The investigators found no significant decrease in labeled cells and concluded that significant regeneration did not occur from an unlabeled fixed progenitor population. This conclusion was somewhat limited by the fact that the investigators could not entirely rule out that the SLC34a1-CreER^{T2} mouse was not transcriptionally active in a putative fixed intratubular progenitor population, although this possibility is unlikely. Finally, the investigators showed up-regulation of STC marker proteins CD133, CD24, KIM-1, and vimentin in genetically labeled tubule cells after AKI. This shows that significant amounts of differentiated tubule cells up-regulate expression of STC markers after injury.

In the second study from our group, Berger et al[15] traced the fate of STCs after AKI (Fig. 3). The investigators took advantage of the fact that PECs and STCs have a similar protein expression pattern. Our group previously generated the first and to date still the only available doxycycline-inducible transgenic PEC-rtTA mouse line that is transcriptionally active in PECs.[19] In the original description of the PEC-rtTA mouse it already was noted that individual scattered cells in the proximal tubule were labeled,[19] suggesting that the PEC-rtTA mouse recapitulates the expression pattern of the specific transcriptional program in PECs and STCs. Indeed, it could be shown that the tubular cells marked by the PEC-rtTA mouse co-express STC markers (KIM-1, annexinA3, SSeCKS, and CD44), proliferate more, and become more numerous in the recovery phase after different tubular cell injuries. From these findings, it was concluded that the PEC-rtTA mouse also marks STCs.

To test whether STCs are fixed progenitor cells, irreversible genetic labeling was induced before AKI

by administration of doxycycline (Fig. 3). However, after the recovery phase, no significant increase of labeled cells was observed. This finding ruled out that STCs are a fixed progenitor population. When inducing the genetic labeling in the PEC-rtTA mouse during the recovery after AKI, significantly more tubular cells were labeled. The increase in STCs occurred much too rapidly (ie, within 24 hours) to be explained by cellular proliferation from only a few pre-existing fixed progenitor cells. Also, STCs appeared throughout the cortex as individual scattered cells and not in continuous clusters, which would be expected if single clones expanded.

Taken together, the results show that STCs are not a fixed progenitor population and that STCs can arise from any surviving proximal tubular cell.[15]

THE STC PHENOTYPE IS NOT ACTIVATED IN TUBULE CELLS IN PHYSIOLOGICAL GROWTH

In previous studies, it already was noticed that under normal conditions, proximal tubular cells undergo cellular divisions while remaining fully differentiated. When normal adult rats received BrdU for 1 week and were analyzed after another week, BrdU-labeled cells were fully differentiated (ie, formed a brush border).[41] In healthy juvenile growing rats, Vogetseder et al[42] showed that cycling tubule cells were fully differentiated similar to noncycling cells during physiological growth in young rats.

Kusaba et al[39] performed the earlier-described "clone size expansion" experiments using cell fate tracking of differentiated tubule cells in healthy juvenile mice. They found that physiological cellular divisions of proximal tubule cells occurred at least in part also from labeled differentiated proximal tubule cells. Finally, our group showed that no STCs could be detected in the kidneys of healthy juvenile mice during physiological growth using the PEC-rtTA mouse.[15]

STCs MAY RENDER THE KIDNEY MORE RESISTANT TO INJURY

STCs may be more resistant to ischemia because they contain significantly fewer mitochondria.[12,13] This suggests that STCs may be able to derive their adenosine triphosphate also from glycolysis, but this still needs further investigation.

In whole-mount tissue culture of explanted human kidneys, the consequences of ischemia were investigated by Hansson et al.[12] For up to 72 hours of organ culture, detaching tubule cells did not show the STC phenotype. On the other hand, tubule cells with the STC phenotype remained anchored to the basal membrane (20% of all tubular cells, while 60% of the STCs survived up to 72 hours). In vitro, it was shown that STCs show a higher resistance to injurious agents compared with all other differentiated cells.[22] In summary, this indicates that the STC phenotype not only may serve regenerative purposes, it also may be more resistant to injuries.

