

Upregulation of CD133 and CD44 as Markers of Resident Renal Progenitor Cells Following BM-MSCs Transfusion for Renal Regeneration

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ABSTRACT

Bone marrow mesenchymal stem cells (BM-MSCs) are very unique type of cells that have multipotency and immunomodulatory-regenerative properties. The aim of the present work is to ensure induction of the renal progenitor regenerative process by MSC infusion through potential upregulation of CD133 as a marker of tubular /parietal epithelial cell/ glomerular proliferation and CD44 as a marker of interstitial/ glomerular regeneration. BM-MSCs withdrawn from selected donors were isolated and further cultured to maximize their proliferation reaching 70-80% confluence. They were infused intravenously (IV) in 19 CKD patients. Renal biopsies from the 19 patients were stained for CD133 and CD44 expression before, 1 and 2 months after IV BM-MSCs injection. There was significant CD133 and CD44 upregulation in the tubulointerstitial compartment related to glomerular urinary and vascular poles as well as parietal and visceral epithelial cells following IV BM-MSCs.

In conclusion, this present work confirms the potential role of IV BM-MSCs in renal regeneration by stimulation of resident renal progenitor cell proliferation.

Keywords

CD133, CD44, Mesenchymal stem cells, Renal regeneration.

Aim of The Work

To ensure induction of the renal progenitor regenerative process by MSC infusion through potential upregulation of CD133 as a marker of tubular /parietal epithelial cell/ glomerular proliferation and CD44 as a marker of interstitial/ glomerular regeneration.

Introduction

Kidney disease is now considered to be a public health priority, owing to its increasing worldwide incidence and associated morbidity and mortality [1]. Podocyte depletion is central in all

renal pathologies causing ESRD. Glomerulosclerosis ensues after loss of 30% of podocytes [2]. The normal renal regeneration is not fully understood. The replacement of nephron cells is probably mediated by inherent residual progenitors with each part regenerated by specific type of progenitors [3-6]. The regeneration of the glomerular podocytes has been believed to be achieved by proximal tubular progenitors at the urinary pole which proliferate and differentiate through the parietal epithelial layer towards the hilar pole. The progenitor CD133+CD24+PDX (podocin)- cells are present at urinary pole, CD133+CD24+PDX+are present between urinary and vascular poles and CD133-CD24-PDX+ mature podocytes are present at vascular pole (CD133 and CD24 are progenitor markers whereas PDX is the marker of mature

podocytes) [7]. They might be directed towards their destination by Macula densa control [8]. The multiplication of mature podocytes may be another source of regeneration with limited capacity to divide in situ [3,4].

Moreover, interstitial progenitors CD44 may contribute to podocyte population being as well directed to this destination by the macula densa and are specified by their renin lineage property and can be characterized and stained differentially from the parietal epithelial type [8]. Furthermore, under pathological conditions bone marrow derived progenitors may contribute to podocyte replacement [9-11]. On the other hand, tubular regeneration is mediated by differentiation of tubular progenitors (CD133+CD24+CD106+ scattered tubular cells) responsible for regeneration of each part of the tubule selectively [12]. The regeneration under pathological conditions may not be sufficient to maintain the full recovery of acute or chronic tear mechanisms requiring possible interventional procedures to regain renal integrity. This could be achieved by transfusion of BM-MSCs which could contribute to the repair process not only by parenchymatization and differentiation (which is probably a limited aspect 1-2%) but mainly via the release of vesicle organelles, cytokines and growth factors which induce the inherent renal progenitor regeneration process [9-11]. BM-MSCs transfusion has been shown to improve the kidney function and aid regeneration in various animal experiments with AKI and ESRD [9-11] as well as clinical trials which were first demonstrated by our work in 2012 [13] and further confirmed by several studies [14].

