Transient Receptor Potential Vanilloid 4 (TRPV4) activation by agonist promotes autophagy and extracellular matrix synthesis through AMPK pathway in rat intervertebral disc

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INTRODUCTION: The intervertebral disc is the largest avascular, low nutrient organ in the human body[1]. Autophagy is an important cell survival mechanism by self-digestion and recycling damaged components under stress conditions, primarily nutrient deprivation[2]. Biologically, disc cells and their extracellular matrix are stimulated by physiological range of mechanical loading, and abnormal loading can result in disc degeneration. However, physicochemical factors and their mechanisms which maintain disc homeostasis have not been fully clarified. One possible mechanosensitive regulator in disc homeostasis is Transient Receptor Potential Vanilloid 4 (TRPV4). TRPV4 is a mechanosensitive Ca^{2+}-permeable channel, which is activated under a physiological mechanical stimulation in disc nucleus pulposus (NP) cells *in vitro*[3,4]. Since the intervertebral disc is exposed to high osmotic and hydrostatic pressure, we hypothesized that TRPV4 contributes to intradiscal homeostasis. Our objective was to elucidate that TRPV4 activation by agonist promotes autophagy and extracellular matrix synthesis in the rat intervertebral disc.

METHODS: ***In-vitro*** **study**: Disc NP cells harvested from 12-week-old male Sprague-Dawley rats were used. (1) Cytotoxicity of rat disc NP cells was evaluated using the Cell Counting Kit-8 (CCK-8) after 24-h treatment of 0–100-nM TRPV4 agonist (Sigma-Aldrich, GSK1016790A) in DMEM with 10% FBS. (2) Ca^{2+} imaging was performed using Fluo-4 AM (Dojindo, #273221-67-3) to detect intracellular Ca^{2+} changes. Ca^{2+} response was measured using a microplate reader (PerkinElmer, EnSpire). (3) To simulate clinically relevant disease conditions, cells were cultured for 24 h in DMEM with 0% FBS or with 0% FBS and 10-ng/ml interleukin-1 beta (IL-1β), a pro-inflammatory cytokine linked to the pathogenesis and severity of disc degeneration. Then, expression of phospho-AMPK (pAMPK), autophagy markers (mTOR, RAPTOR, p70/S6K, LC3-II, and a substrate p62/SQSTM1), extracellular matrix molecules (COL2a1 and Aggrecan), catabolic matrix metalloproteinases (MMPs), anti-catabolic tissue inhibitor of metalloproteinases (TIMPs), apoptosis markers (PARP, cleaved PARP, and cleaved Caspase-9), and senescence markers (p53, p21/CIP1, and p16/INK4a) were assessed by Western blotting. The α-tubulin was used as a loading control. The intensities of the bands were quantified using ImageJ software. Apoptosis was also assessed by TUNEL staining. The *P*-values of < 0.05 were regarded as statistically significant.
In-vivo **study**: Thirty-six 12-week-old male SD rats were used, and TRPV4 agonist and control (dimethyl sulfoxide) were injected into respective discs using a 33-G needle. (4) A rat tail model of disc degeneration induced by temporary static compression was designed[5]. Rat tails were affixed with an Ilizarov-type apparatus with springs between the 8th and 10th coccygeal (C) vertebrae. While DMSO was injected into C9/10 (loaded control) and C12/13 (unloaded control), TRPV4 agonist was injected into C8/9 (loaded experimental) and C11/12 (unloaded experimental). Then, axial force at 1.3 MPa was applied for 24 h and subsequently released. Radiographic degeneration was assessed at 0 and 28 d after compression.

RESULTS: ***In-vitro*** **study**: (1) In rat disc NP cells, TRPV4 agonist significantly decreased cell viability at 20 nM or higher (P<0.05). Therefore, 10-nM TRPV4 agonist was used for subsequent experiments. (2) Intracellular Ca^{2+} level increased immediately after the administration of TRPV4 agonist **(Fig. 1)**. (3) pAMPK expression significantly increased by TRPV4 agonist in DMEM with 0% FBS (P<0.05). In DMEM with 0% FBS and IL-1β, TRPV4 agonist increased pAMPK, LC3-II, COL2a1, Aggrecan, and TIMPs, and decreased mTOR, RAPTOR, p70/S6K, p62/SQSTM1, and MMPs (P<0.05), indicating the promotion of autophagy and extracellular matrix synthesis through AMPK pathway **(Fig. 2)**. TRPV4 agonist also increased PARP, and decreased cleaved PARP, cleaved Caspase-9, p53, p21/CIP1, and p16/INK4a (P<0.05), indicating the suppression of apoptosis and senescence **(Fig. 3)**. Lower proportion of TUNEL positive cells was shown under IL-1β stimulation with agonist treatment relative to the control (P<0.05).
In-vivo **study**: (4) In the loaded, TRPV4 agonist-injected discs, radiographic disc height was significantly maintained compared to the control at 28 d after compression (P<0.05).

DISCUSSION: *In vitro*, the TRPV4 activation by agonist promoted autophagy and extracellular matrix synthesis through AMPK pathway under pro-inflammatory IL-1β stimulation. *In vivo*, intradiscal injection of TRPV4 agonist suppressed radiographic disc degeneration. The TRPV4 agonist could be a new therapeutic agent for intervertebral disc diseases via modulating autophagy.

SIGNIFICANCE: TRPV4 agonist is a potential new therapeutic agent for degenerative, inflammatory disc diseases.

REFERENCES:1. Urban JP et al. *Arthritis Res Ther*, 2003. **2.** Mizushima N et al. *Nature*, 2008. **3.** Takeoka Y et al. *Int J Mol Sci*, 2021. **4.** Takeoka Y et al. *Spine*, 2023. **5.** Hirata H et al. *J Orthop Res*, 2014.

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FIGURES:

Fig.1

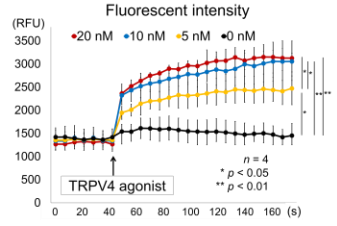

Fig.2

WB Rat disc NP cells in DMEM with 0% FBS (24 h)

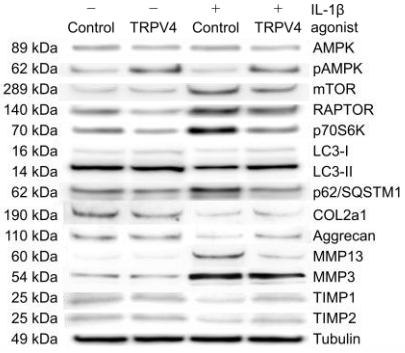

Fig.3

WB Rat disc NP cells in DMEM with 0% FBS (24 h)