Potency of Human Urine-Derived Stem Cells for Renal Lineage Differentiation

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Abstract Kidney is one of the most difficult organs for regeneration. Several attempts have been performed to regenerate renal tissue using stem cells, the results were not satisfactory. Urine is major product of kidney and contains cells from renal components. Moreover, urine-derived stem cells (USCs) can be easily obtained without any health risks throughout a patient's entire life. Here, we evaluated the utility of USCs for renal tissue regeneration. In this study, the ability of USCs to differentiate into renal lineage cells was compared with that of adipose tissue-derived stem cells (ADSCs) and amniotic fluid-derived stem cells (AFSCs), with respect to surface antigen expression, morphology, immunocytochemistry, renal lineage gene expression, secreted factors, immunomodulatory marker expression, *in vivo* safety, and renal differentiation potency. Undifferentiated USCs were positive for CD44 and CD73, negative for CD34 and CD45, and formed aggregates after 3 weeks of renal differentiation. Undifferentiated USCs showed high SSEA4 expression, while renal-differentiated cells expressed PAX2, WT1, and CADHERIN 6. In the stem/renal lineage-associated gene analysis, *OCT4*, *SSEA4*, and *CD117* were significantly downregulated over time, while *PAX2*, *LIM1*, *PDGFRA*, *E-CADHERIN*, *CD24*, *ACTB*, *AQP1*, *OCN*, and *NPISH* were gradually upregulated. In the *in vivo* safety evaluation, renal-differentiated USCs did not show abnormal histology. These findings demonstrated that USCs have a similar MSC potency, renal lineage-differentiation ability, immunomodulatory effects, and *in vivo* safety as ADSCs and AFSCs, and showed higher levels of growth factor secretion for paracrine effects. Therefore, urine and USCs can be one of good cell sources for kidney regeneration.

Keywords Urine-derived stem cells · Kidney · Regeneration · Amniotic fluid-derived stem cells

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1 Introduction

The damaged nephron has a limited capacity to restore activity through the regeneration of missing cells by their surviving neighbors. Stem/progenitor cells have been described in many organs as a multipotent population adjacent to endothelial cells in the microvasculature and demonstrate clonogenic, self-renewing ability and can give rise to terminally differentiated cells of the original tissue. However, renal stem/progenitor population disappears in the adult kidney, possibly due to the loss of its niche. Although recent study showed the presence of renal stem/progenitor cells in adult rat kidney, the mechanism of repairing process by renal stem/progenitor cell in human kidney still remains poorly understood [1]. Therefore, many patients with end-stage renal disease (ESRD) must continue renal replacement therapy, such as peritoneal dialysis or hemodialysis, for the rest of their life [2]. Moreover, current renal replacement therapies are not curative, as they only delay disease progression and prolong patient survival. Although kidney transplantation is a viable therapy for ESRD, most patients face very long waiting times because of the shortage of kidney donors. Therefore, effective methods for renal regeneration are greatly needed.

Recently, stem cell-based therapy has been proposed as a promising option for renal tissue repair [2]. Although resident renal cells may contribute to organ repair, such cell populations are very limited in the kidney, and the number of stem cells that migrate from the bone marrow is also not sufficient [1]. For this reason, exogenous stem cells are needed to achieve renal regeneration. Several groups have demonstrated the use of different *ex vivo* expanded mesenchymal stem cells (MSCs) for the treatment of renal failure [3], and MSCs obtained from adipose tissue, umbilical cord blood, amniotic fluid, and dental pulp have been investigated as potential sources for renal tissue regeneration [3–7]. However, there is still no consensus on the best source of stem cells for kidney regeneration.

Adipose tissue-derived stem cells (ADSCs) offer the widest spectrum of applications for cell-based therapies. However, they are obtained by liposuction, which carries a risk of complications both during and after the procedure. Umbilical cord blood-derived stem cells (UCSCs) show a MSC-like potency; however, cells of the hematopoietic lineage are more easily derived from UCSCs, and they can be only acquired at birth [8]. Amniotic fluid-derived stem cells (AFSCs) possess high proliferative capability and have plasticity that is similar to embryonic stem cells; however, it is becoming more difficult to obtain amniotic fluid because of the development of advanced prenatal diagnostic techniques. Dental pulp-derived stem cells (DPSCs) can be

obtained without adverse health effects and express early markers of MSCs [9], however, they can only be acquired from exfoliated deciduous teeth at a certain time of life.

