Continuous Low-Intensity Ultrasound Reprograms Fibronectin Fragment–Activated Macrophages Toward a Reparative Phenotype

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INTRODUCTION

Macrophages are central regulators of inflammation and tissue repair, capable of shifting between pro-inflammatory (M1) and anti-inflammatory (M2) states depending on their environment. In post-traumatic osteoarthritis (PTOA), fibronectin fragments (Fnfs) generated from cartilage degradation drive persistent M1 activation, contributing to chronic inflammation and tissue damage. Continuous low-intensity ultrasound (cLIUS) has emerged as a potential non-pharmacological approach to modulate immune responses and promote healing, but its effects under physiologically relevant inflammatory conditions remain unclear.

OBJECTIVE

To investigate whether cLIUS can modulate Fnfs-induced macrophage activation by reducing pro-inflammatory responses and promoting an M2-like reparative phenotype, using transcriptomic analysis and differential clustering to identify both gene-level changes and coordinated transcriptional remodeling.

MATERIALS AND METHODS

- THP-1-derived macrophages were encapsulated in 3D alginate hydrogels and polarized to M1 with Fnfs (25 µg/mL, 72 h).
- Experimental groups received cLIUS stimulation (5 MHz, 14 kPa, 20 min × 4 times/day, 3 days).
- Viability was assessed by Live/Dead confocal imaging. RNA was isolated (n=3 replicates/group) for RNA-seq and qRT-PCR validation.
- Differential expression (DESeq2) and differential clustering analyses were performed, followed by Reactome pathway and enrichment analyses.

HYPOTHESIS

We hypothesize that cLIUS enhances STAT6 activation in macrophages, which in turn upregulates M2-associated genes and drives macrophage polarization toward a reparative, M2-like phenotype.

