The primary function of immune system is to provide protection against infection. Mammalian immune system is divided into two basic categories: innate and adaptive immune responses. The innate immune system is the first line of the defensive mechanisms, is nonspecific and acts immediately, whereas adaptive immune system provides antigen specific response, activates humoral and cellular immune responses and exposure to the antigen leads to immunological memory. Although the immune system is designed to be protective, excessive or inappropriate activation of immune cells or cytokines can lead to serious chronic inflammatory disorders. Members of Toll-like and interleukin-1 receptor superfamily (TLR/IL-1R) are part of the innate immune system and play an important role in pathogen recognition and inflammation. It has been shown that SIGIRR (single immunoglobulin IL-1 receptor related molecule) is negative regulator of TLR/IL-1R-mediated signaling. Excessive or uncontrolled activation of TLR/IL-1R is linked to many disorders including autoimmune, chronic inflammatory and infectious diseases, as well as different types of cancer.

Colorectal carcinoma is the second leading cause of cancer related death in Europe. The development of colorectal carcinoma follows a stepwise normal-adenoma-cancer progression, marked by both genetic and epigenetic alterations. Epigenetic changes do not alter the underlying DNA sequence, instead they involve other alternations such as DNA methylation, leading to inactivation of tumour suppressor genes. Although epigenetic disruption was originally thought to only affect transcription, emerging evidence shows that it also regulates alternative splicing.

In our previous studies, we have shown that SIGIRR acts as tumor suppressor in mouse model of colorectal cancer. Our most recent study demonstrated that receptor SIGIRR is frequently inactivated in human colorectal cancer by the increased expression of a novel SIGIRR isoform ($SIGIRR^{\Delta E8}$). $SIGIRR^{\Delta E8}$ transcript is generated
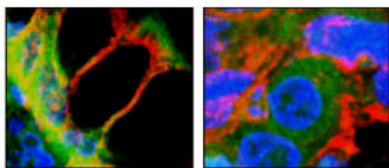
Microscopic picture showing SIGIRR localization in normal and cancer cell (SIGIRR, DAPI, Na⁺K⁺-ATPase)

by an alternative splicing event that excludes the eighth exon of the SIGIRR gene. $SIGIRR^{\Delta E8}$ functions as a dominant negative mutant and blocks full-length SIGIRR's ability to inhibit TLR/IL-1R signaling. $SIGIRR^{\Delta E8}$ traps the full-length SIGIRR protein in the endoplasmic reticulum (ER), preventing its modification by complex glycan and membrane localization.

Integrated analysis of exon and RNA sequencing data from 68 pairs of normal and colon cancer samples indicates that $SIGIRR^{\Delta E8}$ alternative splicing is independent of detected genetic mutations, suggesting an epigenetic mechanism underlying the splicing. Based on this evidence, **we hypothesize that SIGIRR is inactivated during colorectal cancer development through epigenetic alterations that promote the expression of $SIGIRR^{\Delta E8}$, generating potentially oncogenic form of SIGIRR protein that promotes cancer growth. The goal of this proposal is therefore to elucidate the mechanism by which epigenetic alteration results in alternative splicing and inactivation of tumor suppressor SIGIRR.** In proposed experiments we plan to use novel research techniques (global analysis of DNA methylation, metabolic profiling, CRISPR technology, transgenic animals, mouse model of colon cancer, xenograft model), which will allow for detailed investigation of our hypothesis.

Colon cancer is among the most commonly diagnosed cancers of gastrointestinal tract. Every year, there are 450,000 new cases diagnosed in Europe, including 12,000 cases in Poland. The development of the CRCs exemplifies the multistep transformation model of tumorigenesis, therefore it is very important to elucidate the detailed mechanism underlying tumor development, progression and metastasis. Inactivation of tumor suppressor genes is considered to be a critical step during tumor formation. The obtained results might provide novel insights into one of the mechanisms of colon tumorigenesis. While our preliminary data indicates the correlation of $SIGIRR^{\Delta E8}$ with severity of colon cancer, we will further study the potential of this specific epigenetic change of SIGIRR as a diagnostic and prognostic, as well as predictive biomarker in new screening/monitoring and therapeutic strategies.