Dedifferentiation and Proliferation of Surviving Epithelial Cells in Acute Renal Failure

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Abstract. In contrast to the heart or brain, the kidney can completely recover from an ischemic or toxic insult that results in cell death. During recovery from ischemia/reperfusion injury, surviving tubular epithelial cells dedifferentiate and proliferate, eventually replacing the irreversibly injured tubular epithelial cells and restoring tubular integrity. Repair of the kidney parallels kidney organogenesis in the high rate of DNA synthesis and apoptosis and in patterns of gene expression. As has been shown by proliferating cell nuclear antigen and 5-bromo 2'-deoxyuridine labeling studies and, in unpublished studies, by counting mitotic spindles identified by labeling with antitubulin antibody, the proliferative response is rapid and extensive, involving many of the remaining cells of the proximal tubule. This extensive proliferative capacity is interpreted to reflect the intrinsic ability of the surviving epithelial cell to adapt to the loss of adjacent cells by dedifferentiating and proliferating. Adhesion molecules likely play important roles in the regulation of renal epithelial cell migration, proliferation, and differentiation, as do cytokines and chemokines. Better understanding of all of the characteristics resulting in dedifferentiation and proliferation of the proximal tubule epithelial cell and cell–cell and cell–matrix interactions important for this repair function will lead to novel approaches to therapies designed to facilitate the processes of recovery in humans.

In contrast to the heart or brain, the kidney can completely recover from an ischemic or toxic insult that results in cell death. After severe injury, viable and nonviable cells are desquamated, leaving regions where the basement membrane remains as the only barrier between the filtrate and the peritubular interstitium. This allows for backleak of the filtrate, especially under circumstances in which the pressure in the tubule is increased as a result of intratubular obstruction that results from cellular debris in the lumen. In addition, there are rents in the basement membrane itself that result in leakage from the bloodstream into the tubule of molecules such as fibronectin, which may then bind to cells and debris in the lumen, contributing to the obstruction of the tubule (1).

Repair

When the kidney recovers from acute injury, it relies on a sequence of events that include epithelial cell spreading and possibly migration to cover the exposed areas of the basement membrane, cell dedifferentiation and proliferation to restore cell number, followed by differentiation, which results in restoration of the functional integrity of the nephron (2). In the gastrointestinal tract, this sequence of events leading to epithelial repair has been referred to as "restitution." (3). Under normal circumstances, proximal tubule cells divide at a low rate, as evaluated by proliferative cell nuclear antigen (PCNA) and Ki-67 immunoreactivity (4). This cell production balances the loss of tubular epithelial cells into the urine (5). This turnover rate must be under tight control as a small imbalance between cell loss and cell division would soon lead to nephron loss or marked increases in nephron and kidney size over time. This low rate of cell turnover changes dramatically after an ischemic insult in which there is cell death by necrosis and apoptosis and a response to replace these cells. As we have shown in PCNA and 5-bromo 2'-deoxyuridine labeling studies (6) and in unpublished studies, by counting mitotic spindles identified by labeling them with antitubulin antibodies, this response is rapid and extensive, involving many of the remaining cells of the proximal tubule (7). We have interpreted this extensive proliferative capacity to reflect the intrinsic ability of the surviving epithelial cell to adapt to the loss of adjacent cells by dedifferentiating and proliferating. The extent of this process is, in our opinion, too extensive within 24 h of the insult to be due to a subpopulation of resident stem cells if that population is small. In preliminary experiments with bone marrow transplantation, the number of bone marrow–derived cells in the repaired kidney is very small, indicating that the bone marrow is not a significant source to repopulate proximal tubule cells postischemia (Park K-M and Bonventre JV, unpublished data).

Repair of the kidney parallels kidney organogenesis in the high rate of DNA synthesis and apoptosis and in patterns of gene expression. Vimentin, a filament protein that is expressed

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in mesenchymal cells but not in the mature nephron, is detectable in proximal tubules for >5 d after ischemia/reperfusion injury (7). The neural cell adhesion molecule (NCAM), which is expressed in metanephric mesenchyme but not in mature kidneys, is abundantly expressed in proximal tubules 5 d after reperfusion of postischemic rat kidneys (6). Thus, a molecule not expressed by normal mature renal tubule epithelial cells is expressed in proximal tubular cells during recovery from ischemia, recapitulating its expression in early renal development. The mitogenic response may be driven in part by autocrine and paracrine growth factors at the tubular sites of severe injury (8). This mitogenic potential of adult proximal tubule cells has been used as a rationale for the therapeutic use of growth factors to accelerate recovery from acute renal failure (ARF) (9).

