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Isolation and Characterization of Resident Mesenchymal Stem Cells in Human Glomeruli

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In humans, renal resident stem cells were identified within the interstitium, the tubular cells, and the Bowman's capsule. The aim of the present study was to investigate whether multipotent stem cells are present also in the adult human-decapsulated glomeruli and whether they represent a resident population. We found that human glomeruli deprived of the Bowman's capsule contain a population of $CD133^+CD146^+$ cells and a population of $CD133^-CD146^+$ cells expressing mesenchymal stem cell (MSC) markers and renal stem cell markers CD24 and Pax-2. The $CD133^+CD146^+$ cells differed from those previously isolated from Bowman's capsule as they co-expressed endothelial markers, such as CD31 and von Willebrand factor (vWF), were CD24-negative and were not clonogenic, suggesting an endothelial commitment. The glomerular mesenchymal $CD133^-CD146^+$ population (Gl-MSC) exhibited self-renewal capability, clonogenicity, and multipotency. In addition to osteogenic, adipogenic, and chondrogenic differentiation, these cells were able to differentiate to endothelial cells and epithelial cells expressing podocytes markers such as nephrin, podocin, and synaptopodin. Moreover, Gl-MSC when cultured in appropriate conditions, acquired mesangial cell markers such as alpha-smooth muscle actin (α-SMA) and angiotensin II (AT-II) receptor I. The expression of the embryonic organ-specific PAX-2 gene and protein and of donor sex identity when isolated from glomeruli of a renal allograft suggested these cells to be a tissue resident population. In conclusion, these results indicate the presence of a multipotent mesenchymal cell population resident in human glomeruli that may have a role in the physiological cell turnover and/or in response to glomerular injury.

Introduction

Tissue-specific/somatic stem cells are considered to be critical for the growth of the organs during development as well as for their physiological cell turnover. These cells may represent a remnant of embryonic stem cells in the adult tissue, or populations of stem cells homed within the tissues and modified by the local microenvironment. Recently, different populations of progenitors/stem cells have been identified in the adult kidney in rodents and humans [1–5]. In the human adult kidney, the presence of resident progenitors/stem cells expressing the CD133 as a stem cell marker has been detected in the interstitial–tubular compartment and in the Bowman's capsule [1,2]. In rats, by exploiting the retention of BrdU, multipotent stem cells were detected in the papilla whereas committed progenitor cells were detected in tubules [3,4]. Moreover, cells expressing embryonic markers, such as Pax-2, Wnt4, and Wnt1, and the Oct-4 stem cell marker were detected in the tubular compartment [5]. Recently, a population of resident stem cell expressing a mesenchymal phenotype has been isolated in glomeruli of mice [6].

Mesenchymal stem cells (MSC) are characterized by their ability to differentiate into different cell lines of mesenchymal origin [7]. The major reservoir of MSC is the bone marrow (BM) [8]; however, recent data in a murine model have demonstrated that the MSC compartment is more widely distributed. In fact MSC have been detected virtually in all murine adult tissue (spleen, muscle, kidney, lung, liver, brain, thymus) in association with the vessel walls [6].

In human, MSC have been isolated from circulating blood as well as from several tissues such as synovium, adipose tissue, trabecular bone, dental pulp, dermis, and lung [9–16]. However, the presence of MSC in human glomeruli has yet to be investigated.

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The aim of the present study was to investigate whether MSC are present in human adult glomeruli and whether they represent a resident population. Moreover, we evaluated the differentiative potential of MSC derived from glomeruli with particular regards to glomerular cell types, such as podocytes, endothelial cells, and mesangial cells.

Materials and Methods

Isolation and culture of mesenchymal stem cells derived from human glomeruli and from bone marrow

Approval of the study was obtained from the Center for Molecular Biotechnology Institutional Review Board. MSC populations from glomeruli (Gl-MSC) were obtained from the normal portion of cortex from surgically removed kidneys (15 different preparations) from patients with informed consent obtained in accordance with the Declaration of Helsinki. After dissection of the cortex, the glomerular suspension was collected using a standard established technique [17,18]. Briefly after passage through a graded series of meshes (60 and 120 meshes), glomeruli were recovered at the top of the 120 meshes sieve. Glomeruli were then collected at the bottom of a conical tube by spontaneous precipitation (10 min at room temperature) and were devoid of the visceral layer of the Bowman's capsule mechanically, by several rounds of aspiration/expulsion using a 10-mL pipette, and enzymatically, by digestion for 2 min with Collagenase I (Sigma, St. Louis, MO). The removal of the Bowman's capsule by glomeruli and the absence of tubular contamination were confirmed by light microscopy. Glomeruli were then collected at the bottom of a conical tube by spontaneous precipitation in order to remove cells and Bowman's capsules, and were transferred to fibronectin-coated T25 flasks (Falcon; BD Bioscience, Two Oak Park, Bedford, MA). Several culture media were compared. RPMI (Sigma) with 10% fetal calf serum (FCS) (Euroclone, Wetherby, UK), in the presence of 1X ITS (Sigma) and Hepes (free acid, 10 mM) (Sigma) (expansion medium), yielded the achievement and the best amplification of the Gl-MSC and therefore was used in subsequent experiments. Cells were achieved to reach confluence prior to passaging; the interval between passages varied (3–7 days) until passage 4, and from then on was established at around 7 days. The number of population doubling (PD) was calculated by the following equation: n of PD = log2 (N/N_o), where N is the number of cells yielded and N_o is the number of cells plated.

Human MSC were isolated from BM and cultured as previously described [7].

Characterization of glomerular-derived mesenchymal stem cells

Cytofluorimetric analysis was performed as described [1] and the following antibodies, all phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated were used: anti-CD105, -CD29, -CD31, -CD146, -CD44, -CD24, -CD90 (Dakocytomation, Copenhagen, Denmark); -CD73, -CD34, -CD45, -CD80, -CD86, -CD166, HLA-I (Becton Dickinson Biosciences Pharmingen, San Jose, CA); -CD133 (Miltenyi Biotec, Auburn, Alabama); KDR (R&D Systems, Abington, UK); -HLA-II (Chemicon International, Temecula, CA); -CD40 (Immunotech, Beckman Coulter); -CD154 (Serotec, Raleigh, NC) monoclonal antibodies. Mouse IgG isotypic controls were from Dakocytomation. All incubations were performed in 100 µL of phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and 0.1% sodium azide, at 4°C. For each sample, 10,000 cells were analyzed on FACSCalibur cytometer (BD Biosciences Pharmingen). Gating was constructed based on negative controls and compensation controls were included in all analyses performed. Population percentages and numbers were generated for gated populations from each experiment using Cell Quest software (BD Biosciences Pharmingen).

Indirect immunofluorescence was performed on Gl-MSC, undifferentiated or differentiated, cultured on chamber slides (Nalgen Nunc International, Rochester, NY), fixed in 4% paraformaldehyde containing 2% sucrose and when needed, permeabilized with Hepes-Triton X-100 buffer (Sigma). The following monoclonal antibodies were used: anti-vimentin (Sigma), anti-cytokeratin (Biomeda, Foster City, CA), anti-E-Cadherin (Dakocytomation), alpha-smooth muscle actin (α-SMA) (Dakocytomation), anti-synaptopodin (Progen Biotechnik, Heidelberg, Germany). Anti-von Willebrand factor (vWF) (Dakocytomation), anti-Nestin (Chemicon International), anti-Pax-2 (Covance, Princeton, NJ), anti-Nanog, anti-Oct-4, anti-Musashi (AbCam, Cambridge, Science Park Cambridge, UK), anti-podocin, anti-Angiotensin II Receptor 1 (AT1) (Santa Cruz Biotechnology, Santa Cruz, CA) rabbit polyclonal antibodies and pig polyclonal anti-Nephrin (Progen Biotechnik) were used. Omission of the primary antibodies and substitution with nonimmune rabbit, rat, or mouse IgG were used as controls where appropriated. Alexa Fluor 488 anti-rabbit or anti-pig IgG and Texas Red anti-mouse IgG (Molecular Probes, Leiden, The Netherlands) were used as secondary Abs. Confocal microscopy analysis was performed using a Zeiss LSM 5 Pascal Model Confocal Microscope (Carl Zeiss International, Germany). Hoechst 33258 dye (Sigma) were added for nuclear staining.

FISH studies

Gl-MSC were isolated from glomeruli of an explanted kidney from a male donor transplanted into a female recipient. The patient, a female of 45 years, was transplanted with a male graft. The graft was explanted after 9 months due to recurrence of severe nephritic syndrome resistant to therapy with a bioptic diagnosis of focal glomerulosclerosis.

The cultures at subconfluence (70%) underwent an overnight incubation period (16–18 h) with Velbe, 0.01 µg/mL final concentration. The cells were pelleted and the pellet resuspended in hypotonic solution (0.2 g KCl + 0.2 g Na citrate/100 mL). After an incubation at 37°C for 20 min, the cells were fixed in 3:1 methanol–acetic acid and stored for 18 h at –20°C. The metaphase chromosome were prepared by dropping about 50 µL of suspension into clean glass slides and allowed to air-dry before visualization using contrast microscopy.

