Research project objectives

In this project, we aim to investigate the role of CRNDEP, the centrosomal peptide recently discovered by our team. We will also try to identify its protein partners, and to determine its prognostic value in ovarian cancer. In addition, the impact of artificial overexpression or knockdown of the CRNDEP-coding gene on the response of MCF-7 and HEK 293 cells to cytostatics and estrogens will be evaluated as well.

Research methodology

Until recently, the *CRNDE* (colorectal neoplasia differentially expressed) gene was classified as non-protein coding, and all of its transcripts were treated as long non-coding RNAs (lncRNAs). Lately, we have discovered a protein product, CRNDEP, encoded by one of the transcripts. This peptide, consisting of 84 amino acids, seems to be involved in cell proliferation as a component of centrosomes. We also found that CRNDEP is overexpressed in highly proliferating tissues, like intestinal crypts of the colon, spermatocytes, proliferative-phase endometrium and ovarian cancer cells. Our other study revealed that overexpression of *CRNDE* in ovarian cancer cells associates with a poor prognosis in patients treated with the taxane/platinum regimen. Furthermore, *CRNDE* expression is significantly elevated in tumors with the wild-type TP53 protein compared to those with the TP53 accumulation.

Herein, we plan to perform a screening for associations between the aberrant expression of *CRNDE*, and changes in the rate of proliferation and apoptosis, occurrence of abnormalities of the cell cycle, and potential decrease in cellular viability of MCF-7 and HEK 293 cells. The same associations will be also assessed after treating these cells with estrogens and several cytostatics, including nocodazole, paclitaxel, phenformin, griseofulvin, and carcitriol. As we have not yet established daughter cell lines with the stable overexpression or knockdown of *CRNDE*, in the initial phase of this project, we will be transfecting the MCF-7 and HEK 293 cells with *CRNDE*-overexpressing or *CRNDE*-silencing constructs that we already possess. Later, all discovered relationships will be corroborated in newly established stable cell lines, containing expression cassettes for regulable overexpression or knockdown of *CRNDE*, developed from MCF-7 and HEK 293 cells. These daughter cell lines will be created by utilizing the CRISPR/Cas9 technology for targeted editing of the genome in combination with the use of Tet-On expression system. The impact of aberrant *CRNDE* expression on entire transcriptomes of the cells will be evaluated by RNA sequencing on the NGS HiSeq 2500 platform. In addition, the transcriptional activity of individual genes, likely functionally related to *CRNDE*, i.e., *TP53*, *IRX5*, and genes encoding for cyclins, and the estrogen receptor, will be assessed by Real-Time qPCR on the 7500 Fast Real-Time PCR System.

We also intend to evaluate a prognostic value of CRNDEP by determining how alterations of its expression affected overall survival and disease-free survival of 250 ovarian cancer patient treated with the taxane/platinum regimen. This study will be performed by immunohistochemical staining of formalin-fixed, paraffin-embedded tumor sections with the use of CRNDEP-specific antibody.

Lastly, we will try to identify protein partners of CRNDEP by using the method named Strep/FLAG Tandem Affinity Purification (SF-TAP). It allows for a purification of the proteome in its native form, which preserves protein complexes existing in vivo, thus enabling the identification of native protein-protein interactions. Thanks to this two-step purification approach, the percentage of false positive results is significantly lower than in single-step affinity purification methods.

Research project impact

Ovarian carcinoma is the leading cause of death from gynecological malignancies, while the breast cancer is the first most common malignancy in European women. According to the Polish National Cancer Registry, 2547 and 5226 patients died of either ovarian or breast cancer, respectively, in Poland in 2010. Mortality in both diseases is exceptionally high due to either the absence of specific symptoms at early phases (ovarian carcinoma), or various difficulties with its early detection (breast carcinoma). Thus, the majority of patients are diagnosed at late stages, which are characterized by poor prognosis. There is an urgent need for improvement of screening and therapeutic methods. Identification of new markers, potential targets of molecular therapy, could remarkably facilitate the fight against these two neoplasms.

Centrosomes evolve in animals to serve as the main microtubule organizing centers (MTOC), and also to regulate progression of the cell cycle. They are composed of two orthogonally arranged centrioles surrounded by an amorphous mass o proteins termed the pericentriolar material (PCM). As already mentioned, we have lately discovered that accumulation of the TP53 protein in ovarian cancer cells correlates with the decreased expression of *CRNDE*. This observation appears to be supported by the fact that TP53 along with other tumor suppressor proteins, like BRCA1, BRCA2, RAD51 and PARP1, are crucial components of the centrosome. They play roles in the regulation of the centrosome metabolism, whereas mutations and abnormal expression of their genes lead to structural and numeric aberrations of centrosomes.

Centrosomal abnormalities is a well-known phenomenon in various types of cancer. They can be divided into two subgroups, structural of numeric aberrations. Interestingly, both types can be simultaneously found in a tumor. In contrast to normal cells, most tumor cells contain multiple centrosomes, which tend to cause the formation of multipolar mitotic spindles, chromosome segregation defects, and cell death. Nevertheless, many cancer cells divide successfully because they can cluster multiple centrosomes into two spindle poles. Some therapeutics, like the antifungal drug Griseofulvin, or Reduced-9-bromonoscapine (RedBr-Nos), have the ability to disrupt the centrosome clusters, which may drive cancer cells with amplified centrosomes to mitotic catastrophe and apoptosis without affecting normal cells.

Although the *CRNDE* gene is still rather poorly investigated, in all papers available, it is presented as a tumor-promoting factor. Its expression is highly elevated in colorectal cancer, ovarian cancer, and in other solid tumors and leukemias. Other studies suggest its role in promoting cell growth and invasiveness in gliomas through the mTOR pathway, both in vitro and in vivo. It was also reported that treating the colorectal cancer cells with insulin and insulin-like growth factors results in decreased levels of nuclear *CRNDE* transcripts. These repressive effects can be negated by inhibitors against either the PI3K/Akt/mTOR pathway or Raf/MAPK pathway, suggesting that *CRNDE* is a downstream target of both signaling cascades. Moreover, knockdown of *CRNDE* leads to the inhibition of aerobic glycolysis, also known as the Warburg effect, being one of cancer hallmarks. Remarkably, it was demonstrated that the levels of extracellular *CRNDE* transcripts circulating in blood plasma are significantly elevated in patients with colorectal adenomas and adenocarcinomas compared to the control group. The authors concluded that this blood test is able to discriminate between ill and healthy individuals with a high diagnostic sensitivity and specificity.

Considering all these facts, it seems probable that further investigation of the function of *CRNDE* transcripts and its protein product may benefit in improving the future cancer diagnostics, or even in developing novel methods for the molecular therapy of cancers.