Kinetics and characterization of initially regenerating proximal tubules in S3 segment in response to various degrees of acute tubular injury

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Abstract

Background. We examined kinetics and characterization of regenerating proximal tubule (PT) cells after various degrees of tubular injury in S3 segments of PT and assessed label-retaining slow cycling cells in S3.

Methods. PT injury was induced by different doses of uranyl acetate (UA) injection into rats, and initially regenerating PTs were identified by in vivo bromodeoxyuridine (BrdU)-labelling before sacrifice or were examined on vimentin positivity. Next, the ³H-thymidine pulse/chase approach was applied to the early regenerating PTs identified by BrdU-labelling after UA injection.

Results. Low-dose UA induced focal PT depletion and initial BrdU positivity in the proximal three-quarters of the S3 segment of PT. Autoradiography showed the increased number of label-retaining and label-diluted cells in the proximal three-quarters of S3 in rats treated with low-dose UA compared to normal rats. High-dose UA induced almost complete PT depletion in the proximal three-quarters of S3 and less PT depletion in the distal quarter of S3 and initial BrdU+ cells were restrictedly found in the distal quarter of S3. Label-retaining and label-diluted cells were increasingly found in the entire S3 at day 7, but only label-retaining cells remained in similar numbers in the distal quarter of S3 until day 42. Initially regenerating PT cells with any doses of UA expressed vimentin, suggesting dedifferentiated PT cells.

Conclusions. Initially regenerating cells after PT injury in S3 are dedifferentiated pre-existing PT cells, which may scatter throughout S3 and be responsible for focal repair of S3. Some initially regenerating PT cells in the distal S3 showed persistent label-retaining cells at day 42 after high-dose UA insult and contributed to renewal of the entire S3, thus they might be slow cycling cells with responsibility for S3 repair.

Keywords: acute tubular injury; progenitor cells; proximal tubule; regeneration; S3 segment; uranyl acetate

Introduction

Although several studies have focused on regeneration after acute tubular injury, little is known about mechanisms regulating renal epithelial maintenance and remodelling. Extensive morphological analysis of renal repair after acute tubular injury has long suggested that tubular cells are generated by division of surviving, pre-existing tubular cells [1–3]. Recently, extrarenal cells have been shown to contribute to renal repair [4–6], however, the extent to which extrarenal cells actually participate in the repair process is not clear [7]. Moreover, it is not known whether the adult kidney harbour renal epithelial stem cells. Because of the lack of specific markers of tubular stem cells [7], they have been extremely difficult to localize. Because organ-specific adult stem cells have the ability to generate adult tissue and divide cells very slowly, one way to locate them has been to follow cell division and identify a group of slow cycling cells before and after an injury [8]. Recently, Maeshima et al. [9] reported that a distinct population of slow cycling cells exists scattered in at least the S3 segment of the proximal tubule (PT) in the normal kidney, and that they behave as renal progenitor-like cells that participate in renal tubular regeneration in ischaemia/perfusion renal tubular injury in rats.

We previously reported that exposure to 5 mg/kg of uranyl acetate (UA) results in rapid and extensive death/depletion of PT cells in almost the entire S3 segment in rats [10,11] and that repair of the PT in S3...
is rapidly induced and virtually completed 2 weeks after administration of UA [10–12]. In this toxic model of acute renal failure, the initial regenerative response was restricted to PT cells at the distal area of S3, and upstream proliferation of PTs was subsequently noted along the denuded tubular basement membrane [11]. This suggests that the initial regenerating PT cells raised from the distal area of S3 have a large capacity for proliferation. However, it remains unknown whether they actually contribute to the repair of the entire S3 and whether initial regenerating PT cells are raised from the distal area of S3 regardless of the severity and/or area of injury in S3.

The present study is an extension to our previous report [10–12] and was designed to examine kinetics and characterization of regenerating PT cells in S3 after various degrees of acute tubular injury in different areas of S3 by different doses of UA, and to assess label-retaining slow cycling cells in PT by the 3H-thymidine pulse/chase approach.

