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Isolation and Characterization of CD133 Positive Stem Cell from Human Kidney with Renal Cell Carcinoma

Mohammad Reza Abbasi[1], Saeed Heidari-keshel[2], Reza Zahed[3], Gholamreza Behrouzi[2], Reza Roozafzoon[2], Sara Aghazadeh[2], Leilia Aghajanpour[2], Maryam Bashtar[2] and Ahad Khoshzaban[2,4,5*]

[1]*Nephrology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[2]*Stem Cell Preparation Unit, Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences, Iran*

[3]*Department of Emergency Medicine, Imam Khomeini Hospital Complex, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[4]*BioDental Materials Department, Dental Faculty of Tehran University of Medical Sciences, Tehran, Iran*

[5]*Iranian Tissue Bank Research center, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran*

[*]**Corresponding author:** Ahad Khoshzaban, Stem cell preparation unit, Eye research center, Tehran University of Medical Sciences, 1336616351, Tehran, Iran, Tel: +98-2155400003; E-mail: dr_khoshzaban@spu.ir

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Abstract

Background: Stem cells are one of the rare cell populations in each tissue, whose presence is proven in different types of tissues in the human body. The purpose of this study was to carry out simultaneous investigation into renal stem cells, which are similar to stem cell populations (CD133+) in normal part of the human kidney of a patient afflicted with renal cell carcinoma.

Methods: After kidney dissection, its normal part was divided into papilla, medulla, and cortex. Then, the stem cells of each part were separately isolated. Two cell groups (A and B) were considered. Group A contained the MACS-isolated $CD133^+$ cells and group B consisted of the cells, which had not been sorted by any markers. After enzymatic digestion, all isolated cells in both groups were cultured. The cells of each part were analyzed by flowcytometry, proliferation assay, karyotyping and gene expression.

Results: The results of the present study show that $CD133^+$ cells are renal stem cells and they are found in every three parts of the kidney, but their frequency is higher in papilla. The cells in group B expressed cell surface marker CD44 significantly. The high expression of OCT 3/4, NANOG, SOX2 and SCA-1 genes was seen in both groups, but the expression of REX1 gene in group A was 5 times more than in group B.

Conclusion: It seems that $CD133^+$ cells are the most original cell population found in papilla of human kidney with high frequency. The finding has explored a new horizon towards appropriate selection of stem cells subpopulations ($CD133^+$ cell from healthy part of renal carcinoma) to be used in cellular therapeutic approaches in more defined manner.

Keywords: Human renal stem cells; CD133; Sub-population; REX1

Introduction

Dealing with the role of stem cells in the human body can be viewed as a double-edged sword and we can consider an edge as much as it is useful for treatment that the other edge can be harmful as the major cause of disease incidence [1,2]. Therefore, the current work investigated two aspects of stem cells. Tissue-specific stem cells have been found in many organs, including bone marrow, gastrointestinal mucosa, brain, prostate, and skin [3,4]. These cells play a vital role in regenerating these organs and they are suitable cell sources for repairing the organ after injury [5]. The renal stem cells, which are able to self-renew and differentiate into the different types of kidney cell, are similar to tissue-specific stem cells in other organs [3,6]. The extensive studies on stem cell isolation have been presented. For instance, in 2004, Oliver et al. isolated the stem cells from rat kidney papilla and these stem cells were defined as slow-cycling cells [6]. In 2005, Bussolati et al., isolated the stem cells from adult human kidney using cell surface markers ($CD133^+$). He reported that these cells express epithelial and endothelial markers [7]. In 2006, Sandep also isolated the stem cells from the tubules of rat kidney and he found that they show higher self-renewal and differentiation capacity in comparison with $CD133^+$ cells [8]. Maeshima et al. isolated the stem cells from the tubules of rat kidney and he declared that these cells are mostly found in the proximal tubules. These cells could differentiate into renal cells under in vivo conditions [9]. Kitamura et al., isolated the stem cells with high proliferative potential from the proximal tubules expressing Sca-1, Musahi-1, and the markers, which are expressed during early stages of nephrogenesis. These cells revealed a triploid karyotype, but they did not form tumor in NOD/SCID mice [10]. Cancer stem cells possess characteristics associated with normal stem cells, such as self-renewing and differentiation into the abnormal cells. If the accuracy of the cancer stem cell hypothesis is confirmed that cancer stem cells are created from the mutant stem cells, it can be supposed that the cancer stem cells are able to differentiate into different cell lineages. For example, melanoma stem cells can differentiate into mesenchymal lineages, such as adipocyte, osteoblast,

