DESCRIPTION FOR THE GENERAL PUBLIC

The *CRNDE* gene has emerged lately as an oncogene involved in development of the variety of neoplasms. Our team has identified and submitted to GenBank first two complete transcripts of this gene. We also showed that their overexpression is a negative prognostic factor in ovarian cancer patients, associated with increased risk of death and recurrence. Moreover, we proved that one of these transcripts contains an open reading frame encoding the CRNDEP micropeptide, which seems to play a crucial role in a cell division, and is upregulated in cells characterized by a high proliferation rate, both cancerous and normal.

The main aim of this project is to examine the *CRNDE* gene sequence along with its promoter methylation in a group of ovarian tumors with a detailed clinico-pathological characterization and follow-up data available. In addition, we aim to assess expression of CRNDEP micropeptide in the same group of tumors, and determine possible correlations between altered CRNDEP levels and patient survival or tumor response to chemotherapy. Lastly, we would like to investigate a function of this micropeptide by verifying results of our previous studies showing that high overexpression of CRNDEP leads to a formation of stress granules in HeLa cells, and then trying to elucidate the underlying mechanism.

In the present project, extensive studies on a DNA and protein level will be conducted. They will be also supplemented with bioinformatics and statistical analyses. First, evaluation of the *CRNDE* gene sequence will be performed with the use of Ion S5 next generation sequencer (Thermo Fisher Scientific) on 135 frozen ovarian tumors from patients treated with either cisplatin and cyclophosphamide (PC regimen, N=32) or taxanes and cisplatin (TP regimen, N=103). For the same group, the *CRNDE* RNA levels were previously assessed by qPCR, showing a negative impact of *CRNDE* overexpression on survival of the TP-treated patients. In this study, we are going to examine not only the coding sequence of the gene, but also its promoter region, introns and both UTRs. Noteworthy, *CRNDE* shares its promoter with another gene, *IRX5*, and correlated expression of both these oncogenes was found to be a negative prognostic factor in colorectal cancer. Given that, we decided to evaluate the entire *IRX5* gene sequence in our study, too. Apart from identification of mutations, a concurrent analysis of single nucleotide polymorphisms (SNPs) within the *CRNDE/IRX5* locus will be performed. This approach could provide useful information on copy number variations (CNVs), and allow for determination of haplotypes related to poor clinical outcome due to the increased risk of death, recurrence or tumor resistance to therapy.

In the same group of 135 ovarian tumors, we are also going to assess a copy number variation (CNV) of the *CRNDE* gene and a level of its promoter methylation. The CNV study will employ fluorescence in situ hybridization (FISH), a technique widely used in molecular cytogenetics. *In situ* detection of fluorescent signals will give us the opportunity to directly count the number of *CRNDE* copies within the interphase cell nuclei, without a need of using reference genes which may become unstable in cancer cells. In this experiment, a single color CRNDE-specific FISH probe (Empire Genomics) will be utilized. The *CRNDE* promoter methylation status will be assessed with the use of Cells-to-CpG Bisulfite Conversion Kit (Thermo Fisher Scientific) followed by PCR on bisulfite-converted DNA templates and Sanger sequencing.

In this project, we also intend to measure expression of the CRNDEP micropeptide in 250 formalin-fixed, paraffin-embedded (FFPE) ovarian tumor sections from TP-treated patients with complete clinico-pathological characterization and follow-up records. This evaluation will be carried out using an indirect Enzyme-Linked Immunosorbent Assay (ELISA) and the rabbit anti-CRNDEP antibody (Abgent). Apart from measuring CRNDEP expression and correlating it with ovarian cancer prediction and prognosis, we will also try to explain how strong artificial overexpression of CRNDEP in a fusion with either EGFP, DsRed Monomer or the FLAG tag induces oxidative stress in HeLa cells, which was shown in our previous studies. This phenomenon will be examined in several cell lines: HeLa (derived from cervical cancer cells), breast cancer cell line MCF-7, ovarian carcinoma cell lines: TOV-1, IGROV1, SKOV-3, A2780 and colorectal carcinoma cell lines: HT-29, HCT-116. These cell lines differ with respect to TP53 activity and accumulation status, which not only allows for investigation of the underlying cellular mechanism, but also creates opportunity for testing, whether this function of CRNDEP depends on the TP53 status or not. Two assays, ROS-Glo H_2O_2 and GSH/GSSG-Glo (Promega), will be used in this study.

Considering a multitude of scientific reports on oncogenic role of *CRNDE* in various malignancies, one may expect that thorough examination of this gene along with identification of the function, and clinical significance of its products may lead to their usage as sensitive and specific biomarkers of early-stage tumorigenesis or facilitating a selection of the best treatment method. Ultimately, they may even be used as targets for molecular therapy of cancers. Accordingly, levels of one of *CRNDE* transcripts in blood plasma were recently reported as a novel, promising biomarker for diagnosis and prognosis of colorectal cancer.