Spinal cord injury (SCI) causes the most devastating sensory-motor traumas, affecting about 350,000 new patients every year. The injury initiates a cascade of highly destructive secondary events, among which there is degradation of the nerve tissue. As axonal regeneration in the injured spinal cord is not observed in mammals, the consequences of the SCI last throughout the patient's life, leading to permanent disabilities. Currently, there are no effective strategies introduced to reverse the destructive effects of SCI. The only known clinical approach for recovery is based on the training-induced plasticity of the spinal cord circuits. In experimental conditions, however, there are some strategies tested that give some promising results. Novel approaches are based on cell therapies with the usage of progenitor/stem cells cultured specifically *in vitro*. Many of those treatments propose embryonic stem cell grafting, but these approaches raise ethical concerns and their effects are rather limited. Thus, it is important to elaborate on an alternative and more effective approach to SCI therapy.

One of the SCI effects is the disruption of nerve fibers that run along the spinal cord. The innervation derived from serotonergic (5-HT) neurons from the brainstem, which controls locomotor functions, is destroyed. Our team has already demonstrated that transplantation of the fetal brainstem region of raphe nuclei containing 5-HT neurons into the spinal cord below total transection of paraplegic rats results in improvement of their hindlimb locomotor functions, due to restored 5-HT innervation.

In the present project we will go one step further, we aim to generate defined populations of pre-differentiated progenitor cells. We will test the hypothesis that modulation of the *in vitro* culture conditions can result in diverse differentiation of neural progenitor cells (NPCs) and the generation of different types of neurons. Thus, in the proposed project, we aim to establish whether NPCs of various sources (either exogenic – embryonic brainstem origin or endogenic – ependymal spinal cord central canal origin), pre-differentiated under the control of various trophic factors would be able to differentiate to various phenotypes in conditions of *ex vivo* spinal cord slices. Finally, we will determine whether NPCs expanded in defined *in vitro* conditions after intraspinal grafting in adult paraplegic rats can generate cells of region-specific *in vivo* and enhance recovery of lost motor functions.

Respecting the 3Rs principle (Reduction, Refinement, and Replacement), we will carry out the majority of our investigation on cells in culture (*in vitro* conditions). However, being aware that the complexity of cellular and molecular composition can hardly be modeled in a dissociated cell culture, we also plan to use organotypic spinal cord slices in *ex vivo* conditions, which is described as the model of SCI. The organotypic slices provide an optimal model for the first screen of possible ways for restoration of lost monoaminergic innervation using pre-differentiated cell transplantation. Thus, in organotypic spinal cord slices, the grafted cell fate in response to the insult of the injured spinal cord microenvironment will be investigated. We will start our project using *in vitro* techniques, to switch afterward to spinal cord slices in culture (*ex vivo* model). After the *in vitro* and *ex vivo* investigations, we will be able to select the most promising population of pre-differentiated progenitor cells that passed positive verification under *ex vivo* organotypic culture conditions. Such NPCs will be then tested in adult paraplegic rats in the *in vivo* condition. We aim to prove the concept, that modulation of cell fate by targeted differentiation can result in the generation of 5-HT cells suitable for restoration of the missing neurotransmitter system in the spinal cord below total transection.

We plan to perform molecular characteristics of ependymal cells differentiated into neural phenotype in the *in vitro* conditions using RNAseq-based transcriptome analysis. The preliminary analysis of the results of RNAseq-based transcriptome of embryonic NPCs proliferating under different conditions *in vitro vs.* activated spinal cord ependymal cells after spinal cord injury allowed us to propose the TGF β and Wnt signaling pathways - as candidates for modulation that might direct ependymal progenitor cell differentiation into neuronal lineage (ultimately into 5-HT neurons), which will be tested in the proposed project. Understanding the transcriptome characteristics of NPCs directed to various differentiation pathways is important for initiating their differentiation in the predicted direction. Understanding molecular mechanisms involved in the activation, proliferation, and differentiation of the ependymal NPCs after spinal cord injury may contribute to the development of new SCI treatment therapies based on modulating these cells directly in the central canal of the patient's spinal cord.

From a clinical point of view, the ependymal cells have a significant advantage over other multipotent cells (e.g. iPS) because they can be easily and quickly available (the therapeutic window in spinal cord injury is narrow) and their differentiation can be modulated both before and after transplantation. In addition, these cells are already activated at the site of injury and their involvement in regenerative processes allows the aspect of histocompatibility to be omitted. In the future, the sequencing data gathered here may become a valuable source of more universal knowledge for generating novel therapeutic strategies employing microenvironment modification to enhance the endogenous neurogenerative ability of the injured spinal cord.

From a future perspective, employing endogenous spinal NPCs into targeted differentiation could result in reinnervation of the spinal cord *in situ*, a novel procedure that could be a promising treatment for SCI patients.