and chondrocyte. Also, normal stem cells differentiate into luminal, ductal, and myoepithelial lineages in breast cancer [11]. By finding the characteristics of initiator stem cells, valuable information about tumor biology will be obtained, which is important in designing therapeutic approaches. Kidney cancer is one of the rampant urological tumors and 3% of malignant tumors are associated with this disease. This cancer exhibits high metastatic potential and has a higher risk of cancer recurrence. Kidney cancer is resistant to radiotherapy and chemotherapy, so that the only treatment is surgery. Many similarities have been found between normal and cancer stem cells because many molecules, which are expressed in stem cells, are expressed in cancer stem cells of the tissues. In the present study, $CD133^+$ cells were isolated from the normal part of the kidney of a patient who afflicted with kidney cancer. The isolation was performed based on the report presented by Bussolati et al. [7]. They had found that the renal stem cells are $CD133^+$ cells. Although the method applied in this study was based on the method reported by Bussolati and colleagues, some differences were observed, including the specific isolation of $CD133^+$ cells from the papilla, medulla, and cortex separately. The purpose of this study was to carry out simultaneous investigation into renal stem cells, which are similar to stem cell populations in normal part of the human kidney of a patient afflicted with renal cell carcinoma. These two groups underwent flowcytometric analysis for study surface markers of stem cells, reverse transcriptase PCR for the expression of SCA1, Nanog, OCT 3/4, and SOX2 genes, Real-time PCR for the expression of REX1, WNT, and CDX1 genes, karyotyping test, and also differentiation of stem cells into osteoblast and adipocyte. Consequently, we inquired for renal stem cells and the probability for the presence of cancer stem cells and comparing them to each other phenotypically and genotypically in the kidney and also for the probability of the presence of unknown populations.

Materials and Methods

Isolation and culture of renal stem cells

The present study was performed on one 58-year-old patient who suffered from kidney cancer (histopathological types: 23 clear-cell, four papillary, one chromophobe, and two undetermined renal carcinomas). His cancerous kidney was candidate for total resection. After kidney dissection by a surgery team, the kidney was divided into normal and cancerous parts. The cancerous part was completely encapsulated without perforation and invasion into normal part of kidney tissue and pathology analyses were performed on it. In order to transfer the normal part of the kidney to cell culture laboratory in Iranian Tissue Bank (Imam Khomeini Hospital), a specific transfer medium was used. The medium consisted of HBSS, 15 µM HEPES, penicillin, streptomycin, and 0.35 g NAHCO3 [Gibco] at 4°C (pH 7.4). The blood clots and connective tissues were removed from the normal parts as far as possible. Papilla, medulla, and cortex parts were separated from each other under the stereo microscope [Ceti, UK]. Each part (cortex, medulla, and papilla) was placed on three separate plates [Nunc, USA]. PBS, consisting of penicillin, streptomycin, and Amphotericin B [Gibco USA] antibiotics, was added to each plate at ratios of 1x, 2x, and 4x, respectively. Actually, each part was washed with three different ratios of PBS as described above. Each part was separately cut into 2 to 5 mm sections and rinsed twice in 2x PBS. For enzymatic digestion, the tissue sections of each part were added into a 50-ml falcon tube, including 5 mg/mol collagenase BB [Roche, Germany], 0.25% Trypsin/EDTA [Gibco, USA], and 0.05 mg/ml DNase. Then, they were incubated at 150 rpm on the orbital shaker at 37°C for 6 h. The mixture resulting from enzymatic digestion was centrifuged at 2000 rpm and the supernatant was cultured, while the cell deposition on the bottom of the falcon tube was enzymatically digested again. Finally, the liquid, which was obtained from centrifuging and also the cell deposition from the last enzymatic digestion were cultured separately. The substrate [Roche] surface was covered with fibrinonectin and then the isolated cells were cultured on this substrate. The cell culture medium included transferrin, insulin [Sigma, Germany], MCDB-201 [Sigma, Germany], DMEM low glucose, 10-4 M ascorbic acid 2-phosphate, 1000 U/ml selenium [Sigma], 100 U/ml penicillin, 1000 U/ml streptomycin [Gibco], 10 ng/ml EGF [Roche], 10 ng/ml PDGF-BB [Roche Germany], 2% FBS [Gibco USA], and 100 ng/ml LIF (leukemia inhibitory factor). The cells were isolated from the cortex, medulla, and papilla. In the first group (Group A), the isolated cells from each part were separately passed through a MACS column [miltenyi Biotec], including CD133 monoclonal antibody, thereby $CD133^+$ cells were isolated from other cell populations, and then they were cultured. The isolation of cells was performed according to the method of the article written by Minoru Takemoto et al. [7]. In the second group (Group B), the isolated cells from each part were separately direct cultured without selecting any cell surface markers in culture media described above (Figure 1).

