

Doctoral Thesis for the Degree of Doctor of Philosophy, Faculty of Medicine

Characterization of genetic
alterations in ovarian cancer associated with
chemotherapy response

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ABSTRACT

Ovarian cancer is the most lethal of all gynecological malignancies, and exhibit an overall five-year survival rate of only 48% in Sweden. The high mortality in ovarian cancer is largely due to late diagnosis and chemotherapy resistance. Finding predictive markers of chemotherapy response and elucidating the resistance mechanisms would help to individualize and improve treatment of ovarian cancer patients.

With the aim to explore genetic alterations and to search for potential predictive biomarkers of chemotherapy response in ovarian cancer patients, a total of 133 epithelial ovarian carcinomas were investigated genetically. Initially, early-stage tumors of mixed histology from patients treated with carboplatin were analyzed with both metaphase comparative genomic hybridization (CGH) and array CGH. The main finding was that gain in chromosome arm 1q, and more specifically 1q25.1-41, was significantly associated with carboplatin resistance. Additionally, differences in the genetic alteration patterns were detected between the three histologic subtypes serous, mucinous and clear cell. Subsequently, stage III serous ovarian tumors from patients treated with combination therapy paclitaxel/carboplatin were analyzed with array CGH and quantitative real-time polymerase chain reaction (QPCR). Gain in 3q26.2 and losses in the regions 6q11.2-12, 9p22.3-21.3 and Xp22.2-11.1 were found significantly more frequent in the resistant cases than in the sensitive cases. When examining the gene expression of four genes located in these genomic regions, the *EVI1* gene expression differed between samples with gain versus without gain, and exhibited higher expression in the gain group. Furthermore, based on the significant genomic regions, a decision tree was generated and loss in regions 6q11.2-12, Xp11.3 and Xp22.13 was the best combination to classify the tumor material according to chemotherapy response. Next, a patient material treated with combination therapy docetaxel/carboplatin and consisting of advanced stage serous ovarian tumors was analyzed with array CGH. Losses in 8p23.3-23.1 and 8p22 were significantly associated with sensitivity, and gains in six regions in chromosome 9 (9p13.2-13.1, 9q21.2-21.32, 9q21.33, 9q22.2-22.31, 9q22.32-22.33 and 9q33.1-34.11) were significantly associated with resistance. Interestingly, this was a different set of genetic alterations than the paclitaxel/carboplatin material generated, although the two materials exhibit similar clinical features and are given similar therapies. Altogether, specific genetic alterations associated with differential chemotherapy response and patient outcome were identified in these studies. The different chemotherapies were associated with different genetic alterations, which might lead to the establishment of separate predictive biomarkers.

LIST OF PAPERS

This academic thesis is based on the following papers, referred to in the text by roman numerals:

- I **Osterberg L**, Levan K, Partheen K, Helou K, Horvath G
Cytogenetic analysis of carboplatin resistance in early-stage epithelial ovarian carcinoma.
Cancer Genetics and Cytogenetics **163**(2): 144-50. (2005)

- II **Osterberg L**, Levan K, Partheen K, Staaf J, Sundfeldt K, Horvath G
High-resolution genomic profiling of carboplatin resistance in early-stage epithelial ovarian carcinoma.
Cytogenetic and Genome Research (2009)

- III **Osterberg L**, Levan K, Partheen K, Delle U, Olsson B, Sundfeldt K, Horvath G
Potential predictive markers of chemotherapy resistance in stage III serous ovarian adenocarcinomas.
Manuscript, submitted

- IV **Osterberg L**, Levan K, Partheen K, Delle U, Olsson B, Sundfeldt K, Horvath G
High-resolution array CGH reveals specific copy number alterations associated with docetaxel/carboplatin response in ovarian carcinomas.
Manuscript

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ABBREVIATIONS

BASE	bioarray software environment
BAC	bacterial artificial chromosome
cDNA	complementary DNA
CGH	comparative genome hybridisation
CNA	copy number alteration
Cy3	Cyanine 3
Cy5	Cyanine 5
DNA	deoxyribonucleic acid
FIGO	International Federation of Gynecology and Obstetrics
FISH	fluorescence <i>in situ</i> hybridization
FITC	avidin-flourescein isothiocyanate
Mbp	megabasepairs
mRNA	messenger RNA
PCR	polymerase chain reaction
QPCR	quantitative real time PCR
RIN	RNA integrity number
RNA	ribonucleic acid
SCOTROC1	Scottish Randomised Trial in Ovarian Cancer
SRO	smallest region of overlap
TRITC	antidigoxigenin-tetramethylrhodamine isothiocyanate
WHO	World Health Organization

INTRODUCTION

Cancer

Cancer is the leading cause of death worldwide, accounting for nearly 8 million deaths per year, and the number is predicted to rise to an estimated 12 million deaths in 2030 [1, 2]. Of all cancer deaths worldwide, more than 70% occur in low- and middle-income countries. Around 11 million new cancer cases are diagnosed each year worldwide [3]. In Sweden, cancer is the second largest cause of death following cardiovascular disease, and more than 20,000 people die of cancer each year in our country [4, 5]. The number of diagnosed cases in 2007 was 50,100, a number that has almost doubled since the 1970s [4, 5]. This is thought to be partly, but not fully, explained by an ageing population and improved diagnostics. Risk factors for cancer are a large variety of environmental and genetic factors.

Cancer is a generic term for a multitude of diseases that can affect any part of the body. The hallmark of cancer is uncontrolled cell growth, which begins in one cell and in most cases leads to a mass of cells termed neoplasm or tumor. The development of cancer is a multistep process that takes place over many years. A complex succession of events that affect the genome of the cell occur when it transforms from normal to malignant [6]. Genes that directly or indirectly control cell proliferation are altered during tumorigenesis; the most prominent alterations being gain of function of oncogenes and loss of function of tumor-suppressor genes. Identification of the genetic or epigenetic changes in cancer cells and the proteins that the changes affect are useful as diagnostic and prognostic markers as well as molecular targets for therapeutic intervention.

Ovarian cancer

Ovarian cancer is the tenth most common type of female cancer in Sweden, and accounts for 3% of all female cancers [7]. Even though the incidence is low, ovarian cancer is the most lethal of all gynecological malignancies, and exhibit an overall five-year survival rate of only 48% [5]. Ovarian cancer mostly affects postmenopausal women and the median age of diagnosis is 60-65 years [8]. Around 90% of ovarian cancers are sporadic, and less than 10% are hereditary [8]. Ovulation is probably an important co-factor in the development of ovarian cancer since the ovarian surface epithelium undergoes repetitive disruption and repair. Therefore, factors that decrease the number of ovulatory cycles are protective, such as the use of oral contraceptives, pregnancy and lactation [9]. Risk factors except for family history of the disease are: nulliparity, early age at menarche and late age

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at menopause, possibly hormonal infertility treatment and postmenopausal hormone-replacement therapy, and lifestyle factors as smoking and alcohol [9, 10].

Disease symptoms of ovarian cancer are diffuse and nonspecific and sometimes asymptomatic. It is therefore frequently diagnosed in advanced stages, which partly explains the low survival. Ovarian tumors are staged surgically according to the International Federation of Gynecology and Obstetrics (FIGO) system (Box 1; Figure 2) [11], and thorough surgical exploration is important since subsequent treatment is based primarily on the stage of the disease but also on grade. Debulking surgery is performed and optimal cytoreduction is preferred since the prognosis of the disease greatly depends on how much residual tumor is left after primary surgery. Five-year survival is significantly better if none or only tumors less than 5-10 mm is left in place [11]. However, when the tumor is present on the entire peritoneum or grows in an infiltrative fashion this is not always achievable. Survival rates differ greatly between the stages from ~86% in stage I to ~19% in stage IV (Figure 1) [11]. The term early-stage usually refers to stage I and II, and advanced stage refers to stage III and IV. Grade of differentiation is also established for ovarian tumors; grade one is highly differentiated, grade two moderately, and grade three is poorly differentiated. Ovarian tumors primarily spread by overgrowth to other pelvic organs or by direct exfoliation of cells into the peritoneal cavity and then follow the route of the peritoneal fluid. It also spreads via the lymphatics, whereas blood-borne metastasis is less common.

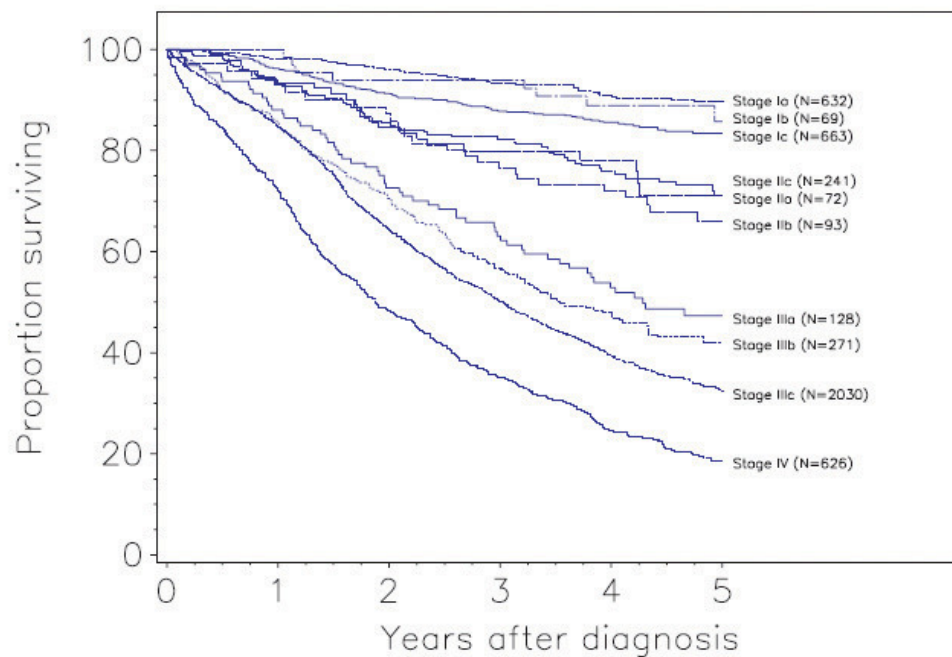


Figure 1. Relative survival by FIGO staging. (This figure was published in [11], copyright Elsevier with permission to reprint (2006).)

