Characterization and Fate of Telomerase-expressing Epithelia during Kidney Repair

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ABSTRACT

After acute kidney injury, mice with short telomeres develop increased damage with reduced proliferative capacity, which suggests an important role for telomere length in kidney repair. The enzyme telomerase reverse transcriptase (mTert) regulates telomere length; embryonic stem cells and certain adult stem cells express mTert, but whether cells in the adult kidney express mTert and whether these cells play a role in renal repair are unknown. Here, we found that telomerase protein and mRNA were highly enriched in renal papilla, a proposed niche of kidney stem cells. Using mTert-GFP reporter mice, we detected mTert in a subset of papillary epithelial cells comprising the collecting duct predominantly but also the loop of Henle. Approximately 5% of mTert-GFP* cells were label retaining, a characteristic of stem cells. mTert mRNA levels increased in renal papilla after ischemia-reperfusion injury, but genetically labeled mTert-expressing papillary cells neither divided nor migrated out of the renal papilla during kidney repair. In summary, these data suggest that cells expressing telomerase reverse transcriptase are not a progenitor-cell population, and they do not play a direct role in kidney repair.


Acute kidney injury, a disease whose incidence is rising in our aging population, contributes to excess morbidity and mortality and substantially increased healthcare costs.1 Understanding kidney repair mechanisms is required to rationally design novel therapeutic approaches to treat this disease. In adult kidney, cell proliferation is very low during homeostasis, but acute injury induces a robust proliferative repair response.2– 4 Each round of cell division shortens telomeres, repetitive DNA sequences at chromosome ends, and loss of telomeric DNA beyond a critical threshold induces cell senescence or apoptosis. Aging and ischemia-reperfusion injury, conditions associated with multiple rounds of cell division, both induce telomere shortening in kidney.5,6 mTert knockout mice, whose proximal tubule telomeres are short to begin with, develop even shorter telomeres after injury. These mice develop exacerbated acute injury compared with wild-type controls, have a deficient proliferative response associated with expression of cell cycle inhibitors, and undergo profound interstitial fibrosis at late time points.6 These observations indicate an important role for telomere length and telomerase activity in kidney repair.7 The precise mechanism by which shortened telomeres impair kidney repair is unclear, however, in part because the relative expression of mTert among different kidney cell types is undefined.

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Although telomerase activity and expression have been localized to self-renewing tissues such as testis, bone marrow, and intestine, with the exception of testis, mTert is expressed at low levels in most tissues and is restricted to discrete subpopulations of cells.8 The identification of telomerase-expressing cells in mouse tissues has been challenging because of the lack of adequate mTERT antibodies and because of low mTert expression making immunohistochemistry and in situ hybridization difficult.9 To facilitate the identification of mTert-expressing cells in mouse tissues, we previously developed mTert-GFP as a reporter system, which marks telomerase expressing embryonic stem cells, hematopoietic stem cells, male germ cells, induced pluripotent stem cells, and a recently identified population of slowly cycling intestinal stem cells.10–13

Telomerase-deficient mice are characterized by a defect in stem cell maintenance and ultimately develop organ failure in highly proliferative tissues including testis, bone marrow, and intestine, suggesting that telomerase activity may be required for stem-cell homeostasis.14 The ability of telomerase to serve as a stem-cell biomarker combined with the mTert knockout mouse kidney phenotype suggest the possibility that a kidney mTert-expressing progenitor population could exist, and its absence in mTert knockout mice exacerbates injury because of the absence of stem cell-mediated repair.

Whether or not an adult kidney stem or progenitor population exists remains controversial.15 We have previously demonstrated using genetic lineage analysis that extratubular cells do not directly contribute to epithelial repair after acute injury.16 More recently we have shown that proximal tubule does not contain an intratubular progenitor, either.17 However, published reports suggest the possible existence of kidney stem cell maintenance and ultimately develop organ failure in highly proliferative tissues including testis, bone marrow, and intestine, suggesting that telomerase activity may be required for stem-cell homeostasis.14 The ability of telomerase to serve as a stem-cell biomarker combined with the mTert knockout mouse kidney phenotype suggest the possibility that a kidney mTert-expressing progenitor population could exist, and its absence in mTert knockout mice exacerbates injury because of the absence of stem cell-mediated repair.