Materials and methods

Subjects

The current work was conducted on 19 CKD patients [diabetic nephropathy, lupus nephritis, TMA, graft IFTA (interstitial fibrosis tubular atrophy), FSGS (focal segmental glomerulosclerosis), hypertensive nephrosclerosis, MPGN (membranoproliferative glomerulonephritis)] selected from Cairo university and Azhar (girls) hospitals (10 males and 9 females). Their ages ranged from 18 to 64 years. Serum creatinine ranged from 0.7 to 9 mg/dL. 24 hours urinary proteins ranged from 0.5 to 7.5 g/day. All received IV BM-MSCs injection. Ultrasound guided percutaneous renal biopsies (16 native and 3 allograft) were taken from all patients before, 1 month and 2 months after IV BM-MSCs injection. Renal biopsies were stained for CD133 and CD44 expression.

BM-MSCs collection, processing, harvesting and injection

BM-MSCs samples were aspirated and collected at two weekly intervals from the selected donors. Under complete aseptic conditions, using a preservative free heparin in a sterile syringe, 100 ml of BM blood was aspirated from iliac crest of the donors. The aspirate was then diluted at ratio 6:1 with Phosphate Buffer Saline (PBS). Thereafter, the diluted cell suspension (35 ml) was carefully layered over 15 ml of ficoll hypaque in 50 ml conical tubes. Mononuclear cells were separated and transferred to a new 15 ml conical tube. The cells were then washed and the mononuclear cells were suspended in a 5 ml complete culture medium containing FCS (1 ml), α -MEM (4 ml), gentamycin (100 μ l), fungi zone (100 μ l), and fibroblast growth factor (2 μ l). The mixture was mixed well

and divided into tissue culture flasks. The flasks were incubated in 5% CO₂ incubator at 37°C for 48 hrs. Since MSCs have this unique phenomenon of plastic adherence, the medium was changed after 24 hrs by throwing the contents (the media and nonadherent cells); another complete medium was prepared as mentioned earlier and the flasks returned to the incubator. These cells were left for another 3–4 weeks in the incubator and the medium was changed every week using the same procedure as denoted earlier. After the third week, the cells were immunophenotypically tested for cell surface markers (CD29 and CD34) using Flowcytometry.

The cells were also examined with an inverted microscope for confluence and morphology; besides, they were counted using a hemocytometer. In case of adequate number of cells (90% confluence), the contents were thrown and the cells were harvested. On reaching such confluence, the media was discarded and each flask was rinsed with PBS to remove any FCS. A volume of 3 ml of pre-warmed trypsin-EDTA solution (0.05%/0.53 μ m EDTA) was added to each flask, and then incubated at 37°C for 10 min. After trypsinization, the cells were dissociated from the adherent flask wall using a scraper in a zigzag manner, followed by gentle tapping to detach the MSCs. Later, the MSCs were re-suspended in 5 ml of complete media. Finally, 15 million MSCs (which were isolated from a single donor) diluted in 5 ml saline were injected into the patient intravenously in two sessions with one week apart.

Immunophenotypic characterization of BM-MSCs

After harvesting and counting MSCs using hemocytometer, the cells were tested for mesenchymal marker CD29 and hematopoietic stem cell marker CD34. Approximately 100 000–200 000 MSCs in DPBS were stained for 20 min at room temperature with 10 μ l of antibody, as determined from the manufacturer's recommendation (mesenchymal markers) CD29 PE and exclusion marker CD34 FITC (hematopoietic stem cell marker). A volume of 2 ml of PBS was added to the MSCs and then the tubes were centrifuged at 200g for 5 min at room temperature. The supernatant was discarded and the labeled MSCs were finally re-suspended in 0.5 ml flow buffer (FACS wash) (5% FBS+95% PBS). The cells were analyzed on a flow cytometer Coulter Elite XL Caliber collecting 10,000 events. As a control, unstained cells were applied first to exclude the effect of autofluorescence of the cultured cells.

