

Down-regulation of TGF-β1 in fibro-adipogenic progenitors initiates muscle ectopic mineralization

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Introduction:

Previously we have demonstrated that stress response-induced high glucocorticoid levels could be the underlying cause of traumatic heterotopic ossification (HO) [1]. However, it is not known how glucocorticoids initiate muscle ectopic mineralization (EM) at a cellular and molecular level. It is well known that HO formation is associated with inflammation [2]. But it is counterintuitive to learn that glucocorticoids, usually regarded as immune suppressants, can cause a hyperinflammatory state leading to muscle EM formation. Therefore, glucocorticoids must target other cell types whose regulatory roles can override those of inflammatory cells during muscle regeneration. This study thus aims to explore the regulatory role of fibro-adipogenic progenitors (FAPs) in glucocorticoid-induced EM.

Methods:

Glucocorticoid-induced EM model was created by systemic administration of a high dose of dexamethasone (DEX) daily for 7 days to mice with muscle injury induced by cardiotoxin (CTX) injection. A PDGFRα-creERT: tdTomato reporter mouse line was used to trace FAPs during muscle regeneration and enable sorting out FAPs for gene expression analysis. A PDGFRα-creERT: TGF-β1^{flox/+} transgenic mouse model was utilized to specifically knock out the *TGF-β1* gene in PDGFRα-positive FAPs.

Results:

We showed that DEX treatment inhibited inflammatory cell infiltration into CTX-injured muscle, but inflammatory cytokine production in the muscle was significantly increased (Figure 1). Accompanying this phenotype, *TGF-β1* gene expression in FAPs was greatly down-regulated, while a number of categories related to the inflammatory response and inflammatory cell chemotaxis were enriched in FAPs after DEX treatment (Figure 2). Transgenic mice that specifically knocked out the *TGF-β1* gene in FAPs exhibited a hyperinflammatory state demonstrated by increased inflammatory cell infiltration into the injured muscle (Figure 3B), and spontaneously developed EM following muscle injury as evidenced by microCT imaging (Figure 3C) and Alizarin Red staining (Figure 3D).

Discussion:

The regulatory role of FAPs in muscle regeneration and maintaining muscle homeostasis is gaining increasing attention [3]. In this study, we demonstrated that DEX treatment down-regulated TGF-β1 expression in FAPs, which led to a hyperinflammatory state both in FAPs and in the injured muscle. By specifically knocking out the *TGF-β1* gene in FAPs, the transgenic mice completely phenocopied this hyperinflammatory state and spontaneously developed EM following muscle injury. Thus, we proved that FAP-derived TGF-β1 is a key molecule in regulating muscle inflammatory

response and subsequent EM, and that glucocorticoids exert their effect via down-regulating TGF-β1 in FAPs.

Significance:

Our results provided a mechanistic explanation for how glucocorticoids induce HO, and shed light on therapeutic strategies for treating muscle EM, i.e., enhancing TGF-β1 signaling in FAPs to inhibit the excessive inflammatory response.

References:

[1] Bone. 2020 July; 163:115355. [2] Curr Osteoporos Rep. 2019 Dec;17(6):387-94. [3] Open Biol. 2021 Dec;11(12):210110.

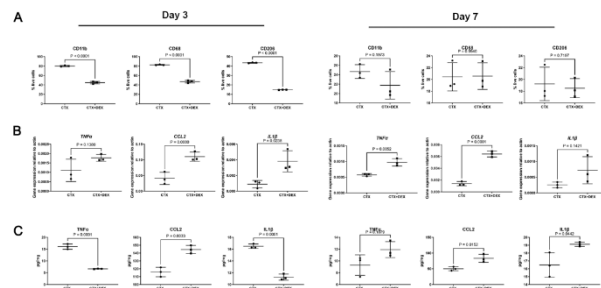


Figure 1. DEX treatment caused a hyperinflammatory state after muscle injury. (A) Flow cytometry analyses of macrophage numbers in muscle tissues in CTX only control group and DEX treatment group at Day 3 and Day 7 time points after DEX treatment. (B) Muscle tissue PCR analysis of inflammatory cytokine mRNA levels. (C) Muscle tissue ELISA analysis of inflammatory cytokine protein levels.

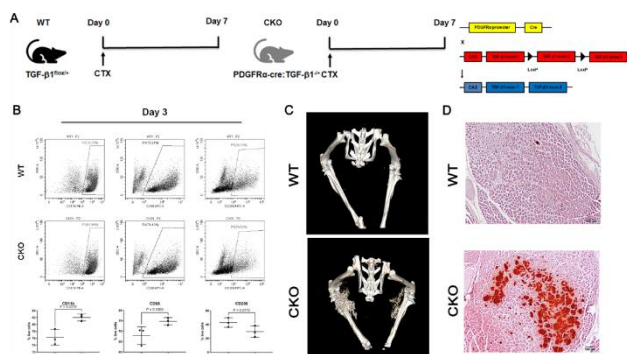


Figure 3. FAPs *TGF-β1* gene conditional knock-out mice spontaneously developed EM following muscle injury. (A) Schematic of experimental design using FAPs *TGF-β1* gene conditional knock-out mice: PDGFRα-creERT: *TGF-β1*^{flox/+} (CKO) mice and sex-matched *TGF-β1*^{flox/+} littermates (WT) were given only CTX injection. (B) Flow cytometry analyses of macrophage numbers in muscle tissues in WT and CKO mice 3 days after CTX injection. (C) microCT (C) and Alizarin Red staining (D) demonstrating EM formation in CKO mice but not in WT mice 7 days after CTX injection.

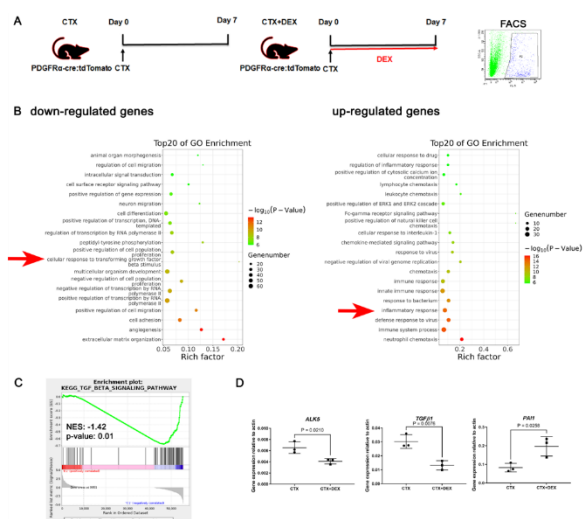


Figure 2. TGF-β1 expression in FAPs was greatly down-regulated after DEX treatment. (A) Schematic of experimental design using PDGFRα-creERT: tdTomato reporter mice. Representative FACS image of sorting out tdTomato positive FAPs for cell sequencing. (B) GO, (C) GSEA (C) and PCR (D) analysis of tdTomato-positive FAPs from CTX only control group and DEX treatment group.