Expression of toll-like receptors in cartilage endplates cells: a role of toll-like receptor 2 in pro-inflammatory and catabolic gene expression

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1. Background

The vertebral cartilage endplate (CEP), vital for intervertebral disc health, is prone to degeneration, linked to chronic low back pain, disc degeneration, and Modic changes. While intervertebral disc cells express toll-like receptors (TLRs) to trigger an immune response, it's uncertain if CEP cells (CEPC) do the same. CEPC, with their higher cell density compared to the disc, are of particular interest.

2. Aims

1. to identify the presence of TLRs and their effect on downstream genes in cartilage endplate cells (CEPCs)
2. to compare the expression of TLRs and downstream activated genes on CEPC from Modic type 1 changes (MC1), MC2 or degenerated non-Modic change (nonMC) cartilage endplates.

3. Methods

RNA was isolated either directly from CEP tissue of patients undergoing spinal fusion surgery or after expansion and treatment in vitro. The treatments consisted of TNF-α, TLR2/6, TLR2/1 ligands, TLR4 ligand, FNf30 for 48h. Specificity of TLR2/Pam2csk4 was tested using the TLR2 inhibitor TL2-C29. qPCR quantified gene expression (TLRs, IL-6, IL-8, MMP1, MMP2, MMP3, MMP9, MMP13). Flow cytometry and microscopy was used to identify TLR2 on a protein level.

4. Results

All TLRs (TLR1-10) were detected when RNA was directly isolated from CEP tissue (Figure 1). TLR7 and TLR8 were lost upon expansion of the cells in vitro (data not shown).

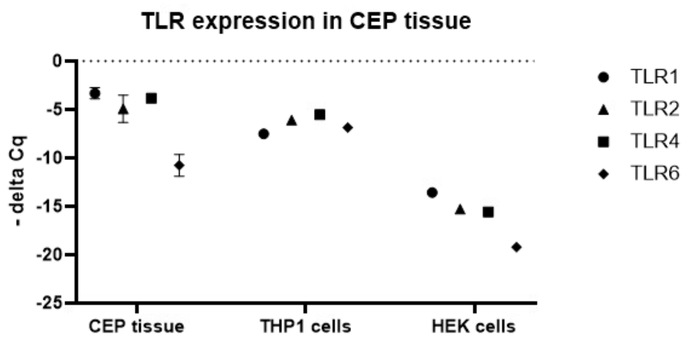

Figure 1. Surface TLRs were measured in CEP tissue, THP1 cells (positive control) and HEK cells (negative control).

Stimulation with TNF-α, Pam2csk4, Pam3csk4, LPS, and FNf30 significantly increased only TLR2 expression, implying its role in inflammatory conditions. Additionally, they upregulated MMP1, MMP3, MMP9, and MMP13, indicating TLR2 signaling triggers degenerative changes (Figure 2).

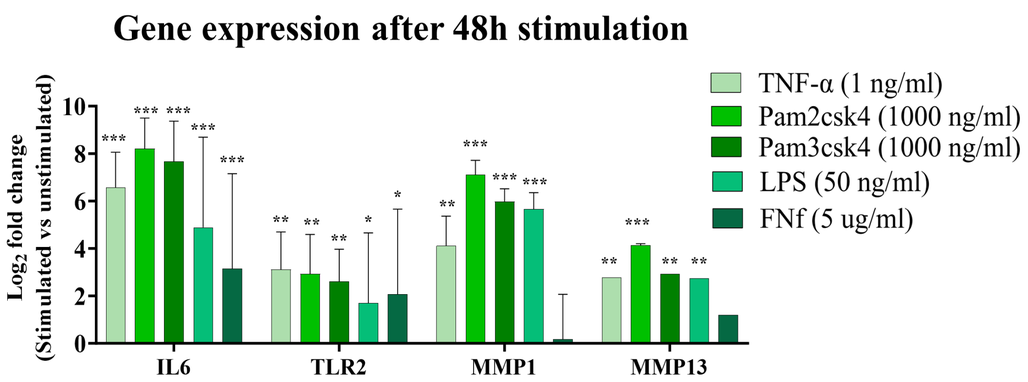

Figure 2. Surface TLRs were measured in CEP tissue, THP1 cells (positive control) and HEK cells (negative control).

Specificity of TLR2/6 ligand Pam2csk4 was tested using TLR2 inhibitor TL2-C29. A concentration dependent inhibition with TL2-C29 was found of previously shown upregulated genes (Figure 3).

