Regenerative Potential of Embryonic Renal Multipotent Progenitors in Acute Renal Failure

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ABSTRACT
Bone marrow– and adult kidney–derived stem/progenitor cells hold promise in the development of therapies for renal failure. Here is reported the identification and characterization of renal multipotent progenitors in human embryonic kidneys that share CD24 and CD133 surface expression with adult renal progenitors and have the capacity for self-renewal and multilineage differentiation. It was found that these CD24/H11001 CD133/H11001 cells constitute the early primordial nephron but progressively disappear during nephron development until they become selectively localized to the urinary pole of Bowman’s capsule. When isolated and injected into SCID mice with acute renal failure from glycerol-induced rhabdomyolysis, these cells regenerated different portions of the nephron, reduced tissue necrosis and fibrosis, and significantly improved renal function. No tumorigenic potential was observed. It is concluded that CD24/H11001 CD133/H11001 cells represent a subset of multipotent embryonic progenitors that persist in human kidneys from early stages of nephrogenesis. The ability of these cells to repair renal damage, together with their apparent lack of tumorigenicity, suggests their potential in the treatment of renal failure.


The development of stem cell (SC) therapies for kidney is in its infancy primarily because of the complexity of this organ.1–3 Two sources of SC have been envisioned in the development of such treatments: bone marrow (BM)-derived SC (BMSC) and adult renal SC (ARSC).1–3 Several studies have examined the possibility that BMSC might be used for renal repair,4–15 and most authors concluded that although BMSC recruitment occurs, repair is predominantly elicited by endogenous renal cells.12–15 The existence of ARSC has been an important matter of debate.1–3,16–22 Recently, we provided evidence for the existence of resident multipotent progenitors in adult human kidney.22 These cells represent a subset of parietal epithelial cells localized to the urinary pole of the Bowman’s capsule and were thus named adult parietal epithelial multipotent progenitors (APEMP).22 APEMP are characterized by the expression of two SC markers, CD24 and CD133, and display regenerative potential in mouse models of acute renal failure (ARF).22

The potential application of blastocyst-derived
embryonic SC (ESC) is heralded as another attractive option for the treatment of renal failure, but their use is limited because of the possibility of teratoma growth. Thus, ESC need to be differentiated in vitro into a specific cell lineage before transplantation, a process that does not warrant the absence of contaminating undifferentiated tumorigenic cells. Fetal kidney–specific stem/progenitor cells may be ideal for tissue replacement because of their inherent organ-specific identity, which obviates the need for directed differentiation.

Development of the mature mammalian kidney results from reciprocal signaling between the branching ureteric bud (UB) tips and the undifferentiated metanephrogenic mesenchyme (MM) that leads to the aggregation and condensation of renal epithelia progenitor cells to form the renal vesicle, which then undergoes transformation in S-shaped body. At this stage, the proximal end of S-shaped body becomes invaded by blood vessels, differentiates into podocytes and parietal epithelial cells, and then generates glomeruli. Simultaneously, the middle and the distal segments of the S-shaped body begin to express proteins that are characteristic of tubular epithelia. The existence of renal embryonic progenitors in the MM is supported by the observation that some MM-derived cells display multidifferentiation potential; however, this putative renal embryonic progenitor has not been identified yet, and specific markers allowing its recovery are unknown.

In this study, we hypothesized that the SC markers that characterize APEMP could also be used to identify renal embryonic progenitors. Indeed, in embryonic human kidneys, coexpression of CD133 and CD24 characterized renal vesicles and S-shaped bodies, but during nephron development, their expression remained selectively localized to cells of the urinary pole of the Bowman’s capsule. Accordingly, CD24+CD133+ renal embryonic cells (REC) progressively decreased during gestation and represented <2% of whole cells in adult kidneys. CD24+CD133+ REC exhibited both self-renewal and multidifferentiation potential. More important, when injected into mice with ARF, they regenerated cells of different portions of the nephron, reduced tissue necrosis and fibrosis, and significantly improved renal function. Of note, CD24+CD133+ REC did not display tumorigenic potential. These results provide the first characterization of embryonic renal progenitors and their possible application for the therapy of renal disorders.

