EUROPEAN JOURNAL OF GYNAECOLOGICAL ONCOLOGY – EJGO (ISSN 0392-2936) publishes original peer reviewed work, preferably brief reports, in the fields of female genital cancers and related subjects, prevention, early detection, epidemiology, pathology, diagnosis, management, and also proceedings of Gynecologic Oncology Society global meetings. The Journal is covered by ISI Journal Master List, Index Copernicus International, Science Citation Index Expanded, Current Contents - Clinical Medicine, Web of Science, Index Medicus/MEDLINE, EMBASE Excerpta Medica, PubMed, MedSci, Pubget, Genamics JournalSeek, Sciencescape, Unbound Medicine, and PubFacts.com. EJGO is issued bimonthly in one volume per year by 7847050 CANADA Inc., Montréal (Canada). Printed in Italy by “Centro Servizi Editoriali S.r.l.” - Grisignano di Zocco - 36040 Vicenza (Italy).
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Cytogenetic analysis of epithelial ovarian cancer’s stem cells: an overview on new diagnostic and therapeutic perspectives

A.S. Laganà, F. Colonese, E. Colonese, V. Sofo, F.M. Salmeri, R. Granese, B. Chiofalo, L. Ciancimino, O. Triolo

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Summary
Ovarian cancer is one of the most frequent solid tumor that shows clearly biphasic behaviour in response to chemotherapy, with the majority of patients who achieved complete remission after the first cycle of chemotherapy, and subsequently present a relapse which, in most cases, leads to death. Epithelial ovarian cancer (EOC) arises as a consequence of genetic alterations that affect the cells of the ovarian surface, which leads to changes that occur through the activation of oncogenes and inactivation of tumor suppressor genes. The progression of EOC is characterized by a series of combined epigenetic aberrations, including the most important of those determined by the loss of methylation of certain regions of DNA encoding genes such as Ras-association domain-containing family 1 (RASSF1A) tumor suppressor, death-associated protein kinase (DAPK) protein kinase associated with the regulation of apoptosis, human sulfatase-1 (hSulf-1) sulfatase, which plays a key role in the regulation of apoptosis, breast cancer 1 gene (BRCA1) tumor suppressor gene, involved in the processes of DNA repair, and HOXA10 (gene required to promote many transcription factors). To date, accumulating evidence suggests that the initial clinical response is due primarily to the therapeutic efficacy of chemotherapy against differentiated cancer cells that constitute the bulk of the tumor, whereas the high rate of recurrence is thought to be due to remaining drug-resistant cells, biologically distinct, identified as cancer stem cells (CSC). Current efforts are focusing on genetic and cytological definition of CSC, to guide the development of new diagnostic, and therapeutic perspectives.

Key words: Epithelial ovarian cancer (EOC); Cancer stem cells (CSC); Initial ovarian cancer cells (OCICs).

Introduction
Ovarian cancer is not a single entity but instead represents tumors of epithelial, germ cell, or sex cord stromal origin. The coelomic epithelium, also called germinal epithelium, represents the site of origin of all epithelial structures of female reproductive tract except for the distal vagina. It is thought that the majority of ovarian neoplasms originate from it. According to WHO classification [1], approximately 90% of ovarian cancer is of epithelial origin. About 5% are germ cell tumors, most likely originate in cells derived from the primitive streak that ultimately migrated to the gonads. Finally, 5% is constituted of sex cord stromal tumors, arising from the ovarian stroma consisting of granulosa cells, theca cells, and fibroblasts. The cells of origin of ovarian cancer has been long debated. The current paradigm is that epithelial ovarian cancer (EOC) arises from the ovarian surface epithelium (OSE). OSE is composed of flat, nondescript cells more closely resembling the mesothelium lining the peritoneal cavity, with which it is continuous, rather than the various histologic types of ovarian carcinoma (serous, endometrioid, and clear cell carcinoma), which have a Müllerian phenotype. Accordingly, it has been argued that OSE undergoes a process termed “metaplasia” to account for this profound morphologic transformation. Recent molecular and anatomopathological studies not only have failed to support this hypothesis but also have provided evidence that EOC stems from Müllerian-derived extraovarian cells that involve the ovary secondarily, thereby calling into question the very existence of primary EOC. This new model of ovarian carcinogenesis [2] proposes that fallopian tube epithelium (benign or malignant) implants on the ovary to give rise to both high-grade and low-grade serous carcinomas, and that endometriotic tissue implants on the ovary can produce endometriosis, which can undergo malignant transformation into endometrioid and clear cell carcinoma. Considering the large amount of new evidence, in the current paper the authors aimed to elucidate the role of ovarian cancer stem cells (CSC) in the origin, progression, and evolution of EOC.
Materials and Methods

A comprehensive review of the literature was conducted in order to investigate the role and the advance in research about CSC in ovarian cancer. A detailed search of the literature was performed using the key words ‘epithelial ovarian cancer’, ‘cancer stem cells (CSC)’, ‘ovarian cancer-initiating cells (OCICs)’. English-language publications in PubMed and Cochrane database were analyzed. The analysis was performed focusing on epidemiology, etiopathogenesis, diagnosis and therapy of EOC, and particularly on genetic mutations.

Classification, epidemiology, and clinical characteristics of ovarian cancers

EOC is the leading cause of death from gynecologic cancer in the United States and is the country’s fifth most common cause of cancer mortality in women. In 2013, it was estimated that 22,240 new diagnoses and 14,030 deaths from this neoplasm would occur in the United States; less than 40% of women with ovarian cancer are cured [3]. The estimated number of new ovarian cancer cases in Europe in 2012 was 65,538 with 42,704 deaths [4, 5]. There is variation in the incidence rate across the continent with a higher incidence in northern European countries. The incidence of ovarian cancer increases with age and is most prevalent in the sixth and seventh decades of life [6]. The median age at the time of diagnosis is 63 years, and more than 70% of patients present with advanced disease. Incidence rates have been declining by approximately 0.7% per year between 1985 and 2001, and by 1.9% per year from 2001 to 2007 [7, 8]. Incidence varies among races, and in the United States the delay-adjusted incidence rate is 52% higher for Caucasians than African Americans [9]. Although these hormonal, reproductive, environmental, and racial and ethnicity factors mildly alter ovarian cancer risk, genetic factors have the most potent impact. Ovarian neoplasms are classified into three groups, according to the tissue of origin: epithelial, stromal and endocrine cells, and germ cells. EOC accounts for over 90% of all ovarian malignancies and is primarily a disease of postmenopausal women. Ovarian cancer is more frequent in industrialized countries, and available epidemiologic data suggest that environmental factors may contribute to development of the cancer, although this remains uncertain [10]. EOC can be classified into distinct morphologic categories: serous, mucinous, endometrioid, clear cell, transitional cell (Brenner tumors), mixed, and undifferentiated type (for prevalence, see Figure 1) Papillary serous histology accounts for 75% of ovarian cancers, and its histological pattern simulates the lining of the fallopian tube. High-grade, poorly differentiated tumors are the majority and are macroscopically indistinguishable from other epithelial tumors. Although no universal grading schema exists for ovarian serous carcinoma, a two-tiered system (low-grade vs high-grade) has received increasing acceptance [11, 12]. Histologic grade is of prognostic significance [11] and may also be of predictive value in that low-grade tumors appear less responsive to chemotherapy than high grade tumors [13-15]. Mucinous tumors histologically resemble endocervical epithelium. They tend to be the largest epithelial ovarian neoplasms, but are prone to remain confined to the ovaries. Endometrioid tumors closely resemble the components of endometrial cancer. The prevalence of endometrioid ovarian cancers has decreased in recent years, likely due to better pathological diagnosis, and currently they account for almost 10% of ovarian cancers. Endometriosis and in particular endometriotic cysts have been implicated as putative precursor lesions to endometrioid ovarian cancer. ARID1A mutations have been detected in endometriotic cysts and in endometrioid ovarian cancer, suggesting a causative role [16]. Clear-cell cancers account for about 5% of ovarian cancers, although the incidence varies worldwide. The prognosis for Stage 1 clear-cell cancers is relatively good. However, advanced stage clear-cell cancers have a worse prognosis than serous ovarian cancers as the tumours tend to be resistant to the standard chemotherapeutic agents used in ovarian cancer. Clear-cell cancers are also strongly associated with endometriosis and a significant proportion carries ARID1A mutations [16]. Transitional cell carcinomas represent less than 1% of ovarian cancers. Primary
Cytogenetic analysis of epithelial ovarian cancer’s stem cells: an overview on new diagnostic and therapeutic perspectives

Ovarian transitional carcinomas are rare but carcinomas with transitional features are quite common. The majority of the latter are variants of high-grade serous carcinomas and exhibit WT1 positivity. Although some series have reported an improved outcome for ovarian transitional cell carcinoma, a recent Gynecologic Cancer Intergroup (GCIG) study reported that, when controlled for other prognostic factors, the outcome for patients with transitional cell carcinoma did not differ significantly from that for patients with serous carcinoma [17]. Undifferentiated carcinoma are rare, and refers to tumors with no discernible histologic differentiation or only minor areas of differentiation. Mixed carcinomas are those containing two or more distinct histologic types of cancer, with each subtype involving at least 10% of the tumor mass. The presence of serous carcinoma or sarcoma as one of the components worsens the prognosis [18]. Borderline EOC (also known as ovarian cancer of LMP, borderline ovarian cancer) is a primary epithelial ovarian lesion with cytologic characteristics suggesting malignancy but without frank invasion and with a clinically indolent course and good prognosis [19]. They comprise about 10%–15% of ovarian tumours. In contrast to patients with frankly invasive ovarian carcinoma, women with borderline disease tend to be younger and are often diagnosed with Stage I disease [20, 21].

Risk factors
The exact cause of ovarian cancer remains unknown but many associated risk factors have been identified. Evidence supports the role of an excess of ovarian stimulation of androgens on epithelial cell in increasing the risk of ovarian carcinoma. This risk could be reduced throw factors associated with a progesterone-mediated stimulation [22]. Progesterone antagonizes estrogen-driven growth in the endometrium, and insufficient progesterone action strikingly increases the risk of endometrial cancer. Epidemiologic studies have identified risk factors in the etiology of ovarian cancer. Woman’s reproductive history appears to contribute significantly to her lifetime risk of ovarian cancer. Family history (primarily patients having two or more first-degree relatives with ovarian cancer), nulliparity, older age (>35 years) at pregnancy confers an increased risk for cancer. Recent data suggest that hormone therapy and pelvic inflammatory disease may increase the risk for ovarian cancer [22-24]. No protective factors have been identified, but it is of note the trend in decreasing incidence of epithelial tumour in women using the oral contraceptive pill over a long period of time [25, 26]. All of these risk factors point to ovulation being correlated with the development of ovarian cancer.

Clinical presentation, diagnosis and prognosis
Early detection is the key to the successful treatment of ovarian cancer, as it enhances the possibility of success of the therapy and it improves the overall survival. However, ovarian carcinoma is rarely diagnosed at an early stage because the disease causes few specific symptoms when it is localized to the ovary. Abdominal discomfort, bloating, and early satiety are the most common symptoms experienced by women with EOC. Patients presenting with such nonspecific complaints may be found to have ascites and a pelvic mass on physical examination. Occasionally an umbilical lymph node metastasis will be present (Sister Mary Joseph’s node) or a pleural effusion will be found. The mass on pelvic examination is frequently firm and fixed, with multiple palpable nodularities in the cul-de-sac [27]. The majority of patients are diagnosed with an advanced disease (Stage III and IV). About 20% of ovarian cancers are found at an early stage, and 94% of these patients live longer than five years after diagnosis. Instead, when the disease is advanced, the five-year survival rate is less than 20% [28, 29]. Regarding the overall survival rate for all types of ovarian cancer, the five-year relative survival was about 36% during the years 1975-1977, and it increased at 45% during 1995-2002 [25]. Lastly, even if patients with advanced disease initially respond to standard chemotherapies, in more than 70% a relapse occurs due to chemoresistance mechanisms, and finally the majority die for the disease [30]. Although screening of asymptomatic women for ovarian cancer is not currently effective, knowledge of ovarian cancer symptoms may help identify patients at an earlier stage. The “Gynecologic Cancer Foundation” and the “American Cancer Society” have suggested guidelines in order to diagnose ovarian cancer at an earlier, more curable stage. Following a full clinical assessment, measurement of serum CA 125 is routinely used to aid diagnosis. Ultrasound examination is the most useful noninvasive diagnostic test. Transvaginal ultrasonography has improved the visualization of ovarian structures, thus improving the differentiation of malignant versus benign conditions [31]. Regarding the utility of serum biomarkers to detect early disease, it is questionable as it is elevated only in about 50% of patients with the International Federation of Gynecology and Obstetrics (FIGO) Stage I disease. In advanced disease, CA 125 is elevated in about 85% of patients. It is not specific for ovarian cancer and raised CA 125 levels may be found in non-gynecological malignancies (e.g. breast, lung, colon, and pancreatic cancer) and benign disease (e.g. endometriosis, pelvic inflammatory disease, and ovarian cysts). Serum CEA and CA 19–9 levels are sometimes measured in situations where it is unclear whether an ovarian mass is of gastrointestinal origin or a primary mucinous ovarian tumour. Even if several protocols have been proposed, also integrating the dosage of different biomarkers, actually, none of these has reached an elevated level of sensitivity and specificity [32]. Surgery is necessary for the diagnosis, staging, and treatment of EOC. Although most are benign, between 13% and 21% of women undergoing surgery for a suspicious adnexal mass will have an ovarian malignancy. Recommendation for surgery depends on the
degree of suspicion that this mass may be malignant; factors that should be considered include age, menopausal status, family history, size and complexity of the mass, associated symptoms, CA 125, unilateral versus bilaterality, and characteristics on ultrasound. Management may include observation with repeat examination, further radiographic imaging, and laparoscopy or laparotomy depending on the clinical circumstances.

**Therapy**

Primary surgical treatment of ovarian cancer has advantages in terms of diagnosis, staging, and tumor debulking. The value of debulking surgery is well established in FIGO Stage III epithelial ovarian cancer [33]. Most women will have widespread disease, therefore surgery alone does not cure the disease. Neoadjuvant chemotherapy prior to surgical debulking proposes to increase the proportion of patients who may be optimally cytoreduced, while decreasing surgical morbidity and mortality [34]. Several retrospective studies have shown that there was no difference in overall survival (OS) or progression-free survival (PFS) for patients with advanced ovarian cancer treated with neoadjuvant chemotherapy compared with primary debulking surgery [35-39]. However, the result of a meta-analysis of Bristow and Chi [40] suggested that neoadjuvant approach was associated with a worse OS and that the definitive operative intervention should be undertaken as early in the treatment program as possible. However a more recent meta-analysis [41] of multiply central randomized trials concluded that survival was similar in patients treated with neoadjuvant chemotherapy followed by interval debulking surgery compared to primary debulking followed by chemotherapy and criticized the meta-analysis of Bristow and Chi [40]. Hence, even if the therapeutic benefit of neoadjuvant chemotherapy followed by interval cytoreduction remains controversial, it is clear that chemotherapy has a decisive role, also in inoperable stages. Regarding surgery techniques and management, initial surgery should be a comprehensive staging laparotomy, including a total abdominal hysterectomy and bilateral salpingo-oophorectomy. For a young patient who wishes to maintain fertility, a unilateral salpingo-oophorectomy may be adequate for select Stage I tumors and or low risk tumors. Surgical cytoreduction is optimal if the residual tumor nodules are less than one cm in maximum diameter or thickness. In addition to total abdominal hysterectomy and bilateral salpingo-oophorectomy, all involved omentum should be removed and suspicious and/or enlarged nodes should be resected, if possible. Those patients with tumor nodules, outside the pelvis (presumed Stage IIIIB) should have bilateral pelvic and para-aortic lymph nodes dissection [42]. Data regarding cytoreduction show that it seems to improve OS [25] and in patients undergoing neoadjuvant chemotherapy, it achieved a better PFS and reduction in the risk of death of 1/3 [43]. Patients who obtained major results were those who experienced a disease-free survival (DFS) >24 months and who underwent a radical cytoreductive surgery [44]. Interesting results in term of responses are also obtained utilizing intraperitoneal chemotherapy with cisplatin [45].

**The role of genetic mutations in the etiopathogenesis of EOC**

Ovarian cancer is the most common cause of death from gynecological cancers in the Western world. There are many issues in terms of early detection and therapy, most of them related to an incomplete and fragmentary knowledge of the molecular basis of pathogenesis and progression of the disease. There is embryological and in vitro evidence that OSE is the origin of ovarian epithelial carcinomas. OSE is a simple mesothelium that overlies the surface of the ovary. It is important to note that the adult OSE and the Müllerian epithelium arise from a common embryonic origin, the coelomic epithelium. In early development, OSE cells form part of the coelomic epithelium and the coelomic epithelium adjacent to the presumptive gonads invaginates to give rise to the Müllerian ducts, i.e. the primordia for the epithelia of the oviduct, endometrium, and endocervix. The relevance of this close developmental relationship between the OSE and the Müllerian epithelia could explain the frequent acquisition of architectural and functional characteristics of the Müllerian epithelia during neoplastic progression of OSE and the similarities between OSE-derived carcinomas and Müllerian epithelial malignancies. OSE cells from ovaries of women with strong familial history of ovarian cancer frequently undergo Müllerian metaplasia in adult life [46]. According to this hypothesis, EOC could be considered as a result of several genetic alterations involving oncogenes and oncosuppressors with critical role in the normal regulation and development of OSE cells (Table 1). Genetic expression changes depending on the histotype of the epithelial ovarian carcinoma: for instance, Wilms tumor protein type 1 (WT1) is characteristic for the serous subtype of ovarian carcinoma and is rarely found in the non-serous subtypes, whereas endometrial carcinomas express estrogen receptors (ER), but not WT1. Finally, clear cell ovarian carcinomas are negative for ER and WT1 expression and they do not hyper-express p53 protein [60]. Moreover, it seems that mutations and/or hyperexpression of three oncogenes, HER-2/neu, c-myc, and KRAS and the oncosuppressor gene p53, as well as BRACA1 and BRCA2 genes, play a key role in etiopathogenesis of this neoplasia [35]. Epithelial ovarian tumor progression is characterized by a series of epigenetic aberrations, the most important of them are determined by methylation of DNA caused by Ras-association domain-containing family 1 (RASSF1A) genes (oncosuppressor), death-associated protein kinase (DAPK, a protein kinase involved in the regulation the apoptosis), human sulfatase-1 (hSulf-1, sulfatases apoptosis-related), breast cancer 1 gene (BRCA1), and HOXA10 (key gene in promoting many transcriptional fac-
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Function(s)</th>
<th>Cytogenetic band</th>
<th>Author(s)</th>
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<tbody>
<tr>
<td>BRCA1</td>
<td>Breast cancer 1, early onset</td>
<td>E3 ubiquitin-protein ligase that mediates in particular the formation of “Lys-6-linked polyubiquitin” chains and plays a key role in DNA repair. It is not clear whether it also mediates the formation of other types of polyubiquitin chains.</td>
<td>17q21</td>
<td>Aunoble et al. [47]</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Breast cancer 2, early onset</td>
<td>Involved in the repair of DNA strand breaks and/or homologous recombination. Might be involved in the S-phase checkpoint if cellular cycle regulation.</td>
<td>13q12.3</td>
<td>Aunoble et al. [47]</td>
</tr>
<tr>
<td>ABCB1</td>
<td>ATP-binding cassette, sub-family B (MDR / TAP), member 1</td>
<td>P-glycoprotein (ABCB1, MDR1) is a well-characterized ABC Human Transporter, which is involved in the &quot;multidrug resistance&quot;.</td>
<td>7q21.12</td>
<td>Ma et al. [48]</td>
</tr>
<tr>
<td>ABCG2</td>
<td>ATP-binding cassette, sub-family G (WHITE), member 2</td>
<td>Transporter that may play an important role in the exclusion of xenobiotics. Seems to play an important role in the phenotype of drug resistance of different tumor cell lines.</td>
<td>4q22</td>
<td>Bapat et al. [49]</td>
</tr>
<tr>
<td>BMI1</td>
<td>Polycomb ring finger oncogene</td>
<td>Component of the Polycomb group (PCG) multiprotein PRC1, a complex required to maintain the repressed transcriptional state of many genes, including Hox genes, throughout development.</td>
<td>10p11.23</td>
<td>Bapat et al. [49]</td>
</tr>
<tr>
<td>NANOG</td>
<td>Homeobox</td>
<td>Transcriptional regulator involved in the homeostasis of embryonic stem cells. Its action promotes pluripotency of embryonic stem cells and prevents their differentiation.</td>
<td>12p13.31</td>
<td>Bapat et al. [49]</td>
</tr>
<tr>
<td>NES</td>
<td>Nestin</td>
<td>May play a role in the trafficking and distribution of proteins of the Intermediate Filaments and potentially other cellular factors to daughter cells during division of the progenitor cells.</td>
<td>1q23.1</td>
<td>Bapat et al. [49]</td>
</tr>
<tr>
<td>POU5F1</td>
<td>POU class 5 homeobox 1</td>
<td>Transcription factor that forms a complex with SOX2 on DNA and controls the expression of a number of genes involved in embryonic development, as YES1, FGF4, UTF1 and ZFP206. Critical for early embryogenesis and the pluripotency of embryonic stem cells.</td>
<td>6p21.31</td>
<td>Bapat et al. [49]</td>
</tr>
<tr>
<td>TP53</td>
<td>Tumor protein p53</td>
<td>It acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological conditions and on the cell type.</td>
<td>17p13.1</td>
<td>Bapat et al. [49]</td>
</tr>
<tr>
<td>ARL11</td>
<td>ADP-ribosylation factor-like 11</td>
<td>Plays a key role in apoptosis. It can act as a tumor suppressor.</td>
<td>13q14.2</td>
<td>Yang et al. [50]</td>
</tr>
<tr>
<td>CD44</td>
<td>Antigen (homing function and Indian blood group system)</td>
<td>Receptor for hyaluronic acid (HA) Mediates cell-cell interactions and cell-matrix through its affinity for HA, and possibly also through its affinity for other ligands, such as osteopontin, collagen, and matrix metalloproteinases (MMP).</td>
<td>11p13</td>
<td>Zhang et al. [51]</td>
</tr>
<tr>
<td>DAPK1</td>
<td>Death-associated protein kinase 1</td>
<td>Serine/threonine kinase-dependent complex calcium/calmodulin which acts as a positive regulator of apoptosis.</td>
<td>9q34.1</td>
<td>Balch et al. [52]</td>
</tr>
<tr>
<td>HOXA10</td>
<td>Homeobox A10</td>
<td>Sequence-specific transcription factor that is part of a regulation system that provides cells with specific functional polarity.</td>
<td>7p15.2</td>
<td>Balch et al. [52]</td>
</tr>
<tr>
<td>RASSF1</td>
<td>Ras association (RalGDS/AF-6) domain family member 1</td>
<td>Potential tumor suppressor. Necessary for &quot;death receptor-dependent&quot; apoptosis. It mediates also the activation of STK4 (serine/threonine kinase 4) during Fas-induced apoptosis.</td>
<td>3p21.3</td>
<td>Balch et al. [52]</td>
</tr>
<tr>
<td>CJD</td>
<td>Dna1 (Hsp40) homolog, subfamily A, member 1</td>
<td>Absent or down-regulated in many cases of advanced ovarian adenocarcinoma, due to hypermethylation and allelic loss. The loss of its expression correlates with an increase in the resistance to antineoplastic drugs, such as cisplatin.</td>
<td>13q14.1</td>
<td>Strathdee et al. [53]</td>
</tr>
<tr>
<td>MLH1</td>
<td>MutL. homolog 1, colon cancer, nonpolyposis type 2</td>
<td>It forms heterodimers with PMS2 (mismatch repair endonuclease) to form alpha MutL, a component of the system of the post-replicative DNA mismatch repair (MMR). DNA repair is initiated by mts alpha (MSH2-MSH6) or mts beta (MSH2-MSH6) that binds to dsDNA mismatch.</td>
<td>3p21.3</td>
<td>Banez de Caceres et al. [54]</td>
</tr>
<tr>
<td>VHL</td>
<td>von Hippel-Lindau tumor suppressor</td>
<td>Involved in ubiquitination and subsequent proteasome degradation through the von Hippel-Lindau ubiquitination complex.</td>
<td>3p25.3</td>
<td>Banez de Caceres et al. [54]</td>
</tr>
<tr>
<td>MMP1</td>
<td>Matrix metalloproteinase 1 (interstitial collagenase)</td>
<td>Matrix metalloproteinases (MMPs), also called matrixins, are zinc-dependent endopeptidases that are the major protease involved in the degradation of ECM (extracellular matrix).</td>
<td>11q22.3</td>
<td>Yuecheng et al. [55]</td>
</tr>
<tr>
<td>SULF1</td>
<td>Sulfatase 1</td>
<td>Show aril-sulfatase activity and a highly specific as endoglucoasamina-6-sulfatase. It can remove the sulfate from the C-6 position of glucosamine in specific subregions of the molecule of heparin.</td>
<td>8q13.1</td>
<td>Staub et al. [56]</td>
</tr>
<tr>
<td>MYD88</td>
<td>Myeloid differentiation primary response gene (88)</td>
<td>Adapter protein involved in the signaling pathway of Toll-like receptor and IL-1, as part of the innate immune response</td>
<td>3p22</td>
<td>Alvero et al. [57]</td>
</tr>
<tr>
<td>NFkB</td>
<td>Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1</td>
<td>NF-kappa-B is a pleiotropic transcription factor that is present in almost all cell types and is involved in many biological processes such as inflammation, immunity, differentiation, cell growth, apoptosis, and tumorigenesis.</td>
<td>4q24</td>
<td>Alvero et al. [57]</td>
</tr>
<tr>
<td>PROM1 (CD133)</td>
<td>Prominin 1</td>
<td>This gene encodes a transmembrane glycoprotein pentaspan. The protein is localized in extramembranous protrusions and often is expressed on adult stem cells, which are thought to function in the maintenance of stem cell properties by suppressing differentiation.</td>
<td>4p15.32</td>
<td>Curley and al. [58]</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homolog</td>
<td>Tumor suppressor. It acts as a dual specificity protein phosphatase, dephosphorylating tyrosine and serine.</td>
<td>10q23.3</td>
<td>Yang et al. [59]</td>
</tr>
</tbody>
</table>
Hypermethylation of the CpG islands of gene promoters is one of the earliest and most frequent alterations leading to cancer. It is an important epigenetic mechanism for gene silencing, which may confer tumor cells of growth advantage. DNA methylation is the addition of a methyl group at the 5'-carbon of cytosine in CpG context, mediated by DNA methyltransferases (DNMTs) [35]. Of note, hystonic DNA-associated protein are involved in several modifications associated with the permissive and repressive transcription of chromatin [61-63]. Hypermethylation of DNA has a promoter function in ovarian carcinogenesis. Aberrant methylation of normally unmethylated CpG islands, located in the 5' promoter region of genes, has been associated with transcriptional inactivation of several genes in human cancer [64]. Normal epithelial surface cells contain the methylated CpG island in their DNA and do not express the MCJ gene (member of the DNAJ family); Strathdee et al. [56] identified a frequent loss of methylation of the MCJ gene in this setting of gynecological cancer. Thus, there is substantial evidence for DNA methylation and epigenetic regulation in tumors influencing sensitivity to platinum-based chemotherapy and patient survival in ovarian cancer, and this might be useful as a predictive tool for outcome and drug-resistance. Finally, oncosuppressor gene silencing as p16INK4a (4a kinase inhibitor), VHL (Von-Hippel Lindau gene on chromosome 3) and hMLH1 (MutL homolog 1, related to hereditary nonpoliposis colorectal carcinoma), that physiologically lead to hypermethylation of the promoters for human carcinomas oncosuppressor, might be a possible target for molecular diagnosis in cell from biological liquids [57]. Molecular alteration might be related not only to genetic alteration but also to the messenger RNA profiles. Recent studies [65] suggest that microRNAs (miRNAs) are involved in the pathogenesis and progression of epithelial ovarian tumors: Wu et al. [66] has found an upregulation of miR-199a, miR-200a, and a downregulation of miR-214 and miR-100. Another important aspect is that the expression of SPARC (secreted protein, acidic, and rich in cysteine) mRNA is decreased or absent in cell lines of ovarian carcinomas. The hypothesis is that the methylation of the SPARC promoter is an important factor for the genesis the progression of ovarian carcinomas [67]. DNA hypomethylation may promote the expression of tumor suppressor genes, while DNA hypermethylation may decrease or stop the expression of tumor suppressor genes and cause the tumor suppressor genes to lose function. This may result in unrestricted cell growth and ultimately lead to tumorigenesis. DNA methylation has many advantages as a biomarker: its relative stability (in vivo and in vitro), functional links to gene expression, and potential to be detected in cell-free DNA from body fluids.

The new horizon of experimental therapies

In order to improve the outcomes in patients affected by ovarian cancer, many drugs are under evaluation. Regardless of chemotherapy, it has been observed that 5-Aza (5-Aza-cytidine) has a role in restoring the functional expression of E-caderin and in reducing the metalloproteinases activity. 5-Aza through stopping the aberrant methylation of DNA, might represent a new therapeutic scenario [58]. Another possible target in ovarian cancer is hSulf-1. It was characterized to be a heparin-degrading endosulfatase that functions to desulfate cell surface HSPGs and negatively modulate growth factor and cytokine signaling [68]. HSulf-1 protein is widely expressed in normal tissue, but inactivated in majority of various human cancers. Re-expression of hSulf-1 in cancer cells effectively results in a decrease of cell proliferation as well as an increase of sensitivity to chemotherapy-induced apoptosis [68]. Therefore, data suggest that hSulf-1 normally functions as a negative regulator in cell proliferation, and an epigenetic therapy directed against hSulf-1 might sensitize ovarian tumors, in order to render them more vulnerable to conventional first line therapies [59]. In addition, interesting data result from Lewis Y (LeY) antigen: a difucosylated oligosaccharide carried by glycoconjugates on the cell surface. Overexpression of LeY is frequently observed in epithelial-derived cancers and has been correlated to the pathological staging and prognosis. The role of LeY antigen as a cancer-associated antigen in tumorigenesis and development gradually arouses more concern. It can enhance the proliferative and adhesive abilities of cells probably through enhancing the level of several growth factors [70]. LeY is a potential therapeutic target for LeY-positive cancers. Anti-LeY mAb have been shown to have excellent specificity and potential therapeutic value in the treatment of prostate [71], breast [72], small cell lung cancer [73], and also in ovarian cancer it may represent a new potential strategy in treating LeY-positive tumors [70]. Recent evidence suggests that ADP-ribosylation factor-like tumor suppressor gene 1 (ARLTS1) may act as a tumor suppressor gene and facilitate chemosensitivity in ovarian cancer cells by acting synergistic with chemotherapeutic agents to induce the apoptosis signaling pathway and regulate apoptosis-related proteins [53]; of note, a study of Ween et al. [74] focused on the assembly of pericellular matrix containing hyaluronan (HA) and versican. Since it has been shown to be a pre-requisite for proliferation and migration of mesenchymal cells, the Authors investigated whether treatment with recombinant versican could induce the formation of a pericellular matrix by ovarian cancer cells and promote their motility, invasion, and adhesion to peritoneal cells in vitro. They also determined whether versican-induced pericellular matrix formation and metastatic cancer cell behavior could be blocked by small HA oligosaccharides. They concluded that the acquisition of a HA/versican pericellular matrix by ovarian cancer cells increases their metastatic potential. HA oligomers can block this mechanism and might be promising inhibitors of ovarian cancer dissemination.
**EOC's stem cells: diagnostic and therapeutical perspectives**

Recent studies suggest that EOCs, like other solid tumors, contain distinct populations of cells that are responsible for tumor initiation, maintenance, and growth. These cells, termed CSCs, display some of the features of normal stem cells and are thought to evade current chemotherapeutic strategies for the treatment of EOCs. The CSC hypothesis provides an attractive cellular mechanism to explain the therapeutic refractoriness, dormant behavior, and relapse of the disease, which poses a major therapeutic challenge in the case of EOC. Recently, an American Association for Cancer Research (AACR) workshop defined CSC as a malignant cancer cell with a stem cell phenotype [75]. Whilst the CSC hypothesis does not specifically address the mechanisms of malignant transformation, it has been suggested that CSCs are the malignant counterparts of normal adult tissue SCs which, due to dysregulated signaling pathways, are unable to maintain stem cell homeostasis. As well as the normal SCs, also CSCs are thought to reside at the top of the lineage hierarchy and give rise to differentiated cells, which themselves have no potential for self-renewal, and therefore do not contribute significantly to tumor growth. Due to their long life, SCs remain in a tissue for longer periods compared to their differentiated progeny, thereby making them more likely to acquire transforming mutations [76]. It has been demonstrated that CD44+CD117+ cells are often present in EOC. There is growing awareness that EOC is genetically and epigenetically different from normal OSE: the co-expression of epithelial and mesenchymal markers in EOC suggests an involvement of epithelial-mesenchymal transition (EMT) in cancer initiation and progression. In addition, ovarian cancer initiating cells (OCIC) are surprisingly correlated with epigenetic mechanisms of gene regulation in normal stem cells [77]. Ovarian CSCs have a characteristic genetic profile that allows to reform the original tumor mass, to confer drug resistance, and to promote recurrence [78, 79]. Others characteristics of CSC identified in EOC are CD44 and MyD88 positivity (Table 2), the NFkB-complex constitutive activation, increased production of cytokines and chemokines, high proliferation, drug resistance, the TNF-alfa apoptosis-mediated resistance, and the ability to recreate the full phenotypic heterogeneity of the parent tumor [80].

Chemotherapy eliminates the majority of tumor cells, but a nucleus of high proliferative cells with the cited characteristics still remains [57]. Of note, Researchers focused on the role of Müllerian inhibiting substance (MIS) and side population cells. It has been found that SP cells form larger tumors and have higher tumorigenic propensity than do non-SP (NSP) cells, but did so in mouse ovarian cancer cell lines (MOV- CAR7). These stem/progenitor cells were inhibited by MIS, whereas the lipophilic chemotherapeutic agent doxorubicin more significantly inhibited the NSP cells [81]. These findings predict that chemotherapeutic agents and MIS may differentially affect populations in human ovarian cancer that are relatively chemoresistant and demonstrate stem cell characteristics. Accumulating evidence suggests that mesenchymal stem cells are recruited to the tumor microenvironment; however, controversy exists regarding their role in solid tumors. An interesting study of McLean et al. [82], identified and confirmed the presence of carcinoma-associated MSCs (CA-MSCs) in human ovarian tumors. These CA-MSCs had a normal morphologic appearance, a normal karyotype, and were non-tumorigenic. CA-MSCs were multipotent with capacity for differentiating into adipose, cartilage, and bone. When combined with tumor cells in vivo, CA-MSCs promoted tumor growth more effectively than did control MSCs. CA-MSCs had an expression profile distinct from that of MSCs from healthy individuals, including increased expression of BMP2, BMP4, and BMP6. Importantly, BMP2 treatment in vitro mimicked the effects of CA-MSCs on cancer stem cells, while inhibiting BMP signaling in vitro and in vivo partly abrogated MSC-promoted tumor growth. These data suggest that MSCs in the ovarian tumor microenvironment have an expression profile that promotes tumorigenesis and that BMP inhibition may be an effective therapeutic approach for ovarian cancer. CD44 is a surface molecule which mediates cell adhesion and migration by binding extracellular matrix components such as hyaluronic acid, osteopontin, or activating receptor tyrosine kinases, which are related with tumor progression and metastasis [83, 84]. Hyaluronic acid bioconjugates with paclitaxel are being studied to enhance selective entry of cytotoxic drugs into human EOC cells expressing CD44 and for its use in intraperitoneal treatment of ovarian carcinoma [85]. Casagrande et al. [86] found that the subpopulation CD44+...
had a high expression of the genes encoding for claudin-4. Because this tight junction protein is the natural high-affinity receptor for Clostridium perfringens enterotoxin (CPE), Authors investigated the sensitivity of ovarian cancer stem cells to CPE treatment in vitro and in vivo. As a result, a high expression of the high-affinity CPE receptor (claudin-4) at both RNA and protein levels in multiple primary CD44+/NF-kB-high/MyD88+ ovarian cancer stem cell populations was demonstrated. These results are consistent with previous reports demonstrating a high expression of the CPE receptors in multiple primary ovarian cancer cell lines characterized by a high resistance to chemotherapy [87], as well as the study evaluating the proteomes of cisplatin-resistant ovarian cancer cells by Stewart et al. [88], who also found claudin-4 as one of the top differentially expressed proteins in cisplatin-resistant ovarian tumors. Moreover, Alvero et al. [89] showed that the phenyl-substituted isoflavone compound, NV-128, can induce cell death through mitochondrial depolarization and mTOR inhibition. CD117, known as c-kit, is a type III receptor tyrosine kinase involved in cell signal transduction. It has a role in cancer initiating cells from primary human tumors, and has been used as stem cell marker for identification and characterization of hematopoietic stem and progenitor cells, of cardiac CD117-positive stem cells in adult human heart, and other mesenchymal stem cells. High expression level of CD117 was observed in ovarian cancers [49]. Chen et al. [90] demonstrated in vitro that human EOC CD44+/CD117+ cells possessed the properties of tumor chemoresistance to conventional therapies, such as 5FU, docetaxel, cisplatin, and carboplatin. An interesting study of Luo et al. demonstrated that CD117+ ovarian cancer cells had the ability to self-renew, differentiate, and regenerate tumor compared to CD117- in xenograft model [91]. Imatinib, a potent CD117 (c-KIT) specific inhibitor, has been used in clinical trials for the treatment of many types of cancer, including EOC [92]. Since CD117 in ovarian carcinoma was associated with poor response to chemotherapy [90], c-KIT could be a therapeutic target of a tyrosine kinase inhibitor. The glycoprotein CD133 is expressed by a number of progenitor cells including those of the epithelium, where it is expressed on the apical surface [93]. Regarding EOC, Ferrandina et al. [94] demonstrated that CD133+(+) cells gave rise to a larger number of colonies and showed an enhanced proliferative potential, compared to CD133(-) cells. The percentages of CD133-1 and CD133-2 epitopes expressing cells were significantly lower in normal ovaries/benign tumors with respect to those in ovarian carcinoma. Both the percentages of CD133-1- and CD133-2-expressing cells were significantly lower in metastases than in primary ovarian cancer. The Authors did not detect any difference in the distribution of the percentage of CD133-1- and CD133-2-expressing cells according to anatomo-pathologic parameters and response to primary chemotherapy. Moreover, using flow cytometry, these Authors reported that CD133-1 and CD133-2 were both expressed in human ovarian tumors at higher frequency than in normal ovaries and metastatic omental lesions. CD133-1 and CD133-2 may be useful, therefore, to select and enrich population of CD133(+) ovarian tumor cells that are characterized by a higher clonogenic efficiency and proliferative potential [76]. In addition, a study of Curley et al. [58] demonstrated that CD133+ cells derived from ovarian tumors were capable of self-renewal and were associated with increased tumor aggression in xenografts. Of note, the association of CD133+ cells and the aldehyde dehydrogenase (ALDH), a useful marked used for solid tumors, has been studied as a possible set of markers to identify ovarian CSCs. Recent data show that the presence of ALDH(+) CD133(+) cells in debulked primary tumor specimens correlated with reduced DFS and OS in ovarian cancer patients [95]. Chefetz et al. [96] investigated the role of NF-kB in the EOC stem cells. Previous data have shown that EOC stem cells are characterized by constitutive NF-kB activity as well as constitutive cytokine secretion [57, 97, 98]. Aurora-A kinase (Aurora-A) is associated with tumor initiation and progression and is overexpressed in numerous malignancies. In this study the Researchers showed that Aurora-A was overexpressed in ovarian cancer cells compared to OSEs, and described the effect of Aurora-A inhibition in EOC stem cells using a specific inhibitor, MK-5108. MK-5108 decreased the growth of EOC stem cells, induced the formation of multi nucleated cells and arrest the cells in G2/M phase. Moreover, MK-5108 abrogated the NFkB activity, as well as cytokine and chemokine secretion in the EOC stem cells [96]. The subpopulation isolation from the SKOV3 cell line offers a suitable in vitro model for studying ovarian CSCs in terms of their survival, self-renewal, and chemoresistance, and for developing therapeutic drugs that specifically interfere with ovarian CSCs [48]. Zhao et al. [99] studied the inhibitory effect of human umbilical cord mesenchymal stem cells infected by an adenoviral vector containing interleukin 12 gene on the proliferation of ovarian carcinoma SKOV3 in vitro and the growth of tumor explants in nude mice. Using this approach they observed an inhibition of the proliferation, an induction of apoptosis of ovarian carcinoma SKOV3 cells in vitro, and a suppression of the growth of ovarian cancer explants in nude mice. More recent studies have been proposed to identify CSC by using approaches based on the expression of markers as POU5F1 (OCT4: octamer-binding transcription factor 4), NANOG (a transcription factor expressed in embryonic stem cells), BMI1 (polycomb ring finger oncogene), NESTIN (intermediate filament type 4 protein), and ABCG2 (ATP-binding cassette sub-family G member 2), that are in compliance with functional in vitro and in vivo assays. It might be beneficial to develop a targeted therapy directed against CSC and to use it in order to improve the effect of conventional chemotherapies. In this setting, an immunotherapeutic procedure has been tested, based on the findings that EOC expresses well-defined target antigens that are capable of stimulating an antitumor immune response [100]. A subset of
OVCA cells with a CD44+ phenotype was isolated in samples from patients with OVCA that possessed CSC properties and the use of immunotherapy using fusions of dendritic cells and OCIC to specifically target the OCIC subpopulations was explored. Fusion cells prepared in this way activated T cells to express elevated levels of IFN-γ with enhanced killing of CD44+ OVCA cells. A combined therapy may represent a promising approach for the treatment of OVCA, since conventional therapies kill the bulk of tumor cells, whereas OCIC-reactive cytotoxic T lymphocytes potentially target the resistant OCIC fraction [101].

Conclusion

In the last years, a growing scientific knowledge about the molecular pathways involved in ovarian carcinogenesis has led to the discovery and evaluation of several novel molecular targeted agents, with the aim to test alternative models of treatment in order to overcome the clinical problem of resistance. Ovarian cancer patients initially respond well to surgical cytoreduction and chemotherapy, but the majority of patients who respond to primary chemotherapy ultimately develop recurrent, usually drug-resistant, disease that is conceivably due to the ability of ovarian cancer stem cells to escape these drugs. Data suggest that EOCs, like other solid tumors, contain distinct populations of cells that are responsible for tumor initiation, maintenance and growth. These cells, termed cancer stem cells, display some of the features of normal stem cells and are thought to evade current chemotherapeutic strategies for the treatment of EOCs. Distinguishing CSC-associated antigen profiles may elucidate novel, more sensitive biomarkers for early detection of EOCs and provide molecular targets for the development of new treatment modalities. It has become imperative to further investigate normal adult ovarian stem cells in order to elucidate their potential role in ovarian cancer onset, since there is an urgent need for better predictive molecular markers that characterize early oncologic transformation to permit earlier detection, to uncover additional therapeutic targets, and to change therapeutic protocol.

References

Syndecan-1 serves as a marker for the progression of epithelial ovarian carcinoma

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Summary
Purpose: Syndecan-1 (SDC-1) promotes the proliferation of cancer cells and plays a role in angiogenesis by binding to a variety of extracellular effectors. The present study was designed to compare the expression of SDC-1 in the normal ovary and in ovarian tumors, to better understand its role in the progression of epithelial ovarian carcinoma (EOC). Materials and Methods: The expression of SDC-1, fibroblast growth factor 2 (FGF-2), and FGF receptor 1 (FGFR1) and their transcripts in 65 samples including the normal ovary, benign tumors, borderline tumor, and ovarian tumors was assessed using immunohistochemistry and the reverse transcription-polymerase chain reaction. The influence of FGF-2 on the expression of SDC-1 mRNA syndecan-1 in a human ovarian carcinoma cell line was determined using an FGF-2-neutralizing antibody. Results: SDC-1 was not detected in normal ovarian tissue but was present in the epithelial cells of benign or borderline tumors and in ovarian adenocarcinomas. The levels of expression were significantly different in ovarian tissues derived from benign or malignant cases. Coordinate stromal expression of SDC-1 and its mRNA was detected at the original site of the tumor, as well as in metastatic foci in the greater omentum of ovarian adenocarcinomas. FGF-2 reduced the level of expression of SDC-1 mRNA when added exogenously to SKOV3 cells. This effect was abolished in the presence of an FGF-2-neutralizing antibody. Conclusion: SDC-1 contributes to the role of FGF-2 in proliferation and angiogenesis but may also play a role in the invasive properties of EOC. To the present authors’ knowledge, this study is the first to report the presence of distinct patterns of expression of SDC-1 in local and metastatic foci in the greater omentum in patients with EOC. These data reinforce the role of the tumor stroma in the invasive properties of ovarian adenocarcinoma and suggest that stromal changes in the expression of SDC-1 may originate from the stroma and contribute to the pathogenesis and metastatic potential of EOC.

