Regulation of postnatal myogenesis by SOXF transcription factors

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Adult skeletal muscle possesses a remarkable regenerative capacity, based on the recruitment of satellite cells, a population of quiescent stem cells located on myofibres within muscle masses. Emerging from a proliferating embryonic progenitor pool, the molecular mechanisms underlying the transition from developmental progenitor cells to postnatal muscle stem cells and the establishment/maintenance of quiescence remain poorly understood. We have performed chronological molecular dynamics of skeletal muscle progenitor and postnatal stem cells spanning embryonic and foetal development, as well as postnatal and adult life up to 18 months of age, deciphering the intrinsic molecular pathways involved in specification and regulation of the satellite cell lineage. We have identified *SoxF* gene family members (Sox7/17/18) as novel key regulators of skeletal muscle homeostasis and skeletal muscle regeneration using single fibres experiments combined with *in vivo* analysis of a compound $Pax3^{Cre/+};Sox17^{GFP/Flox}$ mouse to conditionally ablate Sox17 in the skeletal muscle lineage. A tight regulation of the Wnt/ß-catenin canonical signalling output is required to ensure skeletal muscle regeneration. Sox17 associates with ß-catenin acting as a regulator of the Wnt/ß-catenin signalling pathway in other cell types. Our data suggest that in adult skeletal muscles SoxF factors display a dual activity as both intrinsic regulators of muscle stem cell quiescence and interacting with extrinsic signalling pathways to regulate the expansion of activated muscle stem cells.

Muscle stem cell environment coordinates adult myogenesis during skeletal muscle regeneration

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Adult skeletal muscle regeneration requires the recapitulation of myogenesis by satellite cells/muscle stem cells and their progeny, the myogenic precursor cells. Increasing evidence identifies the cell environment of muscle stem cells as an important regulator of this process. Several cell types have been shown to regulate/control the sequential steps of myogenesis, including macrophages, fibro-adipogenic precursor cells and vessel cells. Each of these cell types sustains adult myogenesis in a strictly regulated way in space and time to provide appropriate cues to myogenic precursor cells, as well as to the other neighboring cells. Indeed, synchronicity of the delivered cues is crucial for efficient muscle regeneration. During the first phase after damage, infiltrating (pro-inflammatory) macrophages, fibro-adipogenic precursor cells and endothelial cells cooperate to ensure the proliferation and expansion of myogenic precursor cells. Later on, once the resolution of inflammation has started, anti-inflammatory macrophages, fibroblasts, endothelial and peri-endothelial cells coordinate their action to sustain the late steps of muscle regeneration including terminal myogenesis and fusion, myofiber growth, angiogenesis and extracellular matrix remodeling, as well as self-renewal of muscle stem cells. A series of secreted factors and intracellular signaling pathways have been shown to participate in the regulation of these sequential processes. They may represent useful targets to enhance the regeneration process in normal muscle regeneration, as well as during degenerative myopathies, were synchronicity of the regenerating cues is lost.

Towards the next-generation *ex vivo* and *in vivo* gene therapy for duchenne muscular dystrophy

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There is an urgent need to develop the next-generation vectors for gene therapy of muscle disorders, given the typical modest advances in clinical trials. These vectors should express substantially higher levels of the therapeutic transgene, enabling the use of lower and safer vector doses. To address this unmet need, a genome-wide computational strategy was used to identify potent muscle-specific transcriptional cis- regulatory modules (CRMs), containing clusters of muscle-specific transcription factor binding sites. These novel elements resulted in an unprecedented increase (up to 400-fold) in skeletal muscle-specific gene expression when delivered using adeno-associated viral vectors (AAV9) in mouse models. These muscle-specific CRMs resulted in high expression levels of human micro-dystrophin (MD1) and follistatin (FST) in immunodeficient dystrophic SCID/mdx mice, consistent with robust functional correction of the dystrophic phenotype and its underlying pathophysiology. As an alternative approach, we explored the use of *ex vivo* genetically modified stem/progenitor cells expressing either the full-length dystrophin coding sequence (11.1 kb). Dystrophic mesoangioblasts from a Golden Retriever muscular dystrophy dog were stably transfected with a large-size piggyBac transposon resulting in expression of full-length human dystrophin. Dystrophic mesoangioblast-like cells were also generated from iPS of DMD patients. These iPS-derived mesoangioblasts, constitute an essentially unlimited supply of stem/progenitor cells that could be genetically corrected using PB transposons expressing the full-length dystrophin. These results pave the way towards improved viral and non-viral gene therapy approaches for DMD (MC and TV are supported by FWO, EU MYOCURE N° 667751, Willy Gepts Fund, Walter Pyleman Fund, VUB IOF).

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