**Subjects and methods**

**Experiment 1**

Male Sprague–Dawley rats weighing 180–230 g (SLC Co., Shizuoka, Japan) were used in the present study. To induce various degrees of acute tubular injury, they received a single intravenous injection of 0.25, 0.5, 2 or 5 mg/kg of UA, and three rats were sacrificed at 2, 2.5, 3, 5 and 7 days after UA injection. Three normal rats served as controls. To identify initially regenerating PT cells, all of the above rats were injected intraperitoneally with 40 mg/kg bromodeoxyuridine (BrdU; Sigma Chemicals Co., St Louis, MO), 1 h before sacrifice. Rats were anaesthetized with intraperitoneal administration of pentobarbital sodium (30 mg/kg), and a blood sample was collected through the abdominal aorta, then both kidneys were dissected out after a brief flush with phosphate-buffered saline (PBS) for histological examination. Serum creatinine level was measured by the enzymatic method (Mizuho Med., Saga, Japan).

**Experiment 2**

To identify label-retaining PT cells after acute tubular injury, another 45 rats with or without UA injection received bolus intraperitoneal injections of 3H-thymidine (120 μCi/100 g body weight; Wako, Osaka, Japan) at 2, 2.5 and 3 days after UA injection (when regenerating, PTs were identified by in vivo BrdU-labelling in experiment 1), then sacrificed at 7, 21 and 42 days after UA injection, and the kidneys were fixed in methacarn after a brief flush with PBS for autoradiography.

**Immunohistochemistry**

For histological examination of renal tissues, 3μm thick sections were stained with periodic acid-Schiff (PAS). A standardized avidin–biotin complex technique was applied to detect the following antibodies: mouse monoclonal antibody against BrdU (Amersham International, Poole, UK) as a cell proliferation marker, mouse monoclonal antibody against cyclin B1 (GNS1, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) as a G2/M-phase marker, and mouse monoclonal antibody against vimentin (clone V9, Sigma). Briefly, 4% paraformaldehyde-fixed sections were deparaffinized and dehydrated. Endogenous peroxidase was blocked by treatment with 3% H2O2 for 30 min, and then the sections were incubated with 10% normal donor serum for 20 min, followed by incubation with primary antibodies overnight at 37°C for BrdU and at 4°C for cyclin B1 and vimentin. After rinsing with PBS, sections were incubated with biotinylated donkey anti-mouse IgG (Chemicon International Inc., CA) for 30 min at room temperature. Then, streptavidin-conjugated peroxidase (Nichirei, Tokyo) was added for 30 min, and then the reaction product was visualized by incubation with diaminobenzidine (DAB). Counterstaining was performed using haematoxylin. For histochemical control sections, the first antibodies were omitted or replaced by normal donkey serum of corresponding animals. Signals in both normal control and experimental sections were negative or negligible.

For double-immunostaining for BrdU and vimentin, tissue sections were first stained for BrdU using the DAB detection system followed by staining with mouse monoclonal antibody against vimentin using the Vectastain ABC-AP kit with vector Red (Vector Laboratories, Burlingame, CA). Biotin-conjugated donkey anti-mouse IgG (Chemicon) was used as a secondary antibody for BrdU, while alkaline phosphatase-conjugated donkey anti-mouse IgG (Chemicon) was used for vimentin. Tissue sections were counterstained with haematoxylin.

** Autoradiography**

The methacarn-fixed sections were dehydrated, air-dried, dipped in NTB2 emulsion (Eastman Kodak Co., Rochester, NY), and placed in light-tight boxes. Following a 3 week incubation at 4°C, slides were developed (D19; Eastman Kodak) and counterstained with haematoxylin and eosin.

**Morphometric analysis**

For morphometric analysis, the thickness of the layer of the outer stripe of the outer medulla (OSOM) in the sections from the kidneys bisected through the longitudinal axis was calculated with a 40× high power objective with the slide on a mechanical stage. This moved vertically between the border of cortex-OSOM and the border of OSOM-the inner stripe of the outer medulla, by counting the number of grid lines of a 1 cm2 eyepiece graticule with 100 equidistant grid lines. Based on the number of the grid line, the layer of the OSOM at the site was arbitrarily divided into four zones of equal thickness (zones 1–4) (Figure 1C).

