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The Spine Journal 15 (2015) 530–538

THE SPINE JOURNAL

Basic Science

Bone marrow mesenchymal stem cells slow intervertebral disc degeneration through the NF-κB pathway

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Received 28 March 2014; revised 9 October 2014; accepted 21 November 2014

Abstract

BACKGROUND CONTEXT: Previous studies have demonstrated the use of bone marrow mesenchymal stem cells (BMSCs) in tissue-engineering treatments to slow or reverse diseased intervertebral discs. Several approaches have successfully used the coculturing of stem cells with disc-native nucleus pulposus cells (NPCs) with the evidence of transformed BMSCs into NP-like cells, increased activity and matrix production by NPCs, or elements of both. The influence of the cytokine transforming growth factor-beta (TGF-β) in the differentiation of BMSCs into NP-like cells and its upregulation in coculture to increase matrix production are well established. However, the role of the inflammatory signaling molecule nuclear factor kappa B (NF-κB) in intervertebral disc degeneration is far less clear, although there is some existing evidence suggesting its role in the pathogenesis and progression of disc disease. A limited number of studies in other pathologies have alluded to the antagonistic relationship between both proteins. To date, there is no such investigation of their dynamic role in coculture of BMSCs and NPCs.

PURPOSE: The purpose of this study was to investigate the relationship of the regenerative effects of BMSCs cocultured with NPCs. The authors hypothesized that as levels of TGF-β increase in the coculture, the levels of NF-κB will concomitantly decrease. This would in turn be reflected by an increase in the expression of messenger RNA markers of the nucleus pulposus matrix that includes aggrecan, Type II collagen (CII), and SOX-9 and an increase in the cellular proliferation.

STUDY DESIGN/SETTING: This study is based on a coculture with the contact of rabbit NPCs and BMSCs.

METHODS: Bone marrow mesenchymal stem cells were cocultured with NPCs at a ratio of 1:1 and compared with BMSC and NPC controls cultured alone. Cell proliferation was evaluated by Cell Counting Kit-8 from 3 to 9 days. Gene expressions of aggrecan, CII, and SOX-9 was assayed by reverse transcription-polymerase chain reaction from 5 to 14 days. Detection of TGF-βl and NF-κB was determined by enzyme-linked immunosorbent assay, and immunohistochemical staining was carried out to evaluate CII synthesis.

RESULTS: After 3 days, cellular proliferation of the cocultured group exceeded that of controls. After 11 days, the expression of SOX-9 in the cocultured group had also exceeded controls. Furthermore, after 14 days, expressions of aggrecan and CII significantly exceeded controls. Immunohistochemical stains of CII in the NPC control group were positive at each point in time and demonstrated strongest expression at 14 days. Coculturing BMSCs with NPCs, therefore, seems to have resulted in the promotion of aggrecan, CII, and SOX-9 gene expressions. Finally, after 11 days, TGF-βl content of the cocultured group significantly exceeded control levels, whereas NF-κB content had significantly lowered.

FDA device/drug status: Not applicable.

Author disclosures: ***CC:*** Nothing to disclose. ***JZ:*** Nothing to disclose. ***XL:*** Nothing to disclose. ***AS:*** Nothing to disclose. ***MM:*** Nothing to disclose. ***ZL:*** Nothing to disclose. ***QS:*** Nothing to disclose. ***JL:*** Nothing to disclose. ***HY:*** Nothing to disclose. ***NE:*** Nothing to disclose.

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http://dx.doi.org/10.1016/j.spinee.2014.11.021

CONCLUSIONS: Coculture of BMSCs may be able to delay NPC matrix degeneration potentially through the concomitant upregulation of TGF-β and the downregulation of NF-κB pathway.

