

Poster

Characterization of new Mrf4 KOs

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ABSTRACT

Motivation: The myogenic regulatory factors (MRFs) are a family of basic-helix-loop-helix (bHLH) transcription factors, essential for skeletal muscle development, homeostasis and regeneration. There are four MRFs: MyoD, Myf5, Mrf4 and MyoG. During this project we will focus on Mrf4.

Mrf4 is first expressed in the mouse embryo at 9.5 days post-coitum (dpc) in undifferentiated cells of the ventral somitic region, extending to other somites by 10 dpc. Its expression is down-regulated from 12.5 dpc, up-regulated in a second foetal phase and then maintained in all adult skeletal muscles. Mrf4 is linked to Myf5 in mammals. The organization of the Mrf4/Myf5 locus, in which the regulatory elements are interdigitated, requires a series of equilibria, to maintain the specific expression pattern of each gene and avoid the cross-interaction between their enhancers and promoters. This complex equilibrium has hindered the study of their individual function, as the modification of some of the elements in the locus affect the expression of not only one but both genes. This is specially evident in the case of previous Mrf4 knock-outs, in which three different phenotypes were obtained varying from birth lethality to complete viability.

We have previously described the effect of Mrf4 Knock-down mediated by iRNA electroporation of skeletal muscle in adult rats. During this project we have performed a preliminary characterisation of three new Mrf4 KO alleles, recently generated in the lab by CRISPR/Cas9 targeting the Mrf4 minimal promoter (MP4 allele), or its first exon (112-KO alleles). With this approach we expect to unravel the role of Mrf4 during mouse embryonic development and in adult muscles.

Methods: To study the effect of inactivating Mrf4 on the expression pattern of the other MRFs we have performed In Situ Hybridisation (ISH) using 10.5 and 11.5 dpc embryos. We will study RNA expression levels by qPCR at four embryonic stages (10.5, 11.5, 12.5 and 16.5 dpc) and we are also looking for possible skeletal phenotypes by bone staining in fetuses at 18.5 dpc. Finally, we are studying how these three different KO affect adult skeletal muscles in normal conditions and an induced atrophy model. We have obtained Soleus, Tibialis Anterior (TA), and Extensor digitorum longus (EDL) leg muscles from adult WT and mutant mice and we are characterising putative fiber type changes using

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