immunodepletion was assessed by fluorescence-activated cell sorting (FACS) analysis of peripheral blood. After 4 weeks, 30 Gy was delivered to the right hindlimb in five fractionated doses to generate limb contracture. The irradiated, contracted limb was then grafted with 200 μl fresh human lipoaspirate and limb extension was measured over the subsequent 8 weeks, at which point skin was harvested for assessment of fibroblast subtypes for FACS and immunofluorescence. A group of mice with radiation-induced groin contracture did not undergo fat grafting and served as the control group.

Results: FACS analysis indicated successful immunodepletion and engraftment by 3 weeks post bone marrow transplantation. At one month following groin irradiation, mice had developed significant right hind limb contracture with significantly reduced limb extension (***(p≤0.0001). Histologically this was paralleled by thickening of the dermis, and substantial expansion of the fibrogenic Prx-1-positive fibroblast subpopulation. While human fat graft volume retention was reduced over 8 weeks following implantation, this was associated with significantly improved in limb extension. The skin overlying the grafted fat showed reduced collagen density, as indicated by trichrome staining, as well as a reduction in the fibrogenic Prx-1-positive fibroblast subpopulation by immunofluorescence imaging, as compared to the control mice.

Conclusion: Here we show that fat grafting improves the extensibility of irradiated and contracted hind limbs and reverses radiation-induced skin fibrosis by both reducing the collagen content and by altering the composition of dermal fibroblast subpopulations. Specifically, fat grafting results in a depletion of the Prx-1-positive fibroblast subpopulation. Further elucidating how this profibrotic fibroblast subpopulation is involved in ventral surface soft tissue fibrosis will facilitate development of novel strategies to treat/prevent debilitating late side-effect of radiotherapy.

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Novel Lineage Tracing System To Identify Site-specific Ectopic Skeletal Stem Cells

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Purpose: Traumatic heterotopic ossification (HO) is the formation of bone outside of the healthy skeletal system after traumatic injury causing decreased range of motion and discomfort in affected patients. Previous research has identified potential progenitor cells of HO, however, this is still contended. Better understanding of the cells contributing to HO will allow for targeted treatment to reduce HO in high-risk patients. It was recently discovered that cells expressing Hoxa11, an embryonic patterning transcription factor expressed exclusively in the zeugopod, is expressed by mesenchymal stem cells (MSCs) into adulthood and all skeletal lineages are marked downstream of these progenitors using a CreERT2 lineage tracing system. Currently, most lineage tracing systems label MSCs throughout the body, making it difficult to determine if genetic deletion is due to systemic or local effects. We hypothesize, using a mouse model of HO that Hoxa11+ MSCs will differentiate into site-specific HO-progenitor cells, and eventual form HO.

Methods: Hoxa11-CreERT2;ROSA-TdTomato mice were treated with tamoxifen at 6 weeks of age, chased for one week, then injured with an established and reproducible HO-forming Achilles’ tenotomy with concurrent 30% total body surface area back burn. Injured and uninjured hind limb samples were harvested one week, three weeks, and nine weeks post-injury, sectioned for immunofluorescent histology, and imaged by confocal microscopy.

Results: Examining the uninjured hindlimb of the Hoxa11-CreERT2;ROSA-TdTomato mouse, tdTomato-marked Hoxa11 lineage-positive cells were found throughout the zeugopod within tendon, enthesis, and bone. These cells were PDGFRα+ (not shown). Three weeks after injury, Hoxa11 lineage-positive cells are found at regions of heterotopic ossification in the burn/tenotomy model, specifically at the cut end of the tendon and calcaneus. After injury, Hoxa11 lineage+/PDGFRα+ cells express chondrocyte marker, SOX9, and pSMAD3, a downstream TGFβ1 signaling pathway transcription factor central to HO formation. Three weeks after injury, condensing chondrocytes within the HO anlagen are Hoxa11 lineage-positive and are colocalized with early bone marker, RUNX2. At nine weeks post-injury, SP7+ osteoblast and RUNX2+ pre-osteoblasts are marked with tdTomato. Hoxa11 cells were also able to
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