DOES TRANSITIONING INTO THE STC PHENOTYPE MEDIATE THE EFFECT OF PRECONDITIONING?

Preconditioning is a general phenomenon of increased stress resistance against injuries in virtually all tissues and all species examined, especially in AKI (for a summary of the extensive literature see Wever et al[43]). Ischemic preconditioning also renders the kidney resistant to subsequent ischemic injury of the kidney.[44]

In early studies, it was noticed that tubule cells become more resistant to the same kind of injury during the course of AKI. When administering gentamycin to rats for prolonged periods of time (40 mg/kg/d), after initial acute tubular injury, recovery occurred despite continued administration of gentamycin.[6,45,46] The acquired resistance against the toxic effects of gentamycin could be overcome by doubling the dose,[45] or by withholding liquids from the animals to induce water retention and urine concentration by the kidneys.[6] Gentamycin insensitivity was shown to be reversible and transient[45] and also could be induced by other types of tubular necrosis/regeneration.[47] Taken together, these findings are consistent with the notion that transient transition into the STC phenotype is associated with a decrease in endocytotic activity (see earlier) and renders the tubule cells more resistant to gentamycin.

The conditioning stimulus is protective when applied to the target organ itself (ie, kidney) or to a remote tissue (eg, transient ischemia to the skeletal muscle of the arm/leg using a simple tourniquet). The protective mechanism of preconditioning has not yet been resolved at the cellular or molecular level, but it involves a soluble factor transported via the blood. Importantly, the time period between the conditioning stimulus and AKI appears to be crucial. In general, the protective effect is greatest when the conditioning stimulus is applied approximately 24 hours before AKI.[43] Transition into the STC phenotype takes place within a similar time frame. It is tempting to speculate that the as yet unidentified soluble factor induces the proximal tubule cells to acquire the STC phenotype, rendering the kidney more resistant to stress signals and ameliorating renal damage.

STCs AS THERAPEUTIC TARGET: WHAT MAY BE GOOD FOR THE TUBULE MAY BE BAD FOR THE GLOMERULUS AND VICE VERSA

We previously have shown that PECs may become activated in different glomerular diseases.[18,48] In all

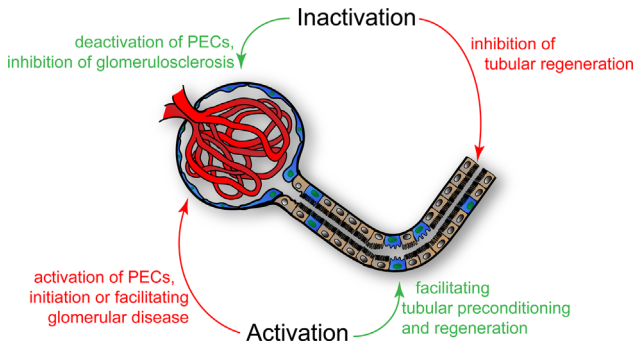

Figure 4. Potentially opposing effects of therapeutic interventions in the glomerulus versus the tubule. Because PECs and STCs have similar transcriptional programs, similar signaling pathways are expected to be activated in both cell types. Activation of PECs is associated with glomerular disease, whereas tubule cells acquiring the STC phenotype (ie, activation) are associated with increased resistance against injury and increased regeneration, and vice versa.

forms of glomerular diseases examined to date, PEC activation contributed to a loss of renal function and progressive scarring. In the tubule, however, transition into the STC phenotype appears to be associated with increased resistance to injury and tubular regeneration. Because activated PECs and STCs express a similar and unique transcriptional profile, it is possible that pharmacologic interventions may exert opposite effects in the glomerulus and tubular system. It may be beneficial to inhibit pharmacologically activated PECs in crescentic nephritis, in which renal function is lost rapidly by tubular obstruction from proliferating PECs.[18,48] The same inhibitor, however, might exert negative effects on tubular cell survival by inhibiting transition into the STC phenotype. Thus, transiently obstructed tubules may degenerate faster and may no longer be able to repair once the therapeutic intervention has induced unblocking of the tubules in the glomerulus (Fig. 4). To treat or even prevent AKI, a pharmacologic agent will be required that pushes tubule cells into the STC phenotype. In the glomerulus, such an agent may activate PECs, and this may be sufficient to induce crescentic nephritis.[49]

