Activation of the Beta-Catenin-mediated CTGF Secretory Pathway via TGF-Beta in Senescent Chondrocytes is a Mechanism for Intervertebral Disc Degeneration

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Learning Objectives

By the conclusion of this session, participants should be able to:

- Describe the basic pathophysiology of intervertebral disc degeneration.
- Discuss the potential molecular mechanisms by which intervertebral disc degeneration is mediated.

Introduction

Intervertebral disc degeneration is an age-related process that begins during childhood. Regions of discs containing chondrocyte-like cells are prone to degenerate, but the mechanism is unknown. Overexpression of Smurf2 under the control of type II collagen alpha 1 (*Col2a1*) promoter has been shown to accelerate age-related intervertebral disc degeneration in *Col2a1-Smurf2* transgenic mice. Notably, increased levels of connective tissue growth factor (CTGF) protein and TGF-ß mRNA have been observed in chondrocyte-like cells in *Col2a1-Smurf2* transgenic mice discs, implicating CTGF in disc degeneration progression. This study aimed to elucidate the mechanism by which Smurf2 overexpression in chondrocyte-like cells results in increased CTGF secretion during disc degeneration in *Col2a1-Smurf2* transgenic mice.

Results

The primary old bovine NP cells were found to be in various stages of senescence. Treatment of these cells with TGF-ß induced rapid accumulation of cytoplasmic ß-catenin, which interacted with CTGF and recruited it to the plasma membrane for secretion (**Figure 1, Figure 2**). This TGF-ß-initiated, ß-catenin–mediated CTGF secretory cascade did not occur in primary young bovine NP cells; however, the cells became senescent after Smurf2 overexpression, thereby allowing the cascade to occur (**Figure 3**).

Methods

Primary old bovine nucleus pulposus (NP) cells were isolated from 3.5-year-old caudal bovine intervertebral discs as substitutes for chondrocyte-like cells in *Col2a1-Smurf2* transgenic mice discs. Primary young bovine NP cells were isolated from 3-month-old caudal bovine intervertebral discs to represent normal cells in wild-type mice discs. The cells were transfected with lentiviruses expressing Smurf2 and GFP. Western blot, immunoprecipitation, and immunohistochemistry were utilized to analyze the downstream effects of TGF-ß treatment on ß-catenin and CTGF in the old bovine NP cells, young bovine NP cells, and young bovine NP cells after gain of Smurf2 function.

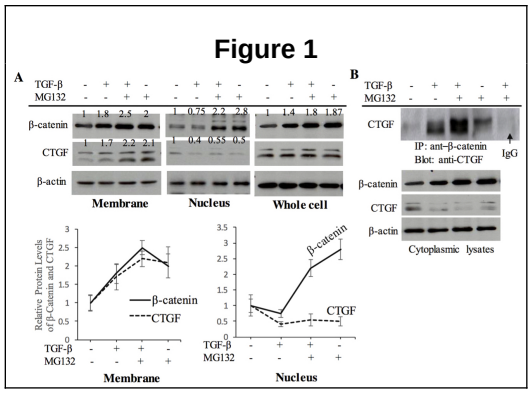

Figure 1: After TGF-ß treatment of old bovine NP cells: (A) ß-Catenin and CTGF protein levels are positively related in the plasma membrane and negatively related in the nuclei. (B) ß-Catenin interacts with CTGF in the cytoplasm.

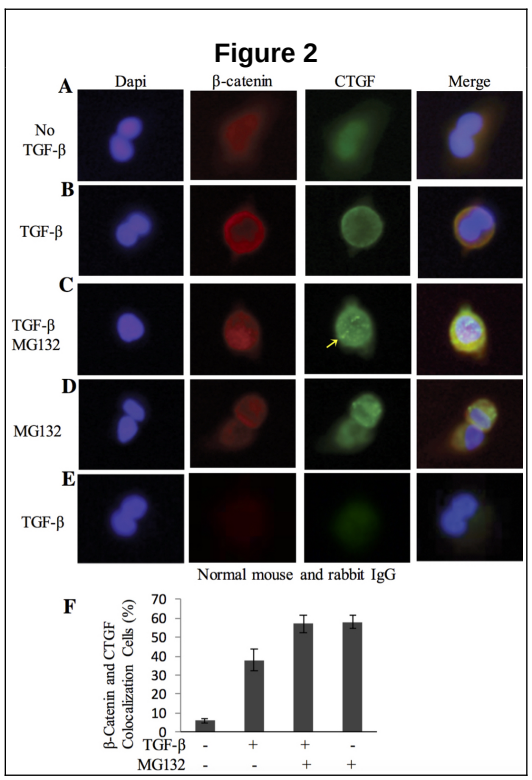

Figure 2: Co-localization of ß-catenin and CTGF in the plasma membrane of TGF-ß treated old bovine NP cells.

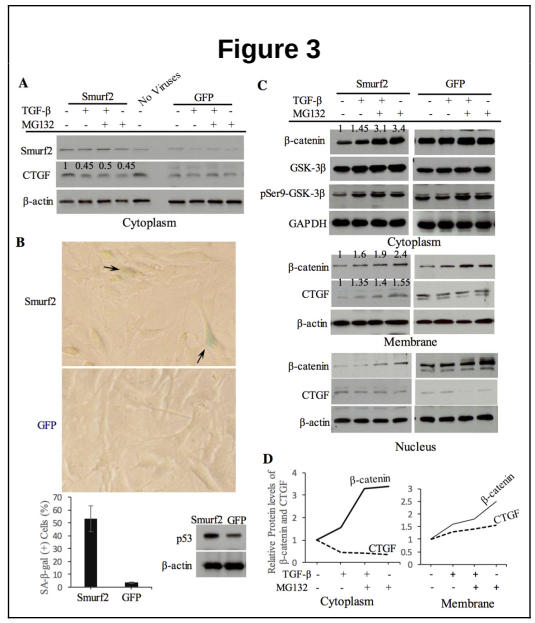

Figure 3: Young bovine NP cells become senescent and obtain characteristics of old bovine NP cells after gain of Smurf2 function.

Conclusions

Smurf2-induced intervertebral disc degeneration in *Col2a1-Smurf2* transgenic mice occurs through activation of the CTGF secretory pathway in senescent chondrocyte-like cells within intervertebral discs.