Genomic markers of ovarian adenocarcinoma and its relevancy to the effectiveness of chemotherapy

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Abstract. Ovarian cancer is the eighth most common cancer and the seventh highest cause of cancer-associated mortality in women worldwide. It is the second highest cause of mortality among female reproductive malignancies. The current standard first-line treatment for advanced ovarian cancer includes a combination of surgical debulking and standard systemic platinum-based chemotherapy with carboplatin and paclitaxel. Although a deeper understanding of this disease has been attained, relapse occurs in 70% of patients 18 months subsequent to the first-line treatment. Therefore, it is crucial to develop a novel drug that effectively affects ovarian cancer, particularly tumors that are resistant to current chemotherapy. The aim of the present study was to identify genes whose expression may be used to predict survival time or prognosis in ovarian cancer patients treated with chemotherapy. Gene or protein expression is an important issue in chemoresistance and survival prediction in ovarian cancer. In the present study, the research group consisted of patients treated at the Surgical Clinic of the Gynecology and Obstetrics Gynecological Clinical Hospital, Poznan University of Medical Sciences (Poznan, Poland) between May 2006 and November 2014. Additional eligibility criteria were a similar severity (International Federation of Gynecolgy and Obstetrics stage III) at the time of diagnosis, treatment undertaken in accordance with the same schedule, and an extremely good response to treatment or a lack of response to treatment. The performance of the OncoScan® assay was evaluated by running the assay on samples obtained from the four patients and by following the recommended protocol outlined in the OncoScan assay manual. The genomic screening using Affymetrix OncoScan Arrays resulted in the identification of large genomic rearrangements across all cancer tissues. In general, chromosome number changes were detected in all examined tissues. The OncoScan arrays enabled the identification of ~100 common somatic mutations. Chemotherapy response in ovarian cancer is extremely complex and challenging to study. The present study identified specific genetic alterations associated with ovarian cancer, but not with response for treatment.

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Introduction

Ovarian cancer is the eighth most common cancer and the seventh leading cause of cancer-associated mortality in women worldwide. It is the second highest cause of mortality among female reproductive malignancies and accounts for 140,200 mortalities each year. The estimated incidence and number of mortalities in the USA from ovarian cancer is 21,980 cases and 14,270 mortalities, respectively, for 2014 (1,2). Ovarian cancer is the fourth most common malignancy in women and is the leading cause of gynecological cancer-associated mortality. Poland is one of the countries with high morbidity rates for ovarian carcinoma. Epidemiological data show steady rise of ovarian cancer incidence. Due to late-onset symptoms, ovarian cancer is mainly diagnosed in an advanced stage. In total, 60-70% of patients present with stage III or IV disease and are therefore associated with poor survival. The International Federation of Gynecology and Obstetrics (FIGO) staging classification in ovarian cancer has an independent prognostic role. The major role of the staging system is not only to provide universal terminology that may be used in different oncological hospitals worldwide, but it also informs us about the prognosis and outcome prediction subsequent to specific treatment. The majority of ovarian cancer patients are diagnosed with late-stage disease as the asymptomatic progression is poorly understood, and an efficient screening strategy is not presently available (3-5). The

current standard first-line treatment for advanced ovarian cancer includes a combination of surgical debunking and standard systemic platinum-based chemotherapy with carboplatin and paclitaxel (6,7). This standard treatment results in >80% response rates and 40-60% complete responses; however, the majority of patients with advanced disease (stages III-IV) will eventually relapse, even with initial disease response. Improvement in survival has also been poor in ovarian cancer. Gene expression-based tools for the prediction of patient prognosis subsequent to surgery or chemotherapy are currently available for certain cancers. The prediction of cancer prognosis using molecular signatures is a popular research field, within which a wide variety of approaches have been considered (7). Popular RNA or protein expression measurement techniques include cDNA hybridization microarrays, end-point and quantitative reverse transcription polymerase chain reaction (PCR), and immunohistochemistry approaches (8). Although a deeper understanding of this disease has been attained, relapse continues to occur in 70% of patients 18 months following the first-line treatment. Therefore, it is crucial to develop a novel drug that effectively impacts on ovarian cancer, particularly one that is resistant to current chemotherapy. The 5-year survival rate of ovarian cancer patients with stage I is 92%. However, patients diagnosed in the late stage have poor prognosis, with a 5-year survival rate of only 19% for stage IV patients. The median progression-free survival time ranges between 16 and 21 months, and the median overall survival time ranges between 24 and 60 months (9,10). Subsequent to repeated cycles of chemotherapy, recurrent ovarian cancer eventually develops resistance to numerous available cytotoxic agents. As a result, studies into the mechanisms of drug-resistance, biomarkers for drug resistance, and the development of new-targeted therapies have been the subject of numerous ovarian cancer studies (11). Although patients receiving standard therapy, including surgical cytoreduction and platinum-based combination chemotherapies, may have an initial favorable response, the majority of patients experience relapse within 5 years (12). Consequently, there is an urgent requirement for novel treatments for this deadly disease.

The aim of the present study was to identify genes of which the expression may be used to predict survival time or prognosis in ovarian cancer patients treated witch chemotherapy. As aforementioned, the presence of resistance to the chemotherapy agent administered dramatically affects the survival of a patient. It is therefore reasonable to expect the gene signatures identified to include genes responsible for chemoresistance, which will affect the mechanism of action of the drug. Gene or protein expression is an important issue of chemoresistance and survival prediction in ovarian cancer. The concept of identifying gene signatures is popular, but requires careful handling to extract the information required for this to be successful. There are certain previous studies that investigated the differing response of different types of ovarian cancer to chemotherapy (13). Identification of biomarkers that can reliably predict drug sensitivity and resistance is extremely important.

Materials and methods

In the present study, the research group consisted of patients treated at the Surgical Clinic of the Gynecology and Obstetrics Gynecological Clinical Hospital, Poznan University of Medical Sciences (Poznan, Poland) between May 2006 and November 2014. Of the 2,000 patients, four who suffered from ovarian serum carcinoma were chosen. Additional eligibility criteria were a similar severity (FIGO stage IIIC) at the time of diagnosis, treatment undertaken in accordance with the same schedule, and an extremely good response to treatment or a lack of response to treatment. Finally, two patients who had an exceptionally good response to treatment and two patients who did not respond to treatment were selected. A detailed description of the therapeutic effects of the patients enrolled in the present study is subsequently reported. Informed consent was obtained from all patients, and ethical approval was provided by the Bioethics Committee of Poznan University of Medical Sciences.

The tissue samples were collected from neoplastic lesions removed during surgery prior to starting drug therapy. The tissues were stored in paraffin blocks.

Case reports

Case 1. Patient 1 (48 years of age) was classified as having a good response to treatment. The patient was referred from a gynecological ward of Gniezno County Hospital (Gniezo, Poland) in October 2007 with a suspected neoplastic process that extended from the ovary, for treatment at the. Surgical Gynecology Clinic of the Gynecological and Obstetrics Clinical Hospital (Poznan, Poland). On admission, vaginal and transabdominal ultrasounds were performed, which showed conglomerate tumors occupying the pelvis. This ovarian tumor had the following dimensions, 7x8 and 6x5.9 cm infiltrated the large intestine (descending colon and anus) and bladder. The level of the marker cancer antigen (CA) 125 was 207 IU/ml in the blood (normal reference values are <35 IU ml). Subsequent to preparation, partial excision of the pelvic tumor, with reconstruction of the walls of the bladder and anastomosis of the proximal descending colon and the rectum was performed. Unfortunately, due to infiltration of the tumor into the left iliac vessels, the whole tumor was not removed Subsequent to a period of recuperation in November 2007, treatment was commenced with first-line chemotherapy, consisting of paclitaxel and cisplatin (intravenous infusion of paclitaxel 175 mg/m^2 and 75 mg/m^2 cisplatin per cycle lasting 3 h with 3 weeks break between chemotherapy cycles) which lasted continuously until February 2008. At the start of this stage of treatment, a lesion in the vicinity of the left iliac vessels were visible on transvaginal ultrasound, 1.0x0.7 cm in size, while the CA125 level was 50 IU/ml in the blood. Subsequent to a cycle of paclitaxel and cisplatin chemotherapy (intravenous infusion of paclitaxel 175 mg/m^2 and 75 mg/m^2 cisplatin per cycle lasting 3 h with 3 weeks break between chemotherapy cycles.), this lesion was invisible and the CA125 level was 13 IU/ml in the blood. At a follow-up in late April 2008, ultrasound examinations found recurrence in the vicinity of the left iliac vessels, with a dimension of 4x4x5 cm and the patient was admitted to the oncology clinic of the Gynecology and Obstetrics Gynecological Clinical Hospital (Poznan, Poland). It was decided to perform surgery to remove the lesion. Considering the high infiltration of the left iliac vessels and subsequent to consultation with a vascular surgeon, the lesion was not entirely removed, leaving a fragment of a tumor measuring