Key words: Syndecan-1; FGF-2; FGFR1; Ovarian carcinoma.

Introduction
Syndecans are members of a family of cell surface proteoglycans that play regulatory roles in wound healing, inflammation, angiogenesis, and neuronal patterning. There are four members of the syndecan family (syndecan-1, syndecan-2, syndecan-3, and syndecan-4), each comprising an ectodomain carrying heparan sulfate- or chondroitin sulfate-rich glucosaminoglycan chains, a transmembrane domain, and a short cytoplasmic tail. The syndecans can bind structural proteins of the extracellular matrix and growth factors, such as basic fibroblast growth factor (FGF-2) [1, 2]. Syndecan-1 (SDC-1) expression is associated with poorly differentiated tumors, and loss of expression of SDC-1 by epithelial cells correlates with poor clinical outcomes in patients with squamous cell carcinoma of the head and neck [3–5], mesothelioma [6], poorly differentiated non-small cell lung cancer [7, 8] and in patients with hepatocellular carcinoma with high metastatic potential [9]. In gastric cancer [10, 11] and in breast carcinoma [12], the stromal expression of SDC-1 correlates with a poor prognosis. In contrast, pancreatic adenocarcinoma cells overexpress SDC-1 compared with normal pancreatic cells [13], indicating that SDC-1 plays different roles in the growth of tumors.

Epithelial ovarian cancer (EOC) is the second most common female genital tract malignancy after endometrial cancer. They account for approximately 15,000 annual deaths in the United States. Patients often present with disseminated disease because they may be asymptomatic. This can be explained by containment of the tumor cells within the ovary, as well as by the early dissemination of tumor cells to the peritoneal cavity. SDC-1 is expressed by ovarian cells and can be detected within the extracellular matrix [14]. The goal of the present study was to clarify the relationship between the expression of SDC-1 and the progression of EOC.
Materials and Methods

Subjects and tissues

Patients’ clinical data included those for age, menarche, menstrual cycle, pregnancy and childbirth history, serum CA125, mass size, clinical stage, and clinical characteristics. The tumors were staged according to the guidelines of the International Federation of Gynecologists and Obstetricians (FIGO) stage, based on surgical and histological assessments. Samples of frozen ovarian tissue were taken from biopsies of patients admitted to the China Medical University’s Shengjing Hospital for Cancer Research during 2003 to 2007. The tissues were cut into five-µm diameter cubes, frozen in liquid nitrogen within one hour of resection, and stored at –80°C. Samples of normal ovarian tissues were obtained from the archive of the Shengjing Hospital of China Medical University. The Ethics Committee of the China Medical University approved this study. Histological samples were reviewed by a histopathologist specializing in gynecological oncology. Samples that were included in the study were classified as those obtained from the normal ovary, benign ovarian tumors, epithelial tumors of borderline malignancy, or primary ovarian adenocarcinomas. Histological grade was assessed as moderately or poorly differentiated.

Immunohistochemistry

The antibodies used for immunohistochemistry were as follows: mouse anti-human syndecan-1 antibody, CD138 Ab-1 (5F7), rabbit anti-fibroblast growth factor 2 (FGF-2), and rabbit anti-FGF receptor 1 (FGFR1) PV9000 kit. Embedded tissue samples were cut into seven-µm sections. The slides were placed in a staining rack at room temperature and deparaffinized with dimethyl benzene and rehydrated in a graded alcohol series. After washing with PBS, 3% hydrogen peroxide was used to block non-specific endogenous peroxidase activity. High temperature and pressure were used for antigen retrieval. After blocking with 10% normal serum, slides were incubated with primary antibody overnight at 4°C. Slides were washed in phosphate-buffered saline (PBS), incubated with secondary antibody, and then treated with PV9000 reagents, followed by staining with 3,3-diaminobenzidine and counterstaining with hematoxylin. Slides were dehydrated in a graded alcohol series and mounted for analysis.

Two independent observers who were not aware of the clinicopathological information inspected all slides. The staining patterns were scored using a semiquantitative scoring system. Scores of 0–3 were given according to the intensity and the percentage of cells stained as follows: 0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from tissues using the RNAout kit by following the manufacturer’s instructions and was stored at –80°C. Samples (100 mg) were mixed with one ml RNAout dissolved in RNase-free water. Primer sequences were as follows: SDC-1, sense: agctgaccttcacactcc and anti-sense: tcggctcctc- caaggagt; FGF2, sense: agggegtgaactcaaaaac and anti-sense: cccaggtcctgttttggat; FGF2, sense: cagggatcctggttgatt; FGFR1, sense: cccgatgcctggttgatt and anti-sense: accatgcaggagatgaggaa; β-actin, sense: gtggggtgcaccaggcacca, and anti-sense: gttggggtgcaccaggcacca. RT-PCR reactions were performed as follows: rcDNA synthesis was performed in a total volume of ten µl (MgCl2, two µl; RNAase free dH2O, three µl; dNTP, one µl; RNase inhibitor, 0.25 µl; avian myeloblastosis virus reverse transcriptase, 0.5 µl; oligo-DT 0.5 µl; RNA, one µl). The conditions used for the procedure were as follows: 42°C for 30 minutes, 99°C for five minutes, and 5°C for five minutes. For PCR amplification of β-actin, FGF-2, and FGFR1, the following conditions were used: total volume, 25 µl (5×RNA Buffer, five µl; dH2O, 13.75 µl; dNTP, two µl; each primer, 0.5 µl; Taq polymerase, 0.25 µl; cDNA, three µl). The PCR protocol for β-actin, FGF2, and FGFR1 was as follows: 94°C for two minutes; 30 cycles of 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 45 seconds; and 72°C for seven minutes. The PCR protocol for SDC-1 was as follows: 94°C for two minutes; 35 cycles of 94°C for 45 seconds, 52°C for 60 seconds, 72°C for 60 seconds; and 72°C for seven minutes. PCR products were electrophoresed using 1.5% agarose gels. Data were analyzed using a GIS-2020 gel image analytical system with β-actin as the standard.

Total RNA was extracted from cultured cells using the RNAout kit by following the manufacturer’s instructions. RT-PCR reactions were performed as follows: Reverse transcription was performed using the reagents described above; the SDC-1 primers 5'-CCCTGA AGA TCA AGA TGG CTC T-3' (sense) and 5'-CCC GAG GTT TCA AAG GTG AAG T-3' (antisense) (563 bp) and the β-actin primers gttggggtgacccagggca (sense) and ctctcaatgtgcagcagattc (anti-sense) were used. PCR reaction conditions for SDC-1 and β-actin were as follows: 30 cycles of 30 seconds at 94°C and 30 seconds at 55°C; and extension for one minute at 72°C. PCR products were analyzed as described above.

Cell culture

SKOV3 cells were cultured in Ham’s F-12 medium supplemented with 10% fetal bovine serum (FCS), 50 IU/ml penicillin, 50 µg/ml streptomycin, and 50 µg/ml L-ascorbic acid (F12/FCS); incubated at 37°C in an atmosphere containing 5% CO2; and used at passages 2–6. SKOV3 cells grown in 12-well plates (2.5 × 104 cells/well) were serum-starved for 48 hours. FGF-2 (100 pg/ml, one ng/ml, 10 ng/ml) or 10% FCS were then added to each well in each group for 24 hours. Media and growth factors were changed daily.

Statistical analysis

Chi-square tests were performed to compare the frequency of SDC-1 staining in benign and malignant cases by using SPSS version 13.0. A p < 0.05 was considered statistically significant. Two independent samples were analyzed as appropriate to compare relative optical densities of benign and malignant cases, and differences between positive staining and negative staining cases were evaluated using SPSS version 13.0. A p < 0.05 was considered statistically significant.

Results

Clinical characteristics of patients

The mean age of subjects was 34.4 years (range, 21–63). The ages of members of the EOC group were higher than those of patients with benign or borderline lesions. The values of serum CA 125 of the EOC group were significantly higher than those of the other groups, with no significant differences in the sizes of foci of patients with EOC compared with those of the other three cases. The most common symptom of EOC was abdominal distension. The majority of the lesions were benign, followed by 60% and 36% frequencies of Stage III and Stages I-II, respectively (Table 1).

The expression of SDC-1, FGF-2, and FGFR1 in local foci of ovarian lesions

SDC-1 was detected in three cases (10%) of benign lesions (two endometrial cysts, one teratoma) and was present around
Syndecan-1 serves as a marker for the progression of epithelial ovarian carcinoma. In one case (25%) of a borderline lesion, staining was present in the cytoplasm of glandular epithelial cells and scored as 1. In eight cases (32%) of EOC (Stage III), staining was localized to membranes and, in seven cases, to the cytoplasm of the glandular epithelial cells. Staining in two cases was localized to the stroma and was assigned scores of 1–2. The expression of SDC-1 was undetectable in normal ovarian tissue (Figure 1).

The expression of FGF-2 was detected in three (10%) patients with benign lesions (one endometrial cyst, two teratomas) and staining (scored 1–2) was localized to the cytoplasm of glandular epithelial cells. In eight (32%) patients with EOC (three, Stage I; five, Stage III), staining was localized to the cytoplasm of glandular epithelial cells (scored 2–3). FGF-2 expression was undetectable in either normal ovarian tissue or in borderline lesions (Figure 2).

Levels of expression SDC-1, FGF2, and FGFR1 in the primary foci of ovarian lesions

The differences in the levels SDC-1 and FGF2 mRNAs between benign lesions and EOC agreed with the results of the immunohistochemical analyses. In contrast, the level of expression of FGFR1 mRNA differed between those of patients with benign lesions and with EOC ($P < 0.05$). There was also a significant difference between staining patterns and the levels of mRNA expression of the positive and negative groups (Figure 3).

The expression of syndecan-1, FGF-2, and FGFR1 in metastatic foci present in the greater omentum

SDC-1 was detected in eight patients with EOC (one, Stage IV; seven, Stage III), among which staining was localized to the glandular epithelium in four cases, the stroma in seven cases (+), and to both the glandular epithelium and stroma in three cases (score for glandular epithelium = 2, score for the stroma = 2–3). The expression of FGF-2 was detected in four patients with EOC (Stage III) and in one

Table 1. — Clinical data

<table>
<thead>
<tr>
<th></th>
<th>Normal (2)</th>
<th>Benign (27)</th>
<th>Borderline (5)</th>
<th>Epithelial ovarian cancer (26)</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>43 ± 12.7</td>
<td>34.4 ± 12.8</td>
<td>42.8 ± 16.7</td>
<td>54.4 ± 9.4</td>
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<td>Menarche</td>
<td>13.5 ± 0.7</td>
<td>14.1 ± 1.8</td>
<td>14 ± 1.2</td>
<td>15.1 ± 2.3</td>
</tr>
<tr>
<td>Cycle</td>
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<td>28.3 ± 2.3</td>
<td>31.6 ± 4.8</td>
<td>29.2 ± 1.6</td>
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<td>Focus</td>
<td>4.2 ± 1.4</td>
<td>7.5 ± 4.6</td>
<td>9.7 ± 6.1</td>
<td>10.5 ± 4.4</td>
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<tr>
<td>Gravidity</td>
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<td>1.7 ± 1.4</td>
<td>1.8 ± 1.1</td>
<td>2.7 ± 1.3</td>
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<tr>
<td>Parity</td>
<td>0.5 ± 0.7</td>
<td>0.6 ± 0.6</td>
<td>1 ± 1.2</td>
<td>1.4 ± 0.9</td>
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<tr>
<td>Serum CA 125 (u/ml)</td>
<td>10.4 ± 4.7</td>
<td>57.8 ± 86.8</td>
<td>132.8 ± 105.8</td>
<td>846.5 ± 623.1</td>
</tr>
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<td>Chief complaint</td>
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<tr>
<td>Asymptomatic</td>
<td>15</td>
<td>3</td>
<td></td>
<td></td>
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<tr>
<td>Abdominal pain</td>
<td>6</td>
<td></td>
<td>5</td>
<td></td>
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<tr>
<td>Menstruation disorder</td>
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<td>2</td>
<td>1</td>
<td></td>
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<tr>
<td>Dysuria</td>
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<tr>
<td>Abdominal distension</td>
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<tr>
<td>Mass</td>
<td>1</td>
<td></td>
<td>3</td>
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<tr>
<td>Anorexia</td>
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<td></td>
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<td>Low-grade fever</td>
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</tr>
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<td>Types of disease</td>
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<tr>
<td>Endometrial cyst</td>
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<tr>
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<tr>
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<tr>
<td>Adenofibroma</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boundary cystadenoma</td>
<td></td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystadenocarcinoma</td>
<td>25</td>
<td></td>
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</tr>
</tbody>
</table>

The expression of syndecan-1, FGF-2, and FGFR1 in metastatic foci present in the greater omentum

SDC-1 was detected in eight patients with EOC (one, Stage IV; seven, Stage III), among which staining was localized to the glandular epithelium in four cases, the stroma in seven cases (+), and to both the glandular epithelium and stroma in three cases (score for glandular epithelium = 2, score for the stroma = 2–3). The expression of FGF-2 was detected in four patients with EOC (Stage III) and in one
patient with a boundary lesion (score = 1–2, with staining localized to the cytoplasm of glandular epithelial cells). The expression of FGFR1 was detected in nine patients with EOC (Stage III) and one patient with a boundary lesion (score = 1–2, with staining localized to the cytoplasm of glandular epithelial cells (Table 2).

The influence of FGF-2 on the expression of SDC-1 in SKOV3 cells

When cultured in the presence of 10% FCS, SKOV3 cells expressed moderate levels of SDC-1 mRNA, which increased with time. When FGF-2 was added to the culture, the expression of SDC-1 mRNA was inhibited and its level
Syndecan-1 serves as a marker for the progression of epithelial ovarian carcinoma.

 did not change with time. When an FGF-2-neutralizing antibody was added, the level of expression of SDC-1 mRNA was restored to that without FGF-2 and became weaker with time (Figure 4).

**Discussion**

Epithelial ovarian cancer can occur in female individuals as young as 15 years; however, the mean age at presentation age is 56 years. The median age for presentation of ovarian adenocarcinoma is between 60 and 65 years. In the present study, the age range for patients with EOC was 21–63 years. Childbearing and contraception reduces the risk of developing ovarian cancer. In the present study, the gravidity and parity of patients with EOC were 2.7 ± 1.3 and 1.4 ± 0.9, respectively, and were not significantly different from their values in the benign group.

The levels of CA125 in serum serve as a prognostic marker for EOC. In the present study, the levels of CA 125 were 57.8 ± 86.8 µl/ml, 132.8 ± 105.8 µl/ml, and 846.5 ± 623.1 µl/ml for benign lesions, borderline adenomas, and EOC, respectively. Because the location and characteristics of the ovary, many patients remain asymptomatic. In the present study, the first common symptom of patients with EOC was abdominal distension (56%), and the five-year survival rate of patients presenting with advanced disease (64%, 16/25) was lower (14%–38%).

**Table 2.** Immunohistochemical analysis of tumor foci in the greater omentum of patients with EOC or borderline ovarian lesions (30 cases).

<table>
<thead>
<tr>
<th>Protein</th>
<th>Site</th>
<th>HSCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDC-1</td>
<td>Glandular epithelium</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Stroma</td>
<td>23</td>
</tr>
<tr>
<td>FGF-2</td>
<td>Glandular epithelium</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Glandular epithelium</td>
<td>20</td>
</tr>
</tbody>
</table>
Syndecans are members of the cell-surface heparan sulfate peptidoglycan family and are ubiquitously expressed in a wide range of cells. They play important roles in a variety of cellular functions, including cell adhesion, differentiation, and migration. SDC-1 was first detected by RT-PCR analysis of mRNAs isolated from patients with myeloma, and its expression by all myelomas was subsequently confirmed by immunohistochemical analysis with a monoclonal antibody [15, 16]. As a cell surface receptor, SDC-1 is intimately involved in normal and pathological events by regulating cell–cell and cell–matrix interactions, cell migration, development, neovascularization, microbial pathogenesis, and tumorigenesis [17-20]. Alterations in SCD-1 expression are associated with strongly aggressive phenotypes of some cancers and indicates poor prognosis of patients with breast, ovarian, and pancreatic cancers as well as in those with gliomas [21-27].

In the present study, using immunohistochemical analysis, the authors detected the expression of SDC-1 in the glandular epithelium in 8/25 cases of EOC with a mean staining score of 1.04. In contrast, SDC-1 was detected in only 3/30 patients with benign lesions of the ovary, with a mean staining score of 0.43. These results are consistent with those described for tissues taken from patients with pancreatic [28] or prostate cancer [29]; however, they do not correlate with the expression patterns of gastric, lung, cervical, and head-and-neck cancers [30-33]. Therefore, SDC-1 might facilitate the development of EOC through its regulation at the transcriptional level because the levels of SDC-1 and its mRNA change coordinately [34].

SDC-1 participates in proliferation, migration, and cell-matrix interactions [35], and localizes to the cell surface as well as intracellular compartments [36, 37]. SDC-1 binds and sequesters growth factors, including members of the fibroblast growth factor family [38] and acts as a coreceptor to facilitate signaling through FGFRs. FGFs stimulate not only mitogenesis but also angiogenesis, which is required for tumors to grow larger than two mm. FGFs mediate their biological effects by binding to FGFRs, which are high-affinity cell-surface receptors with protein tyrosine kinase activity. The most widespread expression was observed for FGFR1 and FGFR2. For example, high levels of immunoreactive FGFR1 were detected in the skin, cornea, lung, heart, placenta, kidney, and urethra, and moderate levels were detected in the testis and ovary. FGFR2, FGFR3, and FGFR4 are expressed at relatively low levels in ovaries [39].

FGF-2 plays an important role in oncogenesis [40-43]. Fujimoto et al. reported that increased levels of the expression of FGF-2 in advanced primary ovarian cancers indicate that FGF-2 may accelerate the growth of ovarian cancer cells [44]. SDC-1 regulates cell growth and differentiation in part by modulating the interactions of growth factors with their cellular receptors [45]. In the present study, analysis of ECOs revealed higher levels of FGF-2 and FGFR1 and their transcripts in the cytoplasm of epithelial cells. It was thought that SDC-1 together with FGF-2/FGFR1 as a coreceptor played a role in the development of EOC. Further, coexpression of SDC-1 and FGF-2/FGFR1 was detected in epithelial cells in only one case of Stage III EOC. In another study, no significant relationship was noted between expression of SDC-1 and FGF-2 in malignant mesotheliomas in vivo [46].

Here, when FGF-2 was added to cultures of SKOV3 cells, the expression of SDC-1 mRNA was inhibited, indicating that upregulation of SDC-1 expression occurred before the level of expression of FGF-2 changed. Metastatic foci in the greater omentum are known to proliferate at higher rates than primary tumor cells, leading to more rapid progression of tumor dissemination. The current analyses of the levels of SDC-1 expression in the glandular epithelium and stroma indicate that SDC-1 likely contributes to the invasiveness of EOC.

Reciprocal interactions between epithelial tumor cells and stroma play a very important role in facilitating tumor cell growth and migration in patients with breast cancer [47]. The induction of SDC-1 expression in reactive stromal fibroblasts creates a favorable microenvironment for accelerated tumor cell growth and angiogenesis. Thus, SDC-1 joins a group of molecules that are aberrantly expressed in the stromal compartment and contribute to carcinoma progression [48]. Cancer-associated stroma may contribute to tumor cell invasion and the development of metastasis. Here, the authors found moderate levels of expression of SDC-1 in stroma in two cases of local foci derived from patients with Stage III EOC. Staining was weakly positive in the cytoplasm of glandular epithelial cells but was not detectable in those of the other groups. The intensity of SDC-1-staining in metastatic foci of the greater omentum in patients with EOC was more frequent and intense compared with the glandular epithelium. The present authors believe it is therefore reasonable to conclude that the changes in the levels of expression of SDC-1 in local versus metastatic foci indicate that SDC-1 plays a role in the invasiveness of EOCs.

The present study also shows that the elevated levels of expression of FGF-2 (5/30) and FGFR1 (10/30) in metastatic foci in the greater omentum indicate more active mitogenesis and angiogenesis than those seen in localized tumors. The current results wherein exogenously added FGF-2 was found to downregulate the expression of SDC-1 mRNA in SKOV3 cells lead the authors to speculate that stromal SDC-1 may arise from ectodomain shedding [49-51] from the cell membranes of EOC cells or from stromal cells. However, only four cases of ECO were positive for SDC-1-staining in local and metastatic foci. Therefore, they conclude that SDC-1 is not shed but is expressed by the stromal cells. Growth factors, such as FGF-2, and the accumulation of SDC-1 within the tumor stroma, may contribute to extensive angiogenesis and stromal proliferation.
Conclusion

In summary, the authors present novel data on the expression of SDC-1 in local and metastatic foci in patients with EOC. These findings implicate and reinforce the contribution of stromal expression and changes in the expression of SDC-1 in the pathology and metastasis of ovarian cancer.

Acknowledgment

This study was granted by the “985” project of Sun Sat-yat University (No. 82000-3321302) to Dr. Lin Ma.

References


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Confluence analysis of multiple omics on platinum resistance of ovarian cancer

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Obstetrics and Gynecology Hospital of Fudan University, Shanghai (China)

Summary
Objective: The study aimed to provide novel insight into the mechanism of platinum resistance of ovarian cancer. Materials and Methods: RNA-seq data ERP000710 were obtained from Gene Expression Omnibus database, including specimens from six platinum sensitive samples and six platinum tolerance samples. The author analyzed the data of the 12 samples as a whole because of the low flux sequencing. Single nucleotide polymorphisms (SNPs) were identified between platinum-sensitive and platinum-tolerant samples using VARSCAN, followed by functional prediction of the SNPs. After processed by Btrim software, the data were subjected to Cuffdiff for the identification of differentially expressed genes (DEGs), followed by function and pathway enrichment analysis. In addition, VARSCAN software was used to detect the specific mutations in platinum tolerance samples, combined with functional prediction of mutations. Results: The author obtained 38 new SNPs after excluding 22 SNP from dbSNP database and 1000 Genomes Project and found ESRP1, LDHA, DDX5, and HEXA were associated with platinum resistance of ovarian cancer. Totally, 290 upregulated and 157 downregulated genes were selected. Biological processes such as immune response, inflammatory response, and response to wounding and pathways such as cell adhesion molecules, calcium signaling, and NOD-like receptor signaling pathways were enriched with upregulated genes. Cell-cell signaling, cell morphogenesis, and basal cell carcinoma pathway were related to downregulated genes. Conclusion: Based on high-throughput RNA-seq data and confluence analysis of multiple omics, the author explored the biological mechanisms on platinum tolerance of ovarian cancer, which may provide new ideas and methods for further research.

Key words: Ovarian cancer; Differentially expressed genes (DEGs); Single nucleotide polymorphisms (SNPs); Function and pathways enrichment.

Introduction
Ovarian cancer is a type of tumor from uncontrolled cell growth in several different parts of ovary. It has a high incidence of morbidity and mortality among all gynecological cancers [1]. Currently, it has been reported that the five-year survival of ovarian cancer is roughly 30%, since a large proportion of cancer was diagnosed at an advanced stage [2]. Previous studies revealed that a number of possible factors are thought to be involved in the cause of ovarian cancer, such as age (especially in the elder infertile women) [3], family history of ovarian cancer or breast cancer [4], and abdominal distension. In addition, as far as we know, hereditary forms of ovarian cancer by far can be caused by mutations in specific genes (most notably BRCA1 and BRCA2) [5]. However, only ten percent of ovarian cancer has a genetic link.

Treatment for ovarian cancer involves surgery, chemotherapy, a combination of surgery with chemotherapy, and sometimes radiotherapy [6]. The kind of treatment depends on many factors, including the type, stage and grade of ovarian cancer, as well as the general health of the patient. Currently, surgery is the first choice for the treatment of ovarian cancer [7] while the developmental combination chemotherapy regimen of MECCA (consisted by mitomycin C, etoposide, cisplatin, and carboplatin) was utilized for the purpose of significantly longer survival after the primary cytoreductive surgery [8]. However, intrinsic or acquired resistance of cell to cisplatin limits the effect of chemotherapy in cancer [9]. Early studies suggest that cisplatin enters the cells (malignant or non-malignant cells from cancer patients) and forms platinum-DNA adduct that reacts with nucleophilic sites in cellular macromolecules [10]. The objective of this adduct is to trigger cell cycle arrest and apoptosis. There are many reasons that may lead to resistance to cisplatin: increased DNA repair ability, decreased intracellular concentration because of decreased drug uptake, and increased reflux or increased inactivation through sulfhydryl molecules [9, 10]. It is reported that about 25% of patients exhibit primary resistance at an early stage of chemotherapy, and for the other 75%, approximately 15% to 20% of them resisted to chemotherapeutic cisplatin adducts after relapse [11]. Consequently, platinum resistance is a core problem in treatment.

For an improved understanding of platinum resistance mechanisms, in the present study, the author screened specific mutations in platinum resistance samples by analyzing
the mRNA expression profile data of ovarian cancer from Expression Omnibus (GEO) database. Also, the author predicted specific mutation functions of platinum resistance samples and discussed the biology functions and pathways of the screened differentially expressed genes (DEGs). This research has a potential to explore platinum resistance mechanism of ovarian cancer, which will provide a theoretical guidance for effective therapies, or for approaches that can improve therapeutic responses to the treatment of ovarian cancer.

Materials and Methods

RNA-seq data of ovarian cancer

The mRNA expression profile data of ovarian cancer were obtained from NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/) Gene Expression Omnibus (GEO) database (access number: ERP000710) [12, 13], and the Platform was Illumina Genome Analyzer IIX. A total of 12 cell specimens from two cases of high-grade serous ovarian carcinoma were acquired before and after clinical platinum resistance developed in patients, including six platinum-sensitive samples and six-platinum tolerance samples. Due to the data deficiency in each sample, the six platinum-sensitive samples were regarded as one group, while the six platinum-tolerant samples were the other group. The author analyzed the information of the 12 samples in the two groups.

Data processing and genome mapping

Btrim [14] software was used to preprocess the raw data. A number of five consecutive bases were treated as a window before the author calculated the average weight. If the average weight was less than 20, the author trimmed the end of the sequence as well as the sequence whose reads length was less than 35 bp. Afterwards, Ensembl GRCh37 was used for reference sequence and tophat2 [15] software was utilized for genome mapping. Only the reads which mapped to specific genome locations were retained.

Screening for new single nucleotide polymorphisms (SNPs)

Samtools [16] was applied for the purpose of mapping the processed data into mpileup format. Then, through software VARSAN [17], the author analyzed the mutations. The mutation selection criteria is zero frequency in the platinum-sensitive samples whereas > 0.2 frequency in the samples of platinum resistance. Moreover, SNPs annotation was obtained after the screening by Seattle [18], followed by removing SNPs from dbSNP database and the 1000 Genomes Project. Retained SNPs were new mutations namely, the specific mutations of platinum-resistant samples.

Functional prediction of specific mutations

In terms of SNPs located on genes, the author queried the functions of SNP located genes in NCBI, and forecasted the possible influence of new SNPs on gene function. As for the SNPs located between genes, according to ENCODE data, were analyzed for the influence they made at genome regulation level.

Differentially expressed genes (DEGs) analysis

Cuffdiff [19] was utilized to identify DEGs. To ensure the results reliability, genes only with the [fold change] value > 2, p-value < 0.05 and FPKM (reads per kilobase of exon model per million mapped reads) ≥ 1 at least in one sample were selected as DEGs.

Table 1. — Function distributions of new SNPs.

<table>
<thead>
<tr>
<th>Function</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intron</td>
<td>10</td>
</tr>
<tr>
<td>Intergenic</td>
<td>5</td>
</tr>
<tr>
<td>Coding-synonymous</td>
<td>5</td>
</tr>
<tr>
<td>Missense</td>
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</tr>
<tr>
<td>Missense-near-splice</td>
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</tr>
<tr>
<td>Utr-3</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
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</table>

Table 2. — Missense SNP loci.

<table>
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<th>Chromosome</th>
<th>Position</th>
<th>GeneList</th>
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<td>5</td>
<td>176764384</td>
<td>LMAN2</td>
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<tr>
<td>7</td>
<td>134851617</td>
<td>C7orf49</td>
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<td>8</td>
<td>95677177</td>
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<td>10</td>
<td>135123755</td>
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<td>12</td>
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<td>15</td>
<td>72645480</td>
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<td>19</td>
<td>6677984</td>
<td>C3</td>
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</table>

Function and pathway enrichment analysis

Gene Ontology (GO) [20] analysis was used for functional annotation of upregulated and downregulated genes using the Database for Annotation, Visualization and Integrated Discovery (DAVID) online tool [21]. Through the gene-annotation enrichment analysis, it is possible to reduce the dimension of the data and increase the likelihood for researchers to identify the most relevant biological processes they need [20]. A p-value < 0.05 and count value > 2 was chosen as the cut-off criterion. Moreover, DAVID online tool was utilized to analyze Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in which DEGs were enriched [22]. The count number > 2 and p-value < 0.05 were chosen as cut-off criteria.

Results

Specific mutations screening of drug resistance samples

Based on VARSAN, the author selected 59 specific mutations of platinum resistance samples. Afterwards, annotation of the mutation sites was obtained, and 22 SNPs from dbSNP database and 1000 Genomes Project were excluded. The author finally obtained 38 new SNPs. The function distributions of the 38 new SNPs are shown in Table 1.

Functional prediction of specific mutations

Initially, the author conducted mutation functional prediction of nine missense SNP loci (Table 2), and found epithelial splicing regulatory protein 1 (ESRP1), lactate dehydrogenase A (LDHA), hexosaminidase A (HEXA), and (DEAD (Asp-Glu-Ala-Asp) box helicase 5) (DDX5) were closely related to platinum resistance. Next, the author analyzed the other 29 new SNPs, and found that these loci did not cause protein structure changes while they were likely to
Confluence analysis of multiple omics on platinum resistance of ovarian cancer

Influence genome regulation level. However, sites with regulatory function were not found in these 29 SNPs through ENCODE data.

**Identification of DEGs**

Based on the Cuffdiff, the author obtained 447 DEGs of ovarian cancer, of which 290 were upregulated DEGs and 157 were downregulated DEGs.

**Function and pathway annotation of DEGs**

To explore the roles of DEGs in platinum resistance in ovarian cancer, the author adopted both GO biological process enrichment analysis and KEGG pathway enrichment analysis to analyze upregulated and downregulated DEGs. The enriched GO categories of up-regulated genes, such as behavior, chemotaxis, immune response, leukocyte chemotaxis, and leukocyte migration are listed in Table 3, while the biological processes with a highly significant correlation with downregulated genes, such as cell-cell signaling, cell motion, cell morphogenesis, and negative regulation of cell proliferation are listed in Table 4. Through KEGG pathway enrichment analysis, the author found four significant pathways that were enriched by upregulated genes, including chemokine signaling pathway, ribosome, cell adhesion molecules (CAMs), calcium signaling pathway, and NOD-like receptor signal-

---

**Table 3. — Top ten ranked biological processes enriched of upregulated DEGs.**

<table>
<thead>
<tr>
<th>Term</th>
<th>Count</th>
<th>Genes</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>GO:0007610—behavior</td>
<td>23</td>
<td>EGR1, CXCL1, S100P, CCL2, IL8, S100A9, OBP2B, CXCL1, CCL2, DDO, PLAUR, CXCL10, SAA2, CCL20, SAA1, CXCL16, GRIN2D, GRP, ADRA1B, CHRN4B, IL1B, CHRN2B, PLAU</td>
<td>5.99E-09</td>
</tr>
<tr>
<td>GO:0007626—locomotory behavior</td>
<td>18</td>
<td>CXCL1, CCL2, IL8, S100A9, CX3CL1, CCL28, PLAUR, CXCL10, SAA2, CCL20, SAA1, CXCL16, GRIN2D, ADRA1B, CHRN4B, IL1B, CHRN2B, PLAU</td>
<td>6.33E-09</td>
</tr>
<tr>
<td>GO:0042330—taxi</td>
<td>14</td>
<td>CXCL1, CCL2, IL8, S100A9, CX3CL1, CCL28, PLAUR, SAA2, CCL20, SAA1, CXCL16, IL1B, PLAU</td>
<td>1.77E-08</td>
</tr>
<tr>
<td>GO:0006935—chemotaxis</td>
<td>14</td>
<td>CXCL1, CCL2, IL8, S100A9, CX3CL1, CCL28, PLAUR, SAA2, CCL20, SAA1, CXCL16, IL1B, PLAU</td>
<td>1.77E-08</td>
</tr>
<tr>
<td>GO:0006955—immune response</td>
<td>26</td>
<td>CXCL1, IFIH1, CCL2, VTCN1, SUSD2, RSAD2, TNFSF14, IL32, CX3CL1, CCL28, TNFSF18, IFI35, CXCL10, IL23A, CCL20, PGLYRP2, IL1B, IL1A, EBI3, ICAM1, IL8, IL1RN, OASL, UNC13D, CXCL16, IFI6</td>
<td>8.57E-08</td>
</tr>
<tr>
<td>GO:0030595—leukocyte chemotaxis</td>
<td>8</td>
<td>CCL2, SAA2, IL8, SAA1, CXCL16, S100A9, IL1B, CX3CL1</td>
<td>1.16E-07</td>
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<tr>
<td>GO:0050900—leukocyte migration</td>
<td>9</td>
<td>ICAM1, CCL2, SAA2, IL8, SAA1, CXCL16, S100A9, IL1B, CX3CL1</td>
<td>1.61E-07</td>
</tr>
<tr>
<td>GO:0060326—cell chemotaxis</td>
<td>8</td>
<td>CCL2, SAA2, IL8, SAA1, CXCL16, S100A9, IL1B, CX3CL1</td>
<td>1.70E-07</td>
</tr>
<tr>
<td>GO:0006952—defense response</td>
<td>24</td>
<td>CXCL1, IFIH1, CCL2, NMI, IL8, S100A8, IL1RN, S100A9, RSAD2, IL32, CX3CL1, CXCL10, LGALS3BP, UNC13D, IL23A, SAA2, CCL20, SAA1, CXCL16, PGLYRP2, SERPINA3, IL1B, SERPINA1, IL1A</td>
<td>1.72E-07</td>
</tr>
<tr>
<td>GO:0006954—inflammatory response</td>
<td>17</td>
<td>CXCL1, CCL2, NMI, IL8, S100A8, IL1RN, CXCL10, UNC13D, IL23A, SAA2, CCL20, SAA1, CXCL16, SERPINA3, IL1B, SERPINA1, IL1A</td>
<td>4.39E-07</td>
</tr>
<tr>
<td>GO:0009611—response to wounding</td>
<td>20</td>
<td>CXCL1, CCL2, NMI, IL8, S100A8, IL1RN, S100A9, PLAUR, CXCL10, UNC13D, IL23A, SAA2, CCL20, SAA1, GRIN2C, SERPINA3, IL1B, SERPINA1, PLAU, IL1A</td>
<td>4.21E-06</td>
</tr>
</tbody>
</table>

DEGs: differentially expressed genes; GO: Gene Ontology; Count: gene numbers; BP: biological process; FDR: false discovery rate.

**Table 4. — Biological processes enriched of downregulated DEGs.**

<table>
<thead>
<tr>
<th>Term</th>
<th>Count</th>
<th>p-value</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0007267—cell-cell signaling</td>
<td>10</td>
<td>0.003291</td>
<td>FGF5, CBLN1, BMP2, CXCL14, APOE, LPAR3, SLC22A3, GJA1, KCNQ2, WNT6</td>
</tr>
<tr>
<td>GO:0006928—cell motion</td>
<td>7</td>
<td>0.03385</td>
<td>NDN, FOXJ1, TUBBP1, TNR, VIM, SCNN1G, ISL1</td>
</tr>
<tr>
<td>GO:0009002—cell morphogenesis</td>
<td>6</td>
<td>0.035021</td>
<td>BMP2, NDN, FOXJ1, TNR, GJA1, ISL1</td>
</tr>
<tr>
<td>GO:0048858—cell projection morphogenesis</td>
<td>5</td>
<td>0.036334</td>
<td>NDN, FOXJ1, TNR, GJA1, ISL1</td>
</tr>
<tr>
<td>GO:0008285—negative regulation of cell proliferation</td>
<td>6</td>
<td>0.036849</td>
<td>BMP2, GPC3, NDN, FOXJ1, APOE, GJA1</td>
</tr>
<tr>
<td>GO:00303030—cell projection organization</td>
<td>6</td>
<td>0.039506</td>
<td>NDN, FOXJ1, TNR, LPAR3, GJA1, ISL1</td>
</tr>
<tr>
<td>GO:0032990—cell part morphogenesis</td>
<td>5</td>
<td>0.041612</td>
<td>NDN, FOXJ1, TNR, GJA1, ISL1</td>
</tr>
<tr>
<td>GO:0048754—branching morphogenesis of a tube</td>
<td>3</td>
<td>0.043402</td>
<td>BMP2, GPC3, MYCN</td>
</tr>
<tr>
<td>GO:0042063—gliogenesis</td>
<td>3</td>
<td>0.043402</td>
<td>FGF5, NDN, SOX11</td>
</tr>
</tbody>
</table>

DEGs: differentially expressed genes; GO: Gene Ontology; Count: gene numbers; BP: biological process.
ing pathway (Table 5). Furthermore, downregulated genes were significantly enriched in basal cell carcinoma pathway (Table 6).

**Discussion**

At present, with the development of high-throughput sequencing technologies and large-scale clinical trials being carried out, pharmacogenetics has played an increasingly important role in the field of cancer chemotherapy. Due to the better understanding of clinical drug resistance-related SNP, researchers have been exploring the reality of the growing list of genetic mechanisms of cancer and chemoresistance [23]. Nowadays, RNA-Seq can carry on the gene expression difference studies in the whole-genome level [24]. Research used this method to show that quantitative analysis is more accurate and reliable, because the analysis testing range is wider of higher repeatability [25]. In addition to analysis of gene expression, RNA-Seq can also detect new SNP, transcripts, splice variants, and provide an allele-specific gene expression [26]. The wider dynamic range and a smaller false positive of RNA-Seq data should be higher than the chip [25]. In this study, RNA-Seq data were used to detect DEGs and SNPs in platinum resistance of ovarian cancer.

It is clear that the mechanism of ovarian cancer resistant to platinum drug is quite complex, and it has a great many unresolved problems that need to be assessed. Studies have demonstrated that DNA repair undoubtedly affects resistance to platinum-based DNA-damaging agents, and processes involved DNA repair that contribute to more than one drug resistance phenotype [10, 27]. ESRP1 belongs to the RNA-binding protein family which is critical in post-transcriptional control of RNAs, such as RNA splicing, mRNA stabilization, and mRNA localization [28]. In this paper, in querying gene functions of SNPs located, the author found that it is not easy for ESRP1 to regulate RNA splicing, and the mutation loci of N260Y was on the RNA recognized motif of ESRP1. It has also been reported that ESRP1 is associated with the cancer metastasis of lung and is considered as a potential therapeutic target for the prevention of metastasis [29]. Thus, we could hypothesize that mutation of N260Y loci in ESRP1 might lead to the destruction of RNA recognition function, and some changes in the form of gene splicing, and then result in the occurrence of drug resistance. In addition, LDHA, DDX5, and HEXA gene were also found to have mutated in platinum resistance of ovarian cancer. Specifically, LDHA catalyzes L-lactic acid and NAD to generate anaerobic pyruvate and NADH; moreover, recent studies have shown that LDHA inhibition contributes to increased apoptosis through production of reactive oxygen in certain cells, and LDHA might play an role in the metastasis of tumors [12, 30-32]. LDHA may mediate platinum resistance by influencing tumor growth and metastasis. DDX5 is a kind of RNA helicase which plays various roles in cell proliferation, and several studies have revealed that it is associated with tumor initiation and progression [33, 34].

The present author obtained a total of 447 significant DEGs, including 290 upregulated DEGs and 157 downregulated DEGs. In the meantime, by GO biological process enrichment analysis and KEGG pathway enrichment analysis, they screened several processes and pathways which may involve in mechanism of ovarian cancer platinum resistance. Of all biological progress that upregulated DEGs enriched in, immune response, inflammatory response and response to wounding appeared to play a crucial role in drug resistance in ovarian cancer cells. Consistently, Interferon regulatory factor 1 (IRF1), an important transcription factor (TF) in the regulation of immune, is linked to platinum sensitivity in high-grade serous ovarian cancer [35]. The involvement of IL-6 in platinum resist-

<table>
<thead>
<tr>
<th>Term</th>
<th>Count</th>
<th>p-value</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa04062: chemokine signaling pathway</td>
<td>9</td>
<td>2.41E-03</td>
<td>CXCL1, CCL2, IL8, CCL20, ADCY9, CXCL16, CX3CL1, CCL8, CXCL10</td>
</tr>
<tr>
<td>hsa03010: ribosome</td>
<td>6</td>
<td>4.82E-03</td>
<td>RPS27, RPL41, RPS29, RPL34, RPL39, RPL13AP5</td>
</tr>
<tr>
<td>hsa04514: cell adhesion molecules (CAMs)</td>
<td>7</td>
<td>6.43E-03</td>
<td>CLDN8, ICAM1, CLDN9, CLDN3, ITGB8, CLDN6, SDC4</td>
</tr>
<tr>
<td>hsa04020: calcium signaling pathway</td>
<td>7</td>
<td>2.41E-02</td>
<td>ADCY9, TNNC1, GRIN2C, GRIN2D, GRPR, ADRA1B, ITPR3</td>
</tr>
<tr>
<td>hsa04621: NOD-like receptor signaling pathway</td>
<td>4</td>
<td>4.42E-02</td>
<td>CXCL1, CCL2, IL8, IL1B</td>
</tr>
</tbody>
</table>

DEGs: differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes; Count: gene numbers.
ance in ovarian cancer has also been validated, and is mediated by upregulation of cellular inhibitor of apoptosis 2 (cIAP-2) expression [36]. In addition, it has been revealed that cell adhesion pathways may contribute to the tolerance in ovarian cancer cells [37]. Appropriate calcium intake is believed to reduce the risk of ovarian cancer [38]. Increasing studies have suggested that NOD-like receptors are associated with reproductive diseases, such as endometrial cancer [39, 40]. The study also suggested that upregulated calcium signaling pathway and NOD-like receptor signaling pathway might be related to the platinum resistance in ovarian cancer.

With the evaluation of detection technology and standardized test results, scientists will be able to detect more DEGs and pathways which are platinum-resistant related in peripheral blood of tumor patients, make a preliminary estimate of the sensitivity to chemotherapeutic drugs, and then design the best treatment plan, which will enable possible individualized chemotherapy with a real significance.

Conclusion

In the present study, based on high-throughput second generation sequencing data, the author used dynamic gene expression profile combined with different levels of biological information. The author identified important mutations such as ESRP1, LDHA, DDX5, and HEXA in this logical information. The author identified important mutations, such as ESRP1, LDHA, DDX5, and HEXA in this logical information. The author identified important mutations, such as ESRP1, LDHA, DDX5, and HEXA in this logical information. The author identified important mutations, such as ESRP1, LDHA, DDX5, and HEXA in this logical information. The author identified important mutations, such as ESRP1, LDHA, DDX5, and HEXA in this logical information.

References


Factors contributing to the low participation rate of Turkish women to a breast cancer screening program in Antwerp, Belgium

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2 Department of Social Medicine and Epidemiology, Antwerp University, Wilrijk (Belgium)

Summary
Objectives: To explore possible factors explaining a low participation rate to breast cancer screening for Turkish women living in Antwerp, Belgium, and to develop ways to increase participation rate. Material and Methods: The authors used focus group discussions with Turkish women to explore their reasons to participate or not to participate in breast cancer screening. Groups consisted of four to six women. Inclusion criteria were: being female, having a Turkish origin, and age between 50 and 69 years. For each focus group, one Turkish women was invited and asked to invite five other women meeting the inclusion criteria. Results: Three focus group discussions with in total 17 women have taken place. Six women had participated to all consecutive invitations for breast cancer screening. One woman had participated once, but not the next time she was invited. Ten women had never participated to screening mammography, although some of them had undergone diagnostic mammography. In all three focus group discussions, insufficient knowledge of the Dutch language, the unavailability of a professional interpreter, being careless about healthcare, and a negative influence of the husband, were the main reasons not to participate in breast cancer screening. Invitation letters are not read because they are in a language the woman does not understand. Less frequently mentioned obstacles were being on a holiday or being sick on the day of the scheduled mammography, fear of pain, considering an examination useless when not having any symptoms, being anxious for a positive result, and the physical distance to the screening center. Receiving an invitation in Turkish and knowing that a person speaking Turkish will be available at the screening center were proposed as possible measures to improve participation. Conclusion: The single most important reason why Turkish women living in Antwerp, Belgium, do not participate in breast cancer screening was a language problem; other reasons were a lack of knowledge concerning breast cancer screening and not worrying about breast cancer. The language barrier in this population of older women can possible be overcome by Turkish speaking personnel at the screening centers.

