FUNCTIONAL CHARACTERIZATION OF TRPV4 AS AN OSMOTICALLY SENSITIVE ION CHANNEL IN ARTICULAR CHONDROCYTES

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Purpose: Transient receptor potential channel vanilloid 4 (TRPV4) is a calcium permeable cation channel that is gated by a number of factors, including osmolarity and temperature. Chondrocytes, the cells in articular cartilage, have been shown to perceive and respond to their osmotic and mechanical environments; however, the molecular basis of this signal transduction is not fully understood. The purpose of this study was to demonstrate the presence and functionality of TRPV4 in porcine articular cartilage.

Methods: TRPV4 protein expression was examined in porcine chondrocytes via Western blotting and immunostaining. TRPV4 mRNA was measured using RT-PCR. Calcium signaling in response to osmotic stress, TRPV4 agonist/antagonist, and various calcium signaling modifying agents was measured via fluorescent imaging of calcium-sensitive dyes. Cell volume change in response to osmotic stress, TRPV4 agonist/antagonist, and the inflammatory cytokine, IL-1, were measured from light microscopy images. Changes in prostaglandin E2 (PGE2), which is a downstream effect of increased internal calcium and IL-1, levels in response to osmotic stress and TRPV4 agonist/antagonist were measured with an immunocytoassay.

Results: TRPV4 was detected in porcine chondrocytes at the protein level. Addition of 4α-PDD, a TRPV4 agonist, caused calcium signaling, which was significantly blocked by the addition of ruthenium red (RR), a TRPV4 antagonist (Fig. 1). Calcium signaling was also inhibited by removal of extracellular calcium or blocking release from intracellular stores. Blocking TRPV4 with RR significantly modified the porcine cellular response to hypo-osmotic stress by decreasing the percentage of cells responding with a calcium signal. Addition of RR also significantly inhibited the volume recovery in response to hypo-osmotic stress, and 4α-PDD alone caused a slight volume increase (Fig. 2). TRPV4 activation was able to prevent the inflammatory cytokine IL-1 from inhibiting this volume regulation after exposure to hypo-osmotic medium (Fig. 2). Hypo-osmotic conditions caused PGE2 release which was blocked by the addition of RR (Fig. 3).

Conclusions: We have shown that TRPV4 is present in articular chondrocytes and that chondrocyte response to hypo-osmotic stress is mediated by TRPV4 activation, which leads to calcium signaling and protects against IL-1 mediated changes in cell volume.

Fig. 1: Percentage of cells responding to stimuli with increases in intracellular calcium. Asterisks denote experimental groups that are significantly different from control within a given osmolarity (chi-squared test, p < 0.05); number signs specify bars within the 380 mOsm group that are significantly different from 380+4α-PDDm (chi-squared test, p < 0.05). Both the 280 and 480 mOsm controls are significantly different from the 380 mOsm control (chi-squared, p < 0.05).

Fig. 2: Volume recovery (maximum-final) after stimulation with different osmotic and chemical stimuli. Bars are mean (+sem). Bars with different letters are significantly different from one another (ANOVA, p < 0.05).

Fig. 3: Mean (+sem) levels of PGE2 in culture medium after treatment with different osmolarities and TRPV4 agonists/antagonists. Asterisk indicates bar is significantly different from all others (ANOVA, p < 0.05).
TRISTETRAPROLIN: A PROSTAGLANDIN E2-RESPONSIVE BIFUNCTIONAL REGULATOR OF CYCLOOXYGENASE-2 EXPRESSION

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Purpose: Prostaglandins (PGs) are phospholipid-derived, hormone-like molecules that serve as homeostatic bioregulators and immune and inflammatory modulators. The rate-limiting enzyme in inducible PG biosynthesis is cyclooxygenase-2 (COX-2). Prostaglandin E2 (PGE2), the major COX-2 catalytic product, serves as a feedback regulator of COX-2 (and other pro- and anti-inflammatory) gene expression. Exogenous PGE2 increases primary human synovial fibroblast (HSF) COX-2 mRNA stability and translation; these effects are dependent on p38 MAPK-responsive cis-acting adenylate/uridylate-rich elements (AREs) in the COX-2 3'-untranslated region (3'UTR).