~0.5x0.5 cm around the left common iliac artery. The next stage of treatment was second-line chemotherapy consisting of cyclophosphamide and cisplatin (intravenous infusion of cyclophosphamide 750 mg/m^2 and 75 mg/m^2 cisplatin per cycle lasting 3 h with 3 weeks break between chemotherapy cycles, which started at the end of May 2008. However, subsequent to 2 cycles of chemotherapy, the patient had a strong anaphylactic reaction to the chemotherapy, which resulted in a change to topotecan (to 1.5 mg/m^2 for 5 days every 3 weeks). The level of CA125 (7 IU/ml) in the blood had decreased to 3 IU/ml at the end of therapy, the baseline was following completion of the topotecan treatment. Chemotherapy was completed in late October/November 2008, with the ultrasound also revealing no pelvic lesions; it was decided to continue treatment on an outpatient basis, with one follow-up every 3 weeks. During a follow-up in late December 2008, a recurrence 7x5x5 cm in size was observed around the left iliac vessels. In addition, the patient experienced deterioration in general condition, including a lack of appetite, weakness and weight loss (12 kg within 7 weeks). At the request of the patient, further treatment was not commenced, and it was decided in consultation with the patient for palliative care to be administered at their place of residence. The patient succumbed in mid-January 2009. At the request of the family, no autopsy was performed.

Case 2. Patient 2 (50 years of age) was classified as having a good response to treatment. The patient presented to the gynecological clinic of the local hospital in Kościan (Kościan County Hospital) in February 2009 subsequent to the accidental detection of a polycystic solid tumor in the pelvic cavity, posterior to the uterus, during abdominal ultrasound. The patient was urgently admitted to the Surgical Gynecology Clinic of the Gynecological and Obstetrics Clinical Hospital in March 2009 and a transvaginal ultrasonography revealed a tumor 9x5x5 cm in size that was in contact with the ascending colon and bladder. The patient reported a history of partial hysterectomy in July 2007. The CA125 level in the blood was 175 IU/ml. Subsequent to preparation, surgery was performed to remove the lesions originating from the right ovary, with the macroscopically unchanged left ovary. Following a period of recovery, first-line chemotherapy consisting of paclitaxel and carboplatin (6 cycles intravenous infusion of paclitaxel, 175 mg/m^2 lasting 3 h, followed by 400 mg/m^2 carboplatin per cycle, with 3 weeks between cycles.) was commenced in mid-April 2009. Throughout the administration of chemotherapy, there were no lesions in the pelvic cavity and the level of the marker CA125 in the blood dropped between 40 IU/ml at the start of chemotherapy and 13 IU/ml at its completion. In the period between September 2009 and February 2013, the patient was admitted to the Surgical Gynecology Clinic of the Gynecological and Obstetrics Clinical Hospital. In March 2013 during a routine follow-up, a pelvic lesion 7x10x5 cm in size was identified in the right ovary. The patient was admitted to the clinic in order to perform surgery to remove the lesion. The CA125 level was 51 IU/ml. Underwent radical changes and the removal of deciding to start at the beginning of March 2013 chemotherapy (3 cycles of intravenous infusion of paclitaxel 175 mg/m^2 and carboplatin 400 mg/m^2 per cycle lasting 3 h with 3 weeks break between chemotherapy cycles). During the third course of chemotherapy, the patient developed an adverse reaction to carboplatin (palmar-plantar erythrodysesthesia) that resulted in carboplatin being replaced by cisplatin (3 cycles of intravenous infusion of 75 mg/m^2 cisplatin per cycle; 3 weeks break between chemotherapy cycles). Chemotherapy was completed in August 2014, and the patient was referred for follow-up. The last follow-up took place in October 2014. No lesions were detected in the pelvic cavity and the level of CA125 in the blood was 10 IU/ml. The patient succumbed to cardiogenic shock in mid-December 2014. At the request of the family, no autopsy was performed.

Case 3. Patient 3 (49 years of age) was classified as being unresponsive to treatment. In October 2009, the patient was admitted to the Department of Gynecology, Konin district hospital (Konin, Poland) due to a pelvic tumor. On admission to the Surgical Gynecology Clinic of the Gynecological and Obstetrics Clinical Hospital, transvaginal ultrasonography revealed a solid lesion with multiple compartments that filled the entire pelvis, with smaller dimensions totaling 12x10x17 cm. The tumor infiltrated the bladder and bowel. There was no point in time at which the point where the cancer lesion came from could be reached. The level of CA125 in the blood was 156 IU/ml. Subsequent to preparation, non-radical resection of the tumor was performed, including the uterus and ovaries, a fragment of the wall of the bladder and a section of the descending colon. Among the surgically reconstructed section, colon end-to-side colon anastomosis was performed. However, a small residual section infiltrating the jejunum was left. Following a period of recuperation in mid-November 2009, first-line chemotherapy consisting of paclitaxel and carboplatin (6 cycles of intravenous infusion of paclitaxel 175 mg/m^2 and 400 mg/m^2 carboplatin per cycle lasting 3 h with 3 weeks break between chemotherapy cycles) was commenced. During the examination prior to the first treatment cycle, lesions were detected in the pelvis and the blood CA125 level was 21 IU/ml. Following 3 cycles of chemotherapy, pelvic free fluid appeared, and the amount of fluid increased in the following cycle. Prior to the last cycle of (February 2010) chemotherapy, a lesion that involved the bladder wall, 2x2x3 cm in size, was observed during the ultrasound. Due to the poor condition and increasing shortness of breath of the patient, the peritoneal cavity was punctured, and over 3 days, 5 l of fluid were removed. Subsequent to another week of hospitalization and further deterioration in the general condition of the patient, further treatment was not administered at the patient's request, and the patient was discharged. Palliative care was administered between discharge (beginning of April 2010) and early June 2010, when the patient succumbed to ovarian cancer.

Case 4. Patient 4 (49 years of age) was classified as being unresponsive to treatment. In November 2010, the patient was referred to Surgical Gynecology Clinic of the Gynecological and Obstetrics Clinical Hospital by a physician, due to the detection of bilateral ovarian tumors by screening ultrasound. On admission, transvaginal ultrasound was performed, and a solid tumor with central vascularization, measuring 2x1x2 cm, was identified in the left ovary, and a multi-element solid tumor located centrally with peripheral vasculature, measuring 4x3x5 cm, was identified in the right ovary. The level of CA125 in the blood was 410 IU/ml. A radical hysterectomy with removal of the two ovaries, tumors and lymph nodes was performed. Following a period of recovery, first-line chemotherapy consisting of carboplatin and paclitaxel (6 cycles of

intravenous infusion of paclitaxel 175 mg/m^2 and 400 mg/m^2 carboplatin per cycle lasting 3 h with 3 weeks break between chemotherapy cycles) was commenced in mid-December 2010. At the starting of chemotherapy, the CA125 level in the blood was 47 IU/ml, and subsequent to the completion of chemotherapy, it was 46 IU/ml. In May 2011, subsequent to finishing the whole course of treatment, the patient was referred to the Surgical Gynecology Clinic of the Gynecological and Obstetrics Clinical Hospital for follow-up. In June 2011, ultrasound examinations observed a lesion 2x2x0.5 cm in size, which gradually widened (between December 2010 and May 2011) to 7x10x6 cm in size. There was also an increase in the level of CA125 in the blood to 211 IU/ml in February 2013. The patient did not agree to the proposed hospitalizations and surgical procedures. In February 2013, a painful lump 2x2 cm in size was observed in the postoperative scar. Subsequent to obtaining consent from the patient to perform the surgery, a localized lesion in the vagina was removed. In addition, a partly invasive bladder recurrence was removed by local resection of the bladder wall, and a tumor located in the subcutaneous tissue, which was identified as metastasis, was also removed. Following a period of recuperation, second-line chemotherapy consisting of paclitaxel and carboplatin (6 cycles of intravenous infusion of paclitaxel 175 mg/m^2 and 400 mg/m^2 carboplatin per cycle lasting 3 h with 3 weeks break between chemotherapy cycles) was commenced in April 2013. Prior to the fourth cycle of chemotherapy, transvaginal ultrasound was performed, and identified a localized bladder lesion 2x1x1 cm in size, which, despite treatment, gradually increased in size over 3 cycles (13 weeks). Subsequent to completion of chemotherapy treatment for the localized lesion (4x4x3 cm above the vagina) and the level of CA125 in the blood increased from the initial 13 IU/ml to 97 IU/ml subsequent to treatment. In April 2014, the patient refused to consent to the subsequent chemotherapy and self-discharged. In December 2014, the patient was presented again to the Surgical Gynecology Clinic of the Gynecological and Obstetrics Clinical Hospital with weight loss and weakness and was immediately admitted for treatment. Subsequent to improvement of blood morphology, renal function and the general condition of the patient, the proposed chemotherapy regimen Caelyx (doxorubicin) (6 cycles of 50 mg/m^2 doxorubicin per cycle, with 3 weeks between chemotherapy cycles) was administered. In total, six cycles of chemotherapy were administered, which did not stop the growth of the localized lesions in the pelvic cavity. At the end of administrations, the dimensions were 7x5x5 cm and CA125 from level had increased from the original 136 IU/ml to 192 IU/ml. In May 2015, chemotherapy was again attempted, with the fourth-line chemotherapy consisting of paclitaxel and carboplatin (6 cycles of intravenous infusion of paclitaxel 175 mg/m^2 and 400 mg/m^2 carboplatin per cycle lasting 3 h with 3 weeks break between chemotherapy cycles), which was stopped after 3 courses due to the absence of treatment effects, and the request of the patient to be discharged and discontinue treatment. During the last follow-up, the lesion was 10x9x8 cm in size and the blood CA125 level was 625 IU/ml. The patient succumbed to ovarian cancer in late November 2015.