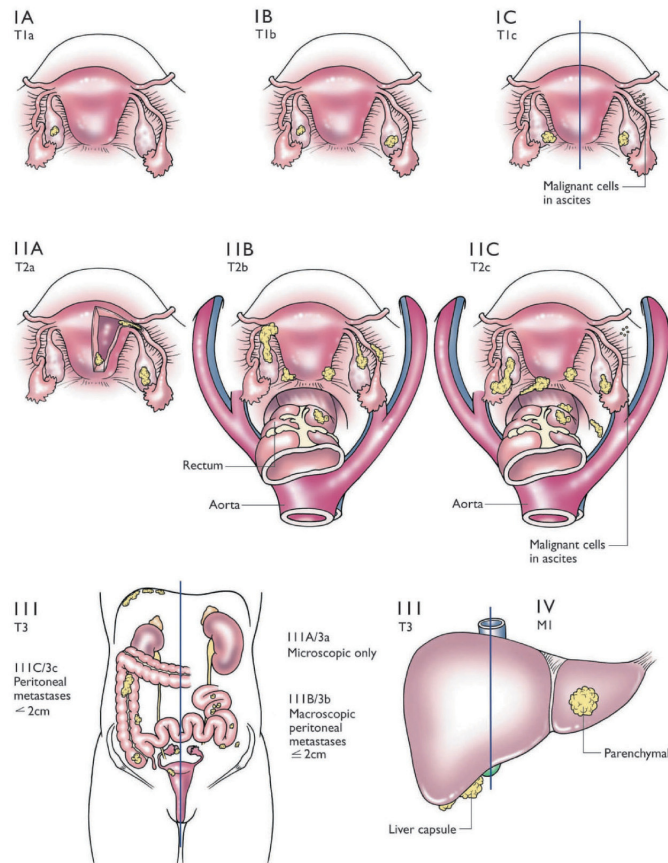


Figure 2 and Box 1. Stage according to the FIGO system. (This figure was published in [11], copyright Elsevier with permission to reprint (2006).)

Box 1.

Stage I – Growth limited to the ovaries

IA – Growth limited to the ovary; no ascites. No tumor on the external surface; capsule intact

IB – Growth limited to both ovaries; no ascites. No tumor on the external surfaces; capsule intact

IC – Tumor either stage 1A or 1B but with tumor on the surface of one or both ovaries; or with capsule ruptured; or with ascites present containing malignant cells or with positive peritoneal washings

Stage II – Growth involving one or both ovaries with pelvic extension

IIA – Extension and/or metastases to the uterus and/or tubes

IIB – Extension to other pelvic tissues

IIC – Tumor either stage IIA or IIB with tumor on the surface of one or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or with positive peritoneal washings

Stage III – Tumor involving one or both ovaries and/or positive retroperitoneal or inguinal nodes. Superficial liver metastasis equals stage III. Tumor is limited to the true pelvis, but with histologically proven malignant extension to small bowel or omentum

IIIA – Tumor grossly limited to the true pelvis with negative nodes but with histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter. Nodes negative

IIIB – Tumor of one or both ovaries with histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter. Nodes negative

IIIC – Abdominal implants more than 2 cm in diameter and/or positive retroperitoneal or inguinal nodes

Stage IV – Growth involving one or both ovaries with distant metastasis. If pleural effusion is present, there must be positive cytologic test results to allot a case to stage IV. Parenchymal liver metastasis equals stage IV.

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The most frequently used prognostic factors of ovarian cancer today are volume of residual tumor after primary debulking surgery, patient age, stage, grade, level of CA-125 and DNA ploidy [12]. The only molecular biologic tumor marker used in clinical practice today is CA-125. It is a serologic marker expressed by 80% of ovarian tumors, and circulating levels of CA-125 reflect tumor burden to some extent and is used to monitor the disease during progression [12, 13]. There is extensive research on biomarkers in ovarian cancer (reviewed in [13, 14]). Probably a panel of markers or profiles, instead of single markers, may be needed to establish useful prognostic factors. Since ovarian cancer is a heterogeneous disease and clinical prognostic factors today are insufficient, the identification of biomarkers could contribute to a better prediction of the clinical outcome for ovarian cancer patients, and facilitate the choice of the most optimal treatment of each individual.

Histology

Around 90% of all ovarian cancers are epithelial, i.e. derived from the germinal epithelium that surrounds the ovaries. The major histologic subtypes of the epithelial tumors are serous, mucinous, endometrioid, clear cell, and undifferentiated carcinomas. These histologic subtypes exhibit the features of various parts of the genital tract. The most common subtype is serous, constituting more than 50% of all epithelial tumors [15]. The vast majority of serous tumors are found in advanced stages (comprising >90% of stage III) and the number of diagnosed cases in early stages are much fewer (Figure 3) [11]. Overall, serous carcinomas exhibit the lowest survival rate with a five-year survival of approximately 40%; mucinous, endometrioid and clear cell all have an overall five-year survival around 60% [11]. However, survival figures of the histologic subtypes vary between the clinical stages. Mucinous carcinomas are most common in stage I, where it has a higher survival rate than the other subtypes. Endometrioid tumors are most commonly diagnosed in early stages, and advanced stages of this histological tumor group have a slightly better survival than the other subtypes. Clear cell tumors are more evenly distributed between stages and have a rather poor prognosis in advanced stages.

Even though the molecular biology differs between the subtypes, and there are several reports on differential chemotherapy response between the subtypes [16-19], all histologic subtypes are given the same therapy. This diversity in ovarian cancer has conducted some researchers to suggest that ovarian carcinoma constitute several distinct disease entities [20].

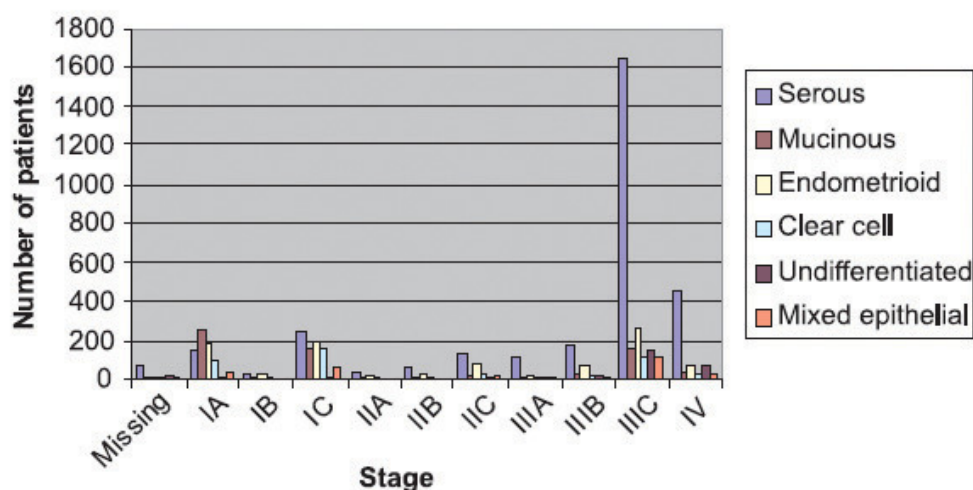


Figure 3. Distribution of ovarian cancer cases according to histologic subtype and stage. (This figure was published in [11], copyright Elsevier with permission to reprint (2006).)

Chemotherapy

Standard treatment for ovarian cancer after surgery is chemotherapy; single-agent carboplatin in early-stage disease and combination therapy paclitaxel/carboplatin in advanced stage disease. In western Sweden, patients with highly differentiated stage IA diploid tumors are the only ovarian cancer patients not given chemotherapy. The remaining stage IA tumors, and IB-IIA tumors are treated with carboplatin as first-line chemotherapy. Patients with stage IIB-IV disease are given carboplatin in combination with paclitaxel as first-line chemotherapy. Response rates at initial combination treatment are high (60-80%) with a large group of patients achieving a complete clinical response [21, 22]. Nevertheless, the majority of the patients relapses with resistant tumors and subsequently die of their disease. Second-line therapy is not considered to be curative but the aim is to control the disease and to improve the quality of life of the patients [23]. A variety of drugs are available for second-line treatment and the choice is based on the time from first-line treatment, previous response and the patient's general state of health.

Carboplatin

Carboplatin is the foundation in ovarian cancer chemotherapy and is widely used in the management of human malignancies; for example testicular, head and neck, and small-cell lung cancer. The platinum compounds, cisplatin and later carboplatin, have been the cornerstone in ovarian cancer therapy since the late 1970s. Carboplatin kills cells by binding to DNA and causing programmed cell death – apoptosis. When entering the cells, carboplatin forms platinum-DNA adducts which triggers cellular signal-transduction pathways, DNA repair systems, cell-cycle checkpoint arrest and ultimately apoptosis [24-

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26]. The initial compound cisplatin exhibit severe toxicities with nephrotoxicity and peripheral neurotoxicity being the most serious [26]. The second-generation analogue carboplatin that has replaced cisplatin in ovarian cancer therapy rarely results in nephrotoxicity and peripheral neurotoxicity. The dose-limiting side effect of carboplatin is myelosuppression, specifically neutropenia and thrombocytopenia [26].

Taxanes

The taxanes, paclitaxel and its analogue docetaxel, are important drugs in cancer chemotherapy and are used in the treatment of ovarian, breast, prostate, and small-cell lung cancer. The drugs bind to the β -tubulin subunit of the tubulin heterodimers, stabilize the microtubules and inhibit the mitotic spindle in the cell [27]. This causes cell-cycle arrest which leads to apoptosis of the cell. However, there are some differences between the two drugs both mechanistically and pharmacologically. Paclitaxel acts in the G₂/M phases of the cell cycle and docetaxel acts in G₂/M/S phases [28, 29]. In addition, docetaxel binds β -tubulin with higher affinity [30], and both drugs cause BCL-2 phosphorylation but docetaxel 100 times more potently [31]. Clinically, paclitaxel and docetaxel exhibit different toxicity profiles (reviewed in [32]). Paclitaxel causes significant neurotoxicity. Docetaxel, on the other hand, causes less neurotoxicity but higher neutropenia which can be managed by the addition of colony-stimulating factor [32]. Docetaxel has largely substituted paclitaxel in combination regimens in breast cancer. In ovarian cancer, the combination paclitaxel/carboplatin was compared to docetaxel/carboplatin as first-line treatment in the SCOTROC1 (Scottish Randomised Trial in Ovarian Cancer) study [33]. The conclusion drawn was that the efficacy was equivalent with similar clinical response rates between the treatment groups, and the major differences between the two regimens were the toxicity profiles [33]. Thus, docetaxel has been suggested as an alternative to paclitaxel in first-line treatment of ovarian cancer [34-36].

The major limitations of the effectiveness of the therapeutic drugs used in ovarian cancer are the dose-limiting side-effects and the development of resistance. Unfortunately, there are presently no tools to predict whether a patient will respond successfully to the chemotherapy or not.

