Dottorato in Medicina Molecolare e dello Sviluppo

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Al collegio dei docenti del dipartimento di Medicina Molecolare e dello Sviluppo

The ankyrin protein family comprises three members: ankyrin R (also called Ankyrin 1), ankyrin B (also called ankyrin 2) and ankyrin G (also called ankyrin 3). Ankyrins R, B and G are adaptor proteins that link integral membrane proteins with the cortical actin-βspectrin cytoskeleton. The ankyrins are mostly ubiquitously expressed, although they are most abundant in erythrocytes, brain and in node of Ranvier and in epithelial cells, respectively. Their transcripts are subjected also to alternative splicing, which generate several and different proteins able to associate with additional components and displaying specific differential subcellular localization.

In striated muscle, a group of small muscle specific isoforms of ankyrin 1 have been identified. Their expression is driven by an alternative promoter located between the exon 39 and exon 40. These small isoforms (ank1.5, ank1.6, ank1.7, ank1.8 and ank1.9) are localized on the sarcoplasmic reticulum membrane, with which they are associated through a transmembrane sequence located at their NH_2- terminal region. Ank1.5 isoform (also called sAnk1), in particular, is the most abundant isoform in muscle cells and it is able to interact with the COOH terminus of Obscurin, creating a molecular bridge between the sarcoplasmic reticulum and the myofibrils of sarcomere.

Recent studies identify the presence of a Single Nucleotide Polymosphism (SNP), called rs508419, located in the internal promoter of small ank1.5, with a significant association with type two diabetes mellitus. This SNP consists in a substitution of a thymine with a cytosine, and this lead to an increase of transcriptional activity of the Ank1.5 promoter, with the consequent higher expression of the protein in the muscle; in fact biopsies of human patient with this mutation share high levels of protein compared to healthy subjects. Moreover, in vitro studies show that

immortalized mouse myoblast cell line, C2C12, transfected with an pEGFP-sAnk1 plasmid show an increased expression of the sAnk1 and a concomitant decrease in glucose uptake.

In order to understand the meaning of sAnk1 overexpression, transgenic animal models have been generated: in the mice the small Ankyrin1.5 expression is under the regulation of a specific muscle protein promoter, the myosin light chain promoter.

In the first year of PhD, i have characterized these animals to understand the levels of sAnk1 expression in wildtype and in transgenic mice; I have isolated soleus, EDL and gastrocnemius muscles from wildtype mice, transgenic and knock out for small Ank1.5, homogenized and prepared lysates to use for western blot to observe the differences of protein expression and quantify them statistically. The results obtained to date, show that the transgenic mice express higher levels of protein then wildtype.

At the same time, i have performed glucose tolerance tests to verify the glycemic curves in transgenic and wildtype mice.

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