The unique properties of stem cells are determined by their ability to self-renew and differentiate. From the point of view of their origin one can distinguish stem cells derived from embryos, such as embryonic stem cells (ESCs), and those that are present within the tissues of adult organisms, e.g. skeletal muscle stem cells - satellite cells. ESCs are pluripotent that means they are able to differentiate into any given cell type and tissue. First one were derived in the 80s of XX century from the inner cell mass of a mouse preimplantation embryo - blastocyst. Since then ESCs serve as an excellent model to study cellular differentiation. They are extensively used to study the gene function, disease development or to test drug toxicity. At the Department of Cytology, Faculty of Biology, University of Warsaw we focus at the skeletal muscles regeneration and ability of various stem cells to improve this process. During progression of diseases affecting skeletal muscle function, such as Duchenne muscular dystrophy, the population of satellite cells, responsible for the repair of damaged tissue, becomes exhausted. For this reason, the regeneration of skeletal muscle fails. Therapies of such diseases could rely on the transplantation of stem cells, for example ESCs. To make this possible it is necessary to fully understand the mechanisms governing differentiation of stem cells. Despite many years of research, knowledge on the differentiation of ESCs is still incomplete, preventing clinical application of these cells. Most of ESC analyzes are carried out using *in vitro* differentiation models. Such approach does not necessarily reflect the *in vivo* behavior of differentiating ESCs. Moreover, it limits the analysis to the early stages of differentiation. Teratomas (gr. *teratos* – monster) serve as a model which allows to analyze early as well as terminal stages of tissue differentiation. These nonmalignant tumors can be formed from ESCs after their injection under the skin of mice. Teratomas are the structures composed of tissues derived from all three germ layers. They are an excellent model for the analysis of differentiation, as during teratoma development fully matured cells and tissues are "produced". This experimental model allowed us to differentiate ESCs to innervated muscle fibers, which cannot be obtained *in vitro*. Using such experimental model I will focus on understanding the role of Pax7 transcription factor during early and most importantly terminal stages of ESC myogenic differentiation and satellite cells formation. During mouse embryonic development Pax7 drives the generation of skeletal muscle precursor cells and also satellite cells present in adult skeletal muscle. Pax7 protein is also involved in the embryonic and fetal myoblasts differentiation. It also acts as anti-apoptotic agent - mice lacking a functional Pax7 showed a considerably reduced number of satellite cells. We have previously shown that lack of functional Pax7 in differentiating ESCs affects proliferation and the early stages of myogenic differentiation. We do not know, however, what would be the consequences of the lack of functional Pax7 during advanced stages of ESCs myogenic differentiation.

The aim of this new project is to determine the role of Pax7 at the early and advanced stages of *in vivo* ESCs myogenic differentiation. To learn that we will focus on: formation, proliferation, and apoptosis of myoblasts; formation of muscle fibers types (fast versus slow), and finally formation of satellite cells in teratomas. Proposed project includes the analysis of control and *Pax7-/-* mouse ESCs already available at the Department of Cytology. I will perform complex analyzes of teratomas derived from *Pax7+/+* and *Pax7-/-* ESCs:

1. Histological analysis of skeletal muscle tissue formed in teratomas.
2. Analysis the expression of key myogenic regulators controlling different stages of myogenesis, including those one that drive the formation of embryonic and fetal myoblasts.
3. Analysis the ability of ESCs to complete myogenic differentiation and to form satellite cells.
4. Analysis of the type of muscle fibers formed in teratomas.
5. Analysis of proliferation and apoptosis in teratomas.

Proposed project will allow us to understand the impact of Pax7 at the advanced stages of myogenic differentiation. The role of Pax7 in the regulation of self-renewal and stem cells differentiation in adult muscles has not been fully explained, yet. There are conflicting data on the Pax7 functions in adult muscles. Thus, obtained results might broaden the knowledge on the functions of Pax7 and could be another step towards understanding myogenic differentiation of ESCs and application of these cells in the treatment of tissues injures and degenerative diseases.