Cross-sectional PTs with severe damage (representing desquamation of tubular epithelial cells associated with focal granulovacuolar epithelial cell degeneration amounting to 75–100% of the section) in the OSOM were defined as necrotic tubules. The number of necrotic tubules, BrdU+/ and cyclin B1+ PT cells was counted in 20 randomly selected fields of each zone in each kidney at 400× magnification.

For proliferation analysis using 3H-thymidine, cells were defined as ‘labelled’ if they contained at least five grains localized over a visible nucleus. The label should be diluted in
proliferating cells (the rapidly cycling cells) but not in slow-cycling cells. Hence, a number of label (grain \(\equiv 2N\)) is the amount of label taken up in S-phase, progenitor cells should retain more than N amount of the grain, if they undergo asymmetrical cell division [13] only once into new progenitor cell and its daughter cell. In our preliminary study, autoradiography of kidneys taken 90 min after a single intraperitoneal injection of 3H-thymidine at 3 days after an intravenous injection of 5 mg/kg of UA showed that the mean grain count of the labelled PTs in zone 1 was about 40 (2N). Thus, slow-cycling 'label-retaining cells' were defined as those containing >20 (N) grains/nucleus and 'label-diluted cells' were defined as those containing 5–10 grains/nucleus in the present study.

The number of PT cells containing 5–10 grains/nucleus, >15 grains/nucleus or >20 grains/nucleus was counted in 20 randomly selected fields of each zone in each kidney at 400× magnification. The mean number at each time point was displayed in histograms.

**Statistical analysis**

Data are expressed as mean±SD. Differences between data sets were determined by one-way analysis of variance

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**Fig. 1.** Proximal tubular injury of S3 after UA injection. (A) Schematic drawing of proximal tubules (S1, segment 1; S2, segment 2; S3, segment 3 and G, glomeruli) in a nephron unit. (B) Photomicrographs of periodic acid-Schiff stained sections, showing proximal tubular injury of S3. (a and b) Rats treated with 0.25 mg/kg of UA at day 2; (c and d) rats treated with 0.25 mg/kg of UA at day 5; (e and f) rats injected with 5 mg/kg of UA at day 2; (g and h) rats treated with 5 mg/kg of UA at day 5. Upper line, border between the OSOM and inner stripe of outer medulla. Asterisks, necrotic tubules; gl, glomeruli. (a–h) ×150. (C) Schematic drawing indicates zones 1–4 in S3. (D) Morphometric analysis of serial changes in the number of necrotic proximal tubules in each zone of the outer stripe of the outer medulla. (a) Rats treated with 0.25 mg/kg of UA; (b) rats injected with 0.5 mg/kg of UA; (c) rats treated with 2 mg/kg of UA; (d) rats treated with 5 mg/kg of UA. Data represent the mean±SD of three animals. *P <0.05, **P <0.01 vs zone 1.
followed by Fisher’s t-test. A $P$ level $<0.05$ was accepted as statistically significant. All statistical analyses were performed using Statview (Abacus Concept, Inc., Berkeley, CA).

Results

Induction of various degrees of PT cell injury in S3

In rats intravenously injected with 0.25 or 0.5 mg/kg of UA, necrotic PT appeared around the corticomedullary junction after day 2.5 and developed in association with focal PT depletion in the proximal three-quarters of S3 (zones 2–4) (Figure 1). On the other hand, in rats treated with 2 or 5 mg/kg of UA, necrotic PT appeared around the corticomedullary junction as early as day 2 and spread to involve the entire S3, and PT depletion was found abundantly in the proximal three-quarters of S3 and to a lesser extent in the distal quarter of S3 (zone 1) (Figure 1). Necrotic PT progressed to maximal PT cell depletion by day 5 in rats at all doses of UA (Figure 1).

Serum creatinine level after various degrees of acute tubular injury

In rats injected with 0.25 or 0.5 mg/kg of UA, serum creatinine level did not significantly increase (Figure 2). On the other hand, in rats treated with 2 or 5 mg/kg of UA, serum creatinine level increased significantly from day 3 until day 7 compared with that in normal control (Figure 2).

