# Isolation of adult rat kidney derived stem cells and differentiation into podocyte-like cells

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## SUMMARY

Studies have shown that adult stem cells can be isolated from different organs. However, no agreement has yet been reached on the identification of adult kidney stem cells. In this study, we have shown that the adult rat kidney contains a population of stem cells that are isolatable and, under suitable culture conditions, these stem cells can differentiate into podocyte. Six adult Wistar rats were used in the present study. Their kidneys were chopped and exposed to collagenase I. Then, with passing through 100 µm mesh, single cells in filtered fraction were cultured in a proliferation medium. After 4 passages, the cells were analyzed by flow cytometry, differentiation and gene expression. Morphological analyses revealed that isolated cells are spindle-shaped, and can differentiate into osteocytes and adipocytes. Flow cytometry demonstrated that these cells expressed cell surface markers CD44, CD90, CD133, c-kit, Pax-2, Oct4, sca-1 and vimentin significantly. Also, the high expression of Wt-1 and Wnt-4 genes was seen in these cells. Moreover, expression of synaptopodin and podocalyxin genes showed differentiation of stem cells into podocyte cells. Our findings demonstrate that isolated cells from the kidney of adult rats are mesenchymal stem cells or progenitor cells and can differentiate to podocyte-like cells.

**Key words:** Stem cell – Kidney – Adult rat – Differentiation – Podocyte

## INTRODUCTION

The detection of specific stem cells of different tissues is a main target of recent investigations and can provide a route to remedy of various diseases such as cardiac diseases and neurodegenerative disorders. Moreover, stem cells probably play a key role in regenerative medicine (Bonaventura et al., 2021; Liu et al., 2016). Several studies have shown that stem cells progeny divide rapidly; however, the turnover term of these cells in the skin and bone marrow is relatively slow (Liu et al., 2016). Also, evidence suggests that stem cells are involved in repairing organs and tissues following injury (Trovato et al., 2020; Nourian Dehkordi et al., 2019). Research has characterized adult stem cells and their niches in different organs such as the bone marrow, skin, liver, intestine, gastrointestinal mucosa, brain and prostate, and

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there is reliable evidence of the presence of such cells in the kidney (Liu et al., 2016; Liu et al., 2020). These cells play a role in normal turnover of the noted organs and can be considered a potential source of cells following organ damage. Renal stem cells (RSCs) are found in the adult kidneys of some organisms like the freshwater teleost and the skate. The renal stem cells can play a role in the formation of new nephron following partial nephrectomy (Gupta et al., 2006; Ahmadi et al., 2020). One of the characteristics of renal stem cells is that these cells can differentiate into different renal and non-renal cells. However, it is important to note that all of these cells are not yet able to differentiate into podocytes. This is a key target, because podocyte damage is one of the main causes of kidney disease (Mora et al., 2012).

Studies have used a variety of detection techniques to find stem cells in the adult mammalian kidney (Kitamura et al., 2005). Due to slow cycling feature of stem cells, researchers have used a bromodeoxyuridine-retaining technique to demonstrate that adult kidneys have progenitor cells (Maeshima et al., 2003), and the renal papilla was introduced as a niche for these cells (Oliver et al., 2004). The RSCs can be the major source of healing following injuries (Lin et al., 2005; Andrianova et al., 2019). Thus, they can be the target for designing new therapeutic approaches (Chen et al., 2008). Although studies have shown the presence of stem cells in adult kidneys, the main methods of isolating these cells and studying their features need more development. The present study attempts to test the hypothesis of existence of RSCs in the kidneys of adult rats and their potential for generating podocyte.

## MATERIALS AND METHODS

### Subjects and ethics statement

Six adult female Wistar rats weighting 190-220 g were used in the present study. The rats were maintained in controlled temperature (22 ± 2°C) with humidity 50-55% and under a 12-h light/dark cycle, with ad libitum access to food and water. All procedures performed in this study were approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (AJUMS). Animal care and handling were done in accordance with National Institutes of Health guidelines.

### Isolation of renal stem cells

Rats were anesthetized with intraperitoneal injection of Ketamin (75 mg/kg) and Xylazine (10 mg/kg). Kidneys were harvested under standard aseptic technique. Then the kidneys were washed in PBS containing 1% penicillin/streptomycin (Gibco, USA) and removed perirenal fat and renal capsule. The kidneys were cut into very small pieces and were re-suspended in 0.3% collagenase type I (Sigma, USA) for 20 minutes at 37°C in shaking water bath. After passing through 100 µm mesh to remove undigested chunks, the filtered fraction containing mainly single cells was centrifuged at 1500 rpm for 5 minutes and re-suspended in proliferation medium that consisted of Dulbecco's modified eagle medium: nutrient mixture F-12 (DMEM/F12, Gibco, USA) with 15% fetal bovine serum (FBS, Gibco, USA), 1% penicillin/streptomycin (P/S) and cultured at 37°C in the presence of 5% CO2. The medium was replaced every three days by fresh medium. The cells were detached using trypsin (0.25%)/EDTA (0.1%) solution (Sigma, USA) and passaged when they reached 70-80% confluence. During isolation and cultivation period, the morphology of cells was monitored by light inverted microscope. After 4 passages, the cells were characterized by flow cytometry, and differentiate into adipogenic and osteogenic lineages.

