

Transcriptional regulatory network underlying connective tissue differentiation during limb development

The musculoskeletal system is composed of muscles, skeletal elements and connective tissues such as tendon and muscle connective tissue. Functional locomotion via this system relies on a precise coordination between its different components. Muscle connective tissue contributes to the elasticity and rigidity of muscles, while tendons transmit forces generated by muscles to the bone to allow body motion. Despite their distinct mesodermal origins, differentiation of muscle and connective tissue progenitor cells are closely related throughout limb development. In contrast to muscle and skeleton, connective tissue patterning and formation remain poorly investigated. In order to identify molecular mechanisms underlying connective tissue formation during limb development, five zinc-finger transcription factors were investigated: OSR1, OSR2, EGR1, KLF2 and KLF4. These transcription factors are expressed in distinct subcompartments of the musculoskeletal system and influence the differentiation of limb mesenchymal cells upon overexpression. To further investigate their roles at the molecular level, several genome-wide strategies were employed in limb mesenchymal explant cultures overexpressing each of the transcription factors. Whole-transcriptome sequencing revealed that the transcription factors share common regulatory functions and positively regulate biological processes related to signal transduction, cell communication and biological adhesion. ChIP-sequencing on histone tail post-translational modifications revealed that the differentially expressed genes were enriched for both active and repressive chromatin signatures at their promoters, suggesting that they are dynamically regulated and might therefore contribute to connective tissue differentiation. Occupancy of each transcription factor was finally investigated via ChIP-sequencing to distinguish between indirect and direct target genes. Altogether, the combination of *in vivo* and *in vitro* data with genome-wide profiling brings molecular insights underlying the differentiation of connective tissue cells. These results provide a framework for future investigations to better understand the interconnectivity between components of the musculoskeletal system.