Identification of initially regenerating PT cells

Initial BrdU+ PT cells were found in zones 2–4 in rats intravenously injected with 0.25 mg/kg of UA, and in the entire S3 in rats treated with 0.5 mg/kg of UA, maintaining a similar distribution by day 5 (Figure 3). In rats treated with 2 or 5 mg/kg of UA, initial BrdU+ PT cells were almost restricted to zone 1 at days 2 to 3, and then BrdU positivity spread to involve the entire S3 (Figure 3). In all groups, BrdU activity reached a peak level at day 5, and then almost subsided at day 7 (Figure 3B). In our preliminary study of in vivo BrdU labelling, BrdU+ PT cells were negligible in rats treated with high doses of UA at day 1. Thus, initially regenerating PTs were considered to appear at days 2–3 in all groups.

Fig. 2. Serial changes in serum creatinine level before and after injection with UA. Open squares, rats treated with 0.25 mg/kg of UA; closed circles, rats treated with 0.5 mg/kg of UA; closed squares, rats treated with 2 mg/kg of UA; open circles, rats treated with 5 mg/kg of UA. Data represent the mean ± SD of three animals. * $P < 0.05$, ** $P < 0.01$ vs normal control; NS, not significant.

Fig. 3. The distribution of BrdU+ cells in the OSOM. (A) Serial changes in the distribution of BrdU+ cells (black nuclei). Uppermost line, border between the cortex and the OSOM; lowermost line, border between the OSOM and the inner stripe of outer medulla. (a) Rat treated with 0.25 mg/kg of UA at day 2; (b) rat treated with 0.25 mg/kg of UA at day 5; (c) rat treated with 5 mg/kg of UA at day 2; (d) rat injected with 5 mg/kg of UA at day 5; gl, glomeruli. (a–d) x100; (B) Morphometric analysis of serial changes in the number of BrdU+ proximal tubular cells in each zone of the OSOM. (a) Rats treated with 0.25 mg/kg of UA; (b) rats treated with 0.5 mg/kg of UA; (c) rats injected with 2 mg/kg of UA; (d) rats treated with 5 mg/kg of UA. Data represent the mean ± SD of three animals. * $P < 0.05$, ** $P < 0.01$ vs zone 1.
**Mode of PT cell repair**

In rats injected with 0.25 or 0.5 mg/kg of UA, PT cells with mitotic figures were detected in zones 2–4 as early as day 2 (Figure 4A). The regenerating PT cells, including cells with mitotic figures, appeared to lift PT cells with a brush-border within the tubular lumen, forming a piled-up appearance or hyperplasia at day 5 (Figure 4B). In contrast, rats treated with 2 or 5 mg/kg of UA showed tubular cells with mitotic figures at the distal zone of S3 (zone 1) as early as day 2. The cells with mitotic figures (Figure 4C) or cells with large oval nuclei without a brush-border (Figure 4D) were sometimes clearly identified at the transient zone between PT and the thin descending limb of Henlé after high-dose UA. Cuboidal cells without a brush-border, which are unlike the cells in the thin descending limb of Henlé, were sometimes found at the transient zone in normal kidneys (Figure 4E). At day 5, spindle-like regenerating PT cells, including cells with mitotic figures, were often found covering the tubular basement membranes in the entire S3 (Figure 4F). In rats receiving any dose of UA, PT depletion was almost substituted by regenerating tubules by day 7.

Cyclin B1+ was used as a G2/M-phase marker and cyclin B1+ PT cells were identified by cellular or nuclear staining patterns [14] (Figure 5A). In rats at all doses of UA, the initial cyclin B1+ PT cells appeared in the same zone of S3 as BrdU+ PT cells but appeared later when compared to BrdU+ PT cells (Figures 3 and 5), indicating that BrdU+ PT cells subsequently divided locally. In rats treated with 0.25 mg/kg of UA, cyclin B1+ cells showed a similar distribution to BrdU+ cells at day 5 (Figure 5Ba), but in rats treated with 0.5 mg/kg of UA, the number of cyclin B1+ cells was larger than that of BrdU+ PT cells in zones 2–4 of S3 at day 5 (Figure 5Bb). On the other hand, in rats injected with a higher dose of UA, the number of cyclin B1+ PT cells gradually decreased from zones 1 to 4 of S3 at days 3 and 5 (Figure 5Bc and d).

