Biomaterials 50 (2015) 56–66

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Nestin$^+$ kidney resident mesenchymal stem cells for the treatment of acute kidney ischemia injury

Mei Hua Jiang [a, b, c, 1], Guilan Li [b, 1], Junfeng Liu [b, d, 1], Longshan Liu [e], Bingyuan Wu [f], Weijun Huang [b], Wen He [g], Chunhua Deng [h], Dong Wang [i], Chunling Li [j], Bruce T. Lahn [b], Chenggang Shi [k, **], Andy Peng Xiang [a, b, l, *]

[a] Cell-gene Therapy Translational Medicine Research Center, The Third Affiliated Hospital, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China
[b] Center for Stem Cell Biology and Tissue Engineering, Key Laboratory for Stem Cells and Tissue Engineering, Ministry of Education, Sun Yat-Sen University, Guangzhou, China
[c] Department of Anatomy and Neurobiology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China
[d] Department of Pathology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
[e] Laboratory of General Surgery, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
[f] Department of Cardiology, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
[g] Department of Geriatrics, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
[h] Department of Urology, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China
[i] Department of Laboratory Medicine, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China
[j] Institute of Hypertension & Kidney Research, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China
[k] Department of Nephrology, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
[l] Department of Biochemistry, Zhongshan Medical School, Sun Yat-sen University, Guangzhou, China

ARTICLE INFO

Article history:
Received 19 November 2014
Accepted 20 January 2015
Available online

Keywords:
Nestin
MSCs
Acute kidney injury
Paracrine
VEGF

ABSTRACT

Renal resident mesenchymal stem cells (MSCs) are important regulators of kidney homeostasis, repair or regeneration. However, natural distribution and the starting population properties of these cells remain elusive because of the lack of specific markers. Here, we identified post-natal kidney derived Nestin$^+$ cells that fulfilled all of the criteria as a mesenchymal stem cell. These isolated Nestin$^+$ cells expressed the typical cell-surface marker of MSC, including Sca-1, CD44, CD106, NG2 and PDGFR-α. They were capable of self-renewal, possessed high clonogenic potential and extensive proliferation for more than 30 passages. Under appropriate differentiation conditions, these cells could differentiate into adipocytes, osteocytes, chondrocytes and podocytes. After intravenous injection into acute kidney injury mice, Nestin$^+$ cells contributed to functional improvement by significantly decreasing the peak level of serum creatinine and BUN, and reducing the damaged cell apoptosis. Furthermore, conditioned medium from Nestin$^+$ cells could protect against ischemic acute renal failure partially through paracrine factor VEGF. Taken together, our findings indicate that renal resident Nestin$^+$ MSCs can be derived, propagated, differentiated, and repair the acute kidney injury, which may shed new light on understanding MSCs biology and developing cell replacement therapies for kidney disease.

1. Introduction

Mesenchymal stromal/stem cells (MSCs) are multipotent progenitor cells that are able to differentiate into osteoblasts, adipocytes, and chondrocytes [1]. In addition to their self-renewal capacity and multipotency, MSCs also possess potent immunemodulatory and paracrine characteristics [2,3]. Furthermore, plenty of preclinical and clinical studies have identified the therapeutic potential of MSCs on kidney protection and repair by their

* Corresponding author. Center for Stem Cell Biology and Tissue Engineering, Key Laboratory for Stem Cells and Tissue Engineering, Ministry of Education, Sun Yat-Sen University, Guangzhou, China. Tel.: +86 20 87335822; fax: +86 20 87335858.
** Corresponding author. Tel.: +86 20 82179168.
E-mail addresses: ericshi412@sina.com (C. Shi), xiangp@mail.sysu.edu.cn (A.P. Xiang).
[1] These authors made equal contributions to this work.

http://dx.doi.org/10.1016/j.biomaterials.2015.01.029
0142-9612/

paracrine, anti-inflammatory, and immunomodulatory properties, such as acute kidney injury (AKI), chronic kidney disease (CKD) or renal transplantation [4–6]. Despites the major reservoir of MSCs in bone marrow, it has been identified that MSCs reside in almost all organs and tissues, these cells in association with the perivascular niche of these organs [7].