SUMMARY AND OUTLOOK

The STC phenotype of proximal tubules represents a transient transcriptional program facilitating regeneration and decreasing the susceptibility against injuries. The transcriptional program likely represents a specific reaction to any kind of cellular injury rather than simple dedifferentiation. STCs proliferate more, are less susceptible to injuries, and express specific markers de novo (Kim-1, vimentin, SSeCKS, annexin A3, and so forth). Because Kim-1 is expressed by STCs, it may be regarded as a marker for kidney regeneration rather than kidney injury. In the proximal tubule, the transgenic doxycycline-inducible PEC-rtTA mouse specifically labels STCs with a higher sensitivity than any of the known STC markers. Thus, the PEC-rtTA mouse represents an important novel tool to specifically mark or manipulate the STC population at any desired time point. Targeting the STC subpopulation of proximal tubule cells therapeutically is a promising novel approach to develop a specific therapy for prevention and amelioration of AKI.

REFERENCES

1. Basile DP, Anderson MD, Sutton TA. Pathophysiology of acute kidney injury. Compr Physiol. 2012;2:1303-53.
2. Gerlach E, Deuticke B, Dreisbach RH, Rosarius CW. [On the behavior of nucleotides and their dephosphorylation degradation products in the kidney in ischemia and short-term post-ischemic re-establishment of blood circulation]. Pflugers Arch. 1963;278:296-315.
3. Uchida S, Endou H. Substrate specificity to maintain cellular ATP along the mouse nephron. Am J Physiol. 1988; 255:F977-83.
4. Porter GA. Risk factors for toxic nephropathies. Toxicol Lett. 1989;46:269-79.
5. Bennett WM. Drug interactions and consequences of sodium restriction. Am J Clin Nutr. 1997;65:678S-681SS.
6. Bennett WM, Hartnett MN, Gilbert D, Houghton D, Porter GA. Effect of sodium intake on gentamicin nephrotoxicity in the rat. Proc Soc Exp Biol Med. 1976;151:736-8.
7. Tanner GA, Sophasan S. Kidney pressures after temporary renal artery occlusion in the rat. Am J Physiol. 1976;230: 1173-81.
8. Parekh N, Esslinger HU, Steinhausen M. Glomerular filtration and tubular reabsorption during anuria in postischemic acute renal failure. Kidney Int. 1984;25:33-41.
9. Stein JH, Lifschitz MD, Barnes LD. Current concepts on the pathophysiology of acute renal failure. Am J Physiol. 1978; 234:F171-81.
10. Cantaluppi V, Quercia AD, Dellepiane S, Ferrario S, Camussi G, Biancone L. Interaction between systemic inflammation and renal tubular epithelial cells. Nephrol Dial Transplant. 2014. (Epub ahead of print)
11. Lindgren D, Bostrom AK, Nilsson K, Hansson J, Sjolund J, Moller C, et al. Isolation and characterization of progenitor-like cells from human renal proximal tubules. Am J Pathol. 2011; 178:828-37.
12. Hansson J, Hultenby K, Cramnert C, Ponten F, Jansson H, Lindgren D, et al. Evidence for a morphologically distinct and functionally robust cell type in the proximal tubules of human kidney. Hum Pathol. 2014;45:382-93.
13. Smeets B, Boor P, Dijkman H, Sharma SV, Jirak P, Mooren F, et al. Proximal tubular cells contain a phenotypically distinct, scattered cell population involved in tubular regeneration. J Pathol. 2013;229:645-59.
14. Tenten V, Menzel S, Kunter U, Sicking EM, van Roeyen CR, Sanden SK, et al. Albumin is recycled from the primary urine by tubular transcytosis. J Am Soc Nephrol. 2013;24:1966-80.
15. Berger K, Bangen JM, Hammerich L, Liedtke C, Floege J, Smeets B, et al. Origin of regenerating tubular cells after acute kidney injury. Proc Natl Acad Sci U S A. 2014;111:1533-8.
16. van Timmeren MM, Bakker SJ, Vaidya VS, Bailly V, Schuurs TA, Damman J, et al. Tubular kidney injury molecule-1 in protein-overload nephropathy. Am J Physiol Renal Physiol. 2006;291:F456-64.

