Generation of Mesenchymal-Like Stem Cells From Urine in Pediatric Patients

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ABSTRACT

Objectives. Mesenchymal stem cells (MSCs) have been widely used for regenerative medicine. Traditionally, the procedures of MSC isolation are usually invasive and time-consuming. Urine is merely a body waste, and recent studies have suggested that urine represents an alternative source of stem cells. We, therefore, determined whether the possibility of isolating mesenchymal-like stem cells was practical from human urine.

Methods. A total of 16 urine samples were collected from pediatric patients. Urine-derived cells were isolated, expanded, and identified for specific cell surface markers using flow cytometry. Cell morphology was observed by microscopy. Osteogenic and adipogenic differentiation potential were determinded by culturing cells in specific induction medium, and assessed by alkaline phosphatase and oil red O stainings, respectively.

Results. Clones were established and passaged successfully from primary cultures of urine cells. Cultured urine-derived cells at passage 3 were fusiform and arranged with certain directionality.

Urine-derived cells at passage 5 displayed expressions of cell surface markers (CD29, CD105, CD166, CD90, and CD13). There was no expression of the general hematopoietic cell markers (CD45, CD34, and HLA-DR). Under in vitro induction conditions, urine-derived cells at passage 5 were able to differentiate into osteoblasts, but not adipocytes.

Conclusions. Urine may be a noninvasive source for mesenchymal-like stem cells. These cells could potentially provide a new source of autologous stem cells for regenerative medicine and cell therapy.

MESENCHYMAL STEM CELLS (MSCS) have been widely used for regenerative medicine, and hold the hope for curing various diseases such as Parkinson disease, type 1 diabetes, myocardial infarction, and tissue injuries. Autologous transplantation of MSCs is both convenient and non-immunogenic, and, therefore, autologous MSCs are the ideal cell source for clinical applications. However, the traditional procedures (eg, skin biopsy or blood extraction) of MSC harvest are usually invasive and time-consuming. Recently, emerging studies have suggested that urine represents an alternative source of stem cells, and this noninvasive method eliminates the complications (eg, local tissue bleeding, infection, and discomfort) for patients [1,2].

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Urine-derived stem cells are likely from the urinary tract, and may represent one of the most promising cell sources in urologic tissue engineering. In this study, we attempted to isolate urine cells from pediatric patients, study their biological characteristics, and evaluate their potential for application in further regeneration research and cell therapy.

MATERIALS AND METHODS

Urine Sample Collection

This study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki), and was approved by the Ethics Committee of the Second Affiliated Hospital to Nanchang University (People's Republic of China). Appropriate written informed consent from all participants was obtained to conduct this study. Mid and last stream urine samples were obtained from pediatric patients without urinary system diseases. Urine samples (100–150 mL each) were collected into sterile bottles of proper volume.

Primary Culture and Cell Expansion

We fully adopted the method of Zhou et al [3] to isolate and culture the urine cells. Briefly, urine samples were centrifuged, and cell pellets were washed with phosphate-buffered saline (containing 100 U/mL penicillin [Hyclone], 100 μg/mL streptomycin [Hyclone], and 500 ng/mL amphotericin B [Sigma]). Cells were counted using a hemocytometer and viability was determined using trypan blue exclusion. The remaining cells were seeded into 12-well tissue culture plates (precoated beforehand with 0.1% gelatin) with the primary mixed medium (composed of high-glucose Dulbecco's Modified Eagle Medium (DMEM)/Ham's F-12 mixture [Hyclone], 10% fetal bovine serum [Invitrogen], 100 U/mL penicillin, 100 μg/mL streptomycin, 2.5 μg/mL amphotericin B, and Renal Epithelial Cell Growth Medium SingleQuot kit supplement [Lonza]). After 4 days of cell growth, half of the primary medium was replaced with the mesenchymal cell expansion medium (composed of high-glucose DMEM medium, 10% fetal bovine serum, 1% glutamine, 1% non-essential amino acid solution [Invitrogen], 100 U/mL penicillin, 100 μg/mL streptomycin, 5 ng/mL recombinant human basic fibroblast growth factor [Peprotech], 5 ng/mL recombinant human platelet-derived growth factor-AB [Peprotech], and 5 ng/mL recombinant human epidermal growth factor [Peprotech]). Thereafter, the medium was exchanged every 2 days. Cell morphology was observed by microscopy. When cell confluence of 80%–90% was reached, cells were replated in 6-well tissue culture plates for further expansion.

