INTRODUCTION

To date, research has shown that progressive breakdown of the extracellular matrix (ECM) is closely associated with disc degeneration. Therefore, biological treatments capable of promoting ECM repair and regeneration have been considered, and clinical trials are underway [1]. Bone morphogenetic protein-7 (BMP7 or OP-1), a member of the transforming growth-factor-β (TGF-β) superfamily, was found to exert potent anabolic effects on chondrocyte differentiation and metabolism via Smad 1/5/8 signaling pathway [2]. But BMP7 induces noggin, through a negative feedback. Noggin, a well known inhibitor of BMP activity, is a vital molecule involved in cephalogenesis and skeletogenesis in a variety of species [3, 4]. Therefore, we hypothesized that noggin is capable of inhibiting BMP7-mediated anabolic effects in bovine disc cells.

Lactoferricin (LfcinB), a peptide 25 amino acid residues, is acquired from lactoferricin via proteolysis by acidic pepsin. LfcinB possesses strong antimicrobial, antivirus, anti-oxidant, anti-cancer activities [5]. Previously, we reported the anabolic and anti-catabolic property of LfcinB in bovine IVD cells.

The aim of the present study is to determine the potential of BMP7 in combination with LfcinB to retard the progression of IVD degeneration. Specifically, we studied the effect of this on bovine IVD homeostasis compared to individual treatments through assessing PG accumulation, PG synthesis, and noggin expression, as well as analyzing the mechanisms by which LfcinB may potentiate BMP7 activity.

METHODS

IVD tissue was obtained from 15-18 months old disc of bovine coccyx. Chondrocytes were isolated from nucleus pulposus, digested by pronase and collagenase, and plated on 12-well plate at 8*10^4 cells/cm² in a 1:1 mixture of DMEM/ Ham’s F12 medium containing 10% FBS. Chondrocytes cultured in serum free media were stimulated with LfcinB or/and chemicals for 24hrs. After cultivation, chondrocytes were harvested for real-time PCR or western blot. The alginate beads culture to supply 3D environment and long term culture was used to dimethylethylene blue (DMMB) assay, particle exclusion assay, and ³⁵S incorporation assay. Analysis of variance was performed using StatView 5.0 software. P values <0.05 were considered significant.

RESULTS

BMP7 not only increases aggrecan gene induction, which is a major component of ECM, but also increases noggin gene induction, a natural BMP antagonist (Fig. 1A). As shown in Fig.1B, BMP7 combined with noggin suppresses aggrecan gene induction. Noggin also suppresses the Sox9 mRNA induction, a critical transcription factor in chondrocytes. These data suggest that BMP7-mediated anabolism in discs is compromised by noggin expression, which is induced by BMP7 itself as a negative feedback mechanism. Therefore, inhibition of BMP7-mediated noggin expression by biochemical treatment can maximize BMP7-mediated anabolic effects on IVD cells. Previously, we showed that LfcinB exerts potential anabolic and anti-catabolic effect on bovine IVD cells.

As shown in Fig. 2 A-D, combined stimulation of BMP7 with LfcinB exerts potential synergistic anabolic effects on IVD cells represented by PG production (A), PG synthesis assay (B) which is correlated with upregulated anabolic gene expression (C). Matrix formation is further visualized by particle exclusion assay (D).

DISCUSSION

We illustrate the potent anabolic effects of LfcinB and BMP7 on bovine IVD cells. Combinational stimulation shows a greater anabolic impact on PG accumulation and synthesis as well as mRNA induction of aggrecan and SOX9 compared to individual treatments. Noggin that serves as a negative regulator on BMP7-mediated anabolism in IVD cells, is effectively suppressed by LfcinB, providing the molecular mechanisms by which the combination of BMP7 and LfcinB shows synergism. Finally, we have observed Sma1/5/8 being activated by BMP7 and LfcinB in IVD cells, which provides another explanation to the synergistic effects seen in IVD cells stimulated by the combination.

REFERENCES


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