Specific Copy Number Alterations Associated with Docetaxel/Carboplatin Response in Ovarian Carcinomas

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Abstract. Background: The continued high recurrence and mortality rate in ovarian cancer is a significant problem and the major obstacle in the treatment of ovarian cancer patients is chemotherapy resistance. Thus, finding predictive markers of chemoresistance and elucidating resistance mechanisms is crucial for individualising treatment and improving survival of ovarian cancer patients. Materials and Methods: Using array comparative genomic hybridisation (CGH), this pilot study analysed the tumour genomes of patients treated with docetaxel/carboplatin as first-line chemotherapy (6 resistant versus 24 sensitive cases). This is the first array CGH study of such material. Results: The study identified genetic alterations specific to chemoresistant (gains in 9p13.2-13.1, 9q21.2-21.32, 9q21.33, 9q22.2-22.31, 9q22.32-22.33 and 9q33.1-34.11) and chemosensitive (losses in 8p23.3-23.1 and 8p22) disease. Additionally, when comparing the results to previously analysed tumour material from patients treated with paclitaxel/carboplatin, the two datasets identified different genetic alteration profiles. Conclusion: Specific genetic alterations were identified and associated with chemotherapy response in ovarian cancer. It will be interesting to investigate these exciting data further in larger independent series of ovarian tumours, and hopefully will contribute to the establishment of predictive markers.

Ovarian cancer is the most lethal cancer in the female reproductive tract, with a five-year survival of around 30% in advanced stage disease (1). Most patients initially respond to chemotherapy, but the majority relapse with chemoresistant disease. Current standard first-line chemotherapy of advanced ovarian cancer is a combination of a platinum agent with a taxane, most frequently carboplatin and paclitaxel. However, the high frequency of resistance and low survival among ovarian cancer patients urge the continuing search for modifications and improvements in the treatment regiments. In addition, toxicity and quality of life is of great importance for the patients. Paclitaxel causes severe toxicity, with neurotoxicity being of particular significance. The second-generation taxane docetaxel, on the other hand, has a different tolerability profile with less neurotoxicity but higher neutropenia in ovarian cancer (reviewed in (2)). Docetaxel is used in the management of a number of solid tumours such as breast, lung and prostate cancer (3). The combination docetaxel/carboplatin was compared to paclitaxel/carboplatin in ovarian cancer in the SCOTROC1 study: their efficacy was equivalent, with similar clinical response rates between the arms, and the major difference between the two regimens was the toxicity profile (4). Thus, docetaxel has been suggested as an alternative to paclitaxel in the first-line treatment of ovarian carcinoma (5-7).

Taxanes exert their cytotoxic effect by binding to β-tubulin and stabilising microtubules, which leads to cell cycle arrest and apoptosis (8, 9). However, mechanistic and pharmacological differences exist between docetaxel and paclitaxel. For example, docetaxel has been shown to bind to β-tubulin with greater affinity, and paclitaxel affects cells during G2/M phases whereas docetaxel acts on cells in S/G2/M phases (10-11). Additionally, docetaxel has longer intracellular retention time and the two drugs are metabolised differently with different pharmacokinetics (12-14). Interestingly, it has been suggested that there is not a complete clinical cross-resistance between the two agents in both in vitro (15) and clinical studies (15-18). Docetaxel showed antitumour activity in paclitaxel-resistant breast and ovarian cancer patients (16-18). Such a difference is of great interest to investigate on a molecular level. Specific markers of resistance and/or sensitivity to paclitaxel and docetaxel, respectively, would be very useful in the clinic.
The present study investigated the genomes of advanced ovarian serous carcinomas from patients treated with docetaxel/carboplatin as first-line chemotherapy, aiming to identify genetic alterations associated with differential chemotherapy response. High-resolution, whole-genome array comparative genomic hybridisation (CGH) was used to scan the tumour genomes for alterations that differed significantly between resistant and sensitive cases. This is the first array CGH study of docetaxel treated ovarian cancer patients, and identified certain genetic alterations associated with chemosensitive and chemoresistant disease respectively. Furthermore, the results were compared with a previous array CGH study of paclitaxel/carboplatin treated ovarian cancer patients to examine whether the genetic alteration patterns differed between the two combination treatments (19).

Materials and Methods

Tumour material. Thirty advanced stage epithelial serous papillary adenocarcinomas of the ovary were analysed with array CGH (Table I). The tumours were collected at the time of primary debulking surgery and stored in –80°C until analysis. All patients were, following surgery, uniformly treated with combination chemotherapy docetaxel/carboplatin. 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. Six patients fulfilled these criteria. Patients were defined as clinically sensitive when they had complete remission after first-line chemotherapy (24 patients). However of these 24 patients, 11 experienced early relapse within two years after first-line chemotherapy (but later than six months). This subgroup was considered when analysing the results.