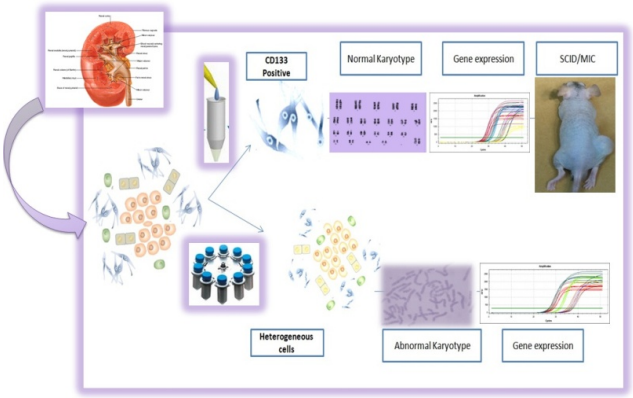

Figure 1: Sample preparation workflow for concurrent analysis of cell separation.

Flowcytometric analysis of renal stem cells

The renal stem cells were first trypsinized and counted. The tubes, containing 105 to 106 cells, were incubated on a rotator shaker for 6 to 10 h, centrifuged at 1000 rpm for 6 min, and 3% human serum was added to cell deposition thereafter. The resultant mixture was placed at room temperature for 30 min. The cells were again centrifuged at 1000 rpm for 6 min and PBS was added to cell deposition. The cell mixture was passed through a nylon mesh and 100 µl of cells was added to each tube with the following antibodies: anti-CD90, anti-CD105, anti-CD166, anti-CD45, anti-CD34, anti-CD133, anti-CD31, and anti-CD44. Next, they were kept at 4°C out of light for 45 min. After washing, the cells were fixed in 100 µl of 10% paraformaldehyde. Finally, flowcytometric analysis [Partec, Germany] was performed by flowing software.

Renal stem cells differentiation into osteoblast and adipocyte

Renal $CD133^+$ cells (passage 3) were used in order to investigate their potential to differentiate into osteoblast and adipocyte cells. For this reason, osteogenic media: DMEM culture medium, consisting of fetal bovine serum [Gibco], 10-7 M dexamethasone, 10 mM β-glycerol phosphate, and 50 μg/mL of ascorbic acid [Sigma], was used. Adipogenic induced media contained DMEM-High glucose, supplemented with 10% FBS, 1% L-glut 1% Pen-Strep, 1 μM dexamethasone, 1 μM indomethacin, 500 μM 3-isobutyl-1-methylxantine (IBMX) and 10 μg/ml human recombinant insulin. The differentiation medium was refreshed twice a week. Twenty-one days after stem cell differentiation, Alizarin red and oil red was used for investigating the induction procedure. Alizarin red staining showed calcium deposition on the surface of osteoblast and oil red to detect lipids in mature adipocytes.

Karyotype analysis of renal stem cells

The cells were first placed in an incubator with 0.1 μg/mL colcemid for 3 to 4 h. Then, the cells were trypsinized and 0.075 M KCl solution was added to them and again they were incubated at 37°C and 5% CO_2 for 20 min. Next, methanol and acetic acid at a ratio of 3:1 (vol/vol) were added for fixing the samples. The cells were dropped on and scattered over the slide surface, and then karyotype analysis was performed on chromosomes.

Proliferation assay by MTT test

A total of 5×10^3 cells (CD133 sorted renal stem cell, unsorted renal cell) in 200 μl cell culture medium were seeded on 96-well cell culture plates. After 12 h, 24 h, 48 h and 72 h incubation, proliferation was assessed with the aid of a MTT, 10 μl of the MTT reagent at the final concentration of 0.5 mg/ml was added to each well and incubated for 4 h in a humidified atmosphere with 5% CO_2 at 37°C. Then, 100 μl of DMSO (sigma, Germany) was added into each well and kept overnight in incubator (Memert, Germany) in a humidified atmosphere with 5% CO_2 at 37°C. Absorbance was measured at 50 nm and 620 nm by pleat reader (biophil, china).

Analysis of Renal Stem Cells Differentiation under *In Vivo* Conditions

All mice, which were used in this study, were monitored by Tehran University of Medical Sciences and they were prepared from experimental models of human cancer in Imam Khomeini Hospital; moreover, animal care and experiments were in accordance with Tehran University of medical sciences animal ethics committee. $CD133^+$ stem cells in the kidney, which had undergone 3 passages, were used for differentiation potential in NOD/SCID mice. Eight hundred cells per gram of body weight of NOD/SCID mice were subcutaneously injected with fibrin matrix in the peritoneal area.