FISH studies were performed using commercial X (green) and Y (red) chromosome centromeric probes and Y classical satellite III heterochromatic probe (Aquarius Cytocell, Cambridge, UK). Probes were prepared and denatured according to the manufacturer's direction. Prepared probes were applied on slide containing denatured metaphases and nuclei (1.45 min in 70% formamide /2× SSC at 70°C) under

a coverslip. Hybridization was performed overnight at 37°C. Then slides were washed once in 0.4× SSC at 72°C for 2 min followed by a second wash of 4× SSC+Tween 20 (Sigma) at room temperature for 5–10 min. Chromosomes and nuclei were counterstained with DAPI and mounted in antifade solution. Samples were analyzed for the identification of male (XY)/female (XX) nuclei and metaphases with a direct microscopic visualization (Nikon epifluorescent microscope). Digital images were obtained using the Cytovision Cytogenetics Image Analysis System.

Immunomagnetical separation, cell sorting, and cell cloning

Cells were immunomagnetically separated from fresh decapsulated glomeruli or from cell cultures at the second or third passage. After dissection and passage through a graded series of meshes, suspension of decapsulated glomeruli was digested for 8 min with Collagenase I (Sigma) and $CD133^+$ cells were isolated by magnetic cell sorting, using the MACS system (Miltenyi Biotech, Bergisch Gladbach, Germany).

$CD133^+CD146^+$ and $CD133^-CD146^+$ cells from the population outgrown from the glomeruli at second and third passages of culture in the expansion medium, were separated by cell sorting (MoFlo; Dakocytomation) and both populations were characterized, cultured, and cloned by seeding of single-cell suspensions in 96-well plates by cell sorting. The number, morphology, and kinetics of resulting clones were analyzed, and some of them were selected for subcloning and differentiation assays.

In vitro differentiation

The adipogenic, osteogenic, and chondrogenic differentiation ability of Gl-MSC or of derived primary and secondary clones was determined as previously described [7]. Briefly, Gl-MSC were cultured with Adipogenic medium (Lonza, Basel, Switzerland) for 3 weeks. To evaluate the differentiation, cells were fixed with 4% paraformaldehyde for 20 min at room temperature and stained with 0.5% Oil Red O (Sigma) in methanol (Sigma) for 20 min at room temperature.

Osteogenic differentiation was assessed by culturing the Gl-MSC in Osteogenic medium (Lonza). The medium was changed two times per week for 3 weeks. To evaluate the differentiation, cells were fixed with 4% paraformaldehyde for 20 min and stained with Alizarin Red, pH 4.1 (Lonza) for 20 min at room temperature.

For chondrogenic differentiation, 2.5×10^5 Gl-MSC were centrifuged in 15-mL conical polypropylene tube (Falcon BD Bioscience) at 150 g for 5 min and washed twice with Dulbecco's modified Eagle medium (DMEM). The pellets were cultured in Chondrogenic medium (Lonza) supplemented with 10 ng/mL of Transforming Growth Factor β3 (Lonza). Medium were changed every 3 days for 28 days. Pellets were fixed in 4% paraformaldehyde overnight, and the paraffin-embedded sections were stained for glycosaminoglycans using 0.1% safranin O (Sigma) and for sulfated proteoglycans with 1% alcian blue.

Endothelial cell differentiation was obtained by culturing the Gl-MSC in the presence of Endothelial Basal Medium (Lonza) with Vascular Endothelial growth factor (VEGF, 10 ng/mL; Sigma) for 3 weeks, as previously described [19]. Capillary-like structure formation on Matrigel (BD Biosciences Pharmingen) was performed as previously described [1].

Podocyte differentiation was obtained by culturing the Gl-MSC for 3 weeks, in the expansion medium plus all-trans retinoic acid (ATRA, Sigma, 20 μmol/L) [20]. The differentiations were performed in the chamber slides coated with type IV collagene [21] (Sigma).

Mesangial differentiation was obtained by culturing Gl-MSC for 6 days, in DMEM high glucose, in the presence of 10% FCS, transforming growth factor (TGF)-β1 (2.5 ng/mL; Sigma) [22], and platelet-derived growth factor (PDGF)-bb (5 ng/mL Sigma) [23]. The differentiations were performed in the chamber slides coated with fibronectin. Mesangial-like cell contraction was evaluated as cell shape change induced by angiotensin II (AT-II). Cell shape change was studied over 2-h period under a Nikon Diaphot inverted microscope with a 20× phase-contrast objective. Cell shape change was recorded using a JVC-1CCD video camera. Image analysis was performed with a MicroImage analysis system (Cast Imaging srl, Venice, Italy) and an IBM-compatible system equipped with a video card (Targa 2000; Truevision, Santa Clara, CA). Image analysis was performed by digital saving of image compared before stimulation and then at 5-min intervals for 2 h. The cell planar surface was calculated by the MicroImage software. The reduction of the planar cell surface >15% was used as a parameter of cell shape change compatible with a cell contraction. The mean cell contraction was indicated. Between 10 and 25 cells were analyzed for each experimental condition and repeated at least four times. Values are given as mean ± SE. Mesangial-like cells, seeded in small Petri dishes (35-mm diameter) coated with Matrigel, in DMEM with 0.25% BSA, were kept in an attached, hermetically sealed plexiglass Nikon NP-2 incubator at 37°C. Cells were stimulated with AT-II (10^{-7} mol/L) (Sigma Aldrich).

Real-Time PCR

Quantitative reverse transcription PCR was performed as described previously [24]. First-strand cDNA was produced from total RNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA 94404). Briefly, 1 or 2 μg of mRNA, 2 μL of RT buffer, 0.8 μL of dNTP mixture, 2 μL of RT random primers, 1 μL of MultiScribe reverse transcriptase, and 4.2 μL of nuclease-free water were used for each cDNA synthesis. After the reverse transcription, cDNA was stored at –20°C.

Relative quantitation by real-time PCR was performed using SYBR green detection of PCR products in real time using a 48-well StepOne™ Real Time System (Applied Biosystems). Sequence-specific oligonucleotide primers (purchased from MWG-Biotech AG, Ebersberg, Germany) were: human nephrin: forward, 5′-CAC GGT CAG CAC AAC AGA GG-3′ and reverse, 5′-GAA ACC TCG GGA ATA AGA CAC CT-3′ [24]; human *PAX-2*: forward, 5′-CCC AGC GTC TCT TCC ATC A-3′ and reverse, 5′-GGC GTT GGG TGG AAA GG-3′; and human β-actin: forward, 5′-TGA AGA TCA AGA TCA TTG CTC CTC-3′ and reverse, 5′-CAC ATC TGC TGG AAG GTG GAC-3′ [1]. Power SYBR Green PCR Master Mix was purchased from Applied Biosystems. Thermal cycling conditions were as follows: activation of AmpliTaq Gold DNA Polymerase LD at 95°C for 10 min, followed by 45 cycles of amplification at 95°C for 15 s and 60°C (for nephrin, Pax-2, and β-actin) for 1 min. To detect the log

phase of amplification, the fluorescence level (quantification of product) was determined at each cycle. The cycle at which the fluorescence reached threshold was recorded, averaged between triplicates, and normalized to the averaged cycle of threshold value for β-actin. Fold change in expression with respect to control was calculated for all samples.

Allogenic peripheral blood mononuclear cell–MSC cocultures

In modified mixed cell cultures, peripheral blood mononuclear cells (BPMC) from healthy volunteers were fractionated on Histopaque-1077 (Sigma) and used as responder cells and allogenic MSC were used as stimulator cells, as described [25,26]. Briefly, Gl-MSC were plated in triplicate onto 96-well plates at 5×10^3 cells/mL in 100 µL of complete media and were allowed to adhere to the plastic for 1–2 h. PBMC were resuspended at 5×10^4 cells/mL and were added to wells (100 µL of volume) either containing or not Gl-MSC, in the presence or absence of the mitogen Phytohemagglutinin (PHA, Sigma) at the final concentration of 2.5 µg/mL. The Gl-MSC to PBMC ratio was 1:10. Human MSCs from BM were used as controls. The culture was maintained for 16–48 h, after this period of culture lymphocyte proliferation was evaluated.

Cell proliferation was studied by DNA synthesis that was detected as incorporation of 5-bromo-2′-deoxyuridine (BrdU) into the cellular DNA using an enzyme-linked immunosorbent assay kit (Chemicon International) according to the manufacturer's instructions. The data are presented as stimulation index (SI) values calculated as described [25]. The experiments were performed at least three times for each point described.

Statistical analysis

Data were presented as the mean ± the standard deviation from three or more experiments. The statistical significance was assessed by two-tailed Student's *t*-test.

Results

Characterization of the cell populations outgrowing from glomeruli

Previous studies demonstrated the presence of $CD133^+/CD24^+$ stem cells in the Bowman's capsule [2]. In order to evaluate whether stem cells are present within the glomerular tuft, human adult glomeruli were devoid of the Bowman's capsule, as described in Materials and Methods (Fig. 1A). Decapsulated glomeruli were placed in culture onto fibronectin in the presence of expansion medium composed of RPMI, 10% FCS, 10 mM Hepes, and ITS. Within 7 days, a glomerular outgrowth of adherent cells was observed (Fig. 1B). Confluence was achieved by days 12–14 when the cell monolayer was detached by trypsin–EDTA treatment. The glomerular remnants were removed by low-speed centrifugation and cells were expanded in the same medium. During the initial culture period there was a morphological heterogeneity in the cell culture (Fig. 1C), but after 3 weeks (fourth passage) the culture became monomorphic with spindle-shaped cells (Fig. 1D). The growth rate was slow during the five early passages, and increased with serial subculture until the 20th passage when the cells were no longer capable of proliferation (100 days of culture, 20–25 PDs). The cell karyotype was studied at the 6th, 10th, and 13th passages without evidence of cytogenetic abnormalities.