The tumours were classified histologically using standard World Health Organization (WHO) criteria and clinical staging and tumour grading was performed according to the International Federation of Gynecology and Obstetrics (FIGO) standards (Table I). All tumours were assessed by one pathologist, according to regional treatment guidelines for gynaecological malignancies in western Sweden. In addition, specimen imprints for cytological evaluation were performed to verify the presence of tumour cells (stained with May-Grünwald-Giems stain). Malignancy was established when at least 70% of the cells were tumour cells. The tumours investigated were collected from patients diagnosed between 2003 and 2007 at Sahlgrenska University Hospital in Gothenburg. The study was approved by the Research Ethics Committee at Sahlgrenska Academy at University of Gothenburg (reference number S154-02). Median age of the patients at initial diagnosis was 65 years (range 47-78 years), and due to the recent time of treatment, the median follow-up time was 27 months (range 8-56 months) among survivors.

Array CGH. Tiling, whole-genome coverage BAC arrays (38,043 BAC clones) were produced at the SCIBLU Genomics Center, Department of Oncology, Lund University, Sweden (http://www.lth.se/sciblu) as previously described (20). BAC clones were mapped to the hg17 build. Array CGH was performed essentially as previously described (20). Normal female reference DNA containing a mix from 10 healthy individuals was obtained from Promega, Madison, WI, USA. Identification of individual spots on scanned arrays was performed with GenePix Pro software 6.0.1.12 (Axon Instruments, Union City, CA, USA), and the quantified data matrix was loaded into the web-based database Bio Array Software Environment (BASE) (http://base.onk.lu.se) (21).

Data analysis. Background correction of Cy3 and Cy5 intensities was calculated using median-feature and median-local background, generating test over reference log2 ratios. Flagged features were removed and spots that had background-corrected Cy3 or Cy5 intensities <0 or >65000 were removed from further analysis. A signal-to-noise filter of ≥5 for both tumour and reference channels was applied to the data. The filtered data were normalised using the popLowess algorithm (22). After normalisation, a smoothing filter was applied with a moving median sliding window of 250 kbp and with adaptive thresholds (22). The CGH-Plotter software (23), as an R (http://www.r-project.org) implementation in BASE, was used for segmentation. The segmentation constant, c, was set to 8. Copy number alterations were determined by comparing the segmented log2 ratios to gain/loss thresholds obtained by an adaptive scaling method (22), using a window size of 2% and a scaling factor of 2. Segments were accordingly designated as gained, lost or not changed, giving a ternary scale (−1, 0, 1), respectively. Using the values given by CGH-plotter, the frequency of copy number changes per tumour was calculated (alterations defined as ≥3 adjacent clones). To facilitate cross-platform comparison, the segmented data were transformed into a virtual probe set with probes spaced at every 50 kbp throughout the entire genome by associating each probe to its closest virtual probe (24).

Statistical analysis. A two-tailed Fisher’s exact test was used to identify gains or losses that differed significantly in frequency between the sensitive and resistant tumour groups. A cut-off value of $p<0.01$

<p>| Table I. Clinicopathological characteristics of the ovarian tumour material from 30 patients. Distribution of resistant (r) and sensitive (s) cases, respectively, is shown in the third column. |</p>
<table>
<thead>
<tr>
<th>Clinopathological characteristics</th>
<th>No.</th>
<th>r/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy response</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Resistant</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Sensitive</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Deceased</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Survivors</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Stage IIC</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>2/19</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td>FIGO grade</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>1/7</td>
<td></td>
</tr>
<tr>
<td>Moderately</td>
<td>19/15</td>
<td></td>
</tr>
<tr>
<td>Poorly</td>
<td>2/1</td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>n.a., Not available.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
was used to reduce the effect of multiple testing in this large dataset. Furthermore, it has been demonstrated that analysing segmented data greatly increases the power to detect true significant associations without increasing the false discovery rate (25). Gains were tested against no gain, losses were tested against no loss and p-values were calculated for each continuous region instead of for each probe.