This study’s purpose is to characterize the molecular factors (i.e., ARE binding proteins, AUBPs) and mechanisms by which PGE2 increases COX-2 mRNA stability and translation in HSFs.

Methods: Gene reporter studies were conducted to screen three AUBPs expressed endogenously in HSFs and shown to bind the COX-2 3'UTR (i.e., AUF-1, HuR, Tristetraplin (TTP)) for their effect on COX-2 mRNA stability/translation. HSFs were cotransfected with a luciferase (Luc) coding for a different AUBP. Luc activity was then quantified by luminometry as a measure of both mRNA stability and translation; results were expressed relative to Luc activity in cells transfected with an empty AUBP vector (i.e., control). AUBP mRNA and protein expression was analyzed by Northern blotting (NB) and Western blotting (WB), respectively. Subcellular localization studies were performed using real-time confocal microscopy of TTP-GFP-transfected HeLa cells; results were confirmed biochemically by WB.

Results: TTP had the most marked and unambiguous effect on Luc-COX-2 3'UTR reporter activity in HSFs (55±15% decrease in Luc activity). HSF TTP mRNA levels were rapidly (20 min) and potently (3.5-fold vs. control) induced by TTP, but displayed a short half-life (1–2 h). Neither TTP-induced TTP transcription nor TTP mRNA decay was mediated by PGE2.

Interestingly, three TTP transcripts of 6.0, 4.0 and 2.2 kb were detected by NB, suggesting the possible existence of additional TTP isoforms and/or splice variants. In support of this possibility, WB analysis of HSF TTP revealed the presence of a second TTP immunoreactive isoform (TTP2, ~60 kDa). Exogenous PGE2 almost completely abolished TTP's nuclear export within 5 min; WB analysis revealed that TTP2 is the shuttling species. The presence of TTP2 is a TTP isoform or splice variant, NB analysis of TTP mRNA was reducing the levels of the known TTP protein (TTP1). To verify if TTP2 is a TTP isoform or splice variant, NB analysis of TTP mRNA was conducted using nuclear and cytosolic RNA extracts; only the 2.2 kb TTP transcript was detected in the cytosol, thus indicating that TTP2 is a post-transcriptionally modified form of TTP1. Analysis of TTP's subcellular localization in HSFs and HeLa cells revealed a predominantly (95%) cytosolic localization. PGE2 promoted TTP's nuclear export within 5 min; WB analysis revealed that TTP2 is the shuttling species. The presence of TTP2 in the nucleus and the fact that it is a zinc-finger protein led us to assess its capacity to influence COX-2 gene transcription. Gene reporter studies revealed that overexpression of TTP could transactivate the COX-2 promoter by ~2-fold.

Conclusions: TTP2 is a novel, uncharacterized post-translational variant of TTP1. Although the exact molecular composition and function of TTP2 are unknown, it appears that TTP2 exhibits distinct nuclear (i.e., transcription) and cytosolic (i.e., mRNA decay/translocation) functions with regards to COX-2 expression. PGE2's influence on TTP2's expression and subcellular distribution make it a strong candidate effector of PGE2-mediated transcriptional and post-transcriptional translational gene regulation.

Osteoarthritis and Cartilage Vol. 16 Supplement 4

MULTI-JOINT RADIOGRAPHIC OSTEARTHRITIS (rOA) PHENOTYPES AMONG AFRICAN AMERICANS (AA) AND WHITES: THE JOHNSTON COUNTY OSTEARTHRITIS PROJECT

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Purpose: Racial differences in OA prevalence have been demonstrated at individual joint sites, but whether there are racial differences in patterns of multi-joit OA involvement is unknown. The current study was designed to assess differences in multi-joit rOA phenotypes among AA and White individuals.

