Original Article
Cytologic changes of ovarian epithelial cancer induced by neoadjuvant chemotherapy

Yiying Wang¹,²,³, Yue Wang¹,²,³, Wenxin Zheng²,³,⁴

¹Department of Obstetrics and Gynecology, Henan Province People’s Hospital, Zhengzhou, China; ²Department of Pathology,College of Medicine, University of Arizona, Tucson, AZ, USA; ³Department of Obstetrics and Gynecology, College of Medicine, University of Arizona, Tucson, AZ, USA; ⁴Arizona Cancer Center, University of Arizona, Tucson, AZ, USA

Received August 12, 2013; Accepted August 30, 2013; Epub September 15, 2013; Published October 1, 2013

Abstract: Objective: Neoadjuvant chemotherapy (NACT) followed by cyto reduction has now become a part of standard care for patients with advanced ovarian cancer. Cytologic changes of the cancer cells induced by NACT, however, sometimes may cause confusion in terms of pathologic diagnosis and therefore inappropriate management. The objective of this study was to characterize the histologic or cytologic features of the ovarian cancers from those patients who received NACT in order to improve the diagnostic accuracy and reduce unnecessary clinical workup.

Methods: Specimens from 120 patients with advanced ovarian cancer who received NACT were studied. All 120 cases had either cytologic samples from ascites (n=108) or fine needle aspiration (n=12) and the diagnosis of consistent with cancers of ovarian origin was made prior to NACT. There were 70 (58.3%) patients received subsequent tumor debulking surgery after NACT. The time frame between NACT and debulking surgery ranged from 28 to 65 with an average of 45 days. Among the 70 cases with cytoreductive surgery, 48 cases containing both pre-NACT cytology/histology and subsequent debulking specimens were suitable for the study. All 48 post-NACT ovarian cancers were reviewed and the characteristic pathologic features in gross were summarized. Microscopic evaluation and immunohistochemical stainings with antibodies against ER, PR, p53, WT1, PAX8, CK7, CK20, and CDX2 were performed to confirm the primary site and histologic type of the cancers.

Results: Grossly, tumor size within the ovaries from those debulking specimens ranged from 2.3 to 6.5 cm in greatest dimension. The cancers were mainly solid (average of 65%) and cystic areas had more or less hemorrhagic appearance. Extensive tumor necrosis and some with fibrosis were present. Microscopically, the non-necrotic cancer cells were arranged in cords, islands and sometimes as scattered single large cells with large amount of eosinophilic cytoplasm with vacuoles. The viable cancer cells contained more or less vacuolated cytoplasm in almost all post chemotherapy cases. Multinucleated tumor giant cells were noted in close to half of the cases. The cancer cells commonly had large hyperchromatic bizarre nuclei with coarse chromatin clumping and sometimes prominent nucleoli. Due to the unusual cytologic changes after NACT, there was a concern of non-ovarian origin or the different histologic type of the cancers. Therefore, immunohistochemical (IHC) staining with the antibodies against ER, PR, PAX8, WT1, CK7, CK20, and CDX2 was performed in all 48 paired cases. The 48 paired samples showed identical immunophenotype in pre- and post-NACT cancers, confirming there was no metastatic or new primary cancer involved in the study.

Conclusions: NACT can apparently induce significant cytologic/histologic changes in ovarian cancer. Aware of such NACT induced changes will be useful to make correct diagnosis for those patients who have received NACT. IHC with appropriate panels of the antibodies will be helpful to aid the diagnosis, particularly when nuclear change is dramatic and the clinical history of ovarian cancer is not available.

Keywords: Ovarian cancer, cytological changes, chemotherapy, immunohistochemical

Introduction

Ovarian cancer (OC) remains one of the leading causes for mortality for gynecologic cancers in the world. With the current treatment modalities, including surgery and combination chemotherapy, there are approximately 70% of OC patients responding to the therapies [1-4]. However, about half of these responded patients are diagnosed as having persistent disease at second-look operation, and additional half may have recurrent diseases afterwards.
Primary tumor debulking (cytoreduction) followed by chemotherapy is considered the standard of care for patients with advanced stage of OC. As an alternative to this practice, NACT followed by cytoreduction surgery, which has been proved to be beneficial to the overall patient survival [5-8], has been used where an accurate cytologic or pathologic diagnosis is required before the initiation of NACT [9, 10]. Therefore, cytologic or pathologic diagnosis plays a critical role in this clinical setting.