Urine is a stem cell source that can overcome all of the disadvantages mentioned above; Urine-derived stem cells (USCs) can be easily obtained without any health risks throughout a patient's entire life, and they can even be collected daily. USCs from voided urine were first investigated [10]. Subsequently, upper urinary tract-derived urine stem cells were investigated and were reported to be more effective based on their high potency and therapeutic utility [11]. Although USCs are a very useful stem cell source that originates from the urinary tract, they have not yet been applied for renal tissue regeneration.

Stem cells can have two therapeutic effects; the first is direct differentiation into renal cells in injured tissue, and the second is an indirect effect on renal regeneration via secreted factors [12, 13]. Secreted factors can regulate cell survival, proliferation, migration, and differentiation; the immune system; extracellular matrix secretion; and cell–cell interactions around the injured tissue [14].

Here, we evaluated the potency of USCs for renal tissue regeneration by assessing the expression of mesenchymal cell surface markers, the efficiency of renal-lineage differentiation, and their immunoregulatory properties and secreted bioactive molecules, and these characteristic were compared with those of ADSCs and AFSCs.

2 Materials and methods

2.1 Cell preparation

The Ethics Committee of Kyungpook National University Hospital approved this study. The cells used in this study were obtained from the Human Resources Bank of Kyungpook National University Medical Center (Code #BP09.OC310.102). Stock USCs from passage 2 were seeded in 100-mm culture plates with a mixed medium composed of keratinocyte serum-free medium (Invitrogen, Carlsbad, CA, USA), DMEM (high glucose), and DMEM/Hamm's F12 (Thermo Scientific, Rockford, IL, USA) in a 2:1:1 ratio with supplements as previously described [11]. ADSCs from passage 2 were cultivated in α -MEM supplemented with 2 mM L-glutamine, 1% penicillin–streptomycin, and 10% FBS (Invitrogen). AFSCs from passage 2 were cultivated in PRIME-XV[®] AFSC Expansion Medium (Cat. no. 91133; Irvine Scientific, Santa Ana, CA, USA). Human renal stem cells (Cat. no. 36100-27; RSCs; Celprogen, Torrance, CA, USA) were used as an indicator cell line for renal lineage induction and were cultured in human kidney stem cell medium (Cat. no. M36100-27S; Celprogen).

2.2 Analysis of stem cell marker expression

Flow cytometric analysis was performed with phycoerythrin (PE)-conjugated mouse monoclonal antibodies specific for MSC markers (CD44 and CD73), hematopoietic stem cell markers (CD34 and CD45), and an immunologic marker (HLA-DR), which were obtained from BD Biosciences (San Jose, CA, USA) according to the manufacturer's instructions. Approximately 1×10^4 cells were measured using a FACS system (BD Biosciences) equipped with CellQuest software. Antibody information is listed in Table 1.

2.3 Analysis of *in vitro* differentiation into renal cell lineages

Cells were seeded at a density of 1×10^5 cells/well in 24-well plates containing growth medium. At 80% confluence, the medium was replaced with renal lineage induction medium (Cat. no. M36100-27DS; Celprogen). The medium was replaced every 3 days, and the experiments were terminated at 3 weeks. Cell morphology was observed every 3 days.

For immunocytochemical (ICC) analysis, cells were cultured for 3 weeks in 8-well chambered slides (Thermo Scientific, Waltham, MA, USA) for staining with the stem cell marker SSEA-4 and the renal lineage markers Pax2, Wt1, and Cadherin-6. Each week, cells were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.2% Triton X-100 for 10 min, and washed 3 times with PBS. After blocking with 5% bovine serum albumin, the cells were incubated overnight with primary antibodies. After removal of the primary antibodies, cells were washed 3 times with PBS and incubated with the fluorescent-conjugated secondary antibody for 1 h. Cells were washed 3 times with PBS and mounted with medium containing DAPI (4'-6-diamidino-2-phenylindole) to detect nuclei (VectaShield, Vector Labs, Burlingame, CA, USA).

