IN SEARCH FOR DNA METHYLATION ABERRATIONS ASSOCIATED WITH SUSCEPTIBILITY TO OVARIAN CANCER

Ovarian cancer (OC) is one of the most lethal cancerous diseases in women. The prognosis of OC mainly depends on its early detection (the 5-year survival rate decreases from ~90% to 30% with late detection). However, due to the asymptomatic nature of OC and the lack of effective tests to detect the early stage of the disease, approximately 70% of women are diagnosed at an advanced stage when the tumor has spread outside of the pelvis and to distant metastatic sites, which cannot be completely removed by surgery. Identification of women at high OC risk and subsequent relevant lifestyle and follow-up strategies may result in early diagnosis and thus reduce the number of OC-related deaths.

OC is characterized by a significant amount of so-called hereditary cases. Germline mutations (present in all body cells, including reproductive cells, and passed down within a family) in already-known cancer-related genes are detected in over 20% of patients. Taking the above into account, OC represents one of the best models to investigate the potential impact of an alternative mechanism, i.e., aberrant DNA methylation, on disease risk. The hypothesis considered in this project is that aberrant DNA methylation of normal tissue is one of the factors contributing, at least in some cases, to the OC risk.

DNA methylation is an epigenetic modification in which a methyl group is attached at carbon 5 of cytosines (5mC), usually in the context of a cytosine followed by a guanine (CpG site). Between 60% and 90% of all CpG sites are methylated in humans, regulating tissue-specific gene expression, repression of repetitive elements, and many other processes. An aberrant methylation pattern is observed in the DNA of many tumors and involves genome-wide hypomethylation as well as hypermethylation of normally unmethylated CpG islands in the promoter regions of tumor suppressor genes. Such hypermethylation, leading to silencing of gene expression, may act as an alternative mechanism of gene inactivation (along with mutations) and represent one of the two hits in Knudson's two-hit hypothesis for oncogenic transformation. Therefore, one of the research goals of the project is to assess the prevalence of aberrant methylation in the promoter regions of several OC-related genes in normal tissue (i.e., blood) in a cohort of OC patients and matched controls, to determine the potential of these abnormalities as novel risk factors for OC. For profiling aberrant DNA methylation, I developed a high-throughput and ultrasensitive methylation panel assay (OVCA MethPanel) covering the promoter regions of nine OC-related genes, which is based on deep amplicon bisulfite sequencing. Thanks to the high coverage (>>1000x), the OVCA MethPanel precisely determines even very low methylation levels in the entire analyzed region, as well as in individual CpG sites. This is extremely important because the vast majority of aberrant DNA methylation in normal tissue is mosaic in nature, meaning it occurs in a small fraction of cells. In parallel, in the search for new candidate DNA methylation aberrations potentially associated with OC, we will perform a whole-methylome analysis. The scale of this approach will enable an analysis of the remaining 98% of CpG sites in the genome that have not previously been analyzed for their association with OC susceptibility. Simultaneous detection of the genetic base and methylation status at each position will also enable the identification of OC-related methylation Quantitative Trait Loci (meQTL), i.e., genetic loci in which genetic variation is associated with changes in the level of DNA methylation at a specific CpG site. A newly designed ultrasensitive methylation panel assay using deep sequencing will validate the CpG sites/regions of aberrant methylation (identified in the whole methylome analysis) in a larger validation cohort. Finally, taking advantage of the applied methodological approaches, we will characterize the nature of identified methylation aberrations, assessing methylation density of CpG sites, homo/heterogeneity of methylation (at the reads/cells level), the extent of methylation (start/end sites), co-occurrence with a genetic variant, allelic specificity, and others aspects. These aspects are important, for example, in predicting the possible impact of aberrant methylation on gene expression (methylation density of CpG sites) or in predicting the early developmental nature of these abnormalities (allelic specificity of methylation).

In summary, the project will contribute to a significant expansion of knowledge on the impact of abnormal DNA methylation in normal tissue on the risk of OC and will provide new potential methylation-related factors influencing OC risk.