Genomic Analysis Of Sox9-lineage Cells During Large-scale Skeletal Injury Reveals A Role For c-FOS/AP-1 In The Initiation Of Repair

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INTRODUCTION: Skeletal stem and progenitor cells (SSPCs) play a crucial role in both the development and repair of bone. Recent research has identified diverse types of SSPCs, distinguished by their specific locations, lineages, and distinctive cell surface markers.[1] However, our understanding of their intrinsic differences and activation mechanisms during bone repair remains limited. We hypothesize the existence of sentinel SSPCs, which when activated, can enhance and expedite the intricate process of bone healing. Recently, while studying a model of large-scale rib regeneration, we identified a rare group of cells marked by *Sox9* expression and comprising a mere 6% of the periosteum. In response to a large-scale resection injury, these cells respond to injury and proliferate, contributing to ~20% of the callus at 10 days post injury.[2] Interestingly, these cells are indispensable during large-scale rib repair, as their removal before injury block the repair process.[3] We hypothesize that these Sox9-lineage cells are specialized to respond to injury and coordinate repair in concert with other callus-building cells.

METHODS: To assess the unique regulatory networks in Sox9-lineage cells before and after injury, we collected Sox9-lineage cells from *Sox9-CreER;R26R-tdTomato* mice via FACS at different time points after large-scale rib resection injury: before injury, 2 days post-injury, and 4 days post-injury. Using RNA-seq, we identified injury-responsive genes (FDR < 0.05) and we combined this with ATAC-seq to assess the chromatin landscape. The proliferation of Sox-9 lineage cells was also assessed by an EdU assay. Experiments using mice were approved by our USC's Institutional Animal Care and Use Committee.

RESULTS: Sox9-lineage cells are some of the first to respond and enter mitosis since, very soon after injury (2 days), nearly half of the actively cycling cells are from the Sox9-lineage. In addition, after injury, RNA-seq assays demonstrate that Sox9-lineage cells express injury-related genes including those for extracellular matrix, growth factors, and cytokines. Assessment by ATAC-seq further indicates that these cells have an intrinsic readiness to activate injury-induced genes as they possess unique distal regulatory elements near those genes even before injury. Using motif analysis, we identified the AP-1 motif as enriched in these open chromatin regions, suggesting that AP-1 factors govern the injury response. Following injury, we observe that new open chromatin regions emerge in Sox9-lineage cells, and that the AP-1 motif is prominently enriched in these peaks. Notably, the expression of c-FOS, a constituent of AP-1, is induced by injury and can be observed as early as 1.5 hours after rib resection.

DISCUSSION: These findings highlight the unique epigenetic characteristics of Sox9-lineage cells, positioning them as sentinel cells that are poised to respond to injury. We also identified AP-1 factors as significant in an SSPC lineage during bone homeostasis and early after injury. Currently, we are characterizing mice that lack *Fos* specifically in Sox9-lineage cells, to further validate the role of c-FOS in Sox9-lineage cell activation post injury.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings contribute significantly by shedding light on the epigenetic traits of Sox9-lineage cells, establishing them as sentinel cells capable of swift injury response. The association of AP-1 factors with SSPCs during bone homeostasis and early injury phases opens new avenues for understanding the regulatory mechanisms in bone repair. Ultimately, this research could pave the way for targeted therapeutic interventions that harness the potential of these specialized cells to enhance bone healing and regeneration.

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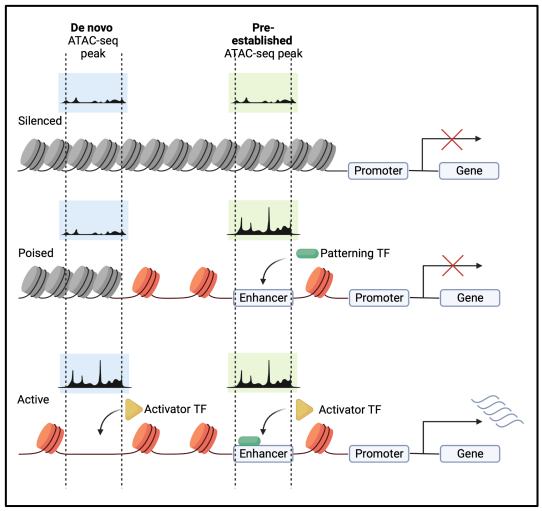

Figure 1. Model for gene regulation in Sox9-lineage cells in response to injury. In cells that do not respond to injury, repair genes are silenced and not expressed (top row). In Sox9-lineage cells, we propose that repair genes are in the poised state with the enhancer open and ready for transcription factor binding (middle row). After injury (bottom row) we propose that transcription of repair genes is activated by such factors as c-FOS (yellow triangle) which will facilitate transcription by both binding at pre-existing enhancers and enhancers that become accessible after injury.