Tnfα Is Correlated With Trpv4 Expression In The Intervertebral Disc

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Introduction: The presence of pro-inflammatory cytokines is correlated with the degree of intervertebral disc (IVD) degeneration and associated with both the matrix breakdown and pain¹. The elevated catabolism induced by pro-inflammatory cytokines can alter both the osmotic and mechanical environment experienced by the native cells and likely contributes to the altered response to mechanical load seen in IVD degeneration². Pro-inflammatory cytokines have been shown to directly alter the mechanobiology of NP cells through modifying aquaporin expression³; however, it remains unclear if other more traditional mechano-sensing proteins, such as integrins or ion channels, are also influence by pro-inflammatory cytokines. The transient receptor potential vallinoid 4 (TRPV4) is a mechano-sensitive calcium permeable ion channel known to play a critical role in regulating the response to dynamic loading in chondrocytes⁴ and is also involved in mediating the response to pro-inflammatory cytokines in other cell types⁵. It is currently unknown if the TRPV4 ion-channel is expressed in the IVD; however, as the IVD experiences similar diurnal fluctuations in osmolarity as cartilage it is likely that TRPV4 may play an important role in mechanotransduction of IVD cells. The aims of this study were to investigate (i) whether the TRPV4 ion-channel is expressed in both human and bovine IVD tissue and (ii) determine whether TRPV4 & integrin α5 expression is influenced by the presence of TNFα in a large animal organ culture model. We hypothesized that TRPV4 is expressed within the IVD and that its expression is influenced by exposure to TNFα. These questions were probed using western blot, immunohistochemistry and a bovine IVD organ culture model.

Methods: TRPV4 expression: The expression of the TRPV4 ion channel was investigated via western blot on isolated bovine and human NP cells and immunohistochemistry on IVD tissue from both bovine caudal IVD and human lumbar IVDs. Western Blot: Bovine NP cells were isolated from 3 bovine tails and cultured in monolayer for 24hrs in standard culture medium (high glucose DMEM, 10% FBS, 50ug/mL ascorbic acid, 1% penicillin/streptomycin) at 5% CO2. Human NP cells were isolated from NP tissue obtained from autopsy and cultured in alginate beads for 72hrs in FBS-free culture medium. Permission was obtained for the use of cadaveric tissue prior to specimen procurement. Following culture, protein was isolated via lysis in RIPA buffer and a western blot specific for the TRPV4 ion channel (1:500 bs-6425R) and beta-actin (1:7500 ab8227) was conducted. Immunohistochemistry: Bovine caudal IVD and Human IVD tissue was fixed in zinc acetate, embedded in methy-methacrylate (MMA) and sectioned (5μm) as previously described⁶. Immunohistochemistry specific for the TRPV4 ion-channel (1:300, bs-6425R) was performed, with omission of the primary antibody used as a negative control. TNFα & TRPV4: To investigate if the presence of TNFα altered the expression of the integrin subunit α5 or the TRPV4 ion channel an organ culture model was used as it most closely models the naturally occurring microenvironment. Bovine IVDs with vertebral endplates retained were separated into two groups,
Dynamic Control (n=4) and Dynamic TNFα (n=4), and were cultured at 37°C & 5% CO2 for 7 days. All IVDs were loaded under dynamic loading (8hrs: 0-0.8MPa @ 0.1Hz & 16hrs of 0.2MPa static compression) using a custom organ culture loading device7. Control IVDs were cultured in standard culture medium and the TNFα group was cultured in control medium supplemented with human recombinant TNFα (100ng/mL). Following culture, tissue was taken for histology. Immunohistochemistry specific for TNFα (1:100, ab66579), integrin sub-unit α5 (1:500, ab112183), and the TRPV4 ion-channel was performed, with omission of the primary antibody used as a negative control. Ten images were captured at 20x within the NP region using an upright light microscope. The percentage of positively stained cells was calculated from each image using ImageJ software and averaged for each sample. A Pearson’s correlation coefficient was used to determine if there was a significant correlation between the percentages of TNFα & TRPV4 expressing cells and between the percentage of TNFα & α5 expressing cells.

Results: TRPV4 expression: There was positive staining for TRPV4 in bovine and human IVD tissue. TRPV4 staining was positive in all regions of the IVD; however, the cartilaginous endplate and the nucleus pulposus regions (Figure 1A&B) stained most prominently. Western blot confirmed that the TRPV4 ion channel was expressed by both bovine and human NP cells (Figure 1C). TNFα & TRPV4: After 7 days of organ culture there was positive staining for both the TRPV4 ion channel and the integrin subunit α5 in all samples (Figure 2A). There was a significant correlation (R²=0.69 / p=0.02) between the % TNFα positivity and the % TRPV4 positivity, however there was no correlation (R²=0.38 / p>0.05) between the % TNFα positivity and the % α5 positivity (Figure 2B).

Discussion: This study investigated (i) if the TRPV4 ion channel is expressed in IVD tissue and (ii) whether the presence of TNFα, a pro-inflammatory cytokine associated with IVD pathology, could alter expression of multiple mechano-sensing proteins. Results demonstrated that the TRPV4 ion channel is expressed in IVD tissue from multiple species, and also highlights that the bovine organ culture model is an appropriate model to investigate interactions between inflammation and mechanobiology within the IVD. Organ culture results demonstrated that there was a positive correlation between the percentage of cells expressing TNFα and TRPV4. This correlation suggested that exposure to TNFα may alter NP cells sensitivity to changes in the microenvironment to which TRPV4 is sensitive, such as diurnal changes in osmolarity and fluid shear. This work supports the hypothesis that inflammatory mediators can alter the way NP cells experience the mechanical environment and proposes another potential mechanism through which TNFα may induces changes in NP cell mechanobiology. As the TRPV4 ion-channel plays an important role in regulating the metabolic response to dynamic loading in chondrocytes future work is required to confirm a similar role in IVD cells. A mechanistic investigation of what factors regulate TRPV4 expression and whether increased TRPV4 expression translates to increased activity is ongoing.

Significance: Identifying the mechanisms by which NP cells sense their mechanical environment, and determining how their response to load is altered with disease will improve understanding of IVD cell mechanobiology and can elucidate potential therapeutic strategies. This works supports the hypothesis that pro-inflammatory mediators can influence the mechanobiology of NP cells through altering the expression of the TRPV4 osmotically sensitive ion channel.
Figure 1: Immunohistochemistry for TRPV4 in (A) bovine and (B) human IVD tissue. (C) Western Blot for TRPV4 in bovine and human NP cells. Scale bar = 50um
Figure 2: (A) Representative images of the TRPV4 and TNFα immunohistochemistry staining in the nucleus pulposus of the Dynamic Control and Dynamic TNFα groups. (B) Correlations between the percentages of TNFα and TRPV4 positive cells and the percentages of TNFα and alpha5 positive cells.