Histopathology

All biopsies (native and transplant) were processed and stained for light microscopy by routine methods. Immunohistochemistry for IgA, IgG, C3 and C4d were performed by Bond III Leica immunostainer. CD133 (Abcam) and CD44 (cell marque) were performed on the Bond III following recommended procedure for each antibody. All transplant biopsies were scored according to the Banff classification criteria.

Quantitation of Immunohistochemical Staining

Granular staining intensity of CD133 and CD44 along the cell membranes was graded from 0 to 3+ as follows: 0, no staining; +/- (0.50), focal weak fine granular staining; 1+, weak fine granular staining along complete luminal surface; 2+, moderate complete

granular staining; 3+, strong coarse and complete granular staining.

Statistics

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 21) as follows:

- Description of quantitative variables as mean, SD and median and interquartile range.
- Description of qualitative variables as number and percentage
- Related-Samples Friedman's Two-Way Analysis of Variance by Ranks test was used instead of repeated measure Anova in non-parametric data ($SD > 30\%$ mean).

Results

Cd133 expression

CD133 stained sections show 0 staining before IV BM-MSCs injection which significantly increased to 0.6 ± 0.3 + staining 1 month after and 2.1 ± 1.1 + staining 2 months after IV BM-MSCs injection (Figure 1). CD133 upregulation was in the tubulointerstitial compartment related to the vascular and urinary poles of the glomeruli after 1 month then in the parietal and visceral epithelium of the glomeruli by 2 months (Figure 2).

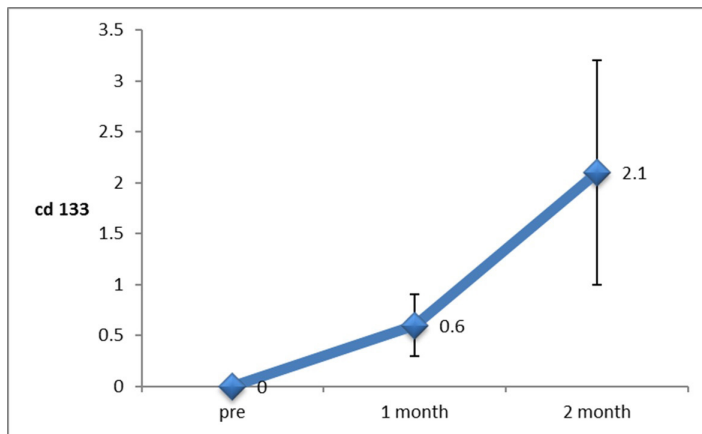


Figure 1: CD133 expression in renal biopsies before and after IV BM-MSCs.

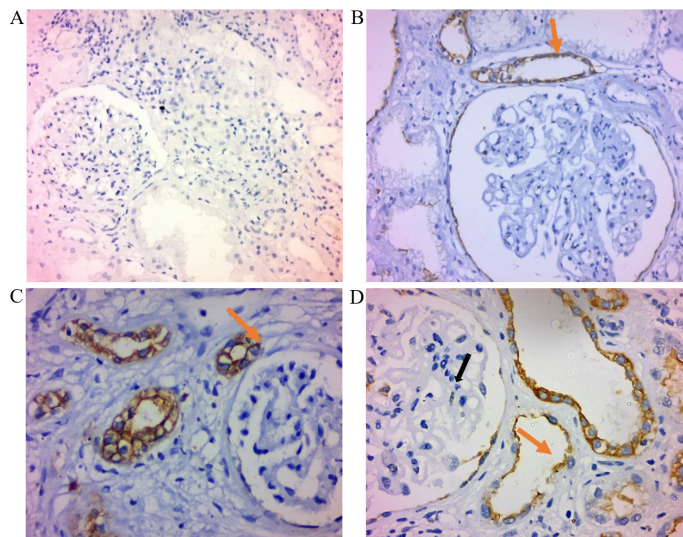


Figure 2: CD133 expression (A) before IV BM-MSCs (B) 1month after IV BM-MSCs, there's CD133 upregulation in a tubule related to vascular

pole of glomerulus (arrow). (C) 1 month after IV BM-MSCs, there 's CD133 upregulation in a tubule related to urinary pole of a glomerulus (D) 2 months after IV BM-MSCs, there's CD133 upregulation in parietal (orange arrow) and visceral (black arrow) glomerular epithelial cells.

