Stem Cell Reports
Article

ISSCR

OPEN ACCESS

Direct Reprogramming of Human Bone Marrow Stromal Cells into Functional Renal Cells Using Cell-free Extracts

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http://dx.doi.org/10.1016/j.stemcr.2015.02.002
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SUMMARY

The application of cell-based therapies in regenerative medicine is gaining recognition. Here, we show that human bone marrow stromal cells (BMSCs), also known as bone-marrow-derived mesenchymal cells, can be reprogrammed into renal proximal tubular-like epithelial cells using cell-free extracts. Streptolysin-O-permeabilized BMSCs exposed to HK2-cell extracts underwent morphological changes—formation of "domes" and tubule-like structures—and acquired epithelial functional properties such as transepithelial-resistance, albumin-binding, and uptake and specific markers E-cadherin and aquaporin-1. Transmission electron microscopy revealed the presence of brush border microvilli and tight intercellular contacts. RNA sequencing showed tubular epithelial transcript abundance and revealed the up-regulation of components of the *EGFR* pathway. Reprogrammed BMSCs integrated into self-forming kidney tissue and formed tubular structures. Reprogrammed BMSCs infused in immunodeficient mice with cisplatin-induced acute kidney injury engrafted into proximal tubuli, reduced renal injury and improved function. Thus, reprogrammed BMSCs are a promising cell resource for future cell therapy.

INTRODUCTION

Cell-based therapies are emerging as one of the most promising approaches of regenerative medicine (Riazi et al., 2009). In the kidney field, the search for a renal-specific stem cell led to the discovery of progenitor cells that protect animals from acute kidney injury (AKI) when systemically infused (Angelotti et al., 2012; Benigni et al., 2010). However, the cell number is a limiting factor, and their biology is far from known. Therefore, other non-renal stem cell sources have been pursued. Derivation of human embryonic stem cells (hESCs) (Thomson et al., 1998) has raised hope because they can give rise to all three germ layers, but progress toward somatic populations has encountered major obstacles, including the risk of cancer and rejection, not to mention the ethical issues involved. The same holds true for induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006), which are similar to hESCs but devoid of at least some of the above problems. The generation of hESC/iPSC-derived mature renal cells (Song et al., 2012) and, more recently, intermediate mesoderm/metanephric mesenchyme (MM) and ureteric bud (UB) renal progenitors (Lam et al., 2014; Lin et al., 2010; Mae et al., 2013; Takasato et al., 2014) has been reported. In principle, patient-specific cells to be used therapeutically could be obtained through reprogramming approaches in which a long-standing interest exists because of the possibility that abundant adult cells can easily be harvested and converted to other cell types (Zhou et al., 2008). In this context, studies have defined sets of transcription factors that can directly reprogram somatic cells into another cell type without passing through the pluripotent state (Ginsberg et al., 2012; Ieda et al., 2010; Karow et al., 2012; Vierbuchen et al., 2010). Using a strategy of re-expressing key developmental regulators in vitro/in vivo, adult cell reprogramming occurs, through which induced cells residing in their native environment might promote their survival and/or maturation (Ginsberg et al., 2012; Ieda et al., 2010; Karow et al., 2012; Qian et al., 2012; Vierbuchen et al., 2010; Zhou et al., 2008).

In parallel with these developments, an intriguing technology for direct cell reprogramming by exposing reversibly permeabilized somatic cells to cell-free extracts has emerged. This method has its origins in the early experiments of Briggs and King, followed by Gurdon (Gurdon, 2006), where a somatic cell nucleus was transferred (SCNT [somatic cell nuclear transfer]) to an enucleated oocyte, resulting in the activation of the somatic cell

nucleus. Cell-extract reprogramming was first demonstrated with extracts of regenerating newt limbs, which promoted cell-cycle re-entry and downregulation of myogenic markers in differentiated myotubes (McGann et al., 2001). Afterward, this approach yielded in-vitro-reprogrammed somatic cells with the extracts from T cells, cardiomyocytes, insulinoma cells, pneumocytes, chromaffin, or embryonic stem cells (Gaustad et al., 2004; Håkelien et al., 2002, 2004; Landsverk et al., 2002; Qin et al., 2005; Qu et al., 2013; Rajasingh et al., 2008).

