Characterizing the functions and molecular mechanisms of VPS4B action in biology of colorectal cancer (CRC) cells and in CRC pathogenesis

VPS4A and VPS4B proteins are enzymes with ATPase activity. Their function in the cell is to cooperate with the ESCRT protein complexes in remodeling of lipid bilayers, which occurs during various cellular processes such as endocytosis, autophagy and cytokinesis. All these processes play important roles in proper functioning of the cell, allowing it to sense environmental signals, take up nutrients, degrade damaged organelles and to divide. In consequence, perturbation of these processes, e.g. due to the lack of one of the ESCRT proteins or VPS4A/B, may contribute to pathologies, like for example tumor development.

Multiple studies have shown that expression of the *VPS4B* gene is perturbed in human cancers, such as lung, liver and breast cancers. Moreover, deletion of one out of two copies of the *VPS4B* gene occurs in almost 70% of human colorectal cancers (CRCs). However, up to date there have been no studies addressing the impact of VPS4B depletion on CRC growth. Therefore, **the general objective of this project is to characterize the functions of VPS4B in biology of human colorectal cancer cells and in CRC pathogenesis.** Specifically, the detailed aims are to:

- identify the function of VPS4B in CRC cells,
- analyze how depletion of VPS4B protein affects growth of CRC cells *in vitro* and *in vivo,*
- analyze the level of VPS4B protein in human colorectal cancer samples.

The experiments will be performed in human CRC cell lines with normal level of VPS4B protein. Expression of the *VPS4B* gene in CRC cells will be transiently silenced using short interfering RNA (siRNA) or permanently inhibited by the *VPS4B* gene disruption.

This project will provide novel data about the molecular functions of VPS4B protein in biology of human CRC cells, as well as contribution of VPS4B to colorectal cancer pathogenesis. The profiling of genes expressed upon VPS4B protein depletion (performed for the first time) will identify the cellular pathways controlled by VPS4B that might be perturbed in colon cancer cells. The analysis of growth of VPS4B-depleted CRC cells *in vitro* and *in vivo* will assess whether decreased level of VPS4B protein may enhance the oncogenic properties of these cells. Finally, the proposed histological studies will characterize the distribution and abundance of VPS4B protein in human healthy colon and CRC tissue samples from patients. In addition, these analyses will also examine the correlation between abundance of VPS4B protein in the CRC tissue sample and the clinical features of a given cancer to find out whether VPS4B protein levels can serve as a prognostic marker of CRC disease.