Poster

Roll of Mrf4 gen in homeostasis of adult skeletal muscle.

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ABSTRACT

Myogenesis is the process that controls skeletal muscle growth during embryonic and postnatal development, and it is orchestrated by a family of four transcription factors (TFs) known as MRFs (Myogenic Regulatory Factors)(1). Our group is working on the function of two of these genes: Mrf4 and Myf5. We and others have established a link between MRFs function and muscle atrophy/hypertrophy (2).

Mrf4 is the only MRF highly expressed in all adult skeletal muscles, but a complete understanding of its function is lacking as previous KOs models affect Myf5 expression in cis (3). Recently, our group has generated two new KO alleles using CRISPR technology (Mrf4 L1/L1 and Mrf4 L2/L2). Preliminary data shows that both alleles develop mild muscle hypertrophy, without affecting Myf5 expression in cis. Experiments in vivo indicate that the two alleles have alterations in muscle metabolism.

This project is focused on elucidating how this TF is involved in muscle growth, function, and homeostasis. Furthermore, as adult satellite cells emerge from embryonic founder cells in which Mrf4 expression was activated, we are also studying Satellite Cell biology and function in the absence of Mrf4.

Surprisingly, we have identified important phenotypic differences between the two alleles; one hypothesis is that one or both may generate a small peptide that modifies the phenotype.

Methods: We dissected the extensor digitorium longus muscle, isolated myofibers from young (3 months) and old (24 months), WT and Mrf4-/- mice, and divided these in three groups: 1- Fibers were fixed and permeabilised for immunostaining with Pax7 antibody to study the myofiber size and content in satellite cells/nuclei; 2- Fibers were plated for bioenergetic analysis using a XF24e Seahorse Analyzer that provides oxygen consumption rate as indicator of mitochondrial respiration as a proxy for metabolic function; and 3- Fibers were plated in proliferation medium to allow their associated Satellite Cells to abandon the niche, migrate and proliferate, and we studied parameters of Satellite Cell proliferation and differentiation.

In order to study the potential effect of small peptides generated by one or both alleles, we generated overexpression plasmids containing the sequences of interest. C2C12 myogenic cells were co-transfected with a GFP-expression vector using the electroporator BTX Gemini and then selected to determine if there were significant differences in proliferation and differentiation

REFERENCES

1. Moncaut, N., Rigby, P. W. J., & Carvajal, J. J. (2013). Dial M(RF) for myogenesis. 280, 3980–3990
2. Moretti I, Ciciliot S, Dyar KA, Abraham R, Murgia M, Agatea L, Akimoto T, Bicciato S, Forcato M, Pierre P, Uhlenhaut NH, Rigby PW, Carvajal JJ, Blaauw B, Calabria E, Schiaffino S (2016). MRF4 negatively regulates adult skeletal muscle growth by repressing MEF2 activity. Nat Commun. 7, 12397.
3. Olson EN, Arnold HH, Rigby PW, Wold BJ (1996). Know your neighbors: three phenotypes in null mutants of the myogenic bHLH gene MRF4. Cell. 85, 1-4