Resistance

Chemotherapy resistance in ovarian cancer is defined clinically and is the basis for the choice of treatment of recurrent disease. First-line response and treatment-free interval are the most important factors to consider before the choice of second-line therapy. The exact definition of resistance varies in the literature. It is thus established that a relapse

within six months after first-line therapy decreases response rates [37, 38]. One classification of resistance used by many researchers is: patients who exhibit steady disease or progressive disease after first-line treatment, or recurrent disease within six months after the last administration of chemotherapy are considered clinically resistant. Patients exhibiting clinical complete remission after first-line treatment and experiencing relapse later than six months after completion of first-line chemotherapy are considered clinically sensitive [34, 39, 40].

The molecular mechanisms that tumor cells use to escape the chemotherapy induced death and to become resistant has been the subject of extensive investigation, but it appears complex and the complete picture is far from unraveled. Resistance mechanisms suggested for carboplatin are: decreased uptake or increased efflux of the drug into the cell, drug inactivation, activation of DNA repair mechanisms, and the avoidance of apoptosis through up- and down-regulations in the complex apoptotic signaling pathways (reviewed in [41]). Mechanisms of resistance proposed for taxanes are: alterations of the target β -tubulin and the microtubules, altered signaling pathways of the cell cycle and apoptosis, and over expression of multidrug efflux pumps such as the P-glycoprotein encoded by the *ABCB1* gene [37, 42]. There have been reports that paclitaxel and docetaxel do not exhibit complete cross-resistance but only partial cross-resistance. Clinical studies in ovarian and breast tumors have shown that docetaxel had antitumor activity in paclitaxel-resistant tumors [40, 43, 44]. In addition, *in vitro* studies have shown incomplete cross-resistance between the two taxane drugs [45, 46].

Probably, multiple resistance-causing mechanisms coexist inside the tumor cells; and it is assumed that genetic or epigenetic alterations are responsible for these mechanisms. Additionally, resistance can be mediated by other factors than tumor-cell-specific, and some models have been suggested [37, 47]. Cytotoxic agents are primarily effective against proliferating cells, and even though tumors proliferate rapidly a proportion of the cancer cells are in a quiescent state (G_0) which makes them more resistant than the cycling cells. In addition, cancer stem-cells have been described to be intrinsically drug-resistant. Different types of resistance models may exist simultaneously in different sub-clones of the same tumor [47].

Genomic alterations and chemotherapy resistance

Since chemotherapy resistance has proved to be very complex, elucidating the various specific mechanisms is effort consuming. A somewhat different approach is to identify genetic alteration profiles that are able to predict chemotherapy response and clinical outcome of each patient, regardless of its importance for resistance mechanisms. Such an

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instrument could identify a high-risk group of patients that should be monitored more carefully or receive a different treatment regiment.

Global genomic explorations of chemotherapy response to the drugs used in ovarian cancer have been performed in several studies, however, predominantly on cell lines. Using metaphase CGH and the further elaboration array CGH, a number of studies have investigated ovarian cancer and chemotherapy response with varying results [48-55]. In addition, several CGH studies have explored ovarian tumor materials with survival as end point and identified various prognostic markers and tumor development markers for ovarian cancer, which is also interesting in the context of chemotherapy response [56-62]. In summary, several genetic alterations are recurrent in ovarian tumor materials and have been associated with tumorigenesis, i.e. gains in 3q, 8q, 20q, and losses in 4q and 8p [63]. Nevertheless, results concerning prognosis and chemotherapy response are more diverse and a range of genetic alterations have been suggested to be of importance.

Taken together, ovarian cancer is a heterogeneous disease exhibiting both intertumoral and intratumoral variations as described in this introduction. Thus, individualized and targeted therapy is proposed to be the future treatment strategy of ovarian cancer patients, and the importance of identifying predictive biomarkers in this approach is emphasized by researchers in the field [64].

AIMS

The overall aim of this research was to explore genetic alterations behind the differential chemotherapy response in ovarian cancer patients, with the ultimate goal to identify predictive markers of chemotherapy response and disease progress.

More specifically, the aims were:

- Characterize cytogenetic alterations associated with carboplatin resistance and histologic subgroup in early-stage ovarian tumors.
- Specify genetic alterations behind differential response to carboplatin and histology in early-stage ovarian tumors.
- Search for genetic alterations that might be useful as predictive markers of paclitaxel/carboplatin response in stage III serous disease.
- Investigate the genetic pattern in tumors from patients treated with docetaxel/carboplatin as first-line treatment, and compare the results to the genetic pattern detected in tumors from patients treated with the routinely used paclitaxel/carboplatin.

MATERIALS AND METHODS

Tumor material

The tumors investigated in this thesis were collected from patients diagnosed between 1993 and 2007 at Sahlgrenska University Hospital, and the study was approved by the local ethics committee. The 133 epithelial ovarian adenocarcinomas were removed during primary debulking surgery at the patient's local hospital, and stored in -80°C until analysis. Local pathologists reviewed biopsies; and in order to ensure uniformity of diagnosis it was also diagnosed by one pathologist at Sahlgrenska University Hospital. The World Health Organization (WHO) criteria were used to classify histology, and clinical staging was performed according to the FIGO standards. The management of ovarian cancer patients was controlled by the western Sweden Clinical Guidelines, and chemotherapy was given subsequent to surgery according to these guidelines [12]. Specimen imprints were stained with May-Grünwald-Giemsa stain and cytologic evaluation was performed on each tumor to verify the adequate presence of tumor cells.

Patients were defined as clinically resistant when they had steady disease or progressive disease after first-line chemotherapy; or recurrent disease within six months after completion of first-line chemotherapy. Patients were defined as clinically sensitive when they had complete remission after first-line chemotherapy, and if experiencing relapse they did so after a treatment-free interval of more than six months. In paper I and II, seven patients included were clinically defined as secondary resistant; these patients did respond to first-line treatment but were considered resistant at recurrence and then died of their disease. Overall, patients with clinical resistance died of their disease, and all sensitive cases, except for in paper IV, survived more than five years from diagnosis.

The selection of tumors was primarily based on the patients' response to chemotherapy. In paper I, 63 tumors from patients treated with carboplatin were analyzed. Early-stage tumors are few, have a high survival and a more mixed histology than advanced stages. We were therefore only able to gather 17 clinically resistant patients, and the tumor group consisted of several histology types (Table 1). In paper II, 32 of the 63 samples previously used in paper I were included, due to lack of tumor material and poor hybridization quality of some samples (Table 1). In paper III, a new set of 40 tumors from patients treated with paclitaxel/carboplatin was analyzed with array CGH (Table 1). In order to refine the tumor material, and since histology generated differences in genetic alterations in paper I and II, only stage III serous tumors were selected. Due to poor-quality RNA in a subset of the samples, only 17 of the 40 tumor samples were analyzed with QPCR. In paper IV an additional 30 tumors from patients treated with docetaxel/carboplatin were

Table 1. Distribution of the tumor materials between the four papers, regarding chemotherapy response, survival, histology and stage. Of the 63 tumors analyzed in paper I, 32 were used in paper II. New independent sets of tumors were used in paper III and IV.

	Paper I	Paper II	Paper III	Paper IV
Resistant	17	12	20	6
primary	10	6	20	6
secondary	7	6	-	-
Sensitive	46	20	20	24
Deceased	17	12	20	6
Survivors	46	20	20	24
Serous	22	11	40	30
Mucinous	17	7	-	-
Clear cell	21	12	-	-
Endometrioid	1	1	-	-
Undifferentiated	1	1	-	-
Stage I	59	29	-	-
Stage II	4	3	-	1
Stage III	-	-	40	22
Stage IV	-	-	-	8
Total	63	32	40	30

investigated. All were serous carcinomas, however due to the small group of patients receiving this therapy stage IV tumors were also included in the analysis (Table 1).

Comparative Genomic Hybridization (CGH)

The CGH technique provides a global analysis of copy number alterations of the whole tumor genome in a single experiment. Copy number gains and losses are detected and mapped. In a typical CGH experiment total genomic DNA is isolated from test and reference cell populations, differentially labeled, and competitively hybridized to a representation of the genome (Figure 4). In the case of metaphase CGH, the hybridization target is normal metaphase spreads, and the location of copy number variations between test and reference DNA are mapped to the physical position on the chromosomes. For array CGH, DNA microarrays are used as the representation of the genome, which makes it possible to map the changes directly onto the genomic sequence. The fluorescence ratio of the test and reference hybridization signals is indicative of the relative DNA copy number in test versus reference DNA. Hybridization of repetitive sequences is blocked by the addition of Cot-1 DNA.

CGH was initially reported by Kallioniemi and colleagues [65] using metaphase spreads. Lately the array format has been developed [66-69] which can provide a number of

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advantages over the use of chromosomes, including higher resolution and dynamic range, direct mapping of aberrations to the genome sequence and higher throughput. The main disadvantage of metaphase CGH is its low resolution, estimated to be around 5-10 Mbp depending on the type of alteration [70]. In array CGH, the resolution is determined by the distance between consecutive clones and the size of the clones. We used bacterial artificial chromosome (BAC) clone arrays. BAC clones vary in length from 150-200 kbp and are spotted onto the arrays after PCR amplification. The tiling BAC arrays used in paper II-IV exhibit a complete coverage of the human genome and provides a resolution around 100 kbp. Limitations of the CGH technique is its inability to detect aberrations that do not result in copy number changes, such as balanced translocations and inversions. Additionally, ploidy changes are not detected by CGH.

Metaphase CGH

Metaphase CGH in paper I was essentially performed as described by Kallioniemi *et al.* [70]. Reference DNA was extracted from blood of a healthy female donor. Tumor and reference DNA were labeled with biotin-16-dUTP and digoxigenin-11-dUTP, respectively. Labeled DNA were hybridized onto normal human metaphase slides prepared from the blood of healthy female donors. Tumor DNA was detected with fluorescent FITC-avidin and reference DNA with TRITC-antidigoxigenin, and images were captured with a CCD camera mounted on a Leica microscope. Further, digital image analysis was performed using the Leica CW4000 software package.

Array CGH

High-resolution tiling BAC arrays used in papers II-IV were produced at the SCIBLU Genomics Center, Department of Oncology, Lund University, Sweden (http://www.lth.se/sciblu/services/dna_microarrays). BAC clones were mapped to the hg17 genome build. Normal female reference DNA containing a mix from ten healthy individuals was purchased from Promega, Madison, WI, USA. Array CGH was performed essentially as previously described by Jonsson *et al.* [71]. Genomic DNA was fluorescently labeled with Cy3-dCTP (tumor sample) and Cy5-dCTP (reference). Labeled DNA were applied to arrays and hybridized for 72 h. Arrays were scanned with Agilent microarray scanner G2505B (Agilent technologies, Palo Alto, CA, USA). The data was processed in the web-based database BASE [72]. After filtering and normalization, the data was segmented using the CGH-Plotter software in BASE. Copy number alterations were determined according to thresholds, and each clone was assigned -1, 0 or 1, giving a ternary segmented data set.

