Summary

DNA methylation changes in ovarian cancer patients.

In our work we studied DNA methylation changes in ovarian cancer. Due to the aggressive nature of this disease, it is very important to obtain detailed mapping of molecular parameters including epigenetics. These parameters could potentially be used in the diagnostics and treatment of ovarian cancer. To begin with, we focused on establishing and optimizing techniques which are suitable for methylation analysis of DNA isolated from paraffin blocks. The next step was to analyze selected CpG loci primarily of tumor suppressor genes.

Formalin-fixed, paraffin-embedded tissue samples from both ovarian adenocarcinomas and normal ovarian tissue were obtained from 109 women (69 patients with ovarian cancer, 40 patients with normal ovarium) treated at the Department of Obstetrics and Gynecology, University Hospital Hradec Kralove. The samples of normal ovary were obtained from patients surgically treated for non-malignant diagnosis. DNA was extracted using a Qiagen DNA extraction kit.

We established and optimized two techniques – MSP (Methylation specific PCR) and MS-MLPA (Methylation Specific Multiplex Ligation-dependent Probe Amplification). We discovered that for methylation analysis of poor quality DNA isolated from paraffin blocks MS-MLPA is more suitable than MSP. This is because MS-MLPA analysis does not require aggressive bisulphite conversion.

Using MSP for TP53 and GATA4 we demonstrated statistically-significant higher promoter methylation in analysed CpG loci of TP53 and GATA4 genes in ovarian cancer patients than in the control group. GATA4 showed statistically-significant higher methylation in the endometrioid tumor type compared with the serous histological type of ovarian carcinoma.

The next part of our study was to investigate the methylation profile of selected genes using MS-MLPA. For analysis in this study we selected two sets from the company MRC-Holland. Using these sets we analysed 47 tumor suppressor genes. We observed significantly higher methylation in genes MGMTa, PAX5, CDH13, WT1, THBS1, GATA5, NTKR1, GATA4 and WIF1 in the ovarian cancer group compared with the control group, while in the ESR1 gene we observed significantly higher methylation in the control group compared with the ovarian cancer group. The methylation frequency of each CpG locus significantly correlated with clinicopathological characteristics including tumor stage, histological grade and histological type of ovarian cancer.

These findings confirm the importance of DNA methylation in ovarian cancer. Since DNA methylation changes are often observed as one of the first changes in carcinogenesis, they could be potentially used as early-stage markers. In the future these epigenetic characteristics could be used in a screening programme for ovarian cancer and treatment, and in individualization of therapy especially in platinum-resistant ovarian cancer.