

## Renal capsule as a stem cell niche

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**Park HC, Yasuda K, Kuo MC, Ni J, Ratliff B, Chander P, Goligorsky MS.** Renal capsule as a stem cell niche. *Am J Physiol Renal Physiol* 298: F1254–F1262, 2010. First published March 3, 2010; doi:10.1152/ajprenal.00406.2009.—Renal resident stem cells were previously reported within the renal tubules and papillary area. The aim of the present study was to determine whether renal capsules harbor stem cells and whether this pool can be recruited to the renal parenchyma after ischemic injury. We demonstrated the presence of label-retaining cells throughout the renal capsule, at a density of ~10 cells/mm<sup>2</sup>, and their close apposition to the blood vessels. By flow cytometry, *in vitro* cultured cells derived from the renal capsule were positive for mesenchymal stem cell (MSC) markers (CD29+, vimentin+, Sca-1+, nestin+) but did not express hematopoietic and endothelial stem cell markers. Moreover, renal capsule-derived cells also exhibited self-renewal, clonogenicity, and multipotency in differentiation conditions, all favoring stem cell characteristics and identifying them with MSC. *In situ* labeling of renal capsules with CM-DiI CellTracker demonstrated *in vivo* a directed migration of CM-DiI-labeled cells to the ischemic renal parenchyma, with the rate of migration averaging 30 μm/h. Decapsulation of the kidneys during ischemia resulted in a modest, but statistically significant, deceleration of recovery of plasma creatinine compared with ischemic kidneys with intact renal capsule. Comparison of these conditions allows the conclusion that renal capsular cells may contribute ~25–30% of the recovery from ischemia. In conclusion, the data suggest that the renal capsule may function as a novel stem cell niche harboring MSC capable of participating in the repair of renal injury.

renal mesenchymal stem cells; cell migration; renal ischemia

THE RECENT CROSS-DISCIPLINARY interest in stem cells has inevitably raised questions related to structures harboring these cells, stem cell niches. The classical examples of such niches are represented by the bone marrow, crypts of the intestinal microvilli, or reproductive organs like ovary and testis, all providing a lifelong reservoir of stem cells capable of self-renewal on the one hand and differentiation toward progenitor and mature cells that are engaged in the maintenance of tissue architecture on the other (14). Accordingly, niche environment facilitates the asymmetric division of stem cells, shields them from systemic signals capable of disrupting their properties, and regulates their abundance. Zhang et al. (28) and Calvi et al. (3) provided a comprehensive view of the bone marrow osteoblastic-hematopoietic stem cell (HSC) niche with its complex regulation by parathyroid hormone and bone morphogenetic protein. These studies have not only spurred the search for other niches presumably present in each peripheral organ throughout adulthood (8) but also identified many postnatal tissues harboring mesenchymal stem cells (MSC) (7).

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In the kidney, Oliver et al. (16) have demonstrated that label-retaining cells are predominantly localized to the renal papilla, the site characterized by reduced oxygen tension, from where they are capable of migrating toward the sites of injury. In addition, Maeshima et al. (13) and Challen et al. (4) advocated the existence of a cortical tubular pool of stem cells, suggesting the existence of another niche in the kidney. Patschan et al. (17) detected nestin-expressing cells, presumably corresponding in part to the MSC, located predominantly in the corticomedullary zone and demonstrated their motility and directed migration toward ischemic renal parenchyma.

One of the structures possessing a low metabolic rate and oxygen tension and minimal fluctuations in physicochemical parameters of the microenvironment and offering mechanical protection by the surrounding adipose tissue is represented by the renal capsule. This thin sheet of connective tissue containing fibroblasts, adipocytes, and blood vessels envelops the kidney and has been considered as an inert barrier separating it from the perinephric tissues. On the basis of the theoretical prediction that this structure may represent a convenient reservoir for pluripotent stem cells, we aimed to examine this possibility. The search was also facilitated by the previous demonstration of the presence of nestin-expressing cells in the renal capsule, which provided an initial insight into this possibility (17). Here we present data on the phenotypic characterization of the cells residing in the renal capsule and provide evidence in favor of label-retaining capabilities of these cells, their close apposition to the blood vessels, and their recruitment to the injured renal parenchyma for potential participation in regeneration of the kidney, all favoring the idea of renal capsule serving as a niche for MSC.

### METHODS

**Animal studies.** The animal study protocol was in accordance with the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee of New York Medical College. Adult FVB/NJ mice and Tie-2/GFP (green fluorescent protein) mice (on FVB/NJ background) were purchased from the Jackson Laboratory (Bar Harbor, ME). Both groups of mice were labeled with bromodeoxyuridine (BrdU) to detect label-retaining cells in the renal capsule, whereas Tie-2/GFP mice were used for isolation and culture of renal capsule-derived stem cells. FVB/NJ mice were also used for ischemia-reperfusion (I/R)-induced mobilization studies. Sca-1/GFP mice were obtained from Dr. M. P. Lisanti (Thomas Jefferson University) and used only to identify Sca-1-GFP+CD29+ cells in the renal capsule. Animals were kept under temperature-controlled conditions and a 12:12-h light-dark cycle, with water and food *ad libitum*.

***In vivo* BrdU labeling.** To locate the cells in the renal capsule that turned over at the slowest rate, we used a modified method for labeling the renal capsule cells with BrdU (Sigma-Aldrich, St. Louis, MO). Briefly, 4-wk-old FVB/NJ mice were subcutaneously injected with a pulse of BrdU twice daily, at 9 AM and 4 PM, for 3.5

consecutive days (50  $\mu\text{g/g}$  body wt) as described previously (16). These mice were then followed for 2 mo (chase period) before I/R experiments or death.