Genetic examination. The proceeding of a genetic examination was performed as previously described (14). Four formalin-fixed paraffin-embedded (FFPE) ovarian carcinoma tissue samples were obtained from the Cancer Pathology Department at Poznan University of Medical Sciences. The FFPE blocks were no older than 5 years.

In order to obtain a high content of cancer cells for DNA extraction, 5-10 sections (5-μm thick) were cut from each paraffin block, and a set of slides was prepared. One slide per patient was then stained routinely with hematoxylin and eosin to identify regions containing a high concentration of cancer cells. Based on this estimation, regions of interest were dissected from the unstained slides. The dissected cells were then put into a 1.5 Eppendorf tube and DNA was extracted using QIAamp DNA FFPE Tissue kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol. Following the extraction, DNA was inspected using NanoDrop spectrophotometer (NanoDrop; Thermo Fisher Scientific, Inc.) and the Qubit 2.0, Quant-iT™ PicoGreen® dsDNA Assay kit (Thermo Fisher Scientific, Inc.). A final concentration of 12 ng/μl DNA in Tris-EDTA buffer (10 mM Tris-HCl, 0.1 mM disodium EDTA, pH 8) was than utilized for the OncoScan® assay (Affymetrix, Inc., Santa Clara, CA, USA). In total, 80 ng of DNA (in 6.6 μl) from each sample were processed. The advantage of the OncoScan assay is possibility of simultaneous identification of copy number alterations, loss of heterozygosity (LOH) and somatic mutations (SMs) in a single experiment. This is possibly due to the use of molecular inversion probe (MIP) technology, and capturing >220,000 small nucleotide polymorphism (SNP) genotypes focused on ~900 cancer locations, distributed across the genome. Another advantage is the ability to identify selected 'hotspot' somatic mutations in nine genes that particularly contribute to the development of various cancers [tumor protein p53, B-Raf proto-oncogene, serine/threonine kinase, KRAS proto-oncogene, GTPase, epidermal growth factor receptor, isocitrate dehydrogenase 1, isocitrate dehydrogenase 2, phosphatase and tensin homolog, phosphoinositide-3-kinase catalytic subunit α (*PIK3CA)* and NRAS proto-oncogene, GTPase]. The experimental procedure includes several steps. Probes were added to the sample DNA, and allowed to anneal at 58˚C overnight (16-18 h) subsequent to an initial denaturation (95˚C for 5 min). Samples was then split into two separate reactions, and proceeded as follows: dATP (A) and dTTP (T) (A/T) were added to one reaction, and dGTP (G) and dCTP (C) (G/C) were added to the second in order to conduct gap fill.

Unincorporated and non-circularized MIPs, as well as the remains of the genomic template, were removed by treatment with exonucleases (Affymetrix, Inc.). The circular MIPs that were gap-filled by the A/T or G/C nucleotides were cleaved using the *Hae*III enzyme, and their linear form was amplified by PCR. Subsequently, the 120-bp PCR product was cut and the smaller (44-bp) fragment containing the specific SNP genotype was subjected for hybridization onto array. Prior to this, samples were mixed with hybridization buffer and injected into the cartridges for 16-18 h at 49˚C and 0.013 x g. Following hybridization, cartridges were removed from the oven, and stained using the GeneChip® Fluidics Station 450 (Affymetrix, Inc.), according to the manufacturer's protocol. Subsequent to staining and washing, arrays were scanned in GeneChip Scanner 3000 7G (Affymetrix, Inc.) and the fluorescence of clusters was measured in order to generate a DAT

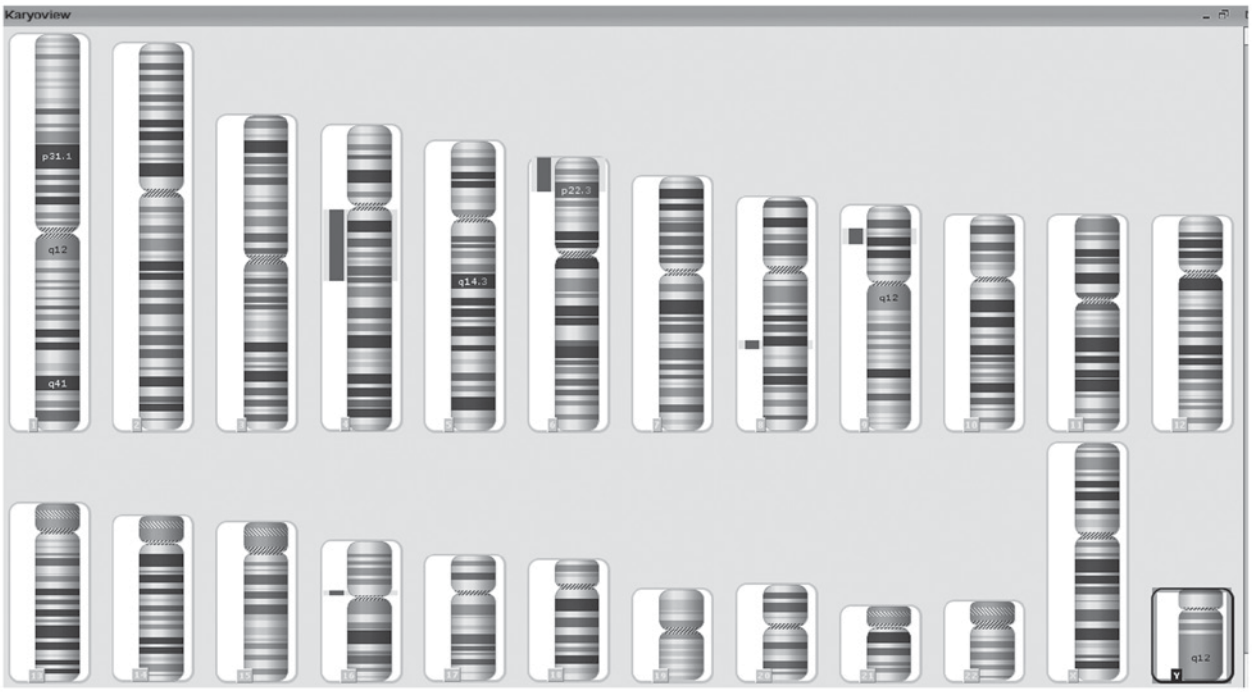

Figure 1. Loss of heterozygosity regions identified in all examined patients. Bars next to the ideogram indicate patients 1-4.

file. Cluster intensities values were automatically calculated using built-in algorithm from DAT files by the Affymetrix GeneChip Command Console software, version 4.0 (Affymetrix, Inc.), and a CEL file was created.