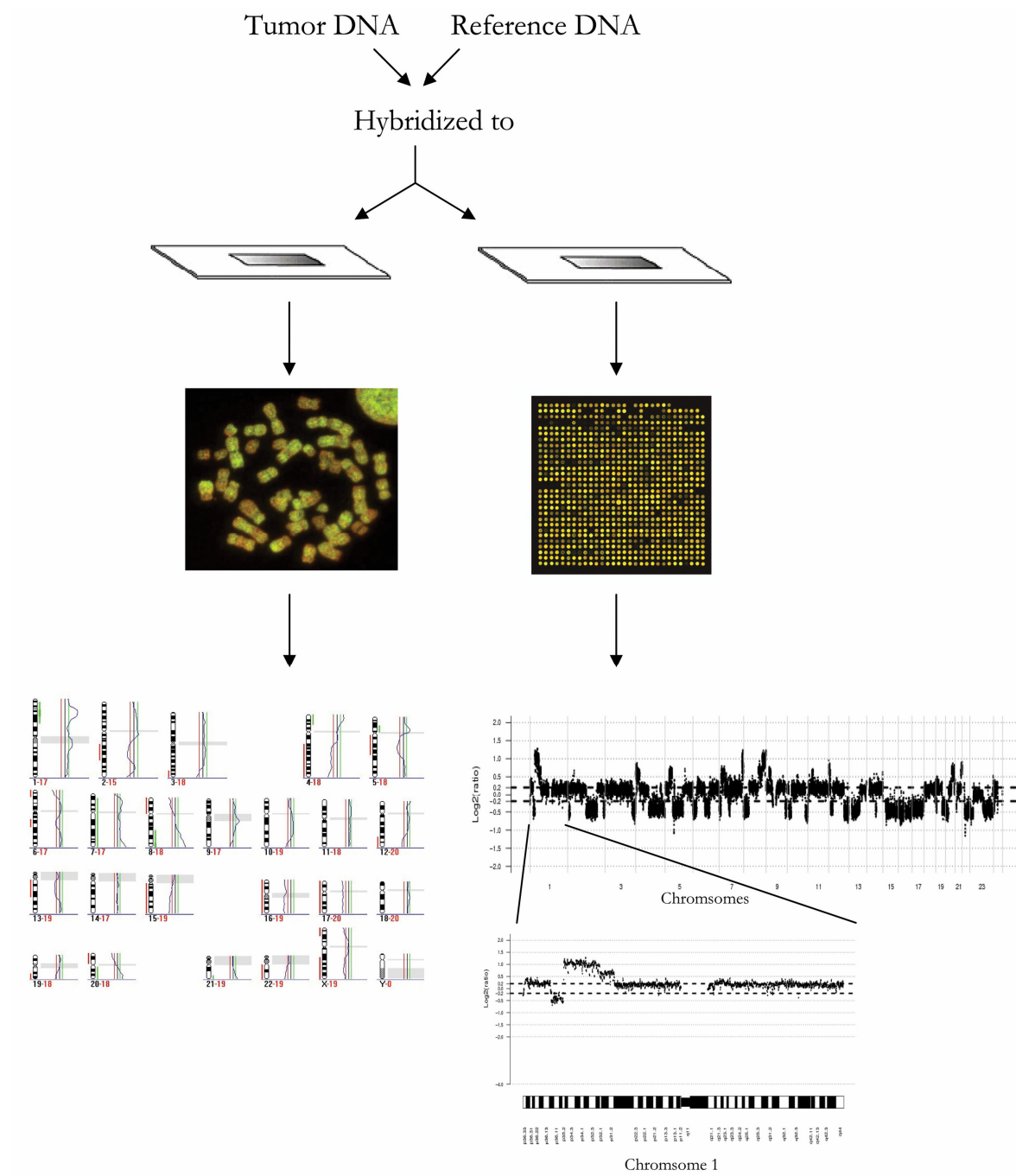


Figure 4. Schematic view of metaphase CGH to the left, and array CGH to the right. Differentially labeled tumor and reference DNA were co-hybridized to glass slides that were coated with metaphase chromosomes or BAC clones, respectively.

CGH statistics

To identify gains and losses that differed significantly in frequency between the tumor groups investigated, two-tailed Fisher's exact test was performed in all four papers. Gains were tested against no gain and losses were tested against no loss. Array CGH generates a vast amount of data and multiple testing is performed, which should always be considered when performing statistical analyses. However, array CGH data has the particular characteristics of being segmented and exhibit a physical dependency in the genome, compared to expression array data for example. This increases the power to detect true significant associations without increasing the false discovery rate [73].

Survival curves were prepared using the Kaplan-Meier method in the SPSS software, version 16 (Superior Software System, SPSS for Windows, Chicago, IL, USA) and *P*-values for the difference between the curves were calculated using the Breslow-Wilcoxon test [74].

Quantitative Real-Time Polymerase Chain Reaction (QPCR)

PCR is a technique for amplifying specific regions of DNA present in a tissue or cells. QPCR amplifies and simultaneously quantifies DNA or RNA sequences semi-quantitatively. Thus, one can detect and quantify the expression of a specific gene of interest. Gene specific primers and light emitting probes are used to amplify and detect the product. In paper III, total RNA was isolated from all 40 tumors. Unfortunately, high-quality RNA was only achievable from 17 of the samples. QPCR was performed as previously described by Partheen *et al.* [75]. Each tumor sample was reverse transcribed from RNA to cDNA in duplicate, and was subsequently analysed in triplicate by real-time PCR. Reference genes *GAPDH* and β -actin were used; both have previously been shown to be stably expressed in ovarian tumor material [75]. When analyzing the QPCR data, samples were grouped according to the corresponding CNA that exhibited significance in the array CGH analysis (*EVII1*, *MDS1*, *SH3GL2*, *SH3KBP1*) or according to chemotherapy response (*ABCB1*). A Student's t-test was performed between the groups.

RESULTS AND DISCUSSION

Carboplatin

In papers I and II we analyzed a primary tumor material from patients treated with single-agent carboplatin. Sixty-three early-stage (I-II) tumors of varying histology were analyzed in paper I. Forty-six were clinically sensitive, and 17 were clinically resistant (10 primary and 7 secondary). In paper II, a subset of 32 of the 63 tumors was further analyzed; 20 clinically sensitive and twelve clinically resistant (6 primary and 6 secondary).

Characterization of chromosomal alterations (Paper I)

The most frequently detected alterations among the clinically resistant cases were gains in 1q, 8q22-qter and 13q21-32, and losses in 8p. When separating the resistant cases into primary and secondary resistant and comparing these to the sensitive, certain cytogenetic regions exhibited significance: gains in 5q14-23 and 13q21-32, and losses in 9q were most frequent in primary resistant cases, and gains in 1q were most frequent in secondary resistant cases (Table 2). Prior metaphase CGH studies of chemotherapy response in ovarian tumor materials are few. Kudoh and colleagues associated gains in 1q21-22 and 13q12-14 with resistance to cisplatin-based therapy in advanced stage ovarian tumors, similar to our results [52]. In agreement with our 5q14-23 finding, Yasui *et al.* and Leyland-Jones *et al.* have identified gains in this region in platinum-resistant cell lines [49, 76]. Additionally, metaphase CGH studies of ovarian tumors in relation to survival revealed gains in 1q and 13q22 to be associated with poor survival, which is in agreement with our results [60, 61]. Furthermore, in a previous study of an advanced ovarian patient material treated with carboplatin-based therapy performed by our group, gain in 1q24-qter was identified as significantly more frequent in tumors from patients who died of cancer than among survivors [62]. However, the concordance between the various studies that have investigated chemotherapy response is quite low, as illustrated by the differing results concerning chromosome 13 (see paper I).

Table 2. The distribution of the cytogenetic alterations that differed significantly when comparing the resistant cases to the sensitive. * Statistical significance with Fishers's exact test, when compared to the sensitive cases.

Region (+ gain, - loss)	Primary resistant (%)	Secondary resistant (%)	Total resistant (%)	Sensitive (%)
+ 1q	30	57*	41	17
+ 5q14-23	40*	0	24	11
- 9q	50*	14	35	17
+ 13q21-32	60*	14	41	20

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High-resolution analysis specifies genetic alterations (Paper II)

With the anticipation to reduce the large cytogenetic regions given by metaphase CGH, we analyzed a subset of the previously studied tumors with the novel high-resolution technique array CGH. The alterations detected with array CGH were still rather large, and the overall copy number alteration (CNA) patterns exhibited a good correlation between the two CGH techniques. Thus, strengthening the results found, although making the search for genes of importance for chemotherapy response more difficult due to the large CNAs.

Unfortunately, we were not able to distinguish between primary and secondary resistant tumors when studying the alteration patterns because of the small group sizes (6 primary and 6 secondary). Nonetheless, when comparing resistant and sensitive cases gains in 1q exhibited significance in this investigation too (Table 3), and the region was narrowed down to 1q25.1-41 with two smallest region of overlap (SRO) at 1q25.2 and 1q32.2 (Figure 5). When scrutinizing the 1q CNAs, the alterations were equally distributed in primary- and secondary-resistant tumors. In an array CGH study by Bernardini and colleagues, gain in 1q42-44 was found overrepresented in resistant tumors from ovarian cancer patients treated with carboplatin-based chemotherapy, similar to our results [53].

A number of alterations were found to be significantly associated with sensitivity, which they were not in the previous metaphase CGH analysis (Table 3). This discrepancy might be due to the smaller number of tumors investigated with array CGH and the methodological differences such as the superior resolution of array CGH. In addition, tumor DNA was extracted from two different (although in close proximity) pieces from the tumors.

Table 3. The regions that exhibited significance when comparing resistant versus sensitive cases with Fishers's exact test. The distribution between the groups and the size of the regions are also shown.

