differentiate into adipocytes and showed colocalization with perilipin and adipocyte morphology.

Conclusions: Our results suggest that Hoxa11+ stem cells differentiate through endochondral ossification into heterotopic bone after injury, highlighting that local cells within the hind limb form the majority of HO bone, rather than circulating progenitor cells. Additionally, this lineage tracing system can be a useful tool to study skeletal stem cells and tendon pathology within the zeugopod.

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Post-Traumatic Limb Immobilization Alters Mesenchymal Stem Cell Fate

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Purpose: Traumatic heterotopic ossification (HO) is a debilitating condition where aberrant bone is formed outside the skeleton due to a fate switch of tissue resident mesenchymal/progenitor cells (MSCs). HO can occur after extremity trauma, burns, and extremity surgeries including amputations and joint replacements. No effective preventive strategies exist as the underlying mechanisms have not been elucidated. Though HO forms at sites of mechanical stress, the role of joint mobilization during extremity trauma, healing, and HO formation has not been clearly defined. We hypothesize that movement is central to HO formation via mechanotransductive signaling, and can provide a basis for improving post-trauma guidelines to prevent HO.

Methods: HO was induced in mice via a dorsal partial thickness burn with concomitant Achilles tenotomy (B/T). Single cell RNA (scRNA) sequencing was performed prior to injury and on 3, 7, and 21 days post-B/T tissue using 10X genomics and downstream analysis with Seurat R package. scRNA sequencing was performed on immobilized mice 7 days post B/T and compared to that of mobile mice at the same time point. Scores were generated for each cell based on correlations with either osteogenic or adipogenic gene signatures in MSCs from mobile or immobilized mice. B/T was performed in mice of 4 groups (n=3/group): forced run, exercised passive range of motion (ROM), ambulated normally (mobile), or immobilized, and hindlimb bone volume was assessed at 9 weeks post-B/T by MicroCT (uCT). Immunofluorescent (IF) labeling for PDGFRα and pFAK, TAZ, or Perilipin-1 was done on 1 week cross sections and quantified (n=3/group).

Results: Single cell clustering showed there are 15 unique clusters, 3 of which are MSC populations with increased expression of mechanotransductive markers such as *Ptk2* (FAK), *Yap1* (YAP) and *Wwtr1* (TAZ). Joint immobilization of the ankle completely inhibited HO formation, therefore, the comparison of mobile and immobile mice was explored. Histology of immobilized mice demonstrated there is decreased mechanotransductive signaling (pFAK and nuclear TAZ) compared to mobile group. Interestingly, we noted increased adipocytes in the immobilized group at 1 week. Comparing scRNA sequencing revealed that MSCs (clusters 2, 3, and 14) from immobile mice correlated with an adipogenic signature compared to mobile MSCs that favored osteogenesis. This finding suggests a cell fate shift towards adipogenesis with joint immobilization.

Conclusion: Hindlimb immobilization plays a significant role altering mechanotransductive pathways which we demonstrate results in an shift in MSC differentiation programming from endochondral ossification to adipogenesis. Immobilization protocols should be considered in patients at high HO risk.

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Reactions of Fibrotic Skin To Fat Grafting In A Rodent Model

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Purpose: The chronic cutaneous fibrosis associated with scleroderma is a significant source of morbidity for patients