perioperative prophylactic IV antibiotics and oral antibiotics after discharge (Group 1) and 29 (34.1%) were given <24 hours prophylactic IV antibiotics only (Group 2). There was no significant difference in demographics (i.e. BMI, age, smoking status, preoperative chemotherapy/radiation) or inpatient treatment of the SSI (Oral/IV antibiotics) between the two groups. In Group 1, 64% (n=36) developed culture positive SSIs, compared to 83% (n=24) in Group 2. Staphylococcus Aureus was the most common bacteria in both groups.

Group 2 demonstrated a significantly increased incidence of gram-positive organisms (46.4% vs 72.4%, p=0.022) and Staphylococcus Aureus (21.4% vs 55.2%, p=0.002). However, there was no significant difference in overall highly virulent (p=0.168), gram-negative (p=0.416), or total isolated organisms (p=0.192) between groups. Interestingly, Group 1 demonstrated an increase in Pseudomonas Aeruginosa SSIs, (14.3% vs 3.4%, p=0.124) but this finding did not reach significance. Implant loss between Groups 1 and 2 (62.5% vs. 62.1%, p=0.969) respectively, was nearly identical.

Conclusion: Our study demonstrates that despite differences in bacterial profiles between the two antibiotic protocols, prolonged postoperative antibiotic use did not provide additional protection against overall highly virulent infections. In addition, the implant loss rate between the two groups was similar. Antibiotic stewardship guidelines against the overuse of prolonged prophylactic regimens should be considered. Further analysis regarding timing of SSIs and antibiotic treatment is warranted.

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Identifying The Myeloid Subpopulation Responsible For Tissue Fibrosis Across Organ Systems Via Machine Learning Parameterization And Predictive Transcriptomics

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Purpose: Fibrosis secondary to ischemia and musculoskeletal polytrauma is a clinical problem common in plastic surgery with limited effective therapeutics currently available. Until recently, high throughput analyses of fibrosis have been untenable. We developed an automated parameterization of fibrosis histology via machine learning (ML) methods capable of distinguishing healthy from fibrotic muscle, correlated with transcriptomic profiles of infiltrating macrophages following musculoskeletal polytrauma. Furthermore, we established a transcriptomic analysis pipeline of multiple muscle and organ fibrosis models using single cell RNA sequencing (scRNAseq) to allow identification of a “fibrotic” macrophage. We hypothesize that inflammatory and fibrotic myeloid cell phenotypes are similar across musculoskeletal and visceral organ systems and that proteomic and transcriptional profiles can be used to predict homeostasis, injury and fibrogenesis.

Methods: Ischemia/reperfusion of the mouse hindlimb with concomitant cardiotoxin injection into the tibialis anterior (IR/CTX) was induced in mice with genetic (LysmCre;Tgfβ1fl/fl) and pharmacologic (TGFβ1-Fc ligand trap) Tgfb1 deletion. H&E, Masson Trichrome, and picrosirius red histology was performed at 1 week post injury (n=3-4/group). Automated quantification of picrosirius micrographs were performed with a novel image processing pipeline resulting in a panel of 13 relevant fiber and branch-point parameters and t-SNE dimensional reduction. Separately, we performed 10X scRNAseq on IR/CTX injured tibialis anterior muscle at homeostasis and 3 days post-injury. For comparison studies, IR/CTX macrophages were isolated and aggregated with additional macrophages from distinct murine data sets for examination of gene expression profiles: wild-type muscle 1-2 days following gluteus muscle crush (data gifted by Regeneron), lung tissue macrophages 14 days after asbestos (GSE127803), and 0, 3, 6 days after LPS (GSE120000) exposure.