### Flow cytometry evaluations

Flow cytometry evaluations for surface markers of RSCs were performed. Briefly, the cells were incubated with anti-rat antibodies in a dilution of (1:100) against CD44, CD133, CD90, vimentin, c-kit, Oct4, sca-1, $Pax_2$ (as positive markers) and CD45 (as a negative marker) overnight at 4°C in the dark condition. All antibodies were purchased from Abcam (Cambridge, UK). Negative and isotype controls were performed. After cell staining, for each reaction 5000 events were counted by a Dako Galaxy flow cytometer and data were analyzed using FlowJo version 8.8.7 software (Treestar, OR).

**Table 1.** Primer sequences for real-time PCR.

| Gene name | Forward sequence | Reverse sequence |
|---|---|---|
| Wnt-4 | TTGGTCAGAGGGTGAGAGGGA | AGTCCAGGTGTGGTGGTTAGGG |
| WT-1 | GTGACTTCAAGGACTGCGAGAGA | TTCTCTGGTGCATGTTGTGATGG |
| Synaptopodin | CCACAGAGGCACATAATG | GGATACAGAGTAGAATAAGAGG |
| Podocalyxin | ACC GGT CCT TAA TTG GTT CC | CCT TTG GCA GTT AGG AGC TG |
| GAPDH | GGATAGTGAGAGCAAGAGAGAGG | ATGGTATTGGAGAGAAGGGAGGG |

### Gene Expression Analysis

To identify isolated stem cells as peronephrogenic cells, the mRNA expression levels of Wnt4, Wt1 as pronephrogenic markers were analyzed by real-time polymerase chain reaction. Bone marrow-derived stem cells were used as control (BMSCs) because these cells do not express peronephrogenic markers.

### Differentiation of RSCs into adipogenic and osteogenic lineage

Renal stem cells at Passage 4 were evaluated for adipogenic, and osteogenic differentiation potential. Cells were plated at $1 \times 10^6$ cells / ml in 6 well plates under proliferation medium (DMEM/F12 and 15% FBS). After reaching ~80% confluency, medium was replaced with appropriate differentiation medium, either adipogenic or osteogenic medium (2 ml / well). The mediums were replaced with fresh medium every 2 days until 21 days. Adipogenic differentiation medium contained DMEM, FBS (10%), 0.5 µM isobutyl-methylxanthine, 1 µM dexamethasone, 10 µM insulin, and 200 µM indomethacin. Osteogenic differentiation medium contained DMEM, FBS (10%), 0.1 µM dexamethasone, 10 µM β-glycerophosphate, and 50 µM ascorbate phosphate. To assess differentiation, cells were fixed with paraformaldehyde (4%) for 30 mins at room temperature and stained with oil red-O for 15-30 mins at room temperature to detect the production of lipid droplets for adipogenesis or alizarin red for 1 h at room temperature in the dark to detect the presence of a calcium deposition for osteogenesis.

### Differentiation of RSCs to podocyte-like cells

For differentiation of RSCs toward podocyte-like cells, cells were grown to confluence in four-well chamber slides and incubated with a podocyte differentiation medium (PDM) that contained DMEM-F12 with 2.5% FBS, 100 mM nonessential amino acids, 100 mM beta mercaptoethanol with the addition of 10 ng/ml of activin A, 15 ng/ml of BMP7, and 0.1 mM retinoic acid. At 10 days of differentiation, cells were assessed by the specific morphology and the gene expression of synaptopodin and podocalyxin by real-time PCR.

### Real-time reverse transcriptase-polymerase chain reaction analysis

Total RNA was isolated from the cultured stem cells using an RNeasy Mini Kit (Qiagen, Gaithersburg, MD, USA) based on the manufacturer's protocol and quantitated with a Nanodrop (Nanodrop Thermo Scientific S.N:D015). cDNA was synthesized by a QuantiTect Reverse Transcription Kit (Qiagen, Gaithersburg, MD, USA). Real-Time PCR was performed using 2X Master Mix including Syber Green (Biofact, South Korea). The forward and reverse primers used are listed in Table 1. GAPDH was used as an internal standard to normalize gene expression levels.

### Statistical analysis

All experiments were performed in triplicate. Statistical analysis of viability data was performed using SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to analyze the mean values statistically at a statistical significance of $P<0.05$.

## RESULTS

### Cell isolation and their morphology

As a source of renal mesenchymal stem cell, after processing of adult rat kidney, primary cells were cultured in vitro condition from kidney as mentioned above. Briefly, after 6 days of culture,

adherent cells were observed, and their number increased rapidly after 15 days by displaying a spindle shape and process resembling fibroblasts and formed a monolayer of about 80% confluence (Fig. 1).

### Flow cytometry analysis

The immunophenotypes of RSCs were analyzed by flowcytometry. Flow cytometric analysis at passage 4 showed that the expression patterns by immunophenotyping undifferentiated renal stem cells revealed cells positive for CD44, CD90, CD133, c-kit, Pax-2, Oct4, sca-1 and vimentin RSCs markers and CD45 was expressed in a small number of cells (Fig. 2, A and B). CD45 is considered hematopoietic surface markers that were not expected to be expressed much in RSCs as confirmed in the present study**.**

### Characterization of RSCs by Real-time-PCR

The results of real time-PCR analyses showed an increase in mRNA expression of Wt-1 and Wnt-4 in RSCs ($P < 0.05$) when compared with bone-marrow-derived stem cells (Fig. 3).