Although the renal resident MSCs have been isolated and shown phenotypic and functional equivalence with bone marrow MSCs, they also demonstrate distinct gene and protein expression profiles [8]. Because tissue resident MSCs might play an important regulatory role of tissue repair or regeneration, the unique functional roles of renal MSCs imply they might be more appropriate as cellular therapeutic agents for the treatment of kidney diseases [9,10]. However, our current understanding of tissue resident kidney MSCs remains limited. Conventionally, kidney MSCs are functionally isolated from renal tissue based on their capacity to adhere to the surface of culture flasks, but the isolated cells do not provide biological information about the starting population [11,12]. Because of the lack of the specific markers for kidney MSCs, the exact nature and localization of MSCs in vivo remain poorly understood. To overcome the limitations of these methods, there is a clear need for specific markers and methods to identify and prospectively isolate kidney MSCs.

The intermediate filament protein, Nestin, is a widely employed marker of multi-potent neural stem/progenitor cells (NSCs) [13]. More importantly, Nestin labels adult stem/progenitor cell populations, indicating that Nestin might be a common marker of multi-lineage stem cells [14]. Méndez-Ferrer et al. firstly reported a stromal Nestin expressing population in bone marrow showing the typical characteristics of MSCs, suggesting Nestin might also become a specific marker for isolating the tissue resident MSCs [15]. Previous studies have shown the Nestin expression in adult and developing kidney [16], including repopulating mesangial cells [17], podocytes [18]. Under specific damage conditions, upregulation of Nestin expression was found in tubular cells, podocytes and interstitial cells [19,20].

To investigate whether Nestin can be used as a candidate marker for identifying the kidney resident MSCs, we report a method for isolating MSCs from the murine kidney, using flow cytometry in combination with in vitro function assays. Moreover, we study the reparative capability and the cellular and molecular mechanism of Nestin$^+$ MSCs in acute kidney ischemia injury model.

2. Materials and methods

2.1. Mice

Homozygous transgenic mice that expressed enhanced GFP under the control of a Nestin promoter (Nestin-GFP, on the C57BL/6 genetic background) were kindly provided by Dr. Masahiro Yamaguchi [21]. C57BL/6 mice were provided by Vital River Laboratories (Beijing, China). All animal studies were carried out in accordance with the guidelines of the Sun Yat-sen University Institutional Animal Care and Use Committee.

2.2. Isolation and culture of Nestin$^+$ cells from the mice kidney

Neonatal Nestin-GFP or C57BL/6 mice kidneys were harvested, the collected kidneys were incubated with Collagenase IV (300 U/ml; Sigma Aldrich, USA) and DNase I (100 U/ml; Sigma Aldrich, USA) in HBSS for 20 min at 37 °C in a shaking water bath. Subsequently, the cell suspensions were digested with 0.25% trypsin (Sigma Aldrich, USA) at 37 °C for 5 min and passed through a cell strainer with a mesh diameter of 40 μm, yielding single cells. The cells expressing green fluorescent protein (GFP) were sorted using an Influx Cell Sorter (BD, USA) and cultured on plastic plates in DMEM/F12 medium (Invitrogen, USA) with 20 ng/ml EGF (Peprotech, USA), 10 ng/ml bFGF (Peprotech, USA), 2% B27 (Invitrogen, USA), 1% N2 (Invitrogen, USA), and 100 IU/ml penicillin/streptomycin (Invitrogen, USA). The cells were cultured at 37 °C under 5% CO_2 and propagated every 2–3 days.

2.3. Colony forming unit-fibroblast assay

For colony forming unit-fibroblast assay (CFU-F), 5000 Nestin$^+$ cells were plated on tissue culture plastic and cultured in above-mentioned cell growth medium. After 10 days of cultivation, the number of colonies containing greater than 50 cells were assessed by inverted microscopy using an Olympus IX71 and quantified with the aid of a DP manager program (Olympus, Japan) [22].

2.4. Cell proliferation assay

Nestin$^+$ cells proliferation was evaluated with Click-iT® EdU cell Fluor Cell Proliferation Assay Kit (Invitrogen, USA). The cells were labeled with 10 μM EdU for 24 h and then washed carefully to remove the dye and staining according to the manufacturer's instructions. Nuclei were counterstained with DAPI (Sigma Aldrich, USA).

Cells were seeded into 12-well plates at a density of 10,000 cells/well. The cells were trypsin with 0.25% trypsin and counted for consecutive 6 days ($n = 3$). Population doubling time (PDT) was determined with the following formula: PDT = days in exponential phase/(log N2 − log N1)/log 2 where N1 was the number of cells at the beginning of the exponential growing phase and N2 was the number of cells at the end of the exponential growing phase [23].

