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BRCA1 gene promoter methylation status in high-grade serous ovarian cancer patients – A study of the tumour Bank ovarian cancer (TOC) and ovarian cancer diagnosis consortium (OVCAD)

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Abstract ***Background:*** Mutations in BRCA1/2 genes are involved in the pathogenesis of breast and ovarian cancer. Inactivation of these genes can also be mediated by hypermethylation of CpGs in the promoter regions. Aim of this study was to analyse the clinical impact of BRCA1 promoter gene methylation status in a homogenous cohort of high-grade serous ovarian cancer (HGSOC) patients.
Methods: The cohort included 257 primary HGSOC patients treated by cytoreduction and platinum-based chemotherapy. DNA was extracted from fresh frozen tissue samples. BRCA1 gene promoter methylation rate was assessed using polymerase chain reaction (PCR).

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Results: 14.8% of patients presented hypermethylation within a selected region of the BRCA1 promoter. The rate of hypermethylation was significantly higher in younger patients (20.8% hypermethylation in the age group ⩽58 years versus 8.7% hypermethylation in the age group >58 years; $p = 0.008$). Optimal tumour debulking could be reached in 63% of patients, without significant differences in the extent of residual disease with respect to the methylation status. No impact of BRCA1 gene promoter methylation status on progression free- and overall-survival rates was found. No significant differences within BRCA1 promoter methylation status between primary and metastatic tissue could be observed. These results on BRCA1 promoter methylation status were also confirmed in a subgroup of 107 patients found negative for BRCA1 exon 11 mutations.

Conclusions: Our data suggest that BRCA1 methylation determines the earlier onset of HGSOC. Furthermore our study supports the idea that BRCAness is not only due to mutations but also to epigenetic changes in BRCA1 promoter gene.

1. Introduction

Ovarian cancer is the most lethal malignancy among gynaecological cancers, with over 140,000 deaths per year worldwide [1]. Most of the patients after receiving optimal tumour debulking and platinum based chemotherapy, will develop recurrence, platinum-resistance and will eventually die from the disease [2].

Recent studies showed that different histological subtypes of ovarian cancer are having different clinical behaviours as also different biomolecular features [3]. High grade serous ovarian cancer is the most encountered histological subtype being characterised by advanced International Federation of Gynecology and Obstetrics (FIGO) stage and decreased overall survival despite increased platinum-sensitivity [4].

The urgent need of new therapeutic targets has led researchers to concentrate the efforts in studying new molecular mechanisms which interfere with the integrity of the balance between oncogenes and tumour suppressor genes and therefore lead to personalised treatment of ovarian cancer patients.

In particular, a specific family of tumour suppressor genes involved in the repair process of damaged DNA (BReast CAncer genes family, BRCA) has been extensively investigated in the last two decades. If mutated, BRCA1/BRCA2 are associated to an increased risk of breast and ovarian cancer. Furthermore, not only mutations will lead to a deficient DNA repair or oncosuppression, but low levels of 'healthy' unmutated gene may cause a functional deficit of the genes. Epigenetic changes might (down) regulate gene expression. In healthy individuals, the regulatory region of the full active BRCA gene is demethylated. Methylation process may cause a downregulation of the protein leading to gene dysfunction [5–12].

The aim of the present study was to analyse the clinical impact of BRCA1 promoter gene methylation status in HGSOC.

2. Materials and methods

2.1. Sample collection

We selected 257 consecutive patients with primary HGSOC subjected to surgical cytoreduction and platinum-based chemotherapy between 2000 and 2011. Further selection criteria were the availability of fresh frozen tumour tissue and a follow-up time of at least 3 years. 207 patients were obtained from the TOC (tumour ovarian cancer) Network. Another 50 patients were obtained from the OVCAD (ovarian cancer diagnosis) project. Five European gynaecologic cancer centres (Berlin, Hamburg, Innsbruck, Leuven and Vienna) prospectively enrolled epithelial ovarian cancer patients into this translational study. The main pathological, surgical and chemotherapy characteristics of the OVCAD patient cohort were published recently [13].

All patients gave their written informed consent before tissue samples were collected. Approval from each local ethics committee was obtained (EK207/2003, ML2524, HEK190504, EK366 and EK260).

Tumour tissue samples were collected at the time of surgery, immediately frozen in liquid nitrogen within 15 min from the removal and then stored at −80 °C till further analysis. All ovarian cancer tissue samples included underwent histopathological assessment to verify histological subtype and high tissue quality. Only specimens presenting at least 50% of tumour area were included in the BRCA1 promoter methylation status analysis. The majority of tissue samples had approximately 80% of tumour area.

2.2. DNA extraction

DNA was extracted from at least 25 mg of fresh frozen tissue specimens using the QIAGEN DNeasy tissue kit (Qiagen GmbH, Hilden, Germany) and following the