**Immunodetection protocol.** Kidneys of FVB/NJ mice previously pulse labeled with BrdU and followed for 2 mo were harvested, fixed in 4% paraformaldehyde (PFA) overnight at 4°C, washed three times with PBS and transferred to PBS containing 30% sucrose (overnight at 4°C), embedded in OCT (Tissue Tek; Sakura Finetek, Torrance, CA), and stored at -80°C until analysis. Cryosections of kidney tissues (10–20  $\mu\text{m}$ ) were used for immunofluorescence analysis. In separate studies, the renal capsules were carefully removed with microforceps and processed separately for detection of label-retaining cells. The capsules were fixed with 4% PFA at room temperature for 20 min and washed three times with PBS. After fixation, the renal capsule was washed with PBS with 1% Triton X-100 and incubated in 2 N HCl for 30 min at 37°C. After that, HCl was immediately neutralized by a 10-min wash with 0.1 M sodium borate and two 2-min washes with PBS. Capsules were then washed again with PBS with 1% Triton X-100 at room temperature and subsequently incubated for 1 h with a blocking solution (5% goat serum, 1 M glycine, 1% Triton X-100, PBS) to reduce nonspecific binding. The renal capsules were stained with Alexa Fluor 488-conjugated mouse monoclonal primary antibody (dilution 1:20; Molecular Probes, Eugene, OR) for 2 h at room temperature in a humidified chamber. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Double immunofluorescence protocols using nestin (Rat-401, Developmental Studies Hybridoma Bank, University of Iowa) and Ki-67 (rabbit polyclonal, Abcam, Cambridge, MA) were used to assess characteristics of BrdU-retaining renal capsule cells. The renal capsules from Sca-1/GFP mice (C57 strain) were also stained with phycoerythrin (PE)-conjugated anti-mouse CD29 (BD Pharmingen), a marker of mesenchymal cell lineage. Images were obtained with a fluorescence microscope (Eclipse TE2000-U) equipped with a digital camera (Spot

mode 4.2; Diagnostic Instruments, Sterling Heights, MI) or a Nikon Eclipse C1 plus confocal microscope and EZ-C1 software version 3.60.

**Isolation and culture of renal capsule-derived MSC.** The adherent cells derived from renal capsules of 3-mo-old male Tie-2/GFP mice (FVB/NJ strain) were prepared as follows. Renal capsules were aseptically removed, minced thoroughly with scissors, and partially digested with collagenase I (1 mg/ml; Sigma) for 60 min at 37°C. The cell suspension was washed with DMEM (Invitrogen) containing 10% fetal bovine serum (FBS; Atlanta Biologicals) and filtered through a 40- $\mu\text{m}$  cell strainer (BD Falcon) to yield a single-cell suspension. MSC were recovered from renal capsule cell suspension by their tendency to adhere tightly to plastic culture dishes, as described previously (18). Filtered renal capsule-derived cells were plated in DMEM containing 10% FBS and penicillin-streptomycin (100 U/ml and 0.1 mg/ml) and allowed to adhere for 24 h. Nonadherent cells were removed, and the culture medium was exchanged every 3 days. After confluence, cells were slowly frozen to -80°C in 7.5% DMSO, after which the cells were thawed and grown to confluence. The freeze-thaw cycle was repeated to remove remaining differentiated cell types, as previously described (27). At confluence, the cells were propagated with 0.25% trypsin-0.02% EDTA solution (Invitrogen) and plated on 10-cm dishes. Cells were used in the studies at passages 8–9.

**Analysis of surface antigen expression of renal capsule-derived cells by flow cytometry.** Cultured renal capsule-derived cells were stained with antibodies and analyzed by FACSCalibur (BD Biosciences). In brief,  $1 \times 10^5$  cells in PBS-1% BSA (wt/vol) were incubated for 1 h at 4°C in the dark with PE-conjugated antibodies (BD Biosciences) against CD31, CD34, Flk-1, c-Kit, Sca-1, CD150, and CD29. Cells were permeabilized with 0.2% Triton X-100 and incubated with monoclonal anti-rat nestin (Rat-401, Developmental Studies Hybridoma Bank, University of Iowa) and monoclonal anti-vimentin (clone LN-6, Sigma) antibodies. These cells were incubated

