Establishing of IL-1β-Induced In Vitro Osteoarthritis Model for Investigating Ferroptosis Inhibition using CRISPR Technology

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INTRODUCTION:

Osteoarthritis (OA) is a degenerative joint disease characterized by cartilage degradation and inflammation, with interleukin-1 beta (IL-1β) playing a key role in its pathogenesis. Recent studies have implicated ferroptosis, a form of regulated cell death driven by lipid peroxidation, in the progression of OA. Acyl-CoA synthetase long-chain family member 4 (ACSL4) is involved in the metabolism of polyunsaturated fatty acids (PUFAs) and has been suggested to play a role in lipid peroxidation and ferroptosis (1). Establishing reliable in vitro OA models by stimulating human chondrocyte cells with IL-1β is crucial to determine if ferroptosis in OA also occurs alongside the usual cell death (apoptosis) and identifying potential therapeutic targets by silencing ACSL4 using CRISPR to reduce lipid peroxidation-induced ferroptosis.

MATERIALS & METHODS:

Primary human chondrocytes were treated with IL-1β to assess cell viability using the AlamarBlue assay and to examine morphological changes. CRISPR-Cas9 technology was constructed by insertion the single guide RNA (sgRNA) targeting ACSL4 into the plasmid.

RESULTS:

Primary human chondrocytes isolated from patients were successfully cultured and characterized. Treatment with IL-1β resulted in a decrease in cell viability with 10 ng/ml IL-1β showing 85% viability at 48hr. Morphological analysis revealed a decrease in the density of chondrocytes, and changes in cell morphology. Successful insertion of sgRNA targeting ACSL4 into the plasmid was confirmed by sanger sequencing, enabling specific targeting of the ACSL4 gene.

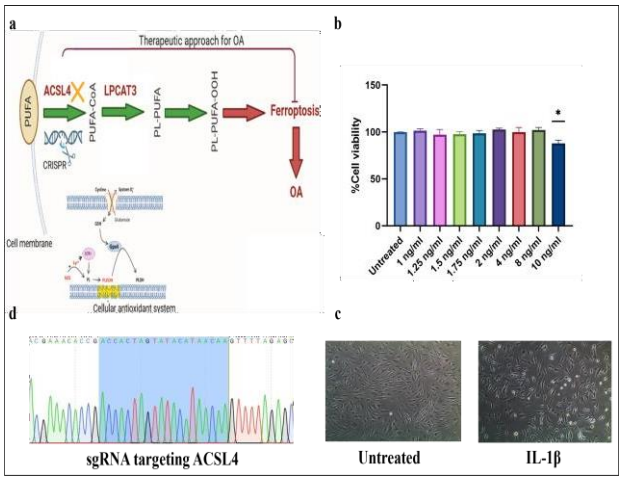

Figure 1: Overview of (a) proposed therapeutic approach for osteoarthritis (OA), (b-c) IL-1β treatment and (d) validation of single guide RNA (sgRNA) construction into the px458 plasmid.

DISCUSSIONS:

Our findings suggest that IL-1β induces an OA-like phenotype in human chondrocytes, as indicated by cell viability and morphology. The successful insertion of sgRNA targeting ACSL4 into the plasmid demonstrates the feasibility of using CRISPR technology to study the ferroptosis in OA pathogenesis.

CONCLUSION:

This model and approach provide valuable tools for further investigating of ferroptosis in OA mechanisms and potential therapeutic interventions.

REFERENCES:

1. Hunter, D. J., March, L., & Chew, M. (2020). Osteoarthritis in 2020 and beyond: a Lancet Commission. The Lancet, 396(10264), 1711-1712.