The role of CCAAT/Enhancer binding protein beta in the regulation of muscle fiber type

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Abstract

Muscle satellite cells are myogenic cells found in mammals and can be induced to proliferate or differentiate in response to external stimuli or injury. Previous work has shown that in satellite cells of resting muscles the transcription factor CCAAT/Enhancer binding protein beta (C/EBPB) is co-expressed with Pax7, a satellite cell marker. At the onset of differentiation, C/EBPB expression is decreased and the master regulator of myogensis, MyoD increases. Expression of MyoD is followed by the up regulation of other important myogenic regulatory factors such as myogenin and terminal differentiation protein, myosin heavy chain (MHC) leading to myoblast differentiation and fusion into myotubes. Muscle types differ mainly by the myosin components. There are 4 different types of MHC isoforms in muscle cells, as well as myosin light chain (MLC) isoforms. MHC isoforms are three fast fibers; MHC type IIA, MHC type IIB and MHC type IIx, and one slow fiber; beta MHC. Using immunohistochemistry, this project will investigate the muscle fiber type on muscle sections obtained from muscle of mice from a conditional C/EBPB knockout, where b is excised in pax7 expressing sat cells. Antibodies against the different MHC isofroms (anti-MHC IIA, anti-MHC IIB, and anti-MHC IIx) were used to detect the relative amount of each protein. Additionally an antibody against laminin-a protein in the basal lamina- was used to show the outer boundary of each muscle section. The results should indicate whether or not there are changes in the ratio of the different myosin heavy chain isoforms, in the wild type as opposed to the mice lacking C/EBPB.

Methodology

- As described in previous work (Marchildon et al., 2012) conditional null C/EBPβ$^{-/-}$Pax7$^{CreER-/+}$ (cKO) mice were generated by crossing a mouse having a C/EBPβ-floxed allele with mice having the Pax7-CreER^{tm} allele (Nishijo et al., 2009).

- The mice were placed under controlled conditions of 22°C, 30% relative humiditiy on a 12 hours light/dark cycle, and given food and water ad libitum.

- To acheive *in utero* activatation of CreER^{tm}, the mice were gavaged with 2.5 mg of tamoxifen (in corn oil) of pregnant dams at E15.5 of pregnancy.

- At postnatal day 56 (p56), tibialis anterior (TA) of the mice was gathered, followed by embedding in Tissue-Tek OCT compound, flash frozen in isopentane and then cooled by liquid nitrogen. Lastly, 8 µm thick sections were obtained.

- Immunohistochemistry was carried out on the sections as outlined in previous work (Bloemberg & Quadrilatero, 2012) using 5 µL of primary Ab SC-71(anti-MHCIIa) , with the following modications done to the procedure;

• Sections were permeabilized with 0.5% Triton X-100 PBS before blocking.

• 5 µL of rat anti-laminin was added along with the primary Ab.

• Secondary Ab cocktail included 6 µL anti-mouse Cy3 and 6 µL anti-rat Cy5, and was incubated overnight.

Results

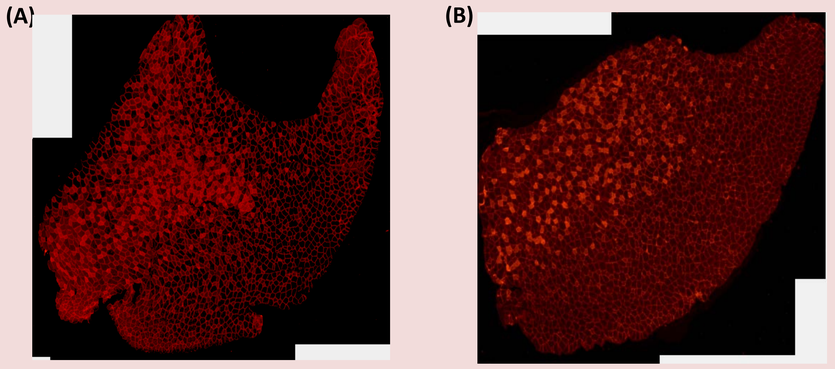

Figure 1. Representative images of mouse TA muscles stained using Indirect Immunofluorescence. Muscle sections shown were incubated with primary antibodies (SC-71) against MHC type IIa. Positive muscle fibers can be seen in red in the TA of (A) Wild type mice (B) C/EBP β knockout mice.

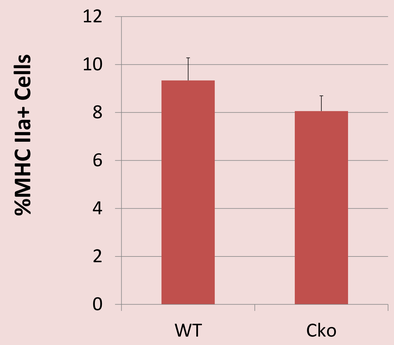

Figure 2. Percentage of MHC IIa obtained from the immunoflourscent staining of TA muscle from p56 wildtype (Wt) and C/EBP β knockout(cKo) mice. Data represents the mean; error bars displayed are the SEM, n=3. P-value, calculated from a two tailed t-test, assuming equal variances is not significant. Abbreviations; C/EBP β – CCAAT/enhancer binding protein β. WT- Wild type. MHCIIa – Myosin heavy chain type IIa.

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Conclusion

- The results show that there are no significant difference between the MHC type IIa fibers in C/EBPβ knockout and wild type mice. Therefore, no link between transcription factor C/EBPβ and the regulation of MHC IIa can be established.

- Future work: A continuation of the research involves staining for MHC IIb and MHC IIx using the same procedure to determine the role of C/EBPβ in their regulation as well as the ratio between fast and slow fibers.

Reference

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