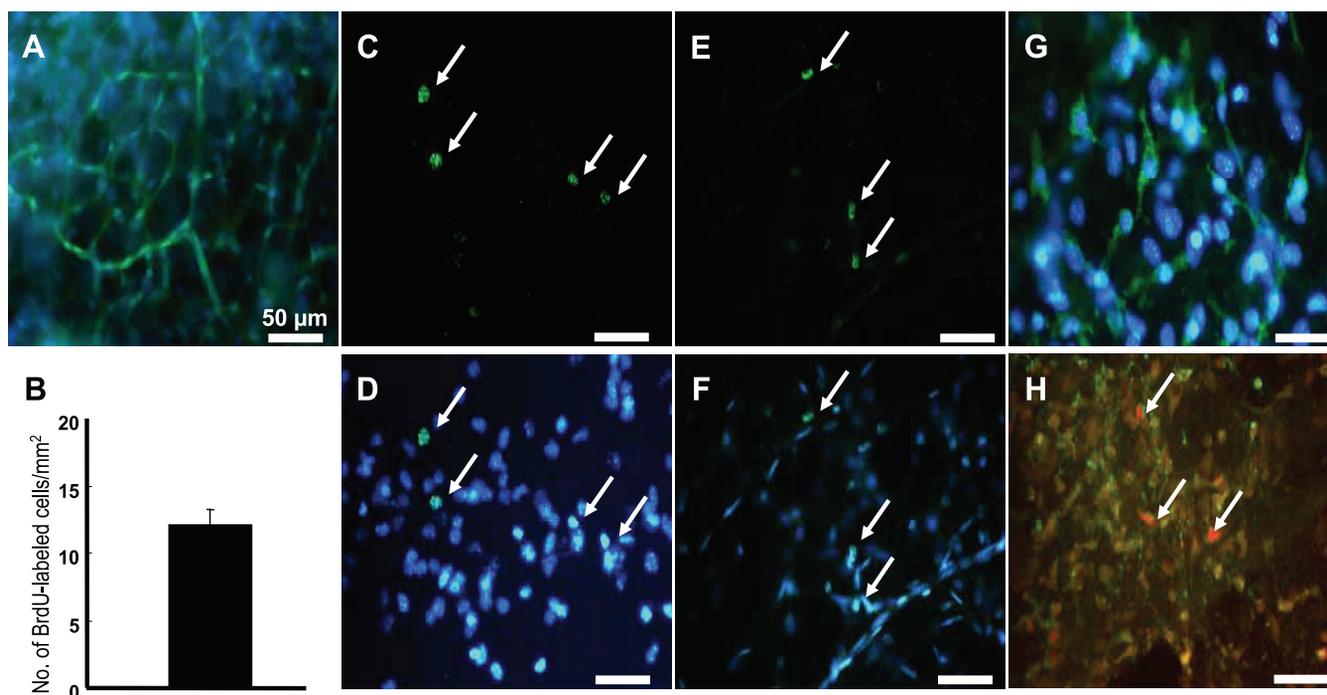


Fig. 1. Microvasculature and label-retaining cells in the mouse renal capsule. Mice were pulsed with bromodeoxyuridine (BrdU) for 3.5 days and followed for 8 wk. Renal capsules were carefully separated from renal parenchyma and analyzed by fluorescence microscopy. *En face* image of the isolated renal capsules from Tie-2/GFP (green fluorescent protein) mice ( $n = 3$ ) and mice pulsed with BrdU ( $n = 5$ ) demonstrated numerous microvasculatures (A) and BrdU-retaining cells scattered throughout the renal capsules (C and D, arrows), respectively. The label-retaining cells showed some predilection for perivascular areas (E and F, arrows). The renal capsules also demonstrated presence of nestin-positive cells (G). After acute ischemia-reperfusion (I/R) injury, some of the nestin-positive cells were positive for Ki-67 (arrows), suggesting active proliferation (H). Scale bar, 50  $\mu\text{m}$ . A: GFP and 4',6-diamidino-2-phenylindole (DAPI). C and E: BrdU-FITC. D and F: BrdU-FITC + DAPI. G: nestin-FITC + DAPI. H: Ki-67-Texas red + nestin-FITC.

with FITC- or Texas red-conjugated goat secondary antibodies (Jackson ImmunoResearch). After each incubation step, cells were washed with PBS-1% BSA (wt/vol) and finally fixed in 1% PFA. Flow cytometry data were analyzed with CellQuest software (BD Biosciences).

**Clonogenic assay of renal capsule-derived cells.** Renal capsule-derived cells were plated at the density of 2 cells/cm<sup>2</sup> on 100-mm dishes coated with 10 μg/ml pronectin (Sigma). Cells were cultured in α-minimal essential medium (α-MEM; GIBCO/BRL, Carlsbad, CA) with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin for 15 days at 37°C and 5% CO<sub>2</sub> without change of medium. The number of formed colonies, defined as conglomerates consisting of at least 50 cells, was counted with a phase-contrast microscope (26).

**In vitro differentiation assays of renal capsule-derived cells.** To determine whether renal capsule-derived cells maintain the multilineage differentiation potential of mesenchymal cells, cells from passages 8–9 were cultured on pronectin-coated dishes. Undifferentiated cells maintained a spindle-shaped morphology. Induction of osteogenic (2), adipogenic (19), and chondrogenic (12) differentiation was performed according to previously reported methods with some modifications. Briefly, confluent cells were grown in serum-free DMEM with insulin, transferrin, and selenium supplemented with hydrocortisone (10<sup>-9</sup> M), thyroxine (10<sup>-9</sup> M), PGE<sub>1</sub> (25 ng/ml; Sigma), and transforming growth factor-β (TGF-β, 5 ng/ml; Peprotech, Rocky Hill, NJ). Osteoblastic differentiation was induced by culturing confluent mouse renal capsule cells for 3 wk in serum-free medium supplemented with 10 mM β-glycerophosphate and 0.3 mM ascorbic

acid (all from Sigma). Culture medium was exchanged every 2 days to overcome the instability of ascorbic acid in neutral pH. Cells were fixed for 15 min in ice-cold PFA before labeling for alkaline phosphatase activity and von Kossa stain for calcified matrix. Adipocytic differentiation was induced in cultured renal capsule cells grown at low density in serum-free medium with 10<sup>-6</sup> M hydrocortisone for 2 wk with medium exchange every 3–4 days. Cells were fixed and stained for lipid droplets with Oil Red O (Sigma). To induce chondrocytic differentiation, confluent renal capsule cells were cultured for 3 wk in serum-free medium supplemented with TGF-β (10 ng/ml), hydrocortisone (10<sup>-7</sup> M), sodium pyruvate (1 mM), 2-phosphate ascorbic acid (1.7 × 10<sup>-4</sup> M), and proline (35 mM) (all from Sigma). Medium was exchanged every 2–3 days for 3 wk before staining with Alcian blue (Sigma). Endothelial differentiation was induced by growth on Matrigel-coated dishes in serum-free medium supplemented with VEGF (10 ng/ml) and FGF-2 (50 ng/ml) (Sigma) for 1 wk (20). Cells were identified by capillary-like tube formation and expression of CD31 by immunohistochemistry.

**In situ renal capsule labeling with CM-DiI and mobilization of renal capsule-derived cells during I/R injury.** Unilateral renal ischemia in male 12-wk-old FVB/NJ mice was performed according to a previously detailed protocol (5). Briefly, renal ischemia was created by clamping the left renal pedicle with microserrafines (Fine Science Tools, Foster City, CA) placed for 30 min. Renal capsules of both ischemic and nonischemic kidneys were carefully coated with red fluorescent CM-DiI (Invitrogen) according to the manufacturer's instructions. After 30 min of labeling, the excess CM-DiI was washed

