# Are renal proximal tubular epithelial cells constantly prepared for an emergency? Focus on "The proliferation capacity of the renal proximal tubule involves the bulk of differentiated epithelial cells"

**Ralph Witzgall**

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# *Are renal proximal tubular epithelial cells constantly prepared for an emergency?* Focus on "The proliferation capacity of the renal proximal tubule involves the bulk of differentiated epithelial cells"

**Ralph Witzgall**

*University of Regensburg, Institute for Molecular and Cellular Anatomy, Regensburg, Germany*

A HUMAN KIDNEY contains approximately one million functional units (the nephrons) that consist of a filter (the glomerulus) and a processing portion (the proximal, intermediate, and distal tubule). The glomeruli produce ~180 liters of primary filtrate every day of which only 1 to 2 liters are finally excreted as urine. It can be easily envisioned that an injury to any portion of the kidney could result in disastrous consequences, and indeed acute renal failure remains a pressing problem in clinical practice and new therapeutic approaches are urgently needed. Theoretically, an injury could strike any part of the nephron, but for reasons only poorly understood the straight portion of the proximal tubule in many cases is most severely affected. Various kinds of injuries may lead to "acute tubular necrosis" (a misnomer because tubular epithelial cells often die by apoptosis), a pathological entity characterized by the loss of tubular epithelial cells and a denuded tubular basement membrane. A number of publications have demonstrated that the proximal tubule can restore its integrity completely. Obviously, however, the regenerative capacity of the proximal tubule sometimes does not suffice or otherwise acute renal failure would not become life threatening. Therefore, one has to wonder whether we can develop strategies to support the regeneration of the tubular epithelium. Under normal circumstances tubular epithelial cells in the adult rat kidney turn over very little, but after an acute injury many mitotic cells have been observed both by proliferating cell nuclear antigen (PCNA) staining (13) and by incorporation of the thymidine analogue bromodeoxyuridine (BrdU) (1). After it was first believed that the surviving epithelial cells dedifferentiate, move through the cell cycle until tubular integrity is restored, go back into $G_0$ phase, and redifferentiate, alternative explanations have been put forward such as the existence of resident renal stem cells or the influx of hematopoietic stem cells from the bone marrow. With the publication of the articles by Vogetseder et al. (10–12), it now appears that the pendulum has swung back to the original interpretation.

Stem cells are defined as self-renewing, slowly proliferating, and undifferentiated cells that possess the capacity to develop into more differentiated cell types. They are called totipotent if they can differentiate into any cell type in our body or pluripotent if they can differentiate into only some cell types. Probably the best characterized stem cell is the hematopoietic stem cell, which not only gives rise to erythrocytes, leukocytes, and blood platelets but (possibly depending on the local milieu) also to a variety of other cell types. Initial studies indeed provided evidence that hematopoietic stem cells can contribute substantially to renal tubular epithelial cells in the noninjured and injured kidney. This was demonstrated by the detection of Y chromosome-positive tubular epithelial cells after the transfer of hematopoietic stem cells from male donors into female hosts (5, 9) and by the detection of β-galactosidase-positive tubular epithelial cells when hematopoietic stem cells were isolated from a special strain of transgenic mice (4, 5). Subsequent studies have cast doubt on these findings and have instead suggested that resident kidney cells are most important for recovery from acute renal injury (2, 3, 6).

What putative renal stem cells look like and where they are located is a matter of debate. Stem cells are felt to be dividing rather infrequently; when they divide, one of the two daughter cells will remain a stem cell, whereas the other one will divide rapidly before differentiating further (so-called "transit-amplifying cell"). Therefore, it has been common practice to administer BrdU into animals for a short period (the pulse period), then wait for some time (the chase period) and isolate those cells that still contain BrdU in their genome (label-retaining cells). This approach is based on the concept that the BrdU label will be diluted so much by consecutive cell divisions that it will no longer be detectable in cells dividing rapidly, whereas in slowly dividing cells it is still visible. Although there may be pitfalls with this idea, it is widely employed. Several articles have made use of the injection of BrdU to identify putative stem cells in the kidney. One article published in 2003 describes the 1-wk long daily injection of BrdU into adult rats (7). After a chase period of 2 wk, BrdU-positive nuclei were found mostly in proximal but also in some distal tubules and collecting ducts (the tubules that take up the urine from the nephrons before it leaves the kidney). When the kidneys were made ischemic after the chase period and allowed to recover for 24 h, the number of label-retaining tubular cells increased, thus indicating that they had divided in the recovery period. Upon double staining of the tissue sections for BrdU and PCNA (a marker for the S-phase of the cell cycle and therefore for actively dividing cells), most PCNA-positive nuclei also still contained BrdU. The authors concluded that the label-retaining cells represent a population of cells with the highest potential for cell proliferation (7). Another publication (8) reported the daily injection of BrdU for 3.5 days into 3-day-old rats and mice. Two or more months later, BrdU-positive nuclei were occasionally seen in glomeruli and tubular epithelial cells in the cortex and medulla, but they were frequent in interstitial cells of the papilla (8).

Now enter the series of articles published by Michel Le Hir, Alexander Vogetseder, and co-workers (10–12). Contrary to the situation in kidneys regenerating from an injury, e.g., after ischemia, when dividing cells show signs of dedifferentiation (13), they found that the vast majority of dividing tubular epithelial cells in 5-wk-old rats showed the same differentia-

Address for reprint requests and other correspondence: R. Witzgall, Univ. of Regensburg, Institute for Molecular and Cellular Anatomy, Universitätsstrasse 31, 93053 Regensburg, Germany (e-mail: ralph.witzgall@vkl.uni-regensburg.de).