Phenotypic Analysis of Urine Cells

Cultured urine-derived cells at passage 5 were harvested and then stained with the specific anti-human antibodies labeled for CD34-FITC, CD166-PE, CD54-FITC, CD105-PE, CD90-FITC, CD13-PE, HLA-DR-FITC, and CD45-PE (BD Pharmingen). Immunoglobulin (Ig)G-FITC and IgG-PE conjugated isotype control antibodies were used to determine background fluorescence. After staining, the cells were analyzed using a FACSCalibur analytical fluorescence-activated cell sorter.

Differentiation Potency Assay of Urine Cells

Cultured urine-derived cells at passage 5 were harvested and then replated in 24-well tissue plates at 5×10^3 cells/cm^2. When cells reached 70%–80% density, the medium was changed to osteoinductive and adipoinductive media (Invitrogen). Subsequently, the medium was exchanged once every 3 days. Osteoinductive differentiation was assayed using alkaline phosphatase staining at day 14, and adipoinductive differentiation was assayed using oil red O staining at day 21.

RESULTS

Isolation and Expansion of Urine Cells in Vitro

Urine samples were collected from 16 pediatric patients (10 males and 6 females). The age of these patients was ranged from 6 months to 4 years. There was no bacterial contamination in any culture samples. Most of the urine cells were terminally differentiated and could be removed when the culture medium was changed. Only a few urine cells could attach to the culture plates, but they exhibited clonal expansion showing 3 to 7 colonies per dish between days 7 and 9, with a cobblestone-like morphology (Fig 1A). Cells reached more than 90% confluence between days 12 and 15 (Fig 1B) and could be passaged. Cultured urine-derived cells at passage 3 were fusiform and arranged with certain directionality.

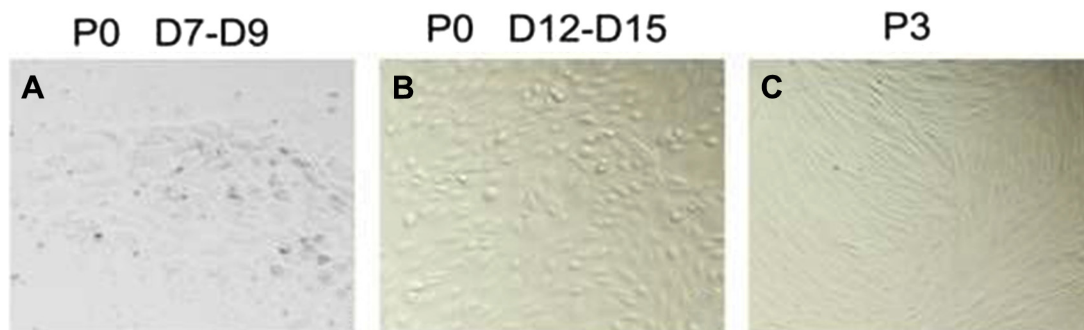

Fig 1. Morphological characteristics of urine cells. (**A**) Representative characteristics of urine cells at passage 1 between days 7 and 9: urine cells exhibiting clonal expansion showed 3–7 colonies per dish with a cobblestone-like morphology; (**B**) representative characteristics of urine cells at passage 1 between days 12 and 15: cells reached more than 90% confluence; and (**C**) representative characteristics of urine cells at passage 3: cells were fusiform and aligned in a certain direction. When cells reached more than 90% confluence, they were arranged in a spiral shape. Abbreviations: D, day; P0, passage 0; P3, passage 3.

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