Non-canonical Wnt signaling (PKC pathway) regulates intervertebral disc cells

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INTRODUCTION
It was previously reported the regulation of Wnt/β-catenin signal in intervertebral disc (IVD). As a result of our previous study, cell senescence of IVD cells was induced by activation of a Wnt/β-catenin signal via the MMP pathway (Arthritis and Rheum 2010). However, it is not yet elucidated about non-canonical pathway include Wnt-Ca2+ and Protein Kinase C (PKC) pathway in IVD. Some groups reported that activation of PKC inhibits of Wnt/β-catenin signal (The FASEB journal express article 2002). PKC is a multigene family that encodes at least 11 distinct isoforms of lipid-regulated protein kinases. Specific isoforms play pivotal roles in several signal transduction pathways that regulate cellular growth, transformation, and differentiation. Thus, we hypothesized that PKC pathway play important roles in IVD, and we focused on this pathway which was non-canonical pathway in IVD.

MATERIALS AND METHODS

Disc harvest: Sprague-Dawley (SD) Rats (12weeks, n=32) were used in this study. Nucleus pulposus (NP) cells were isolated and maintained in DMEM supplemented with 10%FBS supplemented with antibiotics.

Western blot analysis: NP cells were plated in flat bottom 96-well plates and treated with phorbol 12-myristate 13-acetate (PMA) (200nM) for 24h. Cells were incubated with antibodies against β-catenin. Western blot analysis was used to determine the expression levels in the presence of ionomycin and PMA treatment, while PKCs were unchanged in NP cells (Figure.3).

RESULTS
Topflash activity measured following ionomycin and PMA treatment. Ionomycin treatment resulted in induction in Topflash activity but not PMA treatment. There was a dose-dependent change in Topflash activity following treatment PMA (10nM, 100nM or 200nM).

REFERENCES
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