



Kidney Stem Cell Responses in Acute Kidney Injury Repair



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ABSTRACT

The medulla is a proposed stem cell niche in the kidney. In the past, isolation of kidney stem cells has been problematic due to insufficient methods, which have failed to isolate a homogenous stem cell population. This study focuses on isolating and characterizing an endogenous kidney stem cell population from primary mouse medullary interstitial cells and assessing their role in repairing injury. We successfully determined the optimal selective culture conditions for the rapid and efficient isolation of stem cells from the kidney and show the expansion and characterization of a homogenous kidney stem cell population through immunofluorescent staining for seven stem cell markers. We further assessed kidney-repairing capabilities using cell proliferation assays, MTT cell viability assays and wound healing assays. Our results show that our selectively grown stem cells express six known stem cell markers. Results of our wound-healing assay support our hypothesis that medullary stem cells have injury-healing capabilities. This study provides a novel method for the isolation of endogenous kidney stem cells that have injury-repairing capabilities. Our studies reaffirm that the kidney medulla is a stem cell niche.

INTRODUCTION

Millions of patients suffer from acute kidney injury each year, many of whom die. The kidney is a unique organ that is mitotically quiescent but has great capability in repairing itself. Repair of acute tubular injury may occur from within the kidney through the action of intrinsic stem cells residing in the kidney medulla (Gupta, Verfaillie et al. 2006). In the kidney several stem cell niches have been proposed, including in the renal medulla (Oliver, Maarouf et al. 2004). These medullary stem cells have been shown to migrate and proliferate in response to kidney injury. However, there is still much controversy whether these medullary niche cells are truly stem cells and whether they indeed participate in tubular injury repair.

MATERIALS & METHODS

Selective Growth Media and Conditions

Renal intrinsic mesenchymal cells (RIMCs) from a mouse medullary interstitial cell primary culture, which was previously described elsewhere (Moeckel, Zhang et al. 2003). Cell were thawed and plated in Dulbecco's modified Eagle Medium/F12 (DMEM/F12) (Gibco). Knockout Serum Replacement (KSR) (Gibco) was used as a defined serum-free replacement. Some cells were also cultured in the previous base buffers supplemented with N-2(1x) Supplement (Invitrogen, Carlsbad, CA), depicted with corresponding buffer followed by N, i.e. Buffer C-N. Hypersmolarity of culture buffers was generated by adding 12.88 μ L NaCl and adjusted to 400 m Osm/L for each of the base buffers, depicted with corresponding buffer followed by O. Hypoxic conditions were established by culturing cells in the base buffers in 1% O₂, 5% CO₂ and 94% N₂. These two conditions were tested since the renal medulla region is hypoxic and subject to hypertonic conditions (Neuhofer & Beck, 2005). All other cells were incubated in 95% air, 5% CO₂ humidified atmosphere at 37°C.

Immunofluorescent Staining

Secondary antibody against stem cell mark antibodies CXCR4 (ab1670, Abcam), CXCR7 (ab72100, Abcam), CD24 (ab31622, Abcam), CD34 (ab8158, Abcam), Rat-CD44 (#18849, Santa Cruz), Rabbit CD-44/#7946, Santa Cruz), Nestin (#556309, BD Pharmingen) and Pax-7(ab34360, Abcam) are conjugated with FITC (green) Cell nuclei were stained with DAPI (blue) and actin staining with rhodamine phalloidin (Red) (Invitrogen). Images were obtained with a Zeiss fluorescence microscope and analyzed using ImageJ software.

Proliferation Assay: Anti-PCNA(SC-56, Santa Cruz) Western Blot

Wound Healing Assay: NRK 49f cells were grown in Fibronectin (F-1141, Sigma) coated plates to check the supernatant from RIMCs on cell migration. The images taken in 0, 6 and 26 hours after scratch.

MTT Assay MTT(M-2128, Sigma) were added into NRK-49f cells and IMCD cells with/ without supernatant of RIMCs culture to check cell viability.

RESULTS

Through the study of selecting the optimum culture conditions and growth media to efficiently isolate renal intrinsic mesenchymal cells (RIMCs) from a mouse medullary interstitial cell primary culture, we saw an enrichment of stem cells by up to 865%, compared to cell cultures without selective growth conditions. We found that knockout buffer-N (DMEM/F12 +10% Knockout Serum Replacement + N-2(1x)) was the most effective medium to expand the stem cell sub-population (Figure 1).

Immunofluorescent staining revealed, that the enriched cells stain positive for CXCR4 (95%), positive for CXCR7 (56%), positive for CD24 (68%), positive for Nestin (43%), positive for Pax 7 (77%) and positive for several other kidney stem cell markers (Figure 2 & Figure 3).

Wound healing assay shows after 6 hours of culturing there was already visible difference between the control and the supernatant in regard to number of cells migrating into the wound area. Relative to their respective 0 hour areas, the control wound had healed by 15.78% while the supernatant treated cells had healed 16.85%. Furthermore, the distance healed was larger in the control than in the supernatant cultured cells at 6 hour (Figure 6). Preliminary wound healing experiments also revealed a high capacity of these cells to aid in wound repair and cell proliferation (Figure 4).