RESULTS

Human Embryonic Kidneys Contain a High Number of CD24+CD133+ Cells Selectively Localized in MM that Progressively Decrease as the Kidney Differentiates

Total cell suspensions obtained from embryonic kidneys with gestational age of 8.5 to 9 wk exhibited the presence of a conspicuous population (range 35 to 50%) that coexpressed both CD24 and CD133 when assessed by FACS analysis (Figure 1A, left). In embryonic kidneys of 12 to 17 wk of gestational age, the percentage of the double-positive CD24+CD133+ REC significantly decreased to 10 to 20% (Figure 1A, middle), and they represented only 0.5 to 3% of total cells from adult human kidneys (Figure 1A, right).

The localization of CD24+CD133+ REC in human embryonic kidneys (from 8.5 to 17 wk) was then investigated by confocal microscopy. CD24 was strongly expressed in MM-derived structures and in the UB (Figure 1, B and C). Of note, at all stages of embryonic kidney differentiation, CD133 ex-
expression was restricted to a subset of CD24<sup>+</sup> cells (Figure 1, B and C). Double staining for CD133 and E-cadherin, which intensely stains the UB epithelium and its branches, demonstrated CD133 expression only in condensed mesenchyme-derived primordial structures (Figure 1D). Double staining for CD133 and the proximal tubule differentiation marker Lotus Tetragonolobus Lectin (LTA) showed that only immature structures, which were negative or only weakly positive for LTA, expressed CD133 (Figure 1E). When the cells acquired differentiation markers and stained strongly positive for LTA, CD133 expression was absent (Figure 1E). Among MM-derived primordial structures, primary vesicles (Figure 2A), comma-shaped bodies (data not shown), and S-shaped bodies (Figure 2, B and C) expressed CD133.

In S-shaped bodies, strong CD133 staining was observed in the proximal loop, which will give rise to both podocytes and the Bowman’s capsule of the primitive glomerulus, as identified by WT-1 expression (Figure 2B), as well as in the distal loop (Figure 2, B and C); however, when a primitive vascular tuft was evident within the cup-shaped glomerular precursor region, CD133 was detected only in parietal epithelial cells of the Bowman’s capsule (Figure 2D). In maturing glomeruli, CD133 selectively persisted in a subset of cells of the Bowman’s capsule localized opposite to the vascular pole (Figure 2E). Finally, in mature glomeruli, CD133 was selectively maintained in parietal epithelial cells of the Bowman’s capsule localized at the urinary pole (Figure 2F).

**CD24<sup>+</sup>CD133<sup>+</sup>REC Show the Same Phenotypic Markers of APEMP and Exhibit Both Self-Renewal and Multidifferentiation Potential**

CD24<sup>+</sup>CD133<sup>+</sup>REC were recovered from total cell suspensions of digested kidney by using immunomagnetic techniques. FACS analysis showed that such a population homogeneously not only exhibited the presence of CD24 and CD133 but also expressed CD106, CD44, CD54, and CD29, whereas hematopoietic markers (CD34 and CD45) were not detectable (Figure 3A). Recovered CD24<sup>+</sup>CD133<sup>+</sup>REC were continuously grown for 60 to 80 population doublings during a period of 4 mo (Figure 3B). When assessed at 80 population doublings, cells exhibited euploid DNA content (Figure 3C). Cultured CD24<sup>+</sup>CD133<sup>+</sup>REC consistently expressed Bmi-1, Oct-4, and Nanog mRNA, at levels even higher than APEMP, whereas human mesangial cells (HMC) displayed irrelevant levels (Figure 3D) of the same SC-specific transcription factors. The expression of Bmi-1, Oct-4, and Nanog was also evaluated at protein level in human fetal kidneys. Double-label immunofluorescence demonstrated in vivo expression by CD24<sup>+</sup>CD133<sup>+</sup>REC of Bmi-1, Oct-4, and Nanog (Figure 3E).

For assessment of their multidifferentiation potential, CD24<sup>+</sup>CD133<sup>+</sup>REC were cloned by limiting dilution (Figure 4A). Twenty-nine clones obtained from donors of 9 (n = 7), 11 (n = 6), 12 (n = 9), or 14 wk of gestational age (n = 7) were randomly analyzed, and all exhibited the double CD24<sup>+</sup>CD133<sup>+</sup> phenotype (Figure 4B). CD24<sup>+</sup>CD133<sup>+</sup>REC obtained from the same single clone were cultured under conditions favorable for tubulogenic, osteogenic, adipogenic, stromal, or endothelial differentiation. Real-time reverse transcriptase–PCR (RT-PCR) demonstrated that differentiation of single clones toward tubular cells resulted in strong upregulation of mRNA characteristic for different portions of the nephron, such as aminopeptidase A and Na/glucose co-transporter (Na/Glu1), γ-glutamyltransferase (γ-GT), the Na/H exchanger, aquaporin-1 (AQP1), aquaporin-3 (AQP3), the thiazide-sensitive Na/Cl transporter, calbindin D-28k, or the