Key words: Breast cancer screening; Ethnic minorities; Migrants; Prevention; Participation (rate); Belgium; Turkish.

Introduction
Breast cancer is the most common cancer in women and constitutes the most frequent cause of cancer related death of women in Belgium. Since 2001 there is a national screening program in Flanders (the northern region of Belgium) for breast cancer. All women between 50 and 69 years of age are invited biannually for screening mammography. The over-all participation rate in Flanders in 2010-2011 was 50.2%. Some groups demonstrate a lower participation rate, specifically those from ethnic minorities.

Turkish women living in the Netherlands have been noted to participate in only 44%, whereas the general participation rate is over 80% [1]. The causes for this low participation rate are not completely understood. For this study Turkish women living in Antwerp, a city in Flanders with an important Turkish community, have been selected as these represent one of the major groups of ethnic minorities in Antwerp.

Materials and Methods
The authors performed focus group discussions, groups consisted of four to six women with a trained moderator who was also a native Turkish speaker with a medical background (TF). The methodology used was such as described by Morgan [2]. Before the actual discussion, a script had been prepared. Care was taken that at least all questions from the script had been addressed by the end of the discussion. The script included a general presentation of breast cancer screening, then the discussion was opened by informing whether women had, or had not, received the invitation letter from the screening center and how they had reacted to this. Later it was asked why they did or did not participate in the screening and what had influenced this decision, furthermore questions about breast cancer in family or friends were posed. Finally the participants were stimulated to present solutions to eventual barriers to participation, before ending the focus group discussion an open question was asked offering the opportunity to give further comment. Discussions have been tape-recorded but have not been filmed. To document non-verbal communication, an observer noted all non-verbal communications during the focus group discussion.

Inclusion criteria for the selection of focus group members were: being female, aged between 50 and 69, and of Turkish origin. The
The age category is identical to the age women in Flanders receive an invitation for the breast cancer screening program. Women were considered of being of Turkish origin if Turkish was their mother language and they had been born in Turkey. All focus group discussions were performed in Turkish only. For each focus group, only one woman was contacted and she was asked to bring four to five other women with her at the moment of the focus group discussion. All discussions have been completely transcribed and translated into Dutch as this is the mother language of the other members of the research group. Every reason for non-participation to the breast cancer screening program that was given by a woman, received a code.

**Results**

Three focus group discussions occurred. Saturation was reached after the second group discussion, but as a control, a third focus group discussion was held. In the first there were seven participants and the mean age was 57 years. The focus group discussion took 75 minutes and there were no participants with a history of breast cancer. The second group consisted of four women. The focus group discussion took 35 minutes, the mean age of the participants was 60 years and none of the participants had ever had breast cancer. The third focus group consisted of six participants, mean age was 56 years. The discussion took 64 minutes and one of the participants had been diagnosed with breast cancer five years before the discussion. Table 1 presents the participation of these women to screening mammography.

All reasons mentioned by the women not to participate in the breast cancer screening program were reduced to 19 codes and these are presented in Table 2. The most frequently mentioned reason by all three groups were problems with the language and translation. The language problem represented a barrier at the moment the invitation letter arrived. Often Turkish women were unable to read, let alone understand, this letter. Suggestions from the group were to make the letter in Turkish, but other women responded that this has no use as they are analphabetics. The problem with the language also disables the woman even when they finally understand the invitation letter to go to the screening mammography as they have fear not to understand what will be said or asked them. They are also anxious as they think they will not be able to understand the results. All these women are first generation migrants with a very limited knowledge of the Dutch language as they have neither worked nor studied in Flanders. Another problem that was mentioned is that it is another member of the family that selects and reads letters that arrive at home, for instance, the husband or one of the children, and as they are not themselves concerned with this invitation, they immediately drop it. Women stated:

- **Focus Group Discussion 1, participant 3 (FGD1,3):** “we do not want to go to the doctor as we do not know the language”.
- **FGD 3,1:** “we are unable to go if there is not a helper that goes with us because we do not know the language”.
- **FGD 3,4:** “we all are the same, as we do not know the language we do not have the courage and we will not go”.
- **FGD 1,3:** “I have never seen such a letter, other people who live in the house can also have taken the letter”.

The problem of not having an interpreter is closely connected to the language problem. Usually it is not a professional interpreter but family members, such as daughters or husband or sons. If these family members are unable to take free time to go to the mammography unit, they will not go and the mammography will not be taken.

The interpreter also had to read, translate, and explain the invitation letter. Women stated:

- **FGD 1,4:** “no we cannot read, there are daughters for that”.
- **FGD 2,2:** “why we will not go? Because there is no interpreter, we do not understand so we will not go”.

### Table 1. — Participation rate of women to screening mammography

<table>
<thead>
<tr>
<th>Number</th>
<th>FGD 1</th>
<th>FGD 2</th>
<th>FGD 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Always participate to screening mammography</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Drop-out</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Never participated to screening mammography (already had a diagnostic mammography)</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Total amount of women</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>17</td>
</tr>
</tbody>
</table>

**FGD:** Focus Group Discussion.

### Table 2. — Reasons for low participation rate of Turkish women

<table>
<thead>
<tr>
<th>Code</th>
<th>FGD 1</th>
<th>FGD 2</th>
<th>FGD 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Language problem</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Interpreter problem</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Carelessness</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Negative influence husband</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Being already sick</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Being on a holidays</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Not worry about</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Hospital phobia</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Not seen the letter</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Laziness</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Analphabetism</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Transport</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Fear for pain</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Not having any symptoms</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Do not care</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Anxious for positive result</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Having a good mood</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Distance too far</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Already underwent diagnostic mammography</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**FGD:** Focus Group Discussion.
Factors contributing to the low participation rate of Turkish women to a breast cancer screening program in Antwerp, Belgium

Discussion

For this study the authors chose a focus group discussion instead of a questionnaire, because to gain information on this topic, they expected qualitative research to generate more in-depth and useful information on the often very complex reasons of non-attendance. First of all, because of the interaction in a FGD, more reasons will be thought and mentioned compared to the information collected by questionnaire. Moreover, not much is yet known regarding reasons and opinions of breast cancer screening participation in Turkish women in Flanders. FGD are a very good method to gain more insight into explorative research. As far as the authors know, this is the very first study in a population of Turkish migrant women on breast cancer screening participation. In FGD, questions are open and reasons originate from the women themselves and not from the examiner. Focus group discussions constitute qualitative research, hence this study does not provide quantitative data on the relative importance of each particular explanation for non-participation. Data from this study can be used to construct further quantitative analysis.

Women recruited in this study originated from seven different provinces of Turkey. It would be interesting to know the participation rate of Turkish women in Turkey as a comparator. There is no national breast cancer screening program in Turkey [3,4]. One pilot study in Balıkesir in the period 2004 to 2006 demonstrated a 74.2% participation rate [5]. This extremely high value can be explained because the women were first selected, then received an educational program on breast self examination, and the invitation for the mammography was individually delivered at home by a midwife. If the women did not arrive at the moment of mammography, the midwife visited her at home for a second time. Such a very intensive program is not likely to be organized for a complete region let alone a country.

In a Dutch study, the low participation rate for Turkish and Moroccan women was stated to be caused by poor knowledge on screening and on socio-cultural aspects [6]. In Turkey it has been shown that age, education, being married, and having breast cancer in the family were not related to the participation rate for mammography [7]. Women with a lower social economic status and a more traditional and religious view seem to accept disease and look at it as coming from God. In a Turkish study performed in Izmir, the same reasons as in the present study have been described: the lack of symptoms, being careless, and not needing screening as they do not perceive breast cancer as a personal risk [8]. Also
fear of poor diagnosis and pain from the mammography were present as was also noted by our focus groups. In a Turkish study the fear for pain was reduced by education and it was also shown that education can increase “breast awareness” [9-11]. In Flanders no data are known on the prevalence of breast cancer in Turkish migrants versus the autochthonous population. In a Dutch study, the standardized incidence rate for Turkish women as compared on autochthonous Dutch women was only 0.29 [12]. Similar results were obtained in Germany for Turkish women living in Hamburg [13]. A lower incidence of breast cancer in Turkish migrants has also been demonstrated in Australia [14]. Both studies suggesting that breast cancer risk is less in Turkish women. One bias in these numbers can be that women who become sick want to migrate back to their land of origin. Although the frequency of breast cancer in Turkish women seems to be lower, it still constitutes the most frequent cancer in women in Turkey, up to 24% of cancers in Turkish women. A higher incidence of breast cancer is seen in second and third generation migrants. Contrary to the older generations of Turkish migrants who want to return to Turkey when diagnosed with cancer, as they want to die in their own country, the younger generation does not make this move and remains in the country they live in. It has also been suggested that the younger generation has taken over a Western lifestyle that negatively influences the incidence of breast cancer [15-17].

Reasons for not participating in breast cancer screening can be reduced to two major components. The first one is the language problem and all difficulties caused by a language barrier, such as reading the letter and needing an interpreter at the moment of the mammography. The second problem is the lack of knowledge on the disease and on the process of screening, not realizing the differences between screening mammography and diagnostic mammography.

A solution for the language problem is not easy to find. The law in Belgium does not allow any other language than Dutch in the Flemish region to be used for any official document such as an invitation for breast cancer screening. Trying to adapt the language laws has been known to result in major political crisis in the recent past. Even if another language could be used, there is no official list of who is member of the Turkish community or speaks and understands only Turkish. This is difficult because some women from Turkish origin, unable to communicate in Dutch, do have Belgian nationality, while others have only Turkish nationality. In the future this problem will diminish as the younger generation has been working and studying in Dutch. However even then for recently migrated women the problem will persist. At the moment an obligatory language course for newcomers has been introduced and the effect of this is still yet to be seen. Another part of the non-participation could be improved by education, given in small groups in their own language or by their individual family physician. Actually FGDs themselves were educational moments and at the end of the discussion all participants stated that they will take part in the screening the next time. Of course this does not mean that they actually will do this.

Acknowledgements
The study was approved by the ethical committee of Antwerp University.

References

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Pathologic characteristics and prognosis of a rare advanced cervical cancer treated with radical surgery and radiotherapy

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Summary

Objective: To assess the prognosis of rare advanced cervical carcinoma with post-radical-radiation surgery and to compare the clinical value between further surgery treatment group and non-surgery group after radical radiation therapy. Materials and Methods: From January 2002 to July 2010 there were 68 patients with advanced stage cervical carcinoma retrospectively analysed in Maternal and Child Health Hospital of Jiangxi Province. All patients were confirmed by histopathology before treatment, and clinical staging was based on updated 2009 FIGO staging. All patients were Stage Ib2 (local advanced) and more severe. There were 36 patients (29 adenocarcinoma (AC), six adenosquamous carcinoma (ASC), and one undifferentiated carcinoma) classified into observation group that was treated with radical radiation therapy + surgery (total hysterectomy + bilateral salpingo-oophorectomy); other 32 patients (26 AC, five ASC, and one undifferentiated carcinoma) classified into control group that was treated with radical radiation therapy with no further surgery. The radical radiation therapy included external-beam radiation and intracavitary therapy, standard point A dose added up to 85 Gy (these doses are recommended for most patients based on summation of conventional external-beam fractionation and low-dose-rate 40~70 cGy/h brachytherapy equivalents), and 45~55Gy was given to point B. All of the patients were followed up. The average follow-up time was 65.6 months and the survival rate between two groups were compared and analyzed whether there was residual lesion, metastasis, lymph vascular space invasion (LVSI) in the observation group. Results: In observation group there were 15 patients found positive. The positive rate was 41.7% (15/36), in which there nine cases with LVSI and residual foci, four cases with uterus invasion, and one case with only residual foci. Both of the two groups were followed up and the average follow-up time was 65.6 months (range 36~136). In observation group there were 25 cases that have survived until now and the average survival time was 66.6 months (range 36~136 ). Eleven patients died with an average survival time of 10.4 months (range 2~37). In control group there are 22 cases that survived until now (July 2013); the average survival time was 64.4 months (range 36~136 ). Ten patients died with an average survival time of 10.3 months (range 3~28 ). Three cases experienced serious complication in observation group and two cases in control group. There was no significant difference in survival time between the two groups. Conclusion: Due to low efficacy results, post-radical-radiation surgery is not a feasible treatment regimen for rare advanced cervical carcinoma.

Key words: Rare pathological type; Cervical carcinoma; Radical radiation therapy; Surgery.

Introduction

Cervical carcinoma is the second most common gynecologic malignancy and the third most common cause of cancer death in the world, especially in developing countries. Each year there are about 471,000 new cases and 233,000 death cases [1, 2]. While developed countries have witnessed a steady decline in the incidence of cervical cancer since the implementation of cytological cervical cancer screening, it does not occur in developing countries and cervical cancer still occurs in countries where screening has been implemented. In recent years, the incidence of cervical adenocarcinoma (AC), adenosquamous carcinoma (ASC), and other rare pathologic type gradually increased [3-6] while the age of these patients is getting younger and younger [7-10]. It was reported that the prognosis of AC or ASC was not as good as squamous carcinoma (SCC) [11-13]. There are still controversies about its post-radical radiation risk such as residual foci status, lymph vascular space involvement (LVSI), uterine invasion and so on, and it is still inconclusive whether it is necessary to remove the primary tumor site after radical radiation [14, 15].

In the latest NCCN guideline [16] there is no difference in the treatments of AC, ASC, and SCC, and it does not mention whether it is necessary to increase the radiation dose of AC and ASC. In this study 68 patients with advanced stage cervical carcinoma were diagnosed in Maternal and Child Health Hospital of Jiangxi Province. Thirty-six patients underwent radical radiation therapy + surgery. Thirty-two patients underwent radical radiation therapy no further surgery. This study aimed to assess the prognosis of rare pathological type advanced cervical carcinoma with post-radical-radiation surgery and compared the clinical value between further surgery treatment group and non-surgery group after radical radiation therapy.
Materials and Methods

Inclusion criterion
1) All patients were treated in Maternal and Child Health Hospital of Jiangxi Province from January 2002 to July 2010. 2) All of them were confirmed by histopathology. 3) Pelvic examination was taken by more than three gynecologic oncology professors to confirm the clinical stage. 4) All clinical staging was based on updated 2009 FIGO staging. 5) All post-operational specimens underwent comprehensive pathological examinations.

Patients
Sixty-eight patients with cervical carcinoma were studied. There were 36 patients (29 AC, six ASC, and one undifferentiated carcinoma) classified into observation group that treated radical radiation therapy + surgery (total hysterectomy + bilateral salpingo-oophorectomy); In observation group there were four Stage Ib2 cases, one Stage IIa case, 18 Stage IIb cases, two Stage IIIa cases, 11 Stage IIIb cases, and the median age was 46 years (range 32–65). Other 32 patients (26 AC, five ASC, and one undifferentiated carcinoma) classified into control group that were treated with radical radiation therapy with no further surgery. Five Stage Ib2 cases, three Stage IIa cases, 13 Stage IIb cases, one Stage IIIa cases, and ten Stage IIIb cases were in control group and the median age was 47 years (range 32–64).

Both of the two groups were followed up. Follow-up was performed via telephone and outpatient examination. The terminal time was July 2013.

Radiation
Patients were treated with radical radiation therapy plus concurrent cisplatin (DDP 25 mg/m2/week) chemotherapy. Conventional radiotherapy consisted of external beam radiotherapy (EBRT) and high-dose rate intracavitary brachytherapy (HDR-ICBT). Different schedules and doses were administered for Stage IIB and III because of differences in extension of tumor in the parametrium and vagina. EBRT was performed with a dose of

Table 1. — Clinical and post-operational pathology data of 36 patients in observation group.

<table>
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<th>NO.</th>
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<th>Uterine invasion</th>
<th>Follow-up (M)</th>
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P = positive; N = negative; S= survival; D = dead.
Table 2. — Outcome of 32 patients in control group.

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<th>Pathological type</th>
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</table>

S = survival; D = dead.

1.8 Gy per fraction, five times each week. Doses of EBRT at point B were as follows: for Stage IIB, 45 Gy/25 fractions/eight weeks (using a center shield of four cm width) and for Stage III, 50 Gy/28 fractions/eight weeks (the center shield was set up after a whole pelvic irradiation of 30 Gy). The treatment field generally extended from L5 to the inferior border of the obturator foramen. The lateral margin was two cm from the bony pelvis. After two weeks from the initiation of EBRT, patients received ICBT using iridium-192 HDR brachytherapy unit, twice weekly (ovoid and tandem each once a week). Patients with Stage IIB received 50 Gy in ten fractions to point A, whereas patients with Stage III received 35 Gy in seven fractions. Vaginal gauze packing was used to increase the distance from the brachytherapy source to the rectal and bladder walls. Using this HDR-ICBT, the dose constraints for the rectum and bladder were less than 40 Gy for Stage IIB and less than 30 Gy for Stage III. Vaginal mold was supplemented for patients with middle or lower vaginal involvement. A dose of 25–40 Gy/five to eight fractions/two to three weeks was delivered to a depth of 0.5 cm below the vaginal mucosa. Altogether, the sum doses to point A were 50 Gy for Stage IIB and 65 Gy for Stage III; the doses to point B were 60 Gy; the doses for rectum and bladder were not above 40 Gy for Stage IIB and 60 Gy for Stage III.

**Surgical method**

The patients in observation group underwent surgery six to eight weeks after the radical radiation. Extrafascial hysterectomy + bilateral salpingo-oophorectomy was the main surgical method.

**Surgical findings, bleeding volume, and operative time**

Varying degrees of pelvic congestion and edema, connective tissue fibrosis, and adhesions between the pelvic organs were detected in the surgery. The average operative time was 1.5 hours (rang 1.0–2.8) and the average bleeding volume was 95 ml (range 30–200).

**Positivity of post-operative pathology**

The pathologic characters included one of the following risk factors: 1) residual foci; 2) LVSI; 3) uterine invasion.

**Statistical analysis**

Patient characteristics, tumor response, and toxicity were evaluated using descriptive summary statistics. OS were calculated using the Kaplan-Meier method. The survival rate was calculated using binomial distribution. Statistical analyses were performed using SPSS ver. 17.0. A p-value < 0.05 was considered statistically significant.

**Results**

In the observation group, 15 patients were found positive after surgery, of which nine cases with LVSI, four with uterus invasion, and one case with only residual foci. Both of the two groups were followed up, and the average follow-up time was 65.6 months (rang 36–136). In observation group there are 25 cases that survived up to now (July 2013), the average survival time was 66.6 months (rang 36–136). Eleven patients died of which two with pulmonary metastasis, one with intestinal metastasis, three with uncontrolled tumor, and five with recurrence. The median survival time for observation group patients was 10.4 months (rang 2–37). The three-year OS rate was 72.22%. In control group 22 cases survived up to now; the average survival time was 64.4 months (rang 36–130). Ten patients died of which two with pulmonary metastasis, two with intestinal metastasis, one with uncontrolled tumor, and five with recurrence. The median survival time for control group patients was 10.3 months (rang 3–28). The three-year OS rate was 68.75%. The clinical and pathological data of two groups are shown in Tables 1 and 2 in and Figure 1.

**Discussion**

The therapeutic effect of cervical SCC has received recognition in the world, whereby radiosensitivity of carcinoma cells plus chemotherapy increase-sensitivity. Patients with AC (or other rare pathological types) of the uterine cervix have a poorer prognosis than those with squamous cell carcinoma of a similar disease stage [17, 18].
For rare pathological type of cervical cancer, there are no specific standard treatments and also no relevant clinical trials demonstrating the efficacy of radical radiation therapy or surgery.

In the current study, the authors found that surgery after radical radiation failed to improve PFS and OS as compared to radical-radiation-only in patients with rare pathological type of cervical cancer (three-year OS, 68.20% vs. 68.75%, respectively). The median survival time was very close (10.4 vs. 10.3 months). In the present observation of intraoperative findings, varying degrees of pelvic congestion increased the chances of intraoperative bleeding assuredly, tissue edema, and connective tissue fibrosis with different levels of adhesion that also increased the difficulty of the operations and the complications. In the present observation of patients postoperatively, who underwent surgery after radiotherapy compared with those that did not receive radiation therapy had longer postoperative healing time and more postoperative complications, including poor wound healing, lower life quality, and inevitable surgical damage (e.g., ureteral injury). These findings suggested that the surgery after radical radiation may not be effective in improving clinical outcomes in patients with rare pathological type of cervical cancer as compared to radical-radiation-only — not to mention the surgical complications.

The present study showed there were 15 patients (41.7%) positive found in observation group after radical radiotherapy; residue was found in vascular, uterus, and cervix. Regardless of the higher positive rate, there is no point in surgery because of the mediocre survival rate and the poor quality of life. However the study demonstrated that rare pathological type of cervical cancer can have a certain residual rate of tumor after radical radiation, which may increase the risk of recurrence. This may explain the reason of low cure rate of cervical AC. In addition to surgery, can another more effective clinical treatment be found? The present authors did not find similar research that be compared to th present study results and the number of enrolled patients was small which may have resulted in study data bias. In spite of these limitations, the present study indicated that, due to relatively low efficacy, post-radical-radiation surgery is not a feasible treatment regimen for rare pathological type advanced cervical carcinoma. However, in a handful of patients in post-radical-radiation surgery, we can see the merit in young patients who have faster healing time, better treatment effect, and higher quality of life. In patients with a strong desire to operation, the surgery means the end of the tumor.

**References**


Pathologic characteristics and prognosis of a rare advanced cervical cancer treated with radical surgery and radiotherapy

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Stage IVB endometrial cancer: clinical course and survival of patients with single and multiple metastases

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Summary

Objective: Metastatic endometrial cancer (EC) at initial presentation is a rare disease. The present aim was to evaluate prognostic factors and overall survival in patients diagnosed with metastatic EC. Study Design: Using data from the Geneva Cancer Registry, the authors included all patients diagnosed with Stage IVB EC from 1980-2007. Estimates of survival were calculated using the Kaplan-Meier method and compared using the log-rank test. Results: A total of 38 patients were identified. The most frequent metastases were peritoneal or pleural carcinomatosis (66%, n=25) and hematogenous metastases (53%, n=20). Five-year survival rate was 5.7% (95% confidence interval: 0.0 - 13.3), and median survival was 7.6 months. Survival of patients with a single metastasis at the time of diagnosis was longer than for patients with multiple metastases (16 versus two months, respectively; p < 0.001). Conclusion: Metastatic EC is rare disease with very poor prognosis particularly for patients with multiple site metastases.

Key words: Advanced endometrial cancer; Metastatic endometrial cancer; Stage IVB endometrial cancer.

Introduction

Endometrial carcinoma (EC) is the most common gynecologic malignancy in Switzerland with an incidence of 24-25/100,000 women per year and a mortality rate of 3.4/100,000 per year [1].

In almost 90% of cases, EC presents with abnormal vaginal bleeding or discharge leading to an early diagnosis [2-4]. In approximately 75% of EC patients, the tumor is confined to the uterine body corresponding to FIGO Stage I and has a favorable prognosis. For these patients, primary therapy is surgery (total hysterectomy and bilateral salpingo-oophorectomy with or without lymphadenectomy) and the five-year overall survival (OS) rate exceeds 85% [5]. Nevertheless, 3-13% of newly diagnosed EC patients present with advanced stage disease and a five-year survival rate ranging from 0-20% [3, 6, 7]. Stage IV EC accounts for up to 25% of disease-specific mortality during the first year following diagnosis [8, 9].

Stage IVA corresponds to a loco-regional extension to the bladder and/or the rectum and Stage IVB includes EC with distant metastases [10]. Treatment options for Stage IV EC include radiotherapy, chemotherapy, surgery, and hormonal therapy. The low incidence, the heterogeneous clinical presentation, and the poor prognosis contribute to the lack of consensus with respect to optimal management of advanced stage EC patients. Therapeutic strategies for Stage IVB EC patients remain a complex problem for the clinician as these women usually have important comorbidities and are believed to have aggressive forms of the disease and a limited life expectancy. To better understand prognosis, day-to-day treatment practices and OS, the authors conducted a population-based analysis of women diagnosed with Stage IVB EC.

Materials and Methods

The Geneva Cancer Registry collects information from various sources. All hospitals, pathology laboratories, and private practitioners are requested to report every cancer case. Trained tumor registrars systematically extract data from medical and laboratory records and physicians regularly receive enquiry forms to complete missing data. In addition to passive follow-up (routine examination of death certificates and hospital records), the registry regularly assesses survival through an active follow-up performed routinely each year using the files of the Cantonal Population Office, which is in charge of the registration of the resident population. For all dead patients, the registry medical staff systematically consults medical files and/or writes to the practitioner to assess cause of death and code the cause according to the WHO classification. The registry is considered accurate, as witnessed by its very low percentage (<2%) of cases recorded only from death certificates [5]. Recorded data include socio-demographic characteristics, diagnostic circumstances, tumor characteristics, stage of disease at diagnosis, treatment during the first six months after diagnosis, survival, and cause of death.

Searching the data from the Geneva Cancer Registry recorded between January 1980 and December 2007, the authors identified 1,164 women with EC. Patients with uterine sarcomas and malignant mixed Müllerian tumors were not included. A total of 47 patients were diagnosed with Stage IVB EC. Nine cases were
Table 1. — Stage IVB endometrial cancer: patients and tumor characteristics (n=38).

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) median (range)</td>
<td>75 (53-97)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>10 (26)</td>
</tr>
<tr>
<td>Multiparous</td>
<td>17 (45)</td>
</tr>
<tr>
<td>Unknown</td>
<td>11 (29)</td>
</tr>
<tr>
<td>Histologic Type</td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>34 (89)</td>
</tr>
<tr>
<td>Seropapillary, clear cells</td>
<td>4 (11)</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>9 (24)</td>
</tr>
<tr>
<td>G2</td>
<td>10 (26)</td>
</tr>
<tr>
<td>G3</td>
<td>7 (18)</td>
</tr>
<tr>
<td>Unknown</td>
<td>12 (32)</td>
</tr>
</tbody>
</table>

Results

A total of 38 patients were diagnosed with Stage IVB EC, representing 3.2% of all EC cases recorded during the period under study. Patient and tumor characteristics are summarized in Table 1. Median age at diagnosis was 75 years (range 53-97). All patients were postmenopausal and 26% were nulliparous. Endometrioid carcinoma was the most common histological type (89%, n=34).

Symptoms at diagnosis were classified as follows (Table 2): gynecological symptoms (metrorrhagia, spotting, leukorrhea, and vaginal discharge), general symptoms (weight loss, asthenia, fatigue, nocturnal sweating, and dyspnea), abdominal symptoms (abdominal distension, abdominal pain, constipation, diarrhea, nausea, vomiting, and melena) as well as other forms of pain (bone and articulation pain, inguinal pain or pain while walking). Gynecological symptoms were predominant in 61%, while general symptoms were reported in 42% of the cases.

Thirty-six of 38 patients (95%) were diagnosed following a consultation due to symptoms; one patient was diagnosed during an annual examination, and one patient was diagnosed during recovery from an abdominal trauma. Table 2 summarizes the metastatic sites. Hematogenous dissemination was observed in more than 50% of patients (n=20, 53%), including pulmonary (n=10, 26%) and hepatic metastasis (n=9, 24%). Other metastatic sites in the present cohort were: peritoneum, pleura, bones, intestines, lymph nodes, brain, adrenal glands, and spleen.

The authors classified patients into three groups. Group I included 15 patients (39%) who benefited from surgery on the primary tumor with or without other therapies (radiotherapy, chemotherapy, hormonal treatments). Surgery included total hysterectomy and bilateral salpingo-oophorectomy with or without lymphadenectomy. Radical hysterectomy with recto-sigmoid resection was performed on two patients. One patient with peritoneal carcinomatosis underwent cytoreductive surgery and another patient had a lung lobectomy. Group II included nine patients (24%) who received other treatments but no surgical intervention on the primary tumor (i.e. no hysterectomy). Two patients benefited from cerebral metastasis resection and adjuvant brain radiotherapy. Group III included 14 patients (37%) who did not receive any type of treatment (Table 3).

The median survival time was 7.8 months (0.7 - 79 months; mean 14.8 months). The survival curve is plotted in Figure 1. The five-year OS rate was 5.7% (95% confidence interval [CI]: 0 - 13.3). Two patients were five-year survivors, one of whom was alive at 77.9 months of follow-up. One patient was lost to follow-up, three patients died of un-
known causes, and four patients (11%) died from causes other than EC. Table 4 shows the median survival times according to histological grade, symptoms, metastatic site, and treatment. No significant difference in survival was observed between the histological subgroups ($p = 0.108$). The median survival time was eight months for the group with gynecological symptoms and 5.1 months for the group with other types of symptoms, but the difference was not statistically significant. Patients with peritoneal carcinomatosis (with or without other metastases) did not show any difference in median survival (eight months) from those without peritoneal carcinomatosis (7.6 months, $p > 0.05$). Survival inversely correlated with the number of metastatic sites ($p$ log-rank test < 0.001). Twenty-two patients (58%) with a single metastasis had a median survival of 14 months (95% CI: 4.5 - 23.0), while 16 patients (42%) with multiple metastatic sites had a median survival of only two months (95% CI: 0 - 3.8). Five-year survival for a single metastasis was 9.9% versus 0% for multiple metastases (Figure 2). Statistical analysis revealed that the type of treatment was a significant prognostic factor. Median survival was 15.2 months (95% CI: 11.9 - 18.5) for patients in group I, 8.1 months (95% CI: 7.9 - 8.3) for those in group II, and 1.5 months (95% CI: 0 - 3.1) for those in group III ($p < 0.001$). Five-year OS estimates for the three groups were 7.5% (95% CI: 0 - 21.5), 11.1% (95% CI: 0 - 31.6), and 0%, respectively.

Discussion

In the present population, the incidence rate of metastatic EC was 3.2%, within the estimates of 1.3-9% reported in other studies [3, 10, 11]. Stage IVB EC encompasses a small and heterogeneous population with a wide diversity of clinical presentations and performance status, which impairs the identification of significant prognostic factors and explains the lack of standardized treatment protocols for these patients.

Classically, EC manifests by spontaneous and painless vaginal bleeding or discharge during the late reproductive years or in postmenopause. Ayhan et al. reported that up to
92% of Stage IVB patients presented with gynecologic symptoms [12]. In the presented series, only 23 patients (61%) exhibited gynecologic symptoms. Fifteen patients (39%) presented with symptoms related to the metastatic site. The most frequent metastatic sites were the peritoneum, the pleura, the lung, and the liver. The largest series published to date by Eto et al. included 248 Stage IVB EC patients having endometrioid, serous as well as carcinosarcoma, and reported that 77% had intra-abdominal metastases and in 44% of cases the peritoneum was involved [3]. In a series of 55 Stage IVB EC patients, Numazaki et al. reported a 65.5% rate of peritoneal carcinomatosis [13].

The most important prognostic factor in the present cohort was the number of metastatic sites at the time of diagnosis. Patients who had a single metastasis exhibited a better median survival than those who had multiple metastatic sites, 14 months versus two months, respectively (p<0.001). Bristow et al. [8] reported a similar observation.

Recent data in metastatic breast cancer suggest that removing the primary tumor could have a beneficial effect on survival [14-18]. To the present authors’ knowledge, no data support an association between surgical removal of the primary tumor and improved long-term outcome in advanced stage EC patients. In the present series, patients in group I benefited from an advantage in survival compared with patients in groups II and III. Removal of the primary tumor might represent an attractive therapeutic option for a subset of patients unfit for optimal cytoreductive surgery and might offer a potential survival benefit. However, data about the general health status and complete comorbidities were lacking. It is probable that patients with a poor general status or with important comorbidities such as cardiopulmonary disease and morbid obesity were less likely to undergo surgery and constitute a selection bias accounting for the observed survival benefit of surgery.

Conclusions

The present study has several limitations including the small study group sample size, its retrospective nature, and incomplete data regarding patient comorbidities. The major strength was its population-based sampling over a period of 28 years. The present incidence rate may thus be one of the most accurate ever published for FIGO IVB EC. These data reflect the current management and treatment approaches proposed in everyday practice, including patients who did not receive treatment.

In conclusion, metastatic EC is a rare and heterogeneous disease with a great variety of clinical manifestations, ranging from a single metastasis to multiple organ involvement. This study supports the idea that total disease burden plays a central role in survival, as patients having multiple metastases have a poorer prognosis than those having a single one.

References


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Effect of biological behavior and clinical significance of maspin gene on cervical squamous carcinoma SiHa cell

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Summary
Objective: This study was performed to evaluate the effect of mammary serine protease inhibitor (maspin) overexpression on human cervical squamous carcinoma (SCC) SiHa cell proliferation and apoptosis in vitro. Materials and Methods: Recombinant plasmid pcDNA3-maspin was stably transfected into human cervical SCC SiHa cell. Maspin mRNA was determined by RT-PCR, whereas maspin protein was detected by Western blot analysis and immunocytochemistry (IHC). Cell proliferation activity was measured by MTT method. Apoptosis rate and cell cycle distribution were detected by flow cytometry to understand the changes in the cell biological characteristics. Results: The strengthened expression of the maspin gene in the SiHa cell was confirmed by RT-PCR, Western blot, and immunocytochemistry (IHC) (p < 0.05). Suppressed proliferation activity and increased apoptosis rate of SiHa-m (maspin stably transfected) versus SiHa and SiHa-vector cell (SiHa-pc3) were shown by MTT and flow cytometry (p < 0.05). SiHa and SiHa-pc3 had no statistical significance (p > 0.05). Conclusions: The results showed that maspin gene can significantly inhibit human cervical SCC SiHa cell proliferation and effectively slow cancer growth. Maspin may be a new molecular target in the gene therapy of human cervical SCC.

Key words: Maspin; RT-PCR; Western blot; SiHa cell.

Introduction
Cervical cancer is one of the most common malignant tumors of the female reproductive tract. In recent years, patients presenting cervical cancer have exhibited an obvious younger trend. To date, the radical surgery cures, chemotherapy, and radiotherapy are the main treatment modalities to prolong patients’ life. However, we prefer to discover a comprehensive suppressor gene which can inhibit the growth of tumor or promote the cancer cells to apoptosis to cure patients. Mammary serine protease inhibitor (maspin) was first identified by subtractive hybridization as a candidate tumor suppressor protein in normal mammary in 1994 [1]. Maspin is located on chromosome 18q21.3–q23 epithelial cells. In many different cancers, maspin acts as a tumor suppressor that can inhibit motility, invasion, angiogenesis, proliferation, metastasis, and promote the cancer cells to apoptosis [2-7]. The role of maspin has been proposed and extensively studied. However, studies have recently found that maspin expression is downregulated in many tissues, including the mammary gland, gastric, and prostate cancers, but is overexpressed in pancreatic, gallbladder, colorectal, and thyroid cancers, etc. These studies have suggested that maspin may have different activities in different cancers. However, maspin gene function and its molecular aspects in human cervical SCC remain largely unclear. Thus, the present study aims to investigate the effect of the biological behavior and clinical significance of maspin gene on human cervical SCC SiHa cells. In this study, a maspin expression vector was constructed and stably transfected into cultured SiHa cells. The efficacy of the vector with maspin was confirmed by RT-PCR and Western blot analysis. The authors discuss the function of maspin gene in human cervical SCC and its underlying molecular mechanisms by observing the apoptosis rate and proliferation activity.

Materials and Methods
Cell culture and stable transfection
The human cervical cancer SiHa cell line was cultured in DMEM medium containing 10% fetal bovine serum, 100 µ/ml penicillin, and 100 µg/ml streptomycin at 37°C with 5% CO2. The cells were seeded into six-cm Petri dishes (3×10 5 to 5×10 5 cells per well) and stably transfected at 80% confluence with either pcDNA-maspin (overexpressed maspin gene) or pc3DNA (empty vector) using transfection TF reagent, as described by the manufacturer. After 24 hours, fresh medium was added to the cells containing the selection reagent G418 (800 µg/ml). SiHa-pc3 and SiHa-m selection was continued for 39 days, with the medium refreshed every other day. The surviving cells were grown under continuous selection using G418. After six weeks, several clones were created by transfection and the surviving clones were ana-
lyzed by RT-PCR, Western blot analysis, and immunocytochemistry (IHC) to detect maspin expression.

**RNA extraction and RT-PCR**

RNA isolation and RT-PCR were performed as specified. Total cellular RNA was extracted using the acid guanidinium–phenol–chloroform method. The authors successfully synthesized first-strand cDNAs from one μl of total RNA using one μl oligo (dt) primer, two μl buffer, one μl dNTP mixture, two μl DTT, and one μl RNaseH according to the protocol of RT reagent kit manufacturer. Each first-strand cDNA of target genes was amplified under its appropriate parameters. The primer sequence of the target genes for RT-PCR were as follows: maspin upstream primer, 5’-CGCAAGCTTCAAGGATAACTGTGACTCCAGG-3’ and downstream primer, 5’-CAGCTGACTTAAATAGGCGCTATGCCAC-3’.

The integrity, concentration, and purity of the RT-PCR products were analyzed by 1% agarose gel electrophoresis and UV spectroscopy. The entire experiments were repeated at least thrice. GAPDH expression was used for comparative studies.

**Western blot analysis**

Western blot analyses were performed to detect the maspin protein expression after transfection. The cells (SiHa, SiHa-m, SiHa-pc3) were washed twice with PBS, and then centrifuged and homogenized in extraction buffer (one mmol/L PMSF, ten μg/ml leupeptin) on ice for 30 minutes. After centrifugation of the cell suspension at 12,000 × g for ten minutes, protein content of the supernatant was determined by BCA protein assay reagent. The protein lysates were separated in SDS-PAGE loading buffer (2% SDS, 10% glycerin, 0.025% bromophenol blue, 50 mmol/L Tris-Cl, pH 6.8), and then blotted onto nitrocellulose membrane. Proteins were detected using anti-maspin and visualized using anti-rabbit IgG conjugated with HRP and ECL as HRP substrate. The images were captured and analyzed by an image software.

**Immunohistochemistry**

The cells were digested with 0.25% trypsin and seeded into six-cm Petri dishes (1 × 10⁶ cells per well) for two days. Afterwards, the cells were placed in glass slides, fixed with alcohol (or cold acetone) for 20 minutes, washed with 75% H₂O₂-PBS at 37°C for ten minutes, and then sealed with normal goat serum at 37°C for 30 minutes. Cells were treated with anti-maspin at 1:100 dilution in PBS buffer for 40 minutes at RT, and then blocked with normal serum at 37°C for 30 minutes. The cells were placed in glass slides, fixed with alcohol (or cold acetone) for 20 minutes, washed with 0.75% H₂O₂-PBS at 37°C for ten minutes, and then sealed with normal goat serum at 37°C for 30 minutes. Cells were treated with anti-maspin at 1:100 dilution in PBS buffer for 40 minutes at RT, and incubated overnight at 4°C in a humidified chamber. Anti-maspin antibody rabbit IgG was added into the liquid. The slides were then washed with PBS and incubated for 40 minutes with avidin–biotin complex reagent containing horseradish peroxidase. The liquid was washed with PBS thrice for five minutes each, and color development was achieved using DAB substrate diluted with PBS for five minutes. The images were captured with a mining graph system. Four fields of vision were obtained in each group. The images were analyzed by a laser pix image software, and the integrated optical density (IOD) was determined.

**MTT assay for cell growth and viability**

Cell growth and viability were evaluated using MTT assay [8]. The positive cells (SiHa, SiHa-m, SiHa-pc3) were plated into 96-well microtiter plates at a density of 1 × 10⁵ per well in a volume of 0.2 ml. Twelve complex holes were set up in each group and set a hole as control with only DMEM. Cell proliferation was measured at serial time points (every 24 hours for five days) using the MTT cell proliferation kit. The light absorption value was then determined by enzyme linked immune detector with 490 nm wavelength. Cell growth curve was drawn using time as the horizontal axis and light absorption value as the vertical shaft.

**Assessment of cell cycle and apoptosis by flow cytometry**

Cells (SiHa, SiHa-m, SiHa-pc3) were harvested, washed with cold PBS, and then fixed in 75% ethanol at 4°C. Propidium iodide (50 μg/ml) was then added, and the cells were incubated at room temperature in the dark for 30 minutes. The percentage of cells with different DNA contents and cell apoptosis were quantified by flow cytometry. Cell apoptosis rate and cell cycle distribution were analyzed using the ELITE and ModFit LT software.

**Statistical analysis**

Data were analyzed by ANOVA using Statistics Package for Social Science (SPSS) software 10.0. LSD-post-hoc test was employed to assess the statistical significance of the difference between the control and the treated groups. P values less than 0.05 were considered statistically significant, and a p value less than 0.01 was highly significant.

**Results**

**Identification of maspin expression vector**

After restriction endonuclease digestion, a small band of DNA (1200 bp) was detected by electrophoresis (Figure 1). DNA sequencing of the constructs showed the presence of sequences similar to those of target fragments.

**Maspin expression was upregulated in overexpressed transfectants of SiHa cells**

The present authors used recombinant plasmid pcDNA3-maspin to upregulated the maspin gene in SiHa cells. The mRNA and protein expression was detected in the SiHa-m, SiHa-pc3, and SiHa cells by RT-PCR, Western blot analysis, and IHC as described in sections 2.2 to 2.4. Maspin
mRNA expression was reflected by the light density ratio of maspin to GAPDH. The result of RT-PCR showed that the maspin mRNA in SiHa-m cells was significantly increased by 54% and 57% compared with SiHa cells and SiHa-pc3 cells, respectively. No significant difference in expression was found between SiHa and SiHa-pc3 (Figure 2). As expected, the maspin protein expression of SiHa-m was three times more than SiHa cells by Western blot (Figure 3) and the result of IHC also displayed that the level of maspin protein in transfectants of pc3-maspin were increased significantly, contrary to that of the SiHa and SiHa-pc3 groups (Figure 4). However, the level of expression of maspin in cells transfected with pcDNA3 vector was similar to that of normal cells (p > 0.05). These results of the three methods all indicate that the maspin expression vector significantly increased maspin expression in SiHa cells.

Effects of maspin on cell cycle and apoptosis

In the current study, the effect of maspin gene on the SiHa cell cycle and apoptosis was determined, and each assay was performed in triplicate. Flow cytometry analysis showed that the cell apoptosis rates of SiHa-m, SiHa-pc3, and SiHa were 14.8%, 1.6%, and 3.9%, respectively (Figure 5). The data indicated that the apoptosis rate of SiHa-m increased significantly (p < 0.05). There was no significantly change on cell apoptosis rates between SiHa and SiHa-pc3 group (p > 0.05).

The analysis of flow cytometry showed that in SiHa-m the percentages of G1 and S were 67.9% and 18.8%, respectively. Nevertheless in SiHa cells the percentages of G1 and S were 57.0% and 26.7% and in SiHa-pc3 cells were...
Figure 4. — Maspin expression in SiHa, SiHa-pc3, and SiHa-m cells by ABC immune enzyme staining. Immunohistochemical staining for maspin with monoclonal antibody (1:200 dilution). (A) The brown signals represent positive staining for maspin (=400). The value was detected by IOD. (a) Weak positive maspin expression in the SiHa cytoplasm, (b) Weak positive maspin expression in the SiHa-pc3 cytoplasm, and (c) Positive expression of maspin in the cytoplasm and nucleus of SiHa-m. (B) The positivity value of maspin were calculated and illustrated as shown.

Figure 5. — Assessment of cell cycle and apoptosis by flow cytometry. Cell proliferation was restrained, and an obviously increased rate of apoptosis was observed in SiHa-m transfectants compared with SiHa-pc3 or untreated SiHa cells. (A) Flow cytometry of apoptosis assay (a) SiHa-m, (b) SiHa-pc3, and (c) SiHa cells. (B) Cell cycle distribution in (a) SiHa-m, (b) SiHa-pc3, and (c) SiHa cells.
Effects of maspin expression on SiHa cell proliferation

To test whether the maspin gene has a role in the inhibition of growth of SiHa cells, the authors examined its effect on cell proliferation by MTT method. The MTT result was consistent with the flow cytometry analysis at different time intervals after transfection (Table 1 and Figure 6). Statistical analysis showed that the absorbance value of the SiHa-m group was less than those of the SiHa and SiHa-pc3 groups (p < 0.05). According to the three kinds of cell growth curve (Figure 6), the cell growth speed of the SiHa-m-transfected expression vector of maspin was slower than those of the SiHa-pc3 and SiHa (p < 0.05). No significant change was found in the cell proliferation between SiHa and SiHa-pc3 (p > 0.05). These results indicate that the total living cells in SiHa-m are less than those of SiHa and SiHa-pc3, and that maspin strongly restrains the proliferation of SiHa cells.

Discussion

Zou et al. [1] first reported that maspin could significantly inhibit the invasion and metastasis of breast cancer cells in vitro. Many researchers demonstrated that maspin expression is downregulated in many tissues and maspin overexpression can inhibit the growth of tumors. They also confirmed that the basic mechanism of maspin tumor suppressor function was carried by apoptosis. At the same time they proposed that it was modulated through several pathway. Studies have shown that maspin upregulation may cause a retarding cell proliferation in gastric cancer and suppress the survival of lung cancer cells. Prostate cancer cells DU-145 with high maspin expression was more sensitive to apoptosis. It may be modulated through Akt pathway. Several studies demonstrated that maspin overexpression modulates tumor cell apoptosis through the regulation of Bcl-2 family proteins. High levels of maspin can increase Bax expression, inhibit Bcl-2, and can enhance the sensitivity of cell apoptosis through the mitochondrial pathways mediated by Bax [9–14].