At the early passages (first to third) the cells outgrowing from glomeruli were a mixed population containing 31% ± 11% $CD133^+$ and 74% ± 26% $CD133^-$ cells ($n = 15$ preparations) as detected by flow cytometry. Both cell populations expressed CD146 (Fig. 2B). The $CD133^+CD146^+$ and $CD133^-CD146^+$ cells were sorted by FACS at the second or third passage and the sorted populations were characterized and cloned. $CD133^+CD146^+$ cells co-expressed CD31 and vWF (Fig. 2E, 2F), indicating an endothelial phenotype. This $CD133^+$ population did not co-express nestin (data not shown), CD24 and the MSC markers vimentin (Fig. 2G), CD73 and CD166 (data not shown). The same phenotype was observed when the $CD133^+$ cell population was immunomagnetically sorted by the cell suspension of the freshly isolated, digested, decapsulated glomeruli (data not shown). When the cells outgrowing from glomeruli were characterized after the fourth passage in the presence of expansion medium $CD133^+$ cells were no longer detectable.

Sorted $CD133^-CD146^+$ cells were positive for surface markers characteristic of MSC [27], including CD29, CD44, CD73, CD90, CD105, CD166, and were negative for CD133 and specific hematopoietic markers (CD45 and CD34) as well as for endothelial markers (CD31) (Fig. 3A). By immunofluorescence, cells exhibited the expression of the specific MSC

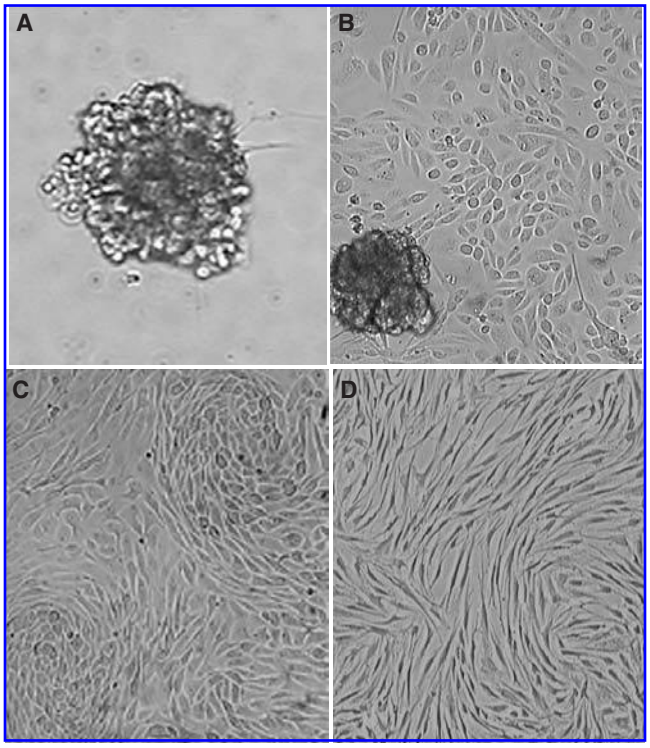

FIG. 1. Morphology of cells outgrowing from glomeruli. Representative micrographs of human adult glomeruli, devoid of Bowman's capsule by collagenase I treatment after (**A**) 24 h, (**B**) 7 days, (**C**) 2 weeks, and (**D**) 3 weeks of culture (200× magnification).

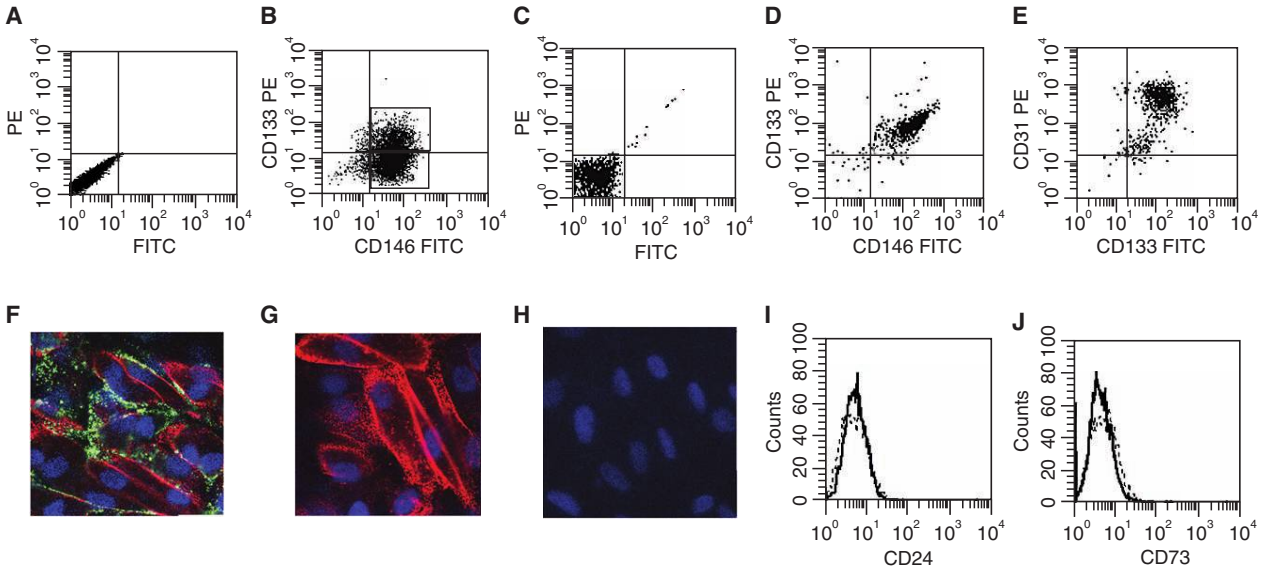

FIG. 2. Characterization of the cells outgrowing from glomeruli at the early passages of culture. Representative FACS analyses of cells outgrowing from glomeruli at second passage of culture: (**A** and **C**) isotype controls, (**B**) analyses of CD133 and CD146 co-expression, and (**D** and **E**) FACS analyses of sorted $CD133^+$ showing the co-expression of CD146 and CD31. (**I** and **J**) FACS analysis showing the absence of CD 24 (**I**) and CD 73 (**J**) by CD133+ sorted cells. Dotted lines indicate the isotypic control. Representative confocal immunofluorescence micrographs showing co-expression of CD133 (Texas Red) and von Willebrand factor (vWF) (Alexa 488, green) (**F**) but absence of vimentin staining (Alexa 488, green) (**G** and **H**) isotypic controls. Nuclear staining was performed with Hoechst dye 33258 (600× magnification).

marker vimentin and of nestin, a marker for neural and liver resident stem cells [28–30] presenting a cytoplasmic filamentous pattern (Fig. 3B). This cell population did not express epithelial markers (cytokeratin and E-cadherin) and other markers normally expressed by specialized glomerular cells as a-SMA that is expressed by mesangial cells, or nephrin that is expressed by podocytes (data not shown), indicating the absence of contaminating glomerular cell. We also evaluated the expression of Nanog, Oct-4, and Musashi transcription factors known to be involved in self-renewal and pluripotency of embryonic and adult stem cells [31–35]. This population that we identified as Gl-MSC showed nuclear–cytoplasm expression of both Nanog and Musashi (Fig. 3B) whereas they did not express Oct-4 (data not shown). Gl-MSC constitutively expressed MHC class I antigens (Fig. 3A) but were negative for class II antigens and for the adhesion molecule CD154 as well as the costimulatory molecules CD80, CD86 and CD40 (Fig. 4A) as described for BM-MSC [36]. In addition, as BM-MSC, which are known to inhibit PHA-induced PBMC proliferation, Gl-MSC induced a significant reduction of PHA-induced PBMC proliferation (Fig. 4B).

To demonstrate the self-renewal capacity of the populations obtained from glomeruli, we carried out experiments of clonogenicity of the sorted $CD133^+CD146^+$ and $CD133^-CD146^+$ cells, by seeding single-cell suspensions in 96-well plates by cell sorting (see gating strategy in Fig. 2B) of cells outgrown from the glomeruli at the third passage of culture in the expansion medium. The cloning efficiency of $CD133^-CD146^+$ was 25.3% ± 5.1%, whereas $CD133^+CD146^+$ were not clonogenic (Fig. 4C). Seeding of single-cell suspensions that were generated from primary clones of $CD133^-CD146^+$ cells generated secondary clones, and seeding of single cells that were generated from secondary clones led to the generation of tertiary clones (Fig. 4C). These data indicated that glomerular $CD133^-CD146^+$ cells were clonogenic and exhibited self-renewal capacity in vitro, whereas $CD133^+CD146^+$ cells were unable to generate clones in these culture conditions. $CD133^-CD146^+$ clones were analyzed by flow cytometry and immunohistochemistry and showed the same mesenchymal phenotype of Gl-MSC derived from the uncloned population (data not shown). When the unsorted cells outgrowing from glomeruli were characterized after the fourth passage in the presence of expansion medium, they all showed a mesenchymal phenotype, indicating that the culture condition was selective for the expansion of Gl-MSC.

