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The renal stem cell system in kidney repair and regeneration

Franca Anglani[1], Monica Ceol[1], Federica Mezzabotta[1], Rossella Torregrossa[1], Emilia Tiralongo[1], Enrica Tosetto[1], Dorella Del Prete[2], Angela D'Angelo[2]

[1]Kidney Histomorphology and Molecular Biology Laboratory and [2]Nephrology Division, Department of Medical and Surgical Sciences, University of Padua, Italy

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1. ABSTRACT

The adult mammalian renal tubular epithelium exists in a relatively quiescent to slowly replicating state, but has great potential for regenerative morphogenesis following severe ischemic or toxic injury. Kidney regeneration and repair occur through three cellular and molecular mechanisms: differentiation of the somatic stem cells, recruitment of circulating stem cells and, more importantly, proliferation/dedifferentiation of mature cells. Dedifferentiation seems to represent a critical step for the recovery of tubular integrity. Dedifferentiation of tubular cells after injury is characterized by the reactivation of a mesenchymal program that is active during nephrogenesis. Epithelial-to-mesenchymal transition (EMT) of renal tubular cells is an extreme manifestation of epithelial cell plasticity. It is now widely recognized as a fundamental process that marks some physiological, such as morphogenesis, as well as pathological events, such as oncogenesis and fibrogenesis. It might be also considered as a key event in the regenerative process of the kidney. Understanding the molecular mechanisms involved in EMT might be useful for designing therapeutic strategies in order to potentiate the innate capacity of the kidney to regenerate.

2. INTRODUCTION

An important, though often underestimated and poorly understood biological defense mechanism, common to many organisms, is the inborn capacity for tissue and organ regeneration. Unicellular and simple pluricellular organisms have a system of regeneration that involves the whole organism, whereas the capacity for regeneration of more complex organisms becomes restricted to particular organs or tissues.

In mammals, continuously renewing tissues such as the intestinal epithelium and epidermis possess great regenerative potential. However, cell turnover phenomena are going on continually in many organs and tissues. There are three, not mutually exclusive, cellular and molecular mechanisms involved in tissue regeneration, i.e. mature cells may proliferate, immature cells (or somatic stem cells) may become differentiated, and circulating stem cells may be recruited and subsequently undergo homing and differentiation. Mature cell proliferation is the simplest regenerative mechanism in parenchymal organs such as the liver.

The kidney shows a strong capacity for regeneration, although the postnatal mammalian kidney is

