

Metastatic Trauma: How Systemic Injury Hijacks The Healing Program Of Skeletal Muscle

Albert Cordoves¹, Alexander Clinton¹, Stephen Chenard¹, Benjamin Asbury¹, Rachel McKee¹, Stephanie Moore-Lotridge PhD¹, Jason Park MD PhD², Jonathan Schoenecker MD PhD¹

¹Department of Orthopaedic Surgery, Vanderbilt University Medical Center, Nashville TN.

²Department of Otolaryngology, Vanderbilt University Medical Center, Nashville TN.

INTRODUCTION:

Orthopaedic surgeons have long recognized that severe trauma can impair healing at anatomically distant sites, suggesting systemic rather than localized pathophysiology. However, it remains unclear whether trauma merely amplifies inflammation or fundamentally reprograms the regenerative machinery of distant musculoskeletal tissues. This study compared normal muscle regeneration after isolated injury with the altered healing response that occurs when the same injury is accompanied by a severe systemic insult.

METHODS:

Eight-week-old male C57BL/6 mice (n = 6 per group) were used to eliminate hormonal variability between sexes. A localized muscle injury was induced in the gastrocnemius via cardiotoxin (CTX) injection to model normal regeneration. To simulate polytrauma, a second group received the same CTX injury plus a 30% total body surface area dorsal burn (CTX + Burn). Imaging by X-ray and micro-CT evaluated dystrophic calcification (DC) within injured muscle. At 7 days post-injury, gastrocnemius muscles were harvested for RNA extraction and bulk RNA-seq. Raw counts were processed with DESeq2 following limma-based batch correction to control for sequencing variance. Principal component analysis confirmed clear group segregation prior to differential testing. Differential expression was analyzed (adjusted p < 0.05; |log₂ FC| > 1.0), and Gene Ontology (GO) enrichment identified pathways altered by systemic trauma. All procedures were approved by the institutional IACUC.

RESULTS:

Mice with CTX + Burn injury developed pronounced dystrophic calcification within the injured muscle, while CTX-only animals demonstrated normal regeneration without mineral deposition. Differential expression testing (adjusted p < 0.05) revealed a broad transcriptomic shift, including upregulation of inflammatory mediators (*S100a8*, *S100a9*, *Il1b*, *Nlrp3*), coagulation and antifibrinolytic genes (*Fga*, *Fgb*, *Serpine1*), and fibrotic regulators (*Tgfb1*, *Colla1*, *Col3a1*, *Acta2*). GO enrichment indicated activation of neutrophil degranulation, cytokine signaling, extracellular-matrix organization, and TGF-β-driven fibrosis, with concurrent suppression of myogenic and metabolic pathways essential for regeneration. This coordinated inflammatory and fibrotic induction suggests an early failure of resolution signaling within injured muscle. Findings were consistent across biological replicates and supported by independent visualization of key transcripts within the dataset.

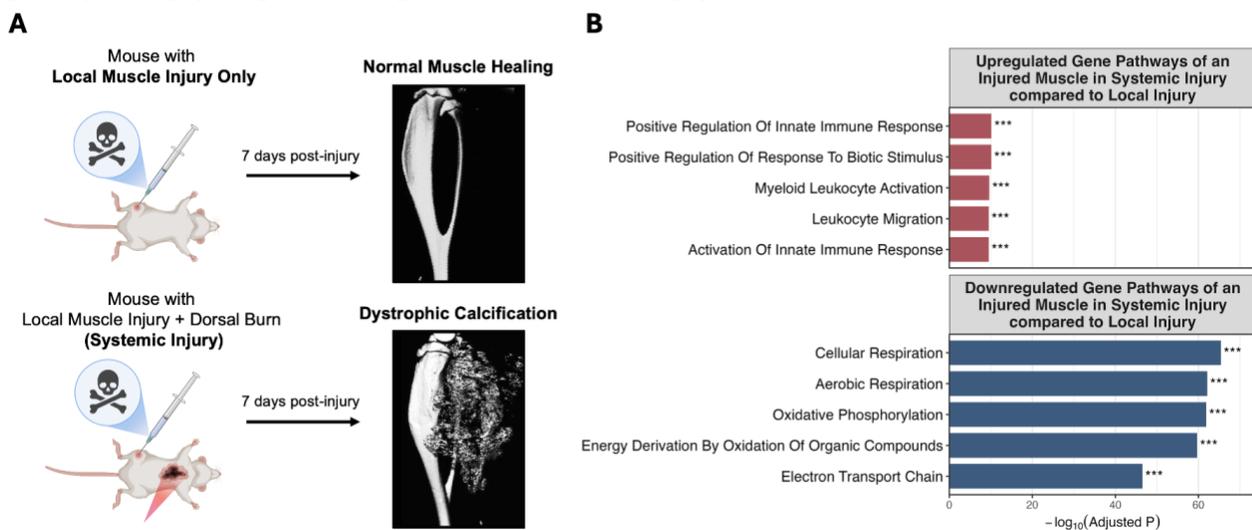
DISCUSSION:

The addition of a remote burn converted a normally regenerative muscle injury into a fibrotic, pro-calcific phenotype. These findings demonstrate that systemic trauma does not simply heighten inflammation, it fundamentally rewires the gene-expression program governing muscle repair, shifting it from regeneration toward chronic fibrosis and mineralization. The resulting transcriptional signature reflects a sustained activation of innate immune and matrix-producing cells, hallmarks of a failed resolution phase. This early dystrophic calcification represents the incipient stage that precedes heterotopic ossification observed in chronic trauma. This model establishes a tractable platform to dissect how systemic inflammatory cues reprogram local tissue repair at early post-injury time points.

SIGNIFICANCE / CLINICAL RELEVANCE:

This study provides the first transcriptomic evidence that severe trauma acts as a systemic disease process capable of reprogramming distant tissue repair. By defining how polytrauma transforms the genetic landscape of muscle healing, these findings establish a mechanistic foundation linking major injury to impaired musculoskeletal regeneration and highlight potential molecular targets to prevent trauma-induced fibrosis and subsequent heterotopic ossification. Together, these findings redefine severe injury as a programmable biological event rather than a local complication, reframing trauma as a systemic disease of failed regeneration.

Figure 1. Systemic injury reprograms the healing response of a remote muscle injury.



(A) Schematic and micro-CT reconstructions 7 days post-injury. Mice with isolated cardiotoxin (CTX) injury showed normal regeneration, whereas those receiving CTX + a 30% total-body-surface-area dorsal burn (CTX + Burn) developed dystrophic calcification (DC) within the injured muscle. (B) Gene Ontology (GO) Biological Process enrichment of batch-corrected RNA-seq data (DESeq2, |log₂FC| > 1.0) shows up-regulation of inflammatory and innate-immune pathways and down-regulation of oxidative and myogenic programs. Enrichment significance determined by Benjamini-Hochberg-adjusted Fisher's exact test; *** adjusted p < 0.001.