Genomic data analysis. CEL files were processed using OncoScan Console software, version 1.1.034 (Affymetrix, Inc.), to recalculate probe intensities into genomic landscape (OSCHP file) as well as a set of QC metrics (MAPD SNPQC and waviness). For each sample, a profile of copy number alterations was created, expressed by numerical values. The LOH profile was created for all samples, assuming a high confidence interval of ≥3 Mbp (ChAS option). The TuScan algorithm was also used for calculation of ploidy (i.e. 0, 66 or 100%). Somatic mutations were evaluated and viewed in the ChAS browser (Affymetrix, Inc.). The reliability of calls for SMs depends on the SNPQC parameter, and therefore it was necessary to obtain ndSNPQC ≥26 ('in-bounds') for all tested samples. The OncoScan assays are able to detect mutations by relying on the signal intensity of designed clusters, which is translated into the mutation score. This algorithm recognizes three basic thresholds for calls, termed 'Undetected' for an absence of SMs, and 'Lower confidence' or 'High confidence' for detected changes. In the present study, the default mutation score thresholds supplied in the software were used.

Results

Genomic studies. Genomic screening using Affymetrix OncoScan arrays resulted in the identification of large genomic rearrangements across all of the cancer tissues. In general, chromosome number changes were detected in all examined tissues. Ploidies were found in three out of four examined samples. Patients 1 and 2 showed incomplete tetraploidy, whereas patient 3 showed incomplete triploidy. Patient 4 showed diploidy, according to the TuScan algorithm, with hypoploidy of chromosomes 13 and 15. The detailed analysis of regions presenting LOH resulted in the detection of 152 LOH segments with a minimum 3 Mbp size (Table I). These findings are shown in Fig. 1, and the location of each altered segment was depicted. Subsequently, unique overlapping regions in patients presenting sensitivity for treatment (patients 1 and 2) vs. patients showing resistance (patients 3 and 4) were assessed. For the first cohort, only 5 segments on chromosomes 4, 6, 8, 9 and 16 were identified (Table II; Fig. 2). Within those regions, 10 cancer genes were identified using the COSMIC database. For the second cohort, 20 regions on chromosomes 3-5, 7-9, 10, 11, 14-16 and 19 were identified. Within the selected segments, 45 different cancer genes were found (Table III; Fig. 3). The identified LOH regions for all patients are presented in Fig. 1.

The OncoScan arrays enabled the identification of ~100 common somatic mutations (Table IV). In the present study, only one mutation was identified, in patient 4. The mutation affected the *PIK3CA* gene and lead to a glutamic acid-lysine substitution (p.E542K, c.1624G>A; Cosmic ID, COSM760). Notably, the mutation was found in cancer tissue that was diploid and was showing only a hypoploidy of acrocentric chromosomes (chromosomes 13, 15, 18 and 22).

Discussion

Ovarian cancer has the highest mortality rate among reproductive cancers and currently ranks as the fifth leading cause of cancer-associated mortalities among women. Despite the improvements achieved in ovarian cancer therapy over previous decades, the overall 5-year survival rate remains

Table I. LOH regions identified in all examined patients.

No.	Sample	Type	Chrom.	Cytoband	Genomic location start	Genomic location end	Size (Kbp)	Gene count
1	1_189975.OSCHP	LOH	1	p21.3	115837919	96311795	19526.124	161
2	4_208156_15.OSCHP	LOH	1	p31.3	89473522	68095206	21378.316	96
3	3_8376_10.OSCHP	LOH	1	p36.23	33275981	7892870	25383.111	392
4	3_8376_10.OSCHP	LOH	1	p36.33	4738355	754191	3984.164	97
5	1_189975.OSCHP	LOH	1	p36.33	33760197	754191	33006.006	524
6	3_8376_10.OSCHP	LOH	1	q23.3	180377339	163377535	16999.804	144
7	3_8376_10.OSCHP	LOH	1	q31.2	197574134	191510124	6064.01	24
8	2_203344_15.OSCHP	LOH	1	q32.1	216605071	200649365	15955.706	180
9	2_203344_15.OSCHP	LOH	1	q43	249212878	237257823	11955.055	102
10	3_8376_10.OSCHP	LOH	2	p21	90245035	42993165	47251.87	302
11	3_8376_10.OSCHP	LOH	2	p25.3	39767074	21493	39745.581	250
12	2_203344_15.OSCHP	LOH	2	q11.2	112928815	101831270	11097.545	79
13	1_189975.OSCHP	LOH	2	q13	141463604	114138191	27325.413	127
14	2_203344_15.OSCHP	LOH	2	q36.1	228157661	224463413	3694.248	18
15	3_8376_10.OSCHP	LOH	2	q36.3	243052331	230641762	12410.569	154
16	2_203344_15.OSCHP	LOH	2	q36.3	243052331	230903874	12148.457	152
17	3_8376_10.OSCHP	LOH	3	p21.31	51927415	46001062	5926.353	143
18	4_208156_15.OSCHP	LOH	3	p21.31	53323914	50248426	3075.488	82
19	3_8376_10.OSCHP	LOH	3	p26.3	11539955	63410	11476.545	69
20	4_208156_15.OSCHP	LOH	3	p26.3	49346130	63410	49282.72	368
21	4_208156_15.OSCHP	LOH	3	q22.3	164972840	138296967	26675.873	147
22	3_8376_10.OSCHP	LOH	3	q25.32	168219437	157426328	10793.109	39
23	3_8376_10.OSCHP	LOH	3	q27.1	197852564	184416008	13436.556	129
24	3_8376_10.OSCHP	LOH	4	p15.1	35668267	29950964	5717.303	1
25	2_203344_15.OSCHP	LOH	4	p16.3	8060637	71565	7989.072	107
26	1_189975.OSCHP	LOH	4	p16.3	49092454	71565	49020.889	278
27	1_189975.OSCHP	LOH	4	q11	190915650	52684890	138230.76	611
28	2_203344_15.OSCHP	LOH	4	q11	190915650	52684890	138230.76	611
29	3_8376_10.OSCHP	LOH	4	q22.3	114068306	97748435	16319.871	90
30	4_208156_15.OSCHP	LOH	4	q24	190915650	103271887	87643.763	337
31	3_8376_10.OSCHP	LOH	4	q26	177478156	119815943	57662.213	207
32	4_208156_15.OSCHP	LOH	5	p14.1	33066481	28142098	4924.383	12
33	3_8376_10.OSCHP	LOH	5	q11.1	68828372	49441965	19386.407	87
34	4_208156_15.OSCHP	LOH	5	q11.2	68828372	51164114	17664.258	83
35	1_189975.OSCHP	LOH	5	q11.2	68828372	52864364	15964.008	77
36	2_203344_15.OSCHP	LOH	5	q11.2	68828372	55081693	13746.679	56
37	3_8376_10.OSCHP	LOH	5	q13.2	90049057	70306677	19742.38	109
38	2_203344_15.OSCHP	LOH	5	q13.2	119919958	70306677	49613.281	206
39	1_189975.OSCHP	LOH	5	q13.2	180698312	70306677	110391.635	749
40	4_208156_15.OSCHP	LOH	5	q13.2	180698312	70306677	110391.635	749
41	3_8376_10.OSCHP	LOH	5	q21.1	106861975	101206368	5655.607	9
42	3_8376_10.OSCHP	LOH	5	q21.3	114957561	107853410	7104.151	33
43	3_8376_10.OSCHP	LOH	5	q22.3	121539398	115180415	6358.983	20
44	2_203344_15.OSCHP	LOH	5	q23.2	124880865	121481182	3399.683	11
45	3_8376_10.OSCHP	LOH	5	q23.3	132783187	129632862	3150.325	34
46	2_203344_15.OSCHP	LOH	5	q31.1	136935228	133568504	3366.724	33
47	3_8376_10.OSCHP	LOH	5	q31.2	142559092	138965375	3593.717	106
48	2_203344_15.OSCHP	LOH	5	q32	150654481	147480079	3174.402	48
49	2_203344_15.OSCHP	LOH	5	q33.1	154336832	150789050	3547.782	21
50	3_8376_10.OSCHP	LOH	5	q33.1	176675423	151738611	24936.812	137
51	2_203344_15.OSCHP	LOH	5	q33.2	180698312	155277214	25421.098	200

Table I. Continued.