However, it is well known that cytologists/pathologists feel difficult to make definitive diagnosis of presence of cancer cells, particularly benign versus malignant cells, high-grade versus low-grade cancers, and gynecologic versus non-gynecologic cancer of origin. The difficulties of such cytologic or histologic diagnosis are commonly encountered from those pre-surgical ascites or pelvic washing specimens or specimens obtained after chemotherapy and they are mainly caused by cytologic changes of the cancer cells induced by chemotherapy, either in the neoadjuvant or post-operative settings [11, 12].

Chemotherapy induced cytologic alterations for the cancer cells have been noticed in many organ systems [13-15]. However, it has not been studied in patients with ovarian cancer, particularly in the setting of NACT. In this study, we compared the cytologic differences induced by NACT in 48 ovarian epithelial cancer cases and summarized the cytologic characteristics of the OCs after NACT. We believe these understanding will be useful in clinical diagnosis and patient management for patients with OC.

Material and methods

Case characteristics

A total of 120 OC patients who received NACT for an apparent advanced stage ovarian cancer between 1979 and 2012 were studied. These included cases from our previous study [9] (n=60) and the remaining from University of Arizona. All 120 cases had either cytologic samples from ascites (n=108) or fine needle biopsies (n=12) and the diagnosis of consistent with cancers of ovarian origin was made. The OC patients had the following clinicopathologic features. Eighty (67%) of the 120 patients had evidence of extra-abdominal tumor spread prior to treatment, 110 (91.7%) had ascites, 40 (33.3%) had unilateral pleural effusion and 16 (13.3%) had bilateral pleural effusions. One hundred and 5 (87.5%) patients with known preoperative serum CA125 levels showed values >500 U/ml, 70 (58.3%) being >1500 U/ml.

After NACT with an average of 45 days ranging from 28 to 65 days, there were 70 (58.3%) patients received tumor debulking surgery. Among these cases, there were 48 cases had both pre- and post-NACT samples for the cytologic or pathologic comparison. By comparing the microscopic features, the results of cytologic/histologic changes induced by NACT were summarized in this study, which has been approved by the Institutional Review Board.

Ascetic sample preparation

Ascetic fluid was tapped from candidates with potential NACT. An average of 50 to 100 ml of ascites was obtained. The samples were treated with Cell-Lite to lyse red blood cells and then spin down in 50-ml sterile plastic tubes. At least one slide with Papanicolaou staining was made from each sample for cytologic examination. A cell block was also made when a cell pellet was visible.

Cytologic and pathologic evaluation

For cytologic samples, the following criteria were used to identify the presence of malignant cells consistent with ovarian primary based on well-established cytologic features published previously [9, 16, 17]: Malignant cells with abundant non-mucinous cytoplasm suggested serous carcinoma; Papillary structures with the above cytologic features consistent with serous carcinoma, particularly when psammoma bodies were seen; Malignant cells with vacuoles or a hint of clear cell differentiation suggested clear cell carcinoma; The presence of prominent nucleoli was looked for as they frequently were present in serous and clear cell carcinomas; When endocervical type malignant cells were present, they were most compatible with a Mullerian or ovarian primary. For biopsy samples, the diagnostic criteria were based on the recent classification described elsewhere [18, 19].

Immunohistochemistry (IHC)

All the 48 paired cases were stained with the following antibodies to compare the similarities
between the pre- and post-chemotherapy cancer samples: estrogen receptor (ER), progesterone receptor (PR), p53, WT1, PAX8, CK7, CK20, and CDX2. IHC for the above antibodies was performed by using the Envision Plus/Horseradish Peroxidase system (Dako, Carpentaria, CA). In brief, sections from paraffin-embedded cell blocks were incubated in hydrogen peroxide and absolute alcohol for 30 minutes to block endogenous peroxidase activity. Antigen retrieval was carried out using pressure cooker pretreatment in citrate buffer (pH=6.0). Tissue sections were subsequently incubated with the primary antibody for 40 minutes at room temperature. After TBS rinses, the tissue was incubated using the Envision Plus secondary antibody for 30 minutes followed by diaminobenzidine for 5 minutes. Appropriate positive (an ovarian cancer known to be positive for PAX8 and a mesothelioma known to be positive for calretinin) and negative (incubation with secondary antibody only) controls were stained in parallel for each round of IHC.

ER, PR, p53, WT1, PAX8, CDX2 were evaluated for nuclear staining, while CK7 and CK20 were cytoplasmic. Immunoreactivity was graded based on the stainings in targeted cells by comparing the H&E morphology. The percentage of the positive cells only with intense staining was counted and recorded for paired samples. The intensity staining was referenced by comparing the positive controls. The concordant staining was defined by 20% or less differences between the paired samples, while discrepancy denoted the staining difference more than 20% of the stained cancer cells.

