Neoplasia • Vol. 7, No. 6, June 2005, pp. 603–613 603

www.neoplasia.com

High-Resolution Mapping of Genomic Imbalance and Identification of Gene Expression Profiles Associated with Differential Chemotherapy Response in Serous Epithelial Ovarian Cancer[1]*

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Abstract

Array comparative genomic hybridization (aCGH) and microarray expression profiling were used to subclassify DNA and RNA alterations associated with differential response to chemotherapy in ovarian cancer. Two to 4 Mb interval arrays were used to map genomic imbalances in 26 sporadic serous ovarian tumors. Cytobands 1p36, 1q42-44, 6p22.1-p21.2, 7q32.1-q34 9q33.3-q34.3, 11p15.2, 13q12.2-q13.1, 13q21.31, 17q11.2, 17q24.2-q25.3, 18q12.2, and 21q21.2-q21.3 were found to be statistically associated with chemotherapy response, and novel regions of loss at 15q11.2-q15.1 and 17q21.32-q21.33 were identified. Gene expression profiles were obtained from a subset of these tumors and identified a group of genes whose differential expression was significantly associated with drug resistance. Within this group, five genes (*GAPD*, *HMGB2*, *HSC70*, *GRP58*, and *HMGB1*), previously shown to form a nuclear complex associated with resistance to DNA conformation–altering chemotherapeutic drugs in *in vitro* systems, may represent a novel class of genes associated with *in vivo* drug response in ovarian cancer patients. Although RNA expression change indicated only weak DNA copy number dependence, these data illustrate the value of molecular profiling at both the RNA and DNA levels to identify small genomic regions and gene subsets that could be associated with differential chemotherapy response in ovarian cancer.

Neoplasia (2005) **7,** 603–613

Keywords: cisplatin, taxol, gene amplification, gene deletion, microarray data mining.

Introduction

Ovarian cancer is the second most frequently diagnosed gynecologic malignancy, and causes more deaths than any other cancer of the reproductive system. The lack of reliable methods of early detection and the absence of specific symptoms result in late-stage diagnosis in 70% of patients. Although initial response rates to conventional chemotherapy among advanced stage patients are high, resistance to chemotherapy remains the primary factor accounting for the low 5-year survival in this patient population [1].

Ovarian cancer chemotherapy most commonly involves a first-line combination of platinum-based compounds plus paclitaxel following cytoreductive surgery. Response to chemotherapy varies among patients, and initial treatment response is often the most important consideration in choosing second-line therapies. The role of CA 125 serum levels as a surrogate

Abbreviations: aCGH, array comparative genomic hybridization; FFPE, formalin-fixed paraffin-embedded; *GAPD*, glyceraldehyde phosphate dehydrogenase; *GRP58*, glucose-regulatory protein 58; *HMGB1*, high-mobility group beta 1; *HMGB2*, high-mobility group beta 2; *HSC70*, heat shock cognate protein 70; PAM, prediction analysis of microarrays; SAM, significance analysis of microarrays; SEOC, serous epithelial ovarian cancer

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[1]This study was supported by a grant award from the Ontario Cancer Research Network (funded through the Government of Ontario), Genome Canada, and the Ovarian Fashion Show Committee (Princess Margaret Hospital). M.B. was supported by the Kristi Pia Memorial Fellowship. The Toronto Ovarian Tissue Bank and Database was funded, in part, by the National Ovarian Cancer Association and the St. George's Society.

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*This article refers to supplementary material, which is designated by "W" (ie, Table W1, Figure W1) and is available online at www.bcdecker.com.

Received 6 December 2004; Revised 21 March 2005; Accepted 21 March 2005.

DOI 10.1593/neo.04760

marker to assess chemotherapy response is well established (reviewed in Ref. [2]). Both the rate of decline as well as the absolute value of CA 125, determined after the first courses of chemotherapy, are generally considered predictors of the final clinical response [3].

Most investigations of drug resistance in ovarian cancer have used anticancer drugs *in vitro* to select for subclones of cell lines with resistance to the selected agent [4–9]. A disadvantage of these approaches is that the cultured cells used are often genomically unstable and may have acquired *in vitro* genetic and epigenetic alterations that are not representative of *in vivo* conditions. In addition, such models primarily address *acquired* drug resistance, and do not provide direct insights into the expression and genomic alterations associated with *intrinsic* drug resistance.