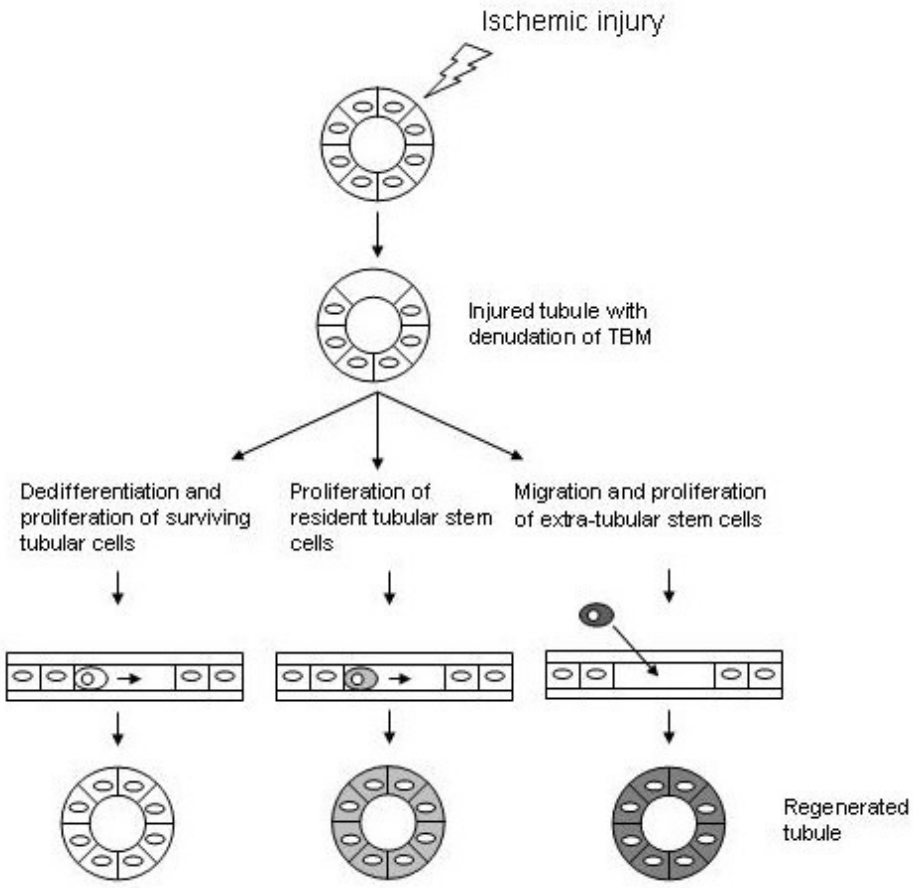

Figure 1. The origin of tubular epithelial regenerating cells in acute renal injury.

unable to generate new nephrons in response to nephron loss. Therefore, the repair processes here discussed, presumably operate where only part of the nephron has sustained damage.

The anatomical and functional recovery of renal integrity after injury is accompanied by the activation of sophisticated processes that have yet to be fully understood, by which damaged tubular cells are completely replaced by normal well-functioning cells that reorganize their architecture to recreate a normal tubule. Although many of the molecular details of this process have been clarified over the last 20 years, the cellular source of newly formed renal epithelial cells is still being debated. Some studies have shown that novel cells derive from the division of differentiated cells (1,2); others have claimed that subpopulations of renal tubular cells act as progenitor cells (3-6); and yet other studies have demonstrated that hematopoietic stem cells and circulating mesenchymal stem cells can repopulate the tubular cell system (7,8) (Figure 1).

Most data on the origin of new tubular epithelial cells in the kidney derive from studies on the regeneration/repair process after tubular necrosis due to ischemic injury.

3. RENAL REPAIR AFTER ISCHEMIC INJURY

The kidney has to filter the blood to remove accumulated toxins and concentrate the urine to prevent dehydration: this dual role generates a high demand for cellular oxygen in a region of relatively low blood flow, making the renal tubular cell particularly susceptible to injury. Acute renal failure (ARF) is the disease in which many different types of damage, including exposure to exogenous toxins, release of endogenous cytokines and toxins, and/or episodes of renal hypoperfusion, cause acute tubular cell necrosis (ATN) via a mechanism involving a reduction in the total or segmental renal blood flow (9).

ATN is the pathological condition recognized in acute renal injury and, in turn, gives rise to deterioration in renal function over a period of hours to days, resulting in the kidney's failure to excrete nitrogenous waste products and maintain fluid and electrolyte homeostasis (acute renal failure, ARF). The recovery of renal function after ARF relies on the appropriate replacement of necrotic tubular segment with a functional tubular epithelium.

In the outer medulla, where tubules have high oxygen requirements, ischemia leads to structural changes

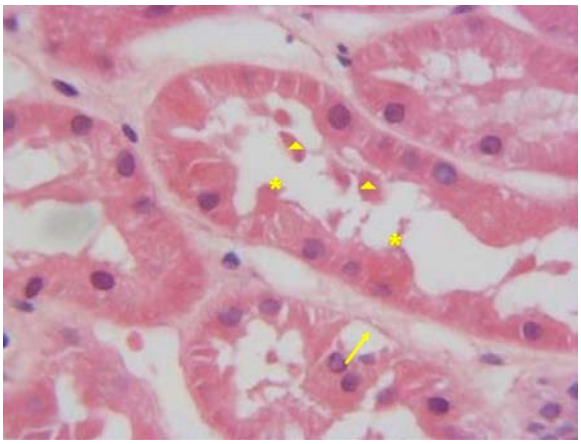

Figure 2. Tubular epithelium degeneration following acute renal injury (Hematoxilin and Eosin stain): high power view (40x) shows necrosis of the tubular epithelium with separation of tubular cells from the tubular basement membrane (↑). In addition to denudation of the tubular epithelium, necrotic epithelial cells are noted in the tubular lumens (▲). Tubular epithelial surface "blebbing" of the apical cell membrane is also shown (*).

in the proximal tubule cells. These include loss of tubular cell polarity, loss of tight junction integrity as a consequence of changes in the actin and microtubule cytoskeleton networks, detachment from the tubular basement membrane, and necrotic and apoptotic cell death (10) (Figure 2). In addition to tubular cell injury, the peritubular vasculature undergoes changes in response to ischemia, with endothelial cell swelling, endothelial junction disruption and detachment of both living and dead cells. The altered peritubular vasculature contributes to the expansion of the initial ischemic insult (11). Restoring tubular integrity is crucial to recovery, but restoring the blood flow once endothelial cell integrity has been regenerated is equally essential.