Keywords: BMSC; NPC; Coculture; IDD; TGF-β; NF-κB

Introduction

Low back pain is one of the leading causes of job-related disability with a cost of at least $50 billion each year in the United States. It is predicted that up to 80% of adults in the United states will experience some form of back pain over their lifetime, with approximately 5% of sufferers becoming chronically disabled [1]. Degenerative disc disease (DDD) is one of the main contributing factors of low back pain. Although majority of patients with early DDD are asymptomatic, progression of DDD can lead to a number of pathologies, including spinal stenosis, segment instability, and spinal nerve compression [2,3]. Conservative management and surgery may alleviate clinical symptoms but are often unable to eliminate the degenerative nature of the disc. For instance, surgery has been shown to improve symptoms in only 65% of patients, leaving about 35% no better or possibly worse [4]. On a cellular level, disc degeneration is characterized by a reduction in the number of nucleus pulposus cells (NPCs) and their extracellular matrix (ECM) products, specifically proteoglycans and Type II collagen (CII), which also serve as phenotypic markers of NPCs [5]. Therefore, therapeutic strategies have focused on increasing NPC cell count and driving matrix metabolism toward increased protein synthesis, which would not only lead to a maintained disc height and an increased loading capacity but also halt or delay the process of disc degeneration [6,7].

Numerous studies have demonstrated the potential of engineering multipotent bone marrow mesenchymal stem cells (BMSCs) toward the repopulation of the degenerating intervertebral disc (IVD) [8]. A number of approaches have been tested, including differentiating MSCs into NP-like cells through the application of TGF-β1, a microenvironment with low oxygen tension, and a three-dimensional cellular culture for proper cell-cell interaction [9,10]. Other methods include the transplantation of MSCs into intervertebral discs with several studies demonstrating the capacity for MSCs surviving and proliferating within the IVD to potentially restore its normal structure and function [11–13].

In addition, there are several studies that demonstrate the upregulation of the biologic and metabolic viabilities of NP cells and differentiating MSCs by coculture. Yammamoto et al. [14] demonstrated that coculture with MSCs and rabbit NPCs induced matrix production in NPCs. They showed that MSCs were effective in activating native NPCs to increase production of the ECM, thus restoring disc height, maintaining an effective swelling pressure, and reinstating the normal biomechanical properties of the disc. Nucleus pulposus cells cocultured with MSCs under direct cell-to-cell contact induced expressions of growth factors tranforming growth factor (TGF-β), insulin-like growth factor (IGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) that lead to cellular proliferation, DNA synthesis, and proteoglycan production in the NPCs. Thompson et al. [15] demonstrated in the previous studies that these growth factors among others were responsible for matrix production by NP cells.

Conversely, Richardson et al. [16] were able to demonstrate that the stem cells themselves showed significant increases in NP marker genes when cocultured with contact to NPCs for 7 days, and this change was regulated by cell ratio. Their work contrasts that of Yamamoto et al. [14], by demonstrating that there was no significant change in NP marker gene expression in either NPCs or MSCs when cells were cultured without contact, regardless of the cell ratio. Their research established a viable method for generating a large population of differentiated stem cells to be used for restoring degenerated intervertebral discs [16]. Indeed, both studies demonstrate the significance of coculture and cell-cell contact in restoring degenerated IVD but offer alternative explanations for enhanced matrix synthesis and increased cellular proliferation.

Past studies by Yang et al. [24] combined the findings from both Yammamoto et al. [14] and Richardson et al. [16] and distinguished itself by suggesting that the mechanisms of interaction between MSCs and NP cells were mediated by secreted factors, not cellular contact, as both cell lines were separated by a membrane to prevent exchange of cellular components. They found that the most significant effect on NPCs was enhancement of cellular proliferation when cocultured in the presence of a small number of MSCs. They also found that to differentiate MSCs into NP-like cells with increased CII expression, MSCs must be in an environment containing numerous NPCs. These studies, among numerous others, establish the role of TGF-β as a major regulatory cytokine to maintain cellular proliferation and matrix synthesis in the disc [24]. However, the function of nuclear factor kappa B (NF-κB), a family of transcription factors that play a central role in mediating cellular response to stress, damage, and inflammation, is not well understood in intervertebral disc degeneration (IDD).

Previous research has shown the connection between the activation of the NF-κB pathway and age-associated disc degeneration, whereas several of these studies demonstrated that blocking this pathway via genetic and pharmacologic