Lorecivivint (SM04690), a Potential Disease-Modifying Osteoarthritis Drug, Inhibits CLK2 and DYRK1A, Novel Molecular Regulators of Wnt Signaling, Chondrogenesis, and Inflammation

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Background

- Upregulated Wnt signaling affects osteoarthritis (OA) pathogenesis by increasing inflammation, subchondral bone formation, and thinning cartilage[1]
- Lorecivivint (LOR), a novel small molecule, has demonstrated OA disease-modifying properties through Wnt pathway inhibition *in vitro* and *in vivo*[1,2]
- The mechanism of action of LOR leading to Wnt pathway inhibition, chondrocyte differentiation, and anti-inflammatory activity is described

Methods

In vitro

- Wnt pathway inhibition was assessed by luciferase reporter assay in SW480 colon cancer cells
- A kinome screen (318 kinases) was performed
- LOR effects on phosphorylation of proteins in human mesenchymal stem cells (hMSCs), chondrocytes, 293T cells, and synovial fibroblasts were measured by Western blot
- LOR effects on splicing were measured in hMSCs by RNA sequencing and PCR
- LOR and siRNA knockdown effects on hMSC Wnt pathway and chondrogenic gene expression were measured using nCounter® panels and qPCR

In vivo

- LOR effects were confirmed in rat knee OA models: **(1) surgical:** anterior cruciate ligament transection with partial medial meniscectomy (ACLT+pMMX) and **(2) inflammatory:** monosodium iodoacetate injection-induced knee OA model (data not shown)

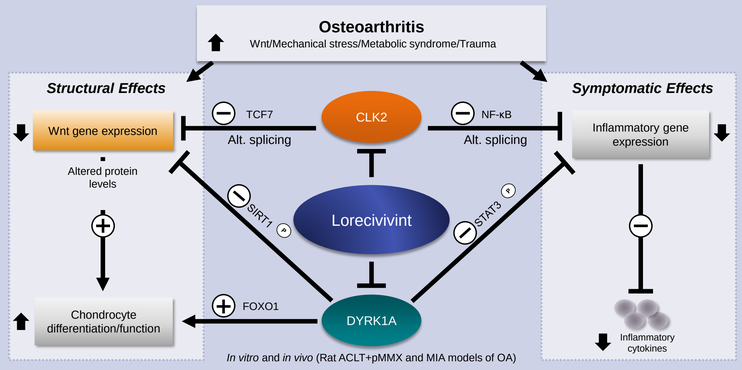
Statistical analyses: One-way ANOVA (multiple groups) and t-tests (two groups)

Conclusions

In vitro* and *in vivo

- LOR inhibited intranuclear kinases CLK2 & DYRK1A, leading to Wnt pathway inhibition
- Inhibition of CLK2 induced early chondrocyte differentiation from hMSCs and inhibition of DYRK1A enhanced chondrocyte function
- Inhibition of STAT3 phosphorylation and NF-κB expression by LOR provided potent anti-inflammatory effects
- Through dual inhibition of CLK2 and DYRK1A, LOR protected cartilage, induced chondrogenesis, and reduced inflammation, supporting its potential for modifying disease and improving signs and symptoms in knee OA

Representation of the dual mechanism of action of lorecivivint



STAT3: signal transducer and activator of transcription 3, SIRT1: sirtuin 1, TCF7: transcription factor 7, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, FOXO1: forkhead Box O1

Results

LOR: A potent inhibitor of the Wnt pathway, CLK2, and DYRK1A *in vitro*

- Luciferase reporter assay identified LOR as an inhibitor of Wnt signaling (IC_{50} = 11 nM). A kinome screen identified CDC-like kinases (CLK2, IC_{50} = 5.8 nM) and dual-specificity tyrosine kinase (DYRK1A, IC_{50} = 26.9 nM) as molecular targets of LOR

Figure 1. LOR inhibited SRSF proteins, Sirt1, and FoxO1 phosphorylation

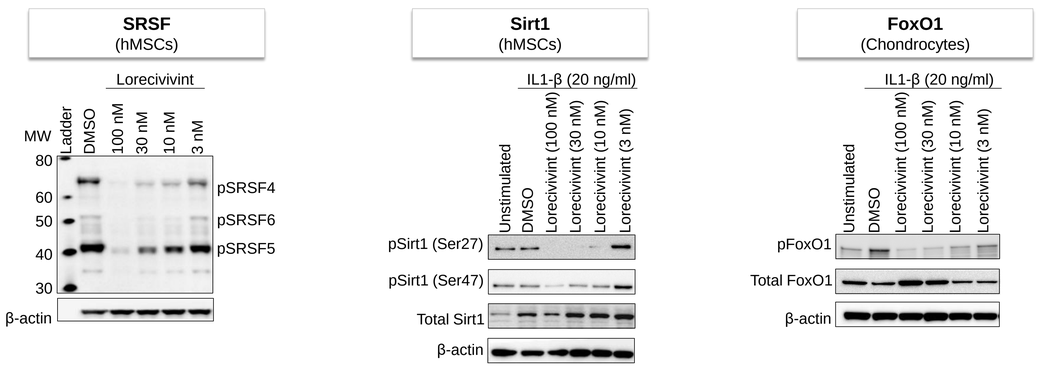


Figure 1. LOR treatment of hMSCs and chondrocytes resulted in decreased phosphorylation of SRSFs, Sirt1, and FoxO1 compared to DMSO