Origin of Gl-MSC

We investigated whether Gl-MSC were a resident renal population rather than a BM-derived MSC localized within the glomeruli. For this purpose, we first tested the presence of markers differentially expressed by resident and BM-derived stem cells. Gl-MSC expressed CD24 (Fig. 5A), that is negative in the BM-derived MSC (Fig. 5B), and it is considered a marker of renal resident stem cells [2,37]. Moreover, the renal origin of Gl-MSC was suggested by the expression of the organ-specific embryonic Pax-2 protein and gene (Fig. 5C and 5E). Pax-2 was not expressed by BM-MSC (Fig. 5D and 5E). Pax-2 is a transcription factor that is expressed by stem cells present in the metanephric mesenchyme [38] as well as by stem cells isolated from adult rat and human kidney [1,5]. Moreover, we isolated Gl-MSC from glomeruli of an explanted kidney from a male donor transplanted into a female recipient. Gl-MSC derived from the transplanted

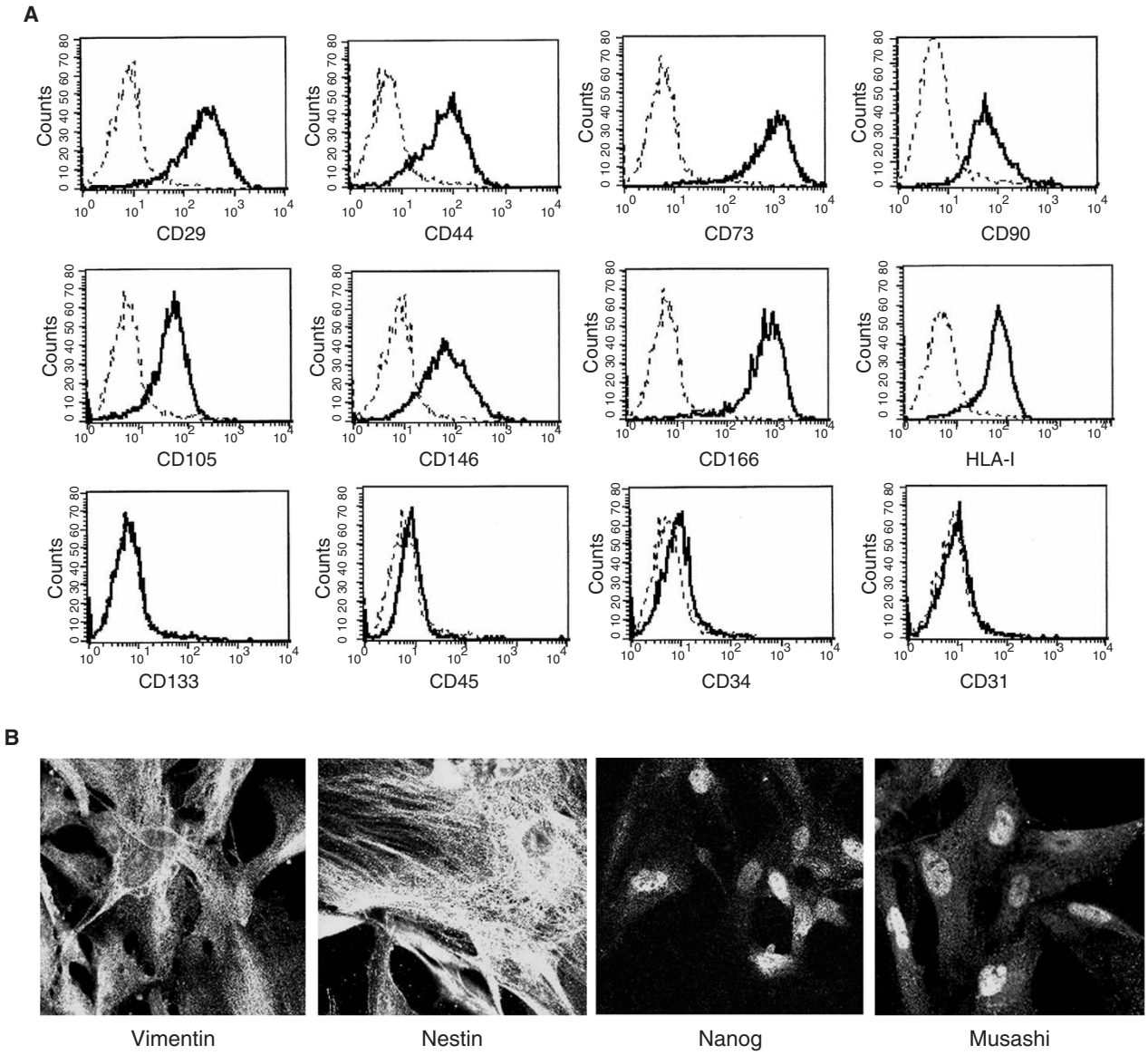

FIG. 3. Characterization of Gl-MSC. (**A**) Representative FACS analyses shows that Gl-MSC were positive for surface markers characteristic of MSCs (CD29, CD44, CD73, CD90, CD105, and CD166), and were negative for CD133 and specific hematopoietic markers (CD45 and CD34) and for endothelial markers (CD31). Dotted lines indicate the isotypic control. (**B**) Representative immunofluorescence micrographs of Gl-MSC stained with antibodies against vimentin, nestin, Nanog, and Musashi (600× magnification). All developed Gl-MSC lines exhibited the same phenotype.

kidney exhibited the same phenotype of Gl-MSC derived from normal kidney (data not shown). To define whether Gl-MSC derived from the BM of the recipient or from the transplanted kidney, the presence of the Y chromosome in Gl-MSC was analyzed at the second and sixth passages. Totally 914 nuclei and 48 metaphases were analyzed, at the second (478 nuclei; 23 metaphases) and at the sixth (436 nuclei; 25 metaphases) passages. The examination in FISH of the hybridization pattern of the dual color X (green)/Y (red) centromeric probes showed 90% of the nuclei with a male red/green pattern (Fig. 5F) and 10% with only one green spot. Probably the nuclei with one green spot derived from male nuclei that loss Y chromosome, an event that frequently occurs in culture. No female nuclei were found (two green spots). The karyotype detected in all 48 metaphases of Gl-MSC was male. The metaphase chromosomes, observed with DAPI used for FISH counterstained, appeared normal (Fig. 5G). These data suggest that Gl-MSC were not derived from the BM of the recipient but rather represent a population resident in the glomeruli of the donor kidney.

The presence of $CD133^-/CD146^+$ co-expressing CD24 in glomeruli was investigated by immunofluorescence. As shown in Figure 6, $CD146^+$ cells co-expressing CD24 were detectable in glomeruli (mean 2.22 ± 0.2/glomerulus $n = 60$) and were CD133 negative. Moreover, CD146 was expressed within the glomerular tuft by endothelial cells. $CD133^+$ cells were mainly observed in the Bowman's capsule.

Multilineage differentiation capacity of Gl-MSC

As the major defining characteristic of MSC is their ability to differentiate into multiple mesenchymal lineages, we

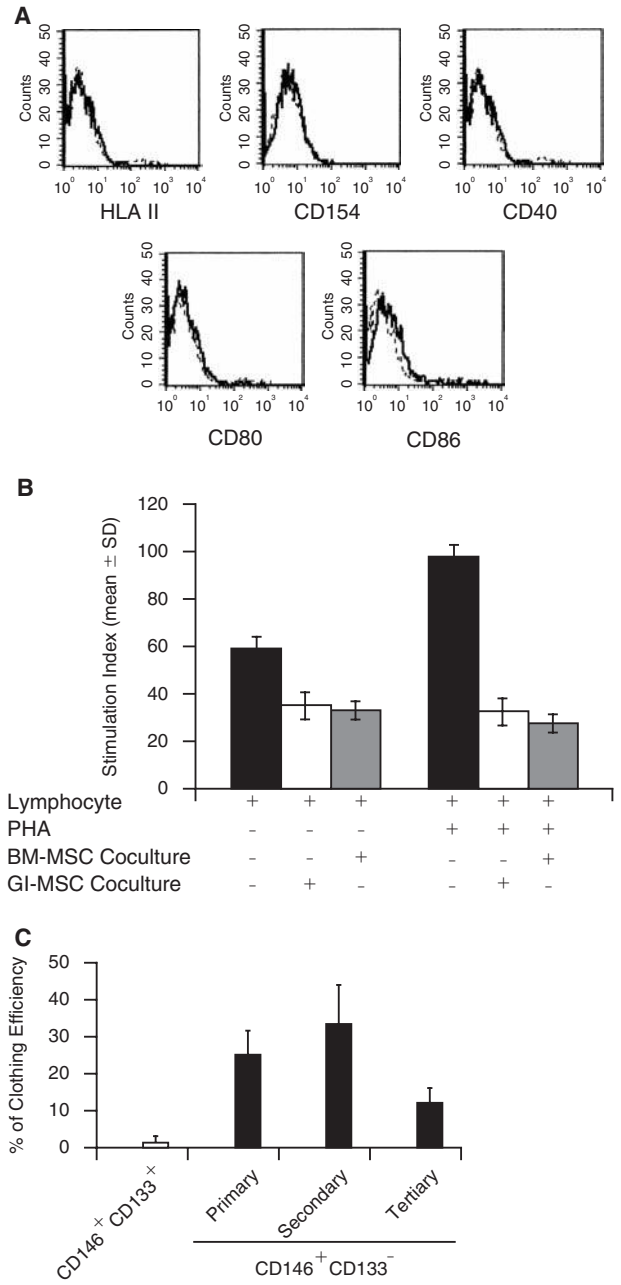

FIG. 4. Immunomodulatory properties of Gl-MSC and cloning efficiency. (**A**) Representative FACS analyses showing that Gl-MSC were negative for class II antigens and the adhesion molecule CD154, as well as for the costimulatory molecules CD80, CD86, and CD40 (dark lines). Dotted lines indicate the isotypic control. (**B**) Addition of BM-MSC or Gl-MSC inhibits PBMC proliferation induced by phytohemagglutinin (PHA). PBMCs (5×10^4/mL) were cultured for 48 h in the presence of 2.5 μg/mL PHA with or without 5×10^3/mL BM- or Gl-MSC. The data are expressed as mean ± SD of triplicates of four separate experiments. $^*P < 0.05$ (Student's *t*-test). (**C**) Clonogenic efficiency is expressed as percent of clones formed in each culture passage.

determined the ability of Gl-MSC to differentiate into specific connective tissue cells. The $CD133^+CD146^+$ population sorted at the second or third passage was unable to differentiate into adipogenic, osteogenic, and chondrogenic lineages under defined culture conditions (data not shown). In contrast, Gl-MSC lines from 15 patients (at passages 4–10) and four primary and four secondary clones showed the same multipotent differentiative ability.