	Region	Resistant (%)	Sensitive (%)	Size (Mbp)
Gain	1q25.1	50	10	1.43
	1q25.2	58	15	0.69
	1q25.3	50	10	0.48
	1q31.3-1q32.1	50	10	2.80
	1q32.1-1q41	50- 58	5-10	10.99
Loss	15q14	0	35	0.62
	15q21.1	0	35	2.72
	15q21.2-15q21.3	0	40	2.57
	15q21.3	0	40	2.31
	15q22.2	0	40-45	4.22
	15q26.3	0	35	3.88
	17q24.1	0	40	0.32
	Xq21.33-q22.1	0	35	3.28

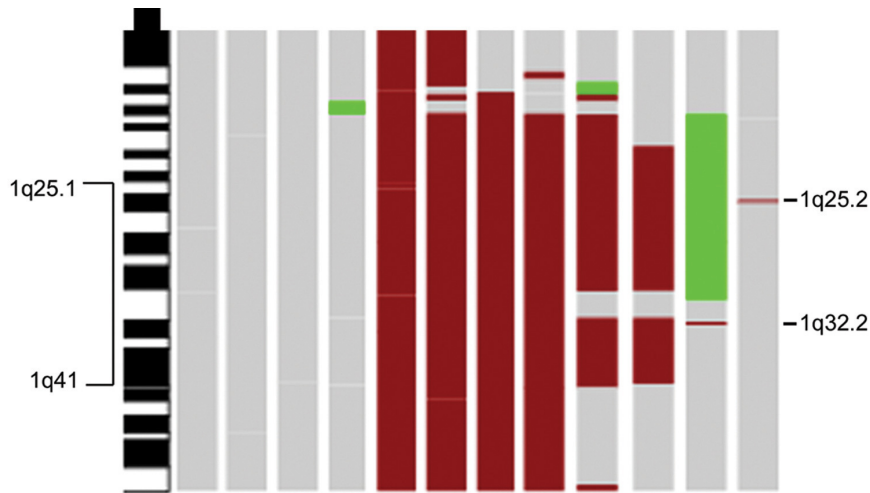


Figure 5. Alterations in chromosome arm 1q in resistant cases. Gains in 1q25.1-41 were significantly more frequent in the resistant versus sensitive samples. The two smallest regions of overlap are shown at 1q25.2 and 1q32.2. Gains are shown in red and losses in green.

Concurrence (Paper I and II)

In both studies, the frequency of altered genome was highest in the primary resistant tumors, and lower in the sensitive tumors. This is in concordance with other studies [52, 53], and probably reflects the higher aggressiveness of these tumors as well as their chemotherapy resistance. Intriguingly, the secondary resistant cases exhibit the lowest frequency of altered genome in both studies. This might partly reflect the fact that these tumors initially did respond to chemotherapy, which is the tumor tissue from which we extracted the DNA. The patient, however, relapsed and then was considered clinically resistant. Whether an undetectable sub clone of the primary tumor causes the relapse or if it is a new event is a matter of speculation. Nevertheless, that a more aggressive, possibly resistant, sub clone survives and proliferates is not unlikely. Such a model has been proposed by respected researchers in the field in excellent reviews [37, 47].

Gains in chromosome arm 1q, and specifically 1q25.1-41, emerged in both CGH investigations of early-stage ovarian tumors from carboplatin treated patients, indicating that the alteration is of interest for ovarian cancer and possibly for carboplatin resistance. The alteration is detected in high frequency in both primary and secondary resistant tumors, of which the patients all died of their disease. Such a finding can be interpreted as a possible driver of primary resistance and a predisposition for secondary resistance. It can also be interpreted to be important for tumor aggressiveness and relapse, features that might be hard to distinguish from resistance. Gains in 1q have been associated with resistance as well as poor survival in ovarian tumors [52, 53, 62]. Kudoh *et al.* detected 1q21-22 and Bernardini *et al.* detected 1q42-44 as potential indicators of resistance to platinum-based therapy, which differs slightly in location from our results [52, 53]. However, these findings altogether invigorate the importance of the 1q region in ovarian

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tumors and imply further evaluation of the region as a possible predictive marker of chemotherapy response.

The complexity of the results obtained emphasizes the difficulties when studying tumors, and also stresses the importance of studying both the primary and the relapse tumor, which is much more difficult to acquire since second surgery is unusual. The CNA pattern associated with chemotherapy response that becomes visible through our and others' reports seems complex. This might partly be explained by the use of varying cell lines in several studies, heterogeneity in tumor materials, small sample sizes and the use of different methodologies.

Histology (Paper I and II)

Serous tumors exhibited a higher frequency of altered genome than the other subtypes in these CGH studies. This is in concurrence to previous reports, and to the lower survival of the serous subtype overall [11, 18, 58]. Further, mucinous tumors displayed the lowest frequency of altered genome in the investigated early-stage tumors, which corresponds to the enhanced survival of the mucinous subtype in early stages.

A number of CNAs differed between the three histologic subtypes investigated. The most prominent findings were gains in 8q associated with serous and clear cell tumors, and the loss in 17q (specified to 17q11.2-12) associated with only the serous subtype. The gains in 8q with the identified SRO at 8q24.22-24.23 were specifically associated with serous and clear cell tumors with a very high abundance in these subtypes (91% and 75%, respectively), whereas absent among mucinous tumors. These findings are in concordance with previous studies and emphasize the heterogeneity of epithelial ovarian tumors [77-79], and it suggests that different subtypes might evolve through different tumor progression pathways.

Histology was rather evenly distributed between the chemotherapy response groups; consequently we reason that histology probably did not influence the results concerning response. However, we were unable to investigate chemotherapy response in relation to histology due to the small group sizes. Differential chemotherapy response between the histologic subtypes has been reported; especially clear cell tumors have been shown to display lower response rates [80].

Combination therapy

In paper III and IV we progressed by examining two additional ovarian tumor materials from patients treated with combination therapy paclitaxel/carboplatin and docetaxel/carboplatin respectively. Since the histological subtypes exhibited different genetic alteration patterns in paper I and II, we solely selected the most common subtype serous in the following investigations. In paper III, 40 primary serous stage III ovarian tumors were analyzed. Twenty tumors were from clinically resistant patients and 20 from clinically sensitive patients. In paper IV, 30 primary serous advanced stage ovarian tumors were analyzed. Six tumors were from clinically resistant patients and 24 from clinically sensitive patients.

Paclitaxel/Carboplatin (Paper III)

In this investigation, gains in 3q26.2 and losses in the regions 6q11.2-12, 9p22.3-21.3 and Xp22.2-11.1 were found significantly more frequent in the resistant tumors than in the sensitive tumors (Table 4).

Table 4. The regions that exhibited significance when comparing resistant versus sensitive cases with Fishers's exact test. The distribution between the groups and the size of the regions are also shown.

	Region	Resistant (%)	Sensitive (%)	Size (Mbp)
Gain	3q26.2	65	20	0.15
Loss	6q11.2-12	40	0	4.85
	9p22.3	45	5	1.05
	9p22.2-22.1	45	5	0.8
	9p22.1-21.3	45	5	1.15
	Xp22.2-22.12	60-65	15	5.5
	Xp22.11-11.3	60-70	15-20	22.85
	Xp11.23-11.1	60-65	10-15	9

The significant region at 3q26.2 is also the SRO among the resistant cases, and a number of samples exhibited gain peaks only in this small region or in the close proximity, highlighting the significance of the region (Figure 6). Gain in 3q26.2 has been found in high frequency in ovarian tumor materials [55, 81] as well as in other solid tumors [82, 83]. The presence of the two genes *MDS1* and specifically *EVII* in the region makes it further interesting since *EVII* is an oncogene and has been associated with paclitaxel resistance [84]. Additionally, a significant correlation between gene copy number and *EVII* gene expression has been reported [85]. When exploring the *EVII* gene expression levels in a subset of the tumor material, we detected a difference between the samples with gain versus those without gain; the group of tumors that previously displayed gain in

RESULTS AND DISCUSSION

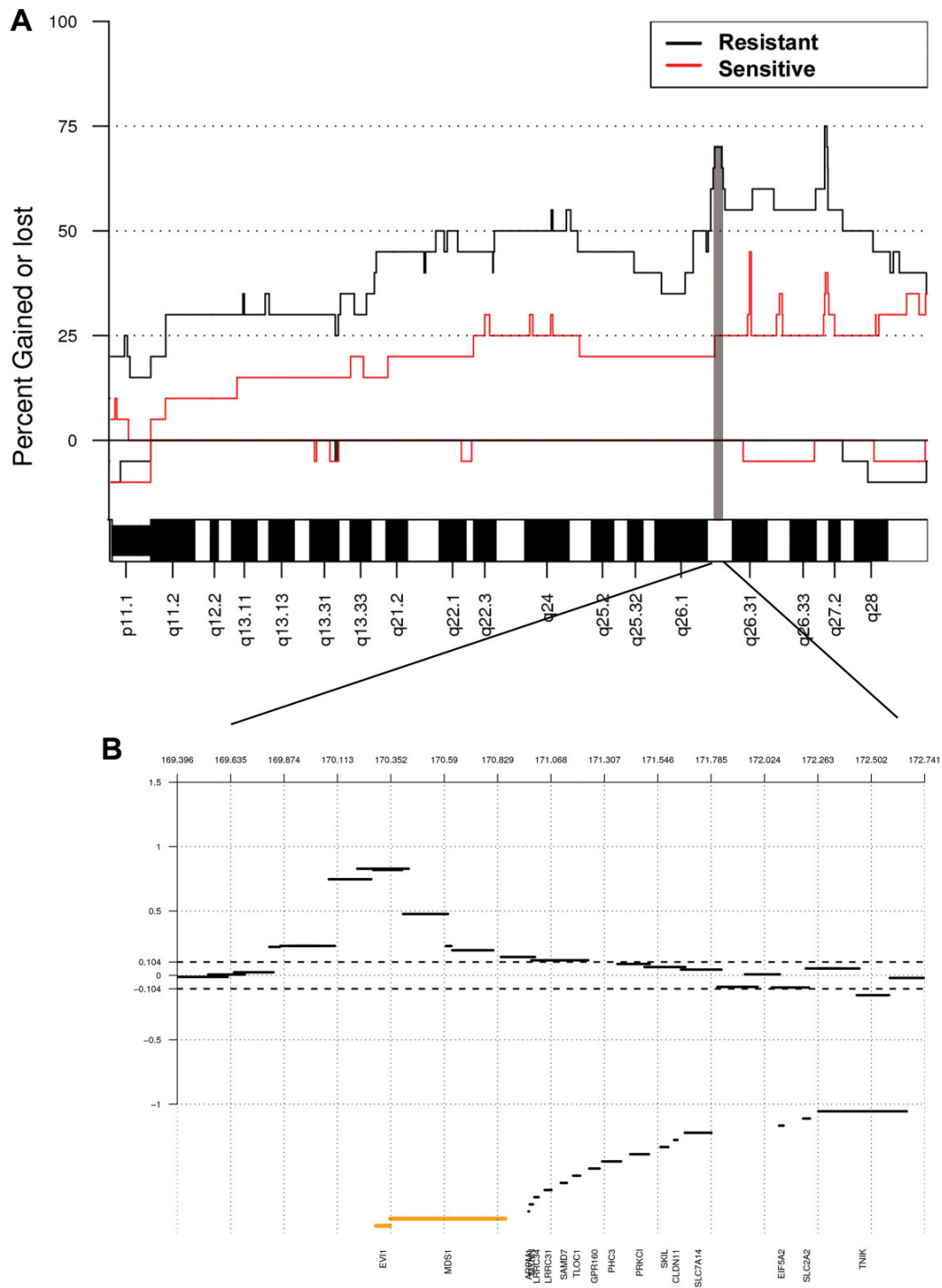


Figure 6. A) Frequency plot of chromosome arm 3q with the significant region at 3q26.2 highlighted in grey. Black line represents resistant cases and red line sensitive cases. B) Example of one case exhibiting a gain peak specifically in the significant region in 3q26.2. BAC clone segments are matched to their size, and all genes in the region are displayed with the ones in the significant region highlighted in yellow.

the region were found to have higher average relative gene expression than the group without gain. Thus, the region 3q26.2 seems to be of significance for ovarian cancer and possibly chemotherapy response and patient outcome; however, further studies are required to elucidate its true role.