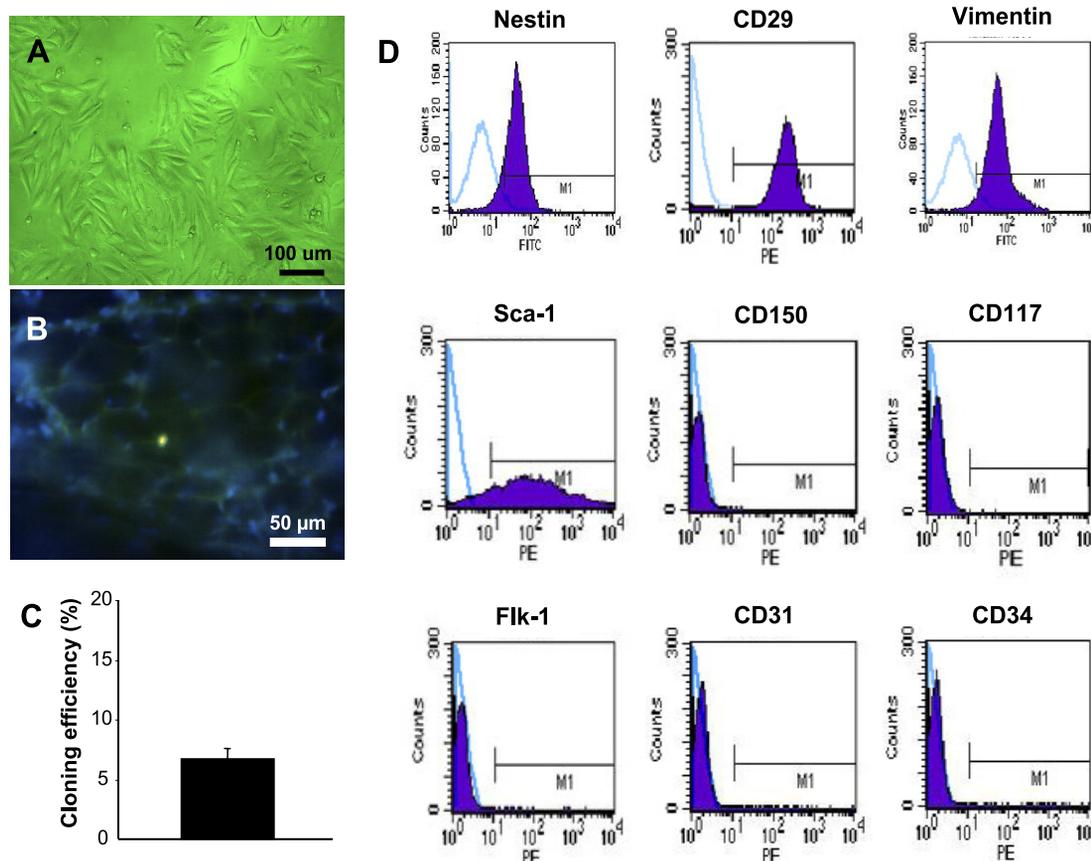


Fig. 2. Characteristics of renal capsule-derived cells. *A*: representative phase-contrast micrograph shows spindle-shaped morphology of cultured renal capsule-derived cells. *B*: merged immunofluorescent image of renal capsule bearing a cell dual-positive for Sca-1/GFP and CD29-phycoerythrin (PE). *C*: cloning efficiency derived from 5 independent experiments was 6.7 ± 0.9%. *D*: FACS analyses show that renal capsule-derived cells were positive for surface markers characteristic of mesenchymal stem cells (MSC) (CD29, vimentin, nestin, Sca-1) and were negative for hematopoietic or endothelial progenitor cell markers (CD31, CD34, Flk-1, CD150, and CD117). The respective isotype controls are also shown (empty histograms).

with sterile PBS. Throughout the procedure, the abdomen was covered with gauze moistened in PBS, and animals were kept well hydrated with saline and at a constant temperature (37°C) with a heated thermoplate (Tokai Hit, Fujinomiya-shi, Shizuoka-ken, Japan). Mice were killed on *days 1* and *3* and *4* wk after renal I/R injury. The kidneys were harvested and fixed in 4% PFA overnight at 4°C, transferred to PBS containing 30% sucrose (overnight at 4°C), embedded in OCT (Tissue Tek; Sakura Finetek), and stored at -80°C. Cryosections (10 μm) were used for immunofluorescent analysis. Mobilization of renal capsule-derived cells during renal I/R injury was identified by detecting red fluorescent CM-DiI-labeled cells in the renal parenchyma by fluorescence microscopy. The renal tissues were also stained for α-smooth muscle actin (α-SMA, clone 1A4, Sigma) and appropriate secondary antibody to identify the possible origin and fate of CM-DiI-labeled cells.

**Functional effect of renal capsule removal in I/R injury.** Bilateral renal ischemia was performed in a separate group of 12-wk-old male FVB/N mice with the experimental protocol described above. For the decapsulation group, renal capsules were gently stripped from both kidneys with microforceps (see Supplemental Data for surgery movie).<sup>1</sup> Serum creatinine was measured on *days 1*, *2*, and *3* after I/R injury in both groups of mice to assess the functional effects of renal capsule removal. Renal capsules were removed in another batch of mice, and their histology and serum creatinine levels were compared with those of a group of mice that underwent sham operation.

**Statistical analysis.** Data are presented as means ± SE, and statistical significance was assessed by two-tailed Student's *t*-test. A *P* value <0.05 was considered to be statistically significant.

## RESULTS

**Immunodetection of BrdU-retaining cells in mouse renal capsule.** Previous studies observed the presence of slow cycling, label-retaining cells in the adult kidney (13, 16, 25). Nestin-GFP-expressing mice showed comparable findings (17). To investigate whether slow cycling cells are also present within the renal capsule, mice were pulse-labeled with BrdU and the presence of label-retaining cells in the renal capsule was investigated by immunofluorescence microscopy. The renal papillary area demonstrated some BrdU-retaining cells, whereas label-retaining cells were rarely observed in the cortical areas (data not shown) as previously described (16). Imaging of the isolated and whole-mounted *en face* renal capsules demonstrated numerous microvasculatures in the renal capsules (Tie-2/GFP mice, *n* = 3, Fig. 1A) along with label-retaining cells scattered throughout the renal capsule (*n* = 5,  $12.1 \pm 1.1$  cells/mm<sup>2</sup>, Fig. 1, B–D). Enumeration of BrdU-labeled cells was performed on the entire recovered renal capsules, and only heavily BrdU-immunostained nuclei were counted as labeled cells. The label-retaining cells showed a predilection for perivascular areas (Fig. 1, E and F). The renal

<sup>1</sup> The online version of this article contains supplemental material.