No.	Sample	Type	Chrom.	Cytoband	Genomic location start	Genomic location end	Size (Kbp)	Gene count
52	2_203344_15.OSCHP	LOH	6	p25.3	21704602	204908	21499.694	116
53	1_189975.OSCHP	LOH	6	p25.3	58770502	204908	58565.594	708
54	3_8376_10.OSCHP	LOH	6	q11.1	69746054	61886392	7859.662	8
55	1_189975.OSCHP	LOH	6	q11.1	170913051	61886392	109026.659	512
56	2_203344_15.OSCHP	LOH	6	q22.32	170913051	126471760	44441.291	261
57	3_8376_10.OSCHP	LOH	6	q23.3	170913051	135739354	35173.697	205
58	4_208156_15.OSCHP	LOH	6	q23.3	170913051	138266430	32646.621	189
59	3_8376_10.OSCHP	LOH	7	p15.3	35873540	21882560	13990.98	118
60	4_208156_15.OSCHP	LOH	7	p22.3	50700153	41420	50658.733	348
61	1_189975.OSCHP	LOH	8	p23.1	26419805	8094762	18325.043	147
62	4_208156_15.OSCHP	LOH	8	p23.1	27024823	8094762	18930.061	148
63	3_8376_10.OSCHP	LOH	8	p23.1	30191040	8094762	22096.278	182
64	1_189975.OSCHP	LOH	8	p23.3	7004147	172416	6831.731	36
65	3_8376_10.OSCHP	LOH	8	p23.3	7004147	172416	6831.731	36
66	4_208156_15.OSCHP	LOH	8	p23.3	7004147	172416	6831.731	36
67	4_208156_15.OSCHP	LOH	8	q11.21	53114569	49845207	3269.362	5
68	4_208156_15.OSCHP	LOH	8	q12.1	117682009	59515755	58166.254	254
69	2_203344_15.OSCHP	LOH	8	q12.3	66046002	62996038	3049.964	12
70	1_189975.OSCHP	LOH	8	q13.3	111154532	71428716	39725.816	192
71	3_8376_10.OSCHP	LOH	8	q24.22	140789847	134986490	5803.357	10
72	2_203344_15.OSCHP	LOH	9	p22.3	24559653	14364589	10195.064	59
73	1_189975.OSCHP	LOH	9	p24.3	33434153	204737	33229.416	138
74	4_208156_15.OSCHP	LOH	9	q21.11	78561334	70984371	7576.963	37
75	1_189975.OSCHP	LOH	9	q21.12	141054761	73134143	67920.618	659
76	3_8376_10.OSCHP	LOH	9	q21.13	99234997	74937502	24297.495	145
77	2_203344_15.OSCHP	LOH	9	q21.13	93599890	79064623	14535.267	67
78	3_8376_10.OSCHP	LOH	9	q31.1	136241639	107839840	28401.799	301
79	4_208156_15.OSCHP	LOH	9	q33.2	141054761	125422864	15631.897	316
80	1_189975.OSCHP	LOH	10	p15.3	32764613	126069	32638.544	188
81	1_189975.OSCHP	LOH	10	q23.1	135434303	82575777	52858.526	437
82	4_208156_15.OSCHP	LOH	10	q23.1	135434303	82843903	52590.4	437
83	3_8376_10.OSCHP	LOH	10	q23.1	114381720	87268004	27113.716	267
84	3_8376_10.OSCHP	LOH	11	p11.2	51575951	46089775	5486.176	59
85	1_189975.OSCHP	LOH	11	p11.2	51575951	48040260	3535.691	18
86	1_189975.OSCHP	LOH	11	p15.5	3789206	192763	3596.443	112
87	4_208156_15.OSCHP	LOH	11	p15.5	27025877	192763	26833.114	361
88	3_8376_10.OSCHP	LOH	11	p15.5	38786252	192763	38593.489	422
89	4_208156_15.OSCHP	LOH	11	q12.2	63386750	60212296	3174.454	108
90	1_189975.OSCHP	LOH	11	q13.4	80566396	70719896	9846.5	108
91	4_208156_15.OSCHP	LOH	11	q14.1	134938847	82560444	52378.403	405
92	3_8376_10.OSCHP	LOH	11	q14.1	93535839	84664703	8871.136	56
93	3_8376_10.OSCHP	LOH	11	q22.1	118473385	99519603	18953.782	155
94	1_189975.OSCHP	LOH	11	q22.3	116216759	108306235	7910.524	62
95	1_189975.OSCHP	LOH	12	p13.33	12919325	189399	12729.926	215
96	3_8376_10.OSCHP	LOH	12	q13.13	133818115	52051129	81766.986	724
97	2_203344_15.OSCHP	LOH	12	q14.1	62234495	59059674	3174.821	3
98	2_203344_15.OSCHP	LOH	12	q21.33	133818115	89779996	44038.119	388
99	4_208156_15.OSCHP	LOH	12	q23.1	133818115	96564524	37253.591	340
100	3_8376_10.OSCHP	LOH	13	q11	111956103	19084822	92871.281	429
101	2_203344_15.OSCHP	LOH	13	q11	115103150	19084822	96018.328	460
102	4_208156_15.OSCHP	LOH	13	q11	115103150	19084822	96018.328	460

Table I. Continued.

No.	Sample	Type	Chrom.	Cytoband	Genomic location start	Genomic location end	Size (Kbp)	Gene count
103	3_8376_10.OSCHP	LOH	14	q11.2	35930195	23299134	12631.061	116
104	4_208156_15.OSCHP	LOH	14	q23.1	107282024	60071277	47210.747	465
105	1_189975.OSCHP	LOH	14	q23.1	99873891	60436201	39437.69	283
106	3_8376_10.OSCHP	LOH	14	q32.2	107282024	100785616	6496.408	170
107	1_189975.OSCHP	LOH	15	q11.2	78938567	22752398	56186.169	617
108	3_8376_10.OSCHP	LOH	15	q11.2	79548077	22752398	56795.679	624
109	4_208156_15.OSCHP	LOH	15	q11.2	102397317	22752398	79644.919	807
110	2_203344_15.OSCHP	LOH	15	q24.2	79167603	75948670	3218.933	42
111	2_203344_15.OSCHP	LOH	16	p11.2	35271725	31842847	3428.878	16
112	3_8376_10.OSCHP	LOH	16	p13.3	23792157	83886	23708.271	366
113	1_189975.OSCHP	LOH	16	p13.3	35271725	83886	35187.839	535
114	1_189975.OSCHP	LOH	16	q11.2	90158005	46461308	43696.697	420
115	3_8376_10.OSCHP	LOH	16	q11.2	90158005	46461308	43696.697	420
116	1_189975.OSCHP	LOH	17	p13.3	22217883	400958	21816.925	399
117	2_203344_15.OSCHP	LOH	17	p13.3	22217883	400958	21816.925	399
118	3_8376_10.OSCHP	LOH	17	p13.3	22217883	400958	21816.925	399
119	4_208156_15.OSCHP	LOH	17	p13.3	22217883	400958	21816.925	399
120	1_189975.OSCHP	LOH	17	q11.1	45863219	25326940	20536.279	472
121	2_203344_15.OSCHP	LOH	17	q11.1	80263427	25326940	54936.487	952
122	3_8376_10.OSCHP	LOH	17	q11.1	80263427	25326940	54936.487	952
123	4_208156_15.OSCHP	LOH	17	q11.1	80263427	25326940	54936.487	952
124	1_189975.OSCHP	LOH	17	q23.2	80263427	58390959	21872.468	320
125	2_203344_15.OSCHP	LOH	18	p11.32	10493077	2063183	8429.894	44
126	4_208156_15.OSCHP	LOH	18	q12.1	78007784	26057436	51950.348	215
127	2_203344_15.OSCHP	LOH	18	q12.2	78007784	36335674	41672.11	172
128	3_8376_10.OSCHP	LOH	18	q12.3	78007784	38349307	39658.477	169
129	1_189975.OSCHP	LOH	18	q12.3	78007784	42908725	35099.059	162
130	1_189975.OSCHP	LOH	19	p13.3	4448843	247231	4201.612	154
131	4_208156_15.OSCHP	LOH	19	p13.3	6222353	247231	5975.122	196
132	3_8376_10.OSCHP	LOH	19	p13.3	9033548	247231	8786.317	277
133	2_203344_15.OSCHP	LOH	19	q13.11	59093239	35366074	23727.165	924
134	4_208156_15.OSCHP	LOH	19	q13.2	56731955	42241444	14490.511	616
135	1_189975.OSCHP	LOH	19	q13.32	59093239	46416646	12676.593	561
136	4_208156_15.OSCHP	LOH	20	p13	16811434	69093	16742.341	139
137	1_189975.OSCHP	LOH	20	q11.22	60126157	34313296	25812.861	250
138	2_203344_15.OSCHP	LOH	20	q13.2	58259236	52721955	5537.281	49
139	3_8376_10.OSCHP	LOH	20	q13.2	60139227	52771260	7367.967	57
140	1_189975.OSCHP	LOH	21	q11.2	48097610	14344536	33753.074	295
141	1_189975.OSCHP	LOH	22	q11.1	51213826	16054712	35159.114	549
142	4_208156_15.OSCHP	LOH	22	q11.1	51213826	16054712	35159.114	549
143	3_8376_10.OSCHP	LOH	22	q11.21	51213826	19939352	31274.474	492
144	2_203344_15.OSCHP	LOH	22	q11.21	51213826	21028945	30184.881	467
145	1_189975.OSCHP	LOH	X	p22.33	58412929	177941	58234.988	396
146	3_8376_10.OSCHP	LOH	X	p22.33	58412929	177941	58234.988	396
147	2_203344_15.OSCHP	LOH	X	q11.1	65127774	61732393	3395.381	12
148	3_8376_10.OSCHP	LOH	X	q11.1	76001785	61732393	14269.392	102
149	1_189975.OSCHP	LOH	X	q11.1	155219364	61732393	93486.971	623
150	4_208156_15.OSCHP	LOH	X	q11.2	67429457	63554561	3874.896	12
151	3_8376_10.OSCHP	LOH	X	q21.31	92806132	88265772	4540.36	3
152	3_8376_10.OSCHP	LOH	X	q25	129607422	125678360	3929.062	18