The mRNA expression of the genes in the significant DNA regions investigated in the study (*EVII*, *MDS1*, *SH3GL2*, *SH3KBP1*) exhibited only weak DNA copy number dependence. This illustrates the general value of molecular profiling at both DNA and RNA levels when studying cancer mechanisms. Concerning our particular study, we unfortunately obtained high quality RNA from only 17 of the 40 tumors, which might influence the results. Nevertheless, finding a genetic profile with reliable predictive potential might be very useful irrespective of the effect on gene- and protein expression.

Based on the significant regions generated by array CGH a decision tree was build for classifying samples as resistant or sensitive (Figure 7). The best combination of classifiers was the regions 6q11.2-12, Xp11.3 and Xp22.13; the tree classified 90% of the cases correct and showed an accuracy of 78% in the cross-validation. As the decision tree had a rather high accuracy on our tumor material we wanted to evaluate its potential in other ovarian tumor materials. We tested the tree on another published tumor material, analyzed with metaphase CGH, which had corresponding stage (III) and histology (serous) to our material, but a different combination treatment (carboplatin, farmorubicine and cyclophosphamide) and survival as end point [62]. The tree classified samples at a lower level (61%) in this material. However, when scrutinizing the tree it classified samples exhibiting alterations in the regions correct in a rather high frequency (88% and 82%), whereas samples without alterations in the regions were inferiorly classified (37%). This suggests the significance of alterations in the specified regions for the outcome of patients with stage III serous carcinomas, and that tumors without alterations in these regions need further characterization. Losses in the X chromosome has been found in cisplatin-resistant cell lines [48, 54]. However, the X chromosome has not been explored to the same extent as the rest of the genome due to the use of male reference in several studies.

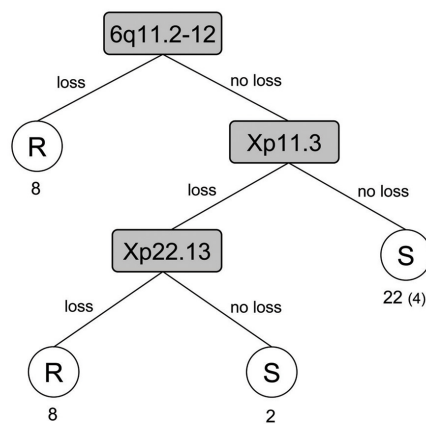


Figure 7. A decision tree based on the significant regions that was generated by array CGH. The regions 6q11.2-12, Xp11.3 and Xp22.13 classified 90% of the tumors correctly. Numbers beneath the circles are the number of cases classified in each group. Numbers in brackets are incorrectly classified cases if any. R=resistant, S=sensitive.

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Taken together, the genetic alterations detected and associated with chemotherapy resistance in this investigation might be possible candidates for predictive makers of chemotherapy response or patient outcome in stage III serous ovarian carcinoma.

Docetaxel/Carboplatin (Paper IV)

In order to explore genetic alterations in relation to docetaxel/carboplatin as first-line treatment of ovarian cancer patients, we performed the same methodological and statistical analysis on 30 tumors from patients treated with docetaxel/carboplatin as we did in paper III. Losses in 8p23.3-23.1 and 8p22 were significantly associated with sensitivity, and gains in six regions in chromosome 9 (9p13.2-13.1, 9q21.2-21.32, 9q21.33, 9q22.2-22.31, 9q22.32-22.33 and 9q33.1-34.11) were significantly associated with resistance (Table 5; Figures 8A and 9A). Loss in 8p is a recurrent aberration found in many solid tumors including ovarian [19, 53, 55, 86-88], and it has been proposed to harbor tumor suppressor genes. Loss in 8p21.1 has been suggested as a predictive marker of chemoresistant disease in ovarian cancer patients [55].

Table 5. The regions that exhibited significance when comparing resistant versus sensitive cases with Fishers's exact test. The distribution between the groups and the size of the regions are also shown.

	Region	Resistant (%)	Sensitive (%)	Size (Mbp)
Loss	8p23.3-23.1	0	67-75	9.45
	8p22	0	67-71	1.5
Gain	9p13.2-13.1	83	13-21	2.1
	9q21.2-21.32	50	0	5.7
	9q21.33	50	0	3.35
	9q22.2-22.31	50	0	1.35
	9q22.32-22.33	50	0	5.25
	9q33.1-34.11	50	0	10.25

Intriguingly, the current investigation identifies a different set of genetic alterations associated with chemotherapy response than the investigation of paclitaxel/carboplatin in paper III, as illustrated in figures 8 and 9. The implication of this discrepancy is intricate to interpret. It might suggest a differential genetic profile behind response to paclitaxel/carboplatin and docetaxel/carboplatin, respectively. If so, such a finding is of great interest and might lead to the establishment of separate predictive markers for the two combination treatments, and would help to individualize therapy of ovarian cancer patients and make docetaxel an option to paclitaxel in first-line therapy. However, this is a small pilot study with short follow-up, a small material and uneven group sizes, which might effect the results and should be taken into consideration. Further studies in independent tumor series or cell lines are required to evaluate the validity of these findings.

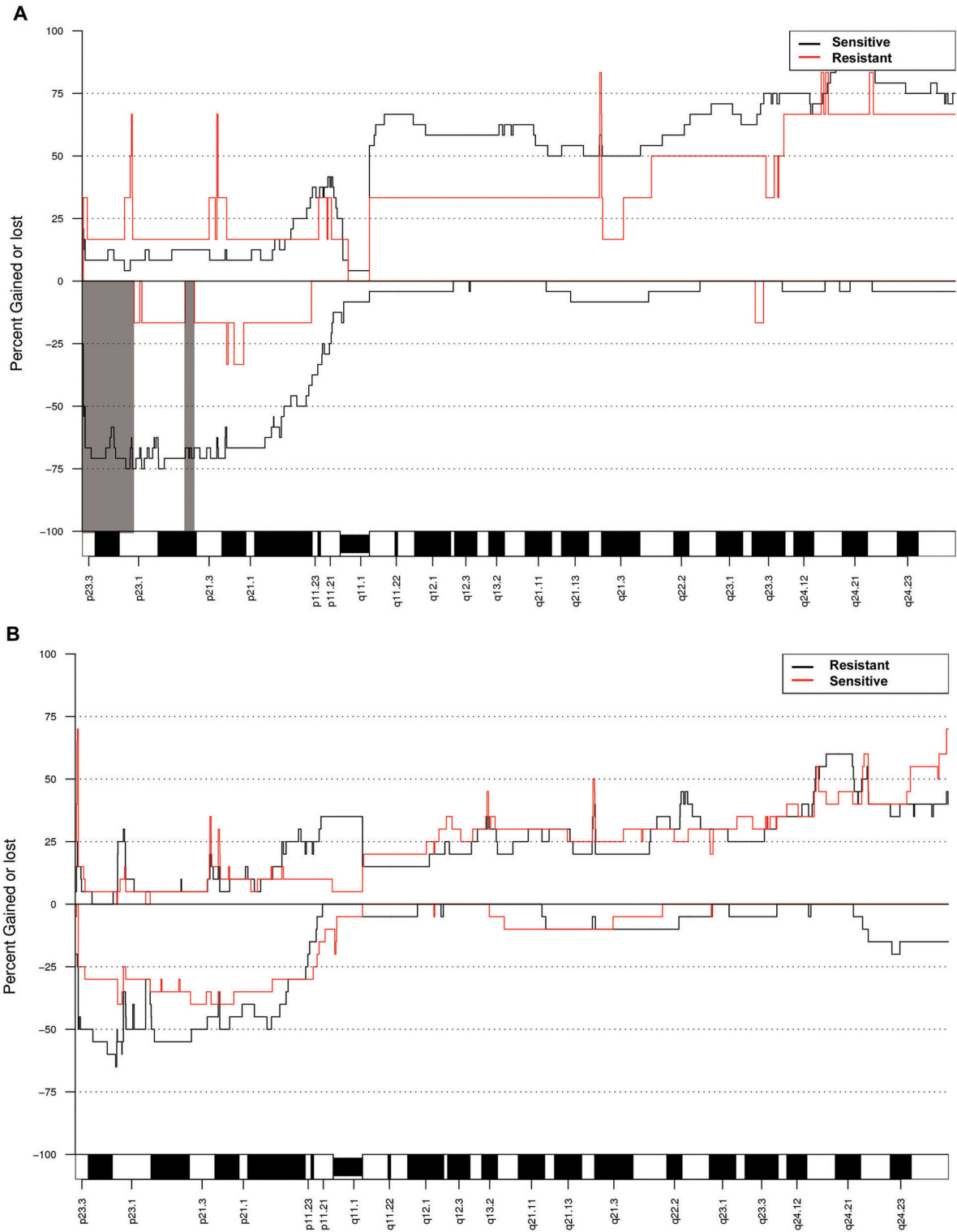


Figure 8. Frequency plots of chromosome 8. A) shows the material from paper IV; resistant (red line) versus sensitive (black line). B) shows the material from paper III; resistant (black line) versus sensitive (red line). Significant regions in the respective study are highlighted in grey. Observe the opposite colorings for resistant and sensitive cases in A and B.

