Regulation of the adult muscle stem niche by Notch signaling

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Adult skeletal muscles can regenerate after repeated trauma, yet our understanding of how adult muscle stem cells (MuSCs) restore muscle integrity and homeostasis after regeneration is limited. In the adult mouse, MuSCs are quiescent and located between the basal lamina, and the myofibre. After injury, they re-enter the cell cycle, proliferate, differentiate and fuse to restore the damaged fibre. A subpopulation of myogenic cells then self-renews for future repair. The paired/homeo-domain transcription factor Pax7 regulates marks perinatal and postnatal MuSCs. When MuSCs are removed from their niche, they rapidly express the commitment marker Myod and proliferate. The basal lamina is rich in collagens, non-collagenous glycoproteins and proteoglycans. How these extracellular matrix (ECM) proteins regulate the satellite cell quiescent niche remains unclear.
Notch signaling is a fundamental cell-to-cell communication pathway that regulates many aspects of metazoan development. This pathway is initiated when Notch transmembrane receptor (on MuSCs) binds to its ligand (eg. Dll1), resulting in the release of the intracellular domain (NICD) and its translocation to the nucleus where it binds to Rbpj on DNA to activate gene transcription. We showed that Notch signaling is critical for the maintenance of the satellite cell quiescence state, as the deletion of *Rbpj* results in loss of MuSCs, and premature differentiation.
Here we investigate the role of Notch signaling via ECM components in the regulation of MuSCs and their niche. A genome-wide ChIP-sequencing of myogenic cells with Rbpj and NICD identified ECM genes such as collagens as Notch signaling target genes. Collagen molecules are typically composed of alpha chains that form homo- or hetero-trimers, via a triple helix domain. NICD/RBPJ binding was found close to two collagen type V genes and *Col5a*3. Three additional enhancers were identified in the 85 kbp genomic interval between *Col6a1* and *Col6a2*, one of which is conserved in humans in sequence and topology, relative to the *hCOL6A1/2* genes.

Characterisation of skeletal muscle stem cell properties in distinct physiological states

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Skeletal muscle stem cells constitute a population of cells with heterogeneous properties. During muscle stem cell quiescence in the adult mouse, we identified a novel dormant cell state, where cells have a lower metabolic activity and delayed completion of the first cell cycle (1). Interestingly, muscle stem cells have a remarkable capacity to survive post mortem (2) and those that survive adopt a dormant state. Metabolism could play a critical role in dictating whether a cell remains quiescent, proliferates or differentiates. Recent findings suggest that alterations in cellular metabolism are related to changes in cell states in many stem cell systems (3). To study the metabolic signature of muscle stem cells in different states we performed transcriptomic and proteomic analysis and we assessed by RT-qPCR the expression of genes involved in various metabolic pathways (glycolysis, oxidative phosphorylation, fatty acid oxidation, etc). To investigate the molecular and functional pathways activated in hypoxic conditions and their connection with metabolic regulation and possibly dormancy and stress-resistance, satellite cell behaviour in hypoxic culture conditions is being assessed. On single myofibers, in which satellite cells are in their natural niche, we assessed whether the satellite cell state might be affected by low oxygen tension. We aim to extend these studies to muscle stem cells in different contexts, including ageing and disease.

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2) Lathil, M., p. Rocheteau, L. Châtre, S. Sanulli, S. Memet, M. Ricchetti, S. Tajbakhsh#, and F. Chrétien# (2012). Skeletal muscle stem cells adopt a dormant state post mortem and retain regenerative capacity. Nature Communications, June 12; 3: 903
3) Folmes C.D., Dzeja p.p., Nelson T.J. & Terzic A. (2012) Metabolic plasticity in stem cell homeostasis and differentiation. Cell Stem Cell 11: 596–606

Characterisation of mouse and human skeletal muscle stem cells by transplantation

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Cell therapies for treating myopathic diseases with healthy donor cells have been proposed. Satellite cells are mononuclear skeletal muscle stem cells that have the ability to self-renew and form new muscle fibers. Although they are strong candidates for cell therapy as determined by studies with mice, however, human satellite cells are considerably less well understood. To investigate the properties of mouse and human satellite cells, we transplanted them into the muscle of immunodeficient *Pax7*$^{DTR/+}$*:Rag2*$^{-/-}$*:gC*$^{-/-}$ mice [1], in which endogenous mouse Pax7+ satellite cells can be depleted by the injection of diphtheria toxin. Upon transplantation, human satellite cells contribute to new muscle formation *in vivo* as assessed by the presence of human lamin A/C integrated in the host mouse muscle fibers and they could occupy the satellite cell niche *in vivo*. Their properties are being further examined including their ability to expand in the niche, and to be re-isolated from host muscle to assess whether human satellite cells have self-renewal properties in mouse. This context allows us to examine to what extent the mouse and human niches are compatible.

[1] Sambasivan, R. et al. Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. Development 138, 3647–56 (2011).