Table 1 Antibodies used for FACS and ICC

Antibody	Company	Dilution
CD44	BD Pharmingen	1:20
CD73	BD Pharmingen	1:20
CD34	BD Pharmingen	1:20
CD45	BD Pharmingen	1:20
HLA-DR	BD Pharmingen	1:20
SSEA4	BD Pharmingen	1:200
PAX2	Abcam	1:1000
WT1	Santa Cruz	1:200
CADHERIN6	Abcam	1:200

Table 2 Sequences of primers used for real-time PCR

Gene	Sequence (5'-3')
OCT4	gga gaa ttt gtt cct gca gtg c aga acc aca ctc gga cca cat c
SSEA4	tcc cag gtt caa gcg att ctc cca aca tgg tga aac gca gtc
CD117	ggc atc atg atc aaa agt gtg aa ccc tcc tgg tcc aca gaa ca
PAX2	cca tta aag cac agc acg tcc gga cca tgt tgc tca ttt tgc
LIM1	ttt gtc tcc gga ttc cca aga tct ggt cgt cat tct cgt tgc
PDGFRA	aaa aag cga agg cgc aat c aat aag ccc ccc ctc aac ct
E-CADHERIN	tgc cca gaa aat gaa aaa gg gtg tat gtg gca atg cgt tc
CD24	atc caa gca tcc tga gca ac ggg cga caa agt gag act gt
ACTB	aaa agc cac ccc act tct ct ctc aag ttg ggg gac aaa aa0
AQP1	tgc act tag cca tga tgg ca tgc aga tgt gat gca ctg gtg
OCLN	aag tgg ttc agg agc ttc ca agt cct cct cca gct cat ca
NPHS1	gct tct gct cct ctc caa tg tct gcc tct gtt gtg ctg ac
GAPDH	aaa tca agt ggg gcg atg ct agc ttc ccg ttc agc tca gg

Total RNA was extracted with TRIzol (Ambion, Carlsbad, CA, USA) according to the manufacturer's instructions. The extracted RNA (2 µg) was used for cDNA synthesis using a DNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The primers were designed with Primer Express Software (Applied Biosystems; primer sequences are listed in Table 2). The assay was performed using the ABI Prism Sequence Detection System 7500 with SYBR Green Polymerase Chain Reaction Master Mix (Applied Biosystems). To analyze the data, the $2^{-\Delta\Delta C_t}$ method of relative quantification was used to estimate copy number. GAPDH was used as an internal control.

2.4 Secreted trophic factor analysis by ELISA

To quantify the growth factors secreted from each renal lineage-induced cell type, culture supernatant was collected for 3 weeks. To measure the protein concentration, the culture supernatant was filtered through centrifugal filters (3-kDa cutoff; Amicon, Billerica, MA, USA), according to the manufacturer's instructions. Then, the

secreted proteins in the supernatant were analyzed by ELISA using VEGF (Quantikine DVE00), bFGF (Quantikine DFB50) and PDGF-BB (Quantikine DBB00) ELISA kits. The optical density was determined using a microplate reader (EL_800; BIO-TEK INSTRUMENTS, Inc., Winooski, VT, USA).

2.5 *In vivo* safety analysis of renal-differentiated MSCs

To evaluate the *in vivo* safety and renal differentiation potency of undifferentiated and differentiated MSCs, cells were implanted into the renal subcapsule of mice. To prepare cells for implantation, 1×10^6 cells of each cell type were centrifuged (1500 rpm, for 5 min) and incubated at room temperature for 30 min. Then, the right kidney of five-week-old male ICR mice (weight, 20 g; Orient Bio Inc., Seongnam, Korea) was exposed through an incision in the back, and the cell pellet was inserted into the renal subcapsule (each group, $n = 5$). Samples were retrieved 4 weeks after implantation. All procedures were performed in accordance with an animal protocol approved by the Yeungnam University Institutional Animal Care and Use Committee (YUMC-AEC2016-003). The histological analysis was performed by an expert pathologist.

2.6 Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD). The statistical significant of differences was determined by *t* test and one-way analysis of variance (ANOVA) with Tukey's post hoc test. Differences were considered significant at a *p* value <0.05 .

3 Results

3.1 Expression of stem cell markers on the cell surface

Flow cytometric analysis showed that most (95% or more) ADSCs, AFSCs, and USCs expressed the MSC markers CD44 and CD73, whereas $<3\%$ of these cells expressed the hematopoietic lineage markers CD34 and CD45 (Fig. 1). In contrast, $<1\%$ of the control renal stem cells expressed these markers.