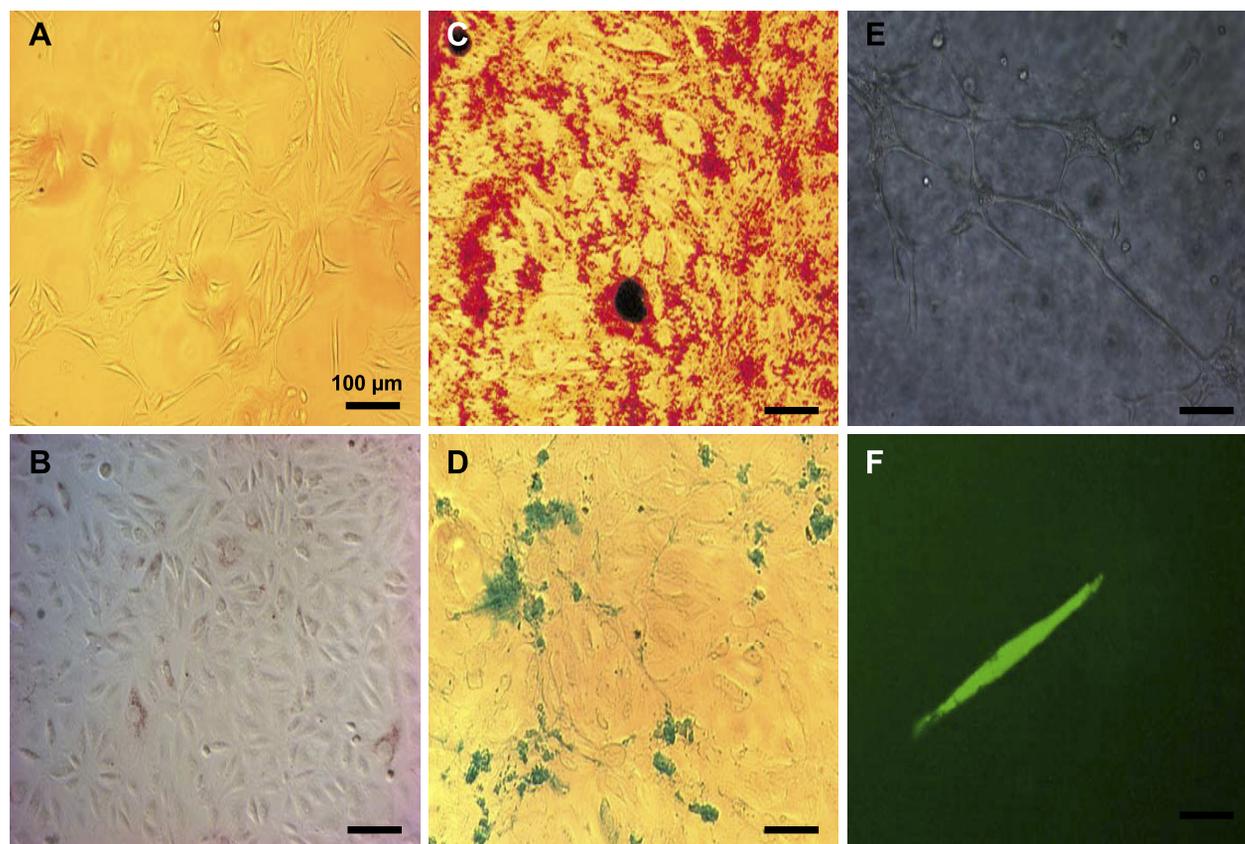


Fig. 3. Multilineage differentiation potential of renal capsule-derived MSC. *A*: micrographs of control cells. *B*: adipogenic differentiation showing the presence of lipid droplets by Oil Red O stain after 2 wk of culture in adipogenic differentiation medium. *C*: calcium deposition detected by von Kossa stain after culture of renal capsule-derived MSC in osteogenic medium for 3 wk. *D*: chondrogenic differentiation shown by formation of pellet positive for Alcian blue after culture in chondrogenic medium for 3 wk. *E* and *F*: micrographs showing spontaneous formation of capillary tube-like structures within 1 wk of culture on Matrigel supplemented with EGM-2 medium (*E*) and rare GFP-positive cells after 4 wk of culture in EGM-2 medium (*F*). Results are representative of 3 independent experiments.

capsules also demonstrated the presence of nestin-positive cells (Fig. 1G). Some of these nestin-positive cells were also positive for Ki-67 after acute I/R injury (but rarely in the intact kidneys), suggesting active proliferation (Fig. 1H and see below).

**Immunophenotypic characterization and self-renewal capacity of renal capsule-derived cells.** The renal capsules were carefully separated from the kidneys and perinephric adipose tissue as described in METHODS. Isolated renal capsules were then collagenase dispersed, and cells were plated on gelatin-coated dishes. After 4 wk in culture, most of the cells derived from renal capsule became spindle shaped (Fig. 2A). FACS analysis showed that cultured renal capsule-derived cells were positive for CD29 (99%), vimentin (97%), Sca-1 (86%), and nestin (87%) but negative for CD31, CD34, Flk-1, CD150, and CD117, identifying them as MSC (Fig. 2B). The self-renewal capacity of the MSC derived from renal capsules was assessed by the limiting dilution method, and the cloning efficiency was

$6.7 \pm 0.9\%$ . These cells were subcultured for >50 generations without loss of proliferative capacity.

**In vitro differentiation of renal capsule-derived MSC.** Because the defining characteristic of MSC is their ability to differentiate into multiple mesenchymal lineages, we examined the ability of renal capsule-derived cells to differentiate into specific interstitial cells. Renal capsule-derived cells could be induced to differentiate into adipocytes, osteocytes, and chondrocytes in the appropriate inductive microenvironment within 2–3 wk (Fig. 3). Moreover, culturing renal capsule-derived cells on Matrigel in EGM-2 medium resulted in capillary tube formation within 3 days. However, very few cells were stained positive for CD31 (endothelial differentiation) even after 4 wk of culture in EGM-2 medium, suggesting a less efficient differentiation into endothelial cells.

**Mobilization of renal capsule-derived cells during I/R injury.** To investigate in vivo whether cells residing in the renal capsule can migrate into the renal parenchyma, fluorescent