Epithelial cell dedifferentiation is a feature of rapidly dividing cells under controlled growth, as in the case postinjury, or noncontrolled growth, as in the case of cancer. This dedifferentiated phenotype in many ways is a reflection of a change in the gene expression pattern of the cell recapitulating the pattern that occurs during kidney development before the mesenchymal-epithelial transition has occurred. Renal mesenchymal cells are dedifferentiated and highly proliferative throughout the developmental period (10). The dedifferentiated phenotype is also likely to be important for the spreading migratory behavior of the viable epithelial cells as they cover the basement membrane during the repair process. The factors responsible for and the significance of reversion to a less differentiated cell phenotype and its relationship to the proliferative and migratory response after renal epithelial cell injury are poorly understood.

The loss of the differentiated phenotype of the epithelial cell of the proximal tubule S3 segment is reflected in a number of ways. The brush border breaks down (11) with blebbing of the apical membrane, fragmentation and internalization, and a rapid change in cell polarity (12). Abnormalities are present in the apical cortical cytoskeleton as reflected by changes in actin localization from apical to lateral cell membrane (13,14). With ATP depletion, cellular free calcium concentration increases, resulting in activation of proteases and phospholipases, which in turn contribute to the disruption of the cytoskeleton and further impair mitochondrial energy metabolism interfering with production of ATP (15). The apical brush border protein villin, an actin bundling and severing protein, appears at the basolateral pole of proximal tubule cells within 1 h after reperfusion (14). Ezrin is dephosphorylated with ischemia resulting in loss of its ability to tether the actin cytoskeleton to the membrane (16). There is dephosphorylation and activation of actin depolymerizing factor, which translocates to the apical region of the cell and enhances microvillar F actin filament severing and depolymerization (17).

ATP depletion of the S3 segment of the proximal tubule (14,18) also results in disruption of cell–cell junctional complexes. Disruption of the tight junction alters both paracellular permeability and cell polarity. The increase in permeability results in backleak of glomerular filtrate. The change in cell polarity has multiple effects resulting from incorrect targeting of membrane proteins. Changes in the localization of Na^+K^+-ATPase, usually confined to the basolateral domain, results in impaired transcellular sodium transport and an increase in intraluminal sodium delivery to the distal tubule. The group IV cytosolic phospholipase A_2, which is activated by ischemia and reperfusion (19), inhibits trafficking of Na^+K^+-ATPase to the cell membrane (20). The enhanced distal sodium delivery may result in afferent arteriole vasoconstriction and reduction in GFR. In transplant recipients with ischemic injury resulting in delayed graft function, Kwon *et al.* (21) demonstrated a striking increase in fractional excretion of both sodium and lithium, which are normally co-transported in the proximal and distal tubules. The observed changes in fractional sodium and lithium excretion coincide with loss of Na^+K^+-ATPase from the basolateral membrane of proximal tubules.

In the postischemic kidney, other genes whose expression is, under normal conditions, otherwise detected at increased levels in the developing kidney fall into a number of functional categories. Some encode growth factors IGF-1 (22,23), fibroblast growth factors (24–27), and hepatocyte growth factor (28,29); others encode transcription factors Pax-2 and Egr-1 (30,31). Some encoded proteins, bcl-2 and bax, have been implicated in apoptosis regulation, a property characteristic of many renal proximal epithelial cells during both development (32,33) and after injury (34). Other growth factors, such as EGF (31,35), and nuclear DNA binding proteins, such as Kid-1 (36), are downregulated in both kidney development and repair after injury. When and how these genes regulate the differentiation state of the cell are ill-defined.