**Regenerating PT and vimentin expression**

In a normal kidney, vimentin as a marker of the mesenchymal phenotype was not expressed in tubular epithelial cells. Double staining of BrdU and vimentin revealed that in rats at all doses of UA, almost all BrdU+ initially regenerating PT cells expressed vimentin at days 2 to 3 (Figure 6A and B) and BrdU+ PT cells and other injured PT cells continuously expressed vimentin at least until day 7 (not shown). In rats injected with 0.25 or 0.5 mg/kg of UA, injured PT cells showed oval nuclei and weak vimentin expression but PT cells lifted-up within the tubules did not show vimentin positivity (Figure 6C). On the other hand, in rats treated with 2 or 5 mg/kg of UA, spindle-like injured PT cells lining the tubular basement membranes showed strong vimentin expression (Figure 6D).

**3H-thymidine-labelled initially regenerating PT cells**

Autoradiography showed labelled cells were found scattered throughout the entire S3 (zones 1–4) at days 7, 21 (Figure 7A and B) and 42 (not shown) in untreated control rats. Most of the labelled cells were label-retaining PT cells (>20 or >15 grains/nucleus), which existed solitarily or sometimes in pairs in the entire S3, and had slightly decreased in number by day 42 (Figures 8A, B and 9A, B). Label-diluted cells that

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**Fig. 4.** Photomicrographs of PAS-stained sections, showing proximal tubular cells in zone 3 (A, B and F) and in zone 1 (C–E) of the OSOM. (A) Rat treated with 0.25 mg/kg of UA at day 2; (B) rat injected with 0.25 mg/kg of UA at day 5; (C and D) rats treated with 5 mg/kg of UA at day 2; (E) normal rat; (F) rat treated with 5 mg/kg of UA at day 5. Arrows, mitosis; arrowheads, oval nuclei; double small arrow, cuboidal cells without a brush border; asterisks, the transient zone between proximal tubules with PAS-positive brush border and the thin descending limb of Henlé. (A–F) ×350.
contained 5–10 grains/nucleus were scant at day 7 and had slightly increased in number by day 42 (Figures 8A, B and 9C), suggesting that some label-retaining PT cells at day 7 underwent cell division in normal kidney over 6 weeks.

In rats intravenously injected with 0.25 mg/kg of UA, labelled PT cells were increasingly found in zones 2–4, with a similar scattered distribution until day 42 (Figures 7C, D and 9). Label-retaining PT cells were significantly increased in number in zones 2–4 and often existed in pairs or clusters, with a similar distribution and number up until day 42 (Figures 8C, D and 9A, B). Label-diluted cells were also significantly increased in number in zones 2–4 at day 7 in pairs or clusters and were slightly decreased at day 42 (Figures 8C, D and 9C).

In rats treated with 0.5 mg/kg of UA, labelled PT cells were increasingly found in zones 2–4 and to a lesser extent in zone 1 at day 7, and decreased in number by day 42 (Figure 9). The number of label-retaining PT cells increased in the entire S3 (Figure 9A and B) and the number of label-diluted PT cells was significantly increased mainly in zones 2–4, and both were decreased at day 42 (Figure 9C).

In rats injected with 2 or 5 mg/kg of UA, labelled PT cells were increasingly found in the entire S3 at day 7 during the repair phase (Figures 7 and 9). Label-retaining PT cells were increased in the entire S3 and to a significantly greater extent in zone 1, mostly in pairs or clusters (Figures 8E, F and 9A, B). They were rapidly and markedly decreased in number in zones 2–4 by day 21, but remained constant in number in zone 1 until day 42 (Figures 8G, H and 9A, B), suggesting that most label-retaining PT cells in zone 1 represent an infrequent cycling population after completion to repair of PT. In contrast, label-diluted PT cells were increased in the entire S3 mostly in clusters, and to a significantly lesser extent in zone 1 at day 7, and were markedly decreased in number in the entire S3 by day 42 (Figure 9C).
In the present study, two types of PT injury in S3 were roughly induced by various doses of UA, and initially regenerating PTs were identified using in vivo BrdU labelling. A lower dose of UA (0.25 or 0.5 mg/kg) induced focal PT depletion, mainly in the proximal three-quarters of the S3. Renal function judged by serum creatinine level did not change significantly with.