Other studies have found that maspin inhibits the development or progression of malignant tumors through some mechanisms, such as the p3 dependent pathway [15, 16]. Many researchers confirmed that maspin expression is downregulated in many tissues; therefore they proposed that maspin gene can be treated as an effective tumor marker in the prognosis, chemotherapy monitoring, and metastasis of cancers. Xia et al. found that decreased maspin expression may significantly enhance the metastatic potential of Stages I and II squamous cell carcinomas, whereas high tumoral maspin expression improves the survival of patients with oral squamous cell carcinoma of the tongue [17, 18]. Hence based on these researches, some drugs or medical technologies that can strengthen the expression of maspin may be used to inhibit the growth of tumor. However, the role of maspin in tumorigenesis remains a matter of controversy. In recent years, many researchers have found that maspin gene is overexpressed in the pancreas, gallbladder, colorectal, etc. Liu et al. performed the research by immunohistochemical staining on pancreatic tissue and demonstrated that more than 90% of cases of ductal adenocarcinoma, as well as all high-grade precancerous lesions (PanIN3) were positive for maspin, and normal pancreatic ducts and low-grade precancerous lesions were usually negative for maspin [19]. Kim et al. compared the pattern of maspin expression in 101 gallbladder cancers, 25 adenomas, and ten normal gallbladder specimens. The positivity of maspin expression was found more than in half of gallbladder cancers, whereas no maspin was expressed in adenomas and normal mucosa of gallbladder [20]. These data show that maspin may play different role in the carcinogenesis, tumor invasion, metastasis, and angiogenesis of cancers. Its relationship to carcinomas opens a new angle to the discussion on its function in cancer. However, it needs more in-depth studies to make them clear.

In the present study, the authors selected the SiHa cell line based on several unique features. First, cervical squamous carcinoma (SCC) accounts for a large percentage (80%–85%) in cervical cancer. Secondly, a large percentage of 90% among these cervical cancer patients are infected with HPV 16. The cervical cancer SiHa cell line contains a large number of copies of the HPV 16 viral genome. Consequently, this study was performed to evaluate the biological charac-
teristics of maspin overexpression on SiHa cell line. The result of FACS confirmed that the apoptosis rate of SiHa-m obviously increased, whereas the percentage of S phase cells decreased significantly. The MTT test also proved that the growth speed of SiHa cells significantly slowed down. Therefore, maspin gene expression in SiHa cells was enhanced, and the apoptosis ability of the tumor was increased. These results suggest that maspin gene could inhibit the invasion of human cervical SCC SiHa cells. Next the present authors will select several cell lines to verify the function of maspin on cervical carcinomas. Meanwhile they will modeling a female nude mouse by seeding cells into its subcutaneous to detect the effect of maspin in vivo. Tumor growth speed, the expression of maspin, and LMVD were detected to evaluate the effect of maspin on tumor growth.

In strategies to treat malignant tumors, increasing attention has been given to gene therapy because of its safety and effectiveness. Thus, an increasing number of researchers have contributed in the research for specific target genes. Studies and achievements in genetics and biology have increased, and treatment strategies against cancer at the molecular level have been developed gradually. Therefore, maspin re-expression may be a new molecular target in the gene therapy for some cancers. Relative studies have investigated the use of maspin as a therapeutic agent against cancer. Zou et al. confirmed that maspin can inhibit cancer growth and metastasis in a breast cancer mouse model through a maspin DNA-liposome therapy. The current study supports the tumor suppressor gene properties of maspin in human cervical SCC. Cervical cancer as the most common of gynecologic malignancies is second only to breast cancer in women and remains one of the most important causes of mortality in women worldwide. If the function of maspin in inhibiting the growth of tumor is confirmed by modeling a female nude mouse by seeding cells which was transfected with maspin, maspin re-expression can be regarded as a new molecular target in the gene therapy of human cervical SCC. Moreover, further studies conducted on this subject would be helpful in elucidating the exact mechanism of maspin.

Acknowledgements

Funding for this work was supported by the Natural Science Foundation of Shandong Province, China (Grant No. ZR2012HL01). The present experiment was performed in the Central laboratory of Binzhou Medical University.

References

Extracellular matrix metalloproteinase inducer (EMMPRIN) remodels the extracellular matrix through enhancing matrix metalloproteinases (MMPs) and inhibiting tissue inhibitors of MMPs expression in HPV-positive cervical cancer cells

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Summary

Purpose of investigation: To study the expression of extracellular matrix metalloproteinase inducer (EMMPRIN), matrix metalloproteinases (MMPs), and tissue inhibitors of MMP (TIMPs) in uterine cervical cancer cell lines in vitro. Materials and Methods: EMMPRIN, MMPs, and TIMPs expression were assessed by Western blot and real-time RT-PCR from cervical carcinoma SiHa, HeLa, and C33-A cells. Results: EMMPRIN recombinant significantly increased MMP-2, MMP-9 protein and mRNA expression in SiHa and Hela cells, but not in C33-A cells by Western blot analysis and real-time RT-PCR. EMMPRIN recombinant significantly inhibited TIMP-1 protein and mRNA levels in SiHa and Hela cells, but not in C33-A cells. There was no difference on the TIMP-2 expression in those cells with the treatment of EMMPRIN recombinant. EMMPRIN RNAi decreased MMP-2 and MMP-9 and increased TIMP-1 expression in SiHa and HeLa cells, but not in C33-A cells. There was no change on the expression of TIMP-2 mRNA levels in SiHa, HeLa and C33-A cells transfected with siEMMPRIN. Conclusions: EMMPRIN may induce MMP-2 and MMP-9, and downregulate TIMP-1 in HPV-positive cervical cancer cells in vitro.

Key words: Extracellular matrix metalloproteinase inducer (EMMPRIN); Matrix metalloproteinases (MMPs); Tissue inhibitors of MMP (TIMP); Cervical carcinoma.

Introduction

Uterine cervical carcinoma is the second most common malignant tumor in women worldwide, corresponding annually to 16% of all cases of tumors in women [1]. It is the most common gynecological malignancy in China and its incidence has recently increased.

Many risk factors have been demonstrated to influence cervical carcinoma, such as HPV infection, an early onset of sexual activity, parity, pregnancy, immunosuppression, and recent sexual partners [2]. Although cervical cancer can be treated with radical surgery with or without radiotherapy and/or chemotherapy, some patients with high risk factors will still have an unfavorable prognosis. The leading cause of cervical cancer death is not the tumor itself, but its metastasis to lymph nodes and distant organs. Therefore, new strategies, such as immunotherapy and molecular-targeted therapy, may prove useful in improving the prognosis of cervical cancer patients. Cervical cancer provides a useful model to study the relationship of matrix metalloproteinases (MMPs) and tissue inhibitors of MMP (TIMPs) to tumor behavior [3].

Tumor invasion and metastasis are key steps in the progression of cancer and involve the degradation of basement membranes (BM) and subsequent remodeling of the extracellular matrix (ECM) [4]. MMPs are thought to play a central role in ECM turnover and degradation. MMP-2 and MMP-9 are found abundantly in cancer tissues. There is growing evidence of their role in tumor progression. MMP-2 (72-kDa type IV collagenase/gelatinase A) and MMP-9 (92-kDa type IV collagenase/gelatinase B) were of particular importance in tumor progression because they are capable of cleaving type IV collagen, the major collagen of the BM [5]. During the last decades, the progress in research showed overexpression of MMP-2 and MMP-9 in cervical cancer, suggesting their prognostic value [6].

*Contributed equally to this work.

Revised manuscript accepted for publication July 3, 2014
EMMPRIN/CD147 is a membrane-associated glycoprotein with two extracellular loop structures, which belongs to the immunoglobulin superfamily [7]. EMMPRIN is highly expressed on the cell surface of various tumors [8]. Recent studies reported that EMMPRIN might promote tumor invasion and metastasis via stimulating MMP synthesis in neighboring fibroblasts and enhance angiogenesis via vascular endothelial growth factor [9]. A recombinant EMMPRIN was shown to stimulate cultured fibroblasts to produce MMP-1, MMP-2, MMP-3, and augment the production of MMP-9 in monocytes [10]. Gene silencing of EMMPRIN by small-interfering RNA was demonstrated to hinder lipopolysaccharide-induced monocyte secretion of MMP-9, indicating a predominant role of EMMPRIN in MMP-9 induction [11].

TIMPs are endogenous inhibitors of MMPs and efficiently inhibit the enzymatic activity by binding to the catalytic domain of the MMPs [12]. The C-terminal domain of TIMPs is important for the binding to MMPs, thereby regulating the MMP activation process. Substantial evidence suggests the importance of the MMPs/TIMPs ratio in tumor tissues [13]. Many studies have shown a 1:1 ratio of MMPs: TIMPs in early cervical cancers. It suggested that tumor progression may select for cells expressing MMPs and do not express TIMPs by promoting tumor cell growth.

The dysregulation of the ECM remodeling may play a role in the invasion and metastases of cervical carcinoma. However, it remains unknown whether EMMPRIN acts to regulate ECM remodeling through modulating the expression of MMPs and TIMPs in cervical carcinoma cells and whether it is related to HPV infection.

The present study was designed to elucidate the expression of the ECM components, including EMMPRIN, MMPs, and TIMPs in vitro. The authors have detected the expression of EMMPRIN, MMP-2, MMP-9, TIMP-1, and TIMP-2 in cultured cervical carcinoma cell lines (HeLa, SiHa, and C33-A cells). To explore the role of EMMPRIN in tumor invasion and metastases in three lines of cervical carcinoma cells, the authors examined the effects of EMMPRIN recombinant protein (972-EMN-050) (50 μg) on the expression of MMP-2, MMP-9, TIMP-1, and TIMP-2 mRNA and/or protein levels in those cells.

Materials and Methods

Cell culture

The present study was approved by the Ethics Committee of Fujian Provincial Cancer Hospital, Affiliated Fujian medical university in China. SiHa, HeLa, and C33-A cells were purchased from a Chinese cell bank. Cells were plated in six-well plates at approximately 2×10^6 viable cells per well in one ml of Dulbecco modified Eagle medium containing 10% fetal bovine serum and cultured at 37°C in an atmosphere of 5% CO₂-95% air in phenol red-free DMEM supplemented with 10% fetal bovine serum.

Statistical analysis

The data were expressed as the mean ± SD from at least three independent experiments. Statistical significance was determined using Student’s t-test and one-way ANOVA. A difference with a p < 0.05 was considered statistically significant.

EMMPRIN expression in three cervical cancer cell lines by western blot analysis

As shown in Figure 1a,b, EMMPRIN protein levels in HeLa, SiHa, and C33-A cell lines were positively expressed compared with control by Western blot analysis (p < 0.05) (Fig. 1a, b). Primary cultured leiomyoma cells were used as positive control. Western blot analysis demonstrated that the transfection of cultured cervical cancer cells with siRNA...
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EMMPRIN (siEMMPRIN) suppressed EMMPRIN protein content compared with the cells transfected with non-specific siRNA control (siControl) cells with the inhibition rate being approximately 60-80% (Figure 1c, d) \((p < 0.05)\).

**Effects of EMMPRIN recombinant on the ECM components in cultured cervical carcinoma cell lines**

The present results showed that by treatment with EMMPRIN recombinant MMP-2 (Figure 2a, b) and MMP-9...
(Figure 2c, d) protein levels were significantly \( (p < 0.05) \) enhanced in HeLa and SiHa cells but not in C33-A cells compared with controls. On the other hand, in EMMPRIN recombinant group TIMP-1 (Figure 3a, b) protein levels were significantly \( (p < 0.05) \) decreased in HeLa and SiHa cells but not in C33-A cells. However, there was no signif-
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In addition, mRNA levels of MMP-2 and MMP-9 were significantly (*p < 0.05) augmented in HeLa and SiHa cells but not in C33-A cells with the treatment of EMMPRIN recombinant (Figure 4a, b) by using real-time RT-PCR. In contrast, TIMP-1 mRNA levels was significantly (*p < 0.05) decreased in HeLa and SiHa cells but not in C33-A cells with the treatment of EMMPRIN recombinant (Figure 4c). There was no change on the TIMP-2 mRNA levels in three cervical cancer cell lines (Figure 4d).

Effects of EMMPRIN RNA interference on the ECM components in cultured cervical carcinoma cell lines

The authors transfected siRNA targeted to EMMPRIN and tested its effect on EMMPRIN, MMP-2, MMP-9,
EMMPRIN was shown to significantly decrease MMP-2 (Figure 5a, b) and MMP-9 (Figure 5c, d) protein contents in HeLa and SiHa cells but not in C33-A cells. On the other hand, mRNA levels of MMP-2 and MMP-9 were significantly decreased in HeLa and SiHa cells but not in C33-A cells transfected with siEMMPRIN (Figure 6a, b) by using real-time RT-PCR. In contrast, TIMP-1 mRNA levels was significantly increased in siEMMPRIN cells compared with siControl cells in HeLa and SiHa cells but not in C33-A cells (Figure 6c). RNAi of EMMPRIN resulted in no difference of TIMP-2 mRNA levels in three cervical cancer cell lines (Figure 6d).

**Discussion**

In the present study, the authors have demonstrated that EMMPRIN, MMPs (MMP-2, MMP-9), and TIMPs (TIMP-1, TIMP-2) were expressed in HeLa, SiHa, and C33-A cervical carcinoma cell lines. Furthermore, a series of studies of EMMPRIN recombinant and RNAi experiments indicated an essential role of EMMPRIN in the regulation of the expression of MMPs and TIMPs in three cultured cervical carcinoma cells.

EMMPRIN is a highly glycosylated cell surface transmembrane protein and expressed as a glycoprotein with a molecular mass of 44-66 kDa due to different degrees of glycosylation of the native protein (~30 kDa) [14]. Li et al. evaluated that EMMPRIN overexpression was found in 28 types of cancers from 14 organs and the haematological system [15]. EMMPRIN appears to participate in the induction of MMP, such as MMP-1, 2, 3, 9, 14, and 15 in fibroblasts surrounding tumor cells [16].

In this research, the authors demonstrated that EMMPRIN was highly expressed in HeLa, SiHa, and C33-A cells. EMMPRIN recombinant can significantly enhance the expression of MMP-2 and MMP-9 in HeLa and SiHa cells, but not in C33-A cells. In the previous research, the authors have reported that EMMPRIN may upregulate MMPs (MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9) and downregulate TIMPs (TIMP-1 and TIMP-2) and collagens (collagen type I and III) in cultured leiomyoma cells [17]. In addition, it was shown that downregulation of EMMPRIN inhibited secretion of MMP-9 and MMP-2 in the cultured T24 cells [18]. Kapral et al. reported that phytic acid can decrease in MMP-2 transcript level, but not MMP-9 and resulted in a strong increase in both TIMP-1 and TIMP-2 expression in colon cancer cells [19].

Zou et al. demonstrated that inhibition of EMMPRIN gene expression via RNAi could reduce tumor cell invasion and tumorigenicity in HO-8910pm EOC cells [20]. Transfection of anti-sense RNA of EMMPRIN into hepatocellular carcinoma cells has been reported to decrease the secretion of MMP-9 and inhibit tumor cells for invasion and metastasis [21]. The present authors assessed the role of the endogenous MMP inhibitor on the anti-invasive effects in HeLa, SiHa, and C33-A cells. They transfected siEMMPRIN in HeLa, SiHa, and C33-A cells and showed that RNAi of EMMPRIN resulted in a significant inhibition of MMP-2 and MMP-9 protein contents and mRNA levels in HeLa and SiHa cell lines but not in C33-A cells. In contrast, the present authors demonstrated that EMMPRIN recombinant inhibited TIMP-1 protein and mRNA levels in HeLa and SiHa cell lines but not in C33-A cells. There was no difference in TIMP-2 expression in those cells. TIMP-1 but not TIMP-2 mRNA levels were significantly increased in siEMMPRIN cells compared with siControl cells in HeLa and SiHa cells but not in C33-A cells. Similar to MMP-1 and MMP-9, TIMP-1 and TIMP-2 have activator protein-1 (AP-1) binding sites in their promoters, and the transcription of TIMP-1 is AP-1 dependent [22]. Therefore, it is suggested that the different responses of TIMP-1 and TIMP-2 expression to EMMPRIN in cervical cancer cell lines may be attributable to the different contributions of AP-1 to the transcriptional activation. Reddy et al. reported that the IL-18 induction of MMP-9 was mediated in part via EMMPRIN and through JNK- and ERK-dependent AP-1 activation and p38 MAPK-dependent NF-κB activation [23]. One of the explanation of the different responses of the TIMP-1 and TIMP-2 expression when treatment with EMMPRIN could be that EMMPRIN may induced MMP-9 through JNK- and ERK-dependent AP-1 activation and inhibited TIMP-1 expression in cultured Hela and Siha cell lines but not in C33-A cells. In a future study, the present authors should detect whether or not AP-1 was induced by EMMPRIN through JNK- and ERK-dependent pathway in HPV-positive cervical cancer cell lines.

The present study suggested a possible association of EMMPRIN with upregulation of MMP-2 and MMP-9 and downregulation of TIMP-1, but not TIMP-2 in HeLa and SiHa cell lines. Strong epidemiologic evidence has linked infection with ‘high-risk’ HPVs, HPV-16, and -18, which infect the anogenital mucosa that progress to the development of cervical cancer. Therefore, the present authors chose SiHa and HeLa cells for this study, which are infected with these virus types. They also included an HPV-negative cervical cancer cell line (C33-A). It is postulated that EMMPRIN may be involved in the MMP-dependent pathway through upregulating MMP-2 and MMP-9 and downregulating TIMP-1 in HPV-positive cervical cancer. Del Toro-Arreola et al., have demonstrated that cell surface expression of MHC class I chain-related chain A (MICA) was higher than cell surface expression of MICB in the HPV-positive cell lines; in contrast, HPV-negative cells expressed lower levels of MICA. Sustained over-expression of MICA at the cell surface of HPV-positive cells, which could promote downregulation of the NK cell functions [24]. However, numerous studies have demonstrated tumor evasion through metalloprotease-induced proteolytic release of MICA and MICB from the cell surface,
which provokes downregulation of NKG2D in NK and T cells. MMP-9 is critically involved in the osteosarcoma-associated proteolytic release of sMICA, which facilitates tumour immune escape [25]. Further study is necessary to elucidate whether EMMPRIN may induce the sMICA expression through NGF-2D receptor by MMP-9-dependent pathway in HPV-positive cervical cancer cell lines.

Conclusion

The authors demonstrated the expression of EMMPRIN, MMP-2, MMP-9, TIMP-1, and TIMP-2 in cultured HeLa, SiHa, and C33-A cells. Furthermore, they provided novel evidence that EMMPRIN may play a vital role in upregulating MMP-2 and MMP-9 and downregulating TIMP-1, but not TIMP-2 in HPV-positive cervical cells. Upregulated EMMPRIN expression may contribute to tumorigenesis, tumor growth, and vascular invasion of HPV-positive cervical cancer. These data suggest that EMMPRIN could become a good marker to predict the prognosis of HPV-positive cervical cancer. Suggest that EMMPRIN could become a good marker to predict the prognosis of HPV-positive cervical cancer and that it could be a promising target for improved cancer therapy in the future.

Acknowledgment

This work was supported in part by Grants-in-Aid for Scientific Research 2009J0504 from the Fujian Natural Science Foundation (from March 2008 to June 2012).

References

Association of EBV and HPV co-infection with the development of cervical cancer in ethnic Uyghur women

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Summary
Objective: Study on the role of Epstein-Barr virus (EBV) and human papillomavirus (HPV) infection in the development of cervical cancer. Materials and Methods: We collected 178 cases of cervical tissue specimens of Uyghur women with cervicitis, cervical intraepithelial neoplasia (CIN I, CIN II-III), and cervical squamous cell carcinoma (CSCC). EBV- and HPV-DNA were detected by PCR of tissue DNA. EBV protein expression was checked by immunohistochemistry. Results: HPV-DNA was detectable in 2.5, 12.5, 68.0, and 96.4% of cases of cervicitis, CIN I, CIN II-III, and cervical cancer, respectively. For EBV-DNA, these numbers were 0, 3.1, 28.0, and 69.6%. There was a significant difference between the groups of cervicitis, CIN II-III, and cancer with respect to both HPV and EBV positivity rates (p < 0.05). Further analysis indicated that cervical lesion pathogenesis was not only accompanied by a gradually increasing rate of HPV or EBV-DNA alone, but also by an increasing rate of HPV-EBV dual infection (r = 0.46; p < 0.01). EBV protein expression was positive in 89.7% of EBV-DNA positive cases (34/39) and 6% of EBV-DNA negative cases (1/17). Conclusion: Cervical cancer development and progression may be closely associated with the dual-infection by HPV and EBV.

Key words: Cervical cancer; Human papillomavirus; Epstein-Barr virus.

Introduction
Cervical cancer continues as a leading female genital cancer, threatening the life of women living in developing countries, where about 80% of all cases occur [1-3]. In China, the highest incidence of cervical cancer has been documented for ethnic Uyghur women in Xinjiang (490–560/100,000), and the mortality is three to four times higher than the average in the country [4-5]. High-risk human papillomavirus (HPV) infection is a necessary but not a sufficient cause of cervical cancer [6-8]. In the present authors’ previous study, they detected a high HPV infection rate in Uyghur women with cervical cancer, with HPV-16 as the most common infection type [9].

Nearly all women will become infected with HPV during their lifetime, but only a minority of these infections will progress to invasive cancer. The existence of long latent period of cancer development after HPV infection suggests the involvement of other etiologies in this malignancy process. Results of previous studies suggested [10-14] that Epstein-Barr virus (EBV) could be a co-factor in HPV associated carcinogenesis and that EBV itself may participate in cervical carcinogenesis. These findings are in contradiction to what has been reported by others [15-18]. The role of EBV in the cervix carcinoma thus remains a topic of great debate. The contribution of EBV to the development of malignancies could not yet been defined clearly. Despite the fact that the implication of HPV in carcinogenesis and prognosis of cervical cancer is well established, the impact of a co-infection with high risk HPV and EBV is still not fully understood. Therefore, the main aim of this study was to investigate the prevalence of EBV infection and HPV-EBV dual infections in cervical tissue samples from Uyghur patients with different cervical lesions such as cervicitis, cervical intraepithelial neoplasia (CIN), and cervical cancer, as well as to evaluate its association with the development of cervical cancer and precursor lesions at the high-risk population.

Materials and Methods
Cervical tissue specimen
A total of 178 formalin-fixed paraffin-embedded (FFPE) cervical tissue specimens from Uyghur patients who had been diagnosed or hospitalized at the Department of Gynecology of First Affiliated Hospital of Xinjiang Medical University between January 2003 and December 2007 were analyzed. Cervical tissue specimens derived from punch biopsies, loop electrosurgical excisions, cone biopsies, and hysterectomies. The pathology slides were reviewed and original histological diagnoses of samples were confirmed by experienced pathologists. The diagnoses were as follows: non-neoplastic cervix (cervicitis), n = 40; CIN, n = 82 (CIN I, n = 32; CIN II-III, n =
50); and squamous cell carcinoma (SCC), n = 56. Patient’s age ranged from 30 to 60 years, with a mean of 48 years. Cervical tissue samples were taken on the date of diagnosis and before initial treatment. All biopsy results were reviewed by two pathologists. Ethical approval for use of all specimens was obtained from the research ethics committee of the First Affiliated Hospital of Xinjiang Medical University.

DNA extraction

The paraffin-embedded tissue sections were deparaffinized with xylene, rehydrated by decreasing concentrations of ethanol and double distilled water (ddH2O), followed by digestion with 100 mg/ml proteinase K. The genomic DNA was extracted by the standard phenol-chloroform (1:1) extraction and ethanol precipitation. Purified DNA was then quantified using a spectrophotometer and stored at -20°C until further use.

Screening HPV and EBV-DNA in tissue specimens

To screen HPV positive samples, the genomic DNA was analyzed with specific primer pairs MY09/11 [MY09: 5′-CGTCCMARRGGAWA CTGATC-3′; MY11: 5′-GCM-CAGGGWCATAAAYATGG-3′, R=A+G, W=A+T, Y=C+T, product length 452 bp] by PCR amplification. Standard PCR was carried out in a total volume of 25 μL using a Taq DNA polymerase, with the following conditions: 94°C for three minutes, followed by 35 cycles of 94°C for 45 seconds, 55°C for 45 seconds, 72°C for 60 seconds, and a final extension at 72°C for ten minutes. Omission of the DNA template occurred in negative controls. To screen EBV-DNA positive samples, the genomic DNA was analyzed with specific EBV DNA PCR primer pairs (5′-CCAGACAGCAGCCAATGTC-3′ and 5′-GGTAGAAGACC-CCC TCTYAC-3′) under the same PCR conditions to amplify a 129 bp fragment. Detection of amplification products occurred by electrophoresis on 2% agarose gel labeled with ethidium bromide and ultraviolet visualization.

Immunohistochemical detection of EBV expression

Immunohistochemical (IHC) staining was performed using a primary antibody recognizing the target protein and an IHC kit containing the biotin-labeled secondary antibody. Briefly, four to five μm-thick sections were cut from the paraffin-embedded tissue blocks. After being dewaxed in xylene and rehydrated in alcohol and distilled water, antigen was retrieved by heating in the microwave oven for 15 minutes at 95°C in EDTA buffer (pH 8.0). After cooling and rinsing in distilled water, endogenous peroxidase activity was blocked by incubating sections for 15 minutes followed by rinsing in 0.01 M PBS (pH 7.4) for ten minutes. Samples were preincubated with a protein blocking solution for ten minutes and the sections were incubated at 4°C overnight in a humid chamber with the EBV coat protein LMP1 specific antibody. Slides were washed three times in PBS and then incubated with a biotinylated secondary antibody for 15 minutes at room temperature. The reaction products were visualized with diaminobenzidine. PBS was used in place of the primary antibody as a negative control and slides were counterstained with hematoxylin, dehydrated, and evaluated under light microscope. The percentage and intensity of positively stained tumor cells in each lesion was investigated by two pathologists who had no knowledge of the patients’ characteristics. A consensus number was reached for each tumor sample between the two investigators. Results were scored on a scale from 0 to 3 by the percentage and intensity of positive cells among tumor cells.

Statistical analysis

All statistical analyses were performed with the SPSS Version 17 software package. All p values were two-sided and the significance level was p < 0.05 or p < 0.01. Mann-Whitney test were used to test continuous variables for differences in scores of DNA- or protein-based EBV detection by PCR or immunohistochemistry among tumor and normal tissues. Fisher’s exact test was used for evaluation of associations with clinical pathological parameters. Linear associations between two continuous variables were quantified by Pearson correlation coefficient.

Results

In this study, HPV infection was highly associated with cervical cancer development in Uygur women. Among cervicitis, CIN (CINI, CINII-III) and cervical cancer patients, the overall HPV positive rate detected by MY09/11 was 2.5, 12.5, 68.0, and 96.4%, respectively. HPV infection rate gradually increased along with the increasing severity of cervical histologic lesions, with a clear tendency of cervicitis < CINI < CINII-III < CSCC (Table 1). HPV infection was significantly higher in genomic DNA of CSCC than in either CIN or chronic cervicitis (p < 0.01, Table 1). Although HPV infection rate seemed to be higher in CINI than in cervicitis, the difference did not reach statistical significance (χ2 = 2.71, p > 0.05). HPV infection rate gradually increased with cervical disease pathogenesis (r = 0.764, p < 0.01). EBV-DNA positivity rate in cervicitis, CIN (CINI, CINII-III) and CSCC patients was 0, 3.1, 28.0, and 69.6%, respectively. EBV infection rate gradually increased with cervical disease pathogenesis (r = 0.606, p < 0.01). The highest EBV positivity rate was observed in Uygur patients with CSCC. The detection rate of EBV was higher in CSCC and CIN than in cervicitis. In addition, EBV was not found in any sample of cervicitis. The differences of EBV-DNA infection rates in different pathologic cervical lesions were statistically significant (p < 0.01). We found that EBV and HPV infection rate behaved similar: cervicitis < CINI < CINII-III < CSCC (Table 1).

Further analysis indicated that cervical lesion pathogenesis was not only accompanied by a gradually increasing rate of HPV or EBV DNA alone, but that it also correlated positively with the increase of HPV-EBV dual infection (r = 0.46; p < 0.01) (Table 1).

Table 1. — Analysis of cervical lesions HPV and EBV-DNA detection.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>HPV</th>
<th>Virus DNA detection</th>
<th>HPV-EBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>40</td>
<td>1(2.5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CINI</td>
<td>32</td>
<td>4 (12)</td>
<td>1 (3.1)</td>
<td>0</td>
</tr>
<tr>
<td>CINII-III</td>
<td>50</td>
<td>34 (68.0)</td>
<td>14 (28.0)</td>
<td>8 (16.0)</td>
</tr>
<tr>
<td>CSCC</td>
<td>56</td>
<td>54 (96.4)</td>
<td>39 (69.6)</td>
<td>39 (69.6)</td>
</tr>
</tbody>
</table>

* = cases of co-infection by HPV and EBV; CV = cervicitis; CIN = cervical intraepithelial neoplasia; CSCC = cervical squamous cell carcinoma. Analysis of the statistical significance among groups by chi-square (χ²) test: HPV, χ² = 108.73, p < 0.01; EBV, χ² = 69.70, p < 0.01; HPV-EBV, χ² = 82.50, p < 0.01.
We analyzed the EBV protein expression on 56 cases of cervical cancer specimens by IHC using an antibody specific to viral envelope protein LMP1, to assess the false positive and false negative rates of DNA-based EBV detection by PCR described above. It was evident that EBV protein expression was located in the nucleus of CSCC cells (Figure 1). EBV protein was detectable in 35 cases among 36 cases positive for EBV DNA (64.3%), whereas only one case was positive for EBV protein in EBV-DNA negative cases (89.7%) (Table 2). If the detection of protein expression by immunohistochemistry is assumed to be the golden standard, the results suggested that EBV-DNA detection by PCR has a certain false positive rate (4/39), and also misses some cases with EBV infection (1/17). Nevertheless, there is no statistically significant difference between the results of the two methods concerning detection of EBV infection ($p > 0.05$), and the coincidence degree of the two methods was very high ($k = 0.80$).

**Discussion**

Around the world, infection is one of the most important causes of cancer [19]. It was estimated conservatively that in the year 2002, 18% of all malignancies were attributable to infectious agents [20].

Molecular epidemiologic evidence originating from studies using polymerase chain reaction techniques has firmly established HPV as a causal factor in cervical cancer development [21-23]. Since high-risk HPV is detected in virtually all cases of cervical cancer, the attributable fraction for this cancer is admittedly 100%. HPV-16 and HPV-18 are the most virulent types and account for approximately 70% of cervical neoplasms [24]. Results of our previous studies have also shown that HPV-16 was the most frequent type in Uyghur patients [9].

In this and previous research, we found that HPV infection rate increased along with the increase in severity of cervical lesions, with a clear tendency of cervicitis < CINI < CINII-III < CSCC.

Although HPV infection is assumed to be required for the development of cervical cancer, some investigators reported that human herpes viruses (HHVs) could act as initiators of HPV carcinogenesis [25]. Human herpes virus 4 (HHV-4) or EBV belongs to the genus lymphocryptovirus of the human γ-herpesvirus family and infects more than 90% of the worldwide adult population [26].

Currently, it is known that EBV is associated with a human benign disease, infectious mononucleosis, and with multiple human malignancies, including nasopharyngeal carcinoma, gastric carcinoma, almost half of the cases of Hodgkin’s lymphoma and B-cell lymphoma in immunocompromised patients [27, 28].

EBV was found in cervical samples and therefore many investigators have attempted to clarify its role in HPV-associated cervical carcinogenesis [29]. In the present study, EBV-DNA positivity rate in cervicitis, CIN (CINI, CINII-III) and CSCC were 0, 3.1, 28.0, and 69.6%, respectively. EBV infection rate gradually increased with cervical dis-
ease pathogenesis (r=0.606, p < 0.01). The highest EBV positivity rate was observed in Uyghur women with CSCC. The analysis showed a clear and statistically significant association between EBV and the development of cervical lesions.

The prevalence of EBV infection in cervical samples has been studied by several researchers: Silver et al. [30] described a prevalence of 20%; Szostek et al. [31] found a prevalence of 22% in HPV-16 positive women, and Voog [32] found a prevalence of 38% in HIV positive women. The overall prevalence of EBV in CN and cervical cancer in this study was 30.3% (54/178), in cervical cancer only, the prevalence of EBV was 69.6%, which is significantly higher than that described in literature. The absence of EBV infection in the cervicitis group and the highest EBV infection rate in the cervical cancer group suggest that EBV infection occurs late in cervical oncogenesis. It may be that the EBV is acting as a cofactor of HPV, in induction of uterine cervix pathology; the suggestion was confirmed by studies of Szkardkiewicz et al. [33].

Preliminary presented results of EBV and HR-HPV co-infection in Irish, North American, Thai, and Japanese SCC cases were recently reported [12, 34]. In the present research, EBV plus HPV in the same specimen were identified in 69.6% of cancer, 16.0% of CINII-III, and 0% of non-neoplastic cervix, the differences being significant. This is particularly noteworthy because of recent experimental evidence demonstrating that EBV and HPV can collaborate to increase proliferation of cultured cervical cells [35]. These findings also confirm that the uterine cervix is a habitat for multiple viral and other infections, some of which have oncogenic potential.

In addition the present results of immunohistochemical detection of EBV expression confirm the location of EBV in the nucleus of the malignant cells. EBV protein expression also was consistent with the presence of EBV-DNA in cervical cancer tissue. We showed that the immunohistochemical assay is a sensitive and simple method for detection of EBV infection in cervical cancer and provides similar results as EBV-DNA detection by PCR. Therefore, the joint application of the two methods for detection of EBV infection could reduce the false positive and false negative rate, and thus improve the accuracy of EBV detection.

In conclusion, Uyghur patients with cervical cancer were HPV-EBV co-infected. The highest rate of HPV and EBV co-infection was found in CSCC, a lesser degree of co-infection was observed in pre-cancerous lesions of the cervix. The cervical cancer development in Uyghur women may be associated with HPV/EBV dual infection, whereby EBV infection is incriminated in cervical cancer progression. However the role of dual infection in cervical oncogenesis needs further investigation.

Acknowledgement

The authors gratefully acknowledge the support of the “Natural Science Foundation of China (NSFC; 81060321)”. The authors are grateful to Dr. Guenter Daxenbichler, Medical University of Innsbruck, Austria, for critical reading of the manuscript.

References


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Human papillomavirus effect on the development of endometrial polyps

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Summary

Aim: Although the association of human papillomavirus (HPV) with warts arising in different parts of the human body has been well-demonstrated, the association of HPV with endometrial polyps has never been studied in the literature up to now. Materials and Methods: Detection of the HPV DNA was carried out by using 13 high-risk HPV real-time PCR Kit and five low-risk HPV real-time PCR Kit. Results: Among 50 endometrial polyp samples, one endometrial polyp sample revealed a positive result for the presence of HPV type 18. Conclusions: This first study in the medical literature investigating the possible effect of HPV on the development of endometrial polyps has demonstrated that HPV might have a role in the development of some of the endometrial polyps. If the present authors’ hypothesis that endometrial polyps caused by carcinogenic HPV types are prone to proceed to endometrial cancer if left untreated is correct, HPV vaccine has a potential to prevent development of at least some of the endometrial polyps and endometrial cancers.

Key words: HPV; Vaccine; Endometrial polyp; Endometrial cancer; Warts.

Introduction

Human papillomavirus (HPV) is a non-enveloped, double-stranded DNA virus belonging to the family, Papillomaviridae. HPV infection is the most common sexually transmitted infection with a prevalence of up to 75% among sexually active adults [1]. Although the infection is mostly asymptomatic, HPV may lead to a diversity of medical conditions including warts and cancer.

Oncogenic HPV infection is a necessary cause of cervical cancer and is strongly associated with other epithelial malignancies such as oropharyngeal, penile, vaginal, vulvar, and anal cancers [2-4]. In those cancers showing associations with HPV, but with less than 100% attributable to HPV, there are most likely multiple pathways leading to cancer, one (or more) of which involves HPV infection. Oncogenic HPV types are also present in precursor lesions of HPV-related cancers.

HPV is also a common causative agent of human warts. HPV has been shown to play role in the development of endocervical polyps [5]. Its association with sinonasal polyps has been reported in several studies [6, 7]. Cutaneous warts are the most common type of HPV induced warts and may arise anywhere in the skin [8]. Although the association of HPV with warts arising in different parts of the human body has been well-demonstrated, the association of HPV with endometrial polyps has never been studied in the literature up to now. This study aimed to investigate the possible role of HPV in the development of endometrial polyps and resultant endometrial cancer. The authors also discussed the possible preventive effect of HPV vaccine on endometrial polyps and cancer.

Materials and Methods

A total of 50 endometrial polyps were studied to detect the possible presence of HPV. DNA isolations were performed from 1-2 mm³ FFPE tissue samples carved with the help of a clean scalpel, by using a DNA blood mini kit and stored at -200°C until the real-time PCR detection step.

Detection of the HPV DNA was carried out by using 13 high-risk HPV real-time PCR Kit and five low-risk HPV real-time PCR Kit, according to the manufacturer’s protocol. The kits screen for 13 high risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and five low risk HPV types (6, 11, 42, 43, and 44). Rotor-Gene Q 6plex was used as the real-time PCR instrument. Analysis of the data was carried out by using Rotor-Gene Q Series Software 1.7. Any samples that had a signal passing the threshold before 45th cycle were considered as positives. The samples that were not considered as positives were checked for the internal control signal, before assessing as negatives.

Any positive samples from real-time PCR part were proceeded to typing part by using genotyping kit HPV GP V.2, according to the manufacturer’s protocol. This kit is intended to carry out the typing of 23 HPV types: 6, 11, 16, 18, 26, 30, 31, 33, 35, 39, 45, 51, 52, 53, 55, 56, 58, 59, 66, 67, 68, 70, 73, and 82. At the end of the experiment, any banding on the strip showed the presence of a specific HPV type.

Results

Among 50 endometrial polyp samples, 49 revealed negative results for the presence of either high-risk or low-risk HPV. One endometrial polyp sample revealed a pos-
itive result for the presence of HPV. Type analysis demonstrated the presence of HPV type 18 effect on this endometrial polyp.

Discussion

More than 100 different types of HPV have been identified up to now [9]. HPV types 16 and 18, classified as high-risk viruses for cervical cancer, are currently considered as human carcinogens, being present in more than 99.7% of cervical carcinomas [10]. In all of the known HPV related carcinomas, the most common types detected are types HPV-16 and HPV-18, which cause more than 70% of HPV-related carcinomas [11-14]. The carcinogenic effect of HPV depends on integration of the virus into the host-cell DNA and expression of the oncoproteins E6 and E7, which antagonize the functions of the tumour-suppressor proteins p53 and pRb, respectively. Through these molecular mechanisms, the virus can transform and immortalize epithelial cells, enabling them to proliferate and form tumours [11-14].

Several authors, beginning with Kirgan et al. in 1990 [15], reported a potential association between HPV infection and colon cancer. HPV antigen was detected in 23% of normal colon specimens, 60% of adenomas, and 97% of carcinomas in this first study. This first and several other studies concluded that HPV infects the columnar mucosa of the colon and that an association exists between HPV and colon neoplasia [16]. Thus, this raises the idea that carcinogenic effect of HPV is not limited to the squamous epithelium as in the case of cervical, oropharyngeal, penile, vulvar, vaginal, and anal cancers [2-4]. Cellular transformation and carcinogenic effect of HPV might also affect the columnar epithelium.

Although the association of HPV with warts arising in different parts of the human body has been well-demonstrated, the association of HPV with endometrial polyps has never been studied in the literature up to now. Such an association would be of clinical importance in two ways. First, endometrial polyps related to HPV, especially oncogenic types, might be susceptible to oncogenic transformation resulting in endometrial cancer. Second, HPV vaccine might prevent development of at least some types of endometrial polyps and endometrial cancer.

Among 50 endometrial polyp samples, only one sample was found to be positive for the presence of HPV. Although this ratio of 2% is not a large proportion, the present study raises the idea that some of the endometrial polyps may originate from HPV infection or HPV might be a cofactor in the development of endometrial polyps. This positive sample was found to be infected with HPV type 18, one of the most common oncogenic types. Then, the question arises: would this endometrial polyp lead to endometrial cancer if it was not removed?

Another important point is that the present authors studied the presence of 13 high-risk and five low-risk HPV types in this study, but there are more than 100 types of HPV identified. Thus the authors might be underestimating the effect of HPV on endometrial polyps by their ratio of 2% in this study.

A last point to discuss is the possible protective effect of the HPV vaccine on endometrial polyps and resultant endometrial cancer. HPV vaccine, either trivalent or quadrivalent, has been shown to be effective against HPV induced conditions. Thus, HPV vaccinated patients might be considered protected against development of HPV-related endometrial polyps. If the present authors’ hypothesis that endometrial polyps caused by carcinogenic HPV types are prone to proceed to endometrial cancer if left untreated is correct, HPV vaccine has a potential to prevent development of at least some of the endometrial cancers.

This first study in the medical literature investigating the possible effect of HPV on the development of endometrial polyps has demonstrated that HPV might have a role in the development of some of the endometrial polyps. The subject requires further studies with greater sample sizes.

References

Human papillomavirus effect on the development of endometrial polyps


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**Introduction**

Radical hysterectomy is one of the most important procedures for cervical cancer treatment [1]. Currently, many techniques are used for radical hysterectomy, such as abdominal radical hysterectomy (ARH), with and without nerve sparing, laparoscopic-assisted radical vaginal hysterectomy (LARVH), total laparoscopic radical hysterectomy (TLRH), and robotic radical hysterectomy [2]. Although these procedures have advantages and disadvantages, most surgeons agree that LARVH is the most ideal because it combines the advantages of both vaginal and laparoscopic approaches [3].

LARVH is composed of both laparoscopic lymphadenectomy and vaginal radical hysterectomy (VRH). The classic VRH, also known as Schauta operation [4], was first described by Schauta at the turn of the 20th century. However, VRH did not gain wide acceptance because of the fatal defects in its inability to resect the lymph node simultaneously. In the 1980s, the development of laparoscopic techniques in pelvic lymph node resection revived the vaginal surgical approaches for cervical cancer treatment [5]. Considerable research has shown that, compared with classic abdominal approach, VRH not only has the same safety and efficacy but also has more advantages in vaginal excision management in patients with morbid obesity or physical complications, as well as shorter hospitalisation, among others [6-8]. However, VRH is performed through the natural channel of the body, and its major difficulty is operative field exposure. VRH is also associated with high risk for urologic complications [9]. These limitations have greatly restricted the application of VRH in clinical settings and are continuously modified through the years.

In the current study, the authors elaborated the VRH modifications. That is, they modified the critical procedures and special equipment to resolve the problem of operative field exposure and avoid urologic complications. The present centre has performed transvaginal operations since 1990 and has developed a series of modified techniques for non-prolapsed uterus. Nearly 20,000 transvaginal uterine operations have been performed in this centre in the past two decades. Since 2004, the authors have developed a modified VRH technique and designed a series of surgical instruments especially for this procedure. These technique and instruments have been proven helpful.

**Materials and Methods**

**Patients**

Between March 2004 and December 2011, a total of 86 women presenting with early cervical cancer at Stages IB1 to IIA1 according to the International Federation of Gynaecology and Obstetrics (FIGO) staging system were treated with modified laparoscopic-assisted radical vaginal hysterectomy (LARVH). Data were collected on operating time, blood loss, ureter separation time, nodal count, hospital stay, and complication recurrence and survival rates. **Results:** All patients successfully completed LARVH. Median operating time was 238 minutes, mean blood loss was 283 ml, median time for ureter separation was 18.5 minutes, median time to post-operative exhaustion was 23 hours, urine recovery was 10.3 days, and median hospital stay was 9.2 days. On average, 23.2 lymph nodes were harvested. Except for one case of left internal iliac vein with intraoperative and postoperative complications, no other major complications occurred, particularly no bladder and ureter injury. Surgical margins were negative in all cases. After median follow-up of 46 months, recurrence rate and overall survival for 84 patients were 3.57% and 97.62%, respectively. **Discussion:** Modified VRH with laparoscopic pelvic lymphadenectomy is an oncologically valid alternative for early stage cervical cancer treatment with minimal intraoperative and postoperative complications. The modification of this procedure and special instruments can enhance the feasibility and the safety of treatment.