THE RESULTS

mTert Is Primarily Expressed in Tubular Epithelial Cells

Because renal papilla is composed of multiple distinct cell types, we next performed costaining to identify cell-specific mTert-GFP expression. mTert was expressed primarily in epithelial cells, with only occasional expression between laminin-positive basement membrane (Figure 2A). The rare interstitial mTert-GFP cells did not colocalize with endothelial cells (CD31), macrophages (F4/80), or pericytes/interstitial fibroblasts (PDGFRβ) (Supplementary Figure 1). Clear colocalization of the epithelial marker E-cadherin and mTert-GFP could be detected in many mTert+ cells (Figure 2B), and mTert-GFP was expressed predominantly in the aquaporin-2-positive collecting duct. Surprisingly, mTert-GFP expression was present in only a subset of these cells (Figure 2C), which were not simply intercalated cells, because many mTert-GFP cells expressed apical AQP2, identifying them as principal cells (Figure 2C, inset). Occasional mTert-GFP cells colocalized with inner medullary and papillary aquaporin-1-positive thin descending limb tubules of the loop of Henle.29 Additionally, occasional mTert-GFP-positive cells were observed in Tamm-Horsfall or NKCC2-positive thick ascending limb (Figure 2, D and E). mTert-GFP cells were much less frequent in outer medulla and cortex, and nearly all were located in aquaporin-2-positive collecting ducts in these regions (Supplementary Figure 2). Parietal epithelial cells have been proposed as a podocyte progenitor niche,24,25 but mTert-GFP-positive cells were never observed in either parietal epithelium or in glomeruli (data not shown).

RESULTS

Selective Expression of mTert in Renal Papilla

To identify kidney cells that express telomerase reverse transcriptase, we initially examined GFP expression in kidneys from adult mTert-GFP transgenic mice, in which GFP expression has been shown to recapitulate endogenous telomerase expression. There was almost no detectable GFP expression in cortex, with minimal expression in the outer medulla, increased expression in the inner medulla, and strongest expression in the renal papilla where approximately 10% of cells were GFP positive (Figure 1, A and B). To investigate whether kidney mTert expression accurately represents endogenous mTert expression, mTert mRNA levels were assessed in cortex and papilla. There was strong mTert mRNA expression in papilla with levels comparable with testis, a tissue known to express high levels of mTert. In contrast, mTert mRNA levels in cortex were undetectable (Figure 1C). To further validate the mTert-GFP mouse in kidney, we compared levels of GFP mRNA with mTert mRNA in cortex and papilla. There was a similar increasing trend for both GFP and mTert mRNA from cortex to papilla (Figure 1D).

Although difficult to measure, human TERT protein levels have been reported to correlate well with telomerase enzymatic activity.26–28 To address this correlation in murine kidney, we measured telomerase mTERT protein levels in lysates from cortex, medulla, and papilla. We could detect telomerase mTERT protein in papilla, with a very weak band present in medulla, consistent with the mTert-GFP and mTert mRNA results (Figure 1E). Taken together, these results indicate that mTert mRNA and protein are strongly expressed in the renal papilla of adult mice and validate the mTert-GFP transgenic as an accurate reporter of kidney mTert expression.

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Figure 1. Telomerase is selectively expressed in the renal papilla. (A) To identify cells that express telomerase, GFP expression in all kidney regions was assessed in the mTert-GFP reporter mouse. Rare mTert-GFP+ cells were seen in the medulla, with the highest concentration observed in the papilla. (B) Quantitation of mTert-GFP+ cells according to kidney region shows that papilla tip and base contain the largest fraction of positive cells, with an intermediate number in medulla, compared with cortex. *P < 0.05, **P < 0.001, each region compared with cortex. (C) Endogenous mTert mRNA is enriched in papilla compared with cortex. RNA was isolated from testis, known to express mTert, or skeletal muscle, which does not express mTert, as well as from kidney cortex or papilla. AQP2 was enriched in papilla and nephrin in cortex, validating the isolation procedure. Endogenous mTert mRNA was detected by RT-PCR in testis and papilla only. (D) To determine whether the relative expression of GFP in the mTert-GFP reporter mouse correlated with endogenous mTert expression levels, RNA was prepared from mTert-GFP mice (n = 3) from either cortex or papilla and subjected to quantitative PCR measurement. Both GFP and endogenous mTert mRNA was over 20-fold higher in papilla compared with cortex. *,*P < 0.05 compared with cortex. (E) To confirm that mTert mRNA reflects tissue telomerase protein levels, kidney lysates (n = 3 mice) were prepared from cortex, medulla, or papilla and probed for mTERT protein by Western blot, using testis as a positive control. A strong 127-kD band corresponding to mTERT protein was highly expressed in papilla, at levels equivalent to that in testis.