The dedifferentiation of renal tubular cells to recapitulate gene expression patterns typical of the developing nephron has major implications for the regulation of renal repair; however, the relationships between proliferation and alterations in the state of cellular differentiation have not been defined. For example, it is not clear to which stage of development the tubule cells revert and how the temporal patterns that evolve may relate to injury, proliferation, and final redifferentiation of the cell. It is not clear to what extent the inductive interactions that occur during kidney development are critical for the repair of the proximal nephron (10).

Dedifferentiation of the epithelial cell may play an important role in spreading and migration of cells over the denuded basement membrane early in the recovery process. In a model *in vitro* of this process, Toback *et al.* (37) scratched cells off the tissue culture plate and monitored the migration of the cells into the denuded area, as well as the gene expression pattern of the cells. After "wounding" the monolayer, there was upregulation of the immediate-early genes encoding Egr-1, c-fos, NAK-1, and gro at 1 h, followed by peak levels of mRNA encoding connective tissue growth factor and c-myc at 4 h. mRNA levels of urokinase-type plasminogen activator and its inhibitor (PAI-1) and heat shock protein-70 were markedly raised 4 to 8 h after wounding. By contrast, mRNA levels for osteopontin, EGF, and hepatocyte growth factor (c-met) receptors, were reduced. NAK-1, PAI-1, and heat shock protein-70 were induced or stimulated only in cells at the wound edge. Adenosine diphosphate, a potent stimulator of cell migration, stimulated expression of urokinase-type plasminogen activator

and PAI-1 after wounding. The RET-glial cell-derived neurotrophic factor pathway stimulates migration of renal epithelial cells (38) and may play a role in regulation of migration, but this has not been studied postischemia. RET-glial cell-derived neurotrophic factor expression is upregulated in renal epithelial cells of the dysplastic human kidney associated with obstruction and high levels of proliferation (39). Another protein, CD44, which is upregulated in the S3 cell with ischemia/reperfusion (40), and its ligands osteopontin and hyaluronic acid, are expressed at wound margins associated with cellular proliferation and migration of injured mucosal and vascular endothelial tissues. CD44 peptide is localized to the basal and lateral cell membranes.

Clearly matrix molecules likely play an important role in the migration that occurs during the repair process. Within 3 h of reperfusion after ischemia, cellular fibronectin is deposited (41). This may stimulate dedifferentiation, based on studies carried out in the skin, gastrointestinal tract, and cornea. At 1 d postischemia, hyaluronic acid is upregulated in the interstitium surrounding regenerating tubules. Osteopontin is upregulated in the proximal tubules after acute ischemic injury (40,42). Immunoreactive osteopontin peptide continues to be localized in those tubules still undergoing repair for as long as 7 d after the injury. At later times after ischemia, laminin isoforms are expressed. It has been proposed that laminin deposition may regulate redifferentiation and repolarization of the epithelium (43).

Cell Adhesion Molecules

Because cell adhesion molecules are of general importance in cell-substratum integrity and signaling, these molecules likely play a role as regulators of migration, differentiation, and proliferation. Cell adhesion and traction allow the cell to pull itself forward. Cell adhesion molecules have been implicated in many aspects of cell injury and repair in the kidney. Integrins tether the epithelial cell to the basement membrane via interaction with the laminin-rich basement membrane. Integrins are heterodimers with one α and one β subunit. Both subunits are type 1 transmembrane glycoproteins with long ectodomains and, with the exception of the vertebrate $\beta 4$ subunit, short intracellular domains (44). The group of integrins that are most widely expressed in the kidney are those that contain the β_1 subunit, the primary family of receptors for extracellular matrix in mammalian cells (45). It seems that the $\alpha_6\beta_1$ integrin is the most important laminin receptor integrin in the adult proximal tubule (45). After ischemia/reperfusion, the normally basal β_1-integrins are relocated to the lateral borders of the cell (1) and the $\alpha 3\beta 1$ integrin is induced (43).