### Differentiation potential into adipogenic and osteogenic

The certain affirmation of multipotency for each cell is the potency to differentiate into more than one cell type. Cells isolated from all kidneys of adult rats displayed the potential to differentiate into varying degrees, as demonstrated by positive adipogenic and osteogenic staining. Adipogenesis was observed with positive oil red-O staining (Fig. 4A) and osteogenic differentiation was showed with alizarin red staining (Fig. 4B).

### Podocyte Differentiation

By day 10, the cells adopted morphological features characteristic of podocyte. We observed large and arborized cells with cytoplasmic processes (Fig. 5A). Moreover, gene expression for podocyte-specific genes such as synaptopodin and podocalyxin was analyzed by qRT-PCR, and the expression of podocyte markers was detected during differentiation (Fig. 5B). Undifferentiated RSCs did not express detectable levels of synaptopodin and podocalyxin that had been observed in RT-PCR analysis.

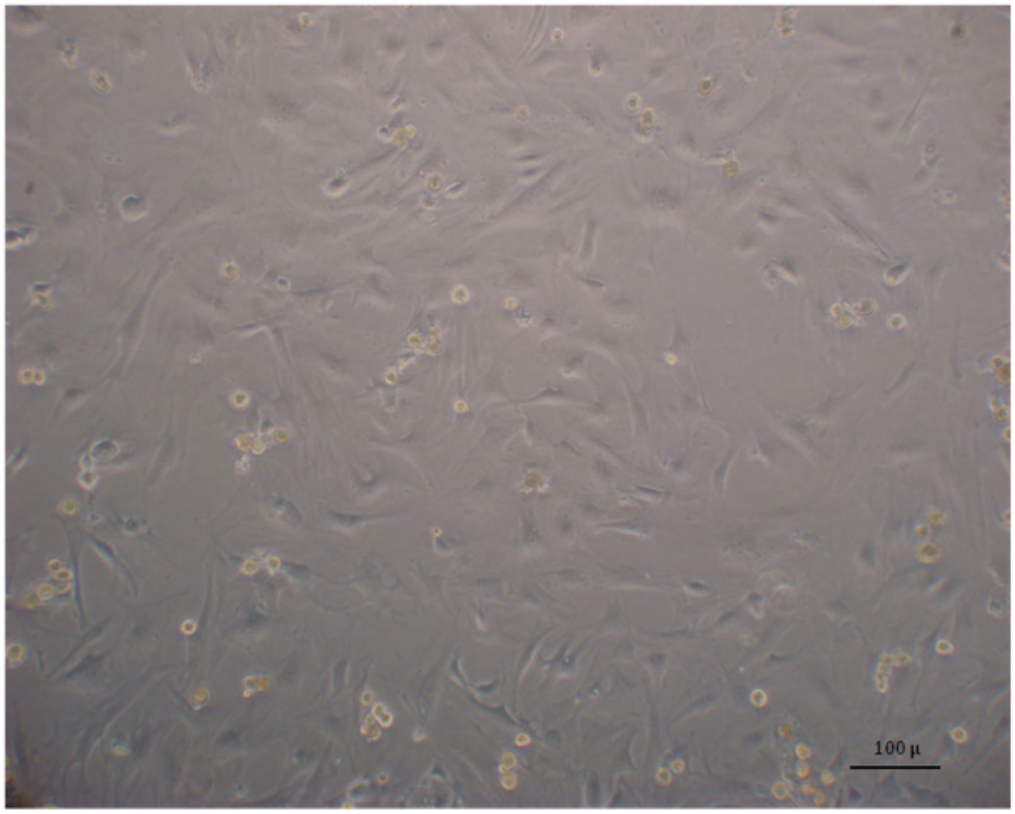


**Fig. 1.-** Characteristics of renal stem cells at passage 4. The cells are monomorphic with a spindle-shaped morphology and slightly similar to fibroblast cells. Scale bar: 100 µm.