Figure 2. LOR modulated alternative splicing *in vitro*

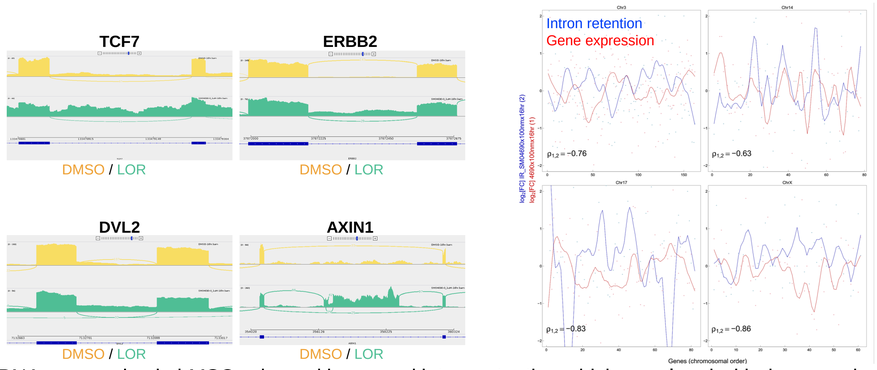


Figure 2. LOR RNA sequencing in hMSCs showed increased intron retention which correlated with decreased gene expression *in vitro*

Figure 3. LOR modulated the Wnt pathway independently of β-catenin

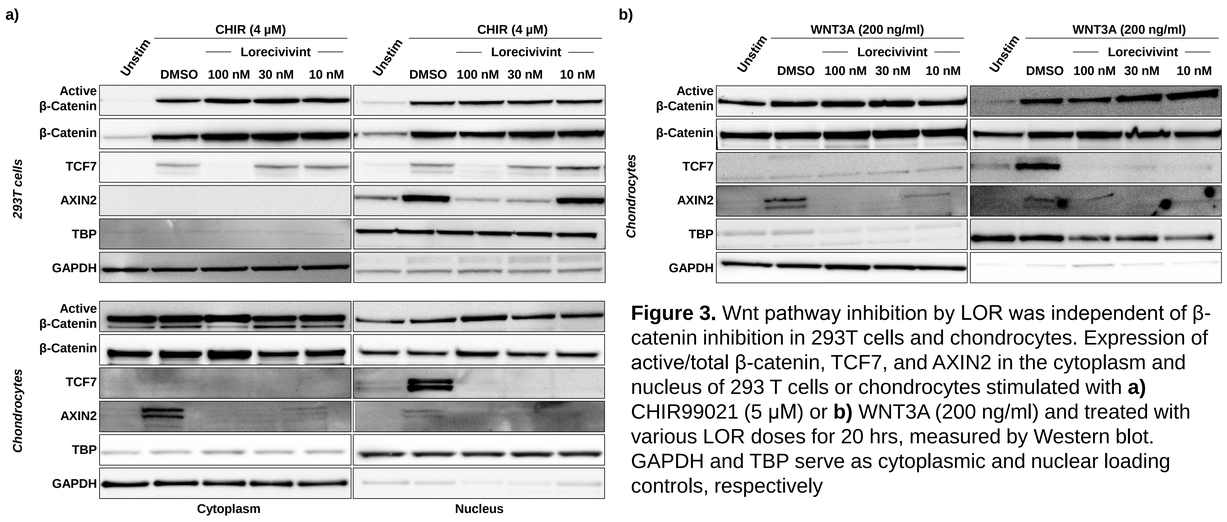


Figure 3. Wnt pathway inhibition by LOR was independent of β-catenin inhibition in 293T cells and chondrocytes. Expression of active/total β-catenin, TCF7, and AXIN2 in the cytoplasm and nucleus of 293 T cells or chondrocytes stimulated with **a)** CHIR99021 (5 μM) or **b)** WNT3A (200 ng/ml) and treated with various LOR doses for 20 hrs, measured by Western blot. GAPDH and TBP serve as cytoplasmic and nuclear loading controls, respectively

