Cancer is one of the leading cause of deaths worldwide. The main cause of cancer development is linked to the ageing of the society and spread of the risk factors, such as improper lifestyle including smoking, poor diet and lack of exercises. Breast cancer has the highest incidence (~1.68 mln new cases) and mortality rate (~0.52 mln cases) among all female cancer patients. Lung cancer is the first cause of cancer-related diagnosis (~1.24 mln) and death (~1.1 mln) in male population. Development of cancer is a consequence of many changes in normal cellular mechanisms involved for example in cell growth and metabolism. Two main groups of genes that must be altered to initiate cancerogenesis are oncogenes and tumor suppressor genes. Human genome encodes so called proto-oncogenes that may be transformed to an oncogene mainly by genetic mutations or increased expression. Proto-oncogenes are involved in the regulation of cell growth and differentiation, thus transformation to oncogene impairs this processes and contributes to cancer development. Tumor suppressor genes are involved in protecting the cells from abnormalities that may lead to cancer. These group of genes is often inactivated in many malignant tumors. Loss of function can be the cause of genetic or epigenetic alterations. Epigenetic alteration refer to any changes observed within a cell that are not caused by changes in the DNA sequence, but have a strong influence on gene activity. One of the most intensively studied epigenetic modification is methylation of cytosine. Methylation is a biochemical process, in which a methyl group is added to cytosine, most often seen in a context of CpG (cytosine-phosphate-guanine) dinucleotide. CpG dinucleotides are abundant in gene regulatory elements such as gene promoters. Methylation within gene promoters is leads to its inactivation. Cancer cells are globally depleted of DNA methylation, but some hyper-methylation occurs within tumor suppressor gene promoters, thus inactivates them and impairs their function. To date, specific mechanisms that are responsible for inactivation of particular suppressor genes are still poorly described. Our aim is to investigate the role of selected members of KRAB-ZNFs (Krüppel associated box-Zinc Finger Proteins) in the modulating epigenetic profile of cancer cells. We are specifically interested in addressing the question whether KRAB-ZNFs may induce epigenetic repression of tumor suppressor genes. We assume that by negative regulation of tumor suppressor genes, cancer-associated KRAB-ZNFs promote progression of cancerous features.

KRB-ZNFs are potent epigenetic repressors. These protein binds to a defined DNA sequence and by formation of multiprotein complex triggers formation of heterochromatin. Heterochromatin is a ‘closed’ state of chromatin environment associated with the transcriptional repression of nearby genes. Our aim is to identify specific genes that are regulated in cancer cells by selected KRAB-ZNFs. We have chosen KRAB-ZNFs that are overexpressed in several cancer types by statistical analyses. These analyses were based on data deposited in TCGA (The Cancer Genome Atlas), one of the largest project aiming at the comprehensive molecular profiling of human tumors. In the next step we will validate these results in laboratory using some basic molecular biology methods like RT-qPCR and Western blot. As a biological material we will use RNA and protein samples from normal and cancer cell lines and tissues obtained from two most common cancer types: breast and lung. Then our goal will be to discover and characterize genome-wide distribution of KRAB-ZNFs deposition sites. To this end, we will enrich DNA fragments bounds by selected KRAB-ZNFs and sequence them using high-throughput method of next-generation sequencing. In the next step, we will prepare cancer cells with decreased expression of selected KRAB-ZNFs utilizing RNA interference technology. This will be followed by phenotypic assays testing cell proliferation, apoptosis, migration and invasion. In this way we will assess the potential of oncogenic properties of selected KRAB-ZNFs. In the last step we want to verify whether the epigenetic and phenotypic effects of KRAB-ZNFs observed in vitro are relevant also in the clinical setting. In order to do so, we will perform a set of statistical analyses correlating expression profile of selected KRAB-ZNFs with clinical outcome of patients.

To our knowledge, this will be the first study that combines molecular biology, genetic engineering, cancer cell biology, epigenetics, bioinformatics and biostatistics to explore the molecular and phenotypic function of cancer-associated KRAB-ZNFs. The proposed workflow of experiments is original and beyond the state-of-the-art. What is more, defining specific factors that may be responsible for inactivation of certain tumor suppressor genes is extremely significant. Our results may be useful not only for expanding the knowledge about molecular mechanisms governing carcinogenesis, but also for the development of the targeted epigenetic anti-cancer therapies.