17. Sagrinati C, Netti GS, Mazzinghi B, Lazzeri E, Liotta F, Frosali F, et al. Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. J Am Soc Nephrol. 2006;17:2443-56.
18. Shankland SJ, Smeets B, Pippin JW, Moeller MJ. The emergence of the glomerular parietal epithelial cell. Nat Rev Nephrol. 2014;10:158-73.
19. Appel D, Kershaw DB, Smeets B, Yuan G, Fuss A, Frye B, et al. Recruitment of podocytes from glomerular parietal epithelial cells. J Am Soc Nephrol. 2009;20:333-43.
20. Witzgall R, Brown D, Schwarz C, Bonventre JV. Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. J Clin Invest. 1994;93:2175-88.
21. Vaidya VS, Niewczas MA, Ficociello LH, Johnson AC, Collings FB, Warram JH, et al. Regression of microalbuminuria in type 1 diabetes is associated with lower levels of urinary tubular injury biomarkers, kidney injury molecule-1, and N-acetyl-beta-D-glucosaminidase. Kidney Int. 2011;79:464-70.
22. Angelotti ML, Ronconi E, Ballerini L, Peired A, Mazzinghi B, Sagrinati C, et al. Characterization of renal progenitors committed toward tubular lineage and their regenerative potential in renal tubular injury. Stem Cells. 2012;30:1714-25.
23. Romagnani P. Family portrait: renal progenitor of Bowman's capsule and its tubular brothers. Am J Pathol. 2011;178:490-3.
24. Zhao X, Zhang Y, Li L, Mann D, Imig JD, Emmett N, et al. Glomerular expression of kidney injury molecule-1 and podocytopenia in diabetic glomerulopathy. Am J Nephrol. 2011;34:268-80.
25. Smeets B, Uhlig S, Fuss A, Mooren F, Wetzels JF, Floege J, et al. Tracing the origin of glomerular extracapillary lesions from parietal epithelial cells. J Am Soc Nephrol. 2009;20:2604-15.
26. Holthofer H, Miettinen A, Lehto VP, Lehtonen E, Virtanen I. Expression of vimentin and cytokeratin types of intermediate filament proteins in developing and adult human kidneys. Lab Invest. 1984;50:552-9.
27. Lazzeri E, Crescioli C, Ronconi E, Mazzinghi B, Sagrinati C, Netti GS, et al. Regenerative potential of embryonic renal multipotent progenitors in acute renal failure. J Am Soc Nephrol. 2007;18:3128-38.
28. Crisi GM, Marconi SA, Rockwell GF, Braden GL, Campfield TJ. Immuno-localization of CD44 and osteopontin in developing human kidney. Pediatr Res. 2009;65:79-84.
29. Ichimura T, Bonventre JV, Bailly V, Wei H, Hession CA, Cate RL, et al. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem. 1998;273:4135-42.
30. Langworthy M, Zhou B, de Caestecker M, Moeckel G, Baldwin HS. NFATc1 identifies a population of proximal tubule cell progenitors. J Am Soc Nephrol. 2009;20:311-21.
31. Gupta S, Verfaillie C, Chmielewski D, Kren S, Eidman K, Connaire J, et al. Isolation and characterization of kidney-derived stem cells. J Am Soc Nephrol. 2006;17:3028-40.
32. Ormos J, Elemer G, Csapo Z. Ultrastructure of the proximal convoluted tubules during repair following hormonally induced necrosis in rat kidney. Virchows Arch B Cell Pathol. 1973;13:1-13.
