Kidney Stem Cell Responses in Acute Kidney Injury Repair

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INTRODUCTION

Millions of patients suffer from acute kidney injury each year, many of whom die. The kidney is a unique organ that is intrinsically quiescent but has great capability in repairing itself. Repair of acute tubular injury in the kidney is a critical event in determining patient outcome and might be used for assessment of renal recovery from warm ischemia.

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 without selectivity. We found that knock-out buffer (MEM/F12 +10% KnockOut Serum Replacement + F-1141) was the most effective medium to expand the stem cell sub-population (Figure 1). Immunofluorescence staining revealed that the enriched cells stain positive for CXCR4 (95%), positive for CXCR7 (95%), positive for CD24 (85%), positive for Nestin (47%), positive for Pax7 (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 18.05%. Furthermore, the distance traveled was larger in the control than in the supernatant cultured cells at 6 hours (Figure 4).

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 culture.

We further enriched these kidney medulla stem cells and characterized their potential to migrate and proliferate in response to kidney injury. However, there is still much controversy whether these medullary interstitial 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 by Rogers et al., were cultured in DMEM/F12 (Gibco) with 10% Knockout Serum Replacement (KSR) (Gibco) and 1% Penicillin/Streptomycin solution (Invitrogen, Carlsbad, CA), depicted with corresponding buffer followed by N, i.e. Knockout Serum Replacement + F-1141 (Sigma). Cells were cultured in serum-free media in 95% air, 5% CO2 humidified atmosphere at 37°C. All other cells were tested since the renal medulla region is hypoxic and subject to injury. However, there is still much controversy whether these medullary interstitial cells are truly stem cells and whether they indeed participate in tubular injury repair.

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 cell yield and poor immortalization. We have 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 homogeneous kidney stem cell population through immunofluorescence staining for seven stem cell markers. We further assessed kidney-repairing capabilities using proliferation assays, MTT cell viability assays and wound healing assays. Our results show that selectively grown stem cells express phenotypic stem cell markers and 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-healing capabilities. Our studies reaffirm that the kidney medulla is a stem cell niche.