Results

Copy number alterations (CNAs) were explored in advanced stage serous ovarian carcinomas using whole-genome high-resolution array CGH. All but one tumour exhibited CNAs detected by array CGH and the rate of alterations was high; the average frequency of altered genome being 46% per tumour. The average size of altered genome per tumour was 1356 Mbp (range 82-2155 Mbp). The vast majority of the tumours exhibited a simplex genome pattern, i.e. rather large low-level alterations. But smaller changes were also numerous and narrow amplifications were present in a number of samples. Low-level copy number alterations dominated and homozygous deletions and high-level amplifications were less abundant. The chromosome arms most frequently altered (>50%) in the ovarian tumour material were +1q, +3q, +8q, +20q, −4q, −8p, −16q, −17p, −22q, −Xpq (Figure 1). The original array CGH data are available in Supplementary file 1 at http://www.oncology.gu.se/Forskning/Publicerade_data/.

Chemotherapy response. When comparing resistant and sensitive samples, some potential marker CNAs with \( p < 0.01 \) were identified (Table II). Loss in 8p23.3-23.1 was found exclusively, and in a high frequency, in the sensitive cases (Figure 2A). The region which is located at the telomere of 8p is 9.45 Mbp in size and contains 96 known genes. Likewise, further down the p arm, loss in 8p22 was also more frequent in the sensitive cases (Figure 2A). When exploring the CNAs in 8p, most losses were rather large and traversed large parts of the p arm. However, the lack of loss among the resistant cases in 8p23.3-23.1 and 8p22 distinguished these two regions. All but two losses were heterozygous.

Further differences between the response groups were found on chromosome 9 (Table II; Figure 3A). Gain in the 2.1 Mbp-sized region 9p13.2-13.1 was more frequent in the resistant tumours. Also, in the q arm, five rather large potential marker regions were found to be gained exclusively in the resistant tumours (9q21.2-21.32, 9q21.33, 9q22.2-22.31, 9q22.32-22.33 and 9q33.1-34.11). The alterations found in 9q were very large gains and extended across all the significant regions and almost the whole arm. The gains in the 9p arm were more heterogeneous, consisted of large alterations as well as smaller gains and 9p13.2-13.1 can be considered as the smallest region of overlap. None of the gains in chromosome 9 were of high amplitude; all were low-level copy number gains.
Figure 2. Frequency plots. A: Frequency plot of chromosome 8 for the 30 ovarian tumours from patients treated with docetaxel/carboplatin in the current study. The black lines represent sensitive cases and the grey lines represent resistant cases. The potential marker regions (p<0.01) are highlighted in grey. B: Frequency plot of chromosome 8 for the 40 ovarian tumours from patients treated with paclitaxel/carboplatin in a previous study (19). The black lines represent resistant cases and grey lines represent sensitive cases. Note the opposite colourings of the lines in figures A and B.

Table II. Potential marker regions. Regions exhibiting p-values below 0.01 when comparing sensitive and resistant cases. The intervals in frequency numbers and p-values are due to CNA gaps inside the significant regions. BAC clones were mapped to the hg17 build.