Many animal models of ischemia/reperfusion injury have given us a better understanding of not only the cellular and molecular mechanisms of ischemic injury, but also more importantly, the tubular regeneration mechanism (12-15). Several days after injury, many surviving tubules recover the brush border, and within 3 weeks of the injury, the lost tubular cells completely replaced by new cells. Proliferation of tubular cells is the main event preceding the recovery of tubular integrity (10).

3.1 Tubular cell proliferation in healthy kidneys

The adult mammalian renal tubular epithelium exists in a relatively quiescent to slowly replicating state, and cell turnover in the healthy adult kidney is very slow. Knowing more about cell proliferation in normal kidneys would help us to understand the regenerative capacity of tubular cells.

Three very recent papers by Vogetseder *et al* (16-18) have addressed this issue. Taking a morphological approach, they demonstrated that cell proliferation in the healthy kidney of young rats relies on the division of differentiated cells and that the bulk of cells in the S3 segment of the proximal tubule participate in cell proliferation. They reasoned that, if cell proliferation relies on stem cells and transit-amplifying progenitor cells (TA cells), then cycling cells should be less well differentiated than their non-cycling neighbour cells. The slow cycling cells, identifiable from their ability to retain the thymidine analog bromo-deoxyuridine (BrdU), were found in the S3 segments. Expression of markers of terminal differentiation, such as basolateral Na-K-ATPase, NaPiIIa and PMP7, and typical tubular cell polarity, indicated that the cells are well differentiated. The authors also found that the proliferative capacity of renal proximal tubular cells stems from a large reserve of cells in G1 phase and thus ensuring a rapid proliferative response when needed (18).

3.2 Tubular cell proliferation in injured kidneys

The S3 segment of the proximal tubule is the part of the nephron most susceptible to ATN, but has great potential for regenerative morphogenesis after severe ischemic or toxic injury. Several publications have demonstrated that the proximal tubule can restore its integrity completely, although there is ongoing debate that how the renal regenerative response is completed and the role of inadequate regeneration following acute injury being a factor in the progression of chronic kidney disease. Viable cells enter into an activated proliferative state characterized by the reappearance of some of the mesenchymal markers detectable during nephrogenesis, e.g. vimentin, neural cell adhesion molecule (N-CAM), PAX2 and basic fibroblast growth factor (19-22). The rapid switch also documents loss of differentiation in cell polarity, reflected by changes in: 1) the actin cytoskeleton from apical to lateral cell membrane, 2) the localization of brush border proteins such as villin at the basolateral pole, 3) the delocalization of Na-K- ATPase, usually confined to the basolateral domain (2). Dedifferentiation seems to be a crucial step in the recovery of tubule integrity and precedes the reconstitution of a well-differentiated morphology. Poorly differentiated cells resembling epithelial precursors proliferate and migrate along the denuded basement membrane of injured tubular segments within a few days of ischemic insult and then redifferentiate into mature tubular cells (1).

The kidney's intrinsic ability to regenerate in response to injury is believed to be sustained by the renotropic system. Hepatocyte growth factor (HGF) is likely to have a leading role in the renotropic system, acting via paracrine and autocrine as well as endocrine mechanisms. In the event of injury, the kidney's stromal cells produce HGF, in response, the epithelial tubular cells up-regulate the specific receptor c-Met, and activate intracellular signals that have a mitogenic, motogenic, morphogenic and anti-apoptotic effect on the tubular cell (23).

Dedifferentiation after epithelial injury suggests that renal regeneration recapitulates some aspects of renal development, because many of the proteins induced in the post-ischemic kidney play an important part in nephrogenesis. In fact, the HGF/c-Met interaction in the

regenerative phenomena echoes the cross talk between epithelium and mesenchyme that represents the key event in morphogenic kidney development (24). It is generally assumed that adult kidney development is a mutually inductive process: the ureteral bud grows out from the caudal part of the Wolffian duct and invades the metanephric blastema. Factors secreted by the ureteric bud induce aggregation of this mesenchyme and its conversion into an epithelium, followed by maturation to form the specialized nephron (25). The induced mesenchyme, in turn, transmits signals back to the ureteric bud that prompt the latter to divide and grow. This reciprocal induction process continues progressively from the deep to the outer cortex, producing the branches of the collecting duct and nephrons.