Surprisingly, there is a paucity of attempts at the reverse reprogramming of adult stem cells toward somatic cells. Human bone marrow stromal cells (BMSCs), also known as bone-marrow-derived mesenchymal stem cells, are adult stem/progenitor cells with self-renewal capacity and restricted potential for generating skeletal tissues, including osteoblast, chondrocyte, adipocyte, and perivascular stromal cells (Bianco et al., 2013; Le Blanc and Mougiakakos, 2012). Whether BMSCs can be used therapeutically is still a matter of debate. Based on their paracrine action rather than differentiation ability, these cells have been used with promising results in different diseases (Le Blanc and Mougiakakos, 2012; Morigi and Benigni, 2013; Reinders et al., 2014; Souidi et al., 2013). No evidence of direct reprogramming of BMSCs into somatic cells is available yet.

Here, we inquired whether human BMSCs could be reverse reprogrammed to acquire a renal tubular epithelial phenotype by using tubular cell extracts. We found that reprogrammed BMSCs (1) acquired an antigenic profile and functional properties of proximal tubular-like epithelial cells in vitro, (2) integrated into developing nephrons ex vivo, and (3) protected mice from AKI.

RESULTS

Morphological and Ultrastructural Characteristics of BMSCs Treated with HK2 Cell Extracts

Human BMSCs were permeabilized with 400 ng/ml streptolysin O (SLO), a concentration that did not affect cell viability. Permeabilized BMSCs exposed to the extract of human proximal tubular epithelial (HK2) cells changed from their usual spindle-shape appearance (Figure 1A) to cobblestone islands within 13–15 days (Figures 1B–1D). During the subsequent 2 weeks, these islands expanded and the formation of "domes" and tubular-like structures (Figures 1E and 1F) similar to HK2 cells (Figure 1H) occurred. This morphological transition did not occur in BMSCs grown in epithelial-specific culture medium, even after 35 days (Figures 1I and 1J). The observed change in morphological appearance of BMSCs treated with epithelial cell extract suggested a phenotypic switch in the cells.

Moreover, transmission electron microscopy (TEM) of cell-extract-treated BMSCs demonstrated brush border microvilli and images of tight intercellular contact (Figures 1K–1M), further confirming that cells acquired a proximal tubular-like epithelial phenotype similar to HK2 cells (Figures 1N and 1O). BMSCs treated with HK2 cell medium did not demonstrate any apical specialization or cell-cell contact, even after 35 days (Figure 1P).

Antigenic Profile of the BMSCs Treated with HK2 Cell Extracts

To explore the renal identity of BMSCs treated with HK2 cell extracts, we studied candidate proximal tubular epithelial cell markers using immunofluorescence microscopy and flow cytometry (fluorescence-activated cell sorting [FACS]) analysis. BMSCs consistently expressed endoglin (ENG; also known as CD105), characteristic of the mesenchymal lineage, and were devoid of the epithelial markers E-cadherin (CDH1) and aquaporin-1 (AQP1) (Figures S1A–S1D). After 8 days, BMSCs treated with HK2 cell extracts weakly expressed CDH1, TJP1 (tight junction protein 1; also known as zona occludens-1), and AQP1 in combination with BMSC markers (Figures 2A–2D). Moreover, after 25 days, BMSCs treated with HK2 cell extracts displayed robust expression of the epithelial markers but substantially reduced expression of ENG (Figures 2E and 2H), which is consistent with the expression profile of HK2 cells (Figures S1E–S1H). Notably, BMSCs that were not converted to cobblestone islands maintained the expression of mesenchymal markers (Figures 2E and 2H, area defined by the dotted line). BMSCs grown in HK2 cell medium, used as control, did not express epithelial markers and retained the expression of ENG (Figures S1I–S1L).

To quantify the BMSC reprogramming process, FACS analysis was performed. By day 8, a cell population of 1%–3% of BMSCs treated with HK2 cell extracts expressed AQP1 and CDH1 (Figure 2I). The highest percentage of reprogrammed BMSCs expressing these tubular epithelial markers was observed after 35 days and averaged 15%–21% (Figure 2I).

To trace the reprogramming process chronologically, we used an EGFP plasmid containing the promoter region of *CDH1* (Genecopeia; HPRM12692-PF02). Human BMSCs were transfected at days 5 and 11 from cell-extract exposure, and CDH1/EGFP-positive cells were assessed 24 hr later (days 6 and 12). At day 6, the EGFP-positive cells had a BMSC-like shape, while at day 12, CDH1/EGFP-positive cells acquired an epithelial-like appearance (Figure 2J). The reprogramming efficiency was ~0.01%–0.02%. Untreated BMSCs grown in HK2 cell medium did not express EGFP at any time points tested (Figure 2J). As expected, several HK2-transfected cells became positive for CDH1/EGFP, with a transfection efficiency of 30% (Figure 2J).

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