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Expression Analysis of Platinum Sensitive and Resistant Epithelial Ovarian Cancer Patient Samples Reveals New Candidates for Targeted Therapies[1]

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Abstract

Ovarian cancer has the highest mortality rate of all gynecologic malignancies. Identification of new biomarkers is highly needed due to its late diagnosis and high recurrence rate. The objective of this study was to identify mechanisms of therapy resistance and potential biomarkers by analyzing mRNA and protein expression from samples derived from patients with platinum-sensitive and -resistant ovarian cancer (total cohort n = 53). The data revealed new candidates for targeted therapies, such as GREB1 and ROR2. We showed that the development of platinum resistance correlated with upregulation of ROR2, whereas GREB1 was downregulated. Moreover, we demonstrated that high levels of ROR2 in platinum-resistant samples were associated with upregulation of Wnt5a, STAT3 and NF-kB levels, suggesting that a crosstalk between the non-canonical Wnt5a-ROR2 and STAT3/NF-kB signaling pathways. Upregulation of ROR2, Wnt5a, STAT3 and NF-kB was further detected in a platinum-resistant cell-line model. The results of the present study provided insight into molecular mechanisms associated with platinum resistance that could be further investigated to improve treatment strategies in this clinically challenging gynecological cancer.

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Introduction

Epithelial ovarian cancer (EOC) accounts for the majority of mortality from gynecological cancers, with diagnosis often at a late stage. Currently, the golden standard of treatment is primary debulking surgery (PDS) followed by platinum-based chemotherapy [1]. Although most patients initially respond to chemotherapy, cancer cells will eventually develop resistance leading to relapse [2]. Despite intensive efforts to improve targeted therapy in EOC, the five-year survival rate is still only 30% for advanced disease [3]. Therefore, increased knowledge about mechanisms of platinum resistance in EOC treatment is needed in search for cure.

Genetically complex and unstable high-grade serous ovarian cancer subtype (HGSC), accounting for approximately 50–70% of EOC, represents the most aggressive histological subtype [4]. A large-scale integrated genomic data analysis for HGSC identified TP53 mutations in almost 96% of tumors. Recurrent somatic mutations were found in nine other genes including NF1, BRCA1, BRCA2,

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RB1 and CDK12, as well as DNA copy number aberrations and promoter methylation events, indicating biological and molecular heterogeneity that should be considered when developing novel therapeutic strategies [5].

While only 10%–15% of ovarian cancer patients carry BRCA1 or BRCA2 mutations in their germline, ~50% of ovarian cancers exhibit a defect in the homologous recombination (HR) repair of DNA [6]. PARP-1 enzyme became an attractive target for chemotherapeutics for its crucial function in single-strand breaks (SSBs) DNA repair mechanism through base excision repair (BER) pathway [7]. The concept of synthetic lethality has been used in genetic studies to determine functional interactions and compensation among genes for decades and has also been exploited in the development of PARP (Poly (ADP-ribose) polymerase) inhibitors [8]. The responsiveness to platinum and PARP inhibitors associates with so called *BRCAness* profile showing independent prognostic value [9,10].

The chemotherapy resistance can arise due to multiple mechanisms, such as drug target alteration, re-activation or amplification of the oncogenic pathway, activation of parallel pathways, increased DNA damage tolerance/repair, and deregulation of growth factor receptors among others [11]. Deregulation of apoptosis and altered phosphorylation (intracellular signaling), as well as metabolic pathways represent the two main biological processes responsible for oncogene-mediated drug resistance in ovarian cancer [12]. In this context, activation of PI3K/AKT cell survival pathway plays a pivotal role with NF-kB and STAT3 as the main mediations of these intracellular events. On the other hand, tumor suppressor genes such as BRCA1, BRCA2, MLH1 and p21 contribute to ovarian cancer drug resistance via alterations in the DNA damage and repair mechanisms, whereas RASSF1, TP53 and TP73 impair the apoptotic machinery for the same outcome [13]. Epithelial-to-mesenchymal transition (EMT) has also been implicated in HGSC invasiveness and chemoresistance, and in vitro studies using ovarian cancer cell lines have shown that more aggressive, mesenchymal-type cells are more resistant to cisplatin treatment [14]. An important signaling cascade involved in EMT is the Wnt signaling, with increasing evidence suggesting that β-catenin-independent pathway via Wnt5a/ROR1/ROR2 has a critical role in EMT and chemoresistance [15–18]. Consequently, therapies targeting these pathways may offer means to overcome drug resistance.