A Subset of mTert-GFP Cells Are Label Retaining
The renal papilla has been proposed to be a stem-cell niche because it is a site of slowly cycling cells that were originally identified by thymidine analog label retention.18 Our identification of the renal papilla as a site of high mTert expression, a stem-cell marker in other tissues, suggested that mTert might also mark a kidney stem or progenitor population. Therefore, we next investigated whether mTert-GFP cells in kidney are label retaining by administering a brief pulse of BrdU to mTert-GFP pups on sequential days at P1, followed by an 8-week chase. This protocol successfully identified label-retaining cells (LRC) in the renal papilla, as originally reported.18 Bromodeoxyuridine (BrdU) LRCs were exclusively epithelial and were most abundant in the renal papilla. These LRCs were predominantly aquaporin-2-positive collecting duct cells, but a minor fraction were either aquaporin-1-positive or NKCC2-positive (Figure 3A), mTert-GFP and BrdU co-positive cells could be identified in all three of these tubule segments (Figure 3B), but they were rare.

Quantitative analysis of papillae from three separate mice revealed that 8.1% of cells were mTert-GFP positive, 16.7% were BrdU-positive, and only 0.4% were mTert-GFP/BrdU co-positive (n = 5026 total papillary cells counted). Thus, of the mTert-GFP cells, 5% were LRCs. These results indicate that LRCs and mTert-GFP cells are heterogeneous and that a minority of mTert-GFP cells within the renal papilla are slowly cycling as reflected by BrdU label retention during the first 2 months of life. In fact, this small fraction is similar to bone marrow, where approximately 5% of hematopoietic stem cells are LRC, and fewer than 1% of hematopoietic BrdU LRC are hematopoietic stem cells.30

Papillary mTert mRNA Is Increased During Repair
A role for kidney stem or progenitor cells in kidney repair after acute injury has been proposed but remains controversial and largely unproven.15,19 To investigate a possible role of mTert-expressing cells in kidney repair, we next assessed the expression pattern of mTert mRNA after bilateral ischemia-reperfusion injury. Indeed, mTert mRNA has been reported as a potential kidney injury biomarker because it was found to be upregulated in mRNA isolated from whole kidney; however, regional differences in mTert expression were not examined.31 In this experiment, serum creatinine was 0.23 ± 0.03 at baseline, it increased to 1.45 ± 0.20 at 24 hours, and it was 1.08 ± 0.08 after 48 hours and 0.78 ± 0.06 after 72 hours (P < 0.01 for 24, 48, and 72 hours after injury compared with baseline, n = 4 per
Kidneys were dissected into cortex, medulla, and papilla at 24, 48, or 72 hours after ischemia-reperfusion. In cortex, we observed a trend of increased mTert mRNA expression after injury that did not reach statistical significance, whereas there was no change in medulla. In papilla, mTert levels increased significantly with peak levels 48 hours after injury (Figure 4).