3.3 Dedifferentiation of tubular cells

Therefore, proliferation occurs together with dedifferentiation in the ischemic kidney, which is in contrast to the situation in the healthy kidney. Vimentin is considered the main marker of dedifferentiation in injured tubular epithelial cells. Vimentin is not expressed in tubular cells of a healthy kidney when active proliferation was induced by lead (II)-acetate treatment (17). Lead salts trigger a strong proliferation of proximal tubular cells without inducing tubular injury; this could possibly be due to the activation of the mitogen-activated protein kinase pathway (26). The functional role of vimentin re-expression in epithelial cells is not entirely clear. Vimentin is a class III intermediate filament component expressed in mesenchymal-derived cells in adult tissues and in many proliferating epithelia during diseases such as cancer (27). In the mature kidney, tubule-interstitium vimentin is exclusively present in the interstititial cells and appears in tubular cells only during the recovery phase following tubular necrosis (28,29). In all these pathological conditions, vimentin is considered a marker of dedifferentiation, and this seems particularly true of the kidney where all epithelial cells of the nephron are derived from the mesenchymal cells of the metanephric blastema. Vimentin is also expressed in tubular cells in culture, where a fully differentiated phenotype has been found coexisting with the cytokeratin cytoskeleton (30,31). It has recently been demonstrated that tubular integrity was recovered after ischemic injury in mice lacking vimentin and was morphologically and functionally similar to the situation in control mice (32-34). The authors suggested that vimentin acts as an essential element in restoring the specific transport function, possibly via the maintenance of membrane physical state, i.e. vimentin may not be a marker of dedifferentiation, but its re-expression may be instrumental to the recovery of the polarity and transport capability of the tubular cells.

The fact that proliferation in healthy kidneys occurs with no dedifferentiation, which is in contrast to the situation in damaged kidneys, suggests that quite different mechanisms may underlie tissue cell turnover and regeneration after injury, possibly resembling those occurring in the liver. The liver has the unique capacity to regulate its growth and mass (liver regeneration), and that is why it would be a good model for studying regenerative mechanisms.

Liver regeneration after partial hepatectomy, relies on the proliferation of hepatocytes, which are highly differentiated and long-lived cells with a remarkable capacity for multiple rounds of replication without dedifferentiation. Hepatocyte proliferation is sufficient to sustain physiological cell turnover and liver regeneration after a sub-lethal damage. When hepatocytes are slow or unable to respond due to toxic injury, cells of various lineages can also differentiate into hepatocytes (35-37). These include: 1) the oval cells located in close proximity to the terminal part of the bile duct, which are presumably the progeny of hepatic adult stem cells (probably as transient amplifying progenitor cells); and 2) bone marrow cells; several experiments have confirmed that hematopoietic stem cells can generate cell lineages in the adult liver. Although how much such cells contribute to liver growth responses is still hard to say (the phenomenon appears to be slow and incomplete). Moreover, in most cases it appears that bone marrow-derived stem cells fuse with resident hepatocytes rather than undergoing transdifferentiation to generate liver cell lineages (38,39)

4. STEM CELLS IN KIDNEY REGENERATION AND REPAIR

If cells that are not yet fully mature are implicated in cell proliferation and recovery after ischemic injury, then a stem cell system should be involved in the kidney too. In fact, the contribution of both kidney specific and bone-marrow-derived stem cells to the phenomenon of renal regeneration/repair has been fairly well documented in several studies.

4.1 The adult stem cell system

The vital function of maintaining tissue homeostasis and integrity throughout life relies on adult somatic stem cells having particular functional features. They need to be highly undifferentiated, so they have none of the typical morphological, structural, molecular and antigenic characteristics of the mature cells of the organ to which they belong. Intrinsic in the stem cell population, is its ability not only to duplicate itself (called self-renewal) but also to produce differentiated cells (24). These two fundamental properties of stem cells - self-renewal and differentiation - derive from two different types of proliferative behavior, asymmetrical and symmetrical division (Figure 3). In invertebrates, each time stem cells divide asymmetrically they produce a single cell identical to its mother (for self-maintenance) plus a differentiated sister cell (transient amplifying precursor, TA cell) with a more limited proliferative capacity that is needed to produce mature cells. Vertebrates also feature a more flexible, symmetrical division process by means of which stem cells can either expand or differentiate in response to specific stimuli. This mechanism acts as a regulatory system: in the event of injury or the onset of pathological conditions, the extracellular environment can modify the size of the stem cell population and the number of its differentiated progeny. The stem cells' capacity for self-renewal is assured by balancing the numbers of symmetrical divisions that generate two stem cells with those developing into two differentiated cells; so there