Reverse transcriptase PCR

RT-PCR was performed in thermocycler [Biorad] in a 25 μl volume using 1 μg mRNA of renal cells for each reaction and RT-GO [Metabion] according to the kit protocol. After cDNA synthesis (according to transcription Metabion kit protocol), which is a template for performing PCR, the reaction mixture was subjected to 30 cycles of denaturation (94°C, 40 sec), annealing (60°C, 30 sec), extension (72°C, 45 sec) followed by 1 cycle of 72°C for 5 min in order to complete the single-stranded template. Moreover, GAPDH and water were considered as the positive and negative controls, respectively. The sequences of primers used for PCR amplification were as follows in Table 1 for osteopontin, osteonectin, alkaline phosphates, Osteocalsin and GAPDH.

After loading, PCR products and DNA ladder were analyzed by electrophoresis in 2% agarose gel and after staining with ethidium bromide; they were evaluated using UVItec gel documentation system.

Real-time PCR

Complementary DNA (cDNA) was synthesized by reverse transcription kit [Invitrogen] and oligo-p(dT) primer (CDX1, REX1, OCT3/4, NANOG, SCA-1, SOX2, and GAPDH) according to the company protocols (Table 1). Then, RT-PCR was performed using cDNA as a template and the primers dasigned with primer design software [Oligo]. The reactions were performed in a volume of 15 μl using 0.25 μg of cDNA and platinum SYBR Green [Invitrogen]. After activating the reaction mixture at 95°C for 15 min, PCR was performed for 45 cycles, consisting of 94°C denaturation for 30 sec, 62°C annealing for 30 sec, 72°C extension for 45 sec, and finally 72°C for 5 min for complete extension.

Gene Name	Sequences 3?5	Tm
Oct3/4	Forward: 5' -CTGAGGGCCAGGCAGGAGCACGAG-3' Reverse: 5' -CTGTAGGGAGGGCTTCGGGCACTT-3'	59
Nanog:	Forward: 5' -TTCCTTCCTCCATGGATCTG-3' Reverse: 5' -TCTGCTGGAGGCTGAGGTAT-3'	61
Sca-1:	Forward: 5' -ACAGCCTGAGCAAGACCTGT-3' Reverse: 5' -ATCATCCACCCCAGAAGTGA-3'	60
Sox2:	Forward: 5' -GGTTACCTCTTCCTCCCACTCCAG-3' Reverse: 5' -TCACATGTGCGACAGGGGCAG-3'	60
GAPDH:	Forward: 5' -CCG CAT CTT CTT GTG CAG TG-3' Reverse: 5' -CTG TGG TCA TGA GCC CTT CC-3'	59
Cdx4:	Forward: 5' -CGTGTGGTCTACACAGATCATCAA-3' Reverse: 5' -GGCTCTGCGATTCTGAAACC-3'	62
Rex1:	Forward: 5' -ACGAGTGGCAGTTTCTTCTTGGGA-3' Reverse: 5' -TATGACTCACTTCCAGGGGGCACT-3'	61
Osteopontin	Forward: 5'- TTTTCTGGATCCTCCATTGC-3' Reverse: 5'-CAAAAGCCATATGCTGCTCA-3'	60
Osteonectin	Forward: 5'- GATGGTGCAGAGGAAACCGA-3' Reverse: 5' TTTGCAAGGCCCGATGTAGT-3'	61
Osteocalsin	Forward: 5'- GACTGTGACGAGTTGGCTGA-3' Reverse: 5'-CTGGAGAGGAGCAGAACTGG-3'	59
Alkaline phosphates	Forward: 5'-CCCAAAGGCTTCTTCTTG-3' Reverse: 5'- CTGGTAGTTGTTGTG AGCAT-3'	61

Table 1: List of primers for PCR and Real time PCR.

The evaluation of reaction product and the melting curve analysis (MCA)-based semi-quantitative real time PCR [Corbett, Qiagen] were performed and GAPDH was utilized as a positive control. All reactions

were done duplicate and the obtained average was calculated by MS Excel.

Results

In the present study, papilla, medulla, and cortex were separated for isolating their stem cells. The isolated cells were passed through a column, containing monoclonal antibody against CD133; thereby $CD133^+$ cells were isolated from other cell populations. Based on the report presented by Bussolati et al. $CD133^+$ cells in the human kidney are renal stem cells [2] and our results show that, the papilla, medulla, and cortex have $CD133^+$ cells. The frequency of $CD133^+$ within the papilla was higher than the medulla and cortex but papilla CD133 positive stem cells were much smaller in terms of size. In addition to the above mentioned method in which the digested cells by collagenase had been selected by $CD133^+$ markers, the obtained cell population was directly cultured without selecting any markers. In group A, the cells were selected based on CD133 markers (Figure 2).