Gl-MSC efficiently underwent osteogenic differentiation after 14 days in osteogenic medium as shown by the Alizarin Red staining of calcium deposits (Fig. 7A). When cultured for 21 days in adipocyte medium, Gl-MSC generated cells containing lipidic droplets (Fig. 7C). In undifferentiated controls, we did not observe any evidence of mineralization or lipidic droplets (Fig. 7B and 7D). Differentiation of Gl-MSC into chondrocytes was obtained using chondrocyte-selective medium [7]. Differentiated pellets harvested at 28 days of treatment stained with safranin O and alcian blue (Fig. 7E and 7F) characteristic for chondrocytic differentiation [7].

We also tested the capacity of Gl-MSC to differentiate, under appropriate culture conditions, in specific cell populations present into the glomeruli, such as endothelial cells, podocytes, and mesangial cells.

To obtain endothelial differentiation, we cultured Gl-MSC for 3 weeks in EBM in the presence of VEGF. Flow cytometric analyses showed the expression of specific endothelial markers, such as CD105, KDR, CD34, and CD31 (Fig. 8A). In addition, when cultured in Matrigel endothelial-differentiated Gl-MSC, and not undifferentiated cells, formed the characteristic capillary-like organization (Fig. 8B).

In the presence of PDGF-bb and TGFβ1, Gl-MSC cells acquired a mesangial-like phenotype as they acquired the expression of α-SMA and of angiotensin 2 receptor 1 (AT1) (Fig. 8C). Shape change, compatible with a cell contraction, was evaluated in response to AT-II. As shown in Figure 8D, differentiated cells underwent contraction after stimulation with AT-II, as it induced a transient reduction of the cell planar surface that was maximal after 20 min of incubation (Fig. 8E).

When cultured for 3 weeks in the presence of 20 μM/L of ATRA, Gl-MSC acquired the expression of specific epithelial markers expressed by podocytes such as cytokeratin, podocin, nephrin, and synaptopodin (Fig. 9A). Nephrin expression was confirmed by Real-Time PCR studies showing that the nephrin transcript was present in Gl-MSC after the differentiation into podocytes and not in Gl-MSC maintained in the expansion medium (Fig. 9B).

In all the experimental conditions the differentiated cells lost the stemness-related markers, such as Nanog, Musashi, vimentin, nestin, CD90, CD146, CD73, with the exception of mesangial- and endothelial-differentiated cells that maintained CD90 and CD146 (data not shown), typically expressed by mature cells. All the Gl-MSC cell lines and clones showed the same differentiation ability.

BM-derived MSC in the same culture condition of Gl-MSC differentiated into endothelial cells or muscle-like cells but not into podocytes-like cells (data not shown).

Discussion

In the present study, we report that adult human glomeruli deprived of the Bowman's capsule contain a resident MSC population. This population exhibited self-renewal capability, clonogenicity, and multipotency. The expression of the embryonic organ-specific Pax-2 gene and protein, and of donor sex identity when isolated from glomeruli of a renal allograft, suggested to be a tissue resident population.

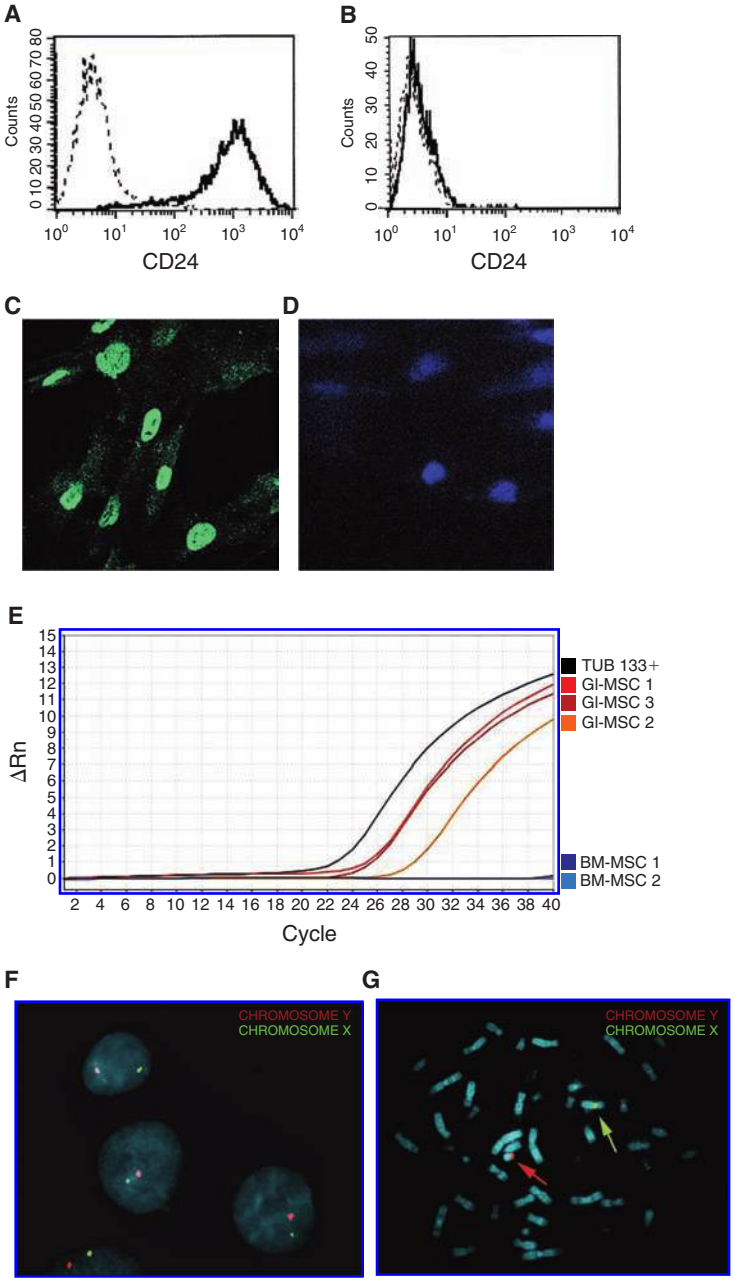

FIG. 5. Origin of Gl-MSC. Representative FACS analyses showing that Gl-MSC (**A**), but not BM-MSC (**B**), are positive for CD24 (dark lines). Dotted lines indicate the isotypic control. Representative immunofluorescence micrographs showing that Gl-MSC (**C**), but not BM-MSC (**D**), are positive for Pax-2 nuclear staining. (**E**) Pax-2 mRNA expression by quantitative real-time PCR of three Gl-MSC and two BM-MSC preparations. $CD133^+$ tubular progenitor cells were used as positive control. (**F**) Representative micrographs of FISH analysis of Gl-MSC isolated from sex-mismatched kidney transplant recipient performed to distinguish cells of donor (male) versus recipient (female) origin. Hybridization pattern of the dual color X (green)/Y (red) centromeric probes showed donor origin (male) of Gl-MSC. (**G**) The karyotype detected in metaphases of Gl-MSC was male. The metaphase chromosomes, observed with DAPI used for FISH counterstained, appeared normal (600× magnification).

Several studies addressed the presence of stem cells in the adult kidney. Oliver et al. [3] identified slow cycling stem cells in the papilla of adult rodent kidneys, based on BrdU retention. Using the same approach, Maeshima et al. [4] identified BrdU-labeled cells in the renal tubules of adult rats and showed the potential of these cells to generate proximal tubules and collecting duct cells or fibroblasts when transplanted into the metanephric kidney. Kitamura et al. established and characterized a distinct population of renal progenitor cells from the S3 segment of the nephron in the rat adult kidney [39]. These cells were shown to be able to self-renewal and to express renal embryonic markers such as Pax-2, Wnt4, and Wnt1. Based on the ability to extrude Hoechst dye, the so-called "side population" with multipotent potential has been identified in mouse embryonic and adult kidney [40]. Recently, Gupta et al. demonstrated the presence in the adult rat kidney of a renal resident population of multipotent renal progenitor cells expressing embryonic stem cell markers such as Oct-4 and Pax-2 [5].

In the human adult kidney, we demonstrated the presence of resident progenitors/stem cells using CD133 as a stem cell marker [1]. The CD133-positive cells were found as rare cells in the interstitium in proximity of proximal tubules and glomeruli, or within tubules and they were shown to undergo renal epithelial and endothelial differentiation both in vitro and in vivo. Recently, Sagrinati et al. isolated and characterized a population of multipotent $CD133^+CD24^+$ cells from the Bowman's capsule of adult [2] and embryonic human kidneys [41].