3.2 Renal lineage differentiation

To investigate the potential of MSCs to differentiate into renal lineage cells, a commercial human kidney differentiation medium was used. In the cell morphology analysis (Fig. 2A), the renal stem cells showed a cobblestone

appearance, and MSCs were generally spindle-like shaped; compared to the ADSCs and AFSCs, USCs were more round shaped. The original morphology of the MSCs gradually changed to a large, round phenotype beginning in week 1 of differentiation, and at this time point, the USCs started to form aggregates, similar to the renal stem cells. Around week 2, the differentiated MSCs were growth arrested and showed cluster-like formations. The cell density in these clusters was highest for the USCs.

To assess the renal lineage differentiation potential of MSCs, renal lineage-specific cell surface markers and genes were assessed by immunocytochemistry (ICC) and real-time PCR, respectively, for 3 weeks. For ICC, the cells were stained with human SSEA4, PAX2, WT1, and CADHERIN-6 antibodies, and examined by fluorescence microscopy. As shown in Fig. 2B–E, expression of the stem cell marker SSEA4 was strong in the stem cell state (on week 0 [0 W]), but diminished in the differentiated phase (on week 1–3). At week 0, PAX2 expression showed a widespread nuclear staining pattern, which gradually coalesced and increased over time, and was highly expressed in the aggregates on week 3. WT1 was expressed in the cytosol, and expression gradually increased over time. CADHERIN-6 was expressed in the cytoplasm in a few cells, and was lower than that in control cells.

In the real-time PCR analysis (Fig. 2F), undifferentiated MSCs expressed *OCT4*, *SSEA4*, and *CD117*, and their expression was downregulated as differentiation proceeded, and at 3 weeks, the differentiated MSCs expressed only baseline levels of these stem cell makers. Conversely, markers of early renal differentiation, such as, *PAX2*, *LIM1*, *PDGFRA*, *E-CADHERIN*, *CD24*, and *ACTB*, were gradually upregulated. In addition, late differentiation markers, such as *AQP1*, *OCN*, and *NPHS1*, showed significant upregulation at week 3 compared to the levels at 1 and 2 weeks. USCs showed expression patterns similar to those of ADSCs and AFSCs.

3.3 Analysis of secreted trophic factors

To determine whether MSCs secrete growth factors during renal lineage differentiation, VEGF, bFGF, and PDGF-bb protein levels were measured using an ELISA kit. Higher levels of these growth factors were secreted at the differentiated phase, and the patterns among the tested MSCs were similar. However, VEGF and PDGF-bb were more strongly expressed by USCs than by ADSCs and AFSCs. The respective levels of these growth factors secreted by USCs in the undifferentiated and differentiated phases were 140 ± 0.50 and 215.75 ± 0.50 pg for VEGF, 0.27 ± 0.23 and 8.22 ± 1.58 pg for bFGF, and 21.37 ± 7.95 and 38.87 ± 3.54 pg for PDGF-bb; Fig. 2G).

Fig. 1 Flow cytometric analysis of cell surface marker expression on mesenchymal stem cells. The values were normalized to the isotype IgG control. Expression of these markers was compared to the levels expressed at week 0 (in the undifferentiated phase before induction of renal-lineage differentiation). For urine-derived stem cells (USCs),

95% or more expressed the mesenchymal stem cell markers CD44 and CD73, whereas <3% expressed the hematopoietic lineage markers CD34 and CD45. *Ctrl* renal stem cells; *ADSC* adipose tissue-derived stem cells; *AFSC* amniotic fluid-derived stem cells; *USC* urine-derived stem cells

3.4 Immunoregulatory properties of USCs

In a FACS analysis of immunoregulatory marker expression, all MSCs showed low expression of HLA-DR, and USCs showed lower expression (1.48%) than ADSCs (2.76%) and AFSCs (2.50%; Fig. 3).

3.5 *In vivo* safety assessment

Renal-lineage differentiated MSCs were implanted into the subcapsule of the kidney. At 4 weeks after implantation, histological analysis showed no abnormal morphology in all implanted kidneys (Fig. 4).

4 Discussion

In this study, we demonstrated that USCs are similar to ADSCs and AFSCs in terms of MSC potency, renal lineage differentiation ability, immunomodulatory effects, and *in vivo* safety, and they showed higher levels of growth factor secretion.