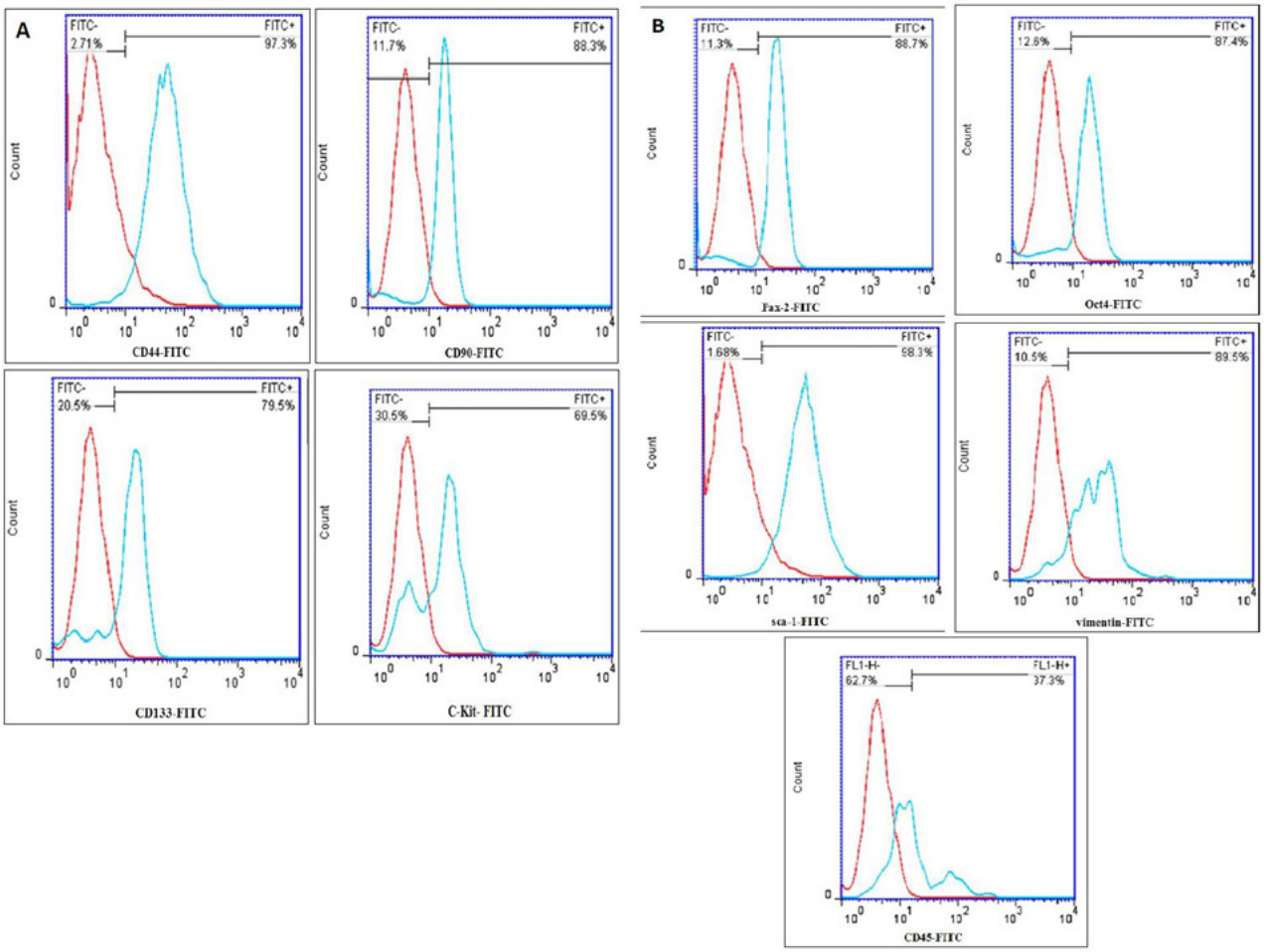


**Fig. 2.- A and B**: Flow cytometry analysis of cell surface markers present on renal stem cells (RSCs).

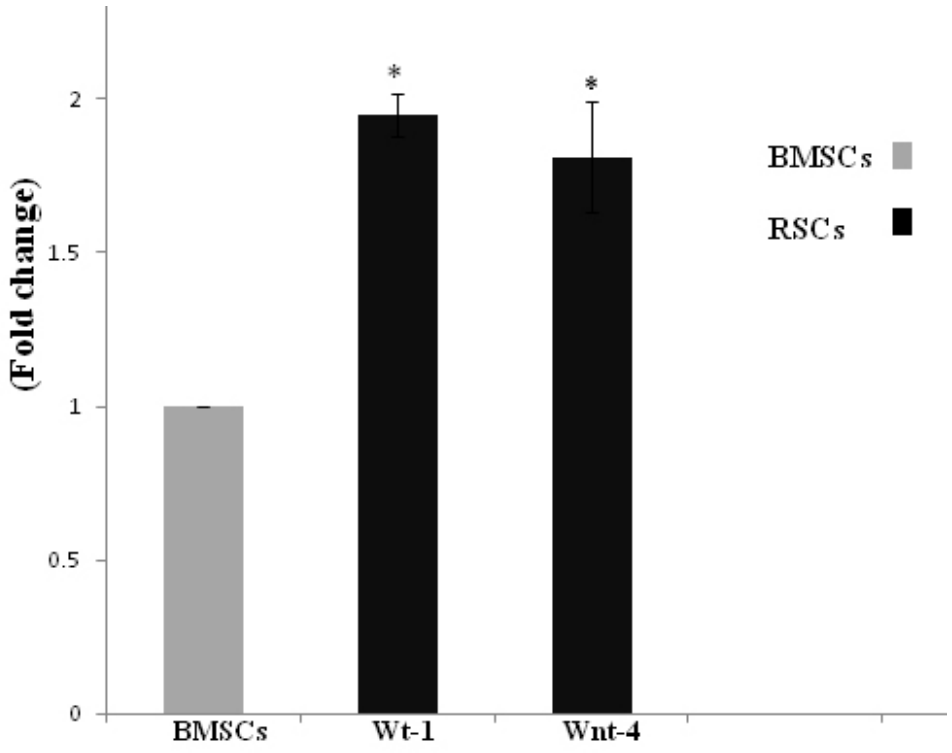


**Fig. 3.-** Real-time PCR analysis for mRNA expression of Wt-1 and Wnt-4 in RSCs (renal stem cells) and BMSMCs (bone marrow stem cells). *$p < 0.05$ compared with BMSCs.

