PhD project thesis for state doctoral exam

Functional and biochemical analysis of extracellular vesicles from ovarian cancer ascites

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Year: 5

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Expected date of completion and defense of dissertation thesis: summer/autumn 2021

High-grade serous carcinomas of the ovaries, fallopian tube and peritoneum (HGSC) is the most common form of the ovarian cancer, with the highest mortality rate of all gynecological malignancies. Lethality of HGSC is caused by early and almost asymptomatic formation of metastases and rapid development of resistance to key therapeutic agents. Despite recent improvements in patients' outcomes, there remains an unmet clinical need to understand the early pathogenesis of HGSC to bring forward new diagnostic and therapeutic approaches.

Ascites often accompanies progression of HGSC. This pathological accumulation of the fluid in the peritoneum, constitutes a complex environment containing diverse cell populations, extracellular vesicles (EVs) and proteins, which affect tumor growth and progression. We took advantage of ascites as an easily accessible source of patient material – both tumor cells and tumor microenvironment in a liquid form.

EVs are nanosized membranous particles serving as conveyors of proteins, lipids and nucleic acids in between cells and can be found in various body fluids, including blood and ascites. EV molecular composition represents a fingerprint of its cellular origin displaying capacity to modulate biological activity in the recipient cell and having a great potential to serve as disease biomarkers. Due to their small size, electron microscopy (EM) remains the most suitable method for visualization of EVs in sufficient resolution. However, manual analysis of individual EVs in EM images is extremely time-consuming and due to inherent nature of EM pictures of EVs, use of common image analysis pipelines and tools is precluded. Therefore, we developed in collaboration two (semi)-automatic software tools for computer-assisted evaluation (both qualitative and quantitative) of EVs in EM images. Both software tools are freely available online for academic use and have been utilized by the EV research community.

WNT signaling pathway is one of key pathways involved in adult homeostasis and its deregulation is often associated with various diseases, such as cancer. It has 2 main branches: canonical (β-catenin dependent) and non-canonical *e.g.* planar cell polarity pathway (WNT/PCP). Using the functional test of WNT signaling inducing capacity (or its surrogate marker – WNT5A protein levels) of ascites, we have identified a subgroup of HGSC patients whose prognosis is better than could be judged from other independent prognostic factors. In detail, for the first time we showed that: 1) Primary patient tumor cells are responsive to WNT signaling pathway induction and that tumors have molecular alterations of WNT/PCP components that can explain sensitivity to WNT5A; 2) Patients whose ascites cannot activate WNT pathway in HGSC cells have less aggressive disease and significantly better outcome; 3) It is active WNT/PCP signaling (and not WNT/β-catenin signaling) which endows HGSC cells with increased self-renewal and ability to migrate and invade and, consistently, inhibitors of WNT pathway block these prometastatic features; 4) WNT5A ligand can be detected in the ascites on protein level and its levels correlate with the ability of ascites to induce WNT/PCP signaling.

Furthermore, we have isolated EVs from malignant and non-malignant ascites and control fluids as well as EVs from conditioned media from various cell lines (ovarian cancer, non-ovarian cancer, non-cancer) and submitted them for mass spectrometry analysis. We believe that this variety of EVs will enable us to find proteins which are specifically enriched in HGSC. These proteins may not only act as important players in HGSC progression but as well serve as potential prognostic/diagnostic/screening biomarkers of HGSC.

To summarize, results obtained during my studies provide novel insights into the process of ovarian cancer progression and may also help in opening new avenues for EV-based biomarkers.