Project title: The role of proteins containing the PIN domain in the regulation of non-coding RNAs.

RNA degradation is one of the mechanisms of post-transcriptional control but also defends eukaryotes against infection with viruses whose genetic material is RNA. In general, the enzymes involved in RNA degradation can be divided into: exonucleases which degrade RNA starting from the 5 'or 3' end, and endonucleases which cut templates in the middle, recognizing specific sequences or structures. In recent years, many proteins containing the PIN domain have been described. Proteins containing such a domain are found in eukaryotes, bacteria and archaea. PIN is the catalytic domain responsible for endonucleolytic cleavage of RNA (PilT N-terminus).

Our group made a significant contribution to understanding the role of the MCPIP1 PIN domain and the role of the protein itself in the regulation of cellular processes. MCPIP1 regulates inflammatory processes as well as cell division, angiogenesis, differentiation and cell death by degrading mRNA and precursor miRNAs. We have shown that MCPIP1 is of great importance in the regulation of skin homeostasis and pathological processes of this organ, including psoriasis and cancer. In preliminary studies, we observed that the skin also contains another protein from this family, MCPIP3. MCPIP1 is present in the granular layer and MCPIP3 in the basal layer. In addition, we observed altered levels of these proteins in Squamous-Cell Carcinoma (SCC), what consequently must be associated with altered levels of RNA as both proteins function as RNases. SCC is one of the most common skin cancers. As our preliminary data also show, these RNases can regulate the level of non-coding RNAs (ncRNAs), which include: miRNAs, circular RNAs, and long non-coding RNAs. These three types of non-coding RNAs are of great importance in the regulation of post-transcriptional gene expression. Non-coding RNAs regulate the level of mRNA in the cell, influence protein synthesis but also regulate each other. For example, long non-coding RNAs can act as "sponges" to trap miRNAs in a cell. Moreover, long non-coding RNAs may contain miRNA templates within their sequence, and their final level in a cell depends on stimuli inside and outside the cell. Circular RNA molecules are relatively new elements. Studies have shown that one of the functions of circular RNAs is modulation of miRNA activity, but also post-transcription regulation and a role in protein translation.

The aim of the project will be to study the interrelationships between RNases and ncRNAs and to evaluate the role of these interactions in the etiology / development of chemically induced mouse tumors. In these studies we will use unique research models - mice with a knockout of the MCPIP1 gene and mice with a knockout of the MCPIP3 gene in keratinocytes. Mice with active genes will serve as a control of the experiment. In our research, we will use modern research methods, such as next-generation sequencing and advanced bioinformatics analyzes. Moreover, selected matrices will be subjected to functional analyzes. Using the human squamous cell carcinoma cell line of the skin (A431) and modern methods of modulating gene expression (shRNA, CRISPR / Cas9), we will check how selected matrices affect the properties of cancer cells: cell cycle regulation, genomic changes, invasive growth, cell migration, chemotaxis, changes in surface receptors, secretion of lytic factors, etc.

Implementation of the project is important for many reasons. First, there is not much information available about posttranscriptional ncRNA regulation. Second, the implementation of the tasks will show for the first time whether MCPIP RNases degrade ncRNA (so far we know that MCPIP1 degrades miRNA). Another important aspect of the project is to demonstrate which ncRNAs have a significant influence on tumor initiation and growth. In addition, the results obtained in this project could prove very important in designing therapies targeting RNA molecules. In addition, the identified RNAs may be used as markers for the detection of early stages of cancer, as well as for predicting the course of disease.