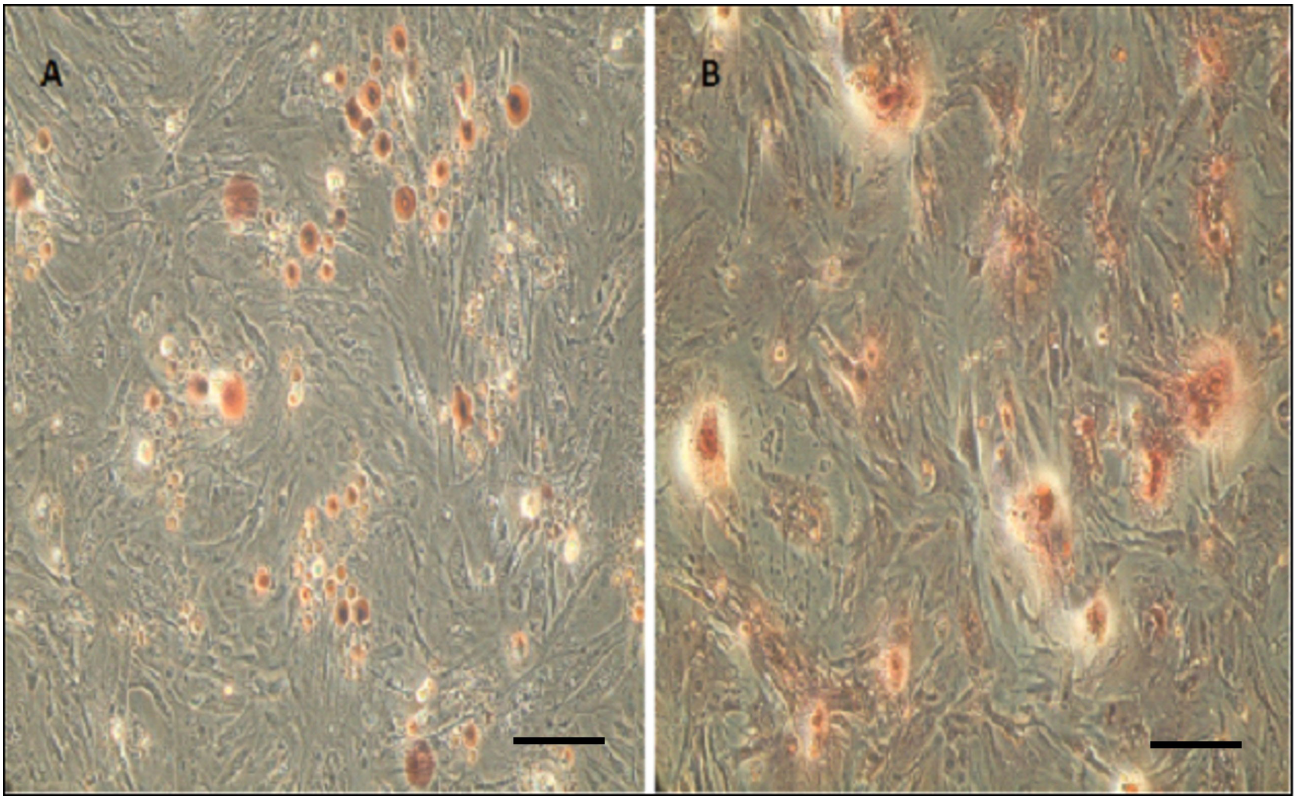


**Fig. 4.-** Photomicrographs representative of the morphological appearance of adipogenic and osteogenic differentiation of RSCs. **A:** adipogenic differentiation after 21 days showing lipid droplets stained with Oil Red; **B:** presence of calcium mineralization after 20 days of induction Alizarin Red staining. Scale bar: 100 µm.

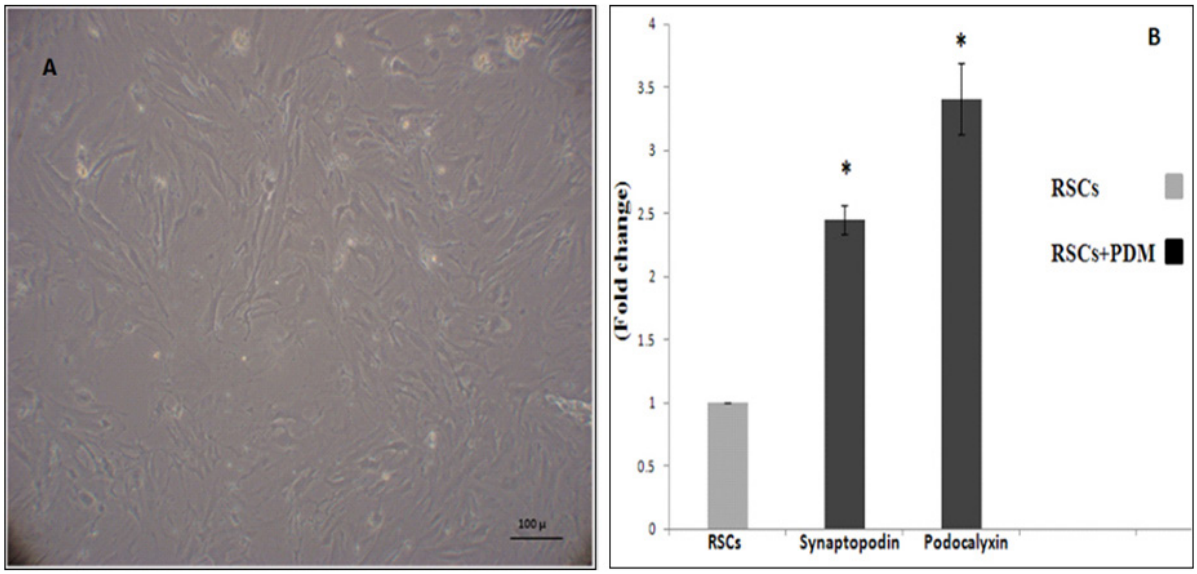


**Fig. 5.- A:** Morphological alterations of RSCs after treatment with podocyte differentiation medium (PDM). At day 10 cells had cytoplasmic extensions with an arborized appearance like podocytes. Scale bar: 100 µm. **B:** Real-Time PCR analysis of podocyte specific genes in cells cultured PDM for 10 days. *$p < 0.05$ compared with RSCs.