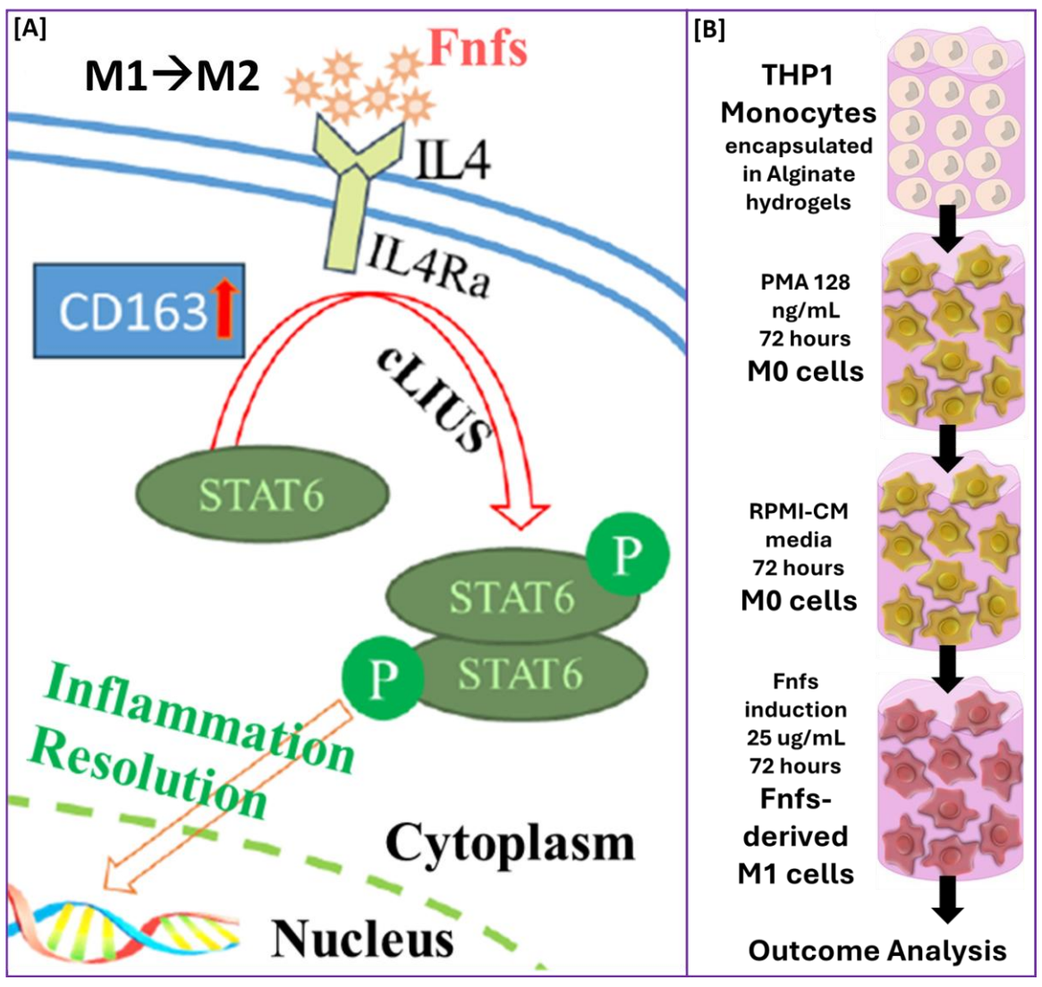

Figure 1: [A] Conceptual design illustrating cLIUS mediated inflammation resolution. [B] Schematics for Fnfs-derived M1 cells from THP1 cells in hydrogel

RESULT

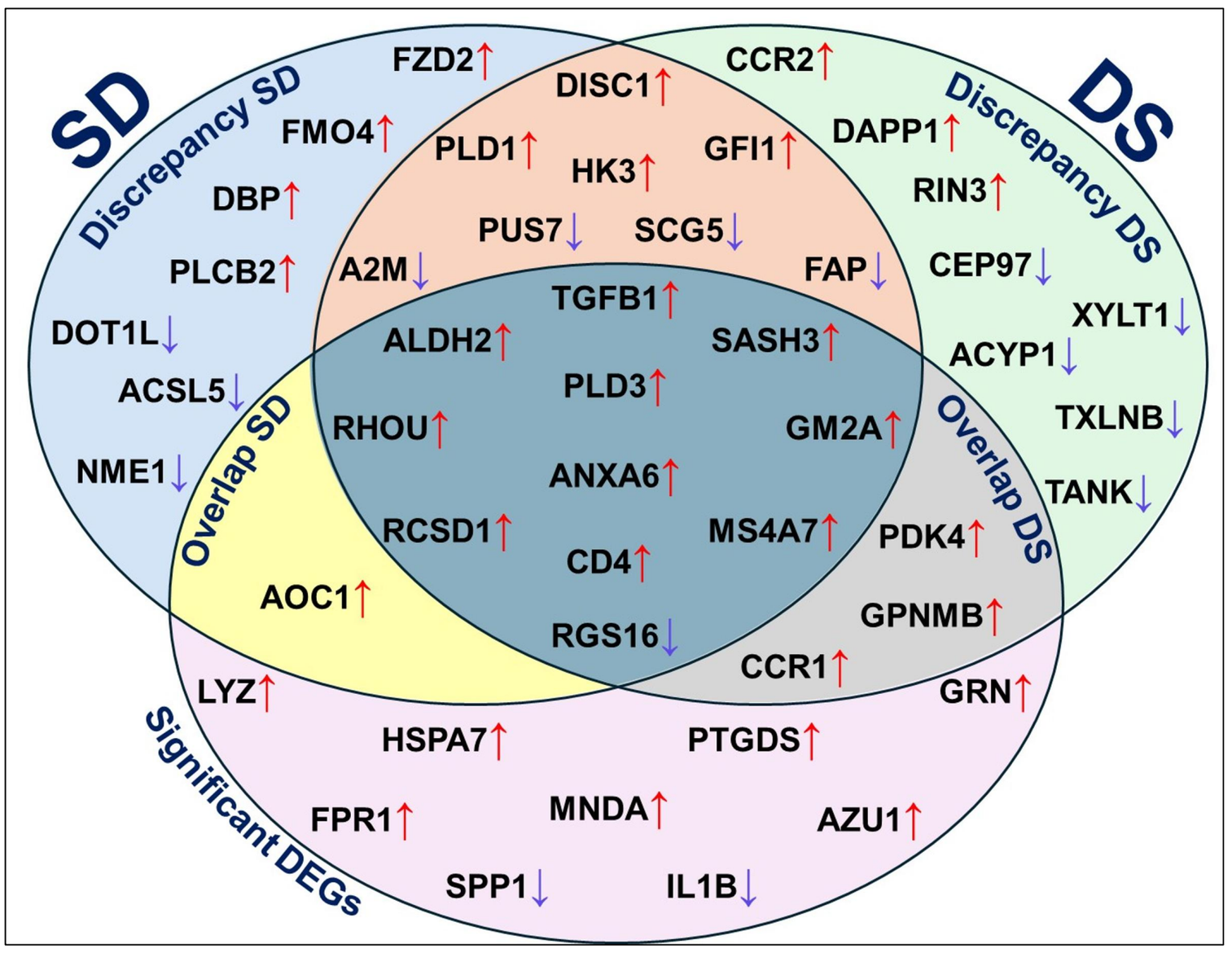

Figure 2. Venn diagram showing overlap of DEG, SD, and DS gene sets. The shared intersection (DEG ∩ SD ∩ DS) represents the most robust candidate genes.