It is interesting that during normal kidney mesonephros development, β_1-integrins localize to all epithelial cell surfaces and become localized only to the basal surface of the cell when the developing nephron elongates and matures (46). The $\alpha_8\beta_1$ integrin is expressed in the kidney. When the gene for α_8 subunit is mutated, there is an abnormality in extension of the ureteric bud, and in most cases, no kidney is formed (47). On the basis of the idea that "exposed" integrins would facilitate cell–matrix and cell–cell interactions in the lumen of the proximal tubule and hence add to the tendency of the luminal contents to obstruct, Goligorsky and Noiri and colleagues (48,49) used RGD peptides to compete with integrin receptors. They found protective effects of these integrins. It is not clear, however, whether the protective effects found are due to the envisioned interactions because Zuk *et al.* (1) found no apical localization of β_1 integrins after ischemia/reperfusion, $\beta 1$ integrin staining was not found on free cells in the lumen of the postischemic proximal tubule. It is possible that the presence of $\alpha_6\beta_1$ integrin has an effect on the differentiation and polarity of the proximal tubule and also has an antiproliferative effect. In human prostate epithelial cells, the loss of this integrin is associated with decreased polarity and acinar formation *in vitro*, properties associated with increased invasiveness of tumors derived from these cells *in vivo* (50). Integrins are important for migration during development (51), and it is possible that the altered localization of integrins in the postischemic proximal epithelial cell (1) could contribute in important ways to the ability of the cell to migrate over the regions of basement membrane exposed by the loss of epithelial cells. Disruption of cell–matrix adhesion would also be expected to result in apoptosis (52), a feature of the postischemic epithelium (2).

Other adhesion molecules are implicated in control of cell mitogenesis, differentiation, anchorage dependence, and apoptosis (1,52). NCAM is a member of the Ig superfamily of proteins that mediate homophilic (NCAM-NCAM) and heterophilic cell–cell interactions (53,54). NCAM has been implicated in the control of cell shape and migration (55) and epithelial polarity (56). In embryonic kidney, NCAM is present in cells of the metanephric mesenchyme (57–59) that are induced to aggregate and to increase in density by the ureteric bud. During conversion to more mature phenotypes, NCAM is rapidly downregulated (59–61). NCAM is expressed in the dedifferentiated cells of Wilms tumors, an embryonic type tumor of the kidney (62). This molecule has been used as a marker for the dedifferentiated phenotype in studies of metanephric mesenchyme (56,61) and in cultured metanephric cells (58). We found that NCAM is detectable by immunohistochemistry in renal vesicles, S-shape bodies, and early tubules (6). There is minimal cellular NCAM expression in tubules of the adult kidney. In postischemic kidneys, NCAM expression is abundant in S3 proximal tubule cells 5 d after reperfusion. As in developing tubules, NCAM is concentrated in basal and lateral aspects of cells that lack expression of two other molecules that characterized the differentiated phenotype of the proximal tubule cell: apical brush border gp330 (63) and dipeptidyl peptidase IV (64). The expression of NCAM is preceded by disassembly of the brush border and proliferation of surviving S3 cells, which is most prominent at 2 d postischemia. NCAM expression persists in some flattened and dedifferentiated cells for up to 7 wk after ischemia.

Another class of adhesion molecules that may be important for the regulation of epithelial cell spreading, migration, differentiation, and proliferation are the leukocyte-endothelial adhesion molecules. There are several classes of leukocyte-endothelial cell adhesion molecules (65), including selectins and integrins. These molecules regulate the intravascular trap-

ping of leukocytes. Integrins interact with Ig-like adhesion molecules such as ICAM and VCAM, which are expressed by endothelial cells and are upregulated after ischemia in response to cytokines. We have shown that anti–ICAM-1 antibodies protect against ischemic renal injury in animals (66) and ICAM-1 knockout mice are protected (67). These leukocytes can generate compounds, such as cytokines and chemokines, which can influence the level of injury, migration, differentiation, and proliferation of kidney epithelial cells. Leukocytes accumulate in the kidney with ischemic renal injury (67,68). Different subclasses of leukocytes are likely to be important at different phases of the injury and repair process in ARF. Infiltration of macrophages and T lymphocytes may predominate over neutrophil infiltrate at a later time after ischemia/reperfusion injury (69). Leukocytes can potentiate renal injury through generation of reactive oxygen species, which can upregulate the expression of adhesion molecules on endothelial cells promoting further leukocyte infiltration. Neutrophils synthesize proteases, including serine proteases, elastase, and metalloproteinases (collagenases and gelatinases), that degrade components of the extracellular matrix. Finally, the leukocyte produces phospholipase metabolites, including various products of cyclooxygenase, lipoxygenase, and the cytochrome p450 enzymes, which are important modulators of vascular tone.