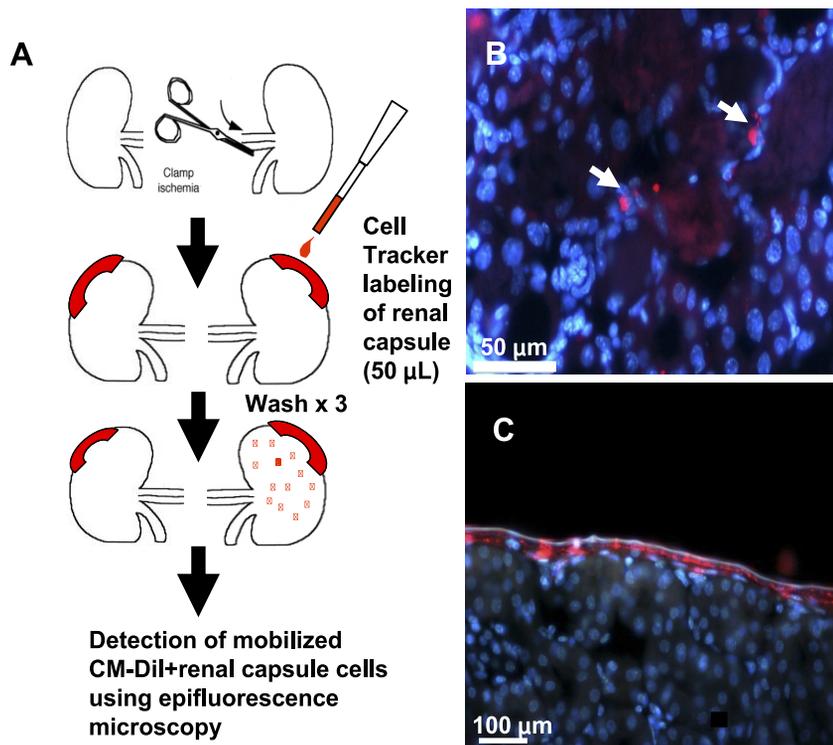
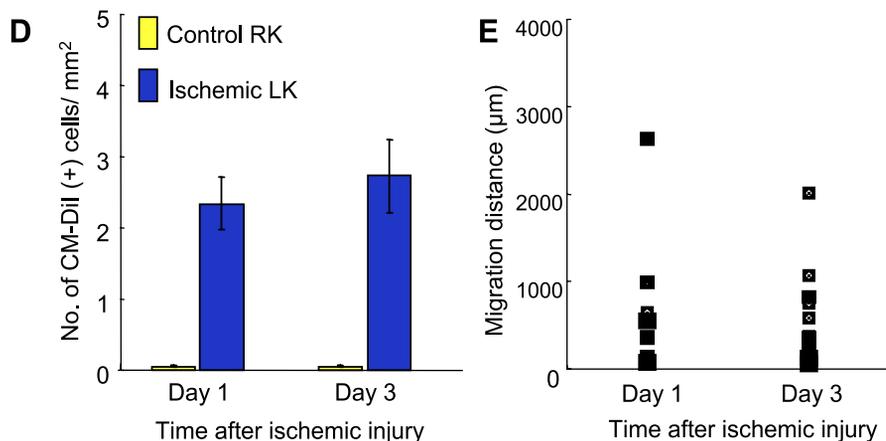


Fig. 4. Mobilization of renal capsule-derived cells into injured renal parenchyma during I/R injury. *A*: schema for in situ labeling of renal capsule with filter paper soaked in the fluoroprobe CM-Dil. *B*: acute I/R injury resulted in migration of in situ CM-Dil-labeled renal capsule cells (arrows) into renal parenchyma, with preferential homing to injured perivascular space. *C*: non-ischemic contralateral control kidney shows CM-Dil labeling confined to renal capsule without any migration. *D* and *E*: degree (*D*) and distance (*E*) of mobilized CM-Dil-labeled cells were comparable between *day 1* and *day 3* after I/R injury. Each result is representative of 4 independent experiments. RK, right kidney; LK, left kidney.



CM-DiI was used as an in situ cell labeling marker. The renal capsules were labeled with CM-DiI through direct contact with filter paper soaked in the fluoroprobe, and unilateral I/R injury was induced in FVB/NJ mice (Fig. 4A). Renal sections were examined by serial immunofluorescence microscopy. Acute I/R injury resulted in migration of in situ CM-DiI-labeled cells (red fluorescence) derived from pre-labeled renal capsules into the renal parenchyma of ischemic, but not contralateral, kidneys (Fig. 4B). In the nonischemic control kidneys, CM-DiI-labeled cells remained confined to renal capsule and showed no migration (Fig. 4C). The number of migrating cells and migratory distances of CM-DiI-labeled cells found in the renal parenchyma were comparable on *day 1* and *day 3* after I/R injury ( $n = 4$  for each time period,  $2.3 \pm 0.4$  vs.  $2.7 \pm 0.5$  cells/mm<sup>2</sup> and  $987 \pm 218$  vs.  $868 \pm 312$   $\mu$ m). This would account for the rate of migration averaging at least 30  $\mu$ m/h,

comparable to what was described ex vivo. These data suggest that cells originating from the renal capsule migrate into the renal parenchyma early after I/R injury.

The kidneys were stained with antibodies for  $\alpha$ -SMA and carefully examined for cells expressing both  $\alpha$ -SMA and CM-DiI. None of the CM-DiI-labeled cells observed on *day 3* after ischemic injury was positive for  $\alpha$ -SMA, suggesting that they are not capsule-derived myofibroblasts. Furthermore, when the fate of CM-DiI-labeled cells was examined at 4 wk, none of them expressed immunodetectable  $\alpha$ -SMA, suggesting that these cells also do not differentiate into myofibroblasts (Fig. 5).

The question that emerged was whether these migrating cells represent a progeny of or original renal capsular stem cells. To address this question, we stained renal parenchyma from control and ischemic kidneys for the expression of the proliferation

