European Journal of Cancer (2013) **49**, 520–530

Available at www.sciencedirect.com

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journal homepage: www.ejcancer.info

Resistance to platinum-based chemotherapy is associated with epithelial to mesenchymal transition in epithelial ovarian cancer

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Available online 13 August 2012

KEYWORDS
Epithelial ovarian cancer
Platinum-resistance
Epithelial to mesenchymal transition

Abstract ***Background:*** The present study is aimed to identify genetic pathways correlated with chemoresistance in epithelial ovarian cancer (EOC).
Methods: We compared the molecular profiles of 23 tumour biopsies of stage III–IV (training set) at primary surgery, before chemotherapy, to the profile from the same patients at second surgery, after several lines of platinum (Pt)-based chemotherapy when the tumours were resistant. In the hypothesis that identified markers were related to Pt-resistance and to prognosis, we validated this signature in 52 EOC taken at primary surgery (validation set) selected to be either very sensitive to the first line therapy, i.e. not relapsing before one year from the end of therapy, or resistant, i.e. relapsing within 6 months from the end of therapy.

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http://dx.doi.org/10.1016/j.ejca.2012.06.026

Results: In the training set, we identified a resistance signature indicative of the activation of epithelial to mesenchymal transition (EMT) by transforming growth factor (TGF)-beta pathway. We then validated this signature in 52 EOC taken at primary surgery (validation set). Some genes involved in EMT, such as *B*MP and *a*ctivin *m*embrane-*b*ound *i*nhibitor (BAMBI), and mir-141 resulted in association with overall or progression free survival.
Conclusion: Some genes involved in EMT were associated to overall or progression free survival, suggesting EMT as vital to the resistance mechanisms.

1. Introduction

In epithelial ovarian cancer (EOC) recurrent disease responds further to chemotherapy, however the progression-free interval becomes shorter after each cycle, as chemoresistance increases until the disease becomes incurable.[1] Although many advances have been made in the understanding of EOC biology, genetic and molecular mechanisms underlying resistance to chemotherapy have yet to be clearly identified.[2] It is still not clear whether resistance is due to subpopulations of resistant cells, possibly with stem cell properties, already existing in the tumour before treatment, or is induced by mutations or epigenetic changes caused by chemotherapeutic drugs. Current knowledge is based on the use of cancer cell lines with acquired resistance to chemotherapeutic drugs. These experimental models have been very useful in identifying mechanisms of drug resistance, although it is unclear whether they recapitulate the situation in patients receiving chemotherapy.[3] To elucidate the biological mechanisms underlying clinical resistance of EOC, we compared gene expression profiles in tumours at diagnosis, and after development of acquired resistance in 23 patients who had relapsed after two or more lines of chemotherapy. We found that resistance was associated with the activation of specific pathways. Thus, we investigated whether these pathways were useful in predicting the sensitivity to first-line therapy in an independent series of 52 EOC patients.

2. Materials and methods

2.1. Patients and samples

Two groups of ovarian cancer biopsies, a training set and a validation set, were selected from a tumour tissue collection of snap-frozen biopsies recruited at the San Gerardo Hospital in Monza (Italy) and routinely stored at −80 °C at the Mario Negri Institute in Milano (Italy). The training set comprises 23 stage III–IV patients from whom tumour biopsies were taken at primary surgery (ovary, PS-O, and/or other anatomical sites, PS-D) and at second surgery for relapse after several lines of chemotherapy (secondary cytoreduction, SCR). All patients had achieved a clinical response to platinum (Pt)-based first line chemotherapy, therefore most of the tumour cells of PS-O can be defined 'sensitive'. On the other hand, the second biopsy (SCR) was taken at second surgery, after two or more lines of chemotherapy, when patients showed progressive disease thus SCR biopsies were made up of tumour cells resistant to Pt-based chemotherapy. In order to verify that molecular differences found could be related to platinum sensitivity, we used as validation set 52 stage III–IV PS-O biopsies, naïve to chemotherapy, with different sensitivity to Pt-based chemotherapy: 20 biopsies (38.5%) were from patients who relapsed within 6 months at the end of carboplatin/taxol therapy and presumably contained a large proportion of tumour cells biologically resistant to Pt (Pt-resistant). Thirty-two biopsies (61.5%) were from patients who relapsed after at least one year from the end of carboplatin/taxol therapy (Pt-sensitive cases). The study protocol for tissue collection and clinical information was approved by the institutional review board and patients provided written informed consent authorising the collection and use of the tissue for study purposes. Detailed information are reported in Supplementary material section. Kaplan–Meier curves depicting the progression free (PFS) and overall survival (OS) for training set and validation set are reported in Supplementary Fig. 1.

2.2. Total RNA and miRNA extraction and purification from human tumour tissues

Frozen tissue specimens (30 mg) were homogenised using the TissueLyser LT (Qiagen, Milan, Italy) and total RNA enriched in miRNA fraction purified using miRNeasy Mini Kit isolation system (Qiagen), following manufacturer's protocols. Total RNA concentration and proteins contamination were determined by Nanodrop spectrophotometer (Nanodrop Technologies, Ambion) and further checked with a 2100 Bioanalyser (Agilent Technologies, Santa Clara, CA, USA); only samples with a RIN number larger than 6 and a Nanodrop A260:280 ratio between 1.8 and 2.1 were further processed and aliquots were stored at −80°C until use.

2.3. Reverse transcription quantitative PCR for RNA and miRNA analysis

Absolute quantification by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and miRNA analysis was as described using primers