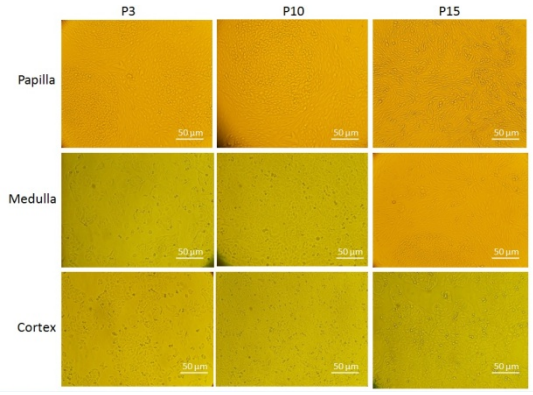

Figure 2: Morphology of CD133 positive renal stem cell at passage 3, 10, 15 by inverted microscope. Scale bar 50 µm.

In group B, the cells were cultured without selecting $CD133^+$ cell surface markers (Figure 3). The proliferation rates (by MTT assay) in group B was much more than group A (Figure 4). In long-term cultured the cortex and medulla CD133 positive cells have been vanishing, so that the duration of each passage, the cell cortex and medulla was 10 days, whereas papilla CD133 positive cells were passage every 4 days required (Figure 4A).

Flowcytometric Analysis

After several passages, flowcytometric analysis was performed on both cell populations in order to express stem cell markers. The markers were as follows: CD133, CD31, CD45, CD34, CD90, CD105, CD44, and CD166. For $CD133^+$ cells in group A, the expression of surface markers, such as CD44, CD105, CD133 and CD90 were positive, but CD34, CD166, CD13, CD31, and CD45 were negative (Figure 5). In group B, for the cells resulting from direct culture, the results of flowcytometric analysis were as follows: CD44, CD166, and CD90 were positive, but CD34, CD133, CD105, CD31 and CD45 were negative (Figure 6). In spite of stem cell markers, the cells in group B showed different patterns of CD markers in comparison with the cells in group A. This represented the presence of a cell population being different from group A. It should be mentioned that CD44 expression was very high in group B and this probably indicates the presence of cancer stem cells.

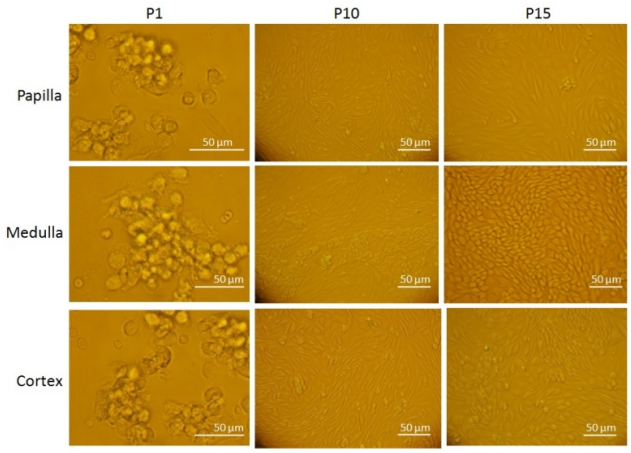

Figure 3: Morphology of un-sorted renal cell at passage 3, 10, 15 by inverted microscope. Scale bar 50 µm.

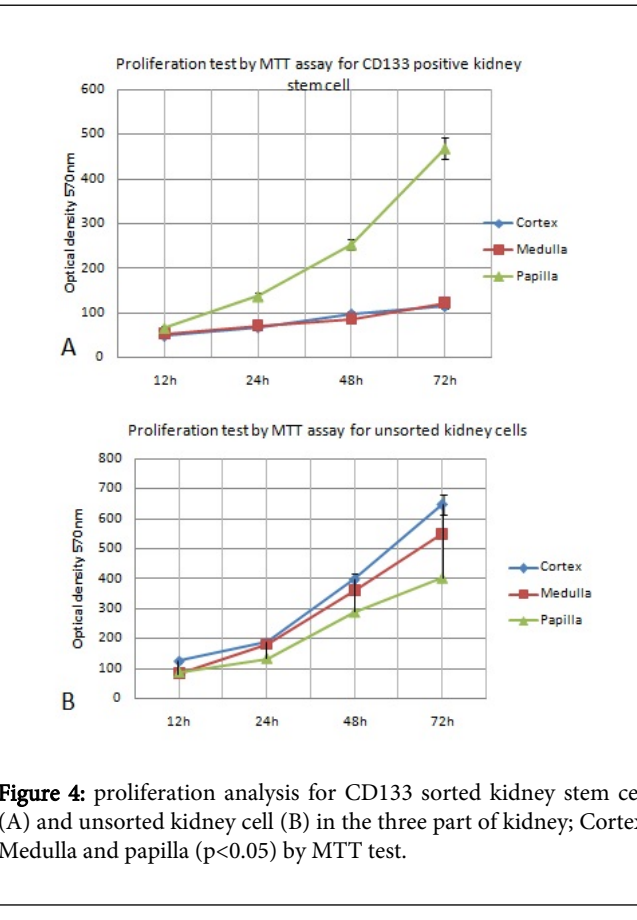

Figure 4: proliferation analysis for CD133 sorted kidney stem cell (A) and unsorted kidney cell (B) in the three part of kidney; Cortex, Medulla and papilla ($p<0.05$) by MTT test.

