

NLRP3 inflammasome suppresses enthesis regeneration through inflammatory and metabolic macrophages-stem cells crosstalk

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INTRODUCTION: Injuries to the enthesis, including rotator cuff tears (RCT) and anterior cruciate ligament injuries, are prevalent among the elderly and physically active individuals. Unfortunately, due to the limited regenerative capacity of the enthesis, the intricate structure and function cannot be fully restored post-injury, resulting in an increased risk of reinjury. Therefore, identifying key factors that impede enthesis regeneration is critical in the field of regenerative medicine. Growing evidence indicates that inadequate enthesis regeneration is strongly associated with the pathological pro-inflammatory niche, characterized by an overabundance of pro-inflammatory macrophages and cytokines, as well as stem cells with diminished regenerative abilities. NLRP3 inflammasome, which is typically activated in immune cells such as macrophages following tissue injury, serves as a crucial bridge between inflammation and tissue regeneration. Upon activation, the NLRP3 inflammasome triggers the release of pro-inflammatory cytokines, including IL-1 β and IL-18, through Gasdermin D (GSDMD)-mediated pyroptosis. Beyond its role in cytokine production, the NLRP3 inflammasome has been implicated in reprogramming cellular metabolism and modulating the release of metabolites that influence regeneration. Nevertheless, the precise role of the NLRP3 inflammasome in orchestrating inflammation and mediating crosstalk between macrophages and stem cells during enthesis repair and regeneration is still unclarified.

METHODS: This study was approved by the Ethical Committee of Shanghai Sixth People's Hospital (Shanghai, China, 2023-KY-171). A total of 6 patients (3 men and 3 women, aged 60-75) diagnosed with full-thickness RCT via magnetic resonance imaging were enrolled in this research. None of the participants indicated any infectious or rheumatoid arthritis. Enthesis remnants on the great tuberosity were collected during arthroscopic rotator cuff repair. Furthermore, hamstring tendons were also collected from 6 other patients (4 men and 2 women, aged 24-41) undergoing anterior cruciate ligament reconstruction and served as healthy controls. The animal experimentation was approved and supervised by the Animal Care and Use Committee of Shanghai Sixth People's Hospital (Animal Experiment Registration number: DWSY2023-0003). 216 (108 male and 108 female) NLRP3 knockout mice (*Nlrp3*^{-/-}) were used to assess the impact of NLRP3 inflammasomes axis on enthesis regeneration. RNA-sequencing, single-cell RNA-sequencing, and untargeted metabolomics were utilized to explore the mechanism of NLRP3 inflammasomes activation on enthesis regeneration. IL-1 β neutralizing antibodies and docosatrienoic acid were employed to investigate the role of IL-1 β and docosatrienoic acid in enthesis regeneration.

RESULTS SECTION: Human tom tendons exhibited significant CD68⁺ macrophage infiltration, along with a significant presence of NLRP3⁺ cells. RNA-sequencing indicated genes related to NLRP3 inflammasome (*Nlrp3*, *Casp1*, *P2rx7*) were upregulated, whereas those related to enthesis regeneration (*Tnmd*, *Gli1*, *Ihh*, *Col2a1*, *Colla2*) were downregulated. NLRP3 inflammasomes were activated in macrophages following enthesis injury, impairing the histological and functional recovery of the injured enthesis. Single-cell RNA-sequencing indicated that *Nlrp3* knockout attenuated pathological inflammation and ameliorated the detrimental effects of IL-1 β signaling crosstalk. Furthermore, untargeted metabolomics revealed that NLRP3 inflammasomes suppressed the secretion of docosatrienoic acid (DTA) that boosts mesenchymal stem cells proliferation. Neutralizing IL-1 β or replenishing docosatrienoic acid accelerated enthesis regeneration.