**Key words:** Radical vaginal hysterectomy; Cervical cancer; Laparoscopic lymphadenectomy.

Statistics (FIGO) underwent modified LARVH in the present centre (Table 1). A total of 84 patients had follow-up visit until March 2013. Data were collected on operating time, pre-, post-, and intra-operative blood loss, nodal count, hospital stay, complication rate, intra- and post-operative complications, and recurrence of disease. This study was conducted with approval from the Ethics Committee of our hospital. Written informed consent was obtained from all participants.

Special instruments for VRH

To overcome the limitation of operative field exposure in transvaginal operations, a series of special instruments for VRH was developed, namely, paravesical space retractor (Figure 1A), vesicocervical space retractor (Figure 1B), vesicocervical ligament retractor (Figure 1C), cervical depressor (Figure 1D), cervical heavy hammer (Figure 1E), and ureteral illuminating catheter (Figure 1F). These instruments are particularly helpful in the critical steps, such as gap separation and ureter knee identification.

Surgical technique

The critical modifications of the procedure are described as follows.

Laparoscopic lymphadenectomy

Firstly, the ureteral illuminating catheter was placed into the bilateral ureter with a cystoscope after the effect of the anaesthesia was achieved. Secondly, laparoscopic pelvic lymphadenectomy was performed according to the protocol introduced by Querleu et al.[6]. Fifteen patients had para-aortic lymph node sampling as their stages were advanced (11 cases at IB2) or as the pelvic lymph node tested positive (four cases). After lymphadenectomy, the uterine artery lying across the ureter was coagulated and cut off. Finally, the operation proceeded to the VRH procedure.

Modified VRH

Vaginal cuff creation

After the side wall of the vagina was pushed away using the vaginal wall depressor, the adequate length of the vagina requiring excision was determined according to tumour size and then grasped using six clamps (Figure 2A). A diluted solution of epinephrine was injected under the vaginal mucosa. Then, a vaginal wall incision was performed around the cervix distally to the clamps. The anterior, posterior, and side part of the vaginal mucosa were mobilised in the direction of the cervix, thus creating the vaginal cuff that was stitched

Table 1. — Age, BMI, parity, clinical FIGO stage, and histological types.

<table>
<thead>
<tr>
<th>Age, BMI, parity, clinical FIGO stage, and histological types.</th>
<th>LARVH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>49.1 (34-66)</td>
</tr>
<tr>
<td>Median BMI (range)</td>
<td>23.8 (19.2-28.6)</td>
</tr>
<tr>
<td>Parity</td>
<td>2.2 (0-6)</td>
</tr>
<tr>
<td>Ib</td>
<td>68</td>
</tr>
<tr>
<td>IIa</td>
<td>18</td>
</tr>
<tr>
<td>Squamous</td>
<td>73</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>11</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 1. — Special Instruments for VRH: A) paravesical space retractor; B) vesicocervical space retractor; C) vesicocervical ligament retractor; D) cervicical despressor; E) cervical heavy hammer; F) illuminant ureteral catheter.
Figure 2. — Main procedures of the authors' modified VRH: A) confirmed the length of the vagina requiring excision and then grasped with clamps; B) creation of the vaginal cuff; C) the vaginal cuff is hung with the cervical heavy hammer; D) blunt dissection of the vesico-uterine space; E) blunt dissection of the rectal-uterine space; F) coagulation and cut-off of the left paracolpium; G) separation of the right para-vesical space; H) exposure of the vesicocervical ligament (a. paravesical space retractor b. vesicocervical space retractor c. vesicocervical ligament retractor); I) facilitation of the sectioning of external and internal side; J) penetration of a small rubber tube and below it the right ureter; K) exposure of the right uterine artery located under the ureter; L) coagulation and dissection of the descending part of the uterosacral ligament; M) Close as possible to the pelvic sidewall to dissect the cardinal ligaments and parametrium tissue; N) pulling of the uterine fundus downward, clamped, and cutting of the sagittal part of uterosacral ligaments; O) closure of the vaginal stump and peritoneum.
hermetically with 7-0 sutures (Figure 2B). All sutures were tied together and hung with the cervical heavy hammer (Figure 2C). This instrument provides great strength for pulling down the uterus without crushing the cervix and helps prevent spillage of the cancer cells. No Schuchardt incision was required in these operations.

**Vesico-uterine space and rectal-uterine space opening**
Tissues attaching the bladder to the cervix and those attached to the rectum to the cervix were sectioned bluntly using curved scissors in the vicinity of the cervix until the vesico-uterine and rectal-uterine spaces were opened (Figures 2D and 2E). The spaces were then bluntly expanded by fingers to push up the bladder and push down the rectum.

**Paracolpium dissection**
The vaginal cuff was pulled to the top left to tighten the right paracolpium and then coagulated. The right paracolpium was cut off using a biclamp. The same procedure was performed on the left side (Figure 2F). The excision of the paracolpium from the vagina in VRH is much easier than that in ARH or TLRH.

**Para-vesical space opening and ureter identification**
The vagina mucosa was clamped at the 11 and 9 o’clock positions on the right side and was pulled using surgical scissors to open the para-vesical space, which has been prepared at the end of laparoscopic lymphadenectomy (Figure 2G). With special retractors placed in the previously dissected spaces, the vesicocervical ligament lying between two retractors was completely exposed (Figure 2H). The ligament was stretched using the “Y”-type vesicocervical ligament retractor placed beneath to facilitate the sections of the external and internal sides of the bladder pillar lobes (Figure 2I). Under the guidance of the previously placed ureteral illuminating catheter, the ureter knee was easily visualised and dissected. A small rubber tube below this instrument was penetrated to facilitate identification and to prevent ureteral damage (Figure 2J). In the current study, all patients underwent Piver III-type radical hysterectomy, in which the cutting off of both the external side and the internal side of the bladder pillars was required to dislocate the ureter completely from its bed.

**Uterine artery management**
The process that exposes the ureter also helps the visualisation of the uterine artery, which is located under the ureter (Figure 2K). The uterine artery is usually cut off in the laparoscopic procedure. However, it had to be pulled down in the current study.

**Pararectal space opening, uterosacral, and cardinal ligament resection**
As the pararectal space was developed, the descending part of the uterosacral ligament was coagulated and dissected (Figure 2L). The cervix was pushed to the opposite side by cervical depressor to stretch the cardinal ligaments and parametrium. Once identified, these parts were clamped, severed, and tied as close to the pelvic sidewall as possible (Figure 2M).

**Anterior and posterior peritoneal reflex and accessory**
The anterior and posterior peritoneal reflexes were opened, the uterine fundus was pulled downward and clamped, and the sagittal part of the uterosacral ligaments was cut (Figure 2N). In the laparoscopic procedure, the utero-ovarian ligaments were sectioned and ligated to reserve the accessories. Otherwise, salpingo-oophorectomy was carried out by ligating and sectioning the infundibulopelvic ligaments. Finally, the specimen was extracted.

**Closure of vaginal stump and peritoneum**
The anterior and posterior vaginal mucosa and peritoneum were closed continuously with sutures (Figure 2O).
Table 3. — Intraoperative and postoperative complications

<table>
<thead>
<tr>
<th>Intraoperative complications</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Bladder trauma</td>
<td>0</td>
</tr>
<tr>
<td>Ureteral injury</td>
<td>0</td>
</tr>
<tr>
<td>Damage of bowel</td>
<td>0</td>
</tr>
<tr>
<td>Damage of great vessel</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Postoperative early complication (occurring within two months)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder dysfunction (urinary retention)</td>
<td>9</td>
</tr>
<tr>
<td>Postoperative morbidity (&gt;38 °C)</td>
<td>7</td>
</tr>
<tr>
<td>Poor healing of vaginal stump</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocyst</td>
<td>1</td>
</tr>
<tr>
<td>Urogenital fistula</td>
<td>0</td>
</tr>
<tr>
<td>Deep vein thrombosis (DVT)</td>
<td>1</td>
</tr>
<tr>
<td>Obturator nerve injury</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. — Follow up data and adjuvant treatment.

<table>
<thead>
<tr>
<th>Total number followed</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median during of follow up (months)</td>
<td>46 (15-108)</td>
</tr>
<tr>
<td>Patients receiving adjuvant radiotherapy</td>
<td>8</td>
</tr>
<tr>
<td>Patients receiving adjuvant chemo-radiation</td>
<td>10</td>
</tr>
<tr>
<td>Mean survival time (months)</td>
<td>89</td>
</tr>
<tr>
<td>Overall survival rate</td>
<td>97.62% (82/84)</td>
</tr>
<tr>
<td>Recurrence rate</td>
<td>3.57% (3/84)</td>
</tr>
</tbody>
</table>

had the criteria of FIGO 1995, refers to the inability to empty the bladder or residual volume of urine of more than 100 ml 14 days after an operation. In the current study, bladder dysfunction was one of the most common postoperative complications. The automatic urine recovery time was longer than that of other reports, and it could be associated with the more thorough transvaginal separation of the ureter. Seven cases of postoperative morbidity and one case of lymphocyst problem had favourable prognosis. No urogenital fistula occurred.

Survival and outcome

Median follow-up was 46 months (15 to 108 months). Two patients were lost in the follow-up. The total recurrence rate in the entire cohort was 3.57% (3/84). All recurrences occurred in the pelvis and received adjuvant chemo-radiation, but two patients died. Three and four patients were recommended to undergo postoperative adjuvant radio-chemotherapy as their final histological results revealed Stage IIB and lymph node positive, respectively (Table 4). The reasons for radiotherapy were lymph vascular space invasion in three patients, adenocarcinoma in two, and deep stromal invasion in three others.

Discussion

This study mainly focused on improving the technology and instruments for VRH, as well as providing data to demonstrate VRH as an effective operation for early cervical cancer treatment. Previous reports have evaluated the validity of LARVH; however, most studies emphasised on operation quality, operation procedure or equipment and did not give full accounts [10-15]. VRH combined with laparoscopic lymphadenectomy is a safe and effective surgical alternative for early-stage cervical cancer patients. VRH has more advantages than ARH in terms of blood loss, hospital stay, cosmetic result, and oncologic prognosis. TLRH is also a safe and effective therapeutic procedure for early stage cervical cancer management because it is characterised by low blood loss and short bowel recovery time. The limitation of TLRH is its difficulty to adequately resect the vaginal cuff according to tumour size, potentially leading to tumour spillage after opening the vagina, and higher stump recurrence [9].

Compared with other radical hysterectomies, VRH offers many distinct advantages [16]. VRH is a simple, safe, and rapid procedure if performed by a well-trained doctor. Moreover, this procedure is applicable to patients with a poor medical condition or those with morbid obesity. VRH also easily manages the extent of vaginal excision. It has low incidence of intraoperative and postoperative complications, as well as quick postoperative bladder and rectal function recovery. These advantages of VRH should be assessed carefully when surgical approach is a choice. However, unlike in other surgical methods, deficiencies are often found in VRH regarding operative field exposure (episiotomy is usually necessary) and critical procedures, such as gap separation and ureter knee dissection [17]. The learning curve of VRH may be longer than that of other surgical methods [18]. To date, only a few hospitals can perform VRH in China and even worldwide. Gynaecologists have been widely concerned about how to make the LARVH more applicable, safer, and easier to learn.

VRH modifications

Firstly, the modification to VRH is the application of an illuminating catheter designed by the authors. The ureteral illuminating catheter contributed greatly in the critical operative procedure (separation of the ureter knee). Prior to VRH operation, the illuminating catheter was inserted into the bilateral ureter under a cystoscope. The catheter shines when switched on, and it enables the easy location of the ureter during the operation. The ureter can also be explored by touching the ureteral illuminating catheter to effectively reduce the risk of ureteral injury. LARVH was previously associated with a higher rate of intra- and postoperative urologic complications than ARH or TLRH because of the difficulty in ureteral dissection [9]. In the current study, the average time for ureter separation was only 18 ± 5 minutes, and no ureter or urinary bladder injury occurred. These findings adequately proved the superiority of VRH. The results also showed that the average range of vaginal excision was 3.25 ± 0.16 cm, and the average length of the excised parametrium was 3.61 ± 0.22 cm. Therefore, the visualisation of the ureteral catheter in VRH is beneficial to remove enough parametrium to meet the radical resection requirement.
Secondly, the special instruments for VRH improved the operative field exposure and lessened the difficulty in the critical procedure. To date, no similar instruments have been reported. The steps in separating the paravesical spaces are designed for VRH. For example, when the vesicocervical ligament is exposed, the vesicocervical space retractor is designed to expose the vesicocervical space, and the paravesical space retractor helps to dissect the paravesical space. The vesicocervical ligament lies between the two retractors. The vesicocervical ligament retractor is designed to press the cervix downward and to have a double-leaf component to hold and stretch the vesicocervical ligament to easily locate the ureter. The cervical depressor also has a double leaf structure to hold the cardinal ligament, and it is used to push the cervix to the opposite side to stretch the cardinal ligament and parametrial tissues. The cervical heavy hammer is designed to provide gravitational pulling power. Although these devices are simple and inexpensive, they greatly assist in critical procedures, such as the dissection of cardinal ligament, parametrium tissues, and ureter. In the current study, all cases had adequate excision of parametrium, and the outcome was satisfactory.

Thirdly, the ureter was lifted using a flexible rubber hose after the ureter was separated. This process avoided ureteral injury. The ureter is more easily separated in VRH than in ARH and TLRH. This finding could be attributed to the fact that the mean automatic urine recovery time was long in the current study.

Finally, the classic or modified Schuchardt incision is unnecessary in the present authors’ operation. This incision enables the widening of the vaginal access to the uterus and its ligaments. However, this procedure leads to bleeding, haematoma, infection, pain, and long-term dyspareunia [4]. Metastases in a Schuchardt incision have also been reported by Bader et al. [19] With the help of special retractors, the operating fields can be exposed efficiently. The Schuchardt incision is unnecessary in the present centre.

In summary, LAVRH is an oncologically valid alternative for cervical cancer treatment. Special instruments can enhance feasibility, safety, and easy performance of this procedure for gynaecologists. Further studies about this technique are needed to underline the promising results of the present single-institutional series.

Acknowledgements

The authors would like to thank Shangwu Yang (PhD) for his assistance in writing the present work. This study was supported by Guangdong Foshan City Technology projects (No. 201008065) to XQH.

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New method: are tumor markers in vaginal-washing fluid significant in the diagnosis of primary ovarian carcinoma?

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Introduction

Ovarian cancer is the seventh most common cancer in women worldwide, with nearly a quarter of a million women diagnosed every year. Despite its relatively low incidence rate, ovarian cancer is an extremely lethal disease. Most patients (75%) present with advanced-stage (III/IV) tumors, for which the five-year survival rate is 30% [1].

The detection of tumor markers has been shown to be an effective and noninvasive diagnostic tool for the diagnosis of ovarian cancer. The serum tumor marker antigens CA 125, CA 19-9, and carcinoembryonic antigen (CEA) are potentially of clinical value for the diagnosis of ovarian cancer [2].

CA 125 is the most commonly-used biomarker for diagnosis and follow-up of ovarian cancer. It is a high-molecular-weight glycoprotein with an elevated serum level (>35 U/ml) in 50-90% of patients with ovarian cancer, depending on the cancer stage. However, the diagnostic performance of serum CA 125 for early-stage ovarian cancer is as low as 25% for Stage I and 61% for Stage II [3].

CEA is a protein that may be elevated in malignancies that produce it, particularly in mucinous cancers associated with the gastrointestinal tract or the ovary. However, some benign conditions have also been associated with an elevated CEA, including cholecystitis, liver cirrhosis, and pancreatitis [4].

CA 19-9 is a mucin protein that may be elevated in ovarian cancer, and it is also used in ovarian cancer management. CA 19-9 levels may be elevated in a variety of other malignant and benign conditions. CA 19-9 may be used as predictive test for the differentiation of ovarian cancer from benign adnexal masses [5].

However, in practice, there is no information available regarding the use of vaginal washings for tumor markers CA 125, CA 19-9, and CEA in the diagnosis of ovarian cancer. The present authors hypothesized that the use of vaginal-washing tumor markers CA 125, CA 19-9, and CEA may increase diagnostic sensitivity and/or specificity in ovarian cancer. Receiver operating characteristic (ROC) curves have been widely used as a standard approach for calculating the sensitivity and specificity of medical diagnostic tests. In this study, the authors aimed to investigate the diagnostic sensitivity and specificity of vaginal-washing tumor markers CA 125, CA 19-9, and CEA by ROC analysis.

Summary

Objective: Ovarian cancer is the seventh most common cancer in women worldwide, with nearly a quarter of a million women diagnosed every year. The serum tumor markers cancer antigens CA 125, CA 19-9, and carcinoembryonic antigen (CEA) are potentially of clinical value for the diagnosis of ovarian cancer. The purpose of this study was to evaluate the diagnostic sensitivity and specificity of vaginal-washing tumor markers CA 125, CA 19-9, and CEA for diagnosis of primary ovarian cancer. Materials and Methods: In the current prospective study, 30 patients with advanced primary ovarian cancer and 30 patients with benign ovarian cysts were enrolled. The vaginal-washing fluid samples were obtained the day before surgery and were immediately centrifuged and stored at -80 °C until analysis. Measurements of CA 125, CA 19-9, and CEA were determined using fully-automated chemiluminescent microparticle immunoassays. Results: The vaginal fluid concentrations of CA 125, CA 19-9, and CEA in patients with primary ovarian carcinoma were significantly higher (p < 0.001) compared to those in patients with benign adnexal masses (p < 0.001). In the ROC curve analysis, the optimal cut-off values for the detection of primary ovarian cancer were >295 for CA 125 (p < 0.001), >101 for CA 19-9 (p < 0.001), and >135 for CEA (p < 0.001). Conclusion: Vaginal-washing tumor markers CA 125, CA 19-9, and CEA are simple, noninvasive, and reliable diagnostic tests for the detection of primary ovarian cancer.

Key words: Vaginal-washing tumor markers; CA 125; CA 19-9; CEA.
Table 1. — Demographic characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Primary ovarian cancer</th>
<th>Benign ovarian cyst</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>30</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>55 (48–64)</td>
<td>51 (45–60)</td>
<td>0.097</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, (kg/m²)</td>
<td>23.5 (21.0–27.2)</td>
<td>22.8 (20.9–27.2)</td>
<td>0.072</td>
</tr>
<tr>
<td>Gravida</td>
<td>3.9±1.1</td>
<td>3.7±1.2</td>
<td>0.609</td>
</tr>
<tr>
<td>Parity</td>
<td>2.5±1.3</td>
<td>2.3±1.1</td>
<td>0.505</td>
</tr>
</tbody>
</table>

Histological type
- Papillary serous cystadenocarcinoma
- Serous cystadenoma

FIGO Stage
- III-IV
- Benign

Ovarian mass size
- 8.9±1.3
- 8.4±1.2
- 0.543

p<0.05 was considered to indicate a statistically significant difference.

Table 2. — Vaginal washing concentrations of CA 125, CA19-9, and CEA in different patient groups.

<table>
<thead>
<tr>
<th>Groups (ml)</th>
<th>CA125 (ng/ml)</th>
<th>CA19-9 (ng/ml)</th>
<th>CEA (ng/ml)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary ovarian cancer</td>
<td>354.5 ± 94.3</td>
<td>128.5 ± 23.2</td>
<td>193.1 ± 29.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Benign adnexal mass</td>
<td>252.6 ± 48.8</td>
<td>82.8 ± 34.8</td>
<td>137.1 ± 41.6</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* p < 0.05 was considered to indicate a statistically significant difference.

Materials and Methods

In the current prospective study, 30 patients with advanced primary ovarian cancer (Group 1) and 30 patients with benign ovarian cysts (Group 2), all treated at the Department of Gynecology’s Oncology Unit at Dicle University and Department of Gynecology at Kocaeli Derince Education and Research Hospital between March 2008 and January 2014, were included in this study. All 60 of these pre- and post-menopausal women, aged 45 years or older, had presented to a gynecologist with a pelvic mass (defined as a simple, complex, or solid ovarian cyst/pelvic mass). None of the patients had history of surgery for tubal ligation. The set of primary ovarian cancer patients (n=30, Group 1) comprised the study group, while the remaining 30 patients with benign ovarian cysts comprised the control group (n=30, Group 2). All of the patients underwent pelvic ultrasonography (transabdominal + transvaginal) performed by an expert gynecological sonographer prior to surgery. All adnexal lesions were described according to the morphological and vascular features as suggested by the consensus opinion from the International Ovarian Tumor Analysis (IOTA) group [6].

The primary ovarian cancer patients were surgically staged and debulked to achieve minimal residual tumor volume via laparotomy. International Federation of Gynecology and Obstetrics (FIGO) criteria were used to stage the ovarian cancer patients [7]. The benign ovarian cysts were removed by laparoscopic surgery. All tissue pathologic analysis of the fallopian tubes, ovaries, and uteri was performed by a gynecologic pathologist. All patients gave written consent, and the study was approved by the local Ethics Committees.

Sampling of vaginal-washing fluid

The vaginal-washing fluid (VWF) samples were obtained one day before surgery. A sterile speculum examination was performed on each patient, during which ten ml of sterile normal saline was injected into the posterior fornix of the vagina and then aspirated from the posterior vaginal fornix with the same syringe. Each vaginal fluid sample was sent immediately to the laboratory, where it was immediately centrifuged at 3,000 rpm for ten minutes and the supernatant was stored at -80°C until analysis. All speculum examinations were performed by the same gynecologic oncologist.

Measurement of vaginal tumor markers

Vaginal-washing testing for CA 125, CA 19-9, and CEA was performed using a fully-automated chemiluminescent microparticle immunoassays (CMIA), according to manufacturer’s instructions, and appropriate controls were included in each run.

Statistical analysis

The results are reported as means ± SD. A t-test was performed for demographic characteristics. The Mann-Whitney U test (SPSS 17.0 statistical software package for Windows) was applied to determine the differences in marker levels. The MedCalc statistical software package was utilized to assess the difference between different areas under the curve (AUC). To evaluate the diagnostic sensitivity and specificity, positive and negative predictive values were calculated at the optimal cut-off. A p < 0.05 was considered to indicate a statistically significant difference.

Results

The demographic parameters and histological types are shown in Table 1. The concentrations of CA125, CA19-9, and CEA in patients with primary ovarian carcinoma were significantly higher (p < 0.001) compared to patients with benign adnexal masses (Table 2). The diagnostic indices for the vaginal-washing tumor markers’ cut-offs are presented in Table 3.

In the ROC curve analysis, the optimal cut-off values for the detection of primary ovarian cancer were > 295 for CA 125 with an AUC equal to 0.81 (p < 0.001) (Figure 1). In the ROC curve analysis, the optimal cut-off values for the detection of primary ovarian cancer were > 101 for CA 19-9 with an AUC equal to 0.87 (p < 0.001) (Figure 2). In the

Table 3. — The diagnostic indices for the vaginal-washing tumor markers’ cut-offs are presented.

<table>
<thead>
<tr>
<th>Markers</th>
<th>ROC area (%)</th>
<th>95% CI</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%) (95% CI)</th>
<th>NPV (%) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 125 (ng/ml)</td>
<td>81.5*</td>
<td>(69.4 - 90.4)</td>
<td>63.3</td>
<td>90.0</td>
<td>86.3</td>
<td>71.0</td>
</tr>
<tr>
<td>CA19-9 (ng/ml)</td>
<td>87.8*</td>
<td>(76.8 - 94.8)</td>
<td>86.7</td>
<td>83.3</td>
<td>83.8</td>
<td>86.2</td>
</tr>
<tr>
<td>CEA (ng/ml)</td>
<td>84.2*</td>
<td>(72.5 - 92.4)</td>
<td>100</td>
<td>66.7</td>
<td>75</td>
<td>100</td>
</tr>
</tbody>
</table>

*p < 0.001 compared with CA125, CA19-9, and CEA. ROC: receiver operating characteristic. CI: confidence interval.
New method: are tumor markers in vaginal-washing fluid significant in the diagnosis of primary ovarian carcinoma?

ROC curve analysis, the optimal cut-off values for the detection of primary ovarian cancer were > 135 for CEA with an AUC equal to 0.84 ($p < 0.001$) (Figure 3).

**Discussion**

Tumor markers are substances measured in blood or other bodily fluids; they are found in normal tissue but may be produced in large amounts when tissue undergoes neoplastic change. They may be products of normal, benign, or malignant tissue on cell surfaces [1-6].

As previously described, serum biomarkers are widely used in ovarian cancer screening and diagnosis, as well as for monitoring treatment response and recurrent disease status in ovarian cancer patients. CA 125 has a sensitivity of 73.2% and a specificity of 79.2% in predicting ovarian malignancy. However, CA 125 is increased not only in cases of ovarian cancer but also in some benign conditions. Isolated serum CA 125 values lack adequate sensitivity or specificity, and a false-positive CA 125 value may result in unnecessary diagnostic work-up or surgery [8].

CEA has been used to monitor colorectal cancer for decades and is reported to be elevated in 30-65% of ovarian epithelial cancers. Tumors of the ovary contain a population of intestinal-like cells that resemble those present in colonic adenomas. Serum concentrations of CEA exceeding five ng/ml are often found in patients with ovarian cancer. Serum CEA elevation occurs more often in mucinous tumors than in serous tumors of the ovary [9].

CA 19-9 is a sialylated antigen, which is expressed in gastrointestinal adenocarcinomas and ovarian cancers. CA 19-9 is also used to monitor disease response to therapy or to detect recurrence in patients with ovarian cancer [10].

The combination of CA 125, CA 19-9 and CEA provides a higher level of discriminatory power than any of these markers alone for distinguishing benign from malignant ovarian masses [11]. Thus, we need simple, reliable, and noninvasive tests for the diagnosis of ovarian cancer. There is no unique and noninvasive gold-standard test applicable in ovarian cancer patients with high accuracy. The purpose of the present study was to determine the effectiveness of utilizing vaginal washings for analysis of tumor markers CA 125, CA 19-9, and CEA in the diagnosis of ovarian cancer, since there is no current literature data on this subject.
The present authors hypothesized that tumor markers excreted from tumor cells, due to regurgitation of tumor cells through the fallopian tube, may be detected in vaginal washing fluid. The present results showed that vaginal-washing concentrations of these three markers were significantly higher in primary ovarian carcinoma than in benign adnexal masses. The optimal cut-off value of 295 ng/ml for the detection of primary ovarian cancer was proposed for CA 125. The sensitivity, specificity, positive predictive value, and negative predictive value of CA 125 were 63%, 90%, 86%, and 71%, respectively. The optimal cut-off value of 101 ng/ml for the detection of primary ovarian cancer was proposed for CA 19-9. The sensitivity, specificity, positive predictive value, and negative predictive value of CA 19-9 were 86%, 83%, 83%, and 86%, respectively. The optimal cut-off value of 135 ng/ml for the detection of primary ovarian cancer was proposed for CEA. The sensitivity, specificity, positive predictive value, and negative predictive value of CEA were 100%, 66%, 75%, and 100%, respectively.

The present authors propose that the sampling of vaginal washings for the tumor markers CA 125, CA 19-9, and CEA is a simple, noninvasive, and reliable diagnostic test for the detection of primary ovarian cancer.

In conclusion, vaginal-washing tumor markers CA 125, CA 19-9, and CEA are found to have high sensitivity, specificity, and positive and negative predictive values in the diagnosis of primary ovarian cancer. The present study demonstrates that the measurement of vaginal-washing tumor markers is a better strategy than utilizing blood-sampling methods.

References


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Human papillomavirus infection among Uyghur women with cervical intraepithelial neoplasia in Xinjiang area

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Introduction

Invasive cervical carcinoma is the second common malignant tumors that threaten the health of female worldwide, with an estimated 493,000 new cases and 274,000 deaths in the year 2002. In general terms, it is much more common in developing countries, where 83% of cases occur and where cervical cancer accounts for 15% of female cancers, with a risk before age 65 of 1.5% [1]. Persistent infection of oncogenic human papillomavirus (HPV) is the pathogenesis of cervical cancer and precancerous lesion, cervical intraepithelial neoplasia (CIN). So far, there are about 100 subtypes of HPV have been identified, of which more than 40 types are known to infect female reproductive tract, according to their pathogenicity,they are classified as high-risk HPV includes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, and so on, types 26, 53, and 66 should be considered as probably carcinogenic [2]. Some recent meta-analysis showed that HPV16 and 18 subtypes are the two common types in Asian women with invasive cervical carcinoma, and pointed out that HPV-52, 58, and 45, 33 subtypes are the common types of Southeast Asia and East Asia, South Asian women with cervical cancer and precancerous lesions [3, 4]. Therefore, oncogenic HPV testing combined with cytological testing was accepted as primary screening in the USA, because of sensitivity and effectiveness [5]. In addition, in view of the wide applications of HPV vaccines in the world, polyvalent vaccine containing HPV16, 18L1 virus-like particle (virus-like particle, VLP) has been licensed, which has been confirmed to be effective in the prevention of the CIN II/III and the efficacy of the vaccine is sustainable for about 42 months since it has been approved [6, 7]. From evidence of clinical trials, these two targeting of HPV (detection and vaccine) are increasingly attractive for cervical cancer prevention worldwide. At present, in Xinjiang, HPV DNA testing has not yet been available in routine screening and HPV vaccine has not been licensed. Evaluating the type-specific data of HPV genotypes in Uyghur women with normal cytology and with CIN in Xinjiang is important as evaluating the potential benefits of vaccination in cervical cancer prevention and the future role of HPV screening. For now, there are only limited data available from Xinjiang on HPV-type prevalence in normal cytology and in CIN.

Summary

Objective: To obtain the baseline data of Uyghur women for human papillomavirus (HPV) vaccination in Xinjiang. Materials and Methods: The authors analyzed the infection and distribution characteristics of HPV genotypes in genital tracts among Uyghur women with cervical intraepithelial neoplasia (CIN) in Urumqi of Xinjiang. A total of 1,431 eligible cases involved in this trial. All cervical samples from these patients were detected for HPV genotype. Results: High-risk HPV was identified in 24.7% of 979 histologically confirmed normal samples and 89.2% of 452 samples with CIN (p < 0.05). The prevalence of one single high-risk type, low-risk type, and multiple HPV types were 74.6%, 10.4%, and 4.2%, respectively. A single high risk HPV infection progressively increased with the severity of cervical lesions significantly (χ2 = 31.53, p < 0.01). While interestingly multiple infection and single low risk HPV infection were decreased with the severity of cervical lesions, and there was significant difference (χ2 = 6.44, p < 0.05; χ2 = 4.85, p < 0.05). The major prevalent high-risk HPV genotypes in 346 samples of CIN II-III were HPV-16, -58, -31, -33, -68, -18, -45, and -39. The comparison of HPV genotype distributions between normal cytology and CIN II-III was analyzed. The estimated risks for progression from viral infection to CIN II-III was highest in HPV-16, -33 (prevalence ratio (PR), 2.62), followed by HPV-31 (2.27), HPV-16 (1.92), HPV-58 (1.62), HPV-18 (1.51), HPV-68 (1.05), and HPV-39 (1.05), suggesting that the six genotypes of HPV-31, -16, -58, -18, -68, and -39 (PR > 1) are higher-risk HPV types in Uyghur women with CIN in Urumqi of Xinjiang. There was no association between multiple infection and cervical lesion progression (0.31, PR < 1). Conclusion: Except for the common HPV-16, -58, -31, -33, -18 in Xinjiang, HPV-68 and HPV-39 may be the oncogenic subtypes to Uyghur female with CIN in Xinjiang. Distinguishing these HPV subtypes may have implications for future cervical screening strategies and vaccine implementation. Multiple infections were not association with an increased risk of high-grade cervical neoplasia.

Key words: Cervical intraepithelial neoplasia; Human papillomavirus; Genotypes; Infection characteristics; Uyghur.
Materials and Methods

Study population

The study subjects consisted of 1,431 Uyghur women in Xinjiang (979 normal cytology, 106 CIN I, 210 CIN II, and 136 CIN III). All of the eligible women enrolled have been living in Xinjiang for more than ten years. Inclusion criteria included the following: continuing irregular vaginal bleeding, bleeding after intercourse, those found on examination to have an unhealthy cervix, or those who visited the hospital for cervical cancer screening. All patients underwent cervical liquid-base cell smears detection and HPV genotyping chip detection. An enrolment questionnaire was completed. All cervical exfoliated cell samples were obtained for cervical liquid-based cytology examination and HPV detection. After the cervical scrapes, women with abnormal cervical smear results underwent colposcopic examination of cervix and cervical biopsy for histological verification was performed. Classification of each cytological diagnosis was based on the Bethesda System 2001. The histological analyses were obtained by two professional pathologists who did not know the HPV status. Final histological diagnosis of the specimens was based on the WHO classification of cervical neoplasia. At last histological diagnosis confirmed 106 as CIN I, 210 cases as CIN II, and 136 cases as CIN III. Then the infection distribution of the HPV types according to the hierarchical diagnosis were evaluated.

HPV sample and genotyping: the collection of cervical cell suspensions for each patient was performed using a plastic cervical swab from ecto-and endocervix of uterus. Each plastic swab was well-mixed with one ml of specimen transport medium and stored immediately at 4°C. All specimens were finally sent to the laboratory for HPV genotyping analysis. The authors used gene amplification and flow-through hybridization technology as an HPV genotyping method, which concluded three processes of extraction of total DNA of cervical cells, gene amplification (PCR), and flow-through hybridization. The experimentation was performed strictly according to the manufacturer’s instruction. The final results of the testing were achieved colourimetric change on the chip under direct visualization. The result was a single or mixed HPV infection. The three steps can detect 21 different HPV genotypes, which were classified as flows: high-risk genotypes: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, low-risk genotypes: HPV 6, 11, 42, 43, 44 genotypes and the common HPV genotypes in Chinese population as HPV 53, 66, CP8304.

Statistical analyses

The data were analyzed Using SPSS for Windows, standard version 17.0 statistical software. To estimate different HPV genotype risk for progression from viral infection to CIN II-III, prevalence ratios (PRs) were calculated. Statistical analysis was carried out with Chi-square ($\chi^2$) test or Fisher’s exact test. A $p$ value of < 0.05 was considered to be statistically significant.

Results

This study included 1,431 Uyghur women (979 normal cytology, 106 CIN, and 346 CIN II-III). The mean age of the study subjects was 36.9 years (range, 18-76) for women with normal cytology, 35.6 years (range, 18-69) for CIN I, 35.9 years (range, 18-73) for CIN II-III. A total of 17 HPV types were detected in women with normal cytology. In CIN II, 15 different HPV genotypes were detected. In CIN I and III, 13 and 14 subtypes were found, respectively.

The characteristics of HPV infection in CIN:

Of the 452 patients with CIN, HPV tested positive in 403 cases (89.2%, 403/452), 92.4% of HPV prevalence in CIN II-III. Single high-risk HPV genotypes infection were detected in 74.6% of the women (337/452), while single low-risk HPV genotypes were detected in 4.2% of the women (19/452). Multiple infections with two and three were found in 10.4% of the women (47/452). Dual infection was present in 37 women, triple infection in ten cases. The present study showed that high-risk HPV genotypes were major genotypes in CIN. A single high-risk HPV infection progressively increased with the severity of cervical lesions significantly ($\chi^2 = 31.53, p < 0.01$). While interestingly multiple infection and single low risk HPV infection were declined with the severity of cervical lesions. There was significant difference ($\chi^2 = 6.44, p < 0.05$; $\chi^2 = 4.85, p < 0.05$) (Table 1).

The distribution characteristics of HPV genotype in cervical intraepithelial neoplasia

The diversity of HPV types was widespread in women with CIN. Of the 21 detectable HPV types, 16 genotypes were identified. The frequencies of main types of HPV in CIN are summarized in Figure 1. The most prevalent high-risk HPV genotypes were HPV-16 (50.4%), followed by HPV-58 (10.7%), HPV-31 (8.4%), HPV-33 (7.9%), HPV-18 (5.7%), HPV-68 (5.2%), HPV-45 (4.7%), HPV-52 (4.2%), and HPV-39 (3.7%). Concerning the low-risk HPV, the distribution HPV-6 (3.7%), HPV-11 (3.0%), other types were much less common.

HPV genotype risk for CIN in Uyghur women in Xinjiang

To assess the progressive potential risk of each HPV genotype, HPV type distribution and prevalence in women with HPV-positive are shown in Table 2. Among Uyghur women with normal cytology, HPV was identified in 24.7% of 979
cases and 89.2% of 452 samples with CIN, (p < 0.05). HPV-16 (26.0%), and HPV-58 (5.4%) were most frequent among Uyghur women with normal cytology, followed by HPV-68 (4.1%), HPV-31, 45(3.3%), HPV-52 (2.5%), HPV-18,-33,-39 (2.1%), HPV-53, -66 (1.6%), and HPV-51 (1.2%). In CIN II - III, HPV-16 was also the most prevalent genotype (50.0%), followed by HPV-58 (8.7%), HPV-31(7.5%), HPV-33 (5.0%), HPV-68 (4.4%), HPV-18 (3.1%), HPV-45 (2.5%), HPV-39 (2.2%), HPV-52 (1.6%), HPV-51 (0.9%), HPV-53 and HPV-66 (0.6%). When compared the HPV genotype distribution between normal cytology and CIN II - III, the authors found that estimated risks for progression from HPV infection to CIN II - III was highest in HPV-33 (PR 2.62), followed by HPV-31 (2.27), HPV-16 (1.92), HPV-58 (1.62), HPV-18 (1.51), HPV-68 (1.05), and HPV-39 (1.05), which suggesting that HPV-33, -31, -16, -58, -18, -68, and -39 (PR > 1) might be the high-risk HPV in Uyghur women in Xinjiang. Although the prevalence of multiple infection increased from normal cytology to CIN II - III, PR was 0.31, suggesting that there was no association between multiple infection and cervical lesion progression.

Discussion

This study represents the characteristics of the distribution and analysis of HPV genotypes in cervical intraepithelia among Uyghur women in Xinjiang region. In this study, the authors also have described the prevalence of a much
wider spectrum of genotypes among Uyghur patients with different grade CIN and normal cytology from Xinjiang region. The present study found that the prevalence of HPV genotype in patients with cervical cancer and precancerous lesions has ethnic and regional characteristics. At present, there are few studies addressing HPV distribution in patients with CIN. A meta-analysis in the Asian population found that the prevalence of HPV in patients with CIN was 76.4% [8]. At the same time, a meta-analysis from Asia suggested that the prevalence of high-risk HPV genotype in patients with CIN was 81.0% [9]. The present study showed that the prevalence of HPV in patients with CIN was 89.2%, which was higher than the two aforementioned studies from Asia, but slightly lower than the prevalence (98.5%) of HPV in patients with CIN in Europe [10]. Another current study which covered Northern to Southern regions in China revealed that HPV type-distribution prevalence in high-grade lesions was 82% [11]. However the present study found that the prevalence of HPV in high CIN was 92.4% in CIN II - III. Importantly variations in HPV prevalence could be caused by the following factors: specimens' quality and storage, as well as HPV type detectable method by different systems. Therefore due to this variability, it is difficult to compare HPV prevalence between studies. The assay used in the present study, may be a reason for the discrepancy.

The present study also revealed that the prevalence of HPV showed a significantly increased trend with increased grade of CIN. As expected, the increased proportion of single high-risk HPV infection was also significantly among CIN I - CIN II / III. While the comparable declined in proportion of single low-risk HPV infection. These findings were similar to those of Sandri et al. [12]. All facts support that high-risk HPV persistent infection was the necessary etiologic factor in cervical carcinogenesis. On the other hand, given cervical lesion precede the development of cervical cancer by several years, the mean duration of low-risk and high-risk HPV infection was four and eight months, respectively [13]. This phenomenon could be explained that the high-risk HPV may persist longer than low-risk HPV. Hence single low-risk HPV infection may be cleared by the body's immune system over time, with which it is not easy to cause persistent infection. From another point of view, it is not the high risk factors to cause the progression of CIN.

In the present study the multiple HPV infections among Uyghur women in Xinjiang area was found in 10.4% in CIN, of which double infection was detected in 8.2%, with triple infection in 2.2%, and quadruple infection had not been detected. Multiple HPV infection was significantly decreased with the severity of cervical lesions. The phenomena might indicate that Uyghur women in Xinjiang intermarry rarely with other nations and are not vulnerable to multiple HPV infection. Other causes may be their special background of life and living habits. While the present data suggested that there was no association between multiple infection and cervical disease progression in Uyghur women in Xinjiang (PR < 1), whether or not multiple infection may be a risk factor for the occurrence and development of CIN and cervical carcinoma have not been confirmed. Some scholars believe that the multiple HPV infections are the risk factors for development of CIN [14, 15], while other scholars suggested that multiple HPV infection was not associated with the progression of cervical lesions [16, 17]. The present results do not support an association of multiple infection with increased severity of CIN. Another study concluded that in all studies of invasive carcinoma, the risk linked to multiple HPV types does not vary significantly from the risk linked to single HPV types [13].

Worldwide studies show that the predominant HPV genotypes of the female with normal cervical cytology were HPV-16, -31, -18 in Europe, HPV-16, -18, -33 in Asia, and HPV-16 in South America [18]. The present study found that the prevalence HPV genotype of the Uyghur women with normal cytology were HPV-16, -68, -31, -45, -52. It is somewhat different because of different geographical areas. A worldwide meta-analysis shows that the distribution of high-risk HPV genotype was different by different geographical areas, it is noteworthy that HPV-31, -52, and -58 genotypes were more prevalent in other areas except for Europe [15]. A study from India showed that the prevalence of high-risk HPV genotype in patients with CIN was 87.5%; the most common HPV genotypes in decreasing order were: HPV-16, -18, -33, -39, -35, and -56 [19]. Another study collected specimens from 17 cities of Europe and discovered that the prevalence of HPV genotypes in patients with CIN II or worse were HPV-16, -33, and -31 [10]. The present study found that the prevalence of HPV genotypes in Uyghur female patients with CIN were in decreasing order: HPV-16, -58, -31, -33, -18, -68, -45, -39, and -52. The common HPV genotypes in CIN II - III were also in decreasing order: HPV-16, -58, -31, -33, -68, and -18, respectively. The present study also found that HPV-16 was the main high-risk genotype in Uyghur women with CIN in Xinjiang. As previously reported, HPV-16 was the most frequent type associated with high-grade cervical lesions and cervical cancer, compared to all other high-risk HPV types [20, 21]. These conclusions may indicate that there is a more rapid progression to high cervical lesion in HPV-16 infected women. Similar to this result, another study had suggested that HPV-16 associated with CIN II could easily lead to progression than CIN II infected with other genotypes [22]. The present data also assessed risks for progression from HPV infection to CIN II-III by different HPV genotypes. The authors found that estimated risks was highest in HPV-33 (PR 2.62), followed by HPV-31 (2.27), HPV-16 (1.92), HPV-58 (1.62), HPV-18 (1.51), HPV-68 (1.05), and HPV-39 (1.05), which suggested that HPV-33, -31, -16, -58, -18, -68, and -39 (PR > 1) might be the high-risk HPV in Uyghur women in Xinjiang. The present authors inferred that in addition to HPV16, 58, 33, 31, and18, HPV-68 and HPV-39 may play important role in Uyghur women with CIN in Xinjiang. However further study is necessary to confirm the real risk related to these.
References


Primary HPV test screening in cervical cancer: a two-year experience of a single screening center in Latina (Italy)


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Summary
Objective: The aim of this study was to evaluate the effect and performance of the new algorithm in cervical cancer screening program in two years’ experience of Latina (Italy). Materials and Methods: The female population was divided into two groups, the first group was referred to PAP test and the second one to hr-HPV test according to national guidelines. Results: In two years the participation mean rate increased among women aged 35-64 compared to women aged 25-34. The primary PAP test positive rate and hr-HPV test positive rate were 4.0% and 5.2%, respectively. The PAP test positive rate among hr-HPV+ women decreased from 2012 to 2013. Women with hr-HPV+/PAP+ were referred immediately to colposcopy and this rate was 1.2%. The predictive positive value for CIN2+ to colposcopy was 10.9% in 2012 and 9.1% in 2013, while the detection rate for CIN2+ was 1.6% in 2012 and 1.4% in 2013. Conclusion: The stratification of the female population leads to a decreased inappropriate therapeutic path while the combination of hr-HPV test with PAP test in woman aged 35-64 lets obtain high levels specificity and sensitivity results.

Key words: Cervical cancer screening; HPV; HC2.

Introduction
Cervical cancer is the second most common cancer in the female population and in Italy it is estimated to affect about 3,500 women/year [1]. The program cervical screening is a health intervention made on an asymptomatic population; it has the purpose of early diagnosis of cervical intraepithelial neoplasia (CIN) [2] before the appearance of symptoms by identifying the disease in the earlier stage of its natural history [1, 3]. The early diagnosis also allows to take appropriate therapeutic treatment promoting healing and reducing cervical cancer mortality [4-7]. Human papillomavirus (HPV) is a key factor, although not sufficient, for the cervical cancer development [8].

The International Agency for Research on Cancer has confirmed the oncogenic potential of 12 HPV types (16, 18, 31, 33, 34, 39, 45, 51, 52, 56, 59, and 59) in the development of cervical cancer, defining these as high-risk HPV (hr-HPV) [9].