<table>
<thead>
<tr>
<th>cytoBand (+ gain, – loss)</th>
<th>bp startPos (BAC)</th>
<th>bp endPos (BAC)</th>
<th>Size (Mbp)</th>
<th>Cases</th>
<th>Resistant (%)</th>
<th>Sensitive (%)</th>
<th>p-Value</th>
<th>No. of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>– 8p23.3-23.1</td>
<td>5000001 (RP11-800L13)</td>
<td>9950002 (RP11-797C19)</td>
<td>9.45</td>
<td>0</td>
<td>67-75</td>
<td></td>
<td>0.0016-0.0051</td>
<td>96</td>
</tr>
<tr>
<td>– 8p22</td>
<td>172500001 (RP11-781O9)</td>
<td>187500002 (RP11-724O9)</td>
<td>1.5</td>
<td>0</td>
<td>67-71</td>
<td></td>
<td>0.0029-0.0051</td>
<td>13</td>
</tr>
<tr>
<td>+ 9p13.2-13.1</td>
<td>368500001 (RP11-101P22)</td>
<td>389500002 (RP11-475B17)</td>
<td>2.1</td>
<td>83</td>
<td>13-21</td>
<td></td>
<td>0.0021-0.0088</td>
<td>24</td>
</tr>
<tr>
<td>+ 9q21.2-21.32</td>
<td>769500001 (RP11-637O13)</td>
<td>826500002 (RP11-796E13)</td>
<td>5.7</td>
<td>50</td>
<td>0</td>
<td></td>
<td>0.0049</td>
<td>20</td>
</tr>
<tr>
<td>+ 9q21.33</td>
<td>8405000001 (RP11-359O6)</td>
<td>874000002 (RP11-107G16)</td>
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<td>50</td>
<td>0</td>
<td></td>
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<td>18</td>
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<tr>
<td>+ 9q22.2-22.31</td>
<td>902000001 (RP11-342P9)</td>
<td>915500002 (RP11-4403)</td>
<td>1.35</td>
<td>50</td>
<td>0</td>
<td></td>
<td>0.0049</td>
<td>8</td>
</tr>
<tr>
<td>+ 9q22.32-22.33</td>
<td>938000001 (RP11-275N7)</td>
<td>990500002 (RP11-128E22)</td>
<td>5.25</td>
<td>50</td>
<td>0</td>
<td></td>
<td>0.0049</td>
<td>59</td>
</tr>
<tr>
<td>+ 9q33.1-34.11</td>
<td>117450001 (RP11-523C3)</td>
<td>127700002 (RP11-3419)</td>
<td>10.25</td>
<td>50</td>
<td>0</td>
<td></td>
<td>0.0049</td>
<td>93</td>
</tr>
</tbody>
</table>
Figure 3. Frequency plots. A: Frequency plot of chromosome 9 for the 30 ovarian tumours from patients treated with docetaxel/carboplatin in the current study. The black lines represent sensitive cases and the grey lines represent resistant cases. The potential marker regions (p<0.01) are highlighted in grey. B: Frequency plot of chromosome 9 for the 40 ovarian tumours from patients treated with paclitaxel/carboplatin in a previous study (19). The black lines represent resistant cases and the grey lines represent sensitive cases. The regions exhibiting significance in the study are highlighted in grey. Note the opposite colourings of the lines in figures A and B.
Additionally, it was investigated whether there was any discrepancy in CNAs between the sensitive cases without relapse versus the sensitive cases with early relapse. Interestingly, one small loss in 14q21.1 (RP11-674M16–RP11-523121) emerged, which was significantly more frequent \( (p=0.0094) \) in the sensitive cases with relapse than in the cases without relapse (67% and 8%, respectively). The region is 4.82 Mb in size and harbours 19 known genes.

**Paclitaxel versus docetaxel.** When comparing the current patient material of ovarian tumours treated with docetaxel/carboplatin to the previously analyzed material of ovarian patients treated with paclitaxel/carboplatin, several differences were revealed (19). Both materials underwent the same technical and statistical analysis in the search for CNAs that may distinguish resistant from sensitive cases. Interestingly, not a single identical CNA was identified when comparing resistant versus sensitive cases in the two different treatment groups. In the previous study investigating paclitaxel/carboplatin resistance, alterations in four regions were associated with resistance, namely gain in 3q26.2 and losses in 6q11.2-12, 9p21.3-22.3 and Xp22.2-11.1. None of these regions emerged in the present investigation. When exploring the regions in the current material, gain in 3q26.2 was generally frequently found (73% in the 30 tumours) but did not differ between the response groups. Losses in Xp22.2-11.1 were also frequently detected in both response groups. Loss in 6q11.2-12, however, was infrequent in the current material and losses in 9p22.1-22.3 were slightly more frequent in the sensitive cases (Figure 3). In contrast, the current material exhibited gains in chromosome 9 as being significantly associated with resistance. Additionally, the regions in 8p that were associated with chemosensitive disease in the current material were common in the previous material (45%), but were, on the contrary, more frequently detected in the resistant cases (Figure 2B).

**Discussion**

Since the mortality rate in ovarian cancer continues to be high and the majority of ovarian cancer patients develop chemotherapy resistance, there is an ongoing search for improvements of the treatment regimens and for the discovery of novel agents. Docetaxel, a second-generation taxane, is used in the management of a number of solid tumours such as breast, lung and prostate cancer (3). In ovarian cancer it is mostly used in second-line chemotherapy, but was shown to be equally efficient in the treatment of ovarian cancer as paclitaxel when combined with carboplatin in first-line chemotherapy (4). The present study investigated the potential genetic alterations behind docetaxel/carboplatin response in a primary advanced stage ovarian tumour material, which is the first array CGH analysis of such a material. CNAs specific to chemoresistant and chemosensitive disease were identified in docetaxel/carboplatin treated specimens. In addition, it has been suggested that there is not a complete cross-resistance between docetaxel and paclitaxel (15-18), i.e. docetaxel had antitumour activity on paclitaxel-resistant tumours. Therefore, the results were compared to previous findings in tumours from patients treated with paclitaxel/carboplatin and different genetic alteration patterns were found.