**Figure 2.** CD24<sup>+</sup>CD133<sup>+</sup>REC localize to renal vesicles and S-shaped bodies, whereas they remain selectively localized to the urinary pole of the Bowman’s capsule during glomerular development. (A) Double-label immunofluorescence for CD133 (green) and E-cadherin (red) shows CD133 expression in a primary vesicle but not in the UB. (B) Double-label immunofluorescence for WT-1 (red) and CD133 (green) showing that in the S-shaped body, CD133 is expressed in the proximal loop (evidenced by WT-1 staining), as well as in the distal loop. Merged signal between WT-1 (red) and To-pro-3 (blue) is shown in pink. (C) Double-label immunofluorescence for vWF (red) and CD133 (green), showing that in an S-shaped body after colonization by vWF-expressing primordial capillaries (red), CD133 is still diffusely expressed. (D) Double-label immunofluorescence for vWF (red) and CD133 (green) showing that at the early capillary loop stage of glomeruli, CD133 is selectively expressed by a subset of cells in the Bowman’s capsule (green). (E) Double-label immunofluorescence for vWF (red) and CD133 (green) showing that in maturing glomeruli, CD133 selectively persists in a subset of cells of the Bowman’s capsule (green) localized opposite to the vascular pole (VP). (F) Double-label immunofluorescence for vWF (red) and CD133 (green) showing that in a mature glomerulus, CD133 persists in a subset of cells in the Bowman’s capsule (green) localized at the urinary pole (UP).
Na channel β (ENaC-β; Table 1). Furthermore, the proximal tubule marker megalin, the distal tubule marker Tamm-Horsfall glycoprotein, and the collecting duct marker ENaC-β were also detectable at the protein level (Figure 4C). After osteoinduction, cloned CD24⁺CD133⁺REC exhibited a strong upregulation of Runx2, osteopontin, and alkaline phosphatase (AP) mRNA levels (Table 1). In addition, they formed AP-positive colonies that developed into mineralized nodules, as assessed by Alizarin Red staining (Figure 4D). Adipogenic differentiation was demonstrated by upregulation of adiponectin and peroxisome proliferator–activated receptor-γ mRNA levels (Table 1) and by Oil Red O staining of intracytoplasmic lipid vacuoles, which were absent from undifferentiated cells (Figure 4E). When cultured under conditions favoring endothelial differentiation, CD24⁺CD133⁺REC exhibited strong upregulation of mRNA levels for KDR, Tie-2, VE-cadherin,
Table 1. mRNA levels of multiple markers after differentiation of CD24<sup>+</sup>CD133<sup>+</sup>REC to tubular cells, osteoblasts, adipocytes, endothelial, or stromal cells<sup>a</sup>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>mRNA Levels Fold Increase&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>Tubular differentiation</td>
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<tr>
<td>Na/Gluc-1 co-transporter</td>
<td>7.44 ± 2.84</td>
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<td>aminopeptidase A</td>
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<td>AQ1P1</td>
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<tr>
<td>AQ1P3</td>
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<tr>
<td>γ-glutamyltransferase</td>
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<tr>
<td>Na/CI transporter</td>
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<tr>
<td>calbindin D-28k</td>
<td>5.10 ± 1.10</td>
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<tr>
<td>ENaC-β</td>
<td>4.15 ± 0.55</td>
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<tr>
<td>Na/H exchanger</td>
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<td>Osteogenic differentiation</td>
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<td>Runx-2</td>
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<td>AP</td>
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<td>Adipogenic differentiation</td>
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<td>adiponectin</td>
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<tr>
<td>KDR</td>
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<td>Tie-2</td>
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<tr>
<td>elastin</td>
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<sup>a</sup>Assessment by quantitative RT-PCR of mRNA levels fold increase for multiple cell type–specific markers after culture of single clones in differentiation media compared with values that were obtained in the same clones before differentiation. Each clone was evaluated for differentiation toward tubular cells, osteoblasts, adipocytes, and endothelial or stromal cells. Data are means ± SEM, as obtained after differentiation of 29 different clones.