**mTert-GFP Cell Number Does Not Change after Acute Injury**

One interpretation of the increased mTert mRNA and enzymatic activity we measured in papilla after acute injury is that it reflects activation of endogenous papillary stem cells that might be recruited to migrate to the outer medulla, the site of injury. If true, there may be an increase in the number of mTert-GFP cells as they divide and migrate to the site of repair in the outer medulla, as has been proposed for putative papillary stem cells. To test this hypothesis, we next subjected...
mTert-GFP mice to ischemia-reperfusion injury and counted the number of mTert-GFP cells in all kidney regions at 24 or 48 hours after injury. BrdU incorporation was assessed in parallel, to verify kidney injury and to ask whether mTert-GFP cells were selectively recruited to proliferate by an injury stimulus. Although there was robust BrdU uptake in cortex and outer medulla, as expected, there was very little cell proliferation in papilla. Only very rare mTert-GFP and BrdU copositive cells could be found in outer medulla, and no copositive cells were observed in papilla (Figure 5A). In agreement with this result, there was no difference in mTert-GFP cell number after injury, in any kidney region, at either 24 or 48 hours after injury (Figure 5B). Similar results were obtained staining for the cell proliferation marker Ki67, rather than BrdU (data not shown). Although we could detect apoptotic cells in the outer medulla 24 hours after injury, there was no apoptosis in the papilla at this time point either (Supplementary Figure S3), consistent with previous reports.18 These results argue against the idea that mTert-GFP expression identifies an adult progenitor population that selectively proliferates after acute kidney injury.

Fate Mapping of Papillary mTert Cells during Repair

The gold standard for assigning lineage relationships among cells in vivo is genetic fate mapping, in which cell-specific expression of a recombinase is used to activate constitutive and heritable expression of an easily detected marker.32 Downregulation of mTert promoter activity during differentiation might compromise our ability to track the progeny of mTert-GFP cells. Therefore we performed genetic fate mapping of mTert cells during kidney repair. We generated mTert-CreER; R26R bigenic mice in which tamoxifen-inducible Cre recombinase fused to the estrogen receptor (CreER) expression is driven by the mTert promoter. Administration of tamoxifen induces permanent recombination of a floxed STOP LacZ reporter allele in cells that express mTert, and we have validated this approach to genetically label intestinal stem cells.13 Control mice were administered vehicle (corn oil), and there was no LacZ expression in kidney, including papilla, indicating that there was no leaky reporter activation (Figure 6A). A single tamoxifen injection in 8-week-old mice (6 mg) led to labeling in papilla primarily, with less labeling in inner medulla and very rare outer medulla labeling, a pattern nearly identical to that of the mTert-GFP mouse.

Genetic labeling of mTert-expressing cells was performed 1 week before ischemia-reperfusion injury in mTert-CreER; R26R bigenic mice. BrdU was administered twice daily thereafter until sacrifice 5 days after the insult when repair is largely

Figure 4. Injury induces mTert mRNA in papilla. RNA was extracted from cortex, medulla, or papilla after ischemia-reperfusion injury. mTert mRNA was measured by quantitative PCR, and although the absolute mTert mRNA levels reflected much higher basal expression in papilla, for comparison data are presented normalized to the uninjured value in each respective kidney region. mTert mRNA was significantly induced in papilla 48 hours after injury. P < 0.05 compared with uninjured, n = 4 mice per time point.

Figure 5. Acute kidney injury does not increase the number of mTert-GFP-positive cells. (A) To determine whether mTert-GFP cell number increased after injury, mTert-GFP mice (n = 3) were subject to ischemia reperfusion injury and sacrificed either 24 or 48 hours after injury. BrdU was administered 3 hours before sacrifice. Representative images for the 48-hour timepoint are shown. Injury-induced proliferation as reflected by BrdU uptake in cortex and outer medulla, but BrdU-positive cells did not colocalize with mTert-GFP expression. Scale bar, 25 μm. (B) On quantification of the number of mTert-GFP-positive cells according to kidney region, ischemia-reperfusion injury did not induce a change in cell number at either 24 or 48 hours after injury, in any kidney region. P = NS between all kidney regions, uninjured compared with injured.
completed. If papillary mTert-expressing cells divided and migrated to the outer medulla to replace proximal tubule epithelial cells that were lost during the injury, there would be an increase in outer medullary LacZ expression. Careful quantification of the percentage of LacZ/H11001 cells according to kidney region was performed in both uninjured and injured kidneys. No difference in the fraction of LacZ-positive cells in any region was observed 5 days after injury (Figure 6, A and B).

Because a subpopulation of mTert-expressing cells might constitute a true kidney progenitor population, and our previous results suggested that only a small fraction of these cells were slow cycling, we next asked whether any mTert-expressing papillary cells selectively divided after kidney injury. We costained for LacZ expression and BrdU in papilla. If any of the mTert-expressing cells had proliferated over the previous 5 days, it would stain positive for both 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal) staining, in the oil-treated mice. Tamoxifen induced strong recombination in papilla, with much smaller numbers of LacZ-positive cells in the inner and outer medulla. After injury and repair, there was no increase in the number of LacZ-positive cells in either the papilla or any other kidney region. 