Statistical analysis

Statistical comparisons of categorical data were carried out using K-square or Fisher exact test, depending upon the sample size. A $P$ value of <0.05 was considered statistically significant.

Results

Cytologic or pathologic diagnosis for the cases with NACT

All slides from the 48 paired cases with both pre- and post-NACT were reviewed under microscope. Based on cytologic/histologic characteristics, we diagnosed that all pre-chemotherapy cytology or FNA samples were consistent with ovarian epithelial cancers. These cancers were further classified into probably high-grade serous carcinoma ($n=40$), clear cell carcinoma ($n=4$), poorly differentiated endometrioid carcinoma ($n=3$), and undifferentiated carcinoma ($n=1$), based on the cytologic and histologic features described previously [9]. All 48 post-NACT surgical debulking cases with at least one resection or biopsy sample containing cancer tissue were reviewed. Histologically, all cancer areas showed more or less cytologic/histologic changes by comparing with the classic histologic features of a particular histologic type of ovarian epithelial cancer (see below). Many of which raised a concern of different histologic

Figure 1. Extensive tumor necrosis after neoadjuvant chemotherapy. This 4.5 cm ovarian mass (A) grossly shows extensive tumor necrosis (the yellowish in color). It is mainly solid with cystic and hemorrhagic areas. Microscopically, more than 90% of the tumor is necrotic. There are only focal isolated islands of viable cancer seen (B).
Neoadjuvant chemotherapy for ovarian cancer

Table 1. Cytologic changes of ovarian cancer induced by neoadjuvant chemotherapy

<table>
<thead>
<tr>
<th>#cases</th>
<th>CTN</th>
<th>CV</th>
<th>LHBN</th>
<th>CChC</th>
<th>MNTGC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGSC</td>
<td>40</td>
<td>22</td>
<td>40</td>
<td>36</td>
<td>38</td>
</tr>
<tr>
<td>CCCa</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>EMD</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>UND</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

HGSC: high-grade serous carcinoma; CCCa: clear cell carcinoma; EMD: endometrioid carcinoma; UND: undifferentiated carcinoma. CTN: coagulative tumor necrosis; LHBN: large hyperchromatic bizarre nuclei; CChC: coarse chromatin clumping; MNTGC: multinucleated tumor giant cells; CV: cytoplasmic vacuolization.

Figure 2. Single large tumor cells in a fibrotic background after neoadjuvant chemotherapy. In the background of fibrosis, there are many single large tumor cells, which are typically 3-4 times larger than non-enlarged cancer cells. These enlarged tumor cells sometimes are multinucleated.

Figure 3. Cytoplasmic vacuolization after neoadjuvant chemotherapy. There are many cytoplasmic vacuoles in the cancer cells. The sizes of such vacuoles vary from fine granular to the size of a nucleus of the cancer cells. The cytoplasm looks eosinophilic when the vacuoles are finely grouping together.

Figure 4. Nuclear change after neoadjuvant chemotherapy. The cancer cells commonly show large hyperchromatic bizarre nuclei with coarse chromatin clumping and sometimes prominent nucleoli. These nuclear changes are also present in Figures 2 and 3.

Type of cancers or metastatic cancer from elsewhere. Such concerns prompted a panel of immunohistochemical staining to aid the differential diagnosis (see below).

Pathologic changes of ovarian epithelial cancer after NACT

All 48 post-NACT ovarian cancers were reviewed and the characteristic pathologic features were as follows. Grossly, tumor size within the ovaries was significantly reduced ranging from 2.3 to 6.5 cm with an average of 4.1 cm in greatest dimension. On cut section, the cancers were mainly solid (average of 65%) and cystic areas had more or less hemorrhagic appearance. Coagulative necrosis ranged from needle size spicules (n=3) to frank yellow dusky masses with irregular shapes up to 5 cm (n=1) (Figure 1) in 25 (52.1%) cases. The tumor consistency was mainly related to the amount of tumor necrosis and fibrosis. The amount of tumor necrosis was documented as <10% (n=24), 10% to 25% (n=16), 26% to 50% (n=6), and >50% (n=2). Fibrosis was more common in tumors containing less necrosis. Hemorrhage and cystic changes were common (Figure 1). Within the fibrotic stroma, the cancer cells were arranged in cords and islands. In vicinity of the tumor islands, scattered single large cells were seen with a size approximately 3 to 4 times larger than non-enlarged tumor cells, mostly in the background of fibrosis (Figure 2). These large cells showed large amount of eosinophilic
cytoplasm and some of them had vacuoles in various sizes (Figure 3). Such vacuolated cytoplasm was seen in all post chemotherapy cases. The cancer cells commonly had large hyperchromatic bizarre nuclei with coarse chromatin clumping (Figures 2 and 3) and sometimes prominent nucleoli were present (Figure 4). Multinucleated tumor giant cells (Figure 4) were noted in 22 (45.8%) of the cases. The cytologic changes after NACT are summarized in Table 1. 