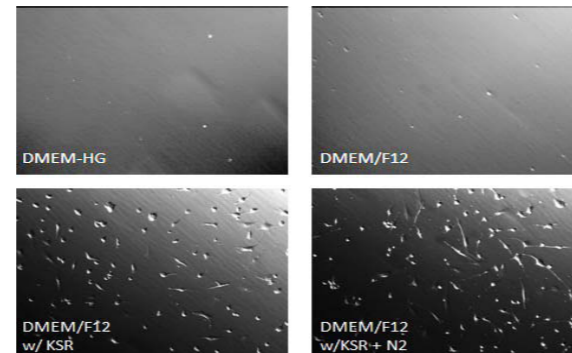


Figure 1. The effect of Knockout buffers(DMEM/F12 w/KSR) on selective growth.

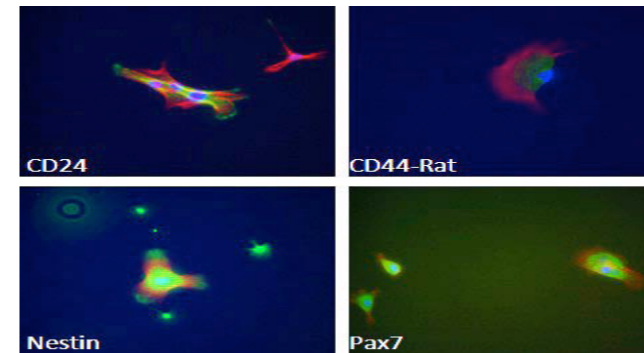


Figure 2. CD24, Pax7 and Nestin stained strongly positive while CD44-rat stained weakly positive (green fluorescence). Cell nuclei labeled with DAPI (blue); actin staining (rhodamine phalloidin) and stem cell marker antibody (green).

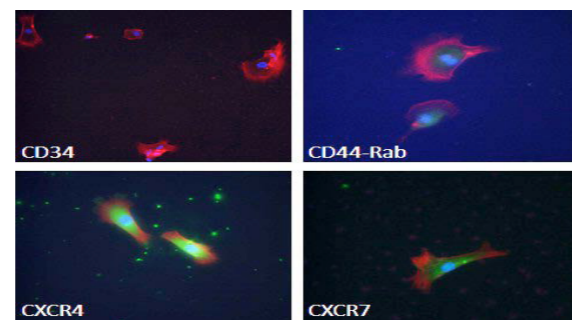


Figure 3. Representative Staining. CD44-Rab, CXCR4, and CXCR7 stained strongly positive while CD34 was entirely negative. Cell nuclei labeled with DAPI (blue), actin staining (red) and stem cell marker antibody (green).

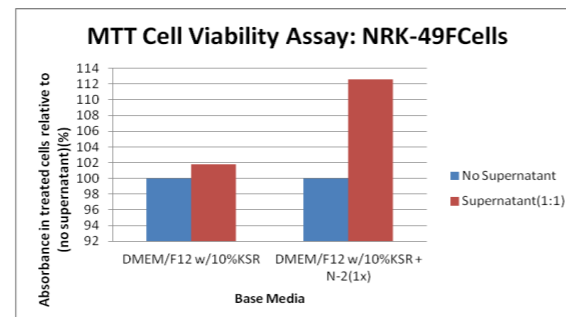


Figure 5. Percentage of viable 49f cells after 24hr treatment in specific media (+/- supernatant). Percentage of viable cells was calculated absorbance of experimental group/absorbance of control group) 100%. A lower percentage corresponded to lower number of cells alive.



Figure 4. PCNA expression of NRK-49f cells when cultured in stem cell supernatant. NRK 49f cells were treated for 48 hours in specific culture conditions.

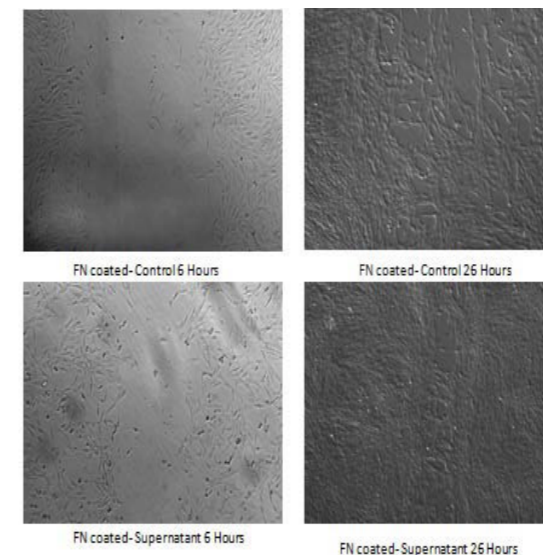


Figure 6. Wound healing assay using NRK-49f cells plated in fibronectin coated cell culture dishes.

CONCLUSIONS

- We have developed a new method to isolate and enrich potential stem cells from the kidney medulla: an efficient and highly selective growth condition for the expansion and enrichment of endogenous kidney stem cells from primary cell culture.
- After enrichment, these cells were characterized and expressed positivity for stem cell markers: CD24, CXCR4, CXCR7, Nestin, CD44 and Pax7.
- Furthermore, these stem cells exhibit phenotypic traits of renal stem cells and have biological effects that benefit kidney injury as demonstrated with PCNA proliferation assay, MTT cell viability assay and wound healing assay.
- It confirms the findings that the renal medulla is indeed a reservoir for endogenous kidney stem cells and can be highly promising for stem cell therapy in protecting and repairing the kidney

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