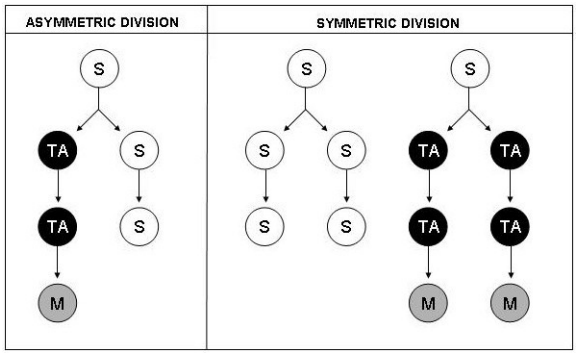

Figure 3. Proliferative behaviours of somatic stem cells: **S** stem cell; **TA** transient amplifying precursor; **M** mature cell.

should be an intermediate compartment of TA cells, in regenerating tissues at least, between the stem cell compartment and the mature cells.

4.2 Bone-marrow-derived stem cells

The hematopoietic stem cells (HSC) remain the best-characterized example of an adult stem cell population and it has long been recognized that a single HSC can reconstitute all hematopoietic cell lineages.

According to a fundamental biological dogma, adult somatic stem cells differ from embryonic stem cells in that they are partially committed; their multipotent capacity is restricted to a certain number of differentiated cell lineages. Some doubts were cast on this conviction, however, in 1998, hematopoietic stem cells proved capable of giving rise to skeletal muscle cells, thus revealing the capacity to transdifferentiate, i.e. the differentiated cells can take on a new identity by switching off one set of lineage-specific genes and activating genes of another, differentiated cell type (40). Needless to say, that studies proposing such a plasticity of adult somatic stem cells remain controversial and existing evidence suggests that *in vivo* transdifferentiation are very rare and that many cases of apparent transdifferentiation are actually fusion events (41).

Three papers published in 2001, demonstrated that during acute or chronic injury in the transplanted kidney, circulating cells are recruited from the peripheral blood for regeneration and tissue repair (42-44). These works provided the first proof that blood cells contain bone-marrow-derived staminal populations - pluripotent HSCs and mesenchymal stem cells or marrow stromal cells (MSCs) - capable of colonizing the kidney and giving rise to mature tubular, endothelial, myofibroblastic and mesangial cells. The idea that bone-marrow contains cells that home to the tubular epithelium has since been further explored and partially confirmed in mice using various models of ischemic injury (45,46). On the other hand, Lin *et al* (47) and Duffield at al (48) demonstrated that bone-marrow-derived stem cells do not contribute significantly to the recovery of epithelial integrity by means of their integration in regenerating tubules. The consensus view is that bone marrow-derived stem cells contribute to renal repair and regeneration via a complex paracrine action , which may be a more protracted response that becomes important in late-stage organ repair (49,50). These studies have shown that the main sources of regenerating tubular cells are resident renal cells.

4.3 Stem cell niches

There is strong evidence to support the existence of tissue-specific stem cells in various organs, for which tissue specific stem cell markers have enabled purification and lineage tracing (51). In contrast, the lack of any definitive stem cell markers in the kidney has hampered the characterization of kidney-specific progenitor populations.

Stem cells tend to be located in deep, narrow tissue niches, where the proliferation that assures self-maintenance occurs. Niches should assure specific environmental cues such as oxygen tension (52,53) and extracellular matrix components (54) providing protective conditions for stem cells. The transient zone is rich in amplifying precursor cells and the differentiated cells lie in increasingly distal cell layers, depending on their stage of maturation. While the correspondence between the directional flux of cell maturation and specific anatomical districts can be found in some highly regenerating organs such as the epidermis, in the kidney, the niche for stem cells has yet to be clearly identified. It may be the inner medulla, a hypothesis based on two considerations: first, that the medulla is the primordial part of the kidney (kidney maturation proceeds centrifugally); second, that hypoxia is greater in the medulla than in the cortex; hypoxic conditions favoring the maintenance of a staminal compartment during kidney maturation. Indeed Oliver *et al* (4,55) demonstrated that in mouse and rat at least, the niche of renal stem cells is the papilla, the innermost part of the inner medulla. Nevertheless, in human kidneys, cells with stem cell characteristics have been found in the cortex (56) and in the Bowman's capsule (57).

