Investigating the Spatial Expression of Putative YAP Target Genes in the Early Stages of Fracture Repair

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Introduction: During bone fracture repair, some of the first responders are progenitor cells in the periosteum, the outermost layer of the bone. Though these periosteal cells are generally quiescent, they proliferate in response to a fracture because of a transcriptional co-regulator called Yes-associated protein (YAP). When a fracture occurs, YAP translocates to the nucleus of periosteal cells, becoming active and binding to other transcription factors to allow for gene expression and subsequent cell proliferation. When YAP is deleted from periosteal cells, they fail to sufficiently proliferate in the early stages of fracture healing, resulting in impaired bone regeneration (Kegelman, 2020). However, the specific proteins YAP transcribes in the context of periosteal cell proliferation remain unknown. We therefore used bulk mRNA sequencing to identify putative YAP target genes involved in this proliferation and spatially mapped the protein level expression of two of these genes in the early fracture callus by immunofluorescence staining.

Materials and Methods: We evaluated protein level expression of BMP4 and IL11 in the fracture callus of 14- to 16-week-old Osterix-conditional YAP/TAZ knockout (KO) mice (n=3) and their wild type (WT) littermates (n=3). We also stained for Osterix and YAP to map the regions where the Osterix-conditional YAP/TAZ knockout took effect. We activated the adult-onset KO in the mice two weeks prior to fracture surgery, then induced femoral fractures using an intramedullary pin for stabilization. We harvested and fixed the intact and fractured limbs four days post-fracture, then decalcified them for two days. We then cryo-embedded the samples in Tissue-Tek OCT resin and cryosectioned them using tape according to previously established protocols (Dyment, 2016). We blocked and permeabilized the sections using 1X PBS with 5% goat serum and 0.3% Triton X-100, then stained them with antibodies corresponding to the proteins of interest. Finally, we used immunofluorescence imaging to determine the location and intensity of the proteins throughout the sample, specifically in the periosteum.

Results and Discussion: Image processing for this project is still ongoing, but we hypothesize that it will reveal reduced protein expression levels of BMP4 and IL11 in the periosteum in KO mice compared to their WT littermates. We also acknowledge that this effect may not be observable in the current sample size. In light of this, data collection is also ongoing.

Conclusions: Our initial troubleshooting resulted in a reproducible protocol for evaluating the protein level expression of putative YAP target genes in the early stages of fracture repair in WT and KO mice. In the event that our hypothesis is correct, we will proceed to testing whether they can rescue the effect of YAP in a KO model. Alternatively, we are continuing to investigate the protein expression of other putative YAP target genes identified by the aforementioned bulk RNAseq.

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