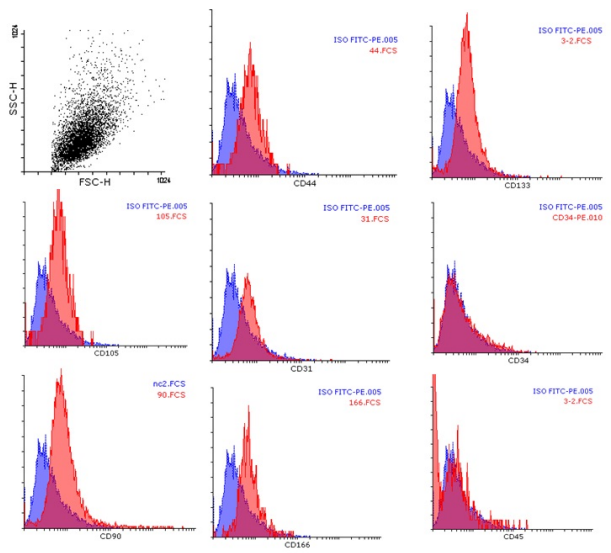

Figure 5: Flowcytometry analysis for stem cell surface marker in CD133 positive cell (group A), blue color indicates the negative area of diagram and red color show that positive area. The results show that, CD133, CD44, CD105, CD90, CD166 were positive and CD45, CD34, CD31 were negative.

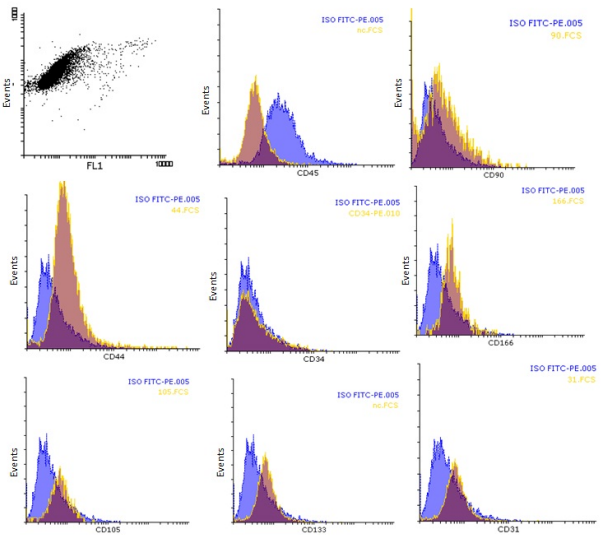

Figure 6: Flowcytometry analysis for stem cell surface marker in un-sorted cell (group B), blue color indicates the negative area of diagram and brown color show that positive area. The results show that, CD44, CD90, CD166 were positive and CD45, CD34, CD31, CD105, CD133 were negative.

Karyotype Analysis

Karyotype analysis was performed on A and B groups for chromosome assay. These cells were in passage 2. In group A, $CD133^{+}$ cells had normal karyotype (46 XY). After 5 passages, the karyotype of renal cells was maintained (Figure 7A). In group B, karyotype analysis was also performed on cells and chromosome abnormality; duplication on chromosome 16 (q23.1) was seen in them (Figure 7B). The results of karyotype analysis represented that the cells in group A were normal stem cells and the cells in group B showed abnormal form.