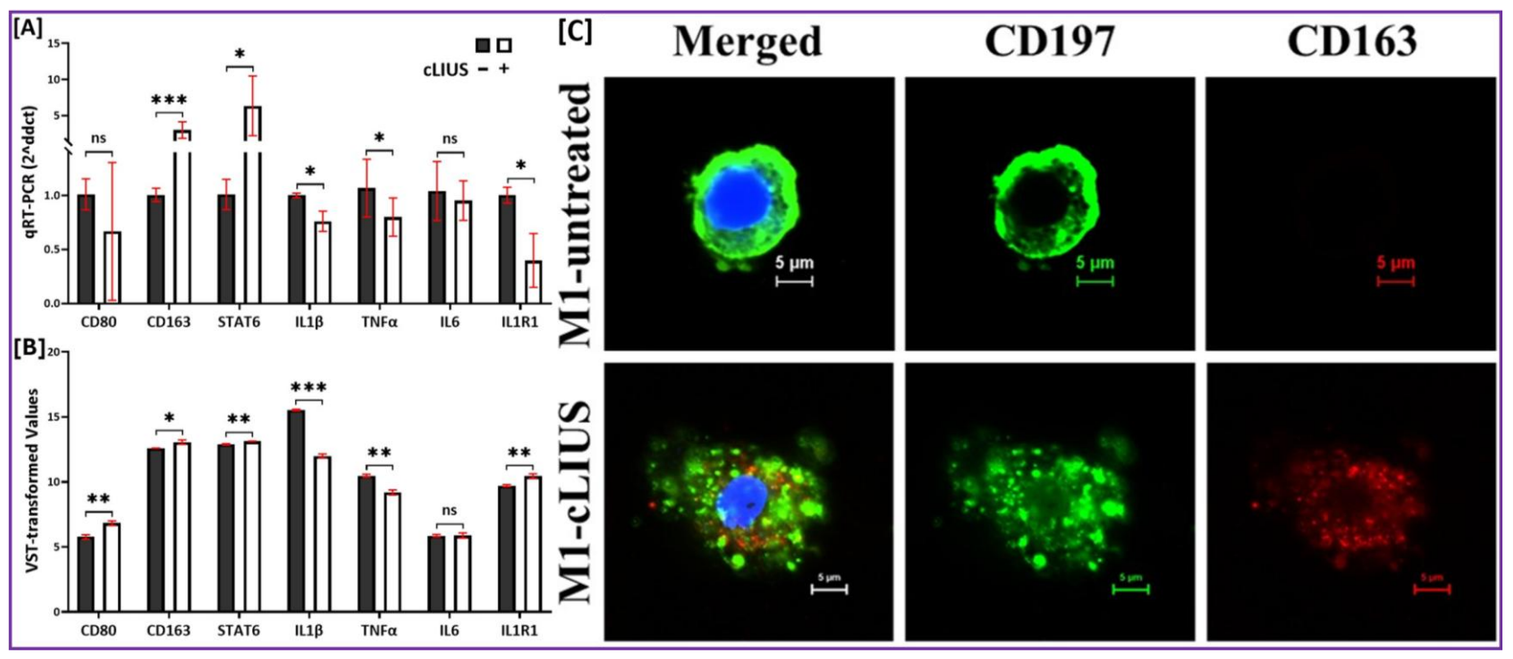

Figure 3. Validation of selected markers by [A] qRT-PCR alongside [B] VST-transformed RNA-seq values. [C] IF images showing M1 marker (CD197) and M2 marker (CD163) in M1 untreated and cLIUS treated cells.

SIGNIFICANCE

- This is among the first studies to evaluate cLIUS as a biophysical modality for inflammation resolution using a transcriptomics-first approach, providing a high-resolution view of global reversal in inflammatory gene programs.
- Mechanical stimulation via cLIUS shifts Fnfs-induced macrophages toward an M2-like reparative phenotype.

ACKNOWLEDGEMENT

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