DISCUSSION
Here, we investigated the hypothesis that telomerase expression in kidney marks a progenitor/stem-cell population. Several lines of evidence suggested that mTert expression might identify adult stem cells in this tissue, including the phenotype of late generation Terc−/− mice with critically short telomeres, which have increased sensitivity to acute kidney injury and decreased repair capacity after acute injury. Because telomerase expression is a biomarker for adult hematopoietic and intestinal stem cells, we reasoned that the heightened sensitivity of these mice to renal injury might result from dysfunctional renal stem cells. Our observation that mTert is selectively expressed in renal papilla, a proposed stem-cell niche, further supported this hypothesis, as did the finding that mTert-GFP was expressed in only a subset of papillary epithelial cells. Finally, the fact that about 5% of mTert-GFP cells were label retaining also was consistent with the notion that mTert-GFP might mark a kidney stem-cell population.

In experiments designed to directly test the functional role of mTert-expressing cells in kidney repair, we found no evidence that these cells divide or migrate during kidney repair.

mTert Is Induced by Osmotic Shock
Telomerase is increasingly appreciated to play important roles in the DNA damage response independent of its established function in telomere maintenance.33–35 We next examined the regulation of mTert expression in primary cultured inner medullary collecting duct (IMCD) cells, because most immortalized cell lines overexpress telomerase and would therefore be unsuitable to examine its regulation.36 IMCD cells expressed high levels of mTert mRNA at baseline, compared with renal cortex and papilla (Figure 7A). Using a hypotonic shock DNA damage model,37 we subjected IMCD cells that were acclimated to culture in media at 520 mOsm to media at 320 mOsm. This rapidly activated the DNA damage pathway, as indicated by phosphorylation of ATM kinase (Figure 7B). This osmotic shock also strongly activated mTert mRNA, as well as protein expression in nuclear lysates, 2 to 4 hours after the shock (Figure 7, C and D).
Acute injury did not induce an increase in mTert-GFP cell number, despite increased papillary mTert mRNA levels. Although the reason for this discordance is unclear, one explanation is that cells that already expressed mTert before injury upregulated expression even further after injury. We found no evidence that papillary mTert-GFP cells in either the tip or the base of the papilla proliferated after injury, and the great majority of proliferating cells in the outer medulla, the site of greatest damage, did not express mTert-GFP. In genetic fate mapping experiments, mTert-expressing cells neither migrated nor proliferated after acute injury, providing conclusive evidence that these are not progenitor cells under the conditions tested and consistent with our recent finding that repair of proximal tubule does not involve specialized progenitors but rather proliferation of injured cells. Renal papilla has extremely low basal rates of cell proliferation, and early exit from the cell cycle has been proposed as an alternative, non-stem cell-based mechanism, to explain the presence of papillary label-retaining cells. The results presented here suggest two possible explanations for the shortened epithelial telomeres and enhanced susceptibility to renal injury of late generation Terc−/− mice. The short epithelial telomeres in these mice may reflect a global reduction in telomere length among all renal cell types because of the absence of telomerase expression during the earliest stages of embryonic development, when telomerase expression is known to be high. Alternatively, telomerase may also be selectively expressed in certain cell types during nephrogenesis, endowing these cells with longer telomeres compared with other kidney cell types. Proximal tubule could be one such cell type because a longer telomere would enhance replicative capacity after injury in the adult. In this scenario, the absence of telomerase activity in Terc−/− mice would lead to preferential telomere shortening in proximal tubule progenitors and therefore adult proximal tubule, potentially explaining their decreased replicative capacity after injury. An examination of telomerase expression and activity during nephrogenesis should resolve these possibilities and requires investigation.