2.5. Flow cytometry analysis

Flowcytometry analysis and sorting were performed on FACS Calibur flow cytometer (BD, USA). The following anti-mouse antibodies were used: Sca-1-APC, CD44-PE, CD106-AF647, CD45-PE, CD11b-PE (eBioscience, USA). A minimum of 100,000 cells was acquired for each analysis.

2.6. Immunofluorescence staining analysis

For immunofluorescence, the cells and kidneys were fixed in 4% PFA and dehydrated in 30% sucrose. The dehydrated kidneys were cut into 10 μm sections. The cells and sections blocked with 10% normal serum for 40 min, and then incubated with primary antibodies overnight at 4 °C in a humidified chamber followed by incubation with then incubated with 488 or 594-conjugated secondary antibodies (Invitrogen, USA) at room temperature for 1 h. The following antibodies were used: anti-Nestin (1:200, Millipore, USA); anti-vimentin (1:500, Abcam, UK); anti-Pax2 (1:100, Abcam, UK); anti-NG2 (1:200, Abcam, UK); anti-PDGFR-α (1:200, Abcam, UK); anti-CD31 (1:200, BD Biosciences, USA); anti-podocin (1:400, Abcam, UK); anti-synaptopodin (1:300, Abcam, UK); anti-E-cadherin (1:200, Abcam, UK). All images were obtained using a Zeiss LSM710 confocal microscope.

2.7. Nestin$^+$ cells differentiation ability in vitro

For osteogenic differentiation, the cells were plated in 24-well plates at a density of 1×10^4 cells/well and cultured in the presence of α-MEM (Invitrogen, USA), 20% FBS, 100 μg/ml ascorbic acid (Sigma Aldrich, USA), 100 nM dexamethasone (Sigma Aldrich, USA), 10 mM β-glycerophosphate (Sigma Aldrich, USA), and 100 IU/ml penicillin/streptomycin. The cells were fed every third day and maintained in culture for 3 weeks. Mineral deposition was visualized by Alizarin Red (Sigma Aldrich, USA) staining for calcium.

For adipogenic differentiation, cells were induced in a DMEM-high glucose medium (Invitrogen, USA) with 100 nM dexamethasone (Sigma Aldrich, USA), 10 μg/ml insulin (Sigma Aldrich, USA), 0.2 mM indomethacin (Sigma Aldrich, USA), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma Aldrich, USA), 10% FBS, and 100 IU/ml penicillin/streptomycin and maintained in culture for 3 weeks. Adipogenic differentiation was analyzed by Oil-Red O (Sigma Aldrich, USA) staining.

For chondrogenic differentiation, cells were cultured in media containing serum free DMEM-high glucose, insulin-transferrinselenious (ITS) acid mix (BD Biosciences, USA), 50 mg/ml L-ascorbic acid 2-phosphate (Sigma Aldrich, USA), 1 mM sodium pyruvate, 0.1 mM dexamethasone (Sigma Aldrich, USA), and 10 ng/ml transforming growth factor β1 (TGFβ1) (Cell Signaling Technology, USA). Medium was changed every 4 days for 3 weeks and then fixed in 4% paraformaldehyde for 15 min, monolayer cells were stained for sulfated proteoglycans with 1% Alcian Blue.

For podocytic differentiation, cells were passed onto dishes that coated with type I collagen (0.1 mg/ml) and incubated in RPMI1640 media containing 5% FBS, 100 IU/ml penicillin/streptomycin, 2 mM glutamine and 10 μM all-trans RA for one week. After immunofluorescence staining for podocin and synaptopodin, the cells were examined via Zeiss LSM 710 confocal microscope (Zeiss, German).

2.8. Reverse transcription and quantitative PCR

The total RNA was extracted from kidney tissue and cells using RNeasy Mini Kits according to the manufacturer's instructions (Qiagen, USA). One microgram of RNA was reverse transcribed using the RevertAid First Strand cDNA kit (Fermentas, USA). Quantitative PCR were performed as described elsewhere [24]. The primer details are shown in Table 1.

2.9. Acute kidney ischemia-reperfusion injury and cells transplantation

To evaluate the recovery of renal function of Nestin$^+$ cells in vivo, Nestin$^+$ cells were washed with PBS and stained with the red fluorescent dye CM-DiI according to the manufacturer's instructions. Seventy-eight C57BL/6 mice ($n = 5$ for each time point in control mice; $n = 8$ for each time point in cell or conditioned media