DISCUSSION: This study elucidates that NLRP3 inflammasome inhibits enthesis regeneration through exacerbating IL-1 β inflammatory crosstalk and inhibiting pro-regenerative metabolic crosstalk between macrophages and stem cells, which may offer promising therapeutic avenues for the treatment of heterogeneous interface tissue injuries.

SIGNIFICANCE/CLINICAL RELEVANCE: This study is rooted in clinical observations, and is significant as it reveals the critical role of NLRP3 inflammasome in enthesis regeneration. More importantly, this study revealed a target that possesses both anti-inflammatory and regenerative effects, which may be superior to commonly used clinical drugs such as glucocorticoids and non-steroidal anti-inflammatory drugs that exert negative effects on tissue regeneration.

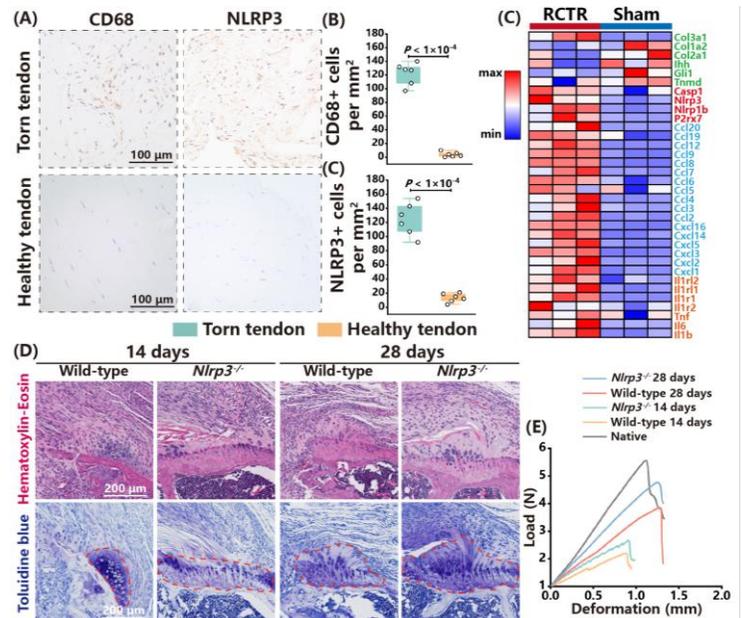


Figure1. NLRP3 inflammasomes are activated at the injured enthesis and impair the histological and functional recovery of the injured enthesis.

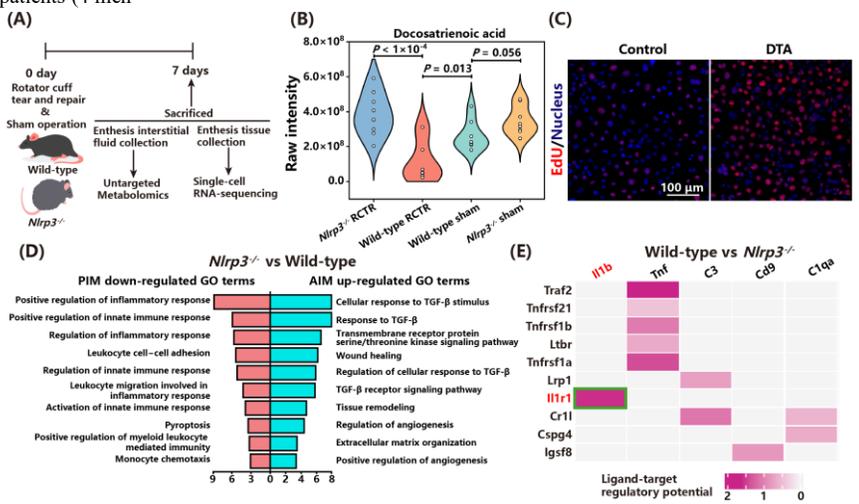


Figure2. NLRP3 inflammasomes exacerbating IL-1 β inflammatory crosstalk and inhibiting pro-regenerative metabolic crosstalk between macrophages and stem cells.