Losses in 8p23.3-23.1 and 8p22 were found exclusively in the sensitive cases and in high frequencies and were associated with sensitivity in the current tumour material (Table II; Figure 2A). When studying the alterations in 8p, the vast majority of the losses were large and extended along most of the p arm. Loss in 8p is a commonly detected alteration in ovarian and other solid tumours (26-30), and has been associated with poor survival in serous ovarian carcinomas (31). 8p23, in particular, has repeatedly been found to be deleted in ovarian and other tumours, suggesting the existence of tumour suppressor genes in the region (32-34). Several candidates have been proposed such as *CSMD1* and *MCPH1/BRIT1*, which both are located in the significant region 8p23.3-23.1 in the current investigation (33, 35). Furthermore, Kim et al. suggested losses in 8p21.1 and 13q32.1 to be predictive markers of chemoresistant disease in serous ovarian carcinomas treated with a platinum-based combination (26). Concerning the present study, it is important to keep in mind the uneven distribution between sensitive and resistant cases when interpreting the results.

Gains in chromosome 9 were associated with resistance in the present study (Table II; Figure 3A). In ovarian cancer, both losses and gains are frequently detected in chromosome 9 (27, 36-38). Concerning chemotherapy response, gain in 9q33.3-34.3 has been associated with paclitaxel/carboplatin resistance (27). In the present study, gains in 9q33.1-34.11 and in the regions 9q21.2-21.32, 9q21.33, 9q22.2-22.31 and 9q22.32-22.33 were associated with docetaxel/carboplatin resistance. In additional concurrence, gain in chromosome 9 was found in a docetaxel-resistant breast cancer cell line (39). Gain in the small region 9p13.2-13.1 was associated with resistance in the current study. In the previous analysis of paclitaxel/carboplatin-treated ovarian carcinomas, losses in 9p22.3, 9p22.2-22.1, and 9p22.1-21.3 were associated with resistance (Figure 3B) (19). This discrepancy between the two chemotherapy regimens is interesting. The heterogeneous alteration pattern of chromosome 9, however, should be the subject of further exploration to elucidate its role in ovarian cancer and chemotherapy response.

Interestingly, loss in 14q21.1 was significantly more common in sensitive cases with relapse when compared to sensitive cases without relapse. The region harbours the gene *PNN* which has been implicated in cell–cell adhesion (40) and suggested to be a tumour suppressor gene (41). These
associations make this finding interesting in relation to metastasis and invasiveness in ovarian cancer.

This is the first array CGH investigation of an ovarian tumour material treated with docetaxel/carboplatin as first-line therapy. Prior investigations on chemotherapy resistance have been performed on paclitaxel and/or platinum resistant tumours or cell lines (26-27, 42); unfortunately with differing results. A previous study analysed an ovarian tumour material of patients treated with paclitaxel/carboplatin and identified gain in 3q26.2, and losses in 6q11.2-12, 9p21.3-22.3 and Xp22.2-11.1 as being significantly more frequent in resistant cases (19). In the present investigation, performing the same analysis on an ovarian patient material treated with docetaxel/carboplatin, a different set of CNAs was identified (losses in 8p23.3-23.1, 8p22, and gains in 9p13.2, 9q21.2-21.32, 9q21.33, 9q22.2-22.31, 9q22.32-22.33, and 9q33.1-34.11) when comparing resistant to sensitive cases. Moreover, when scrutinising the current identified alterations in the previous material and vice versa, concurrence was low and even the opposite pattern was observed for 8p (Figure 2). These findings, which indicate a difference between paclitaxel and docetaxel, are interesting. However, even though both tumour materials were serous papillary carcinomas and of advanced stage, they differed in group size and stage. The current docetaxel material had only 6 resistant cases whereas 24 were sensitive. In addition, a number of stage IV tumours were included compared to solely stage III in the prior paclitaxel material, which also had an even distribution between the response groups (20-20). These factors may have influenced the results and should be kept in mind when interpreting the outcome; the present study is just a pilot study. Moreover, chemotherapy resistance in ovarian cancer is very complex and complicated to study. Still, these results are interesting and worth further exploration and validation in an independent series of ovarian tumours.

In conclusion, genetic alterations specific to chemoresistant and chemosensitive disease were identified in advanced serous ovarian carcinomas treated with docetaxel/carboplatin. Identifying such alterations will hopefully lead to the establishment of predictive markers. Additionally, these results imply a difference in the genetic alteration pattern between paclitaxel and docetaxel response; different predictive markers for paclitaxel and docetaxel could help individualise therapy and make docetaxel an alternative option for paclitaxel-resistant patients in first-line treatment.

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References