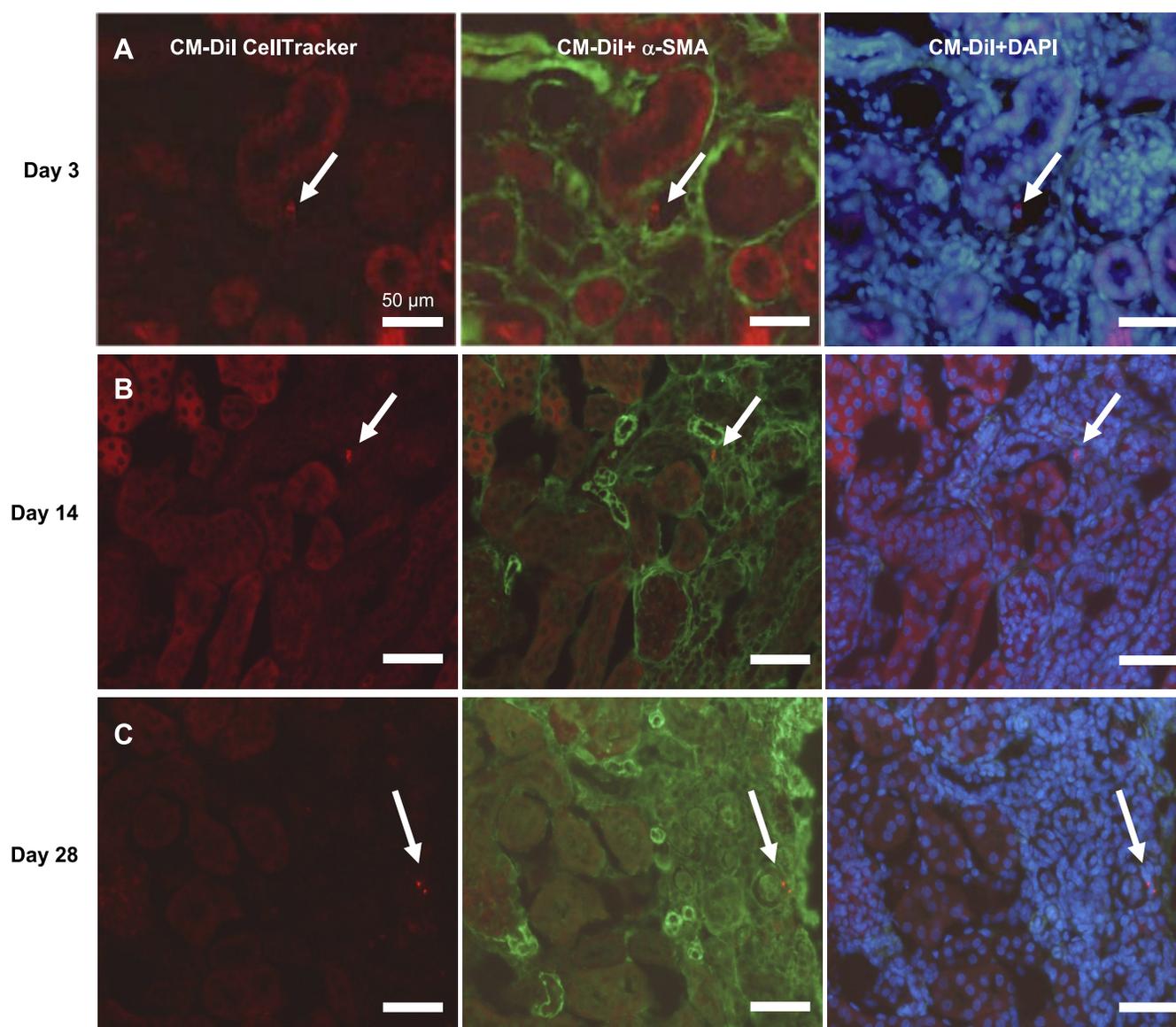


Fig. 5. Fate of mobilized renal capsule-derived cells after I/R injury: representative images of kidney sections of mice that underwent capsular CM-DiI labeling followed by I/R injury and were killed on *days 3, 14,* and *28* after I/R injury as described in METHODS. A–C: kidney sections were stained with  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) and appropriate secondary FITC antibody and carefully assessed by epi-immunofluorescence. None of the CM-DiI-labeled cells (arrows) coexpressed FITC. *Left:* original magnification was  $\times 400$ . Each result is representative of 4 independent experiments.

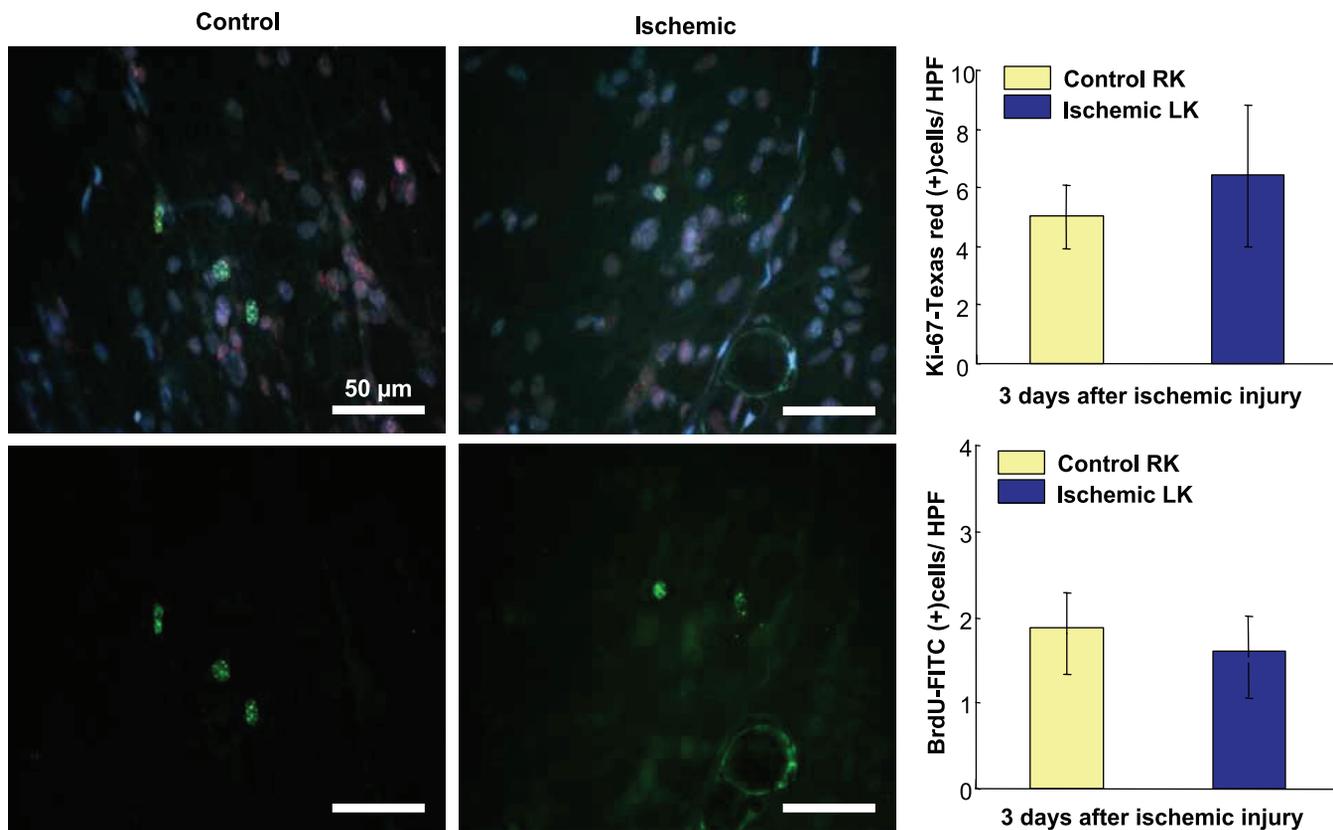


Fig. 6. No detectable proliferation of label-retaining cells in the renal parenchyma after ischemia. *Left*: representative images of proliferating (Ki-67-Texas red positive) (*top*) and BrdU-FITC-labeled (*bottom*) cells in control and ischemic kidneys. *Right*: quantification of the numbers of proliferating (*top*) and label-retaining (*bottom*) cells. Each result is representative of 4 independent experiments. HPF, high-power field.

marker Ki-67 in BrdU pulse-chase experimental mice. As shown in Fig. 6, there was no detectable surge in the proliferation of label-retaining cells in the postischemic kidneys. These data suggest that migrating cells represent, most likely, the original stem cells residing in the renal capsule. The absence of detectable cell division of cells migrated from the capsule to the interstitium may point to the predominantly paracrine progenerative action of these cells; alternatively, it may be argued that the contribution of these cells is relatively small (25–30%) and therefore any potential increase in their proliferation remains below the limits of detection.