Figure 4. Inhibition of CLK2 and DYRK1A reduced Wnt pathway gene expression

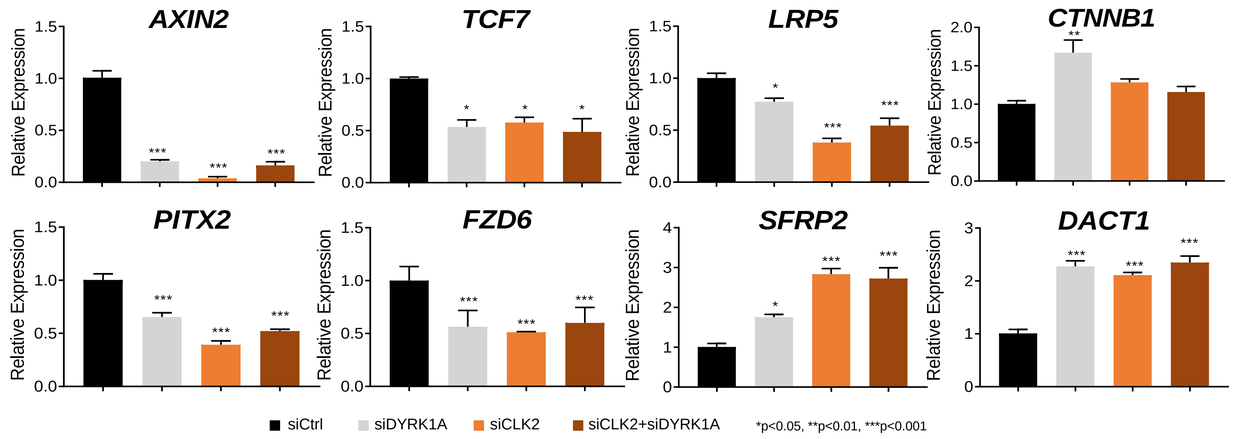


Figure 4. CLK2 and DYRK1A knockdowns led to inhibition of Wnt pathway genes including *AXIN2, TCF7, LRP5, PITX2, CTNNB1,* and *FZD6* and upregulation of secreted Wnt inhibitors *SFRP2* and *DACT1* compared to siRNA controls

Figure 5. Inhibition of CLK2/DYRK1A induced chondrocyte differentiation

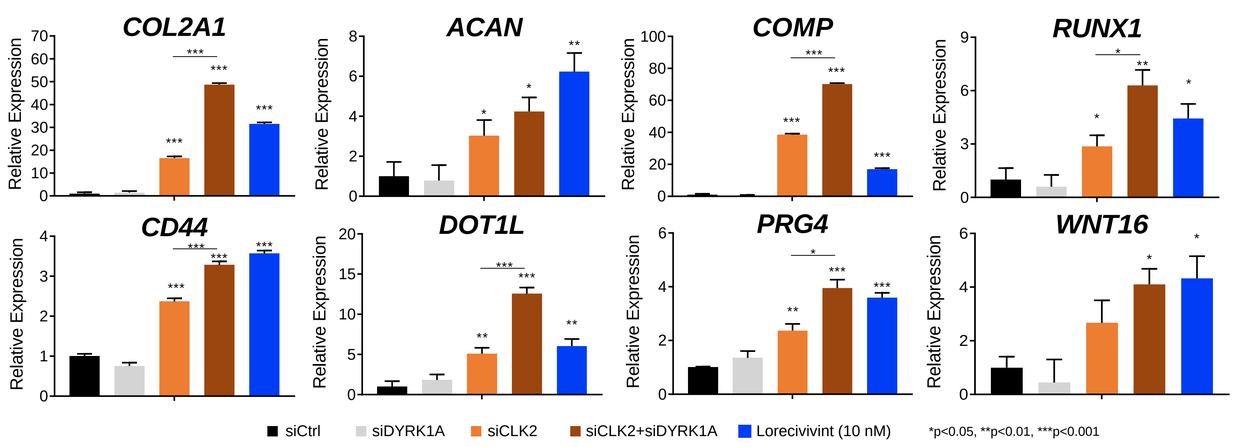


Figure 5. Combined CLK2/DYRK1A knockdown and LOR demonstrated increased expression of chondrocyte genes (*COL2A1, ACAN, COMP, CD44, DOT1L, PRG4, RUNX1,* and *WNT16*) compared to siRNA control or siCLK2 alone

Inhibition of TCF7 induced chondrocyte differentiation (data not shown)

- TCF7 knockdown increased chondrogenic genes (*COMP, SOX9,* and *RUNX1*, but not *RUNX2*) compared to siRNA controls
- LEF1, TCF4, or β-catenin knockdowns did not lead to chondrocyte differentiation

Figure 6. LOR reduced inflammation via inhibition of CLK2 and DYRK1A

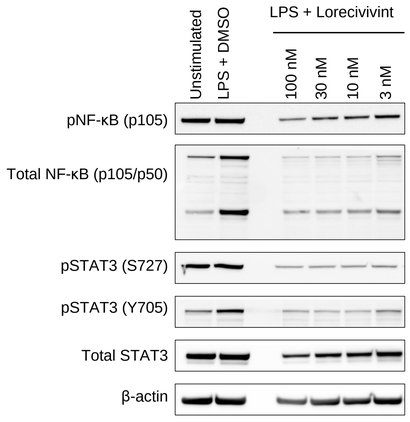


Figure 6. LOR treatment of synovial fibroblasts resulted in decreased phosphorylation of NF-κB and STAT3 compared to DMSO

References: 1. Deshmukh V, et al. *Osteoarthritis Cartilage.* 2017. 2. Deshmukh V, et al. *Osteoarthritis Cartilage.* 2019.

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