4.4 Kidney-specific stem cells

Adult kidney stem cells have been reported in lower organisms such as the *Drosophila* (58), the skate and the freshwater teleost (59). Adult stem cells in higher organisms are thought to have a slow-cycling time (60,61). Labelling with the DNA marker BrdU is one of the methods of choice for identifying slow-cycling cells. When BrdU is incorporated in the proliferating cells in the late stages of organogenesis, only these low cycling cells retain the label. Oliver *et al.* (4) injected 3-day-old rodents with BrdU and showed that several months later; most BrdU positive cells were present in the outer part of the renal papilla that is adjacent to the urinary space. The authors' detailed study of BrdU-retaining cells showed that they were located mainly in the interstitium of the papilla, but some were also within the tubules. Following transient renal ischemia, the labelled cells were rapidly lost from the papilla, indicating that they started to proliferate, then took part in tubular recovery. Papillary cell proliferation occurred even though the renal papilla, unlike other areas of the kidney, displayed no apoptosis after the ischemic insult. Isolation of renal papillary cells also showed that the

cells are multipotent *in vitro*, capable of giving rise to more than one cell type. They were also able to form neurospheres, a characteristic of many organ-specific adult stem cells *in vitro*.

Another way to identify organ specific stem cells relies on the cell's ability to extrude Hoechst 3342 dye (62). Cells with this property, termed side population (SP) cells, have been found in several organs, including the kidney (63-65). SP cells in the adult kidney are thought to represent a progenitor population (65). However, the size, origin, phenotype, and potential of kidney SP cells have been the object of controversy. The SP fraction of embryonic and adult kidneys may represent 0.1-0.2% of the total viable cell population. The immunophenotype and expression profile of kidney SP cells were distinguishable from those of bone marrow SP cells, suggesting that they are a resident non-hematopoietic cell population. Localization by *in situ* hybridization confirmed a primarily proximal tubule location (supporting the existence of a tubular "niche"), but also revealed a considerable heterogeneity, including the presence of renal macrophages. Adult kidney SP cells have demonstrated multilineage differentiation *in vitro* and. their repair capacity was demonstrated in a mouse model of kidney damage induced by adriamycin (66). Reintroducing SP cells into the mouse led to a reduction in the albuminuria/creatinine ratios, but no significant tubular integration, thus suggesting a humoral role for SP cells in renal repair. In this light, SP behave rather like bone-marrow-derived cells.

Selecting a particular cell surface marker of staminality might be useful for isolating stem cells by FACS. Unfortunately, no specific markers for intrarenal stem cells have been recognized so far. However, Bussolati *et al.* (55) used CD133 antigen - a cell surface marker of endothelial progenitor cells, hematopoietic progenitor cells and neuronal stem cells - and isolated a population of CD 133+ and PAX2+ cells from the cortical tissue of adult human kidneys with the capacity to differentiate *in vitro* into either epithelial or endothelial cells. Moreover, following intravenous injection into a mouse with glycerol-induced tubular necrosis, the CD133+ cells were able to home into the injured kidney and become integrated in the tubules. On the other hand, CD 133+ and CD 24+ parietal epithelial cells (PEC) with multipotent features have also been isolated from Bowman's capsule of human adult kidneys (57). Injecting these cells into SCID mice with acute renal failure resulted in the regeneration of tubular structures in different portions of the nephron. More importantly, treating acute renal failure with $CD24^+$ $CD133^+$ PEC significantly ameliorated the morphological and functional kidney damage.

Kitamura *et al* (67) and Maeshima *et al* (68) have reported particularly intriguing findings: both groups isolated stem/progenitor cells from the nephron, but using different approaches. The Kitamura group dissected individual nephrons from adult rat kidneys, separated into segments and cultured them. From the S3 segment, they isolated one clone, designated as rKS56 cells, which revealed the most powerful growth and co-expressed immature cell markers with mature tubular cell markers. These cells showed the characteristic features of stem/progenitor cells, displaying self-renewal, a multiple plasticity restricted to renal epithelial cells and a capacity for regeneration by replacing injured tubular epithelial cells in the ATN model. The rKS56 cells had a cobblestone appearance and expressed pan-cytokeratin and vimentin. The authors reported that mRNA for PAX2, Sca1 and c-kit (considered markers of staminality) was up regulated in these cells by comparison with normal renal epithelial cells (NRK-52 cell line).