**Effect of decapsulation in course of acute kidney injury.** The functional effect of renal capsule removal was assessed by performing bilateral renal ischemia with or without decapsulation. The initial rise in serum creatinine was not significantly different between the control group and the decapsulated group, suggesting that stripping procedures per se did not increase the renal vulnerability to acute ischemic injury. However, there was a modest but statistically significant favorable course of recovery in the control group on *day 3* after ischemic injury versus the ischemia + decapsulation group (% decrease in serum creatinine level from *day 0* to *day 3*:  $46.0 \pm 6.1\%$  vs.  $27.9 \pm 3.5\%$ ,  $P = 0.027$ , respectively; Fig. 7), suggesting *in vivo* significance of renal capsule-derived stem cells.

**DISCUSSION**

The present study provides first evidence that BrdU-retaining, slow-cycling, nestin-expressing cells exist in the renal

capsule and cells cultured from renal capsule express epitopes and intracellular markers characteristic of MSC. Furthermore, cells originating from renal capsule migrate into renal parenchyma after I/R injury, suggesting their participation in renal repair process.

Several criteria need to be fulfilled in describing cell “stemness.” These include a near-unlimited proliferative capacity, clonogenicity, multipotency, and expression of consensus markers. These were all met in the case of MSC derived from

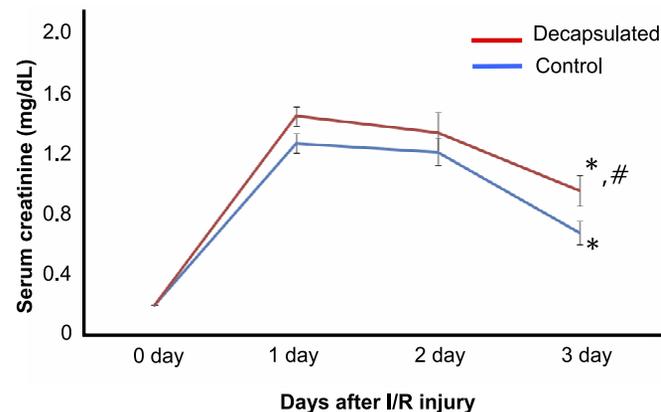


Fig. 7. Effect of decapsulation in the course of acute I/R injury. Mechanical stripping of the renal capsules did not increase the kidney’s vulnerability to acute I/R injury. However, the decapsulated group showed slower renal functional recovery compared with the control group (\*vs. *day 1*, #vs. control group,  $P = 0.043$ ).

the renal capsule. These cells were subcultured for >50 generations without loss of proliferative ability; they possessed clonogenic properties. Cells also showed in vitro ability to differentiate into various interstitial lineages when cultured in induction media (osteogenic, chondrogenic, and adipogenic). In addition, renal capsular cells expressed CD29 (99%), vimentin (97%), Sca-1 (86%), and nestin (87%), all markers of MSC (10).

Recent studies have identified various tissues harboring novel stem cell niches (7, 11, 21, 22). Epicardium, a layer of epithelial cells enveloping the myocardium, has been reported to contain a cell population with stem cell characteristics (c-kit+ and CD34+). Myocardial ischemia induced the epicardial c-kit+ cell proliferation and their differentiation into a myocardial and endothelial phenotype, thus giving rise to myocardial and vascular precursor cells (11). Furthermore, CD24+ CD133+ cells isolated from Bowman's capsule were shown to not only possess stem cell phenotypic characteristics but also provide in vivo renoprotection in functional kidney damage (21). These findings, together with our data, provide support for existence of a renal capsular stem cell niche and potentially open areas for regenerative medicine in renal diseases.

Migration was observed in papillary stem cells (16), in nestin-GFP cells (17), and in renal capsule MSC (this study) after ischemic insult. Among the guidance cues for migration stromal cell-derived factor-1 (SDF-1) plays a prominent role, and ischemic kidney represents a rich source for this chemoattractant (23). The rate of migration is comparable to that measured ex vivo in nestin-GFP mice (17). The fact that MSC residing in the renal capsule are capable of infiltrating renal parenchyma may be indicative of their participation in kidney regeneration, because previous studies performed in our and other laboratories documented the ability of MSC to rescue the kidney after ischemic insult (5, 9, 24). The pericyte-like properties of MSC have been noted and recently summarized (1, 6, 15). In this context, cells in the renal capsule displayed an inconspicuous predilection to the microvasculature, clustering around the vessels in a manner similar to the pericytes. It remains unknown how these cells traverse the capsule-parenchyma boundary in the case of ischemia; however, it is quite possible that they utilize small nutritive arterioles as conduits for this egress.

There are several issues that need future investigation. First, genetic fate tracing analysis would be needed to definitively prove the origin of renal capsule-derived stem cells and their potential progeny infiltrating ischemic renal parenchyma. Second, the in vitro differentiation assays conducted in this study used nonhomogeneous renal capsule-derived cells pooled from culture experiments (not from a single colony). Therefore, the present differentiation results do not demonstrate the true potential of a homogeneous clonally expanded renal capsule-derived cell. However, differentiation was observed in the majority of cells cultured in each of the induction media, suggesting that the bulk of cells have the capacity to differentiate into mesenchymal lineages.

In conclusion, several lines of presented evidence identify the renal capsule as a potential stem cell niche. It serves as a reservoir for MSC, "universal repair cells in adult tissues" (1), which are quiescent under normal conditions but become recruited to the renal parenchyma after renal ischemia. Further

work will be required to firmly establish the origin of these cells and trace their migratory pathways.

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#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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