Adjacent non-cancerous tissue showed dense fibrosis, chronic inflammatory infiltrate, and hemosiderin-laden macrophages. Among the 48 patients, 6 were in reproductive age and known to be pre-menopausal. The residual ovarian tissue was seen, but no developing follicles were identified. Metastasis was seen in 8 (16.7%) pelvic lymph nodes and in 3 (6.25%) peri-aortic lymph nodes. Residual cancer was present in omentum (n=36, 75%), peritoneal biopsy (n=42, 87.5%), bowel wall including small intestine and colon (n=16, 33.3%), liver or spleen capsule (n=11, 22.9%), and diaphragm (n=6, 12.5%).

Immunohistochemical (IHC) staining results

To confirm the nature and rule out metastasis of the cancers, we performed IHC stainings for ER, PR, p53, WT1, PAX8, CK7, CK20, and CDX2. PAX8 was positive in all cases, while CDX2 was all negative. CK7 was diffusely positive in 46 (95.8%) cases, while the remaining 2 were focally positive. CK20 were negative in 40 (83.3%) and focally positive in 8 (16.7%) cases. These CK20 focally positive cases all showed diffuse positivity of CK7 staining. The focally CK7 positive cases showed CK20 negative. WT1 was diffusely positive in 40 (83.3%) cases. ER was positive in 37 (77.1%), while PR was positive only in 5 (10%) cases. The immunostaining for p53 showed diffusely positive (>75% of the tumor cells stained) in 36 (75.0%) cases, while focally positive (<75% positive tumor cells) in 8 (16.7%) cases, and negative in 2 (4.2%) cases. The IHC results were identical in pre- and post-NACT samples. The immunohistochemical staining results from post-chemotherapy samples, together with histologic type of the cancers are summarized in Table 2.

Discussion

NACT for ovarian epithelial cancer is becoming increasingly prevalent within the gynecological oncology community. It is well recognized that subjective improvement in the sense of well-being for those patients who received NACT, compared to those patients who received conventional therapy where the chemotherapy is typically offered after aggressive cytoreductive surgery [7, 20, 21]. However, Prior to the application of NACT, it is essential to have cytologic or histologic diagnosis of the cancer at least consistent or compatible with the ovarian origin to avoid a wrongful chemotherapy agent. In this regard, our previous publication [9] have provided the diagnostic criteria from ascites or fine needle aspiration samples of patients who are suspected for advanced stage ovarian cancer in the clinic. These diagnostic criteria have been widely used in the clinic and have facilitated successful application of NACT for ovarian cancer patients. Patients with ovarian cancer are commonly receiving tumor debulking surgery after NACT. However, NACT induced histologic changes of the ovarian cancers have not been described in the English literature. It is common to see erroneous diagnosis from those patients who received chemotherapy including NACT from ovarian cancer patients [22]. This is mainly due to dramatic cytologic changes after chemotherapy, particularly when...
the history of chemotherapy is not clearly stated, which is quite common in the clinic. This study was designed to address the NACT induced pathologic and cytologic changes for ovarian cancer patients in order to improve the diagnosis and clinical management.