In the present study, we investigated the presence of stem cells within human glomeruli decapsulated to avoid the presence of Bowman's capsule-associated stem cells. We found that cells outgrowing from decapsulated glomeruli at the early passages were composed of two population

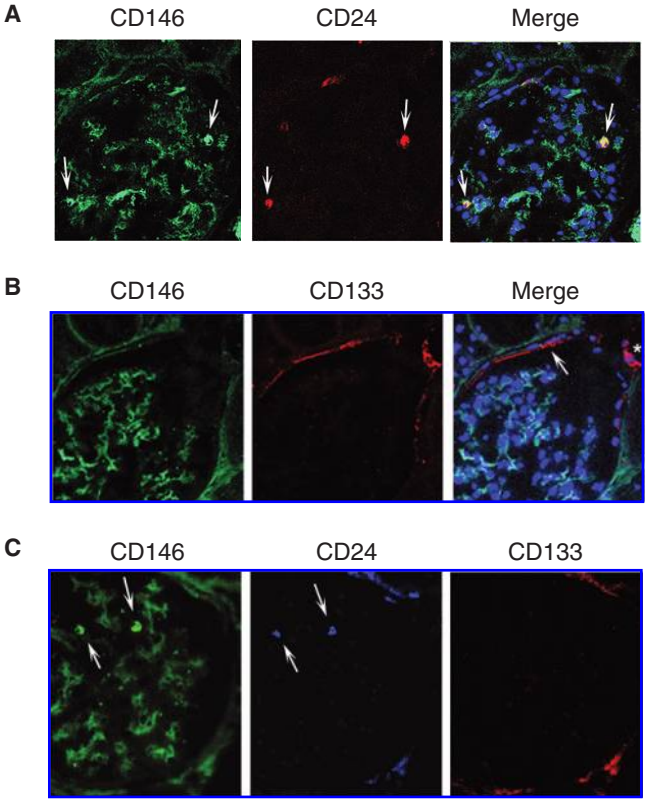

FIG. 6. Immunofluorescence detection of $CD146^+/CD24^+$ cells in human glomeruli. Representative confocal micrograph showing: (**A**) co-expression of CD146 and CD24 in a glomerolus (arrows); (**B**) expression of CD133 within the parietal cells of Bowman's capsule (arrow) and in an epithelial cell of a proximal tubule (*); and (**C**) absence of CD133 expression in glomerular $CD146^+/CD24^+$ cells (arrows). Original 630× magnification.

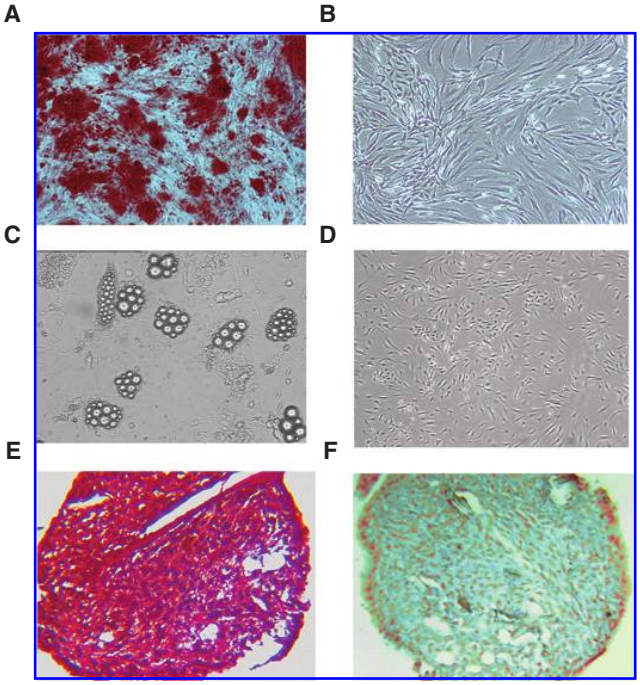

FIG. 7. Multilineage differentiation of Gl-MSC. (**A** and **B**) Representative micrographs of osteogenic differentiation shown by calcium deposition detected by Alizarin Red after culturing Gl-MSC for 14 days in specific osteogenic medium (see Materials and Methods) (**A**), but not in expansion medium (**B**). (**C** and **D**) Representative micrographs of adipogenic differentiation showing the presence of lipid droplets (**C**) after 21 days in adipogenic differentiating medium, but not in expansion medium (**D**) (200× magnification). (**E** and **F**) Representative micrographs of chondrogenic differentiation shown by formation of a pellet positive for safranin O (**E**) and alcian blue (**F**) after culturing Gl-MSC in chondrogenic medium for 28 days (100× magnification). Fifteen different lines and four clones were studied with similar results.

of $CD133^+CD146^+$ and $CD133^-CD146^+$ cells. $CD133^+$ cells sorted from outgrowths of glomeruli or isolated from freshly decapsulated glomeruli were different from those described by Sagrinati et al. in the Bowman's capsule as they did not express the renal stem cell markers CD24 and nestin, were not clonogenic, and co-expressed endothelial markers suggesting their origin from endothelial progenitors. Moreover, $CD133^+CD146^+$ cells did not survive after the third passage. In contrast, the $CD133^-CD146^+$ population that we isolated and cloned from decapsulated human glomeruli was found to be a multipotent population of cells with mesenchymal characteristics similar to the MSC population previously described in glomeruli of mice by da Silva Meirelles et al. [6] or in tubules by Gupta et al. [5]. Recently, Crisan et al. [42] demonstrated a perivascular origin for MSC in multiple human organs using CD146 expression. The expression of CD146 by stem cells associated with vessels may therefore represent a relevant marker for the identification of tissue resident stem cells belonging to the mesenchymal linage. In the present study, we demonstrate that few cells in glomeruli co-express CD146 and CD24, which is considered a renal stem cell marker, but not CD133. Therefore, one can speculate that MSC of perivascular origin are present in the glomerular vasculature and may contribute to the homeostasis of the glomerular tuft.

Being aware of the limitations of the in vitro method on isolation and characterization of stem cells, this remains for the MSC types the main tool for their identification due to their limited number in tissues and by the absence of a selective and specific marker. In this study, Gl-MSC were the only cells outgrown from glomeruli surviving after four cell culture passages and were able to generate long-term cultures. Gl-MSC shared with MSC isolated from human BM and from other adult tissues [9–16,28–30] the expression of CD29, CD44, CD166, CD73, CD90, CD105, CD146, vimentin, and nestin, and the lack of CD34 and CD45 as well as the ability to undergo mesodermal differentiation (osteocytes, chondrocytes, and adipocytes). In addition, the cells expressed the Nanog and Musashi embryonic markers involved in cell maintenance. As BM-derived MSC, Gl-MSC inhibited the PHA-induced proliferation of PBMC. A similar immunomodulatory effect was shown for nontubular Sca-1+lin-multipotent stem/progenitor cells from adult mice [43] and for human MSC derived from

