

Serous ovarian tumors (SOT) represent the most common histopathological type of epithelial ovarian tumors. Ovarian carcinoma (OC), most commonly being of the serous (S) type, is the leading cause of death from malignancies of the female genital tract. Most SOC (90%) belong to high-grade (HG), the others are low-grade (LG). Serous borderline ovarian tumors (SBOTs) are rare (~10% of all ovarian epithelial tumors) and are characterized by intermediate malignant potential and a tendency toward malignant transformation, usually to LGSOC. SBOTs are primarily chemoresistant, while OCs, after standard treatment, usually ultimately recur as chemoresistant. Ovarian tumors usually develop with no symptoms and are difficult to diagnose.

Thus, there is an urgent need to develop new methods for screening, diagnosis, prediction, follow-up and therapy. Tumor microenvironment (TME), with its diverse cellular, non-cellular, protein and regulatory RNA (including long non-coding RNA, lncRNA) complexity, define tumor-derived immunosuppression, and has been shown to play a crucial role in tumor growth, progression and response to treatment. So far, virtually nothing is known on serous borderline ovarian TME, in LGSOCs it is poorly recognized, while in OCs it needs to be better characterized. Moreover, the knowledge on lncRNAs in ovarian tumors is scarce.

We aim to 1) comprehensively characterize the cellular and molecular components of the TME of SBOTs, LGSOCs and HGSOCs, in the clinico-pathological context and chemoresistance, 2) determine the role of lncRNAs in ovarian TME and identify novel lncRNAs, 3) find out if the local immune response parameters and lncRNAs are reflected systemically; 4) examine *in vitro* the functions of the prominent TME lncRNAs and/or other molecules, 5) lay grounds for developing new diagnostic, follow-up and therapy methods

We have a unique, large collection of surgical samples (both fresh-frozen and paraffin-embedded) from previously untreated patients with SBOTs and SOC. The patients have been followed-up for many years, and a thorough clinico-pathological characterization has been performed by experienced pathologist and oncologist. Additionally, our collection comprises frozen samples of normal ovaries/fallopian tubes and a series of prospectively collected paired tumor and the peripheral blood plasma samples from patients with OCs or SBOTs, and the peripheral blood plasma from healthy donors.

A set of the state-of-the-art methods will be employed. Detailed evaluation of transcriptomes extracted from borderline and malignant tumors, will be performed, verified and supplemented with the expression assessed at the protein level. Expression changes of messenger and lncRNAs and of proteins will be analyzed in the context of clinico-pathological and molecular features and chemoresistance.

Transcriptomes will be qualitatively and quantitatively characterized, using the next generation sequencing (NGS) method. After RNA isolation, preparation of cDNA libraries, assessment of their quality, the RNA-seq analysis will be carried out on the NGS HiSeq 1500 platform (Illumina). The subsequent workflow will comprise verification of the NGS results by the NanoString technology at the RNA level, and immunohistochemical confirmation of the cell populations (estimated in the previous step by the bioinformatic tools), and the immunomodulatory proteins in the TME. Immunomodulatory proteins and lncRNAs will also be examined by ELISA and RT-qPCR, respectively, in the matched peripheral blood plasma from a part of patients of each group studied and from healthy donors. The functions of the nominated genes will be evaluated *in vitro* in the OC cell lines, following overexpression/knock-down by the CRISPR/Cas9 system. Changes in the proliferation rate, apoptosis, cellular viability, cell cycle and chemosensitivity (to cisplatin, cyclophosphamide, paclitaxel) will be studied by standard methods. Comprehensive bioinformatic and statistical methods, including tools dedicated to assessing cell population content based on the bulk RNA sequencing analyses and to identify lncRNA targets and functions, will be applied for data analyses.

The novelty of the project lies in a comprehensive molecular and cellular characterization of ovarian TME in the context of the response to treatment, survival, and other clinico-pathological parameters and referred to the characteristics of the normal tissue counterpart, the data not available so far. The following original issues will be addressed: microenvironment hallmarks of SBOTs vs. LGSOCs, expression profiles and functions of lncRNAs in the three examined entities of SOTs, a discovery of new lncRNA molecules. The study will help reveal treatment resistance mechanisms in both SBOTs and SOC, and the influence of local and systemic immune status, and lncRNAs on tumor progression and patient outcome. This is crucial to better understand OT biology, but the project also holds a significant translational potential, as we expect new, potential OT markers to be discovered.

Our large collection of thoroughly characterized OT samples, taken from patients followed-up for many years, enriched with prospectively collected tissue and plasma samples, and normal ovarian/fallopian tissues, secures an excellent material for this study.