<sup>b</sup>P < 0.05.

and von Willebrand factor (vWF; Table 1) and vWF protein (data not shown). For evaluation of endothelial differentiation in vivo, 21 d after culture in endothelial-differentiation medium, CD24<sup>+</sup>CD133<sup>+</sup>REC were labeled with the red fluorescent dye PKH26 and injected subcutaneously in growth factor–depleted Matrigel in SCID mice. After 15 d, Matrigel plugs analyzed demonstrated that PKH26-labeled human cells organized in vivo into vascular structures expressing vWF (Figure 4F). Finally, 15 d after culture in stromal differentiation medium, CD24<sup>+</sup>CD133<sup>+</sup>REC exhibited strong upregulation of mRNA levels for α-smooth muscle actin (SMA), elastin, and calponin (Table 1) and for α-SMA, calponin, or α-smooth muscle myosin heavy chain protein (Figure 4G), suggesting a differentiation toward myofibroblast/smooth muscle cells. Of note, when single-cell suspensions obtained from 11 clones were reconstituted by limiting dilution, the resulting second-round subclones displayed the same capacity for multidifferentiation as the original clone, providing additional evidence that CD24<sup>+</sup>CD133<sup>+</sup>REC are indeed multipotent and exhibit self-renewal in culture. Furthermore, clones obtained from donors of 9, 11, 12, or 14 wk of gestational age exhibited similar self-renewal and multidifferentiation capacity, suggesting that these properties are shared by CD24<sup>+</sup>CD133<sup>+</sup>REC independent of the gestational age (data not shown). Of note, differentiation of CD24<sup>+</sup>CD133<sup>+</sup>REC was associated with a significant downregulation of the SC markers Oct-4, Bmi-1, Nanog, CD133, and CD24 (Supplementary Figure 1), which started when the cells began to acquire markers of differentiated cells. A similar behavior was observed in all of the clones analyzed. A representative parallelism between markers of differentiated cells and the SC markers Bmi-1, Oct-4, Nanog, CD24, and CD133 is shown in Supplementary Figure 2.

**CD24<sup>+</sup>CD133<sup>+</sup>REC Differentiate into Different Types of Resident Renal Cells in SCID Mice that Have ARF**