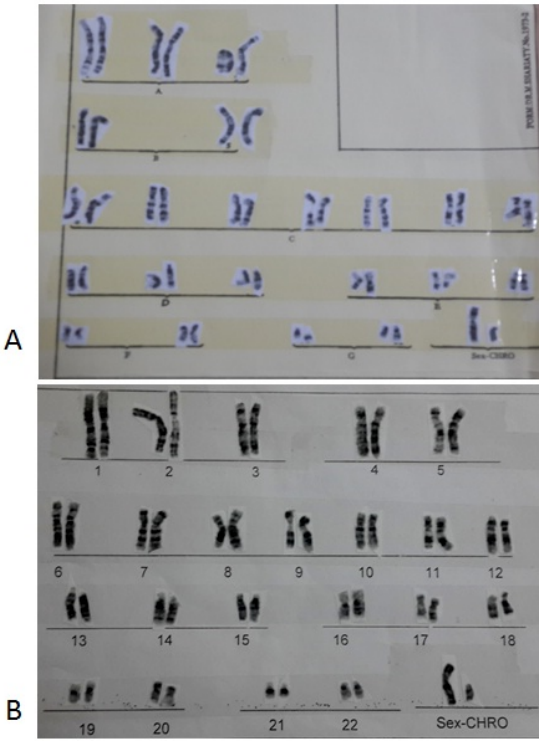

Figure 7: karyotyping ananlysis show those CD133 positive cells were 46XY (A) and karyotype analysis was also performed on unsorted kidney stem cell and chromosome abnormality; duplication on chromosome 16 (q23.1)

Reverse Transcription PCR

The analysis of mRNA expression, which was performed by reverse transcription PCR for Osteopontin, Osteonectin, Osteocalsin and alkaline phosphates genes, indicated that after induction for Osteogenesis media in group A and B, every four genes had been significantly expressed in these cells (Figure 8).

Real Time PCR

In quantitative analysis of mRNA by real time PCR, the quantitative expression of Rex1, WNT, OCT4, SOX2, SCA1, NANOG and CDX1 was investigated and it was found that the expression rate of Wnt and Cdx1 was increased in two groups. This implies the existence of stem cells in both groups, but a decrease in Rex1 expression in group B indicated the existence of cancer stem cells in this group. The expression rate of Rex1 in group A was five times more than group B and in this reaction, GAPDH was considered as a positive control (Figure 9).

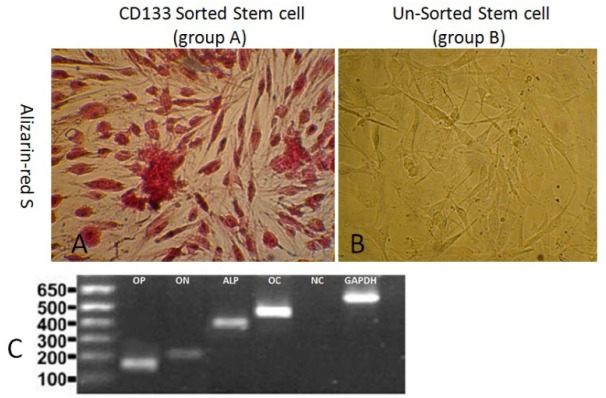

Figure 8: The result of RT-PCR to confirm the isolated stem cells (group A and B): group A can be differentiation to osteoblast cell and alizarin red staining confirms these results but not B group. The genes Osteopontin, Osteonectin, Osteocalsin and alkaline phosphates, columns 6 and 7 are D.D.W and GAPDH considered as the negative and positive controls, respectively.

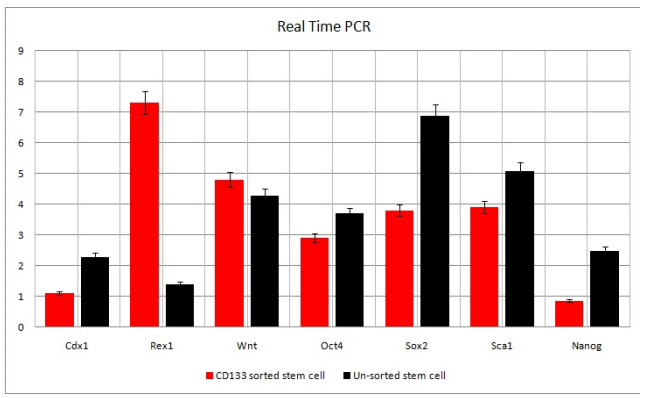

Figure 9: Genes expression results for Rex1, WNT, OCT4, SOX2, SCA1, NANOG and CDX1 by Real time PCR ($p<0.05$).