  

tion markers as the neighboring, nondividing cells (10). If there are stem cells in the kidney, the dividing cells could either represent slowly proliferating stem cells or rapidly proliferating transit-emplifying cells. Therefore, the authors injected BrdU three times daily from *day 4* to *day 7* after birth and analyzed the kidneys 4, 8, 14, and 35 wk later. As expected, the number of label-retaining cells decreased over time (10). Most BrdU-positive cells were present in interstitial cells in the papilla as reported before (8). Out of the label-retaining cells in the tubular epithelium, virtually all of them were as differentiated as the neighboring cells that contained no detectable BrdU (10). Therefore, at least at the level of analysis applied, if the label-retaining cells are stem cells they are highly differentiated. Alternatively, these results can be interpreted such that any regular tubular epithelial cell may be able to divide while at the same time maintaining its high state of differentiation.

This question was tackled in the next publication (11). The continuous application of BrdU over a period of 7 days via an osmotic pump to juvenile rats (4 to 5 wk old) allowed the detection of BrdU predominantly in the straight portion of the proximal tubule (it is not clear why this particular part of the nephron was labeled so strongly). Here almost 50% of the nuclei had incorporated BrdU. When the administration of BrdU was extended to 14 days, 65% of the nuclei stained positive for BrdU. Those cells that had not divided during the 14-day stretch but which now produced Ki-67 (a marker coming up early in the cell cycle) were considered slowly proliferating and therefore may represent stem cells. However, they showed no signs of a low differentiation grade. Are there any fast-cycling cells in the straight portion of the proximal tubule at all? Since the one-time injection of 5-chloro-2′-deoxyuridine (CldU) into juvenile rats and the injection of another thymidine analogue 5-iodo-2′-deoxyuridine (IdU) 15 to 32 h later did not yield any cells containing both CldU and IdU, Vogetseder et al. (11) argue that the gap between consecutive cell divisions is not short. Regrettably, the observation period between the injection of CldU and IdU was not extended, and it is therefore not clear how long the gap actually is, although the authors present indirect evidence that it is at least 1 wk. The essence of these results is that the majority of the cells in the straight portion of the proximal tubule is able to divide, that the gap between consecutive cell divisions is not short, and that putative stem cells (slow-cycling cells) would be highly differentiated and represent a large portion of the epithelial cell population.

How about older rats? Whereas in juvenile rats (4 to 5 wk old) 2.1% and 4.8% of the epithelial cells in the straight portion of the proximal tubule stained positive for the proliferation markers PCNA (11) and Ki-67 (12), respectively, only 0.4% of the cells in this nephron segment synthesized Ki-67 in adult rats (16 to 20 wk old) (12). Surprisingly, a virtually identical percentage of cells (~42%) in both age groups stained positive for cyclin D1, a marker coming up in the $G_1$ phase of the cell cycle but which is absent in quiescent cells. This number was reduced to ~20% in both age groups 36 h after the application of lead acetate, a chemical inducing mitosis without causing tubular injury, while at the same time the number of Ki-67-positive cells increased to 26% in juvenile and to 32% in adult rats, thus indicating a similar proliferation potential in juvenile and adult rats. Furthermore, in control adult rats, virtually all cells in the straight portion of the proximal tubule synthesized the cell cycle inhibitor $p27^{Kip-1}$, but after the administration of lead acetate, many cells lost $p27^{Kip-1}$ and in return stained positive for the hyperphosphorylated (i.e., inactive) form of the retinoblastoma protein, another cell cycle inhibitor (12).

It is amazing that the seemingly so simple experiments published by Vogetseder et al. were not performed earlier. They may be tedious, but performed diligently, and combined with a superb morphology (as one has come to expect from the group of Le Hir and Kaissling), this kind of experiments still yields important insights. Although one has to be careful not to over interpret the results and would like to see them confirmed by additional means, they suggest that many of the epithelial cells in the proximal tubule are not in in the $G_0$ phase but in the $G_1$ phase of the cell cycle (they are positive for cyclin D1). This prolonged $G_1$ phase correlates with the low number of mitotic cells in adult rats and the large gap between consecutive cell cycles (possibly more than 1 wk). Why proximal tubular epithelial cells should be arrested in $G_1$ and not in $G_0$ phase is not clear, there is no obvious reason why the kidney should stay prepared for a sudden emergency all the time. Vogetseder et al. (12) have also provided very good evidence against a (large) pool of stem cells in the proximal tubule, although they leave open the possibility that stem cells exist somewhere else in the kidney (e.g., in the papilla). If that is so, then the progeny of these stem cells in some cases has to travel rather large distances when needed. What remains to be done? The results of Vogetseder et al. should be confirmed in other species such as the mouse, which in addition also offers the opportunity to investigate gene-deficient animals. What about the situation in mice lacking certain cell cycle proteins? Are the cells still in $G_1$ phase, and is the gap between consecutive cell divisions altered? Of course one would also like to know how fast renal tubular epithelial cells are actually proliferating, and not only in the straight portion of the proximal tubule but in other nephron segments as well. Why are dividing cells dedifferentiated after renal injury but not in the healthy kidney? Hopefully somebody will try to answer these questions so that we learn more about an important facet of renal physiology and may ultimately be able to transfer our knowledge into clinical practice.

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I apologize to those colleagues whose work could not be cited due to space constraints.

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