To test the ability of CD24<sup>+</sup>CD133<sup>+</sup>REC to contribute to renal repair, we used an in vivo model of rhabdomyolysis-induced ARF in SCID mice, generated by intramuscular injection of glycerol. CD24<sup>+</sup>CD133<sup>+</sup>REC labeled with the red fluorescent dye PKH26 were injected into the tail vein of glycerol-treated SCID mice at the peak of blood urea nitrogen (BUN) levels (days 3 and 4). As controls, two groups of glycerol-treated SCID mice were administered an injection of saline or an equivalent number of PKH26-stained HMC, respectively. Ten days later, kidneys were harvested and analyzed. Labeled cells were never detected in mice that received saline (data not shown) or in mice that were treated with HMC (Figure 5A), whereas CD24<sup>+</sup>CD133<sup>+</sup>REC spread to the cortex and the medulla of injected mice (Figure 5, B and C, red). In the kidney, PKH26-labeled CD24<sup>+</sup>CD133<sup>+</sup>REC were incorporated into the tubules, where they expressed specific markers of differentiated portions of the nephron, such as the proximal tubule marker LTA (15.68 ± 8.12% of all LTA-stained cells) or the distal tubule/collecting duct marker Dolichos Biflorus Agglutinin (14.48 ± 3.93% of all Dolichos Biflorus Agglutinin–stained cells), which stains distal convoluted tubules, connecting tubules, and collecting ducts (Figure 5, B through F). We next examined the proliferative capacity of CD24<sup>+</sup>CD133<sup>+</sup>REC in vivo. To this end, bromodeoxyuridine (BrdU) was injected daily intraperitoneally into 10 mice that had glycerol-induced renal failure and were treated with PKH26-labeled CD24<sup>+</sup>CD133<sup>+</sup>REC. BrdU-positive proliferating cells were abundantly detected in the kidney at days 4 to 6 after induction of injury. PKH26-labeled tubular cells with BrdU-positive nuclei were observed, demonstrating that CD24<sup>+</sup>CD133<sup>+</sup>REC proliferate in vivo (Supplementary Figure 4). Cells showing PKH26-labeling and CD31 staining were also rarely observed, suggesting that, albeit at low frequency (2.2 ± 0.6%), CD24<sup>+</sup>CD133<sup>+</sup>REC might also acquire the phenotype of endothelial cells (Figure 5G). Furthermore, in SCID mice that were administered an injection of HMC (Figure 5H), HLA-I human antigen expression was never found, whereas human HLA-I antigen was consistently detected in mice that were administered an injection of CD24<sup>+</sup>CD133<sup>+</sup>REC (Figure 5I).
Figure 5. Engraftment of CD24^+CD133^+ REC in SCID mice with ARF and generation of different types of renal tubular cells. (A) Representative micrograph of kidney sections of mice that had induced ARF and were treated with PKH26-labeled HMC cells (red) and stained with LTA (green), showing the absence of red-stained cells. (B) Representative micrograph of kidney sections of mice that had induced ARF and were treated with PKH26-labeled CD24^+CD133^+ embryonic cells (red) and stained with LTA (green). Arrow points to red tubular structures. (C) High-power magnification of another kidney section of mice that had induced ARF and were treated with PKH26-labeled CD24^+CD133^+ embryonic cells (red) and stained with LTA (green). Arrow points to red tubular structures. (D) High power magnification of the kidney section shown in B, which demonstrates regeneration of a proximal tubule structure. (E) Representative micrograph of a kidney section obtained from a mouse that had induced ARF and was treated with PKH26-labeled CD24^+CD133^+ embryonic cells (red) and stained with Dolichos Biflorus Agglutinin (DBA; green), which demonstrates regeneration of distal tubules/collecting ducts structures (arrow). Other tubular structures that are stained with PKH26 but not with the collecting ducts marker DBA are visible. (F) High-power magnification of renal tubules showing PKH26 staining (red) and DBA expression (green). (G) Representative micrograph of a kidney section obtained from a mouse that had induced ARF and was treated with PKH26-labeled CD24^+CD133^+ embryonic cells (red) and stained with CD31 (green), which demonstrates differentiation into endothelial cells (arrow). (H) Double-label immunohistochemistry for cytokeratin (blue) and HLA-I human antigen (red) in kidneys of SCID mice with ARF. Absence of red signal in tubules of a kidney section from a mouse that received an injection of HMC. (I) Human HLA class I–positive cells (red) in cytokeratin-expressing tubules (blue) of SCID mice with ARF after injection of CD24^+CD133^+ embryonic cells. Magnifications: ×20 in A, B, C (zoom 1.7), D (zoom 2.5), E, and F (zoom 3.8); ×40 in G (zoom 2.7), H, and I.

CD24^+CD133^+ REC Induce Complete Recovery of Renal Function and Structure

BUN levels were measured in mice that had glycerol-induced ARF and were administered an injection of CD24^+CD133^+ REC, saline, or HMC. In mice that were treated with saline or HMC, injection of glycerol induced a significant increase in serum BUN, which peaked at day 3, declined at day 7, and stabilized at days 14 and 16 to values significantly higher than those in healthy mice (Figure 6).22,32 By contrast, mice that were treated with intravenous injection of CD24^+CD133^+ REC showed significantly lower BUN values on days 11, 14, and 16 in comparison with mice that were treated with saline or HMC (Figure 6). A detailed statistical analysis of BUN levels performed on day 14 in distinct groups of mice demonstrated that mice that were treated with CD24^+CD133^+ REC displayed BUN levels that were significantly lower than those in mice that were treated with saline or HMC but comparable to those in healthy mice. (Figure 6B). It is interesting that similar results were observed in two distinct groups of mice that were treated with CD24^+CD133^+ REC obtained from a donor of 9 or 13 wk of gestational age (light gray) or CD24^+CD133^+ REC of 9 wk of gestational age (black), mice that were administered saline (red), and mice that were treated with HMC (blue) (n = at least 8 at each time point for each group of treatment). Data are means ± SEM.
**Figure 7.** CD24^+^CD133^+^ REC protect glycerol-treated mice from renal structure deterioration. (A) Representative micrographs of kidneys from healthy mice (left) and from mice that were treated with HMC (middle) or with CD24^+^CD133^+^ REC (right) and stained with periodic acid-Schiff on day 14. (Objective 20x). (B) Representative micrographs of kidneys from healthy mice (left), from mice that were treated with HMC (middle) or with CD24^+^CD133^+^ REC (right) and stained with Masson’s trichrome on day 14. (C, left) Representative micrographs of kidneys from healthy mice stained for α-SMA (green). Nuclei are stained with To-pro-3. (Middle) Representative micrographs of kidneys from mice that were treated with HMC and stained for α-SMA (green) on day 14. Nuclei are stained with To-pro-3. (Right) Representative micrographs of kidneys from mice that were treated with CD24^+^CD133^+^ REC (red) and stained for α-SMA (green) on day 14. Nuclei are stained with To-pro-3. (D, left) Representative micrographs of kidneys from healthy mice stained for TGF-β1 (green). Nuclei are stained with To-pro-3. (Middle) Representative micrographs of kidneys from mice that were treated with HMC and stained for TGF-β1 (green) on day 14. Nuclei are stained with To-pro-3. (Right) Representative micrographs of kidneys from mice that were treated with CD24^+^CD133^+^ REC (red) and stained for TGF-β1 (green) on day 14. Nuclei are stained with To-pro-3. (E) Quantitative comparison of the areas of fibrosis between kidneys obtained from mice that were treated with saline or CD24^+^CD133^+^ REC on day 14 after glycerol injection. Sections were scored for the presence of tubular atrophy (gray) and interstitial scarring (black lines), as described in online data supplements. c versus a, P < 0.001; d versus b, P < 0.001; e versus c, P < 0.001; f versus d, P < 0.001; e versus a, NS; f versus b, NS. (F) Quantitative comparison of fibrotic areas between kidneys obtained from mice that were treated with saline or CD24^+^CD133^+^ REC on day 14 after glycerol injection was performed through measurement of green fluorescence area for α-SMA and TGF-β1 expression by image analysis, as described in online data supplements. c versus a, P < 0.001; d versus b, P < 0.001; e versus c, P < 0.001; f versus d, P < 0.001; e versus a, NS; f versus b, NS. Magnification, ×20.