## DISCUSSION

One of the most complicated and vital organs in the human body is the kidneys, so that despite notable redundancy built in, the gradual deterioration of the renal tissue might induce life threats. In spite of some species,the adult human body cannot generate new nephrons, but it is able to replace individual lost cells (Diep et al., 2011; Little and Kairath, 2016). However, the regenerative capability is limited, and by aging

or developing disease renal function might drop below critical levels (Huling and Yoo, 2017) . Available treatment choices for chronic kidney disease are limited. Some of the lost functions can be restored through dialysis; still, the underlying problem remains effective. Also, whole organ transplants are very effective; however, there is a low supply of organs and the long-term survival rate is blow 40% (Huling and Yoo, 2017; Levey and Coresh, 2012). Regenerative medicine and tissue engineering try to find more efficient treatment choices through using adult renal stem cells. Stem cells obtained from the adult kidney can improve renal engraftment and differentiation so that they can be used for autologous therapies. Several studies have been conducted to find renal stem cells, yet there is no consensus in this field (Huling and Yoo, 2017). In this study, unique cells were isolated from adult rat kidneys that behave consistently with a renal stem cell. These cells are featured with spindle-shaped morphology and self-renewal. The RSCs demonstrate plasticity through the ability of cells to differentiate into cells of adipocytes and osteocytes. These findings are consistent with other works in this field (Gheisari et al., 2009).

In the present study, we isolated the cells that expressed CD133 and Vimentin significantly. Lindgren et al. noted that CD133-positive cells exist in proximal tubule (Lindgren et al., 2011); and, in a recent study, Sagrinati et al. (2006) mentioned that these cells (CD133-positive) in parietal layer of bowman's capsule are adult kidney stem cells. They utilized Vimentin and CD133 as markers of possible kidney adult stem cells.

Here, RSCs were studied using the stem cell markers Pax-2 and Oct-4, so that the both of them were expressed in these cells significantly. Pax-2 is determined as a main regulator in the development of the kidney (Tayyeb et al., 2017) and a transcription factor expressed by stem cells that are found in the metanephric mesenchyme and other stem cells isolated from adult kidneys (Yamamura et al., 2021; Oliver et al., 2002). The Oct-4 as a transcription factor is found in embryonic stem cells and primordial germ cells in adult gonads. It has a vital role in keeping pluripotency of embryonic stem cells and the viability of primordial germ cells. Recent studies have shown that the cells that express Oct-4 are found in adult organs such as kidneys, and they are potential in vivo markers of adult stem cells (Zhong et al., 2022; Rosenberg and Gupta, 2007).

Studies have shown that expression of c-kit and Sca-1 in cells isolated from adult kidneys might have the potential to serve as renal progenitor or stem cells (Kitamura et al., 2005). Our results also showed that RSCs significantly express markers of c-kit and Sca-1.

The CD44 and CD90 are usual markers of mesenchymal stem cells (L.Ramos et al., 2016). We found that cells isolated from adult rats' kidneys had a notable volume of these markers. According to the study by Gupta et al. (2006), RSCs can be considered mesenchymal-like cells, since they express CD44 and CD90. Our results are consistent with these findings.

Moreover, RSCs expressed CD45 at a lower level, and this marker is one of the markers of endothelial progenitor or hematogenous cells (lee et al., 2010), which reject the chance of extrarenal origin of stem cells isolated in the present study.

Real-time PCR analysis showed that stem cells isolated from adult rats' kidneys expressed mRNA Wt-1 and Wnt-4. Based on the molecular events mediating nephrogenesis hierarchy, Wnt-4 is a key autoregulator of the mesenchymal-epithelial transformation that supports tubulogenesis (Tayyeb et al., 2017; Gallegos et al., 2012; Vainio, 2003). In addition, Wnt-4 mediates tubulogensis in the kidney by nancanonical calcium-Wnt pathway (Tanigawa et al., 2011). The Wt-1 is a key marker of early nephrogenesis (Kreidberg, 2010). According to other study, Wnt-4 and Wt-1 are renal epithelial and mesenchymal markers. A metanephric mesenchyme cell line including embryonic renal stem cells demonstrated expression of epithelial and mesenchymal markers (Kitamura et al., 2005).

