Defining the Mechanisms of Stem Cell Activation following Fracture

Yu Liu[1], Simon Lu[1], Yuchen Liu[1], Louis Gerstenfeld[1], Chao Zhang[1], Beth Bragdon[1]
[1]Boston University Chobanian and Avedisian School of Medicine, Boston, MA
Presenting author: bragdon@bu.edu

Disclosures: Authors have nothing to disclose.

Introduction: Mesenchymal stem cells, more recently named skeletogenic stem cells (SSCs) are terms for the stem/progenitor cells that differentiate to the chondrogenic, osteogenic, and/or adipogenic lineages and are involved with bone homeostasis and the early phases of fracture repair. One such SSC and progenitor cell population can be marked by the expression of Prx1 (paired-related homeobox 1). The Prx1 cell population has been identified in various locations including the periosteum and skeletal muscle within limbs. Recently we showed that Prx1 cell populations isolated from the periosteum contained both quiescent and proliferating cells able to form bone tissue while those isolated from the muscle were primarily quiescent and can only contribute to bone tissue formation following activation either after a fracture injury or in response to high levels of Bone Morphogenetic Protein 2. In this study, we assessed the activation and early stem cell commitment of the Prx1 derived cells from the periosteum and muscle following fracture using single cell RNA sequencing. The objective was to 1) define the heterogeneity of the Prx1 cell population within both the periosteum and the skeletal muscle surrounding the fracture site, 2) determine responses directly to fracture injury and 3) compare the muscle and periosteal Prx1 cell population's ability to respond to injury.

Methods: This study was approved by IACUC. Both male and female mice were used (8-12 weeks old). The tamoxifen inducible Prx1 reporter mouse (Prx1CreER;Rosa26tdTomato;Rag) was used to fluorescently tag the Prx1 cell population. Tamoxifen induction to fluorescently tag the Prx1 expressing cells was carried out three days prior to the mice receiving a femoral fracture stabilized via insertion of an intra medullary pin. Non-operated mice served as day 0 controls. Three days post fracture, the developing fracture callus was removed and carefully separated from the surrounding skeletal muscle. The femoral periosteum and surrounding skeletal muscle were then harvested and the tissues enzymatically digested to release the cells followed by cell sorting for fluorescence (dTomato). Single cell RNA sequencing was performed on the positive dTomato cell populations. For single cell sequence data we will primarily use the Seurat V4 R Tool kit for single cell genomics and the Moncocle software tool for pseudotemporal ordering to project cell lineage fates.

Results: Sorting on dTomato (Prx1 cells) identified unique cell populations including clusters of various populations of mesenchymal cells in both the periosteum and muscle tissues. At the homostasis prior to injury, Prx1 was primarily expressed in several unique mesenchymal cells types, in both tissue compartments, including mesenchymal stem cells/skeletal stem cells (MSC/SSCs), tenocyte-like cells and low proliferative progenitors (we call SSC/SSPC). In the periosteum there were considerably more proliferative progenitors (marked by with ki67), differentiated osteoblasts (express DMP1 but not SOST), fibroblasts, and chondrocytes while in muscle there were greater numbers of endothelia and smooth muscle cell that had been labeled. However following fracture in the periosteum there was a large shift of cells to the proliferating progenitors and osteoblast clusters. In the muscle the shift is primarily from the low proliferating stem cells to a very large number of transitional stem/progenitor cell population but very few proliferating progenitors were seen. Pseudotemporal ordering analysis suggested different trajectory of the periosteal Prx1 in the two compartments suggesting that periosteal SSCs undergo proliferative expansion followed by osteogenic differentiation following injury. In contrast, the muscle Prx1 stem cells move to the transitional state and toward endothelial and smooth muscle cell phenotypes.

Discussion: Extending on our prior bulk sequencing studies, our current results showed that the periosteal Prx1 SSC/progenitors cells are highly sensitized to injury response and contained a unique population that could rapidly shift to the proliferating progenitor stage as an early repair response. It was more unexpected that our preliminary analysis suggests that the proliferative stage is restricted to and does not encompass other stages of differentiation. It is also interesting that early osteoblast lineage commitment is at 3 days post fracture. Since the bone marrow was removed prior to cell isolation, we speculate that we captured the distal regions of the callus, which mineralize prior to the majority of the callus tissue. While the periosteal Prx1 cells are able to quickly respond to injury and start proliferating and differentiating as early as day 3, the muscle Prx1 stem cells must first go through a transitional phase. This would be consistent with our prior studies suggesting that the while SSC are present in the muscle that they are in a more quiescent state needing both injury and much larger exogenous morphogenetic stimulus to become activated and contribute to repair.

Significance: We have identified several different potential cell populations (muscle and periosteum) to target for therapeutics to enhance or initiate fracture repair. Key transitions of activation following fracture injury were identified of known stem cell populations isolated from the periosteum and muscle tissue which may be exploited to more effectively target these cells for therapeutic applications.

Acknowledgements: We want to thank the Boston University Chobanian and Avedisian School of Medicine Flow Cytometry Core and Single Cell Sequencing Core for their assistance. This research was supported by grants from NIH NIAMS K99/R00 AR068582 and from BU CTSI 1UL1TR001430

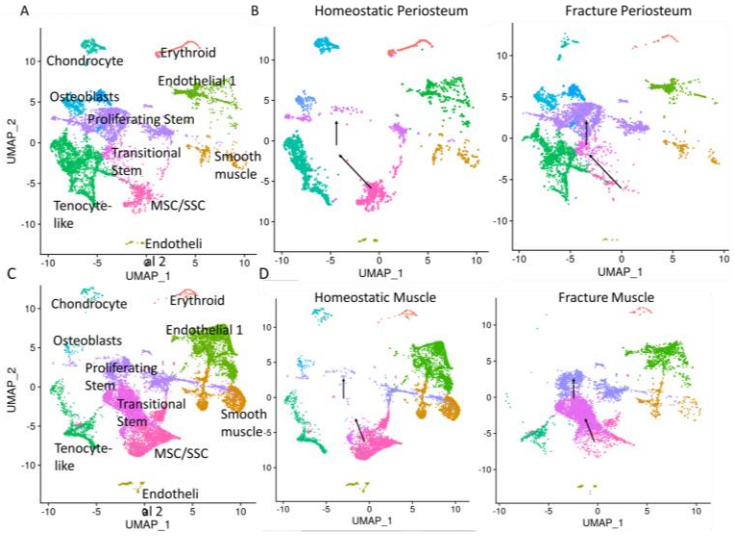

Figure 1. Cluster analysis of the Prx1 cell population found in periosteum and muscle. Prx1 cells were fluorescently tagged three days prior to fracture. Cells were isolated from the various tissues three days post fracture. Prx1 cells were isolated and single cell RNA sequencing was performed. A) Shows color coding of unique cell populations within the periosteum. B) Left panel shows color coding of the individual cell clusters from cells isolated from the non-injured periosteum and (right panel) after fracture. C) Shows the color coding of unique cell clusters of the muscle. D) Left panel shows color coding of the cell clusters of the non-injured muscle and (right panel) after fracture. Arrows highlight shift of cells from homeostatic condition to after fracture.