TLR2 signaling of cartilage endplate cells amplifies inflammation in Modic type 1 changes

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1. Background

Modic Changes (MCs) in vertebral bone marrow are closely tied to cartilage endplate (CEP) damage and accelerated disc degeneration. Discs near MCs release heightened inflammatory cytokines, hastening degeneration, yet the causal link remains unclear. This study explores the presence of Toll-like receptors (TLRs) in CEP cells, investigating their potential role in propagating inflammatory signals from the disc to the bone marrow.

2. Aims

(i) to identify the presence of TLRs and their effect on downstream genes in cartilage endplate cells (CEPCs)

(ii) 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

CEPCs from degenerated discs (6 nonMC, 4 MC1, 4 MC2) were enzymatically isolated from spinal fusion surgery patients' cartilage endplate tissue. Cells were expanded using collagenase P in Dulbecco's modified eagle medium with 10% fetal calf serum, 5% penicillin-streptomycin, and 5% HEPES. They were treated with inflammatory stimuli (TNF-α, TLR2/6, TLR2/1 ligands, TLR4 ligand, FNf30) for 24h or 48h. Pam2csk4 specificity was tested with TLR2 inhibitor TL2-C29. qquantitative real-time polymerase chain reaction measured gene expression (TLRs, IL-6, IL-8, MMP1, MMP2, MMP3, MMP9, MMP13). Statistical analysis used a mixed-effects model and multiple comparisons. TLR2 surface expression was measured with flow cytometry and analyzed with paired t-tests in GraphPad Prism v10.0.2.