LOH, loss of heterozygosity; Chrom., chromosome;

Table II. The chromosomal regions showing chromosomal alterations identified in patients 1-2 showing sensitiveness for chemotherapy.

No.	Type	Segment	Chrom.	Genomic location start	Genomic location end	Size (Kbp)	Cancer genes
1	loh	LOH_2_15.OSCHP	4	52684890	97836479	45151.589	FIP1L1, CHIC2, PDGFRA, KIT, KDR
2	loh	LOH_2_15.OSCHP	6	204908	21704602	21499.694	IRF4, DEK,
3	loh	LOH_2_15.OSCHP	9	14364589	24559653	10195.064	NFIB, MLLT3, CDKN2A
4	loh	LOH_2_15.OSCHP	16	31842847	35271725	3428.878	-
5	loss	Loss1.5_2_15.OSCHP	8	89900441	95759698	5859.257	-

LOH, loss of heterozygosity; Chrom., chromosome.

Table III. The chromosomal regions with alterations identified in patients 3 and 4, who showed chemoresistance.

No.	Type	Segment	Chrom.	Genomic location start	Genomic location end	Size	Genes
1	loh	LOH_3_10.OSCHP	3	63410	11539955	11476.545	SRGAP3, FANCD2, VHL
2	loh	LOH_3_10.OSCHP	3	46001062	51927415	5926.353	SETD2
3	loh	LOH_3_10.OSCHP	3	157426328	168219437	10793.109	MLF1
4	loss	Loss1.0_4_15.OSCHP	4	104892789	126864721	21971.932	TET2, IL2
5	loss	Loss1.0_4_15.OSCHP	4	160026316	190915650	30889.334	
6	loss	Loss1.0_4_15.OSCHP	5	51505664	113875957	62370.293	IL6ST, PIK3R1, APC
7	loh	LOH_3_10.OSCHP	7	21882560	35873540	13990.98	HNRN, PA2B1, HOXA9, HOXA11, HOXA13, JAZF1
8	loss	Loss1.3_3_10.OSCHP	7	23008207	27115718	4107.511	
9	loss	Loss1.7_3_10.OSCHP	7	27127230	32219657	5092.427	
10	loss	Loss1.3_3_10.OSCHP	7	32240424	32817742	577.318	
11	loss	Loss1.0_4_15.OSCHP	8	172416	26170975	25998.559	PCM1
12	loss	Loss1.5_4_15.OSCHP	9	126044009	136147702	10103.693	SET, FNBP1, ABL1, NUP214, TSC1, RALGDS
13	loss	LOH_3_8376_10.OSCHP	10	87268004	114381720	27113.716	BMPR1A, PTEN, TLX1, NFKB2, SUFU, NT5C2, VTI1A, TCF7L2, FGFR2
14	loh	LOH_4_15.OSCHP	11	192763	27025877	26833.114	HRAS, CARS, NUP98, LMO1, FANCF
15	loh	LOH_3_10.OSCHP	11	84664703	93535839	8871.136	PICALM
16	loss	LOH_3_8376_10.OSCHP	14	100785616	107282024	6496.408	
17	loss	Loss1.3_3_10.OSCHP	15	71156952	79214215	8057.263	PML
18	loss	Loss1.5_4_15.OSCHP	16	18069547	19266457	1196.91	
19	loss	Loss1.0_4_15.OSCHP	19	247231	5655792	5408.561	FSTL3, STK11, TCF3, GNA11, MAP2K2, SH3GL1, MLLT1
20	loss	Loss1.7_3_10.OSCHP	19	1550649	8086055	6535.406	

LOH, loss of heterozygosity; Chrom., chromosome.

<50% (15). Therefore, novel agents are necessary to improve the outcomes for ovarian cancer patients. In addition, it is important to understand and define the patients that are likely to be sensitive to treatment and have resistant disease. Ovarian cancer is a lethal gynecological disease that is characterized by peritoneal metastasis and increased resistance to conventional chemotherapies (16). This increased resistance and the ability of the cancer to spread is often attributed to the formation of

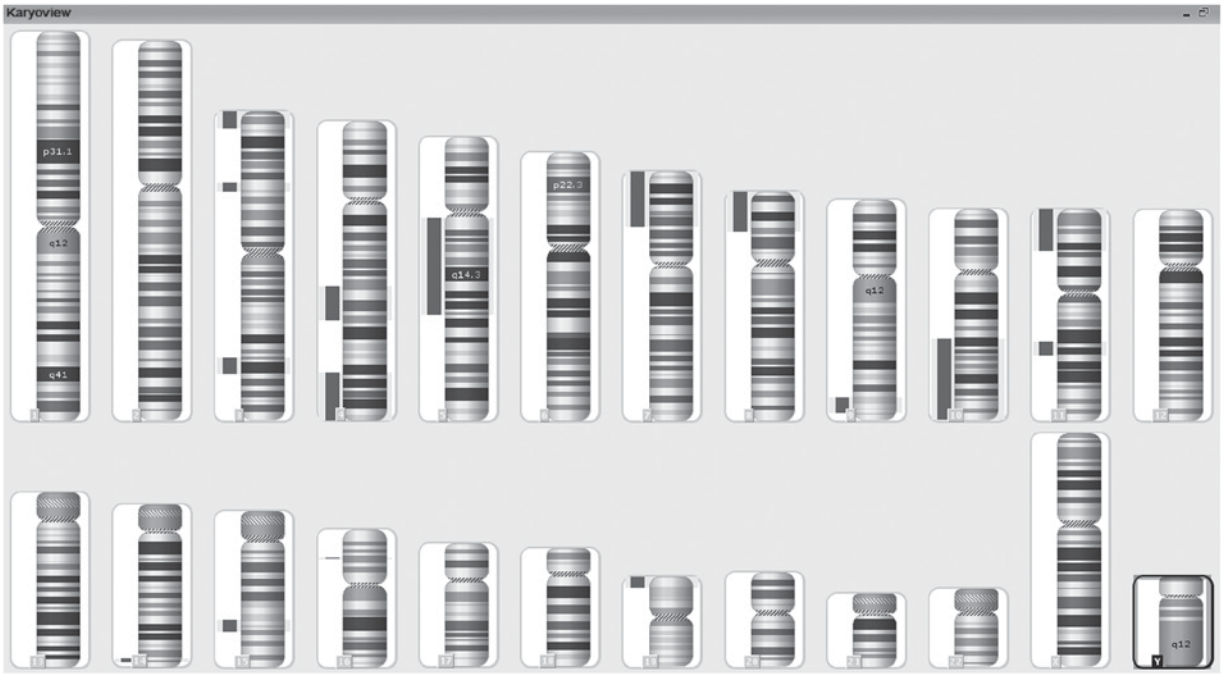

Figure 2. A karyogram showing the chromosomal alterations identified in patients 1 and 2, who showed chemosensitivity.