Results: IR/CTX injury with inhibition of TGF-β1 signaling recapitulated the appearance of uninjured muscle with ML delineating distinct, self-aggregating clusters between WT injury vs. all other groups. While injured and naive muscles were robustly separated, conditional Tgfβ1 deletion and systemic sequestration were observed as intermediate populations. Unsupervised clustering in IR/CTX TA
muscles resulted in 11 (uninjured) and 8 (IR CTX) clusters. Examining for overexpression of gene families in TGFβ1 signaling, we observed stark contrast between uninjured and IR/CTX macrophages suggesting an important role in fibrosis. In order to determine phenotypic resemblance of macrophages between muscular polytrauma vs. crush vs. inhalation injury, we visualized the combination of IR/CTX, muscle crush, and lung macrophages, demonstrating overlap. Furthermore, we observed high levels of Tgfb1 expression in Adgre+ Csf3r− macrophages of muscle crush and lung injury, suggesting a shared myeloid phenotype associated with spatially distinct niches of fibrosis.

Conclusions: ML methods can aid in high-throughput analysis of muscle fibrosis histology. Given transcriptomic similarities between macrophages from IR, muscle crush, and inhalation induced fibrosis, it is possible that these cells may exhibit a conserved mechanism across organ systems and models, elucidating important parallels that could be amenable to ML and identification of important common therapeutic targets.

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An Unrecognized Role Of Ccl5 In Triple Negative Breast Tumor Construction

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Purpose: CCL5 (RANTES) has long been identified as a driver for triple negative breast cancer (TNBC). Numerous studies demonstrate that silencing CCL5 decreases invasion, metastasis, and migration. Similarly, the phenomenon of linearized collagen extending from the tumor body, has been well identified. We hypothesize there is an unrecognized role of CCL5 in depositing linear collagen type VI, identifiable by a juxtacrine relationship between triple negative breast cancer cells and healthy cells.

Methods: Group i (MDA-MB-231, ASCs, and fibroblasts in juxtacrine contact), group ii (ASCs and fibroblasts juxtacrine, MDA-MB-231 in paracrine contact through a 0.2m semipermeable membrane), and group iii (ASCs and fibroblasts in juxtacrine contact) were set up and cultured in normal conditions for 7 days. During this time ECM was deposited, followed by decellularization using NH4OH and Triton-X. ECMs were analyzed using brightfield images, immunohistochemistry, SEM, AFM, and mass spectrometry. An 86-cytokine panel (Ray Biotech) screened cytokines in the media during ECM deposition (day 5 of culture). Cell response to ECM was measured by reseeding MDA-MB-231, and cultured under normal conditions. Immunohistochemistry on human breast tumor sections targeting collagen VI and CCL5 was performed. As an intervention study, monoclonal antibodies against human CCL5, and recombinant human CCL5 were used to block and supply CCL5 in vitro respectively.

Results: Co-culture where all three cell types had juxtacrine contact created ECM linear in format (per SEM and AFM), high in collagen VI (per mass spectroscopy), and was produced under high levels of CCL5 (per cytokine panel). Human breast tumor samples show positive staining for collagen VI and CCL5. These data are in contrast to the healthy cell co-culture, which had rough ECM ultrastructure, lower collagen VI, and was produced in low CCL5 concentration. When CCL5 was blocked in vitro during matrix deposition, the linear matrix formation was disrupted, and collagen VI production was significantly decreased. Furthermore, reseeded cells on the matrix formed without CCL5 had less integrin 1 compared to the matrix formed with unlimited CCL5.

Conclusion: The TNBC co-culture of MDA-MB-231, ASCs, and fibroblasts in juxtacrine contact, reproducibly builds a matrix which high in collagen type VI, linear in format, encourages formation of road-like structures by reseeded cells, and induces high integrin B1 expression of the reseeded cells. This is a yet undescribed role of CCL5, which may help explain the abundant literature on the oncologic benefits of its inhibition.

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Hemangioma Endothelium Loses VE-CADHERIN After Involution: Implications For Cell Death?

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Purpose: Infantile hemangiomas (IHs) are the most common benign endothelial cell (EC) tumor of infancy, arising