ABSTRACT

The present Doctoral Thesis shows the study of the Ankyrin repeat and kinase domain containing 1 (ANKK1) protein in the myogenic lineage during development and in adulthood.

The *ANKK1* gene has been widely related to neuropsychiatric disorders and dopaminergic endophenotypes in the brain. However, the function of its protein is still unknown. The location of *ANKK1* gene in a genomic cluster conserved throughout the evolution thay may be involved in neurogenesis, and the expression of its protein in neural progenitors and its relationship with the cell cycle, have linked *ANKK1* gene to neurodevelopment. ANKK1 belongs to the Receptor-Interacting Proteins family (RIP), whose members participate in the differentiation of several tissues, including muscle tissue. The finding of the location of ANKK1 in murine embryonic myotubes led us to consider the hypothesis of the possible participation of this protein in muscles origin, development and regeneration.

Our results show that ANKK1 is a protein that participates in muscle biology. It is located in myogenic precursors during murine embryonic development and in adult muscle satellite cells. In addition, *in vitro* studies using murine and human myoblasts show a specific pattern of the dynamics of its isoforms: the isoforms ANKK1 kinase (ANKK1-k) and ANKK1 full-length (ANKK1-fl) are expressed in myoblasts and quiescent satellite cells (SCs), whereas only ANKK1-fl is present in myotubes and activated SCs. The nuclear-cytoplasmic shuttle of ANKK1 in myoblasts during early differentiation is blocked by the addition of leptomycin B, which indicates that its exit from the nucleus is mediated by exportins.

In the adult muscle ANKK1 is expressed in the Fast-Twitch muscle fibers type II with glycolytic metabolism. The activation of the glycolytic pathway in murine myoblasts increases *Ankk1* expression. All this confirms the relationship between the expression of ANKK1 and the glycolytic metabolism and explains the specific location of the protein in Fast-twitch muscle fibers. The location of ANKK1 in the muscles of patients with different muscular dystrophies has also been investigated. The myoblasts of patients with Duchenne Muscular Dystrophy (DMD) present an altered expression of ANKK1. The decrease in nuclear ANKK1 in these myoblasts is associated with a more undifferentiated cell stage, defined by the increase in the expression of PAX7. In parallel, in biopsies from patients with different muscular dystrophies, the expression of ANKK1 is associated with regenerative cell populations, that is to say, SCs and regenerating fibers. Regarding the study of its function, we have investigated the participation of ANKK1 in the cell cycle. The overexpression of the polymorphic variants of ANKK1 (A1-A2) in HeLa cells increases the rate of progression of the cell cycle, while overexpression of the catalytically inactive isoform (K51R) decreases it. In all cases, the percentage of cells that reach mitosis is reduced. All this indicates that the expression of ANKK1 affects both the progression of the cell cycle and the number of cells that complete the cycle.
Finally, we have studied the kinase activity of ANKK1. Under the conditions studied, this activity has not been detected *in vitro*. However, given that it is a RIP kinase and its kinase domain is homologous to the rest of the members of the RIP family, we cannot rule out that ANKK1 does not present this activity.

In summary, this Doctoral Thesis shows for the first time the participation of the ANKK1 protein in muscle biology from embryonic development to adult muscle. Thus, we propose ANKK1 as a candidate protein to be studied as a biomarker of muscular disease.

Keywords: ANKK1, satellite cells, myoblasts, myotubes, myogenesis, Fast twitch fibers, muscle regeneration, biomarker.