**CD24^+^CD133^+^ REC Prevent Severe ARF through Enhanced Repair**

Although the peak of BUN levels was observed at day 3, proliferation of endogenous renal tubular cells mostly occurs in the first 48 h and could lead to underestimation of the potential benefit of treatment with CD24^+^CD133^+^ REC. To assess whether CD24^+^CD133^+^ REC might also prevent ARF, additional groups of mice were administered an injection of CD24^+^CD133^+^ REC or of saline 4 and 20 h after glycerol injection. Measurement of BUN levels at days 4 and 6 demonstrated that mice that were treated with CD24^+^CD133^+^ REC showed a significantly reduced severity of ARF in comparison with mice that were treated with saline (Figure 8A). Even in this second series of experiments, complete recovery of renal function (day 14) was observed only in mice that were treated with CD24^+^CD133^+^ REC (Figure 8A). Evaluation on day 4 of gestational age, respectively (Figure 6B). By contrast, mice that were treated with saline showed significantly higher levels of BUN in comparison with mice that were treated with CD24^+^CD133^+^ REC or healthy mice but comparable to those of mice that were treated with HMC (Figure 6B). Accordingly, mice that were treated with HMC showed significantly higher levels of BUN in comparison with mice that were treated with CD24^+^CD133^+^ REC or healthy mice but comparable to those of mice that were treated with saline (Figure 6B). Next, we investigated whether treatment with CD24^+^CD133^+^ REC was also associated with a better preservation of renal structure. A representative evaluation of renal morphology, as assessed with periodic acid-Schiff reagent or with Masson’s trichrome in healthy mice, as well as at day 14 after induction of ARF, in mice that were treated with CD24^+^CD133^+^ REC or HMC is shown in Figure 7. Healthy mice exhibited normal renal morphology (Figure 7, A and B, left), whereas kidney sections obtained on day 14 from mice that had ARF and were treated with HMC (Figure 7, A and B, middle) or saline (data not shown) exhibited focal but large areas of interstitial fibrosis. By contrast, mice that were treated with CD24^+^CD133^+^ REC usually showed normal renal morphology (Figure 7, A and B, right). Sections were scored for the presence of atrophic tubules identified by the thickened tubular basement membranes or for the presence of renal scarring. Quantification of areas of tubular atrophy and tubulointerstitial scarring is shown in Figure 7E. Fibrotic areas were also quantified through measurement of green fluorescence area for α-SMA and TGF-β1 expression by image analysis. In Figure 7, C and D, representative confocal micrographs of a section of kidney parenchyma from healthy mice and mice that were treated with HMC or with CD24^+^CD133^+^ REC (Figure 7, C and D) are depicted. Quantification of α-SMA– or TGF-β1–stained tissue area is shown in Figure 7F.
renal morphology of kidneys of mice that were treated with saline after staining with hematoxylin and eosin (Figure 8B), periodic acid-Schiff (data not shown), and cytoskeleton marker phalloidin (Figure 8D) demonstrated extended areas of necrosis with luminal hyaline casts, tubular cell degenerative changes, and cell loss. By contrast, mice that were treated with CD24+CD133+REC showed only small areas of tissue damage (Figure 8E) that coexpressed tubular markers (Figure 8F), whereas PKH26-labeled CD24+CD133+REC were never observed in association with the few areas of necrosis. A quantitative comparison of necrosis and tissue damage between kidneys obtained from mice that were treated with saline or CD24+CD133+REC is shown in Figure 8G.