Grossly, the tumor size within the ovary is about 4 cm in average. This has been dramatically reduced since a typical ovarian epithelial cancer median size is about 10 to 15 cm prior to the NACT [23]. The histologic effects of NACT include a decrease in the tumor cellularity, which may be extreme so that no or less residual tumor cells are detected. This goes along the large amount of tumor necrosis found in more than 50% of the post NACT specimens in this study. Necrosis could range from needle size spicules to 5 cm in various sizes of geographic appearance. Hemorrhage and cystic changes were common. Fibrosis and hyalinization are also frequent in all post NACT specimens including lymph nodes. Metastatic deposits may be seen in or around these hyalinized areas. These gross changes have also been described in non-ovarian cancers [13, 14]. Therefore, they are not specific for ovarian cancer after NACT. Microscopically, the most dramatic cytologic changes of the ovarian cancer after NACT are the nuclear morphology and cytoplasmic vacuoles. These include hyperchromatic bizarre nuclei and nuclear clumping. Multinucleated tumor giant cells and prominent nucleoli are not always present, but they were found in about 45% of the cases in this study. The tumor cells are large due to increased cytoplasm which may contain vacuoles or eosinophilic granules. These cytoplasmic vacuoles or eosinophilic granules are present in almost every case in this study. Under high power (400X), the vacuoles can be finely granular to largely occupying entire cytoplasmic compartment. The recognition of these changes assumes greater importance in cases where altered tumor cells are present individually [14]. A study comparing histologic grading in pre- and post-NACT specimens did not reveal significant differences in grading [24]. Other studies, however, reported increase in nuclear grade in one-third of cases [25]. Cytologic changes like breast lobular atrophy and cytologic atypia in breast epithelial cells may be seen in non-neoplastic breast parenchyma [26]. However, atypical epithelial changes in benign ovarian or tubal epithelia are not apparent in this study.

The above pathologic characteristics induced by NACT may raise multiple differential diagnosis. First, extensive tumor necrosis with geographic appearance and relatively small cancer size of the ovary will raise a concern of metastatic cancer from gastrointestinal tract. This actually was our main concern when we encountered the specimens at the time of pathology sign-out. Typically, metastatic cancer from gastrointestinal tract shows more or less mucin, while majority primary ovarian epithelial cancers do not contain mucin. This was true in this study. Cancers from gastrointestinal tract metastasized to the ovary will usually not contain hyperchromatic bizarre tumor cells with large amount of vacuolated cytoplasm. In contrast, these cytologic features are classic of ovarian cancers after NACT. In those difficult diagnostic encounters, immunohistochemical stainings are always useful to confirm the diagnosis. The most useful panels we used in this scenario are ER/PR, PAX8, CK7, CK20, p53, WT1, and CDX2. Typically, primary ovarian epithelial cancers are positive for ER/PR, PAX8 and CK7 and negative for CK20 and CDX2. The reverse is true for gastrointestinal cancers. WT1 is helpful to identify ovarian serous cancer, while p53 diffuse staining is of characteristics of high-grade serous carcinoma [27]. We have compared the panels between pre- and post-NACT specimens and found the immunophenotype are basically identical in paired patients. Therefore, the primary ovarian cancers were all confirmed and the possibility of metastasis was excluded. Second, multinucleated tumor giant cells and hyperchromatic bizarre nuclei may be concerned for an undifferentiated carcinoma instead of high-grade serous carcinoma of the ovary. However, areas of predominance of smaller, less cohesive cancer cell clusters and papillae with irregular outlines and many single cells with pleomorphic and syncytial or indistinct cell borders are classic for high-grade serous carcinoma [9]. Third, abundant vacuolated cytoplasm in the cancer cells are of a concern of clear cell carcinoma instead of high-grade serous carcinoma. Based on this study, all 40 high-grade serous carcinoma and all 4 clear cell carcinoma cases showed more or less cytoplasmic vacuoles or even clear appearing cytoplasm after receiving NACT. Based on morphology alone, it is impos-
Neoadjuvant chemotherapy for ovarian cancer

possible to tell the difference between the two. But comparing with pre-NACT slides should readily resolve the issue. Meanwhile, WT1 and p53 staining are reliable biomarkers to differentiate clear cell from the serous carcinoma. The clear cell carcinoma shows negative WT1 and patch positive p53, while high-grade serous carcinoma has diffuse positive WT1 and p53 in the majority of the cases.

In conclusion, a meticulous gross and microscopic examination of post-NACT ovarian specimens is required with ample sectioning to identify the small areas of residual viable tumor. The pathologist should be aware of the possible nuclear and cytoplasmic changes after NACT, which will certainly increase the diagnostic accuracy and cost effectiveness in clinical practice. Immunohistochemistry with a panel of suitable biomarkers may be required to confirm the nature as well as the histologic type of cancer cells. Additionally, our experience suggests that successful use of pathologic/cytologic material to confirm the ovarian cancer diagnosis after NACT requires excellent communication between the clinician and the pathologist.

Address correspondence to: Dr. Wenxin Zheng, Department of Pathology, Department of Obstetrics and Gynecology, College of Medicine, University of Arizona, 1501 N. Campbell Avenue, #5205, Tucson, AZ 85724, USA. Tel: 520-626-6032; Fax: 520-626-1027; E-mail: zhengw@email.arizona.edu

References


Neoadjuvant chemotherapy for ovarian cancer