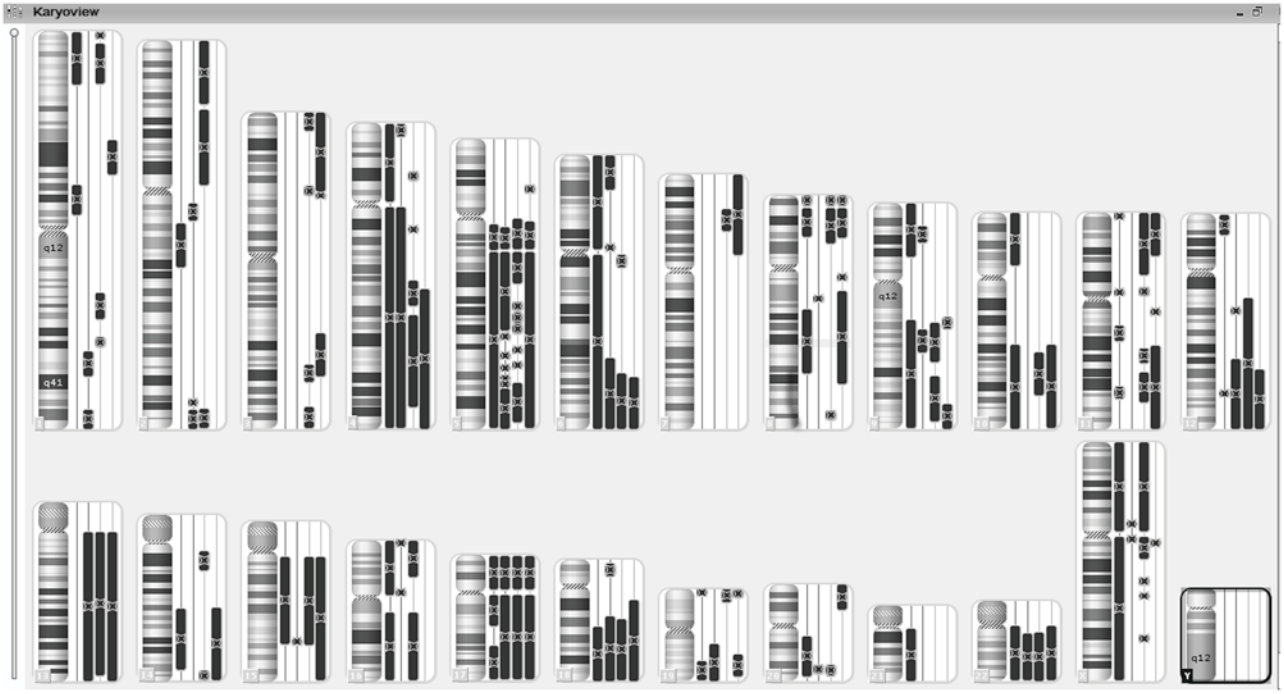

Figure 3. A karyogram showing the chromosomal regions with chromosomal alterations identified in patients 3 and 4, who showed chemoresistance.

multicellular aggregates or spheroids in the peritoneal cavity, which seed to abdominal surfaces and organs (17). Since the presence of metastatic implants is a predictor of poor survival, a better understanding of how spheroids form is critical to improving patient outcome, and may result in the identification of novel therapeutic targets (16). The most widely used tumor marker in ovarian cancer, often considered the 'gold standard', is CA125, which is elevated in 80% of epithelial ovarian cancers (EOCs) (18). CA125 is elevated in 50-60% of patients with stage I EOC and 75-90% of patients with advanced stage EOC (19). The sensitivity of CA125 to identify early stage disease is limited as a screening tool (20). Reliable clinical evidence demonstrates that human epididymis protein (HE4), used alone or in combination with CA125, substantially improves the accuracy of screening and/or disease monitoring (21). HE4, found primarily in the epithelia of normal genital tissues is elevated in EOC (22). HE4 has greater specificity in the premenopausal age group than CA125, since it does not appear to be expressed

Table IV. List of the drivers somatic mutations implemented into Affymetrix OncoScan Arrays.

Mutation	Type	AA change	CDS change	Cosmic ID
NRAS:p.Q61R:c.182A>G	Missense	p.Q61R	c.182A>G	COSM584
NRAS:p.Q61L:c.182A>T	Missense	p.Q61L	c.182A>T	COSM583
NRAS:p.Q61K:c.181C>A	Missense	p.Q61K	c.181C>A	COSM580
NRAS:p.G12V:c.35G>T	Missense	p.G12V	c.35G>T	COSM566
NRAS:p.G12D:c.35G>A	Missense	p.G12D	c.35G>A	COSM564
NRAS:p.G12S/C:c.34G>A/T	Missense	p.G12S‖p.G12C	c.34G>A‖c.34G>T	COSM563‖COSM562
IDH1:p.R132H:c.395G>A	Missense	p.R132H	c.395G>A	COSM28746
PIK3CA:p.E542K:c.1624G>A	Missense	p.E542K	c.1624G>A	COSM760
PIK3CA:p.E545K:c.1633G>A	Missense	p.E545K	c.1633G>A	COSM763
PIK3CA:p.Q546K:c.1636C>A	Missense	p.Q546K	c.1636C>A	COSM766
PIK3CA:p.H1047R:c.3140A>G	Missense	p.H1047R	c.3140A>G	COSM775
PIK3CA:p.H1047L:c.3140A>T	Missense	p.H1047L	c.3140A>T	COSM776
EGFR:p.G719S:c.2155G>A	Missense	p.G719S	c.2155G>A	COSM6252
EGFR:p.G719C:c.2155G>T	Missense	p.G719C	c.2155G>T	COSM6253
EGFR:p.G719A:c.2156G>C	Missense	p.G719A	c.2156G>C	COSM6239
EGFR:p.E746_A750del:c.2235_2249del15	In-frame	p.E746_A750delELREA	c.2235_2249del15	COSM6223
EGFR:p.E746_A750del:c.2236_2250del15	In-frame	p.E746_A750delELREA	c.2236_2250del15	COSM6225
EGFR:p.E746_T751>A:c.2237_2251del15	Deletion In-frame	p.E746_T751>A	c.2237_2251del15	COSM12678
EGFR:p.L747_E749P/del:c.2239_2248>C/G	Various	p.L747_A750>P‖p.L747_E749delLRE	c.2239_2248TTAAGAGAAG >C‖c.2239_2247delTTAAGAGAA	COSM12382‖COSM6218
EGFR:p.L747_T751del:c.2240_2254del15	In-Frame	p.L747_T751delLREAT	c.2240_2254del15	COSM12369
EGFR:p.L747_P753>S:c.2240_2257del18	Deletion In-frame	p.L747_P753>S	c.2240_2257del18	COSM12370
EGFR:p.V769_D770insASV:c.2307_2308ins9	In-frame	p.V769_D770insASV	c.2307_2308ins GCCAGCGTG	COSM12376
EGFR:p.D770_N771insSVD:c.2311_2312ins9	In-frame	p.D770_N771insSVD	c.2311_2312ins GCGTGGACA	COSM13428
EGFR:p.H773_V774insNPH:c.2319_2320ins9	In-frame	p.H773_V774insNPH	c.2319_2320ins AACCCCCAC	COSM12381
EGFR:p.T790M:c.2369C>T	Missense	p.T790M	c.2369C>T	COSM6240
EGFR:p.L858R:c.2573T>G	Missense	p.L858R	c.2573T>G	COSM6224
EGFR:p.L861Q:c.2582T>A	Missense	p.L861Q	c.2582T>A	COSM6213
BRAF:p.V600K:c.1798_1799GT>AA	Missense	p.V600K	c.1798_1799GT>AA	COSM473
BRAF:p.V600E:c.1799T>A	Missense	p.V600E	c.1799T>A	COSM476
BRAF:p.G469E:c.1406G>A	Missense	p.G469E	c.1406G>A	COSM461
BRAF:p.G469A:c.1406G>C	Missense	p.G469A	c.1406G>C	COSM460
PTEN:p.R130G:c.388C>G	Missense	p.R130G	c.388C>G	COSM5219
PTEN:p.R130*:c.388C>T	Nonsense	p.R130*	c.388C>T	COSM5152
PTEN:p.R130Q/fs*4:c.389G>A/delG	Various	p.R130Q‖p.R130fs*4	c.389G>A‖c.389delG	COSM5033‖COSM5817
PTEN:p.R159S:c.477G>T	Missense	p.R159S	c.477G>T	COSM5287
PTEN:p.R233*:c.697C>T	Nonsense	p.R233*	c.697C>T	COSM5154
PTEN:p.P248fs*5:c.741_742insA	Frame-Shift	p.P248fs*5	c.741_742insA	COSM4986
PTEN:p.K267fs*9:c.800delA	Frame-Shift	p.K267fs*9	c.800delA	COSM5809
KRAS:p.A146P:c.436G>C	Missense	p.A146P	c.436G>C	COSM19905
KRAS:p.Q61H:c.183A>T	Missense	p.Q61H	c.183A>T	COSM555
KRAS:p.Q61H:c.183A>C	Missense	p.Q61H	c.183A>C	COSM554

Table IV. Continued.