Discussion

Identification of specific cells population within an organ is an important chore further to explore their potential in tissue defense and organ regeneration. Various methodologies have been developed since last few years but passage various limitations such as heterogeneity and scarcity in getting homogeneous cells population. The results of the present study are based on the presence of stem cells in adult organs. Although the presence of stem cells in the kidney was indicated by some other researchers, in the present study, we aimed at the isolation of stem cells from the cortex, medulla, and papilla of the kidney. In addition, their frequency and characteristics were analyzed. Renal stem cells in the adult kidney, which are able to self-renew and differentiate into the different types of kidney cells, are similar to tissue-specific stem cells in other organs, such as skin, brain, intestine, and stomach. Therefore, further studies into the isolation of stem cells from the kidney have been carried out. For instance, Oliver et al. declared the isolation of stem cells from the papilla of a rat kidney and identified these stem cells as slow-cycling cells, which had had the same characteristics of renal stem cells and expressed epithelial and mesenchymal markers [6]. Bussolati et al. reported the isolation of stem cells from the adult human kidney using the cell surface markers of CD133 cell [7]. These cells express epithelial and endothelial markers. Also, Sandep et al. isolated the stem cells from the tubules of rat kidney and they found them different from $CD133^+$ cells, because of high self-renewal and differentiation potential of CD133 [8]. Maeshima et al. isolated the stem cells from the tubules of rat kidney and demonstrated these cells are mainly found in proximal tubules and they can differentiate into kidney cells under in vivo conditions [9]. Kitamura et al. isolated the stem cells with high proliferative potential from the renal proximal tubules expressing Sca-1, Musahi-1, and the markers, which are expressed at the early stage of nephrogenesis [10]. These cells showed a normal triploid karyotype, but they did not form tumors in NOD/SCID mice. The most important aim to study cancer stem cells in the present study is to be acquaintance with the pathogenesis of renal cell carcinoma and cellular basis of this disease. The level of our knowledge about the differences between normal and abnormal processes and also about detecting them can be very significant. Being acquainted with mechanisms and signals involved in both cell types either in normal or cancer cells makes these two cell types distinguishable and also makes abnormal cells detectable, respectively. Different gene and protein markers have been suggested for identifying cancer stem cells. Due to the commonality of many markers between normal and cancer stem cells, selecting the cell types whether they are normal or cancer is very important for treatment results. The study on cancer stem cells and normal renal stem cells together can yield beneficial results. Although many researchers believe that using stem cells is a key solution for treatment of kidney injuries, determining all molecular characteristics of renal stem cells seems essential before clinical use. It seems that the location of renal stem cells adjacent to toxic materials is effective in gene profile and pushes these cells toward cancer stem cells [12-19]. We found that different cell populations with the characteristics of renal stem cells can be isolated, considering the method for isolation of renal stem cell. This represents the existence of different types of stem cells in the kidney. The techniques for isolating adult stem cells require to be gradually perfected. The results of this study suggest that several cell populations in the human kidney can be isolated as stem cells, but the following questions require accurate analyses and modern techniques in the future: Which cell populations include normal stem cells. Which cells are derived from other cell populations.

It is proposed that normal stem cells can be contrasted completely with cancer stem cells by modern techniques such as proteomics in the near feature. The stem cells were isolated from the cortex, papilla, and medulla of the kidney. Although the frequency of these cells was different in different parts of the kidney, it represented that $CD133^+$ stem cells can exist in different parts of the kidney. On the other hand, the high frequency of isolated $CD105^+$, $CD44^+$, and $CD90^+$ in papilla, medulla, and cortex in group B can be a reason for existing new stem cells in the kidney. With regard to the similarities in some features between stem cells and cancer stem cells and also considering the expression of CD133 marker in cancer stem cells as a significant cell marker, which causes angiogenesis in tumors, it seems that a more specific protein marker should be defined for detecting and isolating renal stem cells. The high expression of some surface markers such as CD105 and CD44 can be a sign for the presence of cancer stem cells in the harvested kidney. The expression of the genes, which were the

indicators of cancer stem cells [19] in cell population and had not been passed through MACS-CD133 column, can imply that these cells are those cancer stem cells of the kidney. In regard to $CD133^+$ cells (Group A), showed normal karyotype (46 XY). The results of the present study indicated that these cells are considered as renal stem cells. However, the cells in group B showed abnormal karyotype in spite of the expression of some genes, such as OCT3/4, NANOG, SCA-1, and SOX2. Consequently, this represented that these cells contain a population of cancer stem cells (Table 2).

Gene	Fold CD133+/Un-sorted	Up-/down-regulation	Main impact of gene
Cdx1	1.1 (2.3)	↓	Stemness genes, Self-renewal and Differentiation
Rex1	7.3 (1.4)	↑	
Wnt	4.8 (4.3)	↑	
Oct4	2.9 (3.7)	↑	
Sox2	3.8 (6.9)	↓	
Sca1	3.9 (5.1)	↑	
Nanog	0.87 (2.5)	↓	

Table 2: Change in the gene expression level of CD133 sorted and un-sorted kidney cell.

Conclusions

The present study has demonstrated the presence of specialized stem cell sub-population within the $CD133^+$ enriched kidney stem cells. Most of the $CD133^+$ cells showed highest co-expression for SOX-2, REX1 and OCT-4 which reveals the existence of embryonic-like stem cells within the human kidney. The finding has explored a new horizon towards appropriate selection of stem cells subpopulations to be used in cellular therapeutic approaches in more defined manner.

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