RESULTS AND DISCUSSION

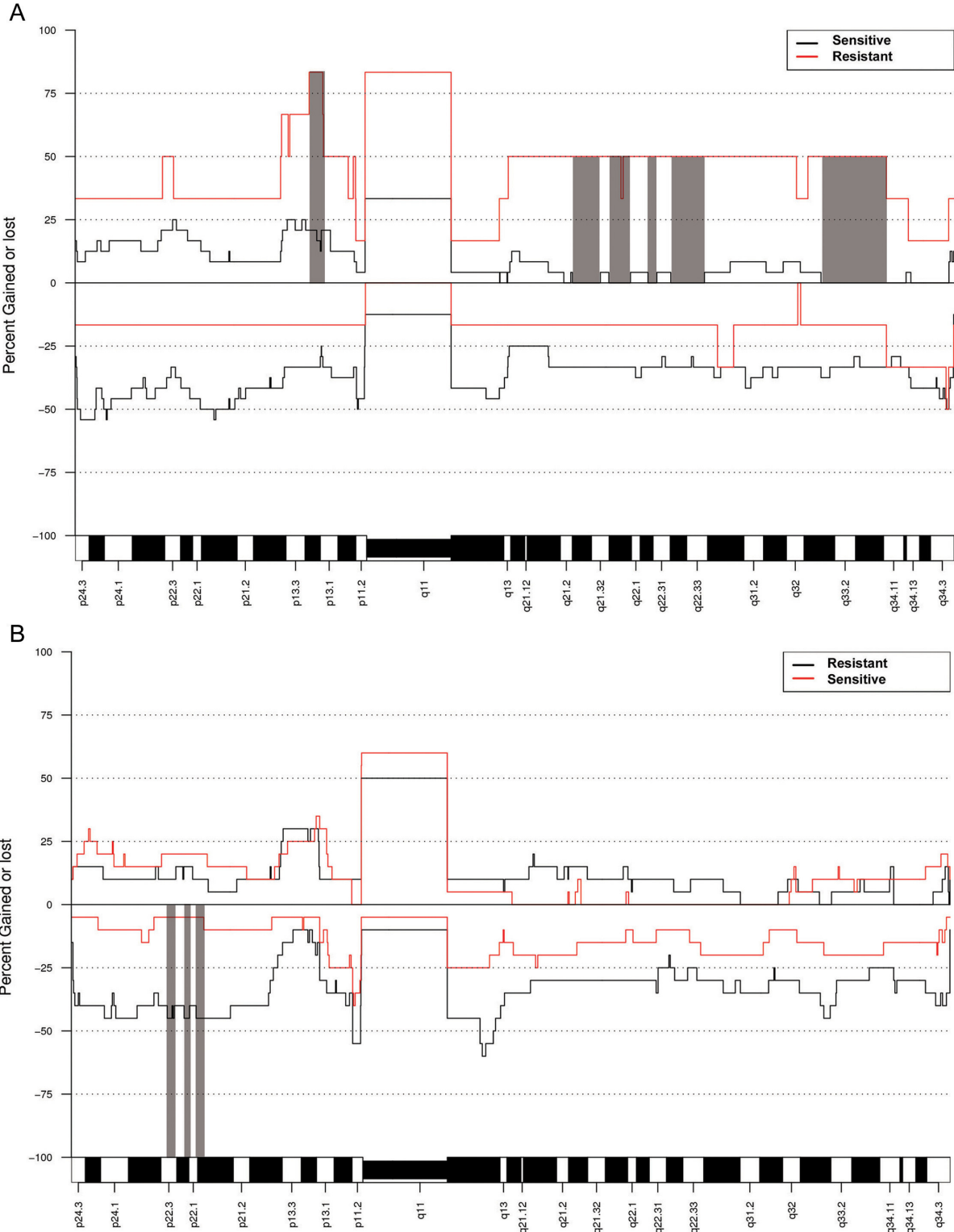


Figure 9. Frequency plots of chromosome 9. A) shows the material from paper IV; resistant (red line) versus sensitive (black line). B) shows the material from paper III; resistant (black line) versus sensitive (red line). Significant regions in the respective study are highlighted in grey. Observe the opposite colorings for resistant and sensitive cases in A and B.

Overall concordance

The overall frequency of CNAs in the three tumor materials investigated with CGH in this thesis exhibit many similarities and some differences (Figure 10). The comparison is of interest since paper I and II contained early-stage tumors with the majority being stage I, paper III contained only stage III tumors, and paper IV contained mostly stage III but also some stage IV tumors. In addition, the paper I and II material had a mixed histology, whereas the later papers had solely serous tumors.

The frequency of altered genome per tumor was on average: 25% in the early-stage material (paper II), 32% in the stage III material (paper III), and 46% in the stage III and IV material (paper IV). The increased frequency of altered genome with increased stage would be expected when considering the nature of tumors. The difference between paper III and IV would therefore be proposed to be due to the inclusion of stage IV tumors in paper IV. However, when scrutinizing the material in paper IV, stage III tumors exhibit 46% and stage IV tumors exhibit 42% on average. Thus, the explanation to the higher frequency of altered genome in paper IV is probably due to other factors.

In the early-stage material, gains in 8q and specifically 8q24.22-24.23, were associated with the serous subtype as discussed above. Gains in 8q were also frequent in the advanced stage serous tumors, especially the 8q24 region, thus strengthening this association. Alterations found recurrently in both the early-stage tumors and the advanced stage tumors, such as gains in 1q, 3q, 8q, 20q and losses in 8p and 17p (Figure 10), might be early events in ovarian tumorigenesis. These regions harbor several genes proposed to be involved in ovarian tumor development; for example the *TP53* gene at 17p13.1, the *PIK3CA* gene at 3q26.32 and the *C-MYC* gene at 8q24.1. Differences between the early-stage tumors and advanced stage tumors concerning overall CNAs were losses in 4q and in the X-chromosome (Figure 10). These alterations were more recurrent in the advanced stage tumors, which is in concordance with other reports on advanced stage serous ovarian tumors [53, 55, 62, 89] and might consequently be late events in ovarian tumorigenesis and contribute to aggressiveness. Altogether, the overall genomic alteration patterns identified in our analyses concur well with previous reports on ovarian tumor materials using similar methodologies [63].

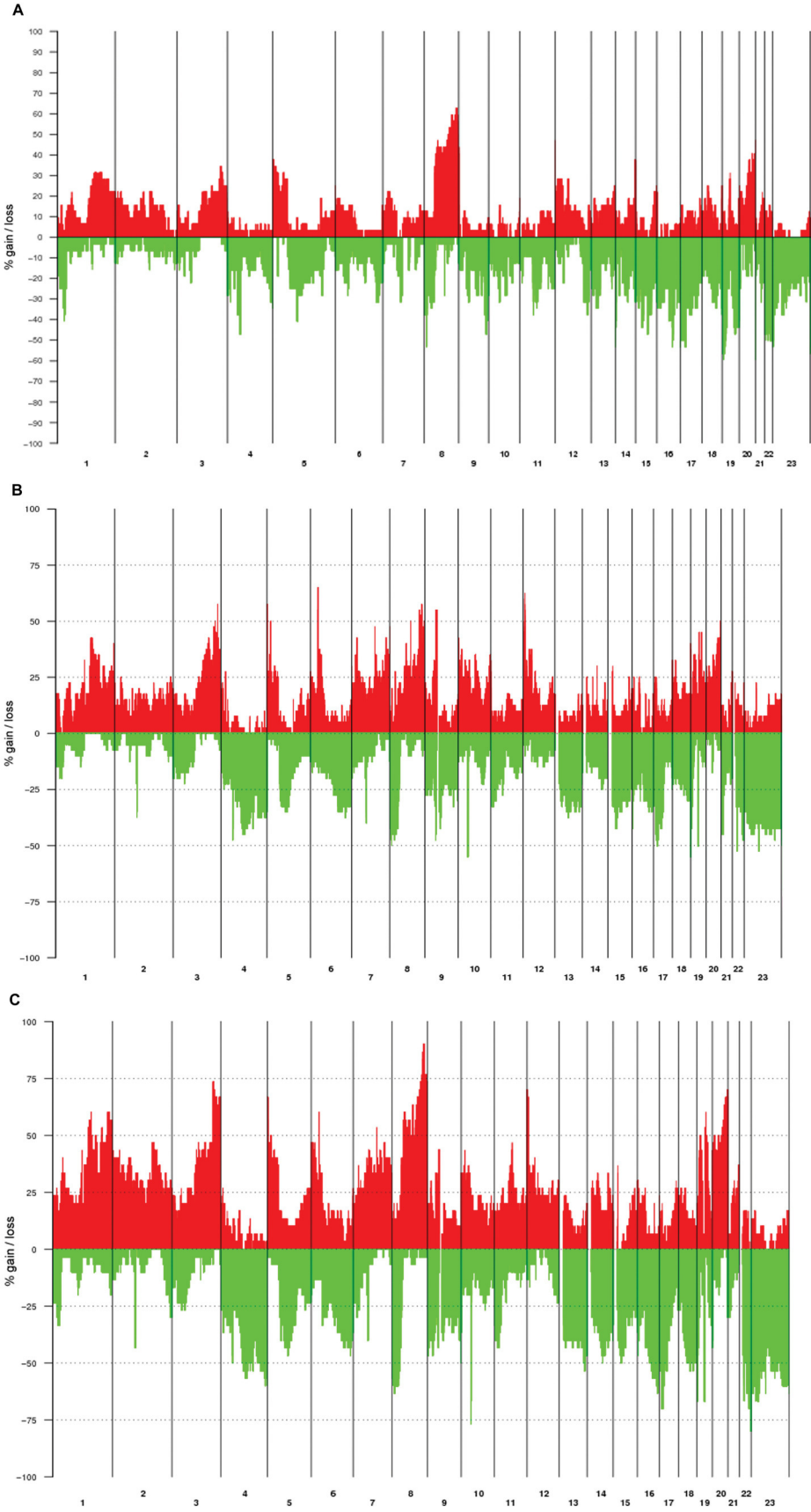


Figure 10. Frequency plots of the total tumor materials in A) paper II with 32 tumors, B) paper III with 40 tumors, and C) paper IV with 30 tumors.

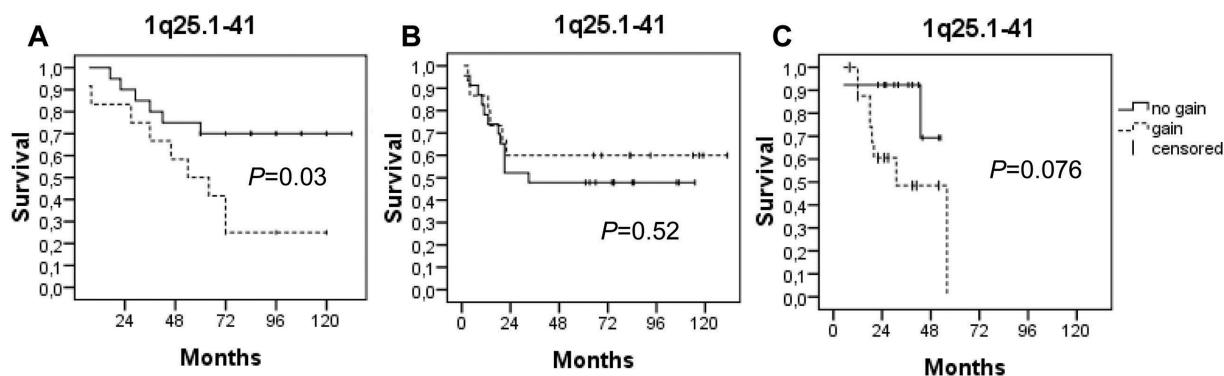


Figure 11. Survival curves of gain in 1q25.1-41 in the three tumor materials: A) the carboplatin material (paper II), B) the paclitaxel/carboplatin material (paper III), C) the docetaxel/carboplatin material (paper IV).