To evaluate the expression of MSC surface markers, we carried out FACS analysis. MSCs are positive for the

representative MSC markers CD29, CD44, CD73, CD90, and CD105, and negative for CD45, CD34, CD14 or CD11b, CD79a, CD19, and HLA-DR [15]. In this study, CD44, CD73, CD34, and CD45 were analyzed to verify the stemness of the ADSCs, AFSCs, and USCs because these markers are included in the minimum criteria for MSC identification [15]. CD44 is a transmembrane glycoprotein that modulates self-renewal, signal transduction, cell–matrix interactions, growth factors, and differentiation [16]. CD44 also induces renal homing of MSCs in the kidney following injury [17, 18]. CD73 is signal transduction molecule in the immune system that mediates cell–cell and cell–matrix interactions [19, 20]. CD34 is a transmembrane phosphoglycoprotein [21], and CD45 is a prototypic receptor-like protein tyrosine phosphatase that regulates signal transduction pathways in immune cells [22], both are considered to be markers for hematopoietic stem and progenitor cells. Like ADSCs and AFSCs, more than 95% of the USCs were positive for CD44 and CD73 antigens, and <1.3% were positive for CD34 and CD45, indicating that USCs are a good source of MSCs.

After evaluating the stemness of the USCs, renal differentiation potency was evaluated. In a morphological analysis, USCs showed a round shape in renal

Fig. 2 Characterization of *in vitro* renal lineage-differentiated cells by morphological, immunocytochemical (ICC), quantitative real-time PCR, and secreted trophic factors analysis for 3 weeks. **A** Representative images from the morphological analysis. The original cell morphology (spindle-like) of the urine-derived stem cells (USCs) gradually changed to a large, round phenotype with a cobble stone-like appearance and cell aggregation. Representative ICC images using SSEA4, Pax2, Wt1, and Cadherin-6 antibodies. **B** In USCs, the stem cell marker SSEA4 was strongly expressed in undifferentiated stem cells, and then diminished in the differentiated phase. **C** Pax2 showed widespread nuclear expression at week 0, and then expression gradually became more localized and increased over time, and it was highly expressed in the cell aggregates at week 3. **D** Wt1 expression in the cytosol gradually increased over time. **E** Cadherin-6 was expressed in the cytoplasm of a few cells. The target proteins are shown in red, and the nucleus was stained with 4,6-diamidino-2-phenylindole (DAPI, blue). **F** Real-time PCR analysis. USCs showed the highest expression of *LIM1*, *CD24*, and *OCLN*. **G** Secreted trophic factor analysis by ELISA. VEGF and PDGF-bb were more strongly expressed in USCs than in ADSCs and AFSCs. The different letters on top of the bars indicate significant differences at $p < 0.05$. *Ctrl* renal stem cells, *ADSC* adipose tissue-derived stem cells, *AFSC* amniotic fluid-derived stem cells, *USC* urine-derived stem cells. (Color figure online)

differentiation medium, which resembled the phenotype of the control kidney stem cells. At around 2 weeks, the USCs formed clusters. However, no kidney tissue-like morphology was observed due to the 2-dimensional nature of the cell culture.

The constituent cells in the clusters were examined by ICC and real-time PCR. To determine the degree and type

of differentiation from stem cells to renal cells, a stem cell marker (SSEA4), and three renal lineage-specific markers (Pax2, Wt1, and Cadherin-6) were assessed by ICC. At 0 weeks, SSEA4 was strongly expressed; then, beginning in week 2, expression significantly diminished. This means that the stem cell characteristics of the MSCs were reduced as differentiation progressed. This phenomenon was

Fig. 2 continued

confirmed by increased expression of the kidney differentiation-related markers Pax2, Wt1, and Cadherin-6. Pax2 is the earliest marker of the intermediate mesoderm, and is detected in renal epithelial precursors [23]. Wt1 is associated with mesenchymal-epithelial transition, induces renal epithelial differentiation, and is a marker for the glomerulus [24, 25]. Cadherin-6 is an indicator of

differentiation into proximal renal tubules. The time-dependent increases in these renal cell markers implies differentiation of the stem cells into multiple renal constituent cells. In particular, Cadherin-6 expression was relatively high in differentiated USCs. This result demonstrated the high differentiation ability of USCs into kidney tubules, because USCs originate from the urinary epithelium [11].