Cd44 expression

CD44 stained sections show 0 staining before IV BM-MSCs injection which significantly increased to 0.5 ± 0.4 + staining 1 month after and 1.8 ± 1.1 + staining 2 months after IV BM-MSCs injection (Figure 3). CD44 upregulation was in the tubulointerstitial compartment related to the vascular and urinary poles of the glomerulus as well as the macula densa after 1 month then in the parietal and visceral epithelium of the glomerulus by 2 months (Figure 4).

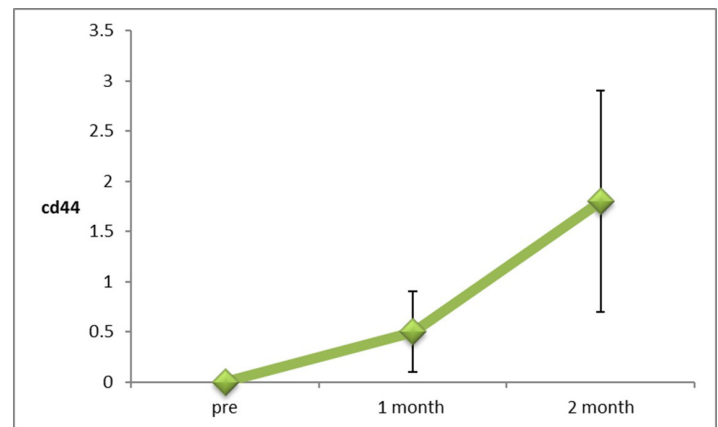


Figure 3: CD44 expression in renal biopsies before and after IV BM-MSCs.

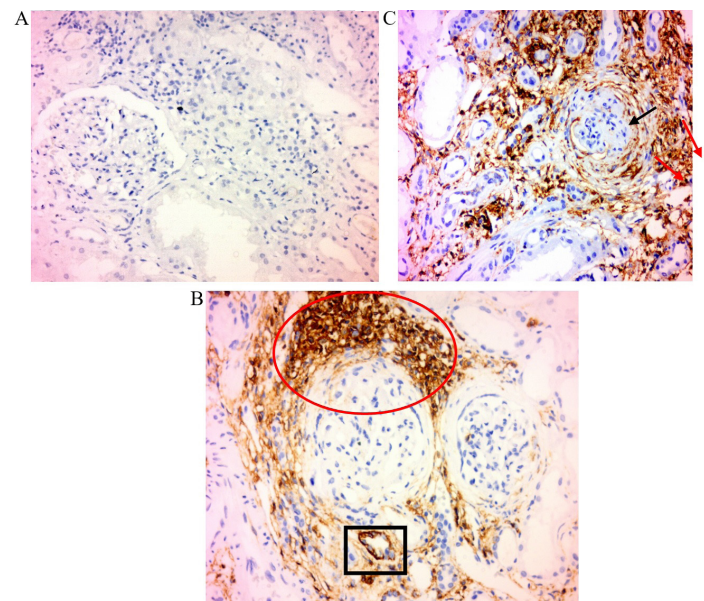


Figure 4: CD44 expression. (A) Before IV BM-MSCs. (B) 1month after IV BM-MSCs, there is CD44 upregulation in tubulointerstitial compartment related to urinary pole of glomerulus (red circle) and macula densa (black rectangle). (C) 2 months after iv BM-MSCs, there's CD44 upregulation in parietal epithelial cells (red arrows) and visceral epithelial cells (black arrow).

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