**Discussion**

In the present study, two types of PT injury in S3 were roughly induced by various doses of UA, and initially regenerating PTs were identified using in vivo BrdU labelling. A lower dose of UA (0.25 or 0.5 mg/kg) induced focal PT depletion, mainly in the proximal three-quarters of the S3. Renal function judged by serum creatinine level did not change significantly with.
Fig. 9. Morphometric analysis of serial changes in the number of $^3$H-thymidine labelled proximal tubular cells in each zone of the OSOM. (A) Label-retaining (>20 grains per nucleus) proximal tubular cells; (B) label-retaining (>15 grains per nucleus) proximal tubular cells; (C) label-diluted (5–10 grains/nucleus) proximal tubular cells; (a) normal rats; (b) rats treated with 0.25 mg/kg of UA; (c) rats treated with 0.5 mg/kg of UA; (d) rats treated with 2 mg/kg of UA; (e) rats treated with 5 mg/kg of UA. Data represent the mean±SD of three animals. *$P<0.05$, **$P<0.01$ vs zone 1.
lower doses of UA. Initially regenerating cells labelled by BrdU in vivo were found sporadically only in the proximal three-quarters of S3 in rats treated with 0.25 mg/kg of UA, and in the distal zone of S3 as well as in rats injected with 0.5 mg/kg of UA from days 2 to 3. BrdU+ PT cells maintained a similar distribution from days 2 to 3. This was followed by cell division, as assessed by mitotic figures and cyclin B1-positive, which restored the focal PT depletion by day 7. The relative number of cyclin B1+ cell to BrdU+ cells was larger in rats treated with 0.5 mg/kg of UA than with 0.25 mg/kg of UA at day 5, suggesting that more divided cells were required to restore the PT depletion in rats injected with 0.5 mg/kg of UA. In contrast, higher doses of UA (2 or 5 mg/kg) induced high magnitude of PT depletion in the proximal three-quarters of S3, and less PT depletion in the distal quarter of S3, and acute renal dysfunction judged by serum creatinine level was found as early as day 3 in rats treated with higher doses of UA. BrdU labelled cells were restricted to the distal quarter of S3 from days 2 to 3. BrdU+ PT cells were found to spread from zones 1 to 4 of S3 by day 5. Cyclin B1 positivity might have followed BrdU+ PT cells, but the number of cyclin B+ PT cells gradually reduced from zones 1 to 4 of S3 at days 3 and 5, suggesting that the regenerating PT cells in the distal zone of S3 may undergo cell division more frequently than those in the proximal zone of S3. The findings suggest that initially regenerating cells in S3 after the UA insult are pre-existing PT cells (surviving PT cells) in the zone of injury regardless of the severity and/or area of PT injury in S3, which may scatter throughout S3 and be responsible for focal repair of S3.

Double immunostaining of BrdU and vimentin revealed that initially regenerating cells in rats treated with any doses of UA expressed vimentin as a marker of the mesenchymal phenotype, suggesting that they are dedifferentiated pre-existing PT cells. In rats injected with any dose of UA, BrdU+ PT cells, possible descendants of initially regenerating PT cells continuously express vimentin at least until day 7. The vimentin+ phenotype is also reported to be associated with development of migratory capacity in other models of wound repair [15]. In rats treated with higher doses of UA, massive proliferation and/or migration might be required to cover large areas of bared tubular basement membrane and, thus, proliferating PT cells may acquire strong vimentin positivity. In contrast, in rats intravenously injected with a lower dose of UA, proliferating PT cells showed weak vimentin positivity, where the regenerating PT would be repairing only focal regions. Very limited areas of PT proliferation might cause the piled-up formation and/or hyperplasia within tubules (Figures 4B and 6C).