In this study, a suitable and specific culture medium (PDM) was used to differentiate stem cells into podocytes. The findings demonstrated that under differentiating conditions, the stem cell isolated from the kidney was able to generate

podocytes. The previous results suggested that, with the appropriate culture medium, stem cells can differentiate into some of renal cells, such as proximal tubule- and podocyte-like cells, as well as non-renal cells, such as osteocytes and adipocytes (Mora et al., 2012). Till now, a number of researches have demonstrated which renal-derived stem cells in vitro can differentiate into podocytes. These reports are usually due to the expression of one or two podocyte markers (Bruno et al., 2009: Ronconi et al., 2009). In appearance, these cells are slightly similar to primary podocytes. Recently, it has been suggested that in order to identify true podocytes in vitro, in addition to expressing specific markers, these cells should show the usual specific appearance characteristics of the podocyte, such as appearance of arborized, high ratio of cytoplasm to nucleus, and obvious cell processes (Shankland et al., 2007). Therefore, according to the contents, the present study results are consistent with previous studies.

## CONCLUSION

This study provides a simplified isolation and characterization procedure for SCs from the adult rat's kidney. Expression of markers in these cells indicated that these cells can be mesenchymal stem cells or progenitor cells that are able to spontaneously differentiate in vitro to cells that have the typical characteristics podocytes. Although the physiological roles of such cells are currently unclear, these cells have the potential to be a valuable resource for the regeneration of kidneys.

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## REFERENCES

AHMADI A, RAD NK, EZZATIZADEH V, MOGHADASALI R (2020) Kidney regeneration: stem cells as a new trend. *Curr Stem Cell Res Ther*, 15(3): 263-283.

ANDRIANOVA N, BUYAN IM, ZOROVA DL, PEVZNER BI, POPKOV AV, BABENKO AV, SILACHEV ND, PLOTNIKOV YE, ZOROV BD (2019) Kidney cells regeneration: dedifferentiation of tubular epithelium, resident stem cells and possible niches for renal progenitors. *Int J Mol Sci,* 20: 6326.

BONAVENTURA G, MUNAFÒ A, BELLANCA CM, LA COGNATA V, IEMMOLO R, ATTAGUILE GA, DI MAURO R, DI BENEDETTO G, CANTARELLA G, BARCELLONA ML, SEBASTIANO CAVALLARO S, BERNARDINI R (2021) Stem cells: innovative therapeutic options for neurodegenerative diseases? *Cell,* 10(8): 1992.

BRUNO S, BUSSOLATI B, GRANGE C, COLLINO F, DI CANTOGNO LV, HERRERA MB, BIANCONE L, TETTA C, SEGOLONI G, CAMUSSI G (2009) Isolation and characterization of resident mesenchymal stem cells in human glomeruli. *Stem Cells Dev,* 18: 867-879.

CHEN J, PARK H-C, ADDABBO F, NI J, PELGER E, LI H, PLOTKIN M, GOLIGORSKY MS (2008) Kidney-derived mesenchymal stem cells contribute to vasculogenesis, angiogenesis and endothelial repair. *Kidney Int,* 74(7): 879-889.

DIEP CQ, MA D, DEO RC, HOLM TM, NAYLOR RW, ARORA N, WINGERT RA, BOLLIG F, DJORDJEVIC G, LICHMAN B, ZHU H, IKENAGA T, ONO F, ENGLERT C, COWAN CA, HUKRIEDE NA, HANDIN RI, DAVIDSON AJ (2011) Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. *Nature,* 470: 95-100.

GALLEGOS TF, KOUZNETSOVA V, KUDLICKA K, SWEENEY DE, BUSH KT, WILLERT K, FARQUHAR MG, NIGAM SK (2012) A protein kinase A and Wnt-dependent network regulating an intermediate stage in epithelial tubulogenesis during kidney development. *Dev Biol,* 364: 11-21.

GHEISARI Y, SOLEIMANI M, ZEINALI S, AREFIAN E, ATASHI A, ZARIF MN (2009) Isolation of stem cells from adult rat kidneys. *Biocell,* 33(1): 33-38.

GUPTA S, VERFAILLIE C, CHMIELEWSKI D, KREN S, EIDMAN K, CONNAIRE J, HEREMANS Y, LUND T, BLACKSTAD M, JIANG Y, LUTTUN A, ROSENBERG ME (2006) Isolation and characterization of kidney-derived stem cells. *J Am Soc Nephrol,* 17(11): 3028-3040.

HULING J, YOO JJ (2017) Comparing adult renal stem cell identification, characterization and applications. *J Biomed Sci,* 24(1): 32.

KITAMURA S, YAMASAKI Y, KINOMURA M, SUGAYA T, SUGIYAMA H, MAESHIMA Y, MAKINO H (2005) Establishment and characterization of renal progenitor like cells from S3 segment of nephron in rat adult kidney. *FASEB J,* 19(13): 1789-1797.

KREIDBERG JA (2010) WT1 and kidney progenitor cells. *Organogenesis*, 6: 61-70.

LEE PT, LIN HH, JIANG ST, LU PJ, CHOU KJ, FANG HC, CHIOU YY, TANG MJ (2010) Mouse kidney progenitor cells accelerate renal regeneration and prolong survival after ischemic injury. *Stem Cells,* 28(3): 573-584.

LEVEY AS, CORESH J (2012) Chronic kidney disease. *Lancet*, 379: 165-180.

LIN F, MORAN A, IGARASHI P (2005) Intrarenal cells, not bone marrow–derived cells, are the major source for regeneration in postischemic kidney. *J Clin Invest,* 115(7): 1756-1764.

LINDGREN D, BOSTRÖM AK, NILSSON K, HANSSON J, SJÖLUND J (2011) Isolation and characterization of progenitor-like cells from human renal proximal tubules. *Am J Pathol,* 178(2): 828-837.

