Effect of Osmotic Stress via TRPV4 Activation on Autophagy and Matrix Synthesis in the Rat Intervertebral Disc

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INTRODUCTION:

Transient Receptor Potential Vanilloid 4 (TRPV4) is a non-selective cation channel responsive to mechanical, thermal, and osmotic stimuli[1)2)]. This study aimed to determine whether osmotic stress regulates extracellular matrix (ECM) metabolism in rat intervertebral disc cells through TRPV4-mediated AMPK activation, mTOR suppression, and autophagy enhancement.

METHODS:

Rat disc nucleus pulposus (NP) cells were cultured in serum-free medium at 200, 300, 400, 500, or 600 mOsm for 12, 24, and 72 h, with or without IL-1β stimulation. Protein expressions of TRPV4, AMPK, mTOR, p70S6K, LC3, p62/SQSTM1, MMP-3, MMP-13, TIMP-1, TIMP-2, Aggrecan, and type II collagen were assessed by Western blotting (WB). Gene expression of ECM-related markers was evaluated by RT-qPCR. TRPV4 involvement was confirmed using the TRPV4 antagonist GSK205. Cell viability was assessed by CCK-8. All the experiments used four independent samples (n=4).

RESULTS:

WB: In hyperosmotic conditions (≥400 mOsm), p-AMPK/AMPK ratio increased and the p-p70S6K/p70S6K ratio decreased at 24–72 h, indicating AMPK activation and mTOR suppression (P<0.05). LC3-II/LC3-I ratio increased and p62/SQSTM1 decreased (P<0.05), consistent with autophagy induction **(Figure1)**. Aggrecan, type II collagen, and TIMPs increased, whereas MMPs decreased, particularly at 72 h (P<0.05) **(Figure2)**. These effects were further accelerated with IL-1β stimulation. Meanwhile, TRPV4 antagonist suppressed osmolarity-dependent AMPK activation, mTOR inhibition, and autophagy (P<0.05).

RT-qPCR: The expression of anabolic genes (Aggrecan, type II collagen, TIMPs) increased, whereas the expression of catabolic genes (MMPs) decreased under the higher osmolarity conditions (P<0.05).

CCK-8 assay: Cell viability remained >80% at ≤500 mOsm but declined significantly at 600 mOsm (68.2%, 64.4%, and 64.2% at 24, 48, and 72 h, respectively).

DISCUSSION:

These findings suggest that hyperosmotic stress promotes ECM synthesis and autophagy in rat disc NP cells via TRPV4-mediated AMPK activation and mTOR suppression.

SIGNIFICANCE/CLINICAL RELEVANCE:

TRPV4-dependent responses to osmotic stimulation may represent a protective mechanism in the intradiscal homeostasis and TRPV4 is a potential therapeutic target for intervertebral disc degeneration.

REFERENCES: 1. Pedersen SF, *Cell Calcium*. 2005 **2.** Liedtke W, *Cell*. 2000

Figure 1

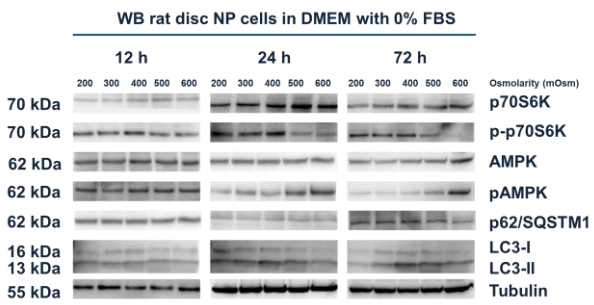

Figure 2