The Maeshima group first demonstrated the presence of slow-cycling cells in rat kidneys, identified as BrdU label-retaining tubular cells (LRTC). These cells were mainly localized in the proximal tubules and actively proliferating in the recovery phase after ischemic injury (3). During tubular regeneration LRTC acts as a source of regenerating cells with an immature phenotype (vimentin-positive cells), that actively proliferate, and then differentiate into epithelial tubular cells. Although the identity of the LRTC has not yet been established, the cells were found to be positive for some nephron segment markers suggesting that they are in a differentiated state. However, the Authors did not rule out the possibility that LRTC were in a partially differentiated state. They could not exclude that there should be some differentiation markers that are expressed in all epithelial tubular cells but LRTC.

Afterwards, using FACS, the authors isolated a population of SP/LRTC cells from tubules of BrdU-treated adult rats that showed phenotypic plasticity, tubulogenic capability and the capacity to become integrated in the developing kidney (68). More interestingly, they found that the phenotypic features of these cells depended on extra-cellular matrix (ECM) components; in particular, the immature phenotype persisted in the presence of factors secreted by metanephric mesenchymal cells during nephrogenesis such as EGF, IGF, and HGF.

5. TUBULAR EPITHELIAL CELL PLASTICITY

As in the liver, therefore, the kidney's capacity for regeneration may be sustained by two different mechanisms: (a) proliferation of the mature tubular cells without dedifferentiation, when the type of injury does not involve necrosis of the tubular segment and denudation of the basement membrane (18,69); and (b) proliferation accompanied by dedifferentiation, possibly due to the proliferation either of a sort of TA progenitor cell in the S3 proximal tubular segment, or of a mature tubular cell that dedifferentiates and then redifferentiates towards the mature epithelial phenotype (2,67-70).

It has been clearly demonstrated that the severity of the injury prompts a different regenerative response from proximal tubular cells. In a rat model of toxic injury induced by different doses of uranyl acetate (UA), using double staining of BrdU and vimentin (as a marker of dedifferentiation), Fujigaki *et al* (70) demonstrated that proliferating proximal tubular (PT) cells showed only weak

expression of vimentin following low doses of UA. In this situation, where renal injury would not be severe, the regenerating cells would be repairing only focal regions. In contrast, the proliferating PT cells of rats treated with higher doses of UA acquired marked vimentin positivity because massive proliferation and migration was required to cover and repair large areas of bared tubular basement membrane. It is suggested that, despite the slow cycling cells not being characterized in terms of any markers of staminality, the regenerating PT cells derived from pre-existing dedifferentiating cells that may lie dormant under normal conditions and only be activated to acquire the features of progenitor cells after significant injury-induced depletion of the PT cells.

Several studies thus suggest that stem/progenitor cells are scattered along the nephron S3 segment and responsible for kidney regeneration. On the other hand, Lin *et al* (47) have provided compelling evidence that mature renal tubular epithelial cells have a leading role in the regeneration process in the post ischemic kidney. They tagged renal tubular epithelial cells with green fluorescent protein (GFP) and demonstrated that, 3 days after ischemia reperfusion injury, GFP-positive cells were incorporating BrdU and expressed vimentin, which indicated that the dedifferentiating cells originated from pre-existing mature tubular epithelial cells. PAX2 has also been seen in the injured area lacking brush border and containing cells that incorporated BrdU. Twenty-eight days after the injury, no vimentin expression was detectable in any tubules and PAX2 was no longer apparent in the proximal tubules. The old paradigm of injured tubules being regenerated via the dedifferentiation of surviving mature tubular cells and their subsequent proliferation and redifferentiation into an epithelial phenotype is now experimentally proven.