The cytology cervical smear (PAP test) has always been considered the first level of investigation in cervical cancer screening. However, it was shown that the molecular hr-HPV test is more sensitive than the PAP test in identifying ≥ CIN2 lesions [10-11]. Hence, the use of molecular methods for the detection of hr-HPV cervical infections, such as primary test in cervical cancer screening programs, has recently been suggested [3, 12, 13]. In these programs hr-HPV test is followed by PAP test only in women with hr-HPV positive test before referring them to colposcopy; this procedure increases the specificity of molecular testing [14-19]. Hence, addressing immediately to colposcopy, all positive hr-HPV women may be the cause of an indiscriminate increase of this procedures and, for this reason, women with positive hr-HPV test are referred to the PAP test [20]. The New Technologies for Cervical Cancer (NTCC) Italian study, carried out to evaluate the performance of the hr-HPV test and showed similar results to those of other cited studies [17, 18]. Moreover, the NTTC study has shown that hr-HPV test does not increase the over-diagnosis of CIN2 lesions compared with cytology in women over 35 years of age, while the over-diagnosis of CIN2 lesions increases in younger woman (< 35 years); therefore this study recommends to carry out the hr-HPV screening not before 35 years of age [10].

The aim of this descriptive study is to evaluate the effect and the quality of the application of the new guidelines on hr-HPV test screening using a two-year experience (2012-13) of a single screening center in Latina (Italy).

Materials and Methods
Study population
The Pathology Unit of ICOT Hospital, Department of Medical-Surgical Sciences and Bio-Technologies, Sapienza University of Rome, Polo Pontino, I.C.O.T, Latina (Italy).
Rome and Screening Unit of Local Health Unit of Latina, have been running a new organized cervical-screening in the Latina district since 2012. The screening program plans to invite each year, about 30% of entire female population, aged 25-64, resident in the Latina district, with the aim of covering the whole population in three years (three years around time). Women aged 25-34 are invited by mail to perform a PAP test while women aged 35-64 are invited by mail to perform a hr-HPV test according to the screening algorithm of the Italian Association of Cervical Screening Programs (GISCi) guidelines [3] during 2012-2013. A double-sampling for PAP test and hr-HPV test was performed on all participating women except for women aged 25-34 who underwent only to PAP test. The screening algorithm is described in Figure 1. The women with a negative PAP test or hr-HPV test were advised to repeat the test after three years. The women positive to hr-HPV test were referred to PAP test; diagnosis were reported according to 2001 Bethesda System [2] evaluated by one cytologist and two pathologists. The colposcopy was performed by two gynecologists of the screening unit. Colposcopic biopsies were read by two pathologists and women with diagnosis of CIN2 or more severe were referred to excisional treatment. The authors present the preliminary data of program screening in the first two years (2012-2013).

Cytology
The cervical cell samples were obtained by using a cytobrush and were put in PreservCyt solution; liquid-based cytology was performed by using the Sure path system. One slide per woman was prepared according to the supplier’s instructions.

Hybrid capture test 2 (HC2)
Exfoliated cervical cells were collected using a cytobrush and eluted in the Sample Transport Medium. First of all, cervical specimens were denatured to disrupt the virus and release the target DNA. The RNA probes were diluted in a probe diluent and once loaded all the samples, calibrators, controls and reagents, the hybridization phase began according to supplier’s instructions. The chemiluminescent reaction was measured by luminometer and the emitted light was measured as RLU. For each reaction were used three negative controls, three positive controls, one quality control for hr-HPV and one quality control for hr-HPV. Samples that showed a RLU ≥ one pg/ml were considered positive.

Results
In 2012 the hr-HPV test was introduced as primary test for cervical cancer screening program. In 2012 there were enrolled 22,862 women (11,484 aged 25-34 and 11,378 aged 35-64), while in 2013 there were enrolled 62,923 women (14,013 aged 25-34 and 48,910 aged 35-64); the screening program involved in 2012 only Latina City and in 2013 the entire district too, hence this accounts for the difference in the number of enrolled women. From 2012 to 2013, 85,785 women were invited and 25,210 were screened (29.4%); women aged 25-34 that underwent a PAP test alone were 4,242 (16.8%) while women aged 35-64 that underwent an hr-HPV test were 20,968 (83.0%). The PAP test positive rate among women aged 25-34 was 4.0% (170/4242) and the hr-HPV positive rate among women aged 35-64 was 5.2% (1092/20968) (Table 1). Among the positive PAP test in 25-34 age group, the most frequent diagnostic category in 2012 was atypical squamous cells of undetermined significance (ASCUS) (48%) followed by low-grade squamous intraepithelial lesion (LSIL) 39%, and high-grade squamous intraepithelial lesion (HSIL) 13%; on the contrary, in 2013 the most frequent diagnostic category was LSIL (63%), followed by ASCUS (30%), HSIL (4%), and atypical squamous cells-high-grade not excluded (ASCH) 3% (Table 2). The hr-HPV positive rate among women aged 25-34 with diagnosis of ASCUS was 83.6% and the positive predic-
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tive value (PPV) for CIN2+ in ASCUS category was 1.2% and 2.2% in 2012 and 2013, respectively.

The overall positivity rate at cytology among women aged 35-64 who were hr-HPV+ was 22.7% (248/1092); particularly, this rate decreased from 2012 to 2013 (27.9% to 21.2%). Among women aged 35-64, the frequency of diagnostic categories was similar in 2012 and 2013; indeed, the most frequent diagnostic category was LSIL (63% vs 65%), then ASCUS (25% vs 23%), HSIL (10.5% vs 8%), ASCH (1.5% vs 3%) and, only in 2013 the authors found two adenocarcinoma (AC) (Table 2). The inadequate PAP test rate was 0.35 and 0 in women aged 25-34 and 35-64, respectively. The referral rate to colposcopy was higher in 25-34 group (3.8%) than in women aged 35-64 (1.2%), while it was the same in both years (1.6%) and the adhesion to colposcopy increased from 2012 to 2013 in both age-classes. The PPV for CIN2+ at colposcopy was similar in 2012 and in 2013 (10.9% to 9.1%) while it was lower among women aged 25-34 than among women aged 35-64 (7.8% to 10.7%). The detection rate (DR) for CIN2+ was higher among women aged 25-34 than among women aged 35-64 (2.8% to 1.1%), while it was similar in 2012 and in 2013 (1.6% to 1.4%) (Table 1). The most frequent histologic category among women aged 25-34 and 35-64 in 2012 and 2013 was CIN1 followed by CIN3 and CIN2 (Table 3). The referral rate to one year for the women aged 35-64 hr-HPV+/PAP- was 3.6% in 2012 and 4.1% in 2013 (Table 1).

Specificity values of hr-HPV test were 96.1% and 95.5% in 2012 and 2013, respectively.

Discussion

This study presents the results of the first two years of HPV screening program of Latina district; it allowed the authors to understand the effects of the introduction of a molecular test in screening for cervical cancer. First of all,
the most encouraging result was the increase in the uptake of hr-HPV test in the screening program. The introduction of this molecular test in place of PAP test was a large change that has not worried the women and it was probably due to the success of information campaign carried out in the district; indeed the present data showed that acceptance to perform the new test was higher than in the group of women aged 25-34 that underwent only PAP test. Moreover the present result was higher than the regional average (29.3%) reported in the previous three years [22]. The hr-HPV positive rate in both years was equal to the value observed in NTCC study where the hr-HPV test was performed on women aged 35-60 [7, 22]. An interesting aspect was the cytological triage of hr-HPV positive cases, not only because the present results were similar to those reported in the literature [7], but mainly because the authors noted that in 2013 there was a decrease in the PAP test positive rate, corresponding to a decrease in the percentage of ASCUS diagnosis. These data were probably due to the gained experience of the operators involved in the reporting of PAP test after hr-HPV test, and these results were expected and suggested by GISCi [3]. The decrease of ASCUS cases is important because minor cytological lesion often regress spontaneously, hence referring all women with ASCUS diagnosis to further examination results in a growth of over-diagnosis, colposcopy, and overtreatment [22]. The present results regarding the inadequate PAP test rate showed that there was a decrease in number from 2012 to 2013 and that the rate was close to zero and lower than the regional average [3]. This has led to a considerable saving in terms of time and costs.

With the introduction of hr-HPV test and cytological triage, it was assumed that there would be an increase in PAP test positive rate and consequently an increase in the number of women referred to colposcopy [3]; in the present study, not only did the authors not observe an increase of PAP test positive rate but moreover, among women aged 35-64, they found that referral rate to colposcopy was lowest than the regional and national average (2.5% to 2.4%) [21, 23]. In the present study this rate in women aged 35-64 was lower in 2013 than 2012, reflecting the decrease of PAP test positive rate and ASCUS rate.

The PPV is an indicator that measures the sensitivity of the test and is calculated as the proportion of women with histological cervical intraepithelial neoplasia grade 2 or worse (CIN2+). In Italy the PPV differs from 2.8% to 52.7% among screening programs [21, 24]. In the present study, PPV values were lower than the regional and national averages (13.5% to 15.3%), however the PPV value of hr-HPV test was higher than PPV value of PAP test. Generally, the screening program with hr-HPV test and cytological triage have a DR for CIN2+ higher than PAP test alone [7, 23]; in the present study the DR for CIN2+ in women aged 35-64 was lower (1.1) compared to the national reference range (2.1 to 3.6) calculated on women with age of 25-64 over the last three years. This difference could be due to the higher age of women undergoing to hr-HPV test and to the different period in which the different screening programs were commenced; indeed, the screening program in Latina district began earlier than the others.

The present authors obtained good results in terms of test specificity using hr-HPV test followed by cytological triage in the screening program; indeed, both in 2012 and 2013 they obtained specificity values near to 96%.

In conclusion, the present data confirm that the early detection of HPV infection using hr-HPV test does not involve an increase in over-diagnosis and consequently an increase in treatments; instead the combination hr-HPV test and cytological triage defines high levels of specificity and sensitivity. Moreover, the new algorithm allows to stratify the population in three groups: women with a very low risk of disease (HPV-/PAP-), women at high risk of disease (HPV+/PAP+), and women with average risk of disease (HPV+/PAP-), to reduce the costs related to referral colposcopy and overall reduce cancer incidence.

Acknowledgments

The authors thank “Fondazione Roma” for the precious support in this research.

References

[1] www.salute.gov.it


[23] Osservatorio Nazionale Screening. Available at: http://www.osservatorionazionalescreening.it/

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DVP parametric imaging for characterizing ovarian masses in contrast-enhanced ultrasound

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Summary
Aim: To evaluate whether parametric imaging with contrast-enhanced ultrasound is an approach capable of for the differential diagnosis of ovarian masses. Materials and Methods: The authors analysed 50 cases of ovarian masses by routine ultrasound and contrast-enhanced ultrasound with a new dedicated parametric image processing software—Sonoliver. The angiogenesis and blood perfusion mode on a digital video recorder were recorded and the morphological characteristics of time-intensity curve (TIC) and dynamic vascular pattern (DVP) curve were subsequently described. The quantity factor, including time to peak (TTP), maximum intensity (IMAX), rise time, (RT), mean transit time (mTT), generated by Sonoliver software were compared in both histological gradings. Results: There were 24 cases (86%) displaying mainly hypo-enhanced with blue imaging in those with benign masses and 15 cases (68%) displaying mainly hyper-enhanced imaging with red in those with malignant masses. The difference was statistically significant \( (p < 0.05) \). DVP curves were unipolar below the baseline in 23 cases (82%) of benign masses and unipolar above the baseline in 15 cases (68%) of malignant masses. IMAX, TTP, and mTT were all significantly higher in those with malignant masses than those with benign ones (all \( p < 0.05 \)), but, no statistical difference in the RT between the two groups was found \( (p > 0.05) \). Conclusions: According to the results, DVP parametric imaging is a new approach capable of differential diagnoses of ovarian masses with contrast-enhanced ultrasound.

Key words: Angiogenesis; Contrast-enhanced ultrasound; Time-intensity curve; Dynamic vascular pattern curve; Ovarian mass.

Introduction
Tumor-associated neoangiogenesis is believed to be a critical requirement for tumor growth and metastasis in ovarian cancer as well as other organs [1]. Therefore, ovarian tumor-associated neoangiogenesis represents the potential to be an early detection target for ovarian cancer, the third leading cause of mortality from female genital cancer. Ultrasound (US) is recognized as the initial diagnostic modality of choice and the least invasive method for the evaluation of most pelvic masses. However, US shows limitation in the detection of small blood vessels and slow flow owing to the lack of reflection from red blood cells and low signal to noise ratio [2-4]. Many studies have shown that US did not improve the sensitivity and specificity of predicting malignancy. Therefore, an in vivo non-invasive method of detecting ovarian tumor-associated neoangiogenesis and blood perfusion is currently an important clinical concern so as to improve the early detection of early-stage disease and have a positive impact on the prognosis of this dreaded disease.

Parametric image processing in contrast enhanced ultrasound (CEUS) is increasingly being recognized as a promising and powerful molecular imaging tool [5]. In CEUS parametric imaging, the assessment of perfusion with ultrasound contrast agents (UCA) is beneficial in many diagnostic indications in molecular imaging, scattering strength of which is orders of magnitude higher than that of red blood cells. Moreover, UCA can be targeted to specific molecular markers through the attachment of appropriate ligands to the surface of the microbubbles and is administered intravenously. Microbubbles could enhance bubble non-linearities of ultrasound waves while not enhancing tissue nonlinearity [6-7]. This feature, by means of contrast-specific imaging software, makes dynamic monitoring of tumor angiogenesis and perfusion become possible. Because of that their size is comparable to red blood cell, currently available contrast microbubbles are able to stay predominantly in the blood circulation and pass through the lung microcirculation [8].

In this study, the authors examined women with suspicious but not clinically obvious malignant ovarian masses who were about to undergo surgery after conventional US. The authors’ aim was to evaluate whether parametric imaging with CEUS is an approach capable of for the differential diagnosis of ovarian masses.

Materials and Methods
Patient selection
Fifty consecutive patients ages 24 to 70 years (average ± SD, 44 ± 12) who were diagnosed to have an ovarian mass that was difficult to confirm by conventional US in the present hospital from January 2009 to December 2011.
This study was approved by the Ethics committee, and written informed consent was obtained from each patient. All patients were scheduled for surgical treatment after evaluation by gynecologic oncologists and radiologists and were screened for contraindication, particularly pregnant or lactating women, interventional therapy or chemotherapy preoperative, cardiac shunts, recent history of thrombosis, and hypersensitivity to this ultrasound contrast agent.

**Contrast-enhanced ultrasound examination**

Patients were examined using Logiq 9 color Doppler ultrasonic diagnostic apparatus with a curved 4C transducer (between 1.0–4.0 MHz). An intravenous bolus injection within 3-5 s of 2.4 ml of SonoVue was used, which consists of microbubbles containing sulfur hexafluoride (SF6) gas encapsulated within a phospholipid monolayer shell, followed by a flush of five ml of saline.

First of all, a baseline US examination is recommended to scan the whole pelvic cavity and record the ovarian mass size, echogenicity, border, and color Doppler flow distribution. Then, determine the optimum scanning section and switch to Real-time CEUS mode with low mechanical index (MI) ranging from 0.1 to 0.15. As soon as the intravenous bolus injection of SonoVue, the real-time enhancement pattern of contrast agent inside the tumor was observed for three to five minutes and the imaging video was recorded in Dicom format for later analysis. Patients were required to maintain supine position and minimize inspiration extent the overall process.

**Analysing of parametric imaging in contrast-enhanced ultrasound**

The information contained in a dynamic sequence of enhancement can be exploited as a diagnostic aid by means of a new dedicated parametric image processing software Sonoliver quantitative software. This software generates a processed sequence by subtracting the mean pixel signal processing obtained in a reference region from the original pixel signal processing, a processing called dynamic vascular patterns (DVP) [9-11]. Such a processing can be used for enhancing the differences in perfusion kinetics between malignant and benign ovarian masses, which generates a sequence of images in warm and cold colors by subtracting the mean pixel-value obtained in a reference region from the original pixel values. DVP processing was designed to help clinicians to confirm their diagnoses of benign and malignant masses. The DVP parametric images were interpreted ac-

![Figure 1. — Screenshot of Sonoliver at time to peak (TTP) of an epithelial ovarian cancer. (A) Three regions of interest (ROIs) are drawn: delimitation ROI (blue border), analysis ROI (green border), and reference ROI (the blue border minus the green border). (B) The DVP parametric images are interpreted according to warm-and-cold color. (C) Time-intensity curve (TIC). (D) DVP curve, with the healthy ovarian tissue taken as reference.](image-url)
DVP parametric imaging for characterizing ovarian masses in contrast-enhanced ultrasound

According to warm-and-cold color (dark blue - light blue – yellow – orange - red), indicating that the perfusion intensity increased in turn (Figure 1B).

The SonoLiver software was designed for real-time evaluation of tissue perfusion obtained by CEUS examination, which also provides an objective quantification of perfusion parameters. Three regions of interest (ROIs) are outlined in the angiogram: (1) delimitation ROI, a blue border delimiting the region of the entire region to be analysed; (2) analysis ROI, a green border sketching out the contours of suspicious lesions; (3) reference ROI, the blue border minus the green border region-wide (Figure 1A). Then the automatic motion compensation of the software was used to correct the respiratory motions. At the same time, the system automatically drew out the time-intensity curve (TIC), from which a series of semi-quantitative perfusion parameters is extracted and analysed, and the DVP curve, with the healthy ovarian tissue taken as reference (Figures 1C, D).

The parameters obtained in this study included: time to peak (TTP); maximum intensity (IMAX), which was the percentage ratio of intensity of ROIs in lesions and ROI reference at the highest point of the perfusion process; rise time (RT), from 10% to 90% of IMAX; mean transit time (mTT) corresponding to the center of gravity of best-fit function of echo-power.

DVP parametric images were read by a clinician experienced in ultrasound who was blinded to the nature of the lesions. Finally, diagnoses made in this way were compared against the biopsy to calculate efficacy scores.

Statistical Analysis
Statistical analyses were carried out using SPSS version 13.0. The differences of measurement data were compared with the t test while the counting data were using Chi-square test. All data were described as mean ± standard deviation. A value of $p < 0.05$ indicated statistical significance.

Results
Most benign ovarian masses are cystic, with clear borders. The performance for malignant masses are mainly mixed or solid, with irregular borders. In the group of benign ovarian masses, the mean diameters before CEUS (7.2 ± 3.4 cm) and after CEUS (9.7 ± 3.0 cm) were not statistically significantly different ($p = 0.398$). In the group of malignant ovarian masses, the mean diameters was significantly higher ($p = 0.006$) after CEUS (9.7 ± 3.0 cm) than before CEUS (8.5 ± 2.9 cm).

The majority of benign tumor blood vessels located peripherally in focus, with regular figure, and the intra-tumor with no enhancement or homogeneous enhancement. On the contrary, blood vessels in malignant tumors located penetrating or central and became coarser, with increasing tortuosity, branching patterns, vascular loops, and shunts.

Figure 2. — (A) The DVP parametric images displays mainly hypo-enhanced with blue imaging in those with benign masses. (B) DVP curves are unipolar below the baseline in benign masses. (C) The DVP parametric images display mainly hyper-enhanced imaging with red in those with malignant masses. (D) DVP curves are unipolar above the baseline in malignant masses.
The difference was statistically significant (p < 0.05), but, no statistical difference in the RT between the two groups was found (p > 0.05) (Table 1).

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Number</th>
<th>IMAX (%)</th>
<th>RT (s)</th>
<th>TTP (s)</th>
<th>mTT (s)</th>
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<td>44±16</td>
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</tr>
</tbody>
</table>

ROI: region of interest, IMAX: maximum intensity, RT: rise time, TTP: time to peak, mTT: mean transit time.

Table 1. — Quantitative parameters in the ROIs of 50 cases of ovarian masses (x ± s).

In CEUS imaging of 50 cases, there were 24 cases (86%) displaying mainly hypo-enhanced with blue imaging in those with benign masses, including: four cases of internal non-enhanced imaging (two cases of inflammatory encapsulated fluid showed no internal enhancement and two cases of endometrial cysts showed ring-like enhancement in cystic wall) (Figure 2A). What is interesting is the mainly hyper-enhancement with red imaging in four cases of benign masses. They were, respectively, two cases of teratoma and two cases of ovarian fibroma. Meanwhile, there were 15 cases (68%) displaying mainly hyper-enhanced imaging with red and seven cases (32%) displaying mainly hypo-enhanced imaging with blue in those with malignant masses (Figure 2C). The difference was statistically significant (p < 0.05).

Twenty-three (82%) of the 28 benign masses were correctly classified as benign on the basis of that the DVP curves were unipolar below the baseline while malignant ones were opposite (Figure 2B). That is, 15 cases (68%) of malignant masses, DVP curves were unipolar above the baseline (Figure 2D). Respectively, the other five cases (18%) of benign masses were two cases of teratoma, one case of fibroma, two cases of corpus luteum cyst hemorrhage, of which DVP curves were unipolar above the baseline. Moreover, the other six cases (27%) of malignant masses were above the baseline at the perfusion period and early clearance, and reduced to below the baseline during the middle-late clearance. One case (5%) of malignant mass was unipolar below the baseline during the whole process of the CEUS.

IMAX, TTP, and mTT were all significantly higher in those with malignant masses than those with benign ones (all p < 0.05), but, no statistical difference in the RT between the two groups was found (p > 0.05) (Table 1).

Discussion

The significant differences of vascular anatomy and hemodynamics between the benign and malignant ovarian masses supplied pathophysiological basis of imaging studies. Use of CEUS has fully revealed the malignant characteristics of tumor angiogenesis and reflected the enhancing process of real-time dynamic observation of tumor microvessel perfusion on the pathological basis of characteristic of blood vessels inside the tumour, which has proved the discrimination between benign and malignant tumors [11-14]. In the present study, the authors have demonstrated that tumor angiogenesis can be visualized by targeted contrast-enhanced ultrasound imaging using ultrasound contrast agent microbubbles and related quantitative parameters were extracted from TIC. Meanwhile, TIC from ROIs generated a complex shape that was applied to tumours in an attempt to compare the echoes in analysis ROI and reference ROI. At the same time, the DVP parametric images read by Sonoliver software, which can be used as a tool for enhancing the differences in perfusion kinetics between focal lesions and normal ovarian organs.

In the present study, vascularity and enhancement patterns were intuitively reflect by warm-and-cold color (warm color stands for hyper-enhancement, cold color indicates the opposite). The majority of DVP parametric images showed hyper-enhancement in malignant masses (68%, 15 of 22) and hypo-enhancement in benign masses (86%, 24 of 28). This observation is consistent with early reports, indicating that malignant ovarian masses which had abundant blood stream would lead to large flow of ultrasound contrast agent microbubbles, whereas benign ovarian masses had light vascularity. However, in the above cases, the circle enhancement was shown in the cystic wall, which was different from that in malignant tumors. As a result, the DVP parametric images of benign and malignant ovarian masses seldom partially overlapped.

With regards to hyper-enhancement with red imaging in four cases of benign masses, two cases were teratomas, attributing to the increase of blood supply from increased thyroid and glial elements, angiogenesis. However, the other two cases with hyper-enhancement of benign masses were ovarian fibroma, of which enhancement was flocculent, sparse and slow. This was different from that of malignant masses. Concerning the hypo-enhanced imaging with blue, perhaps these were associated with liquefactive necrosis of malignancy. The aforementioned show the limitations in the present study. That is, some benign ovarian tumors with abundant blood supply might show the same ultrasound characteristics as the malignant tumors. In addition, although there were morphological and distributional difference of microvessels between benign and malignant lesions, their number and enhanced performance might overlap.

Difference between the original flow signal and the reference flow signal were obtained by DVP curve and reflected different vascular signatures of ovarian masses compared with contrast agent uptake in adjacent tissues. In the present study, analysis of DVP curve of contrast enhancement dynamics confirmed that the malignant masses, which were unipolar above the baseline, were hyper-enhanced with rich blood vessels. Also, the hypo-enhanced were corresponding to unipolar below the baseline. Six cases of malignant masses with bipolar DVP curve illustrated that hyper-enhancement in the during the perfusion period were followed by hypo-enhancement during the mid-
dle-late clearance. Indirectly, the DVP curve reflected the intensity change of the contrast agent microbubbles and hemodynamic differences. These findings suggested that DVP parametric imaging may enhance the individual diagnostic confidence in the subjective analysis of ovarian masses.

A published study showed that RT and TTP were associated with the number of microbubbles considering RT and TTP reflected the contrast agent arrival velocity, IMAX reflected the blood flow, and mTT indirectly responded to the emptying process of the contrast agent [15]. In the present study, there was significant difference in TTP of ovarian masses in two histological gradings, which suggested that the number of microbubbles accessing the vascular bed of malignant masses is much more than that of benign ones and reflect the richness of the malignant vascular. Current data showed that there were significant differences between TTP and mTT of ovarian masses in both histological gradings. The major feature of malignant vascular tumor is arteriovenous fistula, vascular deformation, arteriovenous shunt, multi-branch blood supply leading to large flow, and fast speed of microbubbles. Furthermore, current data showed that there were no significant differences in RT between both histological gradings, perhaps associated with less samples.

Time-intensity curves may provide an objective measure to demonstrate the more rapid enhancement of tumor relative to normal parenchyma. Three-dimensional presentation and other postprocessing image enhancements may increase the conspicuity of cancers. In the final analysis, a simplified protocol will be needed with clear enhancement of malignant foci if enhanced transrectal sonography is to be generally applied in screening for prostate cancer.

The ability to identify malignant tumor with a noninvasive method is important to decide the need for surgery and on the type of surgery required. The present pilot study demonstrated that the DVP curve contained a wealth of quantitative information and objectively reflected distribution of contrast agent in the normal and diseased tissue and the dynamic changes of perfusion. In conclusion, DVP parametric imaging is a new approach capable of differential diagnoses of ovarian masses with contrast-enhanced US.

Acknowledgments

The authors would like to thank Prof. Chen Wen-wei, Department of Ultrasonic Imaging, Renmin Hospital of Wuhan University, for his technical assistance.

This work was supported by the Population and Family Planning Commission of Hubei Province, China (No. JS-2013001) and by the Natural Science Foundation of Hubei Province, China (No. 2010CDB06903).

References


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Assessment of primary radical hysterectomy and neoadjuvant chemotherapy followed by radical hysterectomy in Stage IB2, IIA bulky cervical cancer

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Summary

Objective: Uncertainty concerning the treatment of Stage IB2-IIA (bulky) cervical cancer is still continuing. In this study, an analysis of Stage IB2-IIA (bulky) cervical cancer was performed. The efficacy of primary radical surgery and neoadjuvant chemotherapy followed by a radical surgery was investigated. Materials and Methods: Medical data of 50 patients who were diagnosed with Stage IB2-IIA (bulky) cervical cancer and treated between 2002-2009 were retrospectively assessed. In the radical surgery group, radical hysterectomy + bilateral pelvic + para-aortic lymphadenectomy were performed. In the neoadjuvant chemotherapy group, a combination of cisplatin/topotecan or paclitaxel/carboplatin was given to the patients and then radical surgery was performed. Each group was evaluated individually. Prognostic factors were determined and survival rates were compared between the groups. Results: Radical surgery after neoadjuvant chemotherapy was performed in 21 and primary radical surgery in 29 patients. Median follow-up time was 36.0 ± 14.0 months. Average of the tumor size before treatment was 50.2 ± 7.6 mm. In the radical surgery after neoadjuvant chemotherapy group, lymphovascular space invasion (LVSI) and tumor size (before and after treatment) were determined to be significant factors for each of disease-free survival (DFS) and overall survival (OS). On multivariate analysis, tumor size (before treatment) was found to be an independent prognostic factor for both of DFS ($p = 0.006$) and OS ($p = 0.010$). No significant difference in survival periods was observed among the groups. Conclusion: There was no significant superiority among the two treatment options. Nonetheless, further studies are needed to compare the multimodal approaches in these stages of cervical cancer.

Key words: Stage IB2-IIA (bulky) cervical cancer; Neoadjuvant chemotherapy; Radical hysterectomy.
### Table 1. — Patient characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PRH n (%)</th>
<th>NACT-RH n (%)</th>
<th>( p )</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>53.3 ± 9.9</td>
<td>52.9 ± 8.8</td>
<td>0.882</td>
<td>53.1 ± 9.4</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB2</td>
<td>21 (71.4)</td>
<td>15 (72.4)</td>
<td>0.765</td>
<td>36 (72)</td>
</tr>
<tr>
<td>IIA</td>
<td>8 (28.6)</td>
<td>6 (27.6)</td>
<td></td>
<td>14 (28)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5 (17.2)</td>
<td>1 (4.8)</td>
<td>0.768</td>
<td>6 (12)</td>
</tr>
<tr>
<td>2</td>
<td>21 (72.4)</td>
<td>18 (85.7)</td>
<td></td>
<td>39 (78)</td>
</tr>
<tr>
<td>3</td>
<td>3 (10.3)</td>
<td>2 (9.5)</td>
<td></td>
<td>5 (10)</td>
</tr>
<tr>
<td>Number of removed lymph nodes</td>
<td>35.8 ± 12.6</td>
<td>36.2 ± 16.0</td>
<td>0.415</td>
<td>36.0 ± 14.0</td>
</tr>
<tr>
<td>Tumor size before treatment (mm)</td>
<td>49.8 ± 7.8</td>
<td>50.7 ± 7.4</td>
<td>0.661</td>
<td>50.2 ± 7.6</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>25 (86.2)</td>
<td>20 (95.2)</td>
<td>0.438</td>
<td>45 (90)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>2 (6.9)</td>
<td>0 (0.0)</td>
<td></td>
<td>2 (4)</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>2 (6.9)</td>
<td>1 (4.8)</td>
<td></td>
<td>3 (6)</td>
</tr>
<tr>
<td>Recent status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive free disease</td>
<td>21 (72.4)</td>
<td>15 (71.4)</td>
<td>0.549</td>
<td>36 (72)</td>
</tr>
<tr>
<td>Alive with disease</td>
<td>1 (3.4)</td>
<td>0 (0.0)</td>
<td></td>
<td>1 (2)</td>
</tr>
<tr>
<td>Died</td>
<td>7 (24.2)</td>
<td>6 (28.6)</td>
<td></td>
<td>13 (26)</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>61.9 ± 35.4</td>
<td>36.2 ± 16.0</td>
<td>0.827</td>
<td>36.0 ± 14.0</td>
</tr>
<tr>
<td>Stromal invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No n (%)</td>
<td>0 (0.0)</td>
<td>5 (23.8)</td>
<td>0.019</td>
<td>5</td>
</tr>
<tr>
<td>&lt; 1/2 n (%)</td>
<td>12 (41.4)</td>
<td>8 (38.1)</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>&gt; 1/2 n (%)</td>
<td>17 (58.6)</td>
<td>8 (38.1)</td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

Patients’ archival files were reviewed. Their demographic, clinical and surgical characteristics, pathological findings, and treatment modalities were evaluated. Patients were divided into two groups: PRH group and NACT-RH group. Fifty patients, 29 from the PRH group and 21 from the NACT-RH group, were included.

Clinical stage was determined by clinical evaluation, magnetic resonance imaging (MRI), and chest X-ray. Intravenous ptyelography, cystoscopy, and proctosigmoidoscopy were performed as deemed appropriate. Tumors were staged according to the International Federation of Gynecology and Obstetric (FIGO) 1995 criteria. In the PRH group, type III hysterectomy + bilateral salpingo-oophorectomy (except one patient whose ovaries were preserved) + bilateral pelvic para-aortic lymphadenectomy (BPPLND) were performed. Para-aortic dissection was applied to the renal vein on the left side and to the gonadal vein on the right.

In the NACT-RH group, a combination of cisplatin/topotecan or paclitaxel/carboplatin was given to the patients. The combination of cisplatin/topotecan was repeated every 28 days. Cisplatin (50 mg/m²) was given in the first day and topotecan (0.5 mg/m²) was given in the first three days. On the other hand, the combination of paclitaxel (175 mg/m²)/carboplatin (5 AUC) was repeated every 21 days and both of the agents were applied in the first day.

Patients underwent radical surgery following three cycles of NACT. The same surgical procedure described for the PRH group was performed. Postoperative ART was given to the high-risk patients in both groups. High-risk patients were defined as those who had at least one of the following major findings: positive lymph nodes, parametrial invasion, positive surgical margin and ≥ four cm tumor size, or two or more of the following intermediate findings: lymphovascular space invasion (LVSI), > 1/2 stromal invasion, and two to four cm tumor size.

Follow-up controls were made every three months in the first two years and, subsequently, every six months until the fifth year and then annually. Disease-free survival (DFS) was considered as the period between the operation time and relapse or recurrence dates. Overall survival (OS) was considered as the period between the pathological diagnosis and death dates. Survival times were expressed in months. Each group was assessed individually. Their prognostic factors were determined and then survival rates were compared between the groups.

### Statistical methodology

SPSS 17.0 Evaluation Version (Statistical Package for Social Sciences) software package was used in the statistical analysis of the data. Categorical variables were compared using Chi-square or Fisher test. Continuous variables with normal distribution were analyzed using the t test. Mann Whitney U test was used in the analysis of the continuous variables with abnormal distribution. Univariate analysis of survival rates were carried out by the Kaplan-Meier method and multivariate analysis by the Cox regression method. Log rank test was performed to compare the survival curves between groups. For all tests, \( p < 0.05 \) was considered statistically significant.

### Results

A total of 50 patients were enrolled for this study: 29 were in the PRH and 21 in the NACT-RH group. Patients’ characteristics are demonstrated in Table 1. The average age for all patients was 53.1 ± 9.4 (31 - 73) years. Mean of follow-up period was 36.0 ± 14.0 (4 - 119) months. There was no significant difference between groups according to age, stage distribution, grade, removed lymph nodes’ number, tumor size before treatment, histological type, and follow-up period.

There were 29 cases in the PRH group and the mean age of these patients was 53.3 ± 9.9 (31 - 73) years. The average of follow-up period was 61.9 ± 35.4 (4 - 119) months. Twenty-five (86.2%) of the cases were with squamous carcinoma histology. Mean of the tumor size before treatment was 49.8 ± 7.8 (40-70) mm. FIGO stage was IB2 in 21 (71.4%) cases and bulky IIA in eight (28.6%) patients. Mean number of the removed lymph nodes (LN) was 35.8 ± 12.6 (15-64) and mean number of the metastatic ones was seven. As adjuvant treatment, only RT was given to 11 (37.9%) patients and chemoradiotherapy (CRT) was administered to ten (34.5%) patients.
During follow-up, recurrence was determined in eight (27.6%) cases and DFS was 15 (2-58) months in these patients. Recurrences were pelvic in five and extra-pelvic in three cases (one liver, two liver + lungs). Patients with recurrence were treated with RT (two cases) or CT (six cases). Seven of these patients died and OS in these patients was 29.7 (4-61) months. According to the pathological assessment, neither positive parametrial involvement nor positive surgical margin was found. As pathological risk factors parametrial involvement, LN metastasis, positive surgical margin, LVSI, and endometrial involvement were evaluated. No significant impact of these factors was determined on DFS or OS (Table 2).

A total of 21 patients were included in the NACT-RH group and their mean age was 52.9 ± 8.8 (31 - 71) years. The average of follow-up period was 36.2±16.0 (11-11.5) months. Except one adenosquamous carcinoma case, all the 20 cases were in squamous carcinoma histology. A regimen of cisplatin/topotecan was administered to 17 patients and paclitaxel/carboplatin to the other four patients. Before NACT, mean tumor size was 50.7 ± 7.4 (40-70) mm and after NACT it decreased to 35 mm. Clinical stage was assessed as FIGO IB2 in 15 (72.4%) and bulky IIA in six (27.6%) cases. Mean number of dissected LN was 36.2 ± 16.0 (14 - 73) and mean number of the metastatic ones was

### Table 2. Pathologic findings of PRH group.

<table>
<thead>
<tr>
<th>Pathological risk factors</th>
<th>Recurrence</th>
<th>DFS (months)</th>
<th>OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative n (%)</td>
<td>Positive n (%)</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>Positive n (%)</td>
<td>Positive n (%)</td>
<td>p</td>
</tr>
<tr>
<td>Parametrial involvement</td>
<td>21 (72.4)</td>
<td>8 (27.6)</td>
<td>0.692</td>
</tr>
<tr>
<td>LN metastasis</td>
<td>19(76)</td>
<td>2(50)</td>
<td>0.135</td>
</tr>
<tr>
<td>Positive surgical margin</td>
<td>21(72.4)</td>
<td>8(27.6)</td>
<td>0.135</td>
</tr>
<tr>
<td>LVSI</td>
<td>9(90)</td>
<td>1(10)</td>
<td>0.316</td>
</tr>
<tr>
<td>Endometrial involvement</td>
<td>16(76.2)</td>
<td>3(37.5)</td>
<td>0.382</td>
</tr>
</tbody>
</table>

### Table 3. Treatment and pathological features of NACT-RH group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Recurrence</th>
<th>DFS (months)</th>
<th>OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NACT regimen</td>
<td>Cisplatin + topotecan</td>
<td>57.3 ± 36.4</td>
<td>60.8 ± 31.7</td>
</tr>
<tr>
<td></td>
<td>Paclitaxel + carboplatin</td>
<td>55.7 ± 39.5</td>
<td>55.7 ± 39.5</td>
</tr>
<tr>
<td>Parametrial involvement</td>
<td>57.1 ± 35.9</td>
<td>59.8 ± 32.3</td>
<td>0.382</td>
</tr>
<tr>
<td>LN metastasis</td>
<td>61.5±36.0</td>
<td>64.5±31.8</td>
<td>0.172</td>
</tr>
<tr>
<td>Positive surgical margin</td>
<td>37.5±30.9</td>
<td>39.2±29.7</td>
<td>0.382</td>
</tr>
<tr>
<td>LVSI</td>
<td>75.1±24.5</td>
<td>75.1±24.5</td>
<td>0.355</td>
</tr>
<tr>
<td>Positive</td>
<td>40.6±37.6</td>
<td>45.9±33.2</td>
<td>0.382</td>
</tr>
<tr>
<td>Endometrial involvement</td>
<td>65.1±34.1</td>
<td>67.1±30.8</td>
<td>0.105</td>
</tr>
<tr>
<td>Negative</td>
<td>36.8±35.0</td>
<td>41.7±31.5</td>
<td>0.382</td>
</tr>
<tr>
<td>Positive</td>
<td>72.5±26.1</td>
<td>72.8±25.4</td>
<td>0.014</td>
</tr>
<tr>
<td>Tumor size (mm)</td>
<td>75.2±39.2</td>
<td>77.2±35.9</td>
<td>0.094</td>
</tr>
<tr>
<td>≤50</td>
<td>72.5±26.1</td>
<td>72.8±25.4</td>
<td>0.014</td>
</tr>
<tr>
<td>&gt;50</td>
<td>32.0±36.9</td>
<td>38.6±32.5</td>
<td>0.382</td>
</tr>
<tr>
<td>Tumor size (mm)</td>
<td>68.1±29.8</td>
<td>69.1±28.2</td>
<td>0.061</td>
</tr>
<tr>
<td>≤30</td>
<td>68.1±29.8</td>
<td>69.1±28.2</td>
<td>0.061</td>
</tr>
<tr>
<td>&gt;30</td>
<td>34.8±38.9</td>
<td>41.3±34.1</td>
<td>0.382</td>
</tr>
</tbody>
</table>

### Table 4. Multivariate analysis of the clinicopathological prognostic factors.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVSI</td>
<td>0.113</td>
<td>0.022 - 1.500</td>
</tr>
<tr>
<td>Depth of stromal invasion (superficial)</td>
<td>0.643</td>
<td>0.238 - 2.426</td>
</tr>
<tr>
<td>Depth of stromal invasion (deep)</td>
<td>0.989</td>
<td>0.000</td>
</tr>
<tr>
<td>Tumor size before treatment (mm)</td>
<td>0.006</td>
<td>1.710 - 25.427</td>
</tr>
<tr>
<td>Group</td>
<td>0.784</td>
<td>0.256 - 2.797</td>
</tr>
</tbody>
</table>
After surgery, adjuvant RT was applied to ten (47.6%) patients and CRT to four (19.0%) patients. During follow-up six (28.6%) recurrence cases were reported. DFS was calculated as 11.2 (1 - 25) months in these patients. Recurrences were determined in the pelvis in two cases. The remaining four cases' recurrences were multicentric. Recurrences were treated with RT (two cases) or CT (four cases). All patients with recurrence died and their OS was 20.7 (11 - 30) months. All patients had no parametrical or surgical margin involvement, but there were positive LN in four (19%) and LVSI in 11 (52.4%) patients according to the pathological report. Whereas LVSI had significant effect on survival rates, LN involvement had none (Table 3).

While DFS and OS were found to be 58.7 ± 38.6 months and 61.9 ± 35 months in the PRH group, it was determined as 57.1 ± 35.9 months and 59.8 ± 32.3 months in the NACT-RH group, respectively. During follow up period OS rates were calculated as 75.9% in the PRH and 71.4% in the NACT-RH group. There was no significant difference in recurrence, DFS or OS between the two groups (Figures 1, 2). Among various clinicopathological factors, only tumor size before treatment had significant impact on the DFS and OS according to the multivariate analysis (Table 4).

Discussion

Efficacy of radical surgery and RT or CRT treatments in the early stage cervical cancer are similar. Five-years OS rates are about 90% for both treatment modalities [5, 6]. Recent studies have reported that patients treated with NACT-RH had close rates of OS [7, 8]. Ability of protection of the ovarian functions, less sexual dysfunction, and the possibility of keeping RT as treatment option for recurrences are important advantages of surgery in the early-stage cervical cancers. However, the probability of receiving ART in the LACC is still high. Yessaian et al. reported that ART was performed to 52% of Stage IB2 cervical cancer patients after radical surgery [9]. Also, a study by Finan et al. showed that ART was needed for 72.3% of Stage IB2 after radical surgery [10]. Further, ART was used to 84% of > four cm Stage IB2-IIA in a study by Landoni et al. [11]. NACT was suggested to shrink tumor volume as well as decrease metastasis. Thus, surgery would be more convenient and necessity for RT would be reduced. Hereupon, survival would be improved also. A 22-44% response rate (especially in the LACC cases), reduced metastatic LN, and increased DFS rates were reported with NACT treatment modality in the cervical cancers. Nevertheless, despite approximately 25 years of experience with NACT, its efficacy in the cervical cancer has not yet been elucidated. While some studies supported this approach, [12,13] others did not [14,15]. Complete clinical response obtained with NACT ranged between 0-50% [14, 16, 17]. NACT is given in two to four cycles before surgery and various agents have been described for this purpose. Platin-based regimens are widely used because of their well-known cytotoxic activity in the cervical cancer [18]. In the SNAP 01 and SNAP 02 named, multicentric, randomized phase III study, cisplatin/ifosfamide/paclitaxel (TIP) and cisplatin/ifosfamide (IP) combinations were compared and clinical response was found to be significantly improved with TIP protocol (9% vs 20%) [19]. Park et al. have used paclitaxel/cisplatin combination for three cycles as neoadjuvant therapy in Stage Ib2-IIb cervical cancer. After ten days from the last cycle, patients were evaluated with clinical examinations and MRI. Complete and partial
response rates were found to be 40% and 51%, respectively [20]. Cisplatin + vinkristine + bleomycin (VPB) combination was administered to the patients with ≥ Stage Ib2 cervical cancer in a study conducted by Bermudez et al. Authors have indicated that the response was less than 50% in the Stages Ib - Iib and >50% in Ib2, Iia [21]. In the present study, cisplatin/topotecan and carboplatin/paclitaxel protocols were used and the response was similar to the two protocols. Complete response rate of neoadjuvant CT in the present study was 33.3%.

Various survival rates with NACT treatment modalities in the LACC are reported. Five-year DFS and OS ranged between 29-80% and 21-81%, respectively [8, 12, 15, 16, 22, 23-26]. Aoki et al. compared NACT-RH and PRH treatment modalities in Stage IB-IIB cervical cancer and they recorded that survival rates, surgical, and pathological risk factors were improved in the NACT-RH group [16]. Similar results were reported by Namkoong et al. [27]. Nevertheless, Serur et al. stated that surgical and pathological risk factors were improved in Stage Ib2 patients, but survival was not [8]. On the other hand, according to a retrospective study by Behtash et al., no positive impact was obtained with NACT in the early-stage cervical cancer [15]. Furthermore, NACT-RH vs PRH was assessed in a prospective phase III GOG study and authors declared that surgical, pathological risk factors, and survival was not improved in Stage Ib2 tumors with NACT arm. Five-years OS was 60.7% in the NACT and 63.3% in the PRH arm [14]. Also, in the present study no significant difference was noticed in DFS (p = 0.877) or OS (p = 0.827) rates among PRH and NACT-RH groups. Variability of the reported results and studies’ not being homogeneous in terms of stage make it difficult to understand the real effect of NACT. Survival obtained from NACT arms was found to be lower in the advanced stages [27, 28]. Results of the studies conducted with only Stage IB2 are variable also [7, 8, 14]. Uncertainty created by the clinical staging might be the main reason for this condition. Applied NACT protocol also can be considered as another reason. However, most of the NACT regimens are platin-based, so it is believed that its effect on survival is minimal [22].