In the present study, the technique of ³H-thymidine pulse-labelling was applied to the initially regenerating cells at days 2, 2.5 and 3 after UA insult and label-retaining cells were identified after a chase period. Generally, label-retaining cells can be considered as slow cycling cells under steady-state conditions, which is one of stem/progenitor cell characteristics [16]. However, it is not clear how long the chase period should be for organs with a slow turn-over rate, such as the kidney [17]. According to our preliminary study, a maximal amount of grains taken up in each PT cell was about 40, thus label-retaining slow cycling cells should contain more than 20 grains after asymmetrical cell division once they behave as stem/progenitor cells [18]. However, it is not known how often the target cells underwent cell division in response to the tubular injury. Therefore, we evaluated cells with both >20 and >15 grains/nucleus as label-retaining cells, and the results were similar. We found that (i) label-retaining cells were scattered throughout the S3 segment of PT in normal rats over 6 weeks; (ii) in response to the insult by a lower dose of UA, label-retaining cells were increasingly found in the proximal three-quarters zone of S3 until day 42 after UA. Label-diluted cells, which are possibly daughter cells of label-retaining cells, were also detected, and were responsible for rapidly focal repair of PTs, and (iii) in response to the insult by higher doses of UA, both label-retaining and label-diluted cells were increasingly found throughout S3 at day 7 during the repair phase, and then rapidly decreased in number, but only label-retaining cells remained in similar numbers in the distal zone of S3 after the recovery phase until day 42.

In the present study, we confirmed the findings of Maeshima et al. [9] that label-retaining slow cycling cells are scattered throughout S3 in the normal kidney. The insult induced by a lower dose of UA may activate pre-existing PT cells and resulted in an increase in the number of label-retaining cells scattered in the S3 to contribute to focal repair of PT cells. Label-retaining PT cells detected in the zone of injury until day 42 were considered as slow cycling cells, but they could be just cells that proliferated during the repair and were divided a few times before death since focal PT depletion may require limited areas of PT proliferation. On the other hand, in the initial stage of the repair process, after higher doses of UA, label-retaining slow cycling, pre-existing PT cells in the distal zone of S3 might expand, and their daughter cells might migrate and/or proliferate upward of S3 with prominent mitotic activity, and re-epithelialize the damaged entire S3 segment because most initially regenerating PT cells labelled were raised from the distal zone of S3. In addition to massive proliferative capacity and the ability to produce daughter cells, this population of PT cells in the distal S3 could retain >20 grains over nucleus relative constant until day 42, suggesting that they undergo very infrequent cell division there after completion to PT repair and that their daughter cells mainly contribute to renewal of the proximal three-quarters of S3. Though further investigation should be required, it is tempting to speculate that they might be dormant under normal conditions and might only become activated to acquire a progenitor cell character after significant depletion of PT cells in the proximal three-quarters of S3. Label-retaining cells were scattered in the proximal three-quarters of S3 after PT...
recovery, similar to the pattern in a normal kidney. It is possible that some migrated label-retaining cells from the distal S3 may finally localize scattered throughout S3. A longer period of chase to dilute out their label would be necessary to distinguish label-retaining slow cycling cells from non slow cycling cells in the present study.

Stem/progenitor cells localize in the stem cell niche, which are thought to comprise a unique microenvironment capable of maintaining the undifferentiated phenotype and pluripotent differentiation of stem cells [19,20]. Being unlike initially regenerating PT cells in the zones 2–4 of S3, label-retaining, initially regenerating PT cells in the zone 1 of S3 are spatially restricted to the distal zone of the S3 segment or transition zone between PT and the thin descending limb of Henlé. In normal kidneys some cells, located in the transition zone, were cuboidal without a brush-border and showed early mitotic figures after high-dose UA (Figure 4C), raising the hypothesis that they might be progenitor-like cells with maintaining undifferentiated phenotype, residing there as a stem cell niche under normal conditions. It is also not clear whether PT cells in the distal zone of S3 are UA-resistant or whether lower doses of UA do not reach the distal S3 sufficiently well to induce PT toxicity. The precise localization (microenvironment) as well as the morphology of label-retaining cells concentrated in the distal zone of S3 is now under investigation in our laboratory.

In conclusion, initially regenerating cells after PT injury in S3 are dedifferentiated pre-existing PT cells regardless of the severity and or area of PT injury in S3, which may scatter throughout S3 and be responsible for focal repair of S3. However, some PT cells in the distal zone of S3 show persistent label-retaining cells even after providing daughter cells which contribute to renewal of the entire S3, thus they might be slow cycling cells with responsibility for S3 repair.

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References


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