Fig. 2 continued

The ICC results were verified by real-time PCR. The pluripotent and multipotent markers *OCT4*, *SSEA4*, and *CD117* were highly expressed at week 0, and then were gradually downregulated, meaning that USCs have high stem cell potency, and stem cell gene expression decreased as differentiation proceeded, as is observed in other MSC lines. In the renal-lineage differentiation marker analysis,

USCs showed similar potency to AFSCs. Having a renal differentiation potential similar to that of fetal-derived AFSCs is important, because then these high quality autologous USCs can be used for kidney reconstruction. Moreover, USCs had the highest expression levels of *LIM1*, *CD24*, and *OCLN*. Like Pax2, LIM1 is a nephrogenic intermediate mesodermal marker [26]; CD24 is a

Fig. 2 continued

Fig. 3 Flow cytometric analysis of immunomodulatory marker (HLA-DR) expression on MSCs. USCs showed the lowest HLA-DR expression, indicating that USCs do not trigger an immune

reaction. HLA-DR, major histocompatibility complex, class II, DR; ADSC adipose tissue-derived stem cells, AFSC amniotic fluid-derived stem cells; USC urine-derived stem cells

Fig. 4 *In vivo* safety analysis of renal-differentiated cells. Renal lineage-differentiated USCs were implanted into the subcapsule of the kidney. Four weeks later, a histological analysis was performed. No

abnormal morphology was observed in the implanted kidneys. Ctrl renal stem cells, ADSC adipose tissue-derived stem cells, AFSC amniotic fluid-derived stem cells, USC urine-derived stem cells

renal progenitor cell marker [26]; and OCLN is a mature renal epithelial cell marker. These results indicate that USCs can directly and terminally differentiate into several renal cell types.

When renal damage occurs, the first step in repair is preventing the fibrotic response, and the second is stem cell mobilization. The mobilized cells facilitate renal regeneration by differentiating into renal cells or secreting bioactive molecules to restore the tissue architecture [1]. The secreted factors are HGF, EGF, bFGF, VEGF, BMP-7, PEG-2, TGF- β , PDGF-bb, and IGF-1 [27]. Regarding the therapeutic mechanism of stem cell-mediated renal regeneration, some of the induced MSCs directly differentiate into renal cells in the injured tissue, while the remaining cells may have indirect effects on renal regeneration via

secreted factors [28]. The main beneficial effects of induced MSCs may be paracrine effects [29]. ELISA showed that secretion of these growth factors was enhanced in the differentiated phase, and USCs secreted significantly more VEGF and PDGF-bb than ADSCs and AFSCs. Evaluation of the type and concentration of secreted factors is important for elucidating the contribution of stem cells to renal regeneration. VEGF is predominantly expressed in glomerular podocytes, distal tubules, and collecting ducts epithelial cells, and it is an essential mediator of glomerular recovery in proliferative glomerulonephritis [30]. bFGF is expressed when the kidney is damaged [27], and it induces aggregation of metanephrogenic mesenchymal cells and activation of renal cell proliferation [31, 32]. PDGF-bb is upregulated during kidney injury, and it stimulates

mesangial cell proliferation [33]. Based on the activities of the factors secreted by USCs, we can assume that these cells could contribute to renal regeneration through an indirect mechanism.

For successful transplantation of allogeneic stem cells, HLA-DR expression should be low [34]. HLA-DR is an MHC class II cell surface receptor, and it plays a central role in immunity. The low expression HLA-DR levels in USCs indicate that these cells do not trigger an immune reaction. Furthermore, implantation of renal-differentiated USCs into the subcapsule of the kidney did not lead to tumor formation, demonstrating that these cells are safe for *in vivo* use. The *in vivo* safety of undifferentiated USCs was already reported by Chun et al. [11].

In conclusion, USCs are similar to ADSCs and AFSCs in terms of their MSC potency, renal-lineage differentiation ability, immunomodulatory effects, and *in vivo* safety, and they secrete higher levels of growth factors for a potentially stronger paracrine effect. Therefore, USCs are a good option as a source of stem cells for kidney regeneration.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical statement All procedures involving animals were performed in accordance with the ethical standards of our institution and following an animal protocol approved by the Yeungnam University Institutional Animal Care and Use Committee (YUMC-AEC2016-003).

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