The neural stem cells have an ability for self-renewing and a potential to generate different cell types of the central nervous system like neurons, astrocytes and oligodendrocytes. The neural stem cells proliferate, migrate and differentiate to develop the complex neural network during brain development. The proliferation results in increased number of cells and migration determines their proper location. In the end, the differentiation is responsible for the production of cells that build the nervous system in the correct proportion. Thus, proliferation, migration and differentiation merges the individual cells of the nervous system in a well-defined network to enable them to interact properly, what is necessary for proper brain development. The previous studies have shown that the abnormalities in neural stem cells functioning may lead to brain lesions development, including benign tumors. The benign tumors are observed in many neurological disorders and tuberous sclerosis complex (TSC) is one of them. TSC is an inherited, multisystem disorder that give rise to several neurological symptoms e.g. epilepsy, autism spectrum disorders and intellectual disability, which are linked to brain lesion occurrence called hamartomas. Genetically, TSC is described by mutation in genes TSC1 (hamartin) or TSC2 (tuberin) that encodes TSC1-TSC2 protein complex. The decreased activity of TSC1-TSC2 protein complex leads to the hyperactivation of mTOR signaling pathway, a major cell growth and proliferation controller. The previous studies in mice have revealed that decreased activity of TSC1-TSC2 protein complex leads to improper migration, proliferation and differentiation of neural stem cells that results in brain lesion development similar to those observed in TSC patients. Importantly, the use of mTOR inhibitors counteracts the formation of some of these lesions.

The migration and proliferation processes are regulated by many different proteins. These proteins must localize on the cell surface of neural stem cells in order to communicate with adjacent cells or response to environmental cues, what determines their proper functioning. The presence of these crucial proteins on the cell surface is a dynamic process regulated by endocytosis that enables them to enter the cell.

The previous studies conducted in our laboratory have shown that mTOR kinase is a positive regulator of endocytosis. However, it is not known how this process is regulated in neural stem cells with decreased activity of TSC1-TSC2 protein complex. Our preliminary results have indicated that endocytosis is upregulated in non-neuronal cells derived from TSC patients, but it is not clear whether this change contributes to pathological changes observed in the nervous system of these patients. My preliminary experiments have shown that the localization of many proteins crucial for proper migration and proliferation, and thus proper brain development, is disturbed in human neural stem cells with decreased activity of TSC1-TSC2 protein complex. At the same time, human neural stem cells with decreased activity of TSC1-TSC2 protein complex showed aberrant cell proliferation and migration. *Hence, we hypothesize that altered localization of proteins important for migration and proliferation, regulated by endocytosis, leads to disturbances in the functioning of neural stem cells, resulting in abnormal brain development*. **Consequently, the aim of this project is to identify improperly internalized proteins important for the proliferation and migration of human neural stem cells with a decreased activity of TSC1-TSC2 protein complex.** The aim will be accomplished by (i) the identification of proteins differentially expressed on the surface of human neural stem cells with a decreased activity of TSC1-TSC2 protein complex and the selection of candidate proteins essential for proliferation and migration; (ii) the investigation of endocytosis of selected candidate proteins in human neural stem cells with a decreased activity of TSC1-TSC2 protein complex and (iii) the investigation of rescue effect on migration and proliferation of human neural stem cells with a decreased activity of TSC1-TSC2 protein complex by the manipulation with a protein level of selected candidates.

We expect results of this project to provide a novel insight into the surface localization of proteins in TSC neural stem cells model. Moreover, describing the endocytic events in TSC neural stem cells will help to better understand improper neural migration and proliferation that cause pathological phenotype in TSC. The advances will provide novel information about underlying neurological abnormalities in TSC, which may contribute, in the future, to improvement of current treatment of TSC and other mTOR-related neurodevelopmental disorders.