33. Houghton DC, Hartnett M, Campbell-Boswell M, Porter G, Bennett W. A light and electron microscopic analysis of gentamicin nephrotoxicity in rats. Am J Pathol. 1976;82:589-612.
34. Ysebaert DK, De Greef KE, Vercauteren SR, Verhulst A, Kockx M, Verpooten GA, et al. Effect of immunosuppression on damage, leukocyte infiltration, and regeneration after severe warm ischemia/reperfusion renal injury. Kidney Int. 2003;64:864-73.
35. Humphreys BD. Kidney injury, stem cells and regeneration. Curr Opin Nephrol Hypertens. 2014;23:25-31.
36. Perazella MA, Moeckel GW. Nephrotoxicity from chemotherapeutic agents: clinical manifestations, pathobiology, and prevention/therapy. Semin Nephrol. 2010;30:570-81.
37. Skinner R. Chronic ifosfamide nephrotoxicity in children. Med Pediatr Oncol. 2003;41:190-7.
38. McCampbell KK, Wingert RA. Renal stem cells: fact or science fiction? Biochem J. 2012;444:153-68.
39. Kusaba T, Lalli M, Kramann R, Kobayashi A, Humphreys BD. Differentiated kidney epithelial cells repair injured proximal tubule. Proc Natl Acad Sci U S A. 2014;111:1527-32.
40. Biber J, Hernando N, Forster I, Murer H. Regulation of phosphate transport in proximal tubules. Pflugers Arch. 2009;458:39-52.
41. Maeshima A, Yamashita S, Nojima Y. Identification of renal progenitor-like tubular cells that participate in the regeneration processes of the kidney. J Am Soc Nephrol. 2003;14:3138-46.
42. Vogetseder A, Karadeniz A, Kaissling B, Le Hir M. Tubular cell proliferation in the healthy rat kidney. Histochem Cell Biol. 2005;124:97-104.
43. Wever KE, Menting TP, Rovers M, van der Vliet JA, Rongen GA, Masereeuw R, et al. Ischemic preconditioning in the animal kidney, a systematic review and meta-analysis. PLoS One. 2012;7:e32296.
44. Park KM, Chen A, Bonventre JV. Prevention of kidney ischemia/reperfusion-induced functional injury and JNK, p38, and MAPK kinase activation by remote ischemic pretreatment. J Biol Chem. 2001;276:11870-6.
45. Elliott WC, Houghton DC, Gilbert DN, Baines-Hunter J, Bennett WM. Gentamicin nephrotoxicity. I. Degree and permanence of acquired insensitivity. J Lab Clin Med. 1982;100:501-12.
46. Gilbert DN, Houghton DC, Bennett WM, Plamp CE, Reger K, Porter GA. Reversibility of gentamicin nephrotoxicity in rats: recovery during continuous drug administration. Proc Soc Exp Biol Med. 1979;160:99-103.
47. Elliott WC, Houghton DC, Gilbert DN, Baines-Hunter J, Bennett WM. Gentamicin nephrotoxicity. II. Definition of conditions necessary to induce acquired insensitivity. J Lab Clin Med. 1982;100:513-25.
48. Smeets B, Moeller MJ. Parietal epithelial cells and podocytes in glomerular diseases. Semin Nephrol. 2012;32:357-67.
49. Sicking EM, Fuss A, Uhlig S, Jirak P, Dijkman H, Wetzels J, et al. Subtotal ablation of parietal epithelial cells induces crescent formation. J Am Soc Nephrol. 2012;23:629-40.
50. Berger K, Schulte K, Boor P, Kuppe C, vanKuppevelt TH, Floege J, et al. The regenerative potential of parietal epithelial cells in adult mice. J Am Soc Nephrol. 2014;25:693-705.