The question of the role that telomerase expression plays in kidney papilla remains open. One possibility is that mTert expression marks a dormant kidney stem-cell population that is only recruited under special conditions or an injury more specific to collecting-duct. Such facultative stem cells have been identified in the bile duct of the pancreas, and indeed we reported that mTert expression identifies a dormant, Lgr5-negative intestinal stem-cell population. Alternatively, our in vitro data presented here suggest that telomerase expression in collecting duct reflects an individual cell’s DNA repair response. Papilla is a harsh environment, with high osmolarity and elevated levels of oxidative stress, compared with other kidney regions. Telomerase may therefore play a role in the repair of double-strand breaks, which have been detected almost exclusively in renal papilla. This nonhomologous end-joining repair pathway involves the DNA repair heterodimer Ku86/Ku70 in IMCD cells, and in other systems Ku70 directly associates with telomerase providing a biochemical link between telomerase and IMCD cell DNA repair. Further support for this hypothesis is the observation that the deacetylase Sirt1 is selectively expressed in the inner medulla and papilla, and it also is a positive regulator of telomere length, like telomerase. Our observation that osmotic shock, which activates the collecting duct DNA damage response, increased mTert mRNA and protein provides strong support for this possibility.

In summary, the results of this study favor the traditional

Figure 7. Osmotic stress induces mTert in primary IMCD cells. (A) mTert mRNA was strongly expressed in primary IMCD cultures compared with renal papilla, as assessed by RT-PCR. (B) Hypotonic shock is known to induce cell stress and DNA damage. In IMCD cultures, hypotonic shock of cultures adapted to 520 mOsm rapidly induced expression of ATM, a component of the DNA damage response. (C) mTert mRNA was significantly upregulated in primary IMCD cells after hypotonic shock, as assessed by quantitative PCR. **P < 0.01 compared with IMCD cultures maintained in 320 mOsm, three separate experiments were performed from two independent IMCD cell preparations, with the average mTert values presented. (D) mTert protein is also induced 2 hours after hypotonic shock in primary IMCD cells, as assessed by Western analysis of nuclear lysates.
model of endogenous kidney repair in which surviving epithelial cells dedifferentiate and proliferate without the need for a renal stem or progenitor population. Further studies are needed to better define the role of telomerase in the collecting duct DNA-damage pathway.

CONCISE METHODS

Animal Experiments

All of the mouse studies were performed according to the animal experimental guidelines issued by the animal care and use committee at Harvard University. mTert-GFP and mTert-CreER transgenic mice were maintained on a 129S1/SvIMJ background. Ischemia-reperfusion surgery was performed as described with minor modifications. In brief, 8- to 10-week-old male mice were anesthetized with pentobarbital sodium, and body temperatures were controlled at 36.5 to 37.5°C throughout the procedure. Kidneys were exposed through flank incisions, and mice were subjected to ischemia by clamping the renal pedicle with nontraumatic microaneurysm clamps (Roboz, Rockville, MD), which were removed after 27 minutes. Sham groups underwent the entire procedure with the exception of renal pedicle clamping. 1 ml of 0.9% NaCl was administered intraperitoneally 2 hours after surgery.

BrdU (Sigma-Aldrich) was made fresh and injected at 50 mg/kg intraperitoneally. The mice were anesthetized, sacrificed, and immediately perfused via the left ventricle with ice-cold PBS for 2 minutes. Kidneys were fixed in either formalin or paraformaldehyde and embedded in either paraffin or optimal cutting temperature (Sakura Finetek), respectively. Serum creatinine was measured using a Beckman Creatinine Analyzer 2 by the Jaffe rate method.

For lineage tracing, 8-week-old male mTert-CreER; R26R bigenic mice were given a single pulse of intraperitoneal tamoxifen (6 mg) or corn oil vehicle 7 days before unilateral ischemia surgery. These mice then received intraperitoneal BrdU twice daily (50 mg/kg, intraperitoneally) until sacrifice 5 days after ischemia-reperfusion.

Immunofluorescence Microscopy and Quantification

Immunofluorescence staining was performed on formalin-fixed, paraffin-embedded 4 μM paraffin sections, with the exception of Figures 1A, 6A, and 6C, which was performed on frozen sections. Antigen retrieval on paraffin sections was performed using heated Antigen Retrieval kits (Roche). Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining was performed using the In Situ Cell Death Detection Kit (Roche). Immunofluorescence images were obtained on a Nikon Eclipse 90i or a Nikon C1 D-Eclipse confocal microscope using standard procedures.