Cytokines and Chemokines

Cytokines have been implicated in lung tissue remodeling. In this organ, TNF-α and IL-1β stimulate macrophages to produce matrix metalloproteinase-9 (MMP-9) and stimulate bronchial epithelial cells to produce extracellular matrix glycoproteins such as tenascin (70). Leukocytes produce TGF-β (71), which influences cell growth, cell differentiation, and cell chemotaxis. TGF-β1 has been previously shown to promote a migratory and adherent transformation of monolayers of renal proximal tubule cells in primary culture. TGF-β1 promotes an increase in the production of proteoglycans and a higher ordered structure of the cytoskeleton of the proximal epithelial cell, effects important for regulation of the adhesive migratory response of these cells as well as the DNA synthesis rate response to both EGF and TGF-β1 (72).

Chemokines and selectins (73) are upregulated by inflammatory cytokines, such as IL-1 and TNF-α. Chemokines recruit and, upon adhesion, activate leukocytes. Circulating or locally produced TNF-α may contribute to leukocyte infiltration. Infusion of a TNF-α binding protein decreases bioactivity of TNF-α and neutrophil infiltration and preserved renal function, suggesting that local TNF-α synthesis may be an early and pivotal event in renal ischemic/reperfusion injury (74). Reactive oxygen species produced on reperfusion of the ischemic kidney may also upregulate chemokine expression. Transgenic mice that overproduce the antioxidants intracellular and extracellular glutathione peroxidases (75) have less histologic injury and preserved renal function after 32 min of ischemia followed by 24 h of reperfusion. Neutrophil infiltration was less marked in the transgenic animals compared with wild-type controls. Thus, ischemia/reperfusion injury involves a multifactorial inflammatory response initiated by factors that result in leukocyte infiltration, which can lead to tissue edema and compromise microvascular blood flow, ultimately leading to cell apoptosis or necrosis. Chemokines have been implicated in proliferation, chemotaxis, and remodeling in other tissues and may have similar effects on the renal epithelium. Monocyte chemotactic protein-1 stimulates human retinal pigment epithelial cell migration in a dose-dependent manner (76). CXC chemokines induce hepatocyte proliferation and have been proposed to be important for liver injury, repair, and regeneration (77).

Kidney Injury Molecule-1

We have cloned kidney injury molecule-1 (Kim-1) from rats, mice, and humans. Kim-1 is markedly upregulated on the proximal tubule in the postischemic rat kidney (78). Structurally, Kim-1 is a member of the Ig gene superfamily most reminiscent of mucosal addressin cell adhesion molecule 1 with an extracellular Ig and mucin domain. The Kim-1 ectodomain is shed into the extracellular milieu of human 769-P (human kidney adenocarcinoma cells) and HK-2 (human kidney proximal tubular cells) cell lines, which express Kim-1 under normal culture conditions (79). Human KIM-1 protein is expressed by the proximal tubule of humans with ARF, and the ectodomain appears in the urine of patients with ARF (80). Urinary KIM-1 protein levels were more reliable than levels of urinary γ-glutamyltransferase, alkaline phosphatase, and total protein as a biomarker for tubule injury in ARF. Kim-1 is expressed on the apical membrane, and it is possible that it is involved in regulation of spreading, migration, and/or regulation of proliferation and differentiation of the proximal epithelial cells

Conclusions

In summary, during recovery from ischemic/reperfusion injury, surviving tubular epithelial cells dedifferentiate and proliferate, eventually replacing the irreversibly injured tubular epithelial cells and restoring tubular integrity (2). Renal epithelial cells have a powerful capacity to proliferate and replace cells that have been lost from the epithelium as a result of the injury. Better understanding of all of the characteristics resulting in dedifferentiation and proliferation of these cells and cell–cell and cell–matrix interactions important for this repair function will lead to novel approaches to therapies designed to facilitate the processes of recovery in humans.

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