**CD24+CD133+REC Do not Exhibit Tumorigenic Potential**

The possibility that injection of human CD24+CD133+REC could induce teratoma formation in NOD-SCID mice was also assessed. For evaluation of their tumorigenicity, 1.5 × 10^6 cells were injected subcutaneously in each of 10 NOD-SCID mice. When examined at 3 mo after CD24+CD133+REC injection, none of the 10 mice showed the appearance of any type of tumor.

**DISCUSSION**

Regenerative biology draws on the understanding of normal developmental processes. Indeed, it is generally believed that adult stem/progenitor cells represent a residual population directly derived from the organ-specific embryonic progenitor that is involved in organogenesis during fetal life.1,33,34 We recently identified in adult human kidneys APEMP, a population of multipotent progenitor cells characterized by coexpression of two SC markers, CD133 and CD24.22 This prompted us to evaluate whether coexpression of CD133 and CD24 might be useful to track down multipotent kidney stem/progenitor cells during embryonic life. It is interesting that in the early phases of nephrogenesis, coexpression of CD24 and CD133 characterized renal vesicles and S-shaped bodies, but during glomerular development, their expression remained restricted to a subset of cells in the Bowman’s capsule. In agreement with their putative nature of stem/progenitor cells, CD24+CD133+REC expressed high levels of the SC-specific transcription factors Oct-4,30 Nanog,30 and Bmi-131 and exhibited self-renewal, as well as multidifferentiation potential in vitro, as assessed on progenies of single-cell–derived clones. CD24+CD133+REC could generate degeneration (black), and cell loss (gray) as described in online data supplements. **P < 0.001. Magnification ×20 in A, B, C, D (zoom 1.5), E (zoom 1.5), F (zoom 2.2).
ate tubular cells of different portions of the nephron, as well as extrarenal cell types, such as adipocytes and osteoblasts, indicating their nature of multipotent progenitors, and even endothelial cells, as well as stromal cells, consistently with previous observations suggesting that MM cells can also act as progenitors of both renal endothelium and stroma.27,28,35 It is interesting that after tubular differentiation, CD24⁺CD133⁺REC could acquire some markers of collecting duct cells, which is in agreement with the described capacity of MM to generate also cells of connecting tubules, which exhibit phenotypic features of collecting duct cells.36 The existence of a putative MM cell with SC properties was already known,26 but the nature and surface markers of this population had not yet been established.1,27,28 Converging evidence suggests that this putative cell subset should be particularly enriched in kidneys of 8 wk of gestation.37 Indeed, fetal kidney tissue obtained from 10 to 14 wk of gestation maintains the property to generate de novo functional nephrons but generates a smaller number of mature glomeruli and tubuli than kidneys of 8 wk of gestation.37 Accordingly, the results of this study suggest that CD24⁺CD133⁺REC are enriched in the kidneys of 8 to 9 wk of gestation, substantially decrease during 10 to 14 wk of gestation, and represent <2% of whole renal cells in adults. It is interesting that in both fetal and adult kidney, CD24⁺CD133⁺ cells persist as parietal epithelial cells localized at the urinary pole of the Bowman’s capsule, supporting the concept that CD24⁺CD133⁺ cells found in adult human kidney22 represent a subpopulation of renal embryonic progenitors preserved from the early stages of nephrogenesis. Thus, the urinary pole of the Bowman’s capsule may represent an SC niche, which is a specific site in adult tissues where SC reside.38 In agreement with this hypothesis, ESC, once differentiated toward renal tubular cells, selectively migrated to the tubuloglomerular junction after injection into developing kidneys39; however, because the MM generates the cortical portion of the nephron, whereas most of the renal medulla directly derives from the UB, the kidney should contain at least two SC niches. Accordingly, other authors have suggested the existence of an SC niche in the renal papilla.16,17