LITTLE MH, KAIRATH P (2016) Regenerative medicine in kidney disease. *Kidney Int,* 90(2): 289-299.

LIU D, CHENG F, PAN S, LIU Z (2020) Stem cells: a potential treatment option for kidney diseases. *Stem Cell Res Ther*, 11: 249.

LIU QZ, CHEN XD, LIU G, GUAN GJ (2016) Identification and isolation of kidney-derived stem cells from transgenic rats with diphtheria toxin-induced kidney damage. *Exp Ther Med,* 12(3): 1651-1656.

MAESHIMA A, YAMASHITA S, NOJIMA Y (2003) Identification of renal progenitor-like tubular cells that participate in the regeneration processes of the kidney. *J Am Soc Nephrol,* 14(12): 3138-3146.

MORA CF, RANGHINI E, BRUNO S, BUSSOLATI B, CAMUSSI G, WILM B, EDGAR D, KENNY SE, MURRAY P (2012) Differentiation of podocyte and proximal tubule-like cells from a mouse kidney derived stem cell line. *Stem Cells Dev,* 21(2): 296-307.

NOURIAN DEHKORDI A, MIRAHMADI BABAHEYDARI F, CHEHELGERDI M, RAEISI DEHKORDI S (2019) Skin tissue engineering: wound healing based on stem-cell-based therapeutic strategies. *Stem Cell Res Ther*, 10: 111.

OLIVER JA, BARASCH J, YANG J, HERZLINGER D, AL-AWQATI Q (2002) Metanephric mesenchyme contains embryonic renal stemcells. *Am J Physiol Renal Physiol,* 283: F799-809.

OLIVER JA, MAAROUF O, CHEEMA FH, MARTENS TP, AL-AWQATI Q (2004) The renal papilla is a niche for adult kidney stem cells. *Am J Physiol Renal Physiol,* 114(6): 795-804.

RAMOS T, SÁNCHEZ-ABARCA LI, MUNTIÓN S, PRECIADO S, PUIG N, LÓPEZ-RUANO G, HERNÁNDEZ-HERNÁNDEZ A, REDONDO A, ORTEGA R, RODRÍGUEZ C, SÁNCHEZ-GUIJO F, DEL CAÑIZO C (2016) MSC surface markers (CD44, CD73, and CD90) can identify human MSC-derived extracellular vesicles by conventional flow cytometry. *Cell Commun Signal,* 14: 2.

RONCONI E, SAGRINATI C, ANGELOTTI ML, LAZZERI E, MAZZINGHI B, BALLERINI L, PARENTE E, BECHERUCCI F, GACCI M, CARINI M, MAGGI E, SERIO M, VANNELLI GB, LASAGNI L, ROMAGNANI S, ROMAGNANI P (2009) Regeneration of glomerular podocytes by human renal progenitors. *J Am Soc Nephrol,* 20: 322-332.

ROSENBERG ME, GUPTA S (2007) Stem cells and the kidney: where do we go from here? *J Am Soc Nephrol,* 18: 3018-3020.

SAGRINATI C, NETTI GS, MAZZINGHI B, LAZZERI E, LIOTTA F, FROSALI F, RONCONI E, MEINI C, GACCI M, SQUECCO R, CARINI M, GESUALDO L, FRANCINI F, MAGGI E, ANNUNZIATO F, LASAGNI L, SERIO M, ROMAGNANI S, ROMAGNANI P (2006) Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. *J Am Soc Nephrol,* 17: 2443-2456.

SHANKLAND SJ, PIPPIN JW, REISER J, MUNDEL P (2007) Podocytes in culture: past, present, and future. *Kidney Int,* 72: 26-36.

TANIGAWA S, WANG H, YANG Y, SHARMA N, TARASOVA N, AJIMA R, YAMAGUCHI TP, RODRIGUEZ LG, PERANTONI AO (2011) Wnt4 induces nephronic tubules in metanephric mesenchyme by a non-canonical mechanism. *Dev Biol,* 352: 58-69.

TAYYEB A, SHAHZAD N, ALI G (2017) Differentiation of mesenchymal stem cells towards nephrogenic lineage and their enhanced resistance to oxygen peroxide-induced oxidative stress. *Iran J Kidney Dis,* 11(4): 271-279.

TROVATO L, NARO F, D'AIUTO F, MORENO F (2020) Promoting tissue repair by micrograft stem cells delivery. *Stem Cells Int*, 2020: 2195318.

VAINIO SJ (2003) Nephrogenesis regulated by Wnt signaling. *J Nephrol,* 16: 279-285.

YAMAMURA Y, FURUICHI K, MURAKAWA Y, HIRABAYASHI S, YOSHIHARA M, SAKO K, KITAJIMA S, TOYAMA T, IWATA Y, SAKAI N, HOSOMICHI K, MURPHY P, TAJIMA A, OKITA K, OSAFUNE K, KANEKO S, WADA T (2021) Identification of candidate PAX2-regulated genes implicated in human kidney development. *Sci Rep*, 11: 9123.

ZHONG C, LIU M, TAO Y, WU X, YANG Y, WANG T, MENG Z, XU H, LIU X (2022) Pou5f1 and nanog are reliable germ cell-specific genes in gonad of a protogynous hermaphroditic fish, orange-spotted grouper (*Epinephelus coioides*). *Genes,*13: 79.