5.1 The EMT process as a manifestation of tubular cell plasticity

Epithelial-to-mesenchymal transition (EMT) is a complex and extreme manifestation of epithelial plasticity (71). It is now widely recognized as a fundamental process marking physiological (morphogenesis) and pathological events (oncogenesis and fibrogenesis). EMT is a particular feature of epithelial cells that, under specific circumstances, are able to lose their epithelial characteristics and acquire a new phenotype by activating the mesenchymal program. While it is considered an important mechanism underlying epithelial degeneration leading to tissue fibrosis (72), EMT was never associated with the regenerative process occurring in the kidney during acute injury, despite the transitional steps characterizing EMT being identical to those characterizing tubular epithelial regeneration. This may be explained by the lack of any experimental proof indicating of the fate of mesenchymal cells arising from this process or of whether EMT is a transitional or a final phase of epithelial plasticity. In addition, the experimental models supporting EMT are based on chronic injuries, for the kidney at least. It is worth noting, however, that the best *in vivo* model documenting the EMT process as an important mechanism underlying renal fibrosis, is the experimental model of unilateral ureteral obstruction (UUO), which can be considered a model of acute renal injury. UUO is characterized by a strong inflammatory component, as in ischemia/reperfusion injury, that induces a rapid progression to tubulointerstitial fibrosis in the obstructed kidney (73).

We have very recently reported evidence supporting that tubular cell during the EMT process dedifferentiated by recapitulating some aspects of renal development (74). Using gene expression profiling on large-scale oligonucleotide microarrays, we demonstrated that the EMT process induced by TGFβ1 (one of the main activators of the EMT program on a variety of epithelial cells) in primary human tubular cells, involves the up-regulation of morphogenetic and developmental genes, such as Sox 11, GADD45B, N-cadherin, Activin A, CTGF, FGF1/5, Angiopoietin, Natriuretic peptide precursor B, Calcitonin receptor and Caldesmon 1, and that several intracellular pathways are involved, e.g. TGFβ1/SMAD, MAPK, WNT, JAK/STAT, calcium signaling pathway, the cell cycle and apoptosis, whose involvement in kidney development and nephrogenesis is well known. Our results thus indicate that the recapitulation of embryological programs may be an integral part of the EMT process and suggest that, through this process, tubular cells may be able, under appropriate environmental cues, to redifferentiate not only back to an epithelial type (regeneration) but also towards another cell type, i.e. myofibroblasts (degeneration-fibrosis).

6. SUMMARY AND PERSPECTIVE

Work by ourselves and others seem to suggest that the kidney's need for a real stem cell compartment is less important than the cells' phenotypic flexibility, as in the case of other mesenchymal tissues. The plasticity of tubular cells resembles that of adult mesenchymal cells, which are long-lived and constantly exposed to the extracellular matrix. The matrix provides a microenvironment that helps the cells to maintain a differentiated or undifferentiated state, i.e. the cell plasticity of this tissue seems to serve the same purpose as the multipotency of adult stem cells - without the need for a real stem cell compartment. Self-renewal has also been claimed to be less important than multipotency and phenotypic flexibility in mesenchymal tissue physiology, meaning that environmental cues can reverse their commitment and differentiation (75). In this light, it may be that cells of mesenchymal origin, like the renal tubular cells, are capable of inter-conversion from one cell type to another even when they are in a later stage of maturity than adult stem cells.

Understanding how mesenchymal cells arise from epithelial cells could have a strong impact in unveiling the mechanism behind epithelial cell plasticity underlying kidney regeneration and repair. The comprehension of the cellular and molecular events involved in renal tubule regeneration is indispensable for the design of cell-based and other therapeutic strategies in order to potentiate the innate capacity of the kidney to regenerate its nephrons after an injury.

7. ACKNOWLEDGEMENTS

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Abbreviations: ARF: Acute renal failure; ATN: acute tubular necrosis; TA: transit amplifying; BrdU: bromo-deoxyuridine; N-CAM: neural cell adhesion molecule; HGF: hepatocyte growth factor; HSC: hematopoietic stem cell; MSC: mesenchymal stem cell; SP: side population; LRTC: label retaining tubular cell; UA: uranyl acetate; PT proximal tubular cell; GFP: green fluorescent protein; EMT: epithelial-mesenchymal transition; UUO: unilateral ureteral obstruction

Key Words: Renal Stem Cells, Acute Renal Injury, Acute Tubular Necrosis, Kidney Repair, Kidney Regeneration, Dedifferentiation, Tubular Cell Proliferation, Tubular Cell Plasticity, EMT, Review

Send correspondence to: Franca Anglani, Laboratorio di Istomorfologia e Biologia Molecolare del Rene, Divisione di Nefrologia, Dipartimento di Scienze Mediche e Chirurgiche, Policlinico, via Giustiniani n° 2, 35128 Padova, Italy, Tel: 390498212155, Fax: 390498212151, E-mail: franca.anglani@unipd.it

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