Effect of Intervertebral Disc Degeneration on Nucleus Pulposus-Derived Stem Cells

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INTRODUCTION

Intervertebral disc (IVD) degeneration and chronic lower back pain are a major health problem. Despite decades of research, a fundamental, multidisciplinary, mechanistic understanding of disc degeneration is lacking and, consequently, robust clinical therapies that target underlying causes, rather than symptoms, are still in the earliest stages of development. It was speculated that stem cells (SCs) maintain homeostasis of tissues with a limited regeneration capacity. Changes in the differentiation of resident SCs can represent a loss of homeostasis and diminished regeneration of the tissue. We previously showed that SCs reside in human degenerated nucleus pulposus (NP) and healthy rat NP [1]. Here we hypothesized that IVD degeneration affects the quantity, proliferation, and differentiation potential of SCs in the NP. To pursue this hypothesis we isolated NP-SCs from healthy and degenerated samples of NP and evaluated the cells’ proliferation rate and potential to differentiate into NP-like cells and other mesenchymal lineages.

METHODS

Disc degeneration was induced in a mini-pig model by creating an annular injury under fluoroscopic guidance. Degeneration was verified using MRI 6 weeks after surgery. NP-SCs were isolated from healthy and degenerated discs. The freshly isolated cells were tested with a colony-forming unit (CFU) assay. Colonies with more than 20 cells were counted. Surface mesenchymal stem cell (MSC) marker expression was examined using FACS analysis and immunohistochemistry (IHC). The proliferation rate was evaluated by cell counts made using Trypan blue exclusion and BrdU incorporation. Cell differentiation into osteogenic, chondrogenic, and adipogenic lineages was assessed in vitro using von Kossa staining, GAG quantification with DMMB assay, and Oil red O staining, respectively. In addition, NP-SCs were differentiated into NP-like cells by culturing them in alginate beads supplemented with TGFβ1 in conditions of hypoxia (2% oxygen) and normoxia for 14 days. Differentiation was evaluated using GAG quantification with a DMMB assay.

RESULTS

NP-SCs were isolated from healthy and degenerate IVDs. A significantly higher rate of proliferation was demonstrated in cells from degenerated discs (degenerated-NP) by using cell counts (Fig. 1) and a BrdU assay (Fig. 2), and a higher rate of CFUs as well. Freshly isolated SCs derived from healthy discs (healthy-NP) exhibited a higher rate of MSC markers in the FACS analysis. MSC marker expression was verified using IHC in healthy NP tissue (Fig. 3). Differentiation assays showed that NP-SCs derived from healthy and degenerated discs have the ability to differentiate into the osteogenic, chondrogenic, and adipogenic lineages without significant differences between the two groups. Healthy NP-SCs showed a higher differentiation capacity to NP-like cells than degenerated NP-derived, in conditions of hypoxia but not normoxia (Fig. 4).

DISCUSSION

These results indicate that disc degeneration has a clear effect on SCs residing in the NP. Cells derived from degenerate discs exhibit higher proliferation abilities that are likely caused by the degeneration process in the discs. However, although these cells maintain their differentiation abilities, they have a lower capability of differentiating into NP-like cells. These findings can shed light on the degeneration process taking place in the IVD, elucidating what is the role of SC in this process and explaining why SCs are not able to reverse the degeneration.

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REFERENCE