Active and also productive research in the field of cancer therapy has led to an improved understanding of the molecular mechanisms, providing insight into the development of cancer. This new data has led to the development of new treatment options for cancer patients, including targeted therapies and associated biomarker tests that can select which patients are most likely to respond [19]. The aim of this study was to identify candidate genes and their molecular pathways involved in the pathogenesis of ovarian cancer associating with platinum resistance. Overcoming the paucity of obtaining large collection of tumor samples available for molecular profiling, we investigated differences in mRNA and protein expression between ovarian cancer samples derived from two clinically and molecularly distinct patient cohorts namely high PARP/platinum-sensitive and low PARP/platinum-resistant HGSC cohorts. This comparison aimed at distinction of two cohorts with extremely different clinical behavior. Finally, this analysis led to identification of GREB1 and ROR2 that showed significant differential expression profile between the two groups. Our data suggest new predictive biomarkers for ovarian cancer drug resistance development warranting further investigations.

Materials and Methods

Study Cohort and Tissue Samples

The study was carried out at the University of Tampere and Tampere University Hospital (TAUH), Tampere, Finland. The study protocol was approved by the Ethics Committee of TAUH (identification code ETL-R11137).

The microarray study cohort consisted of 12 HGSC patients who participated in a prospective study addressing PARP enzyme activity in fresh ovarian cancer tumor samples [20]. The selection of this patient subcohort was based on PARP values (high PARP/low PARP, cut off 203 pg/ml, which corresponded to the median value of PARP) and platinum sensitivity/resistance (treatment response), with no differences in respect of age and FIGO (International Federation of Gynecology). Platinum sensitivity was defined as no recurrence within 12 months after the completion of first-line platinum-based chemotherapy. Patient characteristics are presented in Table 1.

The validation cohort of the microarray data consisted of all the patients (n = 53) that participated in the previous prospective study [20]. The median follow-up time of patients was 31 months. The validation cohort is described in Table 2.

For further investigation of ROR2 in EOC, a retrospective subcohort was chosen from the study cohorts described above consisting of a subgroup of patients who had not received NACT (neoadjuvant chemotherapy) and were divided in two categories based on treatment response, i.e. either platinum-sensitive or platinum-resistant (Table 3).

The tumor tissue samples were collected at surgery, two samples approximately 0.5 cm were chosen at the operation room from macroscopically visible tumor and were snap-frozen with liquid nitrogen and stored in −70 °C. The findings from the corresponding archival surgical tumor specimens were assessed by experienced pathologists as part of routine diagnostics at the Department of Pathology at TAUH.

Table 1. Characteristics of the Study Patients in the Microarray Cohort (n = 12)

Characteristic	Platinum Sensitive * n (%)	Platinum Resistant * n (%)
All	6	6
PDS †	5	2
NACT ‡	1	4
PARP § low	0	6
PARP § high	6	0
Age		
mean (SD)	65	62
median (range)	63 (46–78)	62 (55–79)
Grade 3 ¶	6 (100%)	6 (100%)
Stage #		
FIGO st I	0	0
FIGO st II	0	0
FIGO st III and IV	6	6
Histology		
serous	6 (100%)	6 (100%)
PFS ** (months)	28	3.5

* Sensitivity defined as relapse or event-free interval> 12 months after completion of platinum based 1st line therapy.

† PDS - primary debulking surgery.

‡ NACT - neoadjuvant therapy.

§ PARP - PARP activity in fresh frozen tumor tissue was assessed by an enzymatic chemiluminescense assay in a previous study [20]; the cut-off level for high PARP activity was set to 203 pg/ml corresponding to median value.

¶ Grade – Grade 3 represents high grade tumors.

FIGO - International Federation of Gynecology.

** PFS - progression free survival.