In recent years, cytogenetic study of solid tumors has been directed toward the identification of recurrent chromosomal rearrangements and patterns of copy number imbalance that may pinpoint genomic regions involved in cancer initiation, progression, drug resistance, and patients' outcome [10,11]. Molecular cytogenetic methods such as spectral karyotyping and comparative genomic hybridization (CGH) have provided useful insights concerning genomic alterations in ovarian cancer [12,13]. However, because metaphase CGH has a resolving power of 10 to 20 Mb [14], it has not been possible to determine genomic imbalance patterns within cytobands. Recently, genomic and cDNA arrays (reviewed in Ref. [15]) have provided more detailed maps of genomic copy number alterations in tumors and, in due course, will provide comprehensive maps of genomic imbalance in a variety of tumors [16–18]. Moreover, high-resolution maps of copy number imbalance are now being integrated with expression profile data to identify clinically relevant subsets of genes based on concomitant alterations at the DNA and RNA levels [19–23]. Microarray expression profiling has been utilized in a number of recent studies in ovarian cancer (reviewed in Ref. [24]). However, no study to date has performed parallel microarray expression and array comparative genomic hybridization (aCGH) analyses to address genomic imbalance and concurrent expression alterations associated with intrinsic drug resistance in ovarian cancer.

Materials and Methods

This study was designed in three phases (Figure 1). In the first phase, a 2- to 4-Mb genomic interval aCGH map of genomic imbalance in 26 serous epithelial ovarian cancer (SEOC) tumors was generated. In the second phase, statistical analysis of aCGH data sets was used to identify cytobands in which imbalance was associated with drug resistance. In the third phase, gene expression profiles were obtained from a subset of tumors, patterns of gene expression associated with drug response were identified, and a concordance analysis of the relationship between genomic imbalance and expression levels was performed. Finally, expression microarray prediction analysis was carried out to identify a subset of classifier genes that could predict chemotherapy response in ovarian cancer patients.

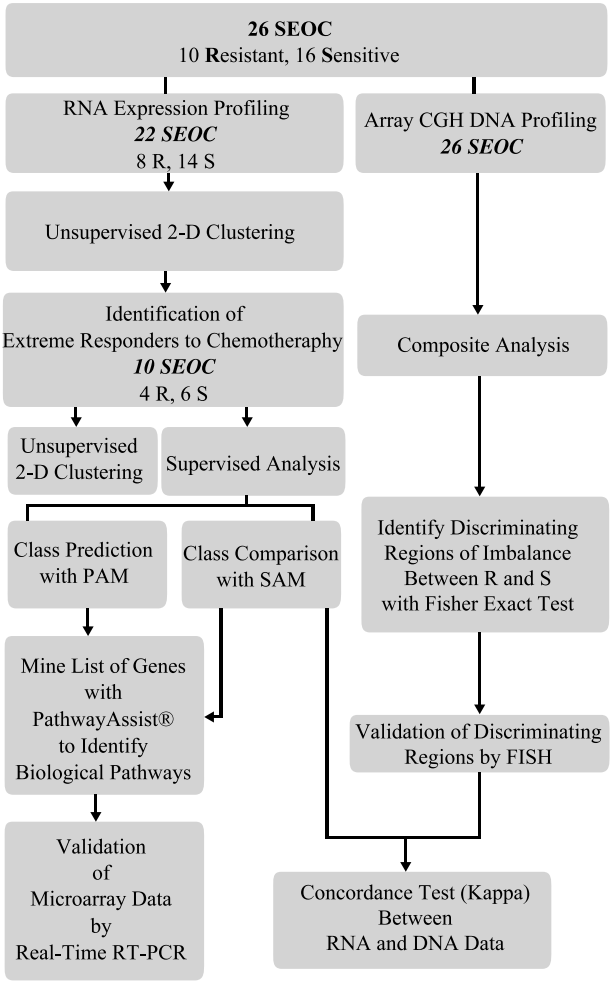

Figure 1. *Flowchart of the experimental design.*

SEOC Tumor Samples

Snap-frozen tumor tissue samples from 26 sporadic SEOC tumors naïve to chemotherapy were selected from the Toronto Ovarian Tissue Bank and Database. No patient included in this study had a family history of either breast or ovarian cancer. All samples were acquired according to the institutional guidelines of the Research Ethics Board. The tumor specimens selected for this study contained at least 75% tumor content as assessed by the surface area of histology slides corresponding to the snap-frozen tissues (the available clinical data are summarized in Table 1). Patients received standard SEOC chemotherapy (carboplatin + taxol). To be classified as sensitive, CA 125 values from patient tumor samples had to meet two criteria. First, the CA 125 values had to fall to below the normal reference ($\sim$35 U/ml) within three cycles of chemotherapy, regardless of the initial baseline. Second, the values had to remain below the normal reference of a period of at least 6 months from the initiation of chemotherapy. Using these criteria within our group of samples, 16 met the criteria for sensitivity and 10 were thus classified as resistant. Due to the accepted variability of CA 125 values, especially in those classified as resistant, a subset of samples was used for a more detailed class comparison. In this group of six sensitive and four resistant samples, the resistant tumors displayed