Rates of recurrence and survival for LACC depend on several factors including lymph node metastasis, surgical margin status, parametrial infiltration, deep stromal invasion, LVS1, and tumor size. Even though LN status does not change the stage in the cervical cancer; it has an important effect on prognosis and decision of the adjuvant therapy. Metastatic lymph nodes were associated with lower survival rates in many studies [29, 30]. There was no significant difference in DFS and OS according to the LN status in the present study. This result can be explained by the small number of patients and the fact that patients were in early stages.

Deep stromal invasion is associated with increased recurrence rate and decreased survival period [31, 32]. Salmal et al. found that recurrence rate was 13% in 77 cases who had > ten mm stromal invasion compared with 4% of 119 cases had < ten mm stromal invasion. Researchers stated that stromal invasion was a significant factor affecting prognosis [31]. The difference of stromal invasion between the two groups was statistically significant in the present study (p = 0.019). However, this observation was not confirmed by the multivariate analysis for DFS or OS.

Kristensen et al. have investigated the prognostic significance of the tumor size on survival and they detected that a five-year survival was 94% in the < two cm tumors and 47% in the ≥ four cm tumors [32]. In accordance with the literature, by the multivariate analysis, the present authors determined that tumor size was a significant prognostic factor for their cases.

Conclusion

In this study, no significant superiority was observed between the PRH and NACT-RH treatment options. Lack of sufficient randomized controlled studies, poor prognosis of the patients whose surgery could not be applied after NACT, and the necessity of ART after NACT for a substantial proportion of the patients were important factors which evidently decreased the tendency to apply NACT. Nevertheless, there is a need for further studies to compare the multimodal approaches in these stages of cervical cancer.

Acknowledgments

Authors thank Professor Naki Tütüncü and Dr. Reyhan Khatib for editing this paper.

References


The presence of advanced lesions and associating risk factors for advanced cervical carcinoma in patients with atypical squamous cells of undetermined significance

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Summary

Purpose of investigation: To characterize histopathological status, high-risk human papillomavirus (hr-HPV) infection status, and associated risk factors in patients with atypical squamous cells of undetermined significance (ASCUS). Materials and Methods: Cervical biopsies obtained from 130 ASCUS patients were subjected to histopathological examination and hr-HPV testing. Associations between advanced lesions and hr-HPV load or age were analyzed, and the confounding factors for high-grade cervical lesions were identified. Results: Cervical biopsies from ASCUS patients had a wide range of pathological states, ranging from normal to invasive cervical carcinoma. High-risk HPV infection was significantly associated with advanced cervical lesions in ASCUS patients; hr-HPV infection and the number of gestations were risk factors for developing advanced cervical disease. Conclusions: A significant portion of ASCUS patients harbor advanced cervical lesions. The number of gestations and hr-HPV infection can increase the risk of developing advanced cervical lesions in ASCUS patients.

Key words: Atypical squamous cells of undetermined significance(ASCUS); Cervical cancer; High-risk human papillomavirus; Viral load; Risk factors.

Introduction

Cervical cancer is the third most common cancer worldwide, with 529,800 diagnoses and 275,100 deaths in 2008 alone [1]. Approximately 85% of cervical cancers occur in developing countries [1]. The incidence and mortality rates of cervical cancer have dramatically decreased in recent years due to advances in diagnostic and testing, especially in developed nations [2]. These advanced methods include the Pap smear and the ThinPrep Pap test. The Bethesda system (TBS) was established in 1988 as a way to unify diagnostic terminology for reporting cervical cytological test results. It was subsequently revised in 1991, and again in 2001, and gives a scaled ranking of cervical abnormalities [3]. Abnormal results based on TBS include: atypical squamous cells (ASC); low grade squamous intraepithelial lesion (LGSIL or LSIL); high grade squamous intraepithelial lesion (HGSIL or HSIL); squamous cell carcinoma; atypical glandular cells not otherwise specified (AGC-NOS); atypical glandular cells, suspicious for AIS or cancer (AGC-neoplastic); and adenocarcinoma in situ (AIS). Within the category of ASC, there are atypical squamous cells – cannot exclude HSIL (ASC-H), and atypical squamous cells of undetermined significance (ASCUS) [4]. Often diagnosed in the cervical biopsies of patients with abnormal cytological tests are cervical intraepithelial neoplasia (CIN). These premalignant cervical lesions are graded into four groups: normal, CIN I, CIN II, and CIN II [5].

ASCUS are interpreted as undetermined significance since their phenotype is clinical enough to be attributable to reactive changes but benign enough to lack a definitive diagnosis of squamous intraepithelial lesion (SIL), either quantitatively or qualitatively. Since its introduction by the TBS, ASCUS has been problematic for several reasons. First, many scenarios can lead to an ASCUS diagnosis, either due to technical limitations such as poor specimen quality or processing, or simply the nature of the samples. For example, samples with mature intermediate-type or orangeophilic cytoplasm and those with atypical metaplasia will both be categorized as ASCUS. Second, ASCUS is a very unreliable diagnosis due to the broad spectrum of parameters and lack of uniformity [6]. Despite the lack of diagnosis standard, ASCUS accounts for a significant portion of the abnormalities observed in cervical cancer screening [7]; in the U.S., more than two million women were diagnosed with ASCUS. There is no consensus for the optimal management of patients following an ASCUS diagnosis. Typically, however, patients are tested using follow-up procedures including...
additional cytological testing, colposcopies, and human papillomavirus (HPV) testing. The proper course of action remains contentious, with some arguing that ASCUS patients are over-treated as observed by normal follow-up pathological assessments and others arguing these patients are under-diagnosed with some ASCUS patients do harbor CIN III or even invasive carcinoma [8]. These factors, combined with the observations that patients with an ASCUS diagnosis, are at a higher risk of developing cervical cancer than patients with normal cytological diagnosis [9]. Highlight the importance of properly characterizing the histopathological status of the cervical biopsies from ASCUS patients.

The main cause of cervical carcinoma and cervical precancerous lesions is HPV infection, specifically high risk strains (hr-HPV) [10]. hr-HPV strains are classified according to their tumorigenic potential and include approximately 13 strains. Several additional factors associated with incidence of cervical cancer also correlate with an increased chance of hr-HPV infection. These factors include adolescent intercourse or pregnancy, multiple parity, smoking, using oral contraceptives or intrauterine devices (IUD), multiple sex partners, abortion history, other sexually transmitted diseases, a high body mass index, and low incomes [11, 12]. Moreover, reproductive tract infection (RTI) and hr-HPV infection also induce ASCUS [13].

In this study the authors sought to characterize the histopathological status of ASCUS and identify cervical cancer-related risk factors in ASCUS patients. To accomplish this, they tested 130 ASCUS patients for hr-HPV infection and followed-up with histopathological examination. Each patient’s social economical status, sexual activity, and medical history were also obtained, and the association between the cervical lesions grade and specific risk factors were analyzed. These data revealed that hr-HPV viral load correlated with the pathological grade of cervical lesions, and that hr-HPV infection and the number of gestation are the confounding factors to the risk of developing advanced cervical lesions. These findings can help identify ASCUS patients with a high risk of developing cervical cancer and facilitate the development of optimal management strategies in treating these patients.

Materials and Methods

Patient characteristics

A total of 130 patients were recruited from inpatient and outpatient clinics in the First Hospital of Jilin University between January 2011 and November 2012. All patients were diagnosed with ASCUS according to the current TBS classification in a liquid-based ThinPrep Pap test. Inclusion parameters included sexually active, non-gravid, and no history of radio/chemotherapy or symptoms of acute genital tract inflammation. The median patient age was 38 years (range from 20 to 66; mean 38.85 ± 8.75).

Patients were informed of the study objectives and design and signed consent forms prior to enrollment. All patients completed a questionnaire inquiring the following information: age, occupation, pertinent clinic symptoms, age of first intercourse, number of sexual partners, numbers of gestation and parity, mode of delivery, the presence of IUD, and history of genital warts in themselves and their partners. The results from this questionnaire were exclusively used for this study. Each patient received a pathological examination and an hr-HPV test. The First Hospital of Jilin University ethics committee approved the study design.

Colposcopy and cervical biopsy

Colposcopies were performed only if the patients had been free of any vaginal operation or sexual intercourse for at least 24 hours. In a dorsal lithotomy position, the vulva of each patient was examined for any suspicious lesions, a speculum was placed in the vagina, and 3% acetic acid was applied to the cervix using cotton swabs for one minute. Any white areas following acetic application or those that had abnormal vascular patterns were considered a higher priority for sampling. Three experienced pathologists conducted histopathological examinations and the histopathological classifications of the biopsies were made according to previous studies [14, 15].

hr-HPV test

For each patient, cervical tissue samples were collected using a cytobrush with slight rotation in the endometrial canal following spatula scraping. The spatula and brush were then dropped into a collection tube. HPV DNA was isolated and detected using a Hybrid Capture 2 High-Risk HPV DNA Test Kit, a kit that assays for 13 hr-HPV species (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). The threshold for a positive HPV test was 1.0 pg/ml.

Statistical analysis

For data collection, EpiData 3.02 was used to build a database. The data were analyzed by Statistical Package for the Social Sciences (SPSS) version 17.0. A chi-square (\(\chi^2\)) test was used to compare the histopathological status in each age group (\(\alpha = 0.05\)). A Mann-Whitney test was used to compare the association between histopathological status and hr-HPV virus load. To identify independent variables that affect the histopathological status of the cervical biopsies from ASCUS patients, unconditional logistic regression analysis (\(a_{\text{input}}=0.05, a_{\text{output}}=0.10\)) was performed. In the regression analysis, the histopathologic grade, with a threshold of CIN II, was defined as the dependent variable. All pathological, demographic, and socioeconomic factors collected were used as candidate independent variables. Since occupation, clinic symptoms, mode of delivery, and history of genital warts are multi-categorical independent variables, dummy variables were set in SPSS.

Results

Patient characteristics

Of the 130 patients diagnosed with ASCUS according to the current TBS classification, 31 were normal or had inflammatory diseases (23.90%); 29 were CIN I (22.30%), 14 were CIN II (10.80%), 55 were CIN III or cervical carcinoma in situ (42.30%), and one had invasive cervical carcinoma (0.80%).

Correlation between age and histopathological status

To analyze the association between age and histopathological status, patients were stratified two groups: < CIN II containing patients who have a normal, inflammation, or CIN I diagnosis; and ≥ CIN II containing patients with CIN II, CIN III, cervical carcinoma in situ or invasive cervical
The presence of advanced lesions and associating risk factors for advanced cervical carcinoma in patients with atypical squamous cells etc.

The ASCUS cytological category is problematic, mainly due to its ambiguity and lack of consensus on diagnosis and management for patients with an ASCUS diagnosis. Since ASCUS is diagnosed in a considerable portion of cervical smear cases every year [7], and there is evidence that ASCUS patients have a higher risk of developing cervical cancer [9], it is important to better understand the pathological variations and to identify risk factors associated with advanced cervical lesions in ASCUS patients. Here, the present authors used pathological examination of cervical biopsies to assess the histopathological status of 130 ASCUS patients and found over 50% of the patients harbor lesions of CIN III or higher grades. Compared to previous reports [16], the present study had a higher percentage of ASCUS patients with advanced lesions. This difference may be explained by the present authors’ clinical standard, which classified patients with CIN II-III with gland involvements as CIN III. The present findings supported the need for close follow-up for ASCUS patients.

HPV infection is rather common in young women. HPV infection is a main cause of cervical cancer, and 99.7% of

Table 1. — Histopathological status of ASCUS patients.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Case number (%)</th>
<th>&lt; CIN II</th>
<th>≥ CIN II</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>19 (14.62)</td>
<td>11 (8.46)</td>
<td>8 (6.15)</td>
</tr>
<tr>
<td>30-39</td>
<td>50 (38.46)</td>
<td>23 (17.09)</td>
<td>27 (20.77)</td>
</tr>
<tr>
<td>40-49</td>
<td>47 (36.15)</td>
<td>17 (13.08)</td>
<td>30 (23.08)</td>
</tr>
<tr>
<td>50-59</td>
<td>12 (9.23)</td>
<td>8 (6.15)</td>
<td>4 (3.07)</td>
</tr>
<tr>
<td>≥60</td>
<td>2 (1.54)</td>
<td>1 (0.77)</td>
<td>1 (0.77)</td>
</tr>
</tbody>
</table>

χ² = 4.983; p = 0.289.

Table 2. — Correlation between hr-HPV viral load and histopathological status in ASCUS patients.

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>N</th>
<th>Hr-HPV viral load</th>
<th>Mean rank</th>
<th>Sum of ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; CIN II group</td>
<td>27</td>
<td>43.56</td>
<td>1176.00</td>
<td></td>
</tr>
<tr>
<td>≥ CIN II group</td>
<td>99</td>
<td>68.94</td>
<td>6825.00</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>126</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. — Unconditional logistic regression analysis of risk factors for developing high-grade (≥ CIN II) cervical lesions in ASCUS patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>S.E.</th>
<th>Walds</th>
<th>p</th>
<th>AOR</th>
<th>95.0% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of pregnancy</td>
<td>0.353</td>
<td>0.172</td>
<td>4.243</td>
<td>0.039</td>
<td>1.424</td>
<td>1.017-1.993</td>
</tr>
<tr>
<td>hr-HPV infection</td>
<td>2.746</td>
<td>1.061</td>
<td>6.694</td>
<td>0.010</td>
<td>15.585</td>
<td>1.946-124.792</td>
</tr>
</tbody>
</table>

regression process, the number of gestations and the presence of hr-HPV infection were found to have significant associations with the outcome in the regression model (Table 3). Both of these factors as independent variables also had a good overall fit by χ² test (χ² = 4.523, df = 1, p = 0.033) and Hosmer-Lemeshow analysis (χ² = 4.314, df = 4, p = 0.365). The percentage of correct prediction of the model was 53.80%, and the Nagelkerke R² value was 0.136. The risk of developing high-grade (≥ CIN II) cervical lesions in ASCUS patients was associated with the number of gestations, with an adjusted odd ratio of 1.424 (Table 3). The impact of being hr-HPV positive on a patient’s risk in developing high-grade (≥ CIN II) cervical lesions was also significant, with an adjusted odd ratio of 15.585 (Table 3). Together, these results demonstrated that the number of gestations and hr-HPV infection are confounding factors for the development of high-grade cervical lesions in ASCUS patients.

Discussion

Finally, the authors attempted to identify independent variables that may contribute to the risk of developing advanced lesions in ASCUS patients using the demographic information collected at the time of recruitment. To assess the potential impacts of these independent variables on the histopathological status of the cervical lesions, the authors performed an unconditional logistic regression using the established histopathological group status (< CIN II and ≥ CIN II) as dependent variables. Using a forward stepwise
cervical cancer cases are HPV positive [17]. To date, there are at least 13 carcinogenic, or high-risk HPV strains [18]. HPV infection of cervical epithelial cells, specifically the expression of the E6 and E7 genes, can lead to cervical abnormalities, ranging from ASCUS to invasive carcinoma [12].

The authors found hr-HPV DNA present in 90% of ASCUS patients, and these hr-HPV-positive patients had a ~15-fold increase in the risk of developing high (≥ CIN II) grade lesions. Consistent with previous reports [19], the present authors demonstrated that in ASCUS patients, hr-HPV viral load was significantly higher in patients with advanced cervical lesions (Table 2).

In addition to HPV infection, other physiological and social factors associated with increased risk of HPV infection in women have also been reported to correlate with the development of high grade cervical lesions. For example, women between 30 and 35 year old are more likely to be diagnosed with cervical carcinoma. The present authors therefore tested for a correlation between histopathological status and age in ASCUS patients. However, they found no significant correlation between the two factors. Therefore, they recommend that ASCUS patients, regardless of their ages, should undergo further cervical evaluation.

The number of gestations has been associated with increased risk of cervical cancer. For example, women with seven or more full-term pregnancies were found to have a four-fold increase in the risk of developing cervical cancer than nulliparous women [20]. In addition, multiple parity was reported to be correlated with invasive cervical carcinomas [21], yet fewer pregnancy was correlated with a decrease in cervical cancer incidence and mortality [22].

Several mechanisms have been proposed for such correlation: first, high levels of estrogen can be immune suppressive, therefore increase the patients’ susceptibility to hr-HPV infection [23, 24]. Second, elevated progesterone levels, also a hallmark of pregnancy, can upregulate E6 and E7 expression and suppress T-cell function, again increasing the patients’ susceptibility to hr-HPV infection [25]. It has also been suggested that in some cases, the positive correlation between multiple gestations and parities and cervical cancer may be partly due to inadequate sterilization of medical devices used during child birth or abortion, which can cause iatrogenic HPV infection [26]. Consistent with such observation, here the present authors identified the number of gestations as a confounding factor to the risk of developing high-grade cervical.

Having sexual intercourse during adolescence increases the risk of having persistent HPV infection, and in turn developing cervical carcinoma. Women who have their first sexual intercourse before turning 17 years old have two- to three-fold higher risk of developing cervical cancer than those having their first intercourse after 20 years of age [27]. In addition, since having multiple sexual partners is often associated with unconsented intercourse with HPV-positive partner during adolescence [28, 29], it can also cause an increase in the risk of having HPV infection, and consequently cervical cancer. The present study demonstrated no significant association between high-grade cervical lesions and the age of first sexual intercourse or the number of sexual partners. The present authors reasoned the lack of association observed here may be due to sampling bias: only a very small number of patients in the study had sexual intercourse during adolescence, and most patients only had one partner as they grew up under the transitional culture influence in the 1960-1970s in China.

In conclusion, the present authors have conducted histopathological examination on cervical biopsies from ASCUS patients and analyzed the risk factors associated with the presence of advanced cervical lesion. Their findings revealed that a significant portion of ASCUS patients had high-grade cancerous lesions and suggested that HPV infection and gestation history are confounding factors to the risk of having advanced cervical lesions. These results are consistent previous studies and further support the use of hr-HPV testing in patients with ASCUS. In addition, the finding that HPV infection and gestation history are confounding factors to having advanced cervical lesions may help in developing optimal follow-up strategies for managing ASCUS patients.

Acknowledgements

This study was supported by the grant from the Science and Technology Development Project of Jilin Province No. 20120735 and the Research Funds of Jilin University.

References

The presence of advanced lesions and associating risk factors for advanced cervical carcinoma in patients with atypical squamous cells etc.


The construction of cDNA library and the screening of related antigen of ascitic tumor cells of ovarian cancer

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Summary

Objective: To construct the cDNA library of the ascites tumor cells of ovarian cancer, which can be used to screen the related antigen for the early diagnosis of ovarian cancer and therapeutic targets of immune treatment. Materials and Methods: Four cases of ovarian serous cystadenocarcinoma, two cases of ovarian mucinous cystadenocarcinoma, and two cases of ovarian endometrial carcinoma in patients with ascitic tumor cells which were used to construct the cDNA library. To screen the ovarian cancer antigen gene, evaluate the enzyme, and analyze nucleotide sequence, serological analysis of recombinant tumor cDNA expression libraries (SEREX) and suppression subtractive hybridization technique (SSH) techniques were utilized. The detection method of recombinant expression-based serological mini-arrays (SMARTA) was used to detect the ovarian cancer antigen and the positive reaction of 105 cases of ovarian cancer patients and 105 normal women’s autoantibodies correspondingly in serum. Results: After two rounds of serologic screening and glycosides sequencing analysis, 59 candidates of ovarian cancer antigen gene fragments were finally identified, which corresponded to 50 genes. They were then divided into six categories: (1) the homologous genes which related to the known ovarian cancer genes, such as BARD 1 gene, etc; (2) the homologous genes which were associated with other tumors, such as TM4SFl gene, etc; (3) the genes which were expressed in a special organization, such as ILF3, FXR1 gene, etc; (4) the genes which were the same with some protein genes of special function, such as TIZ, C1D gene; (5) the homologous genes which possessed the same source with embryonic genes, such as PKHD1 gene, etc; (6) the remaining genes were the unknown genes without the homologous sequence in the gene pool, such as OV - 189 genes. Conclusion: SEREX technology combined with SSH method is an effective research strategy which can filter tumor antigen with high specific character; the corresponding autoantibodies of TM4SF1, C1D, TIZ, BARD1, FXR1, and OV - 189 gene’s recombinant antigen in serum can be regarded as the biomarkers which are used to diagnose ovarian cancer. The combination of multiple antigen detection can improve diagnostic efficiency.

Key words: Ovarian tumors; CA - 125 antigens; Double hybrid system technology; Gene library; Serological test.

Introduction

Among all ovarian neplasias epithalial ovarian tumor is the first cause of death. It can produce initial curative effect by adopting surgery to reduce the tumor cells and platinum chemotherapy. However, 70% of the advanced patients may result in treatment failure due to the drug resistance which be caused by chemotherapy [1, 2]. It is of important significance to discover the marker for screening early ovarian cancer and the targets of immunotherapy [3]. This research adopts the technology of serological analysis of recombinant tumor cDNA expression libraries (SEREX) and suppression subtractive hybridization technique (SSH) to screen and identify the ovarian cancer antigen with higher sensitivity and specific degree. Recombinant expression-based serological mini-arrays (SMARTA) methodology was used to detect the positive reaction of corresponding antigen [4-6]. These studies lay the foundation for the search of a new biomarker of ovarian cancer and targets of immunotherapy.

Materials and Methods

Materials

The following materials were utilized in this study: activation of agarose gel 4B column material of potassium bromide; isopropyl -β-D and IPTG; the extraction kit (Poly real mRNA AT Tract System Prime – a – 1000) and mark System(Prime-a-Gene); CDNA synthesis kit (ZAP – cDNA), cloning kit (ZAP - cDNA Gigapack III Gold) and coli phage cracking liquid; CDNA cut kit (PCR – Select), chromatography laurel (CHROMA SPIN); IgG of alkaline phosphatase sheep tag anti human (Fc), the IgM of alkaline phosphatase people (μ chain), and 5Br,4chlorine,3indole phosphate/nitrogen BcIP/NBT.

The source of specimen

(1) The specimen, which was used to construct the cDNA library, was from the ascitic tumor cells of four cases of ovarian serous cystadenocarcinoma (two cases of Stage IIc, two cases of Stage IIIa), two cases of ovarian mucous cystadenocarcinoma (Stage IIIa), and two cases of ovarian endometrial carcinoma (Stage IIIb) in patients. (2) The mRNA, which was used to prepare the SSH probe, originated from the cancer tissues and the contralateral normal ovarian tissue which was chosen from one case of ovarian serous cystadenocarcinoma (Stage Ia) of patient. (2) The mRNA, which was used
to prepare the SSH probe, originated from one case of cancer and the contralateral normal ovarian tissue of the patient with ovarian serous cystadenocarcinoma (Stage Ia). (3) The serum for screening the cDNA library originated from eight cases of autologous serum which were constructed to cDNA library, and other five cases of oligogenic serum with the high of IgG which diagnosed the ovarian cancer by pathological examination. (4) The oligogenic serum, which was used for SMARTA, originated from 105 patients with ovarian cancer (not including the oligogenic serum of screening cDNA library). The patients were aged 14-75 years and the average was 47 years. Among them, epithelial carcinoma cases were 85 (54 cases of serious carcinoma, 25 cases of mucinous carcinoma, five cases of endometrial carcinoma, one case of undifferentiated carcinoma); non-epithelial carcinoma were 20 cases (nine cases of germ cell tumor, 11 cases of sex cord stromal tumor); I- II period were 35 cases, III ~ IV period were 70 cases; well-differentiated carcinoma were 24 cases, poorly differentiated carcinoma were 63 cases, and differentiated unknown were 18 cases. All the aforementioned specimens were collected from the untreated patients who were confirmed by pathological examination in People’s Hospital. (5) The serum of 105 cases of healthy women, who underwent a physical and without history of tumorigenesis, were considered the normal controls. Their age was 20 ~ 70 years, and the average age was 43 years. The blood serum was stored at 4°C. The entire process lasted two hours. The serum was stored at -80°C partially after it was frozen completely and centrifuged for 15 minutes at 2,000 r/min. Specimen collection was occurred after signed informed consent of the patients.

The construction of cDNA library

The ascites were collected under aseptic conditions. The cells were inoculated in culture flasks after centrifugation. Then the nucleated cells of the non-tumor were removed under gradient centrifugation. After more than 90% of tumor cells were confirmed by cytological examination, Poly AT Tract System 1000 kits were used to extract mRNA according to the manufacturer’s instructions. The synthesis of cDNA’s first chain used the ZAP - cDNA kit, which contained the enzyme loci of Xho I. The construction and amplification of CDNA library adopted the cloning kits of ZAP - cDNA Gigapack III cold, according to the manufacturer’s instructions. The original library was titrated and the storage capacity was calculated according to the number of plaques. The original library was amplified and stored at -70°C.

cDNA library screening through SEREX technology

(1) The adsorption of serum: the serum which was used to screen the cDNA library, was absorbed by the nitrocellulose membrane (NC) which was soaked with E. coli phage cracking liquid. The nonspecific antibody, which reacted to the host bacteria and serum protein phage, was then eliminated. (2) The screening of cDNA library: a diameter of 150 mm flat plaque was spread to prepare bacteriophage plaque after 600 μ host bacteria was infected by 8 x 104 pfu phage. The library was induced and expressed by using the NC which was disposed by the IPTG. The NC membrane was sealed with a closed liquid of 1% of BSA. Then the c reacted to serum of 1:50 dilution at home temperature two hours after adsorption. It reacted to the goat-anti-human IgG and IgM which were marked by 1:10 000 dilute alkaline phosphatase for one hour at room temperature after washing membrane. Then they were put in the BCIP/NBT liquid to conduct developing in a dark place. The positive plaque was picked and stored at 4°C. According to the above method, the obtained positive plaque was screened with sub-cloning to obtain monoclonal. When the subclone was screened, 2 NC membranes with the liquid of IPTG were used to induce and transfer membrane one after another. The first membrane was induced for four hours and the second membrane was induced for eight hours, with the second membrane reacted with IgM2 directly. The bacteriophage plaque, which had a positive reaction to the serum of patients and a negative resistance reaction to IgM2, was positive for the second screen of cDNA library. The positive plaque was picked and stored at 4°C. (3) The internal shear and plasmid extraction of bacteriophage: the host E. coli XL1 - Blue - MRF phage was infected by positive monoclone phage and auxiliary phage ExAssist together and was sheared inwards. The product was centrifuged. The filamentous phage pBluescript, which completed the internal shear existed in the supernatant fluid. (3) The pBluescript phagocytos was transferred into SOLR and spreaded in the AGAR plate of 37 °C overnight. In order to enlarge cultivation, single colonies were selected, and the plasmid DNA was extracted to identify the the size of the screened antigen gene (exogenous insert fragments) after it was cut by EcoR I and Xho I.

Screening of differentially expressed genes with SSH method

The ovarian cancer tissue which was used in SSH and the first and second chain of the matching of normal ovarian tissue were compounded by the cut kit of PCR - Select cDNA. Firstly, the connection of cDNA in the tumor tissue with the joint was treated as the detector to perform the subduction, and then the gene segments that expressed specifically by the tumor were obtained, which indicated that the subduction belonged to positive subduction. Finally, when the negative subduction was conducted by the connection of cDNA in the normal ovary with the joint, the gene segments that expressed specifically by the normal ovarian tissue were achieved. The cDNA fragments, which were obtained by SSH, were purified via CHROMA SPIN chromatography column. After purification and quantitative analysis of ultraviolet spectrophotometer, it was marked by the Pime - a - Gene marker system.

Spot hybridization method and screening of ovarian cancer antigen with nucleotide sequence analysis

The positive plasmid DNA, which was screened by SEREX technology, was applied to the nylon membrane after the denaturation and it was hybridized with the gene fragment, which was prepared by method of SSH, by using the high performance liquid of hybridization kit. Hybridization resulted as positive when it was hybridized with the normal gene fragment. Conversely, the plasmid, whose hybridization resulted as negative, was the one that owned the nucleotide sequence in the differentially expressed ovarian cancer. The plasmid DNA was evaluated by EcoR I and Xho I double enzyme. The plasmid with the fragment was detected by nucleotide sequence. The results were analyzed through similarity comparisons and bioinformatic analysis so that the related ovarian cancer antigen gene could be obtained.

The detection of allograft cancer antigen and autoantibody in serum by using the SMARTA method

(1) The adsorption of serum: the z-ZAP of light phage was transfected to XL1 - Blue - MRF7 of host bacterium. When they were amplified and cultivated overnight at 37°C, ten-ml coupling buffer was added. After 12 hours of oscillation and elution, the elution liquid was finally collected. The E. coli with not completely pyrolysis is broken by ultrasonic. According to the instructions manual, it was coupled with the 4B material of agarose gel which was activated by potassium bromide after the quantitative analysis of protein through ultraviolet spectrophotometer. After the serum of 96 of ovarian cancer patients and the serum of 96 of normal controls, which were waiting for the inspection, they were diluted with dihydroxy methyl amino (1:10) which contained 1% BsA, and mixing the cracking liquid column of E. coli. The oscillation at 4°C was maintained overnight. Then the serum was collected after the elution.
(2) The serological detection: the reaction between the related antigens of ovarian cancer which was screened (including positive phage liquid of related antigen gene of ovarian cancer) and the serum of waiting for detection showed that it was positive when the spot color with the positive of phage liquid was stronger than the spot (no-load phage liquid) with the negative control of phage liquid. It reacted positive between the related antigen and corresponding autoantibody response of serum, namely ovarian cancer antigen and corresponding autoantibodies in serum (IgG and IgM with the markers of alkaline phosphatase which were used to distinguish them).

Statistical methods

Data processing and analysis were performed utilizing MATLAB7.0 software. The comparison of count data were analyzed by the Chi-square test \( (\chi^2) \). Logistic regression analysis was used to assess the value of conjoint analysis of the related antigen in the diagnosis of ovarian cancer.

Results

The construction and amplification of cDNA library

The original capacity of cDNA library was 1.8 x 106 pfu/ml. After the amplification, the capacity was 8.0 x 109 pfu/ml, namely, the cDNA library of phage owned the high performance.

cDNA library screened by SEREX technology

In the first round of serological screening, 261 of bacteriophage plaques were achieved. Then, the second round of subcloning was conducted. After two rounds of screening, 245 of positive monoclonal phages were obtained and they accepted the internal shear immediately. They all get the single colony with pBluescript phage. The recombinant plasmid DNA was extracted after expanding each colony. The 245 of positive plasmid clones, which were appraised by enzyme digestion, includes the exogenous inserted fragment. The size of fragment was 0.5~2.0 kb.

Spot hybridization and the analysis of nucleotide sequence

The 245 plasmid DNA and the SSH probe of marking, which were screening by two rounds of serological, was detected by spot hybridization. The results showed that 59 plasmids had significantly positivity in the cut hybridization probes and had negative reaction in the reverse cut hybridization. It was preliminarily confirmed that these 59 plasmids stand for the candidate antigen genes that were related to ovarian cancer.

The nucleotide sequencing results of 59 plasmids were sent for the similarity comparisons and bioinformatics analyses. It was found that the antigen gene fragments of 59 candidates of ovarian cancer represented 50 genes, which were divided into six categories: (1) the homologous genes of the known related gene of ovarian cancer; (2) the homologous genes of related gene of other tumor; (3) the genes were expressed in some special groups; (4) the homologous genes of some protein gene with special function; (5) the homologous genes of the source of embryonic genes; (6) the unknown genes with no homologous sequence and comparison in the gene pool of GeneBank.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Serum carinoembryonic (n=105)</th>
<th>Normal serum (n=105)</th>
<th>( \chi^2 )</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>Positive rate (%)</td>
<td>Case Positive rate (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TM4SF1</td>
<td>30</td>
<td>28.57</td>
<td>10</td>
<td>9.52</td>
</tr>
<tr>
<td>C1D</td>
<td>23</td>
<td>21.90</td>
<td>7</td>
<td>6.67</td>
</tr>
<tr>
<td>BARD1</td>
<td>23</td>
<td>21.90</td>
<td>5</td>
<td>4.76</td>
</tr>
<tr>
<td>FXR1</td>
<td>24</td>
<td>22.86</td>
<td>8</td>
<td>7.62</td>
</tr>
<tr>
<td>OV-189</td>
<td>33</td>
<td>31.43</td>
<td>14</td>
<td>13.33</td>
</tr>
</tbody>
</table>

Table 2. — The recombinant antigen of ovarian cancer antigen gene and the positive rate of corresponding IgG autoantibody response in serum.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Serum carinoembryonic (n=105)</th>
<th>Normal serum (n=105)</th>
<th>( \chi^2 )</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>Positive rate (%)</td>
<td>Case Positive rate (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIZ</td>
<td>27</td>
<td>25.71</td>
<td>9</td>
<td>8.57</td>
</tr>
<tr>
<td>FXR1</td>
<td>29</td>
<td>27.62</td>
<td>12</td>
<td>11.43</td>
</tr>
<tr>
<td>OV-189</td>
<td>19</td>
<td>18.09</td>
<td>8</td>
<td>7.62</td>
</tr>
</tbody>
</table>

Serology detection of the related antigen of ovarian cancer

SMARTA method was used to detect the reaction of corresponding autoantibody in serum, with which 50 cases of related antigen of ovarian cancer was compared with the serum of 105 cases of ovarian cancer patients, and the serum of 105 cases of normal control women, respectively. The positive rate, which the recombinant and fusion antigen of phage of TM4SF1, C1D, BARD1 gene, FXR1, OV – 189 genes were compared with the corresponding IgG autoantibody response in the cancer serum and the normal serum, respectively, showed a statistical significance \( (p < 0.01) \). When the recombinant antigens of gene TIZ, FER1 together with OV-189 and the related positive rate of IgM autoantibody reaction in the tumor serum were compared with the normal serum, respectively, the difference showed statistical significance \( (p < 0.05) \), as reported in Tables 1 and 2.

The reaction conditions of recombinant antigen of ovarian cancer antigen gene and corresponding autoantibody in serum of ovarian cancer patients in different clinical pathological characteristics: the recombinant antigen of FXR1, OV - 189 gene and the positive rate of corresponding IgG autoantibody response of Stages I–II of ovarian cancer patients in serum was higher than Stages III–IV. They were compared respectively with each other and showed statistically significant differences \( (p \text{ values were } 0.042, 0.025) \). The recombinant antigen of OV - 189 gene and the positive rate of corresponding IgG autoantibody response of ovarian cancer patients with well differentiated in serum was higher.
than middle low differentiation. The comparative difference was statistically significant \((p = 0.001)\), as shown in Table 3. The recombinant antigen of TIZ, FXR1 gene and the positive rate of corresponding IgG autoantibody response of Stages I–II of ovarian cancer patients in serum was higher than Stages III–IV. They showed statistically significant differences \((p\) values were 0.021 and 0.021), as shown in Table 4.

The value of the corresponding autoantibody, which was in correlation with antigen that related to ovarian cancer, was analyzed and applied in the diagnosis of ovarian cancer. The results were as follows: combining the clinical value of IgG and IgM autoantibody in the diagnosis of ovarian cancer (which were produced by the recombinant antigen of this six gene: M4SF1, C1D, TIZ, BARD1, FXR1, and OV-189) with Logistic regression analysis, the diagnostic value of autoantibody repertoire combined with CA125 was analyzed. As a result, when conjoint analysis of relevant IgG autoantibody, which was in correlation with the recombinant antigen of gene TM4SF1, C1D, TIZ, and FXR1 was conducted, and more than three of them were predicted as positive autoantibody repertoire, the diagnostic results of the sensitivity, specificity, and accuracy of ovarian cancer were 68\%, 81\%, and 75\%, respectively, in the diagnosis of ovarian cancer. Sensitivity, specificity, and accuracy were 85\%, 80\%, 81\%, respectively, and sensitivity and accuracy were obviously improved combining CA125 with the spectrum of autoantibodies.

### Table 3. — The corresponding IgG autoantibody in serum of ovarian cancer patients and the positive rate of recombinant antigen of ovarian cancer antigen gene in different clinical pathological characteristics.

<table>
<thead>
<tr>
<th>Clinicopathological features</th>
<th>Total cases</th>
<th>TM4SF1 Positive rate (%)</th>
<th>C1D Positive rate (%)</th>
<th>FXR1 Positive rate (%)</th>
<th>OV-189 Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinicopathological features</td>
<td>Clinically positive cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial</td>
<td>85</td>
<td>25</td>
<td>29.41</td>
<td>19</td>
<td>22.35</td>
</tr>
<tr>
<td>Non-epithelial</td>
<td>20</td>
<td>6</td>
<td>30</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II period</td>
<td>35</td>
<td>11</td>
<td>31.43</td>
<td>6</td>
<td>17.14</td>
</tr>
<tr>
<td>III-IV period</td>
<td>70</td>
<td>16</td>
<td>22.86</td>
<td>15</td>
<td>21.43</td>
</tr>
<tr>
<td>Pathological differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>24</td>
<td>3</td>
<td>12.5</td>
<td>5</td>
<td>20.83</td>
</tr>
<tr>
<td>Middle and low differentiated</td>
<td>63</td>
<td>25</td>
<td>39.68</td>
<td>20</td>
<td>31.75</td>
</tr>
</tbody>
</table>

Note: “\(a\)” signifies the comparison with III-IV period, \(p < 0.05\) “\(b\)” signifies the comparison with middle low differentiation \(p < 0.05\).

### Table 4. — The corresponding IgM autoantibody in serum of ovarian cancer patients and the positive rate of recombinant antigen of ovarian cancer antigen gene in different clinical pathological characteristics.

<table>
<thead>
<tr>
<th>Clinicopathological features</th>
<th>Total cases</th>
<th>TIZ Positive rate (%)</th>
<th>FXR1 Positive rate (%)</th>
<th>OV-189 Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinicopathological features</td>
<td>Clinically positive cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial</td>
<td>85</td>
<td>22</td>
<td>25.88</td>
<td>22</td>
</tr>
<tr>
<td>Non-epithelial</td>
<td>20</td>
<td>5</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II period</td>
<td>35</td>
<td>16</td>
<td>45.71 (a)</td>
<td>18</td>
</tr>
<tr>
<td>III-IV period</td>
<td>70</td>
<td>10</td>
<td>14.29</td>
<td>12</td>
</tr>
<tr>
<td>Pathological differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>24</td>
<td>10</td>
<td>41.67</td>
<td>10</td>
</tr>
<tr>
<td>Middle and low differentiated</td>
<td>63</td>
<td>19</td>
<td>30.16</td>
<td>21</td>
</tr>
</tbody>
</table>

Note: “\(a\)” signifies the comparison with III-IV period, \(p < 0.05\).

### Discussion

Ovarian cancer is one of the three largest tumors in gynaecology and has the worst prognosis, because its onset is concealed by a lack of specific signs and symptoms. Patients that are first visited are already in an advanced stage \([7]\). Currently the diagnosis of ovarian cancer mainly relies on conventional physical and pathological examination, due to the lack of more effective means \([8, 9]\). Therefore searching for the specific antigen of ovarian tumor, or related antigen for the early diagnosis of ovarian cancer has important theoretical and practical significance.

Since 1991, when Boon \([10]\) used the T cell clone technology to find and identify the first melanoma antigen, there are a variety of ways to screen the tumor antigen. Sahin et al., \([11]\) in 1995, founded the technology of Serological Analysis of Recombinant cDNA Expression Libraries (SEREX).

The basic steps of this technology include the establishment of cDNA expression library; screening the cDNA expression library with the serum of patient; positive cloning.
The construction of cDNA library and the screening of related antigen of ascitic tumor cells of ovarian cancer

This study used SEREX technology and a variety of pathological ascites tumor cells to construct a cDNA library, and combining with the SSH method to screen the related antigen of ovarian cancer to ensure the diversity of antigen gene and improve the sensitivity. After screening analysis, 59 candidate antigen gene fragments of ovarian cancer represented 50 genes. These proteins of genes encode protein involved in cancer gene, the zinc finger protein and the protein on the surface of cell membrane, and some functions of unknown protein. Such as BARD1 is a protein, with a certain ring structure domain, which is related to the tumor suppressor genes BRCA1. The gene mutations of BARD1 can destroy the structure of the compound of BRCA1/BARD1 and lead to the tumor [12]. The recombinant antigen of TM4SF1 gene is a transmembrane protein [13]. The recombinant antigen of TIZ gene is a zinc finger protein which can restrain the related factor 6 (TRAF6) of tumor apoptosis and participate the regulation of TRAF6 for osteoclast differentiation [14]; OV - 189 is a protein of unknown function. The role of these proteins in the development and mechanism of ovarian cancer need further research.

SMARTA owns many features, such as high flux, good repeatability advantages, and so on. This experiment detected the serum of related antigens of 50 ovarian cancers by using SMARTA. The results show that the comparison is remarkable between the recombinant and fusion antigen of phages of TM4SF1, C1D, BARD1, FXR1, OV - 189 gene and the positive rate of corresponding IgG autoantibody response in serum of cancer and normal serum. The comparison was also remarkable between the recombinant antigen of phages of TIZ, FXR1, OV - 189 gene and the positive rate of corresponding IgM autoantibody response in serum of cancer and normal serum. These show that the occurrence and development of ovarian cancer in each stage has its related antigens with specific open expressions; searching for their autoantibodies can provide the important serum markers for diagnosing and screening. In addition, the value of a single of tumor markers is limited for cancer diagnosis, combining multiple markers that can improve the sensitivity and accuracy of the diagnosis [15]. The related antigen of ovarian cancer by joint analysis and the positive results of corresponding IgG, IgM antibody can greatly improve the screening of ovarian cancer and accuracy of diagnosis. In conclusion, this study provides a solid foundation for exploring the effect of related antigen of ovarian cancer in diagnosis, immune treatment, and the application of disease detection.

Acknowledgements

This programme was supported by Science and Technology Department of Henan Province, with fund number 22102310113.

References


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Do we understand the pathophysiology of endometrial cancer?

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Summary

Endometrial carcinoma is the fourth most common cancer in UK women. Previous literature describes local, haematological or lymphatic dissemination to common sites including vaginal vault, lungs, liver, bones and brain. The authors present two unusual cases of endometrial cancer metastases to the psoas major muscle and laparoscopic port sites. Case 1 involves a 71-year-old female who underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy and peritoneal washings (TAH, BSO, PW) for Grade 1 endometrial cancer, Stage 1B. Three years later she presented with lower back and right hip pain, with MRI imaging revealing psoas muscle metastases. Case 2 describes a 60-year-old female who underwent laparoscopic-assisted vaginal hysterectomy (LAVH), BSO+PW for Grade 1 endometrial cancer, Stage 1B. Three years postoperatively she presented with a lateral abdominal mass overlying the laparoscopic port site scar, which was Grade 1 endometrial cancer on biopsy. These rare metastatic locations question our traditional understanding of the pathophysiology of endometrial carcinoma.

Key words: Endometrial cancer; Dissemination; Psoas major muscle; Laparoscopic port site.

Introduction

Endometrial carcinoma is historically associated with local, haematological or lymphatic dissemination to structures including the vagina, lungs, liver, bone, and brain [1]. Studies have shown that one-fifth of patients with endometrial carcinoma have extra-uterine disease in these sites, while 10% of those with confined endometrial disease have positive pelvic lymph nodes at diagnosis [2]. The present case reports detailing unusual endometrial carcinoma metastases to the psoas major muscle and anterior abdominal wall associated with laparoscopic port sites question our current understanding of endometrial cancer spread and raise the possibility of alternative dissemination mechanisms. This would have significant implications upon both surgical approach (eg: open versus laparoscopic) and treatment regimes.

Case Report

Case 1

A 71-year-old female, para 2 (normal deliveries) presented to Gynaecology Fast Track Clinic with a two-week history of postmenopausal bleeding per vagina, having gone through menopause at age 50. She had never taken hormone replacement therapy (HRT) and underwent routine recall for cervical smears. She suffered from essential hypertension and her examination findings were unremarkable. She was a non-smoker and had no family history of breast or gynaecological malignancy. Transvaginal ultrasound (US) revealed a raised endometrial thickness of 17.3 mm with a right ovarian cyst measuring 2.3 cm. There were no adnexal masses or free fluid. CA-125 tumour marker was normal. Hysteroscopy showed an area of suspicious, vascular endometrium and endometrial biopsy revealed moderate dysplasia and intraepithelial neoplasia. The diagnosis was endometrial adenocarcinoma of likely endometrioid subtype. The patient underwent TAH, BSO+PW. Histology revealed Grade 1 endometrial carcinoma, Stage 1B. Adjunctive endometrial radiotherapy was administered.