For BrdU/mTert-GFP quantitation, n = 4 mTert-GFP mice were pulsed with BrdU. At 8 weeks, the kidneys were harvested, and 10 high power fields from multiple sections were quantitated. β-Galactosidase staining was performed as described on 7 μM, paraformaldehyde-fixed frozen kidney sections then counterstained with nuclear fast red (Sigma-Aldrich) and mounted. In some cases, BrdU immunofluorescence staining was performed after β-galactosidase staining. For quantitation, six 20× or 40× fields from cortex, outer medulla, inner medulla, or papilla were counted from each kidney (n = 3).

Quantification of mRNA by Real-Time Quantitative PCR

Total RNA was isolated from snap frozen kidneys with RNeasy Mini kit (Qiagen). 300 ng of total RNA was reverse transcribed with the iScript cDNA Synthesis Kit (Bio-Rad). Real-time PCR was performed by SYBR Green PCR Master Mix (Applied Biosystems) for quantification of mRNA expression using GAPDH as the internal control. The PCR was performed on 1/200th of the RT product using the following primer pairs: mTert forward: 5’-AGAAAAACCTTCTCTCAGCA-3’, reverse: 5’-ATGAGAGCAAGCAAGCAGAT-3’; GFP forward: 5’-CACATGAAGGCGACGACGT-3’, reverse: 5’-ATGGTGATGCGCCGATTC-3’; AQP2 forward: 5’-GGGCTTCAATTACTCGCTC-3’, reverse: 5’-GGGTGCCGACCGGTTGAG-3’; nephrin forward: 5’-ATCCAGGGTG-GAGCGACGCTG-3’, reverse: 5’-GGCTGACACCTCGGTTCG-3’, and GAPDH forward: 5’-CATGTTCAGTATGACTCCTG-3’, reverse: 5’-GGCTTCACCCCATTTGATGT-3’.

Cell Culture

Primary mouse IMCD cultures were prepared as described. Briefly, minced fragments of medulla and papilla from 16 mouse kidneys were digested in Collagenase Type I (Sigma), suspended in renal epithelial growth media (Cambrex, Watersville, MD), and grown at 37 °C in 5% CO2 until confluent. Some cells were adapted to high osmolarity medium using NaCl in stepwise fashion, exactly as described. Osmotic shock was accomplished using pre-equilibrated medium. The cells were used within the first three passages.

mTERT Western Analysis

Lysates from kidney cortex, medulla, or papilla were prepared using a radioimmune precipitation assay buffer. Nuclear lysates were prepared from IMCD cells by hypotonic lysis and centrifugation. The proteins were separated by standard techniques, and mTERT protein was detected using a monoclonal antibody (mouse, 1:500, catalog number 5181, Abcam).
Statistical Analyses
The results are presented as the means ± SEM except as indicated and were analyzed by paired t test among groups. A P value of less than 0.05 was considered significant, and all of the experiments were repeated at least twice. All of the mouse experiments included at least n = 3 mice per group.

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DISCLOSURES
None.

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Supplemental Data

Characterization and Fate of Telomerase Expressing Papillary Epithelia During Kidney Repair

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Figure S1. mTert-GFP cells do not colocalize with interstitial cell types.  

A. Endothelial cells (CD31) do not express GFP in the mTert-GFP reporter mouse.  
B. Interstitial pericytes/fibroblasts do not express mTert-GFP. Pericytes/fibroblasts are identified by PDGFRβ immunofluorescence (red).  
C. mTert-GFP is not expressed in macrophages, identified by F4/80 staining (red).
Figure S2. Expression of mTert-GFP in cortex and outer medulla. In contrast to papilla, there was reduced mTert-GFP expression in outer medulla, and very rare expression in cortex. Expression remained epithelial and was almost exclusively localized to aquaporin-2-positive medullary and cortical collecting ducts. Scale bars, 25 µM.
Figure S3. Apoptotic cells in outer medulla compared to papilla after ischemia reperfusion injury. In paraffin sections from kidney 24 hours after renal injury, TUNEL staining reveals numerous apoptotic cells in the outer medulla. No apoptotic cells are detectable in papilla, consistent with previous reports.\textsuperscript{18} Scale bars, 50 µM.