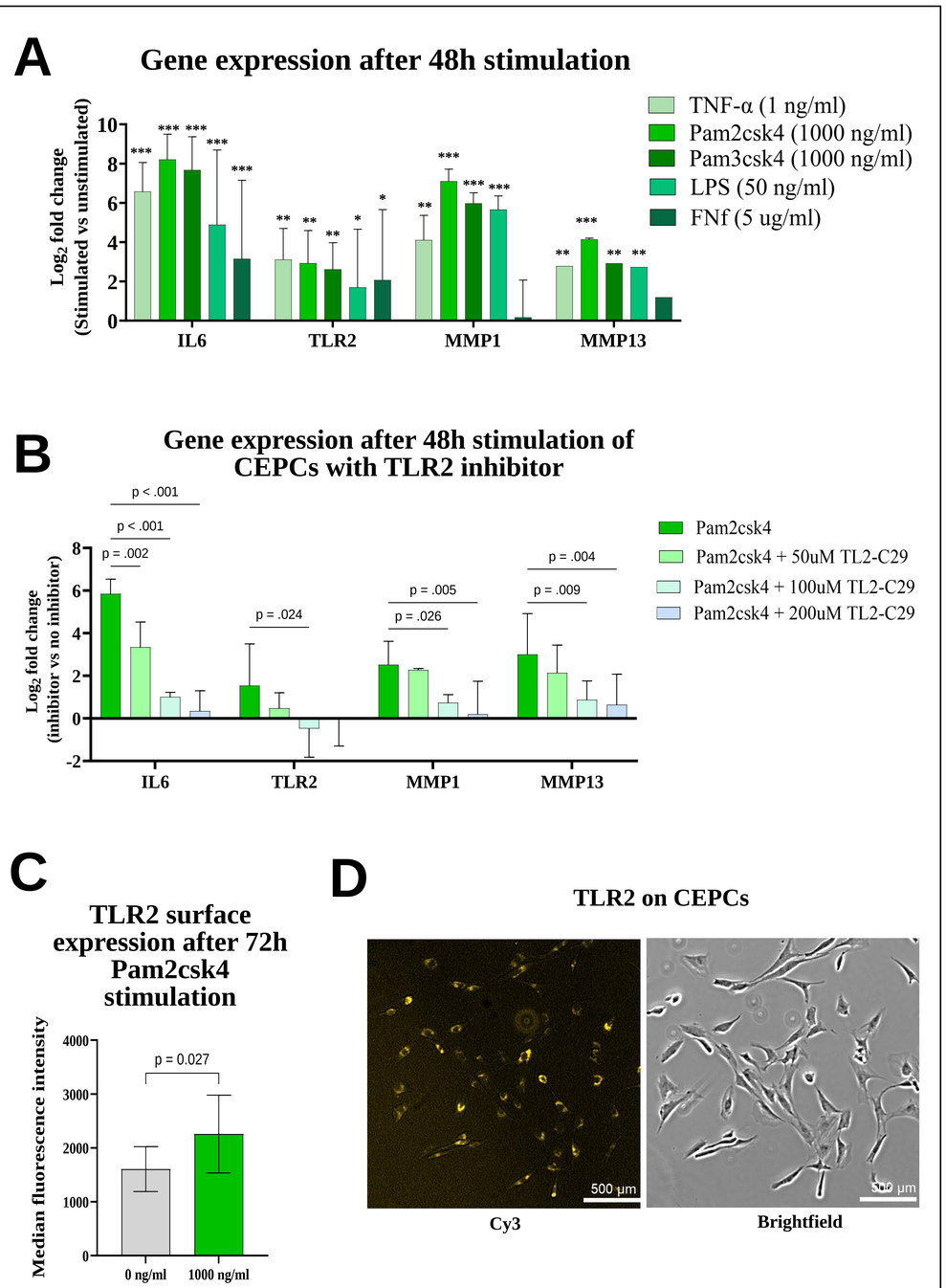

Figure 1. (A) Gene expression of CEPCs treated with TNF-a, Pam2csk4, Pam3csk4, LPS and FNf30 shown as log2 fold change to unstimulated. **(B)** Inhibition of gene expression upregulation by Pam2csk4 through TLR2 inhibitor. **(C)** Flow cytometry measurement of TLR2 on CEPCs after 72h incubation with Pam2csk4. **(D)** Microscopy images of TLR2 after 48h Pam2csk4 stimulation.

4. Results

Untreated CEPCs exhibited gene expressions for all TLRs except TLR8 and TLR9. Stimulation with TNF-α, Pam2csk4, Pam3csk4, LPS, and FNf30 significantly increased only TLR2 expression (Figure 1A), implying its role in inflammatory conditions. Pam2csk4 and Pam3csk4 also upregulated MMP1, MMP9, and MMP13, indicating TLR2 signaling triggers degenerative changes (Figure 1A). TL2-C29 concentration-dependently inhibited the upregulation of TLR2-responsive genes (except MMP9), confirming TLR2 mediation (Figure 1B). Flow cytometry showed a significant increase in TLR2 on the cell surface upon Pam2csk4 stimulation (p = 0.006) (Figure 1C), highlighting the potential significance of TLR2/6 signaling. Fluorescent staining of surface TLRs on CEPC further verified their presence (Figure 1D).

CEPCs were grouped into nonMC, MC1, and MC2 (n = 6 + 4 + 4). MC1 CEPC had significantly higher TLR2 expression (p = 0.029) and slightly higher TLR6 expression (p = 0.070) than nonMC CEPC (Figure 1D). Pam2csk4 stimulation upregulated TLR2 expression in MC1 almost double as much as in nonMC CEPC (p = 0.076, fold change = 3.92 ± 0.84) (Figure 1E).

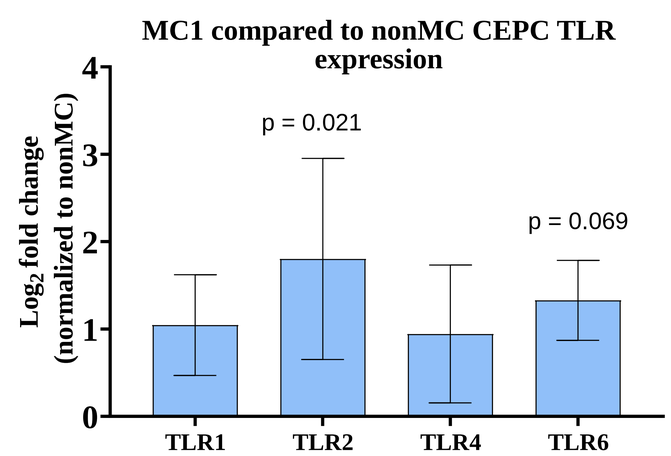

Figure 2. Comparison of TLR expression of CEPCs of MC1 compared to nonMC without prior stimulation.

5. Conclusion

This study showed that CEPCs, especially in MC1, express TLRs, notably TLR2. CEPCs can induce TLR2-mediated inflammation, and in an inflammatory environment, TLR2 and TLR6 expression rises, amplifying responsiveness to TLR2/6 ligands. This mechanism may significantly amplify inflammation in degenerated discs. Targeting TLR2 emerges as a potential strategy to impede MC1 development adjacent to degenerated discs.