Of note, CD24⁺CD133⁺REC engrafted into the kidney of SCID mice that had glycerol-induced ARF and significantly improved their altered renal function. In injured kidneys, CD24⁺CD133⁺REC mostly regenerated proximal and distal tubules and collecting ducts, but, albeit at low frequency, they could also engraft into vascular structures, in agreement with their nature of multipotent progenitors. The low frequency of vascular engraftment is probably related to the fact that in a model of rhabdomyolysis-induced ARF, renal tubule represents the predominant site of tissue injury. More important, early injection of CD24⁺CD133⁺REC substantially prevented functional renal damage, providing an accelerated repair of tubular tissue necrosis; however, the possibility that minimized injury and scarring in the areas of kidney that incorporated administered SC was also related to a paracrine effect of the injected cells also has to be considered. Taken together, the results of this study provide the first example of cell therapy of renal injury by using a selected population of renal embryonic progenitors. These results also suggest that CD24⁺CD133⁺ cells display SC properties independent of their embryonic or adult origin; however, after transplantation of CD24⁺CD133⁺REC in SCID mice with ARF, approximately 15% of the tubular cells were donor derived, whereas after injection of CD24⁺CD133⁺ adult cells, only approximately 6% of tubular cells exhibited human origin,22 suggesting that CD24⁺CD133⁺REC display a substantially higher potential of tissue engraftment and regeneration than CD24⁺CD133⁺ APEMP. Of note, despite their high regenerative potential, CD24⁺CD133⁺REC described here did not seem to exhibit tumorigenic risk. Thus, the ability of embryonic CD24⁺CD133⁺REC to repair renal damages, together with their seeming lack of tumorigenic potential, provides another option for cell therapy of renal disorders.

Concise Methods

Antibodies
See online data supplements.

Tissues
Embryonic kidneys from 8.5 to 17 wk of gestation were obtained from 17 patients who underwent a therapeutic or voluntary abortion. Legal abortions were performed in authorized hospitals, and informed consent was obtained from each patient. The use of human fetal tissues for research purposes was approved by the Committee for investigation in humans of the Azienda Ospedaliero-Università Careggi, Florence, Italy (protocol no. 6783-04). Normal kidney fragments were obtained from 10 patients who had localized renal tumors and underwent nephrectomy, in accordance with the recommendations of the Ethical Committee on human experimentation.

Isolation and Culture of CD24⁺CD133⁺REC
CD24⁺CD133⁺REC were obtained and cultured as detailed in online data supplements.

Cell Cultures
Primary culture of HMC and APEMP were obtained as described previously.22,40

Confocal Microscopy
Confocal microscopy was performed by using a LSM510 META laser confocal microscope (Carl Zeiss, Jena, Germany), as described previously.22,41

Real-Time Quantitative RT-PCR
Taq-Man RT-PCR was performed as described previously.22,41,42 Primers and probes are detailed in online data supplements.

Flow Cytometry
Flow cytometry was performed as described previously.41

Immunohistochemistry
Immunohistochemistry was performed as described previously.39,42
In Vitro Differentiation
Tubulogenic, osteogenic, adipogenic, stromal, or endothelial differentiation of CD24⁺CD133⁻ REC-derived clones was induced as detailed in online data supplements and described elsewhere.²²,⁴¹,⁴³

In Vivo Differentiation
In vivo differentiation of CD24⁺CD133⁻ REC-derived clones toward endothelium was evaluated as detailed in online data supplements.

Xenograft in SCID Mice Model of ARF
Models of rhabdomyolysis-induced ARF in SCID mice and injection of CD24⁺CD133⁻ REC, HMC, or saline were performed as described previously.²²,⁴⁴ Animal experiments were performed in accordance with institutional, regional, and state guidelines and in adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. For details, see online data supplements.

Renal Morphology
See online data supplements.

Evaluation of Tumorigenicity
For assessment of the tumorigenicity of CD24⁺REC-derived clones toward endothelium was evaluated twice a week for 3 mo. The results are expressed as means ± SEM. Comparison between groups was performed by the Mann-Whitney test or the Wilcoxon test, as appropriate.

Statistical Analyses
The results are expressed as means ± SEM. Comparison between groups was performed by the Mann-Whitney test or the Wilcoxon test, as appropriate. P < 0.05 was considered to be statistically significant.

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DISCLOSURES
None.

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See related editorial, “Stem Cells and the Kidney: Where Do We Go from Here?” on pages 3018–3020.