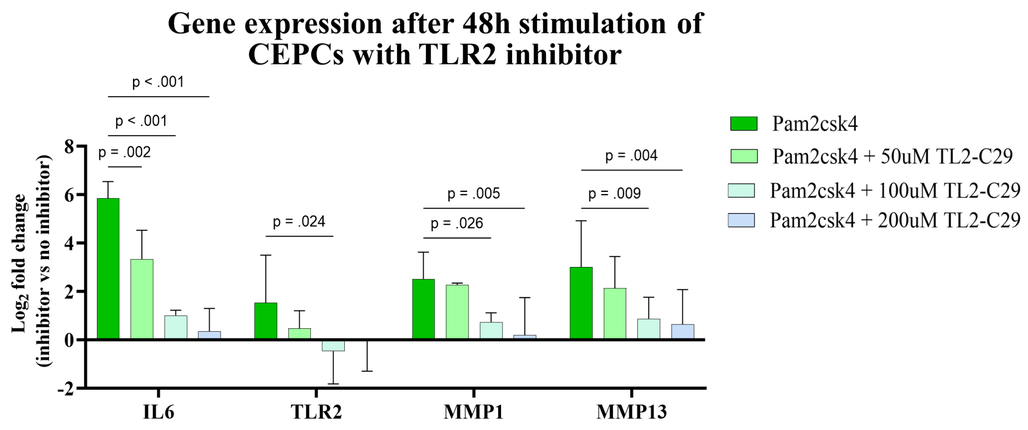

Figure 3. TL2-C29 treatment of the cells prior to Pam2csk4 stimulation caused concentration independent inhibition of genes as measured by qPCR. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison testing.

Flow cytometry showed a significant increase of TLR2 upon Pam2csk4 but not upon Pam3csk4 stimulation (Figure 4), highlighting the potential significance of TLR2/6 signaling. Fluorescent staining of surface TLRs on CEPC further verified their presence (Figure 5).

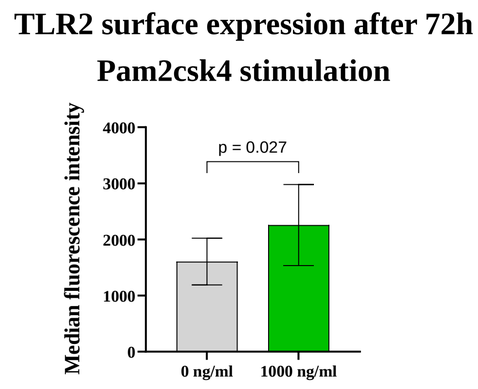

Figure 4. Flow cytometry measurement of TLR2 on CEPCs after 72h incubation with Pam2csk4.

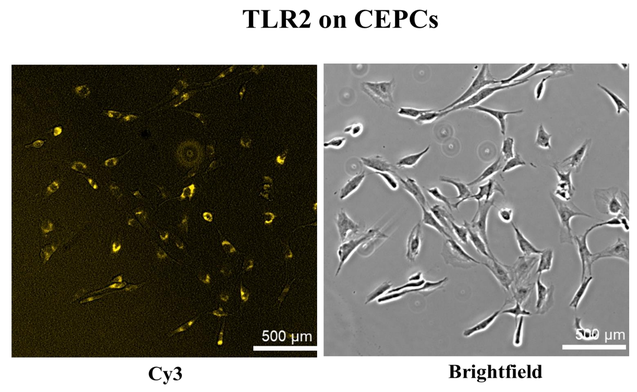

Figure 5. Microscopy images of TLR2 after 48h Pam2csk4 stimulation.

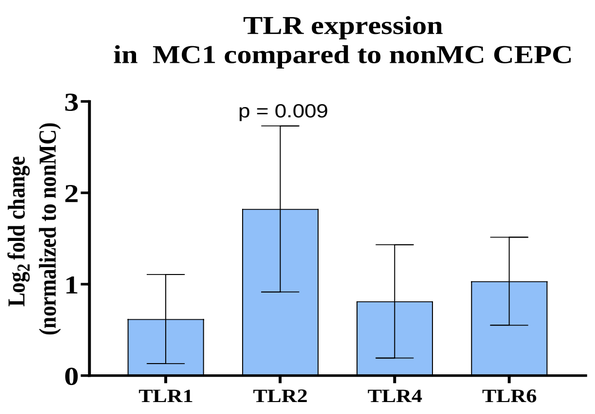

Figure 6. TLR expression ,measured by qPCR on CEPC compared using multiple t-tests on log_2 fold changes.

Grouping of CEPC into MC1 and nonMC found a significantly higher TLR2 expression (p = 0.029) and slightly higher TLR6 expression (p = 0.070) in MC1 CEPC (Figure 6).

5. Conclusion

- CEPC express TLRs
- TLR2 is increased in MC1 CEPC
- Inflammation increases expression of TLR2, enhancing responsiveness to TLR2 ligands
- TLR activation on CEPC induces the release of pro-inflammatory cytokines and MMPs.

TLRs, particularly TLR2, on CEPC may play a pivotal role in both degenerative disc disease and Modic changes, indicating new potential therapeutic targets.

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