Mutation	Type	AA change	CDS change	Cosmic ID
KRAS:p.Q61K/K:c.180_181TC>TA/AA	Missense	p.Q61K	c.181C>A‖c.180_181TC>AA	COSM549‖COSM87298
KRAS:p.G13D:c.38G>A	Missense	p.G13D	c.38G>A	COSM532
KRAS:p.G12D/V:c.35G>A/T	Missense	p.G12D‖p.G12V	c.35G>A‖c.35G>T	COSM521‖COSM520
KRAS:p.G12A:c.35G>C	Missense	p.G12A	c.35G>C	COSM522
KRAS:p.G12C/S:c.34G>T/A	Missense	p.G12C‖p.G12S	c.34G>T‖c.34G>A	COSM516‖COSM517
IDH2:p.R172K:c.515G>A	Missense	p.R172K	c.515G>A	COSM33733
IDH2:p.R140Q:c.419G>A	Missense	p.R140Q	c.419G>A	COSM41590
TP53:p.R306*:c.916C>T	Nonsense	p.R306*	c.916C>T	COSM10663
TP53:p.R282W:c.844C>T	Missense	p.R282W	c.844C>T	COSM10704
TP53:p.R273H/L:c.818G>A/T	Missense	p.R273H‖p.R273L	c.818G>A‖c.818G>T	COSM10660‖COSM10779
TP53:p.R273C/S:c.817C>T/A	Missense	p.R273C‖p.R273S	c.817C>T‖c.817C>A	COSM10659‖COSM43909
TP53:p.R249S:c.747G>T	Missense	p.R249S	c.747G>T	COSM10817
TP53:p.R248Q/L:c.743G>A/T	Missense	p.R248Q‖p.R248L	c.743G>A‖c.743G>T	COSM10662‖COSM6549
TP53:p.R248W:c.742C>T	Missense	p.R248W	c.742C>T	COSM10656
TP53:p.G245S/C:c.733G>A/T	Missense	p.G245S‖p.G245C	c.733G>A‖c.733G>T	COSM6932‖COSM11081
TP53:p.Y220C:c.659A>G	Missense	p.Y220C	c.659A>G	COSM10758
TP53:p.R213*:c.637C>T	Nonsense	p.R213*	c.637C>T	COSM10654
TP53:p.R196*:c.586C>T	Nonsense	p.R196*	c.586C>T	COSM10705
TP53:p.H179R:c.536A>G	Missense	p.H179R	c.536A>G	COSM10889
TP53:p.C176F:c.527G>T	Missense	p.C176F	c.527G>T	COSM10645
TP53:p.R175H:c.524G>A	Missense	p.R175H	c.524G>A	COSM10648
TP53:p.Y163C:c.488A>G	Missense	p.Y163C	c.488A>G	COSM10808
TP53:p.V157F:c.469G>T	Missense	p.V157F	c.469G>T	COSM10670

at high levels in benign conditions (23-25). The strongest risk factor of developing ovarian cancer is a family history of breast and ovarian cancer. It is known that ~15% of ovarian cancer patients in the Polish population carry mutations in the *BRCA1* and *BRCA2* genes (26). A small number of cases are also associated with Lynch syndrome and mutations in *hMLH1*, *hMSH2*, *hMSH6*, *PMS1* and *PMS2* in mismatch repair genes (27). Chemotherapy resistance is a common problem faced by patients diagnosed with EOC (28,29). Currently there are no specific or sensitive clinical biomarkers that may be implemented to identify chemotherapy resistance and provide insight into prognosis. Resistance of tumors to chemotherapeutic drugs remains a major clinical challenge for ovarian cancer treatment. The limitations of clinical chemotherapy have been ascribed primarily to mechanisms that mediate drug resistance at the cellular level (30). Previous studies suggest that tumor cells have the ability to regulate genes that help to export, decrease uptake, or increase the metabolism of chemotherapeutic drugs. Newer data also suggest that interactions between tumor cells and the surrounding microenvironment allow for increased resistance of tumor cells to chemotherapy (31). It has been observed that although 40-60% of patients achieve a complete clinical response to first-line chemotherapy treatment ~50% of these patients relapse within 5 years and only 10-15% of patients presenting with advanced stage disease achieve long-term remission (32). It is hypothesized that the high relapse rate is, at least in part, due to resistance to chemotherapy, which may be inherent or acquired by altered gene expression. The patient response to chemotherapy for ovarian cancer is extremely heterogeneous and there are currently no tools to aid the prediction of sensitivity or resistance to chemotherapy and allow treatment stratification (8). Such a tool may markedly improve patient survival by identifying the most appropriate treatment on a patient-specific basis. A clinically applicable gene signature capable of predicting patient response to chemotherapy has not yet been identified. Research into a predictive model, as opposed to a prognostic model, may be highly beneficial and aid the identification of the most suitable treatment for patients. Although it has not yet been accomplished, progress within the field suggests that the development of a predictive model is possible (8). There is considerable variability between the approaches and success of existing studies in the literature, and there have been high levels of variation in the explanatory genes identified (13). The present study hypothesizes that, if more attention is paid to selecting the patients included, to control for treatment history, these gene signatures may be simplified and

models that are able to predict the response to treatment may be developed.

Targeting molecular signatures, as well as signal transduction pathways for tumor sensitivity and resistance is essential for treatment and improving overall survival in patients with ovarian cancer (33). At present, an efficient molecular diagnostics for patients has not been established. The major goal of the present study was to reveal molecular hallmarks associated with, or even responsible for, the response of a patient to standard treatment. This knowledge facilitates the design and implementation of new therapies based on the genetic defect type. The identification of molecular signatures associated with chemo-response is a recent area of investigation. In ovarian adenocarcinoma, the OncoScan microarray technology has been performed to find genetic markers and locations that would be relevant in the prediction of response to chemotherapy. The OncoScan assay is efficient for the analysis of FFPE samples (14).

For the purposes of the present study, patients were divided into two categories, according to responsiveness to chemotherapy. In microarray analysis, the distribution of specific genetic factors between patients was compared. Significant variances in the occurrence of rearrangements were detected for both amplifications (gains) and deletions (losses). Deletions were more frequent in patients showing chemoresistance (14 losses) than in patients presenting with chemosensitivity (1 loss). However, none of the deletions were present in both patients in the same group. This discrepancy between the two patients in each cohort shows a high genetic heterogeneity of tumors. Detailed mapping data also revealed information on the LOH. The LOH phenomenon is of particular importance since it enables the tracing of loss of the normal alleles of tumor suppressor genes, to determine the tumor phenotype. Therefore, locations presenting high frequency of LOH are attractive candidates for harboring tumor suppressor mutations. In the present study, similar amounts of LOH were present in the two cohorts. In addition, the majority of the samples showed LOH at several loci. Numerous loci with LOH were common between the two cohorts. However, certain LOH were typical for patients with resistance to chemotherapy or patients presenting with chemosensitivity. Regions of typical LOH for chemosensitivity were located on chromosomes 4 (p16.3, q11) and 6 (p25.3) in the present study, whereas LOH associated with loci 3p21.3, 3p26.3, 6q23.3 and 11q14.1 were found exclusively in the chemoresistant cases.

The assessment of LOH in EOC focused on the role of genes located on the short arm of chromosome 3 (3p) in the development of disease. Deletions in regions 3p21.3 and 3p26.3 are common for such cases (34).

LOH in 6q23.3 affects the genes *MYB*, *TNFAIP3* and *ECT2 L*. Only *TNFAIP3* has been implicated in the inhibition of programmed cell death is and suggested to be a tumor suppressor gene (35). At present, the function the remaining genes is not associated with the pathogenesis of ovarian cancer. Furthermore, Shridhar *et al* (36) reported that deletion of the 6q23.3 region, which commonly presents LOH in ovarian cancer.

Notably, the commonly mutated genes for EOC, namely: *CDH1; PRKN; BRCA1/2*; and *AKT1* were not identified in the present study. However, in patient 4, who showed chemotherapy resistance, a somatic *PIK3CA* mutation was identified. Mutation in this gene has been previously associated with ovarian cancer (37). Certain studies have confirmed that the PIK3CA/Akt/mammalian target of rapamycin pathway is commonly dysregulated in ovarian cancers (38,39).

Chemotherapy response in ovarian cancer is a complex and unpredictable process that determines the course of the disease. In the present study, genetic regions associated with ovarian cancer that may play an important role in the context of treatment response were identified. However, additional studies on a larger cohort of patients are required, in order to reveal crucial pathways and molecular determinants that directly influence the disease course and its aggressiveness.

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