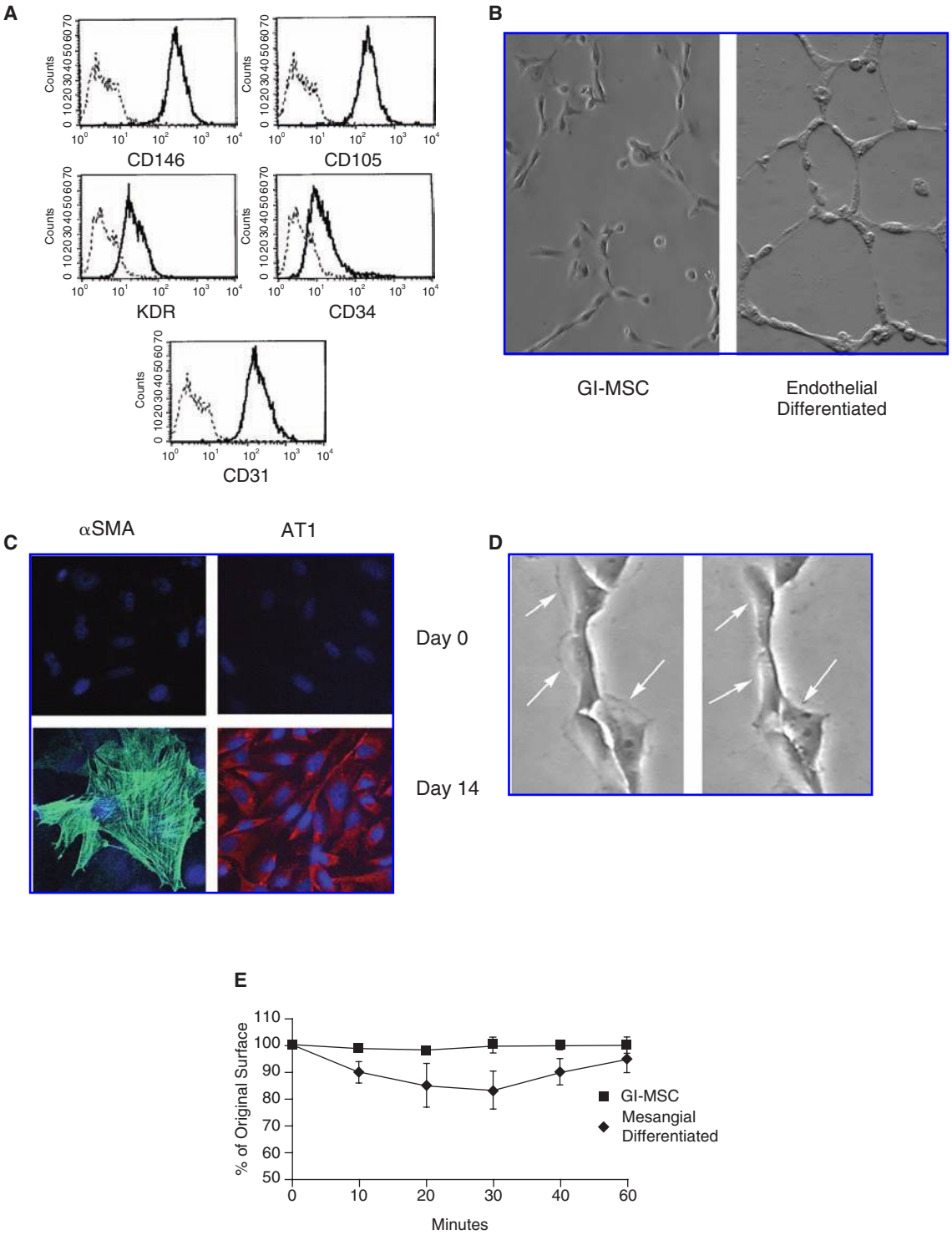

FIG. 8. Gl-MSC differentiation toward endothelial and mesangial phenotypes. (**A**) Representative FACS analyses of Gl-MSC after 3 weeks of culture in endothelial differentiation medium showing the expression of the endothelial markers KDR, CD34, and CD31 (dark lines). Dotted lines indicate the isotypic control. (**B**) Representative micrograph showing spontaneous cell organization in ring-like structures on Matrigel, within 4 h of endothelial-differentiated Gl-MSC, but not of undifferentiated Gl-MSC. Magnification: 200×. (**C**) Representative immunofluorescence micrographs of Gl-MSC, before (Day 0) and after (Day 14) culture in mesangial differentiation medium, stained for alpha-smooth muscle actin (a-SMA) and angiotensin 2 receptor 1 (AT1). (**D**) Micrographs representative of differentiated mesangial-like cells seeded in Petri dish coated with Matrigel, before (*left*) and after (*right*) 30 min of stimulation with angiotensin II (AT-II) (10^{-7} mol/L). Morphological changes (arrows) of differentiated cells, consistent with cell contraction, were observed (200× magnification). (**E**) Kinetics of change of mesangial-like cells shape after stimulation with AT-II. Cell retraction is expressed as the reduction of the planar surface evaluated before stimulation. Values are given as mean ± SD of three different experiments.

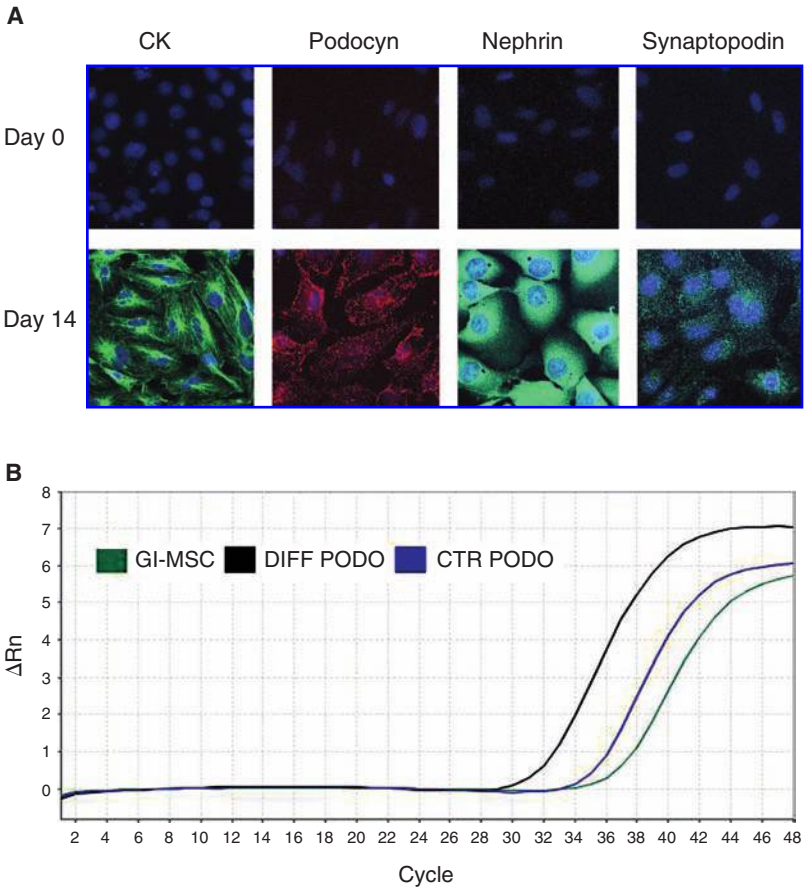

FIG. 9. Gl-MSC differentiation toward podocyte phenotype. (**A**) Representative immunofluorescence micrographs of Gl-MSC before (Day 0) and after (Day 14) culture in the presence of all-trans retinoic acid (ATRA), stained for cytokeratin (CK), podocin, nephrin, and synaptopodin. Nuclear staining was performed with Hoechst dye 33,258 (600× magnification). Fifteen different lines and four clones were studied with similar results. (**B**) Nephrin mRNA expression by quantitative real-time PCR: representative plot of undifferentiated Gl-MSC (green), podocytes differentiated Gl-MSC (black), and a control immortalized podocyte cell line (blue). Three cloned cell lines were tested with similar results.

heart, spleen, and perirenal fat [44]. Recent studies focused on the relevance of these immunomodulatory properties of MSC in different clinical settings [45].

The origin of MSC within adult organs has been debated. In the presence study, we found that Gl-MSC presented the phenotypic characteristics of resident renal stem cells. In fact, Gl-MSC, at variance of BM-MSC, expressed the CD24 that is considered a marker of renal resident progenitors [2,37] and the Pax-2 embryonic organ-specific transcription factor [1,5,38]. The expression of Pax-2 has been previously shown to be a marker of renal resident stem cell populations [1,5,38]. Moreover, the presence of Gl-MSC of donor sex identity in a renal allograft provided evidence for the presence of a MSC population that resides locally within the adult human glomeruli. This result is in agreement with previous evidence for tissue resident MSC provided in human lung from studies of lung transplantation [16]. Gl-MSC may be an embryonic residue or BM-derived cells localized in the kidney and modified by the local environment to acquire characteristics of resident stem cells.

The renal commitment of Gl-MSC is shown by their ability to differentiate into endothelial cells and into specific glomerular lineages such as podocyte and mesangial-like cells under appropriate culture conditions. Indeed, Gl-MSC, a variance of BM-MSC, when cultured in the presence of ATRA and type IV collagen, were able to differentiate in epithelial cells expressing markers specific for the slit diaphragm of podocytes such as nephrin, synaptopodin, and podocin. Concerning the differentiation of Gl-MSC into glomerular mesangial cells, this was obtained by culturing the cells in the presence of a TGFβ1 and PDGF-bb combination, which has been reported to induce smooth muscle cell differentiation in multipotent adult progenitor cells derived from BM [22]. Moreover, Gl-MSC showed immunomodulatory properties as they inhibited proliferation of PHA-stimulated PBMC, similar to the BM-MSC [25–27]. The immunomodulatory activity of Gl-MSC could be relevant for inflammatory glomerular diseases.

In conclusion, the results of the present study demonstrate the presence in adult human decapsulated a population of resident multipotent progenitors expressing a mesenchymal phenotype with the potential to contribute to the turnover of the different glomerular specific cell types. Further studies are needed to evaluate whether Gl-MSC may be exploited in regenerative therapy of glomerular diseases.

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Author Disclosure Statement

S.B., B.B., and G.C. are named inventors on a related patent application.