Methods: We conducted a cross-sectional analysis using data from the Johnston County Osteoarthritis Project (n = 1600, 67% women, 32% AA). Hand rOA was defined as (1) Kellgren-Lawrence (KL) grade ≥ 2 in at least 3 joints, including DIPs, PiPs, or CMCl; (2) at least 2 involved joints in the same joint group; (3) at least one involved DIP in digits 2–5; and (4) bilateral distribution. Tibiofemoral (TFJ) or hip joint rOA was defined as a KL grade ≥ 2, patellofemoral OA (PFOA) an osteoarthritis grade ≥ 2; and lombosacral spine rOA as an osteoarthritis grade ≥ 1 and disc space narrowing at the same vertebral level. Frequencies were calculated for all joint sites. Generalized estimating equations (GEE) were used to investigate racial differences in OA phenotypes simultaneously, adjusting for correlation among joints of the same individual. Logistic regression was used to assess multi-joit rOA phenotypes as separate outcomes (i.e. hand/knee, hand/hip) in a subset of individuals with complete data for rOA at the hand, TFJ, PFJ, hip, and knee (n = 834), with exact methods used for outcomes with small cell sizes. Analyses were also adjusted for age, gender, and BMI.

Results: The mean (SD) age of the sample was 63 (11) years. Overall, 27% of the participants had hand rOA, 33% had TFJ rOA, 49% had PFOA, 30% had hip rOA, and 49% had rOA of the lombosacral spine. In unadjusted analyses by race, Whites more frequently had any hand rOA (35% vs 11%, p < 0.0001) and rOA of the spine (52% vs 44%, p = 0.03); AAAs more frequently had TFJ rOA (39% vs 30%, p = 0.0003); and no racial differences were seen for hip or PFOA. Results were similar in adjusted analyses by race (using GEE) except that there was no racial association for spine involvement. In unadjusted analyses of multi-joit rOA phenotypes, compared to those with no rOA at any site (15% of the subset), AAs had significantly decreased frequencies of isolated hand or PFOA involvement compared to Whites, as well as less frequent combinations of joints including the hands (hand/TFJ, hand/hip, hand/TFJ/hip, Table). After adjustment, AAs were less likely to have any combination of involved joints that included the hands (hand only, hand/TFJ, hand/hip, or hand/TFJ/hip) compared to Whites. AAs had 50% increased odds of TFJ and hip involvement together, but this did not reach statistical significance (Table).

Conclusions: Multi-joit rOA phenotypes differ by race, with AAs more likely than Whites to have multiple large joint OA involvement. Definitions of generalized OA which emphasize hand involvement, commonly used in Whites, may not identify AA individuals with multi-joit OA.

Table 1: Unadjusted frequencies and adjusted odds ratios for multi-joit rOA phenotypes, by race

<table>
<thead>
<tr>
<th>Joint site(s)</th>
<th>AA n=231 (%)</th>
<th>White n=603 (%)</th>
<th>Unadjusted OR</th>
<th>Adjusted OR*†</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>50 (22)</td>
<td>78 (13)</td>
<td>0.001</td>
<td>0.05 (0.32)</td>
</tr>
<tr>
<td>Hand only</td>
<td>0 (0)</td>
<td>33 (6)</td>
<td>1.00</td>
<td>1.00 (1.00)</td>
</tr>
<tr>
<td>TFJ only</td>
<td>19 (8)</td>
<td>22 (4)</td>
<td>0.409</td>
<td>1.45 (0.64–3.28)</td>
</tr>
<tr>
<td>PFJ only</td>
<td>35 (15)</td>
<td>116 (19)</td>
<td>0.004</td>
<td>0.61 (0.35–1.07)</td>
</tr>
<tr>
<td>TFJ/PFJ only</td>
<td>10 (4)</td>
<td>10 (2)</td>
<td>0.354</td>
<td>1.39 (0.56–4.48)</td>
</tr>
<tr>
<td>Hand/TFJ</td>
<td>20 (9)</td>
<td>114 (19)</td>
<td>0.001</td>
<td>0.28 (0.14–0.57)</td>
</tr>
<tr>
<td>Hand/hip</td>
<td>4 (2)</td>
<td>23 (4)</td>
<td>0.016</td>
<td>0.34 (0.07–1.25)</td>
</tr>
<tr>
<td>TFJ/hip</td>
<td>58 (25)</td>
<td>77 (14)</td>
<td>0.520</td>
<td>1.92 (0.88–3.61)</td>
</tr>
<tr>
<td>Hand/TFJ/hip</td>
<td>24 (10)</td>
<td>101 (17)</td>
<td>0.001</td>
<td>0.40 (0.19–0.85)</td>
</tr>
</tbody>
</table>

*p value for AA vs White, unadjusted frequencies, †aOR for AA vs White, adjusted for age, gender, and BMI, † by exact logistic model due to small cell count