Gain in 1q, and specifically 1q25.1-41, was frequently detected in the early-stage carboplatin material and differed significantly between resistant and sensitive tumors. The alteration is also frequently detected in the advanced stage tumor materials (Figure 10), but did not differ between the response groups as additionally illustrated by the survival curves in figure 11. This might suggest gain in 1q to be a potential predictor of carboplatin response in early-stage tumors but not in advanced stage tumors.

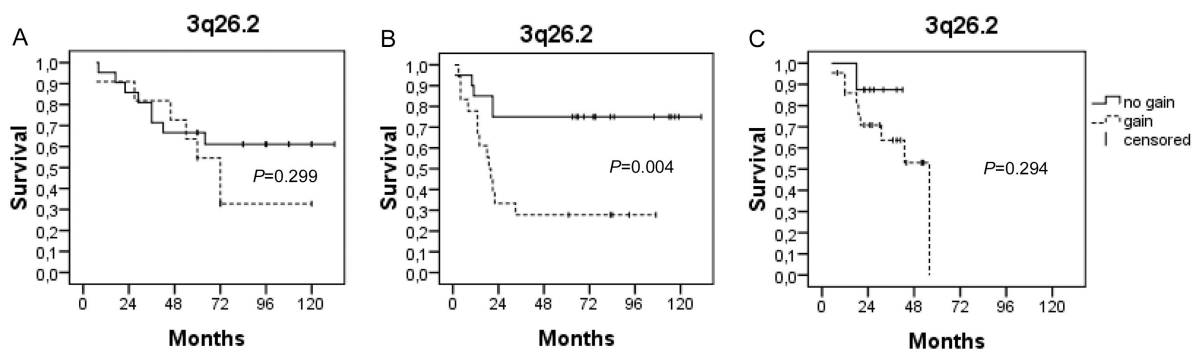


Figure 12. Survival curves of gain in 3q26.2 in the three tumor materials: A) the carboplatin material (paper II), B) the paclitaxel/carboplatin material (paper III), C) the docetaxel/carboplatin material (paper IV).

Gain in 3q26.2 exhibited significance in paper III and the CNA was associated with resistance to paclitaxel/carboplatin. Gain in 3q26.2 was also frequently found (73%) in paper IV, however with no difference between the response groups. Though, follow-up time in this material is short and group sizes uneven, which adds an uncertainty. In the paper II material, gain in 3q26.2 was less frequent (34%) and did not differ significantly between the response groups. The impact of gains in 3q26.2 on survival in the investigated tumor materials is illustrated in figure 12. Taken together, gain in 3q26.2 is a recurrent alteration in ovarian cancer as detected by others [55, 81], and is here associated with resistance to paclitaxel/carboplatin therapy in stage III serous carcinomas.

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CNAs have been examined by CGH in ovarian cancer in a large number of studies [63] and several have focused on chemotherapy resistance using metaphase CGH or array CGH [48, 51-55]. Various CNAs have been suggested as predictive markers in these studies, unfortunately with low concordance. Both cell lines and tumor tissue have been studied, and the variations in the findings might be explained by the use of various cell lines, heterogeneous tumor materials, different resistance classifications, and different CGH platforms. Additionally, different chemotherapy drugs and treatment regimens have been studied which also might influence the results. In table 6A, published CGH studies on ovarian tumor materials with chemotherapy response as endpoint are compiled to illustrate the variations in CNAs detected. An overview of the different CNAs that exhibited significance in papers I-IV is additionally shown in figure 6B. None of the significant regions overlap between the three materials in this thesis, except for loss of 9q that was associated with resistance in paper I, whereas gains in 9q21.2-34.11 was associated with resistance in paper IV. The difference might be due to that the tumor materials in paper I and IV differ in stage, histology, treatment and array CGH platform.

Table 6. A) Published reports that examined ovarian tumor materials with CGH and with chemotherapy response as endpoint. B) Comparison of the genetic alterations that exhibited statistical significance in the four papers.

CNAs associated with resistance	CNAs associated with sensitivity	Chemotherapy	No. of tumors	Stage	Histology	Technique	Study
A							
+1q21-22, +13q12-14		cisplatin/doxorubicin/ cyclophosphamide	28	IC-IV	Mixed	mCGH	Kudoh <i>et al.</i> 1999
-1p26.33, -1p36.13, +1q42-44, -6p22.1-21.2, -7q32.1-34, +9q33.3-34.3, +11p15.2, +13q12.2-13.1, +13q21.31, +17q24.2-25.3, -18q12.2, -21q21.2-21.3		carboplatin/paclitaxel	26	IIC-IIIC	Serous	aCGH	Bernardini <i>et al.</i> 2005
-4q34.2, -4q35.2, +5p15.33, -6q15, -8p21.1, -8p21.2, -11p15.5, -13q14.13, -13q14.2, -13q32.1, -13q34, +14q11.2, -16q22.2, -17p11.2, -17p12, -22q12.3		platinum-based combination	17	IIIC	Serous	aCGH	Kim <i>et al.</i> 2007
B							
+1q, +5q14-23, -9q, +13q21-32		carboplatin	63	I-IIA	Mixed	mCGH	Paper I
+1q25.1-41	-15q14-26.3, -17q24.1, -Xq21.33-22.1	carboplatin	32	I-IIA	Mixed	aCGH	Paper II
+3q26.2, -6q11.2-12, -9p22.3-21.3, -Xp21.33-22.1		paclitaxel/carboplatin	40	III	Serous	aCGH	Paper III
+9p13.2-13.1, 9q21.2-34.11	-8p23.3-23.1, -8p22	docetaxel/carboplatin	30	IIC-IV	Serous	aCGH	Paper IV

In this research project, we begun by examining chemotherapy response to single-agent carboplatin and subsequently progressed to examining combination therapy paclitaxel/carboplatin and docetaxel/carboplatin. The intention behind this was to be able to detect possible differences, if they exist, between chemotherapy resistance to the different treatment regimens. The genomic alterations that exhibited statistical significance when comparing resistant to sensitive cases in the three tumor materials respectively differed (Table 6B). Whether this is a manifestation of the different therapies given or the influence of some other factors is obviously hard to conclude, and need further studies to be elucidated. It might suggest that the different chemotherapy regimens may have different predictive markers. Such predictive markers would be very useful in the clinic and help individualize the treatment of the patients. However, since all patients with chemotherapy-resistant disease die, it is difficult to separate molecular markers significant for poor survival and tumor aggressiveness from markers of resistance. Still, finding molecular biologic differences between tumors from patients that have different outcomes but the same clinical features is of great importance. Identifying a high-risk group of women could lead to a special surveillance of these patients and a different treatment regiment.

In all investigations in this thesis we examined chemo-naïve primary tumors. The rationale for this was that in the clinic at diagnosis and therapy, the primary tumor tissue is available for analysis. Thus, identifying predictive markers in the primary tumor would be a useful tool in the clinical situation. In addition, we selected mainly intrinsically resistant tumors that exhibited resistance already at primary chemotherapy and compared them to sensitive tumors from patients with more than five-year survival. This was done with the intention to refine the analysis and increase the probability to find genetic alterations of importance for chemotherapy response. Nevertheless, as described in the introduction, most ovarian cancer patients initially respond to chemotherapy, and the majority subsequently relapses and then exhibit resistance. Thus, most ovarian cancer patients are not obviously intrinsically resistant, but probably acquire resistance during or after therapy. The ultimate way to study chemotherapy resistance in ovarian cancer would therefore be to analyze both the primary tumor and the relapse in order to elucidate the molecular changes responsible for the acquired resistance. Secondary surgery, however, is rarely performed which renders this strategy difficult. Another approach would be to collect cells from ascites drained from the patient, which is performed regularly and consequently much easier to achieve.

CONCLUDING REMARKS

In this thesis work we detected specific genetic alterations in ovarian tumors that were associated with differential chemotherapy response and patient outcome.

- Gain in 1q, and specifically 1q25.1-41, was significantly associated with carboplatin resistance in early-stage ovarian tumors, but not combination therapy resistance in advanced stage tumors. It is therefore suggested as a potential predictive marker of carboplatin response in early-stage ovarian tumors.
- The gene *EVI1* and its locus 3q26.2 are probably of importance for ovarian cancer and possibly for chemotherapy response.
- Losses in regions 6q11.2-12, Xp11.3 and Xp22.13 are a good combination to predict chemotherapy response and clinical outcome in stage III serous ovarian tumors and should be evaluated further.
- Genetic alteration profiles differed between the different treatment regimens investigated. This together with the heterogeneous nature of the disease suggests the future establishment of a range of predictive markers for ovarian cancer patients.

FUTURE PERSPECTIVES

As described in this thesis, ovarian cancer is a complex disease and chemotherapy resistance a complex phenomenon. The mechanisms behind chemotherapy resistance are far from elucidated, and several non-overlapping predictive markers have been suggested.

The research presented in this thesis contributes with a small piece to the gigantic jigsaw puzzle of chemotherapy resistance and ovarian carcinogenesis, and much work remains to be done before overcoming resistance and obtaining the major improvements in survival we greatly demand. The results need further evaluation in independent tumor materials before any reliable conclusions can be drawn about the impact of the genetic alterations detected. Primarily, investigations on DNA should be performed to validate the findings. It would also be interesting to perform gene expression microarray on the tumor samples used in this thesis, in order to search for a gene list with predictive potential. Establishing DNA biomarkers would be a useful tool in the clinical routine. DNA is compared to RNA more stable and easy to handle, and simple and cost-effective tests could be designed in the form of custom made small DNA arrays or the utilization of the well established FISH technique for example.

Future analyses of both primary and relapse tumors from the same patient would be an ideal way to study chemotherapy resistance in ovarian cancer. However, since secondary surgery is rarely performed it is not easily achieved. Nevertheless, many ovarian cancer patients are drained for ascites in a palliative purpose, and obtaining secondary tumor cells that way is an alternative. Additionally, cell lines are always easily accessible and should be studied in parallel and in addition to patient samples.

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