References

1. Bussolati B, S Bruno, C Grange, S Buttiglieri, MC Deregibus, D Cantino and G Camussi. (2005). Isolation of renal progenitor cells from adult human kidney. Am J Pathol 166:545–545.
2. Sagrinati C, GS Netti, B Mazzinghi, E Lazzeri, F Liotta, F Frosali, E Ronconi, C Meini, M Gacci, R Squecco, M Carini, L Gesualdo, F Francini, E Maggi, F Annunziato, L Lasagni, M Serio, S Romagnani and P Romagnani. (2006). Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. J Am Soc Nephrol 17:2443–2456.
3. Oliver JA, O Maarouf, FH Cheema, TP Martens and Al-Awqati Q. (2004). The renal papilla is a niche for adult kidney stem cells. J Clin Invest 114:795–804.
4. Maeshima A, S Yamashita and Y Nojima. (2003). Identification of renal progenitor-like tubular cells that participate in the regeneration processes of the kidney. J Am Soc Nephrol 14:3138–3146.
5. Gupta S, C Verfaillie, D Chmielewski, S Kren, K Eidman, J Connaire, Y Heremans, T Lund, M Blackstad, Y Jiang, A Luttun and ME Rosenberg. (2006). Isolation and characterization of kidney-derived stem cells. J Am Soc Nephrol 17:3028–3040.
6. da Silva Meirelles L, PC Chagastelles and NB Nardi. (2006). Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 119:2204–2213.
7. Pittenger MF, AM Mackay, SC Beck, RK Jaiswal, R Douglas, JD Mosca, MA Moorman, DW Simonetti, S Craig and DR Marshak. (1999). Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147.
8. Prockop DJ. (1997). Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276:71–74.
9. Zvaifler NJ, L Marinova-Mutafchieva, G Adams, CJ Edwards, J Moss, JA Burger and RN Maini. (2000). Mesenchymal precursor cells in the blood of normal individuals. Arthritis Res 2:477–488.
10. De Bari C, F Dell'Accio, P Tylzanowski and FP Luyten. (2001). Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 44:1928–1942.
11. Zuk PA, M Zhu, H Mizuno, J Huang, JW Futrell, AJ Katz, P Benhaim, HP Lorenz and MH Hedrick. (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7:211–228.
12. Nöth U, AM Osyczka, R Tuli, NJ Hickok, KG Danielson and RS Tuan. (2002). Multilineage mesenchymal differentiation potential of human trabecular bone-derived cells. J Orthop Res 20:1060–1069.
13. Gronthos S, M Mankani, J Brahim, PG Robey and S Shi. (2000). Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA 97:13625–13630.
14. Young HE, TA Steele, RA Bray, J Hudson, JA Floyd, K Hawkins, K Thomas, T Austin, C Edwards, J Cuzzourt, M Duenzl, PA Lucas and AC Black Jr. (2001). Human reserve pluripotent mesenchymal stem cells are present in the connective tissues of skeletal muscle and dermis derived from fetal, adult, and geriatric donors. Anat Rec 264:51–62.
15. Sabatini F, L Petecchia, M Tavian, V Jodon de Villeroché, GA Rossi and D Brouty-Boyé. (2005). Human bronchial fibroblasts exhibit a mesenchymal stem cell phenotype and multilineage differentiating potentialities. Lab Invest 85:962–971.
16. Lama VN, L Smith, L Badri, A Flint, AC Andrei, S Murray, Z Wang, H Liao, GB Toews, PH Krebsbach, M Peters-Golden, DJ Pinsky, FJ Martinez and VJ Thannickal. (2007). Evidence for tissue-resident mesenchymal stem cells in human adult lung from studies of transplanted allografts. J Clin Invest 117:989–996.
17. MacKay K, LJ Striker, S Elliot, CA Pinkert, RL Brinster and GE Striker. (1988). Glomerular epithelial, mesangial and endothelial cell lines from transgenic mice. Kidney Int 33:677–684.
18. Ohse T, JW Pippin, MR Vaughan, PT Brinkkoetter, RD Krofft and SJ Shankland. (2008). Establishment of conditionally immortalized mouse glomerular parietal epithelial cells in culture. J Am Soc Nephrol 19:1879–1890.
19. Rafii S, D Lyden and R Benezra. (2002). Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? Nat Rev Cancer 2:826–835.
20. Vaughan MR, JW Pippin, SV Griffin, R Krofft, M Fleet, L Haseley and SJ Shankland. (2005). ATRA induces podocyte differentiation and alters nephrin and podocin expression in vitro and in vivo. Kidney Int 68:133–144.
21. Perry J, S Tam, K Zheng, Y Sado, H Dobson, B Jefferson, R Jacobs and PS Thorner. (2006). Type IV collagen induces podocytic features in bone marrow stromal stem cells in vitro. J Am Soc Nephrol 17:66–76.
22. Ross JJ, Z Hong, B Willenbring, L Zeng, B Isenberg, EH Lee, M Reyes, SA Keirstead, EK Weir, RT Tranquillo and CM Verfaillie. (2006). Cytokine-induced differentiation of multipotent adult progenitor cells into functional smooth muscle cells. J Clin Invest 16:3139–3149.
23. Suzuki A, H Iwatani, T Ito, E Imai, M Okabe, H Nakamura, Y Isaka, M Yamato and MM Hori. (2004). Platelet-derived growth factor plays a critical role to convert bone marrow cells into glomerular mesangial-like cells. Kidney Int 65:15–24.
24. Collino F, B Bussolati, E Gerbaudo, L Marozio, S Pelissetto, C Benedetto and G Camussi. (2008). Pre-eclamptic sera induce nephrin shedding from podocytes through endothelin-1 release by endothelial glomerular cells. Am J Physiol Renal Physiol 294:F1185–F1194.
25. Di Nicola M, C Carlo-Stella, M Magni, M Milanesi, PD Longoni, P Matteucci, S Grisanti and AM Gianni. (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 99:3838–3843.
26. Aggarwal S and MF Pittenger. (2005). Human mesenchymal stem cells modulate allogenic immune cell response. Blood 105:1815–1822.
27. Chamberlain G, J Fox, B Ashton and J Middleton. (2007). Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 25:2739–2749.
28. El-Helou V, J Dupuis, C Proulx, J Drapeau, R Clement, H Gosselin, L Villeneuve, L Manganas and A Calderone. (2005). Resident nestin^{+} neural-like cells and fibers are detected in normal and damaged rat myocardium. Hypertension 46:1219–1225.
29. Mayer EJ, DA Carter, Y Ren, EH Hughes, CM Rice, CA Halfpenny, NJ Scolding and AD Dick. (2005). Neural progenitor cells from postmortem adult human retina. Br J Ophthalmol 89:102–106.
30. Herrera MB, S Bruno, S Buttiglieri, C Tetta, S Gatti, MC Deregibus, B Bussolati and G Camussi. (2006). Isolation and characterization of a stem cell population from adult human liver. Stem Cells 24:2840–2850.
31. Hatano SY, M Tada, H Kimura, S Yamaguchi, T Kono, T Nakano, H Suemori, N Nakatsuji and T Tada. (2006). Pluripotential competence of cells associated with Nanog activity. Mech Dev 122:67–79.
32. Cavaleri F and HR Scholer. (2003). Nanog: a new recruit to the embryonic stem cell orchestra. Cell 113:551–552.
33. Tai MH, CC Chang, M Kiupel, JD Webster, LK Olson and JE Trosko. (2005). Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. Carcinogenesis 26:495–502.
34. Potten CS, C Booth, GL Tudor, D Booth, G Brady, P Hurley, G Ashton, R Clarke, S Sakakibara and H Okano. (2003). Identification of a putative intestinal stem cell and early lineage marker;musashi-1. Differentiation 71:28–41.
35. Sugiyama-Nakagiri Y, M Akiyama, S Shibata, H Okano and H Shimizu. (2006). Expression of RNA-binding protein Musashi in hair follicle development and hair cycle progression. Am J Pathol 68:80–92.
36. Le Blanc K, C Tammik, K Rosendahl, E Zetterberg and O Ringdén. (2003). HLA expression and immunologic properties

of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 31:890–896.
37. Challen GA, G Martinez, MJ Davis, DF Taylor, M Crowe, RD Teasdale, SM Grimmond and MH Little. (2004). Identifying the molecular phenotype of renal progenitor cells. J Am Soc Nephrol 15:2344–2357.
38. Oliver JA, J Barasch, J Yang, D Herzlinger and Q Al-Awqati. (2002). Metanephric mesenchyme contains embryonic renal stem cells. Am J Physiol 283:F799–F809.
39. Kitamura S, Y Yamasaki, M Kinomura, T Sugaya, H Sugiyama, Y Maeshima and H Makino. (2005). Establishment and characterization of renal progenitor like cells from S3 segment of nephron in rat adult kidney. FASEB J 19:1789–1797.
40. Challen GA, I Bertoncello, JA Deane, SD Ricardo and MH Little. (2006). Kidney side population reveals multilineage potential and renal functional capacity but also cellular heterogeneity. J Am Soc Nephrol 17:1896–1912.
41. Lazzeri E, C Crescioli, E Ronconi, B Mazzinghi, C Sagrinati, GS Netti, ML Angelotti, E Parente, L Ballerini, L Cosmi, L Maggi, L Gesualdo, M Rotondi, F Annunziato, E Maggi, L Lasagni, M Serio, S Romagnani, GB Vannelli and P Romagnani. (2007). Regenerative potential of embryonic renal multipotent progenitors in acute renal failure. J Am Soc Nephrol 18:3128–3138.
42. Crisan M, S Yap, L Casteilla, CW Chen, M Corselli, TS Park, G Andriolo, B Sun, B Zheng, L Zhang, C Norotte, PN Teng, J Traas, R Schugar, BM Deasy, S Badylak, HJ Buhring, JP Giacobino, L Lazzari, J Huard and B Péault. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3:301–313.
43. Dekel B, L Zangi, E Shezen, S Reich-Zeliger, S Eventov-Friedman, H Katchman, J Jacob-Hirsch, N Amariglio, G Rechavi, R Margalit and Y Reisner. (2006). Isolation and characterization of nontubular sca-1+lin- multipotent stem/progenitor cells from adult mouse kidney. J Am Soc Nephrol 17:3300–3314.
44. Hoogduijn MJ, MJ Crop, AM Peeters, GJ Van Osch, AH Balk, JN Ijzermans, W Weimar and CC Baan. (2007). Human heart, spleen, and perirenal fat-derived mesenchymal stem cells have immunomodulatory capacities. Stem Cells Dev 16:597–604.
45. Locatelli F, R Maccario and F Frassoni. (2007). Mesenchymal stromal cells, from indifferent spectators to principal actors. Are we going to witness a revolution in the scenario of allograft and immune-mediated disorders? Haematologica 92: 872–877.

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