Postoperatively, the patient received regular follow-up according to national guidelines; every three months for the first year then six monthly for five years. Three years postoperatively the patient’s general practitioner requested urgent review due to worsening lower back and right hip pain. CT and MRI scans were arranged to exclude recurrence and bony metastases. Imaging revealed a well-defined abnormality within the right psoas muscle measuring 7 x 4 cm beginning at the level of L3 and extending down the psoas muscle with scalloping of L4 vertebra. (Figure 1) There was no significant lymphadenopathy and no evidence of disease elsewhere. Provisional differential diagnoses included haematoma, abscess or sarcoma.

Multi-disciplinary team (MDT) discussion advised CT guided aspiration of the psoas mass, with two aspirations revealing inflammatory changes with no evidence of malignancy. Progressive back and hip pain prompted a repeat MRI scan which confirmed right psoas muscle metastases with para-aortic lymphadenopathy. The patient underwent radiotherapy (50 Gy in 25 fractions) to the psoas mass but the disease progressed clinically and radiologically with an enlarging para-aortic/retroperitoneal mass. Further paraspinal radiotherapy (ten Gy in five fractions) and two cycles of chemotherapy (carboplatin and paclitaxel) yielded little bene-
The patient was commenced on medroxyprogesterone 200 mg BD and died in a hospice shortly after developing malignant spinal cord compression.

Case 2
A 60 year-old female who had been post-menopausal for 8 years presented to the Gynaecology Fast Track clinic with a 2 week history of bleeding per vagina. She had never taken HRT, had always received routine cervical smears and had no significant family history. She suffered from type two diabetes mellitus, hypertension and mild osteoarthritis. She was a non smoker and her examination was unremarkable.

Transabdominal US revealed thickened, echogenic endometrium measuring 17 mm with normal appearances of the ovaries, with no pelvic mass or free fluid. Hysteroscopy showed areas of atypical and vascular endometrium with no polyps or fibroids. Endometrial biopsy revealed atypical endometrial hyperplasia. Invasive adenocarcinoma could not be excluded.

The patient underwent an uncomplicated LA VH, BSO+PW. Histological examination diagnosed Grade 1 endometrial adenocarcinoma, Stage 1B. Intraoperatively, a 12-mm midline port and 2 5-mm lateral ports were utilized with a peritoneal drain placed for 12 hours postoperatively. Uterus delivery was not difficult. The fascial layer of the midline port was closed with vicryl and glue applied to the lateral incisions.

Regular postoperative follow up commenced every four months for two years then six monthly for five years. Three years post-operatively the patient was referred with a possible hernia overlying the left laparoscopic port site (created during LAVH). A CT showed a well-defined abnormality within the right psoas muscle measuring 7 x 4 cm beginning at the level of L3 and extending down the psoas muscle with scalloping of L4 vertebra.

Figure 1. — A well-defined abnormality within the right psoas muscle measuring 7 x 4 cm beginning at the level of L3 and extending down the psoas muscle with scalloping of L4 vertebra.

MDT advised needle core biopsy of the mass, which concluded metastatic endometrioid adenocarcinoma. The anterior abdominal wall lesion was excised with clear margins.

One year later, a suspicious vaginal vault area was biopsied and revealed recurrent Grade 1 endometrial adenocarcinoma of papillary type. CT imaging additionally revealed lymph node recurrence, with a 2.6 x 2cm soft tissue mass near the external iliac node. Radiotherapy (45 Gy in 25 fractions) and brachytherapy (selectron treatment) was administered to the vaginal vault. This yielded a mixed response; reducing the size of the lymph node and vaginal vault lesions but growth in the rectus lesion (not incorporated into the field). The patient was diagnosed with incurable disease and medroxyprogesterone 200 mg BD commenced.

The rectus sheath lesion continued to progress reaching 8 x 7 cm, likely aided by the patient’s use of tamoxifen, which she was advised to cease. Due to the associated pain, palliative radiotherapy (45 Gy in 25 fractions) was administered yet the mass continued to increase in size. She developed a pulmonary embolus and underwent community palliative care.

Figure 2. — A 3.7 x 3.8 cm mass arising from the left anterior abdominal wall with no evidence of recurrent pelvic or lymphatic disease

Discussion
Endometrial cancer accounts for 5% of new cancer diagnoses in UK women with an incidence of 26 cases: 100,000 females, steadily increasing since the 1970s [3]. Contributing factors include increased obesity, use of HRT, and changes in reproductive behaviour (nulliparity/fewer children). Ninety percent of females are >50 years at diagnosis and 10% patients with post-menopausal bleeding have endometrial cancer [3]. Bohkman described two distinct types of endometrial cancer; type 1 (80% cases) associated with obesity and endometrial hyperplasia, with a five-year survival rate of 85%. Type 2 endometrial cancer (eg: serous, clear, small cell) has a poorer prognosis (50% five-year survival), metastasize early, and are not estrogen-associated [4].

Type 1 endometrial cancer characteristically spreads locally (vaginal vault) and via the lymphatic system, with
disease generally localised to the pelvic nodes [5, 6]. Further lymphatic spread involves the common iliac and para-aortic nodes (8% incidence), with the latter having a poor prognosis [6]. Hepatic and pulmonary metastases occur by haematological or lymphatic (three-times more commonly) spread [7]. Type 2 endometrial carcinoma has a greater predilection for lymphatic dissemination, with resultant higher rates of extra-uterine disease at presentation [5].

There are few literature reports on laparoscopic port site endometrial cancer metastases (PSM) [8,19], yet these reports highlight deficiencies in our understanding of endometrial carcinoma pathophysiology. In 1961 Thomas et al. [20] described the principle of tumour cell contamination of surgical wounds yet little advance has been made in our understanding of the exact mechanisms. Factors encouraging spread include incision of the tumour; blood vessels or lymphatics, or direct contact between the tumour and abdominal wall intraoperatively. These principles were surmised by the tumour cell entrapment theory of 1989 [21], recognising the potential for tumour cell spread via:

- Free intra-peritoneal tumour emboli.
- Fibrin entrapment of intra-abdominal tumour emboli on traumatised peritoneal surfaces.
- Blood clots containing viable cancer cells remaining in the abdomen postoperatively.
- Growth of viable cells encouraged by growth and tissue repair factors involved in wound healing.

PSM occur in 1-1.9% of surgery for gynaecological malignancies [11,15], typically presenting 21 months postoperatively (range 7-48 months) [8,13]. PSM and intra-or retroperitoneal tumour spread is not exclusively associated with advanced or high-grade malignancies [13] as supported by the present case reports. In agreement with other studies [9] the lateral port at which PSM occurred in the present case report was not used for specimen retrieval. It is improbable that haematological, lymphatic or direct contact is solely responsible for these metastatic sites. Wang et al. [19] concur that haematological dissemination cannot underly PSMs, considering that the lungs receive most venous drainage from the gynaecological organs, and the abdominal wall receives only a small percentage of cardiac output. Consequently, isolated abdominal wall recurrence without pulmonary metastases is unlikely.

One proposed etiological mechanism involves trans-tubal spread into the peritoneal cavity, a theory strengthened by Cressman et al. [2] who reported that 52% patients with positive PW had no evidence of extra-uterine disease. Stewart et al. [22] described a strong correlation between intra-luminal tumour cells within the fallopian tubes and positive PW and peritoneal metastases. Trans-tubal spread is certainly important for the dissemination of type II endometrial carcinoma [23, 24].

Some suggest that retrograde, trans-tubal dissemination may occur as early as diagnostic hysteroscopy, with Revel et al. advising fluid-based hysteroscopy should be avoided if the TVUS is highly suspicious of malignancy [25-28].

Diurdievic et al. [29] describe the only other case of psoas muscle endometrial cancer metastases, additionally reporting metastases in the anterior and lateral pelvic wall. Lonnefors et al. reported PSMs to most commonly occur in the specimen retrieval port [11].

At laparoscopic surgery, tumour cells may directly seed on the damaged rectus sheath at the port site, encouraged by repeated changes of instruments with traumatic movement into and out of the abdomen; specimen retrieval and the pneumoperitoneum itself seeding tumour cells [8-19]. Small incisions (eg: laparoscopy) are proposed to promote tumour growth more successfully than larger incisions [9,12]. Suggestions to overcome PSM include gas-free laparoscopic surgery; avoidance of power cutting devices; use of retrieval bags; correct trochar placement with minimal trauma; suturing ten- to 12-mm trochar sites; instrument and port-site lavage with iodine or chemotherapeutic agents; initiating adjuvant therapy promptly and including the port sites within the radiation field [8-19,30].

Laparotomy wound recurrences of endometrial cancer are extremely rare, yet conflicting data exists comparing open and laparoscopic surgery [9-14, 31]. A Cochrane review [32] compared the two approaches and found no difference in wound recurrence. However the included studies involved only early endometrial cancer, so these findings may not be generalizable for more advanced disease. Some authors argue that systematic closure of the abdominal wall layers in open surgery produces lower wound recurrences [14]. It is proposed that data showing no difference between open and laparoscopic surgery is skewed by non-uniform trial designs, small sample sizes, inadequate representation of all endometrial cancer grades/stages, and short follow up durations which miss presentations of wound recurrence [14, 17, 33].

A multi-factorial etiology for PSM including the tumour type is most likely responsible, considering that gallbladder adenocarcinoma is well reported to recur at primary port sites [10,11]. Equally, colorectal adenocarcinomas have been linked to PSM, with 50% occurring at the specimen retrieval site [9]. However, similar to endometrial cancer, a Cochrane review of 2008 found no difference in wound recurrence rates comparing open and laparoscopic surgery for early (non-metastatic) colorectal cancer [34].

Conclusion

In conclusion, the present case reports highlight deficiencies in our understanding of endometrial cancer dissemination mechanisms with significant implications on surgical approach.
References


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Endometrial cancer in unicornuate uterus: a case report

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Summary

Purpose of Investigation: Müllerian anomalies have not been implicated as a significant risk factor for the development of cervical, uterine, and ovarian cancers; in the present literature, there are only a few reports of endometrial cancer arising in patients with Müllerian abnormalities. To the best of the authors’ knowledge, this is the first reported case of endometrial cancer arising in a patient with unicornuate uterus. Case Report: A 69-year-old Caucasian woman underwent clinical examination and office hysteroscopy with endometrial biopsy because of abnormal post-menopausal bleeding. The diagnosis was endometrial cancer in unicornuate uterus, hence the patient underwent total hysterectomy with pelvic lymphadenectomy. Conclusion: Uterine malformations and genetic disorders may cause a delayed diagnosis of gynaecological cancers. Gynaecological examination in asymptomatic patients and differential diagnosis in abnormal uterine bleeding patients should be considered.

Key words: Endometrial cancer; Unicornuate uterus; Diagnosis; Therapy.

Introduction

Uterine Müllerian malformations represent a heterogeneous group of congenital anomalies resulting from the underdevelopment of the Müllerian ducts, disorders in their fusion, and/or alterations in septum reabsorption. Prevalence of uterine malformations is estimated to occur in 0.4% (0.1–3%) of the general population [1, 2].

The 1988 classification of Müllerian malformation by the American Fertility Society (AFS) defines the unicornuate uterus as a condition where the uterus is formed from only one of the paired Müllerian ducts, while the other duct does not develop or only in a rudimentary fashion [3, 4]. Two main techniques for the diagnosis of these malformations combine the study of the uterine fundus and cavity: magnetic resonance imaging (MRI) and three-dimensional (3D) ultrasound [5, 6].

Uterine Müllerian malformations are related to many gynecological diseases, in particular to infertility and recurrent miscarriage [7], while a small number of evidences of the association between uterine malformation, such as unicornuate uterus, and genital tumors exist [8-16]. Therefore, a few documented cases of cervical carcinoma arising in unicornuate uteri have been described [8], whereas endometrial malignancy occurring in conjunction with this Müllerian anomaly has not been reported.

In this paper the authors report the case of an endometrial cancer in a unicornuate uterus.

Case Report

A 69-year-old, Caucasian woman came at our attention in February 2013 at the Department of Gynaecology, Obstetrics and Reproductive Science of the Second University of Naples because of a post-menopausal abnormal uterine bleeding occurring a few months earlier. She had been in menopause for about 20 years and had never undergone surgery. She had two spontaneous deliveries and one spontaneous miscarriage. The woman was affected by HCV-related liver disease.

Clinical examination revealed healthy cervix and vagina. Uterus was mobile and deflected to the right. No adnexal masses were palpable. The woman underwent transvaginal ultrasonography, which showed an irregularly formed uterus with almost normal size for her age (52×35×46 mm), but the endometrium was thickened: 11 mm.

Subsequently, the woman underwent office hysteroscopy with endometrial biopsy, with a pathological report of endometrial adenocarcinoma, endometrioid type. MRI was performed to evaluate myometrial infiltration and abdominal diffusion and showed a unicornuate uterus constituted only by its right side (Figure 1). The examination indicated a marked myometrial infiltration at the level of the fundus. The other pelvic organs did not appear to be affected by cancer infiltration. No iliac or retroperitoneal lymphadenopathy was detected. Thus, the woman underwent abdominal total hysterectomy, bilateral adnexitomy, bilateral pelvic lymph node dissection, peritoneal washing, and removal of a peritoneal lesion. At the time of surgery, it was determined that the patient had a unicornuate uterus, with the left side of the uterus completely absent.

Macroscopic examination revealed a unicornuate uterus (size: 9×4.5×2.5 cm) with diffusely thickened endometrium for the presence of a vegetating lesion occupying almost the entire uterine cavity; right ovary was sized 3×2×1.5 cm and right fallopian tube...
long five cm; left ovary was sized 3×2×1 cm with fallopian tube long four cm.

Microscopic examination revealed a well-differentiated (G1) endometrioid adenocarcinoma of the endometrium, infiltrating the myometrium for more than a half. The examination also revealed the presence of neoplastic vascular emboli. Cervix, adnexa, and peritoneal fluid resulted free from disease. The peritoneal lesion revealed to be a calcific concretion. Twenty-one lymph nodes were examined and all of them resulted negative for malignancy. Cancer Stage was FIGO IB. After discharge and an oncological consultancy, the patient is now undergoing a standard follow-up.

Discussion

As a small number of evidences of the association between uterine malformation and genital tumors exist [8-16], Müllerian anomalies have not been implicated as a significant risk factor for the development of cervical, endometrial, and ovarian cancers. Therefore, endometrial malignancy, which is the most common malignant tumor of the female genital tract in developed countries [17], occurring in conjunction with unicornuate uterus has not been reported.

At present, a few reports of endometrial cancer arising in patients with Müllerian abnormalities exist. In particular, six cases of endometrial cancer in didelphys uterus [10-13] and three cases of endometrial cancer in bicornuate uterus [14-16] have been described.

To the best of the present authors’ knowledge, this report represents the first case of endometrial cancer developing in a unicornuate uterus, whereas some cases of cervical and ovarian cancers arisen in unicornuate uterus have been described [8, 9].

This case illustrates the importance of existence of uterine abnormalities in the differential diagnosis when evaluating postmenopausal bleeding. The prevalence of these anomalies may be higher than reported due to the asymptomatic nature of some of these cases, as was noted in the present case, in a woman who had three pregnancies and was 69-years-old when the uterine anomaly was diagnosed secondary to the diagnosis of carcinoma. This emphasizes the importance of a careful physical examination, radiographic evaluation, and/or sonographic and MRI if required [18].

References

Endometrial cancer in unicornuate uterus: a case report


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Isolated brain metastasis from uterine cervical cancer: a case report and review of literature

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Summary

The metastasis of cervical cancer to the brain is usually associated with systemic involvement such as lungs, liver and bones, and isolated brain metastasis is very rare because the primary mechanism of spread is by hematogenous dissemination of tumor cells. Although a few cases of isolated brain metastasis from cervical cancer have been reported, isolated brain metastasis from neuroendocrine cell carcinoma, which is characterized with aggressive and early metastatic features, has never been reported. A 44-year-old woman with cervical cancer composed of large neuroendocrine cell carcinoma was diagnosed with isolated brain metastasis at eight months after the definitive treatment with surgery followed by adjuvant chemotherapy. Careful evaluation would be needed during follow-up for the patients with cervical cancer with aggressive histologic type.

Key words: Cervical cancer; Neuroendocrine carcinoma; Isolated metastasis; Brain.

Introduction

Brain metastases from primary cervical cancer are rare. According to previous reports, brain metastasis from cervical cancer is usually associated with systemic metastases or accompanied by local disease with or without systemic involvement [1-7]. Although a few cases of isolated brain metastasis have been reported in squamous cell carcinoma or adenocarcinoma of the cervix [1, 8, 9], isolated brain metastasis from neuroendocrine carcinoma, which is a highly aggressive and early metastatic cell type, has not yet been reported until now. This report presents a case of isolated brain metastasis from neuroendocrine carcinoma of the uterine cervix.

Case Report

A 44-year-old woman presented with vaginal bleeding which had begun two months prior. Pelvic examination revealed a four-cm exophytic cervical mass. A punch biopsy was taken and pathologic diagnosis was squamous cell carcinoma. On thorough staging workup including pelvic magnetic resonance imaging (MRI) and positron emission tomography-computerized tomography (PET-CT), the clinical stage was determined to be IIA2 and there were no metastatic lesion in distant organs. The patient underwent a radical hysterectomy and pelvic and para-aortic lymphadenectomy. Histologic examination revealed a mixed large-cell neuroendocrine cell carcinoma and poorly differentiated squamous cell carcinoma with lymphovascular space invasion (Figure 1). No metastases were found in the 82 resected lymph nodes. Three courses of adjuvant chemotherapy (5-FU 5,000 mg/m² + cisplatin 50 mg/m²) were given because of positive lymphovascular invasion and the aggressive histologic subtype. She was closely monitored with follow-up appointments every three months. Eight months after definitive treatment, the patient came to the present emergency center with complaints of headache, dizziness, and fine motor incoordination. Multiple metastatic brain tumors were found on brain MRI (Figure 2). There were no metastatic sites on pelvic examination, chest, abdomen and pelvis CT, or PET-CT. She was diagnosed with an isolated brain metastasis from uterine cervical cancer. She was given whole brain radiation therapy of 3,000 cGy in 16 fractions. After treatment, her neurologic symptoms were resolved and she has survived with no evidence of disease for seven months.

Discussion

The mechanism of metastasis from the cervical cancer to the brain is by hematogenous spreading of tumor cells involving through liver, lung, and bones [4]. Therefore, isolated brain metastasis without other systemic disease is extremely rare. Table 1 shows review of published reports about brain metastases from cervical cancer. Of 42 cases, only four cases showed isolated brain metastasis. Histologic types of four cases were squamous cell carcinoma in two cases [1, 9] and adenocarcinoma in other two cases [1, 8]. Thus, to the authors’ knowledge, the present case is the first report of isolated brain metastasis from neuroendocrine cell carcinoma which was mixed with squamous cell carcinoma of the cervix. Neuroendocrine carcinoma is an aggressive histologic type and usually showed concomitant systemic involvements at the time of brain metastasis [4, 5]. Once metastasis to the brain occurs, prognosis is very poor, with most patients surviving less than six months in spite of therapy [10]. However, early detection and treatment may improve the quality of life in these patients. Neuromaging should be considered in patients with cervical
Table 1. — A review of literatures on brain metastases from uterine cervical cancer.

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Stage(n)</th>
<th>Histology(n)</th>
<th>Initial treatment</th>
<th>Interval</th>
<th>n</th>
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<th>Histology(n)</th>
<th>PD or local recurrence</th>
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<td>AC(1)</td>
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<td>Agrawal et al. [10]</td>
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n, number of cases; Interval, interval from initial diagnosis of cervical cancer to diagnosis of brain metastasis(months); PD, persistent disease after initial treatment; AC, adenocarcinoma; SCC, squamous cell carcinoma; AS, adenosquamous cell carcinoma; NEC, neuroendocrine carcinoma; Un, undifferentiated type; Unk, unknown; S, surgery; RT, radiotherapy; CCRT, concurrent chemoradiotherapy; L, lung; Li, liver; B, bone; O, other sites (including breast, skin, pancreas, and distant subclavian, cervical, mediastinal, and thoracic lymph nodes)

'cases of uncontrolled primary disease including peritoneal metastasis, pelvic cavity recurrence, and para-aortic lymph node metastasis.
cancer developing neurologic symptoms, even if PET-CT was negative because PET-CT would not be useful for detecting metastasis to brain. In summary, if the histologic type of primary cervical cancer is unusual and aggressive, as neuroendocrine carcinoma, more careful evaluation should be needed during follow up.

Conclusion

Isolated brain metastasis from cervical cancer is extremely rare because metastasis usually occurs by hematogenous spreading of tumor cells with other systemic involvement. Neuroendocrine carcinoma shows aggressive features associated with concomitant systemic involvements at the time of brain metastasis. However, clinicians should be aware of a possibility of isolated brain metastasis in case of complaining neurologic signs or symptoms by patients, although there would be no evidence of pelvic or systemic disease. Early detection and treatment may improve the quality of life in these patients.

References


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Granular cells tumor of the vulva: an exceptional entity

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Summary

Introduction: Granular cells tumor (GCT) is a rare tumor that develops on skin and soft tissues. Only 1-2% of these tumors present malignant behavior. Material and methods: The authors report three cases of GCT and review the management of these tumors. Case report: The first case is a 73-year-old woman who was diagnosed with an atypical GCT. She was treated with local excision and then presented a local recurrence 21 months after the surgery. The other two cases are 60- and 58-year-old women diagnosed with a benign GCT. They were treated with single excision; both patients underwent second surgery due to margin affection. Conclusions: Management of GCT is not clear nowadays. The careful selection of patients with poor prognostic factors is very important. Follow-up for early diagnosis of local recurrence and metastases of these tumors is of utmost importance.

Key words: Vulvar cancer; Granular cells tumor; S-100 protein.

Introduction

Granular cells tumor (GCT) is a rare tumor develop on skin and soft tissues cells. Its histogenesis is disputed, although the presence of S-100 in the immunohistochemical analysis of the specimen [1,2], suggests that it may derive from Schwann cells.

The tumor typically appears as a solitary and asymptomatic nodule, usually located in the skin of the trunk and the mucosa of laryngopharynx, especially in the tongue [3]. Rarely it locates in the vulva, in which cases the labia majora is the most frequent situation [1].

Malignant behavior has been reported in 1-2% of these tumors [1, 4], although local recurrences are described in up to 25% of the cases [5]. Late diagnosis of malignant forms is common, with primary and multicentric or metastatic lesions in organs such as lung, liver or bone [6,7].

The authors report three cases of vulvar GCTs, only one of which presented atypias.

Case Report

Case 1
A 73-year-old female presented with a solid and asymptomatic mass in the pubis, observed during a routine gynecologic revision. Three months earlier a three-cm nodule was observed in the same location by other specialist, suspicious of lipoma with no pathologic diagnosis.

The lesion was adhered to the skin, but mobile in deep layers, without lymphadenopathies or other vulvar lesions. A biopsy of the mass confirmed the diagnosis of granular cells tumor, without evidence of atypia at that moment. The patient underwent excision of the tumor. The pathological report informed of the presence of polygonal cells with eosinophilic cytoplasm, cells groups with nuclear atypia and rare focal mitosis, forming cords separated by connective tissue tracts. Immunohistochemical study presented diffuse expression of S-100.

Follow-up revisions were performed every six months with vulvar and vaginal cytologies. Twenty-one months after surgery, the patient presented a three-cm sized nodule, showing firm consistency and skin retraction in the same location as primary tumor. Again, the patient underwent total excision of the mass with free margins. No adjuvant treatment was administered. After 60 months of follow-up, the patient remains asymptomatic and with no evidence of tumor.

Case 2
A 60-year-old woman consulted for a two year painless vulvar mass, with occasional pruritus. The exploration showed a 0.5 cm tumor in the right labia majora. The lesion was extirpated and pathological exam did not show malignancy signs, but revealed tumor infiltrated surgical margins. A second surgery was performed in which surgical margins were extended. After one year of follow-up the patient remains asymptomatic.

Case 3
A 58-year-old woman presented an asymptomatic small vulvar mass. The three-mm nodule was located in right labia majora. Surgery was declined at first visit because of the lack of signs of malignancy. One year later the patient presented with a painful five-mm tumor at the same location. The nodule was extirpated with local anesthesia and the pathological report informed of a granular cells tumor with surgical margins affected. A second surgery was performed to leave free surgical margins. Patient remains asymptomatic after only one month follow-up.

Discussion

The diagnosis of gynecologic GCT is difficult due to the fact that they frequently appear as an asymptomatic mass and rarely locate in the vulva. However, occasionally these tumors present symptoms like pruritus or pain [1].

Revised manuscript accepted for publication April 3, 2014

XXXVI, n. 5, 2015
doi: 10.12892/ejgo2665.2015
clinical history and physical examination while biopsying all the suspicious lesions found, are essential for the cor-
drect diagnosis [1].

Moreover the histological diagnosis of malignant behav-
ior is not clear, although an increased mitotic activity, pres-
ence of tumor necrosis, Ki67 > 10%, and positive p53 might predict malignancy [4]. The most predictive criteria of malignant behavior could be the presence of atypical mi-
toses [8].

Clinically, aggressive behavior may be suspected when 
the patient presents tumor size greater than four cm, local recurrence, rapid tumor growth or invasion of adjacent tis-

Pathological diagnosis of these tumors is difficult due to 
its rarity. Thus, experienced pathologists are needed [3]. 
Microscopically the tumor is characterized for the presence 
of eosinophilic cells, with ovoid or polygonal morphology 
and positive S-100 protein [9].

Among all GCTs, just 1-2% present malignant behavior, therefore an appropriate follow-up is mandatory. There are reported two benign cases with an unusual clinical course [1] and a case of a young woman that developed an invasive GCT in the vulva after a previous benign GCT [10].

Lymphatic dissemination of the tumor is commonly ob-
served in case of malignancy. Some authors have re-
ported up to 70% of regional node positivity in these 
cases [6, 8]. Therefore correct staging of the disease is 
needed by sonography (lymphatic and hepatic metas-
tases), chest X-ray (pulmonary and osteoblastic metas-
tases), and thorax-abdomino-pelvic CT-scan (hepatic, 
pulmonary, and osteoblastic metastases). However, it is 
unclear what kind of patients would benefit from each 
technique [6].

On the other hand, treatment should include wide ex-
cision, with free margins, in every case [1, 6, 8]. In case of malignancy, a bilateral inguinal lymphadenectomy must also be performed [6, 8]. The role of radiation ther-

apy or chemotherapy is not clear, although GCTs are con-
sidered radio-resistant. Results after chemotherapy are 
poor [8]. In the present first case, the authors performed 
a total excision of the tumor without lymphadenectomy, 
since just diffused pattern of cells with atypical nuclear 
and focal mitosis were found. Moreover, they did not find 
yany evidence of inguinal involvement or other vulvar le-
sions. However, they found a local recurrence, widely ex-
cised again, which seemed a controversial option but 
valid according to the literature. In the second and third 
cases, because of the absence of malignant histological

signs, only excision of the lesion with free margins was 
performed.

The follow-up of these patients should be based on sev-
eral risk factors as those defined by Fanburg-Smith et al. 
[4]. Furthermore, the possibility of aggressive behavior, 
particularly in recurrent or multifocal GCTs should be con-
sidered [1, 10]. The three patients treated by the authors 
present no evidence of disease after surgery, although fol-

low-up revisions are underway.

In conclusion, there is a need of protocols for the diag-

nosis, treatment, and follow-up of GCTs. Although there is 
a lack of evidence, publications suggest that in the cases of 
malignant histotype, a wide excision of the lesion with in-
guinal lymphadenectomy must be performed. Adjuvant 
treatments have not shown any benefit.

References


[9] Steffansson K, Wollmann RL.: “S100 protein in granular cell tu-


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Primary malignant melanoma of the cervix: a case report

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1 Department of Obstetrics and Gynecology, 2 Department of Pathology, Okmeydani Training and Research Hospital, Istanbul (Turkey)

Summary
Primary malignant melanomas of the uterine cervix are extremely rare tumors. Diagnosis is confirmed by immunohistochemical methods and by exclusion of other primary sites of melanoma. The treatment of this condition is not yet standardized and the overall prognosis of these patients is very poor. The authors report a case of primary malignant melanoma of the uterine cervix.

Key words: Primary; Malignant melanoma; Cervix.

Introduction
Malignant melanoma, which is relatively common neoplasm of the skin and mucous membranes, constitutes 1% of all cancers [1]. It is rare neoplasm of the lower genital tract, being approximately 3% to 7% of all cases. The majority these tumors arise on the vulva and then on the vagina. Historically all malignant melanomas of the cervix were thought to be metastatic, however, after the discovery of melanocytes in the cervix in 1959, it was recognized that primary malignant melanoma of the cervix exists as a separate entity [2, 3]. The clinical history includes vaginal bleeding, vaginal discharge, and postcoital bleeding. Diagnosis is confirmed by histological examination using special staining and immunohistochemical study [4]. The authors report a case with primary malignant melanoma of the uterine cervix.

Case Report
A 66-year-old, multiparous woman was admitted to the present clinic with the complaint of postmenopausal bleeding for two months. Her gynecological examination revealed a dark, fragile, polypoid mass with increased vascularity measuring 4 x 2 cm on the anterior lip of the cervix (Figure 1). There were no abnormal skin, vulvar, and vaginal lesions. The uterus was normal in size, mobile, and there was no adnexal mass. Biopsy of the lesion was performed. The histopathologic diagnosis was malignant melanoma of the cervix (Figure 2). Immunohistochemical stains revealed strong reactivity for vimentin, S-100 protein, Melan-A, and HMB-45 (Figure 3). There was no sign of immunoreactivity for epithelial markers, like cytokeratin and EMA.

Chest X-ray, pelvic, and abdominal MRI were performed preoperatively and no metastasis was detected. The patient underwent radical hysterectomy, bilateral salpingo-oophorectomy, and bilateral pelvic lymphadenectomy. The patient was referred to department of oncology in the postoperative period and radiotherapy was planned.

Discussion
Primary melanoma of the cervix is extremely rare. Cervix is usually involved secondarily as a result of local extension from vagina or vulva or as a result of hematogenous dissemination from a primary melanoma located elsewhere in the body [5]. Etiology of cervical melanoma is either migration from the neural crest or melanocytic differentiation from preexisting local dendritic cells [6].

Cervical malignant melanoma usually remains asymptomatic until it ulcerates and becomes infected, causing vaginal bleeding or discharge [7]. Diagnosis is easily made by simple inspection with a speculum and confirmed by histological examination and by immunohistochemical staining with S100 and HMB45 [8]. Norris and Taylor have suggested the following diagnostic criteria:
1) the presence of melanin in the normal cervical epithelium; 2) the absence of melanoma elsewhere in the body; 3) the demonstration of junctional change in the cervix; 4) the metastases according to the pattern of cervical carcinoma [9].

There is no consensus on optional management strategy after diagnosis. The most preferred surgical method appears to be radical hysterectomy with or without pelvic lymphadenectomy. There is lack of evidence on the efficacy of postoperative radiotherapy or chemotherapy. Postoperative chemotherapy is another plausible option in these patients since many develop metastatic disease. Radiotherapy can be useful in the palliation of an inoperable patient or as an adjuvant therapy [10].
As a result, primary cervical melanoma should be kept in mind for differential diagnosis of cervical neoplasms, and besides histological examination, immunohistochemical study is useful for the definitive diagnosis. Whichever modality is used for treatment, prognosis for primary malignant melanoma of the uterine cervix is poor and unpredictable.

References


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Introduction
Aggressive angiomyxoma (AAM) is an uncommon benign mesenchymal tumor preferentially arising from the connective tissue of the pelvic and perineum of adults. The tumor was first described in 1983 [1]. It has been found to affect women in more than 90% of cases, usually in the second to fourth decade. Pre-operative diagnosis is difficult, because it is often mistaken clinically for other benign conditions, such as Bartholin's cyst, leiomyoma, fibroepithelial polyp, lipoma, abscess, or hernia [2, 3]. Because of the high rate of local recurrence, preoperative diagnosis, was followed with no recurrence. CT and MR imaging demonstrated a well-defined mass, which displaced adjacent structures. Attenuation of the mass was less than that of muscle on unenhanced CT, and a swirling or layering internal architecture was found using both enhanced CT and T1-weighted MR imaging. In one patient, a layering internal architecture was seen on unenhanced CT images. MRI demonstrated the relation of the tumor to the pelvic floor better than CT. The authors concluded that both CT and MRI show the characteristic imaging pattern and trans-diaphragmatic extent of these tumors, and the diagnosis should be considered in any young woman presenting with a well-defined mass arising from the pelvis or perineum.

Case Report

Case 1
A 34-year-old woman presented with an eight-year history of increasing intermittent abdominal pain after a cesarean section. Ultrasonography revealed an asymmetrical mass. At surgery, a 12×3×3 cm mass was identified to the left of the rectum and the rectovaginal septum, closely adherent to the rectum and vagina. The tumor was completely removed and reported as AAM.

Three years later, the patient presented with a recurrent tumor. CT showed an 11×5×7 cm cystic mass with a characteristic swirled internal architecture in the left pelvis. During surgery, a soft mass was identified to the left of the rectum and the rectovaginal septum, adherent to the urinary bladder, vagina, and vesical pelvic fascia. The mass was removed as much as possible. The woman has since been on regular follow-up for five years with no recurrence of the disease.

Case 2
A 39-year-old woman presented with a pelvic mass revealed by pelvic MRI during a routine medical examination. The lesion was found lying in the left ischio-rectal space (Figure 1A, B), although the patient had reported no symptoms. Then the patient underwent excision of the lesion via the transabdominal approach. During surgery, the tumor had a smooth surface and was found to be elastic and soft. The resected tumor was 15×8×3.5 cm, with histological examination confirming AAM.

After three months, a follow-up MRI demonstrated a 2.5×6 cm recurrent tumor in the same space as the original one (Figure 1C). Since the patient was unwilling to have another operation, she received an angiographic embolization. Subsequent imaging confirmed a significantly reduced tumor (Figure 1D). As the patient was asymptomatic, conservative management was undertaken.

Case 3
A 31-year-old woman presented with a vaginal wall cyst and had a vaginal wall cystectomy in another hospital in 1999. Four years later, she presented to the present institute with a recurrence. Physical examination revealed a 5×4 cm cystic mass in the anterior vaginal wall under abdominal pressure. The patient received an anterior vaginal tumor excision, and an AAM diagnosis was made.

After another six years, the woman presented to the present hospital again with a complaint of discomfort and swelling in the

Summary
The purpose of this study was to evaluate the value of CT and MRI in aggressive angiomyxoma (AAM) of the pelvis. A series of four cases from three institutions are reviewed. Among the four cases, three were initially misdiagnosed, and local recurrence necessitated reoperation or angiographic embolization. The fourth case, with accurate preoperative diagnosis, was followed with no recurrence. CT and MR imaging demonstrated a well-defined mass, which displaced adjacent structures. Attenuation of the mass was less than that of muscle on unenhanced CT, and a swirling or layering internal architecture was found using both enhanced CT and T1-weighted MR imaging. In one patient, a layering internal architecture was seen on unenhanced CT images. MRI demonstrated the relation of the tumor to the pelvic floor better than CT. The authors concluded that both CT and MRI show the characteristic imaging pattern and trans-diaphragmatic extent of these tumors, and the diagnosis should be considered in any young woman presenting with a well-defined mass arising from the pelvis or perineum.

Key words: Aggressive angiomyxoma; Pelvic organs; CT; MRI.
lower abdominal. On examination, a soft cyst measuring 10×10 cm was palpated in front of the uterus. Ultrasonography revealed an asymmetrical mass. CT showed a 10×16×17 cm mass traversing the pelvic diaphragm.

The patient underwent abdomino-perineal excision of the vulval and retroperitoneal mass. With an attempt to enucleate the mass, it was found that part of the mass was in the vagina with no obvious gap between the mass and the bladder. Subsequently, a portion of the bladder wall and vagina were resected, and as much of the mass as possible was removed. Histopathology was reported as AAM. Unfortunately, the patient was lost to follow-up because she never returned for a check-up.

Case 4

A 51-year old woman presented with a pelvic mass, detected during a routine medical examination. Color ultrasonography revealed a hypoechoic mass in the right pelvis. CT showed an intrapelvic mass above the pelvic diaphragm. Unenhanced CT
showed a hypo-attenuating mass with a layered internal architecture (Figure 2). Intravenous contrast material enhanced the strands within the tumor. According to the distinctive imaging appearances, a diagnosis of AAM was suspected preoperatively.

At surgery, the mass was located to the right of the rectum and the rectovaginal septum, closely adherent to the rectum and pelvic floor. Wide excision of the tumor was done and reported as AAM. The patient has been followed for eight months and is free from recurrence of the disease.

**Discussion**

AAM of the perineum or pelvis is a rare condition which predominately occurs in women of reproductive age, exhibiting a peak incidence in the fourth decade of life [4]. AAM typically arises mainly from the connective tissues of the perineum or lower pelvis, rather than directly from the pelvic viscera [5]. Owing to its slow
growth pattern, patients are often asymptomatic, and the tumors are often discovered incidentally during routine pelvic check-up or with radiographic imaging [4]. Although it is histopathologically categorized into benign neoplasm, the tumor is still called aggressive because of its high rate of local recurrence, where it can even invade the bladder, bowel or pelvic bone. AAM is generally a locally aggressive tumor, as distant metastasis has only been reported in three cases [2, 6]. Histopathology is definitely the gold standard for diagnosis of AAM. On gross examination, AAM is unencapsulated. It is soft to rubbery, poorly circumscribed, and may have finger-like projections that extend into neighboring tissues [7]. The histological appearance of AAM is characterized by a mixture of stellate or spindle cells within a strong myxoid background of collagen fibers and small vessels with thick walls [8]. Mitotic figures are unusual but are not atypical if present [2]. No specific immunohistochemical marker has been found for AAM. Although most AAMs express estrogen receptor, progesterone receptor, desmin, vimentin, and smooth muscle actin, and is invariably negative for S-100, they are usually of little assistance in distinguishing AAM from its diagnostic mimics [9].

Ultrasound imaging usually reveals a hypoechoic soft tissue mass that may even appear cystic [10]. On sonography, it is homogeneously hypoechoic and well demarcated with multiple thin, echogenic internal septa [11]. There are several distinctive radiological features of AAM on CT and MRI that have been identified in the literature [12]. On cross-sectional imaging, AAM typically appears as a well-defined mass, displacing rather than invading adjacent organs with preservation of fat planes as shown in the present cases. The tumor usually traverses the pelvic diaphragm. CT appearances are variable, including a well-defined homogenous mass which is hypodense relative to muscle, a hypo-attenuating solid mass with a contrasting swirling internal pattern or a predominantly cystic appearing mass with a solid component. In case 4 of the present series, the internal layering pattern was seen clearly in enhanced CT. MRI is the best radiological modality for diagnosis. Characteristic appearances on MRI include iso-intensity relative to muscle on T1-weighted images and hyper-intensity on T2-weighted images, with avid and heterogenous contrast enhancement after administration of intravenous contrast, highlighting a distinct swirling or layering internal pattern. The hypo-density on CT and the hyper-intensity on T2-weighted MRI are likely due to the tumor’s abundant loose myxoid matrix and high water content, while the avid enhancement reflects its high vascularity. The appearance of swirling or layering strands in the tumor is a characteristic diagnostic feature which is found in about 83% of the patients [13]. With CT, the swirled appearance is usually evident only with enhanced scans, but a layering internal architecture was seen on unenhanced CT images in the fourth patient. These are of slightly lower intensity than the remainder of the tumors in T2-weighted-MRIs and are also obvious after enhancement with gadolinium. The cause of this appearance is not very clear but may relate to the fibrovascular stroma that develop in tumors that are stretched as they protrude through the pelvic space or diaphragm (Figure 2). Aside from accurately diagnosing the tumor, MRI can be more helpful than CT in planning the surgical approach by defining the relation of the tumor with the anal sphincter, urethra, bladder and pelvic side-wall, and in clinical follow-up of patients with a recurrent tumor.

The first line of therapy for AAM is complete surgical excision with tumor-free margin, although achieving resection of tumor-free margins is difficult, owing to the infiltrative feature of the tumor and the absence of a definite capsule [2]. However, because of the rarity of AAM, there are no national or specialty guidelines for its management [14]. The high recurrence rate may be due to incomplete surgical excision, which may in turn be attributed to an incorrect initial diagnosis. In the present report, the first three patients were misdiagnosed and as a consequence, had recurrence within three months to six years. The fourth patient, in whom the preoperative diagnosis was correct, underwent a complete excision of the tumor without recurrence. A review of more than 100 cases, however, refuted this by showing that patients with clear resection margins were just as prone to developing recurrences [15], and an incomplete removal is supported by recent literature in vulvar AAM [16].

Other treatment modalities for AAM include chemotherapy, radiation therapy, angiographic embolization, chemoembolization, and hormonal therapy. These are sometimes used as adjuvant therapies for a residual tumor, in the treatment of recurrence, or as a help in succeeding resection by shrinking the neoplasm, and making it easier to distinguish from surrounding normal tissues [3, 17]. In case 2, subsequent imaging showed that the tumor gradually shrunk significantly. However, chemotherapy and radiotherapy are usually thought to be less suitable options because of their low mitotic activity, and angiographic embolization is also controversial [18]. Furthermore, an alternative blood supply may cause a recurrence after the initial response to embolization, necessitating a longer follow-up observation period. AAMs in women are reportedly hormone-responsive tumors. Hormonal therapy is found to have a significant role in the treatment of extensive or recurrent AAM that is estrogen receptor positive. However, hormonal therapy also have long-term adverse effects including menopausal symptoms and bone loss [14].

In summary, AAM is a rare tumor that affects the pelvis or perineum. The tumor has a distinctive imaging appearance of swirled or layered internal architectures after enhancement on CT and MRI. Surgery is potentially curative with recurrence usually due to incomplete resection. The multiplanar images of CT and MRI can reveal extension of
the tumor and are thus valuable in determining the optimal surgical approach.

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Introduction

Due to the advancement at imaging technologies and health care services ovarian tumors that reach massive sizes are rarely seen. Many life-threatening complications such as; severe hypotension, increased venous return and cardiac failure, respiratory problems and intestinal distension can be encountered during the management of patients with massive ovarian tumors [1,2]. Most complications arise during the operations or after removal of the mass and are associated with rapid changes in the body circulation and pulmonary edema.

We present a giant mucinous borderline ovarian tumor weighing 42.5 kg and managed via mid-line laparotomy incision followed by controlled cyst aspiration and total excision of the ovary. We also discussed the problems encountered during the intraoperative and the postoperative period.

Case Report

A 61-year-old nulliparous, postmenopausal patient was admitted to the present emergency department with complaints of abdominal swelling and dyspnea. The patient’s body weight was 113 kg, height 145 cm, abdominal circumference 163 cm, and BMI 53.7 kg/m² (Figure 1). Abdominal ultrasonography showed a thick-walled multicystic large mass with thick septation, with an unidentified origin. Intra-abdominal organs could not optimally be evaluated due to the presence of the large mass. The abdominal computed tomography of the patient showed a cystic mass of 47x43x30 cm in size, with minimal solid areas inside, and was suspected to originate from the ovaries (Figure 2). The blood tests were: Hb: 8.7 gr/dl, Hct: 26%, serum CA 125: 106 IU/ml and CA 19.9: 2,021 IU/ml. Other tumor markers and biochemical tests were at normal range.

The patient underwent laparotomy with a ten-cm long mid-line incision. A compress was placed between the cyst wall and the abdominal wall to prevent spillage of cyst fluid into the abdomen. The cysts tension was reduced after suctioning about seven liters of cyst contents by using a Verres suction needle connected to the aspirator. The needle was then removed and an incision of 0.5 cm in diameter was made at the same place. An aspirator tip was placed through that incision and about 21 more liters of brown, clear, dark, and odourless cyst fluid was aspirated gradually (500 ml/min). When the authors were aspirating the cyst fluid, controlled reperfusion was performed by the anesthesiology team so persistent hypotension did not occur during the operation. The exploration of the lower abdomen showed that the mass was originating from right ovary. The authors were unable to reach the back of the mass due to adhesions, so the incision was extended to the left side of the abdomen and the cyst was aspirated another 6 liters. The aspirated fluid was sent to the pathology department for further analysis. After the cyst was aspirated and the tension was reduced, the cyst wall was elevated with two clamps to prevent leakage of the cyst fluid. An incision was made in the cyst wall and the aspirator tip was placed through that incision and about 10 more liters of brown, clear, dark and odourless cyst fluid was aspirated. The cyst wall was then sutured with 2/0 Vicryl sutures. The thick septation was then resected. The appendix was then dissected from the cyst wall and the cyst wall was removed to reach the back side of the mass. The mass was then dissected from the peritoneum and the ureters. The vessels in the mass were ligated and the ovary was removed. The total weight of the mass was calculated to be 42.5 kg. Postoperatively, the patient was discharged on her postoperative seventh day.

Key words: CA 19.9 antigen; Mucinous cystadenoma; Ovarian neoplasm.
H. Güraslan, L. Yaşar, M. Ekin, C. Kaya, H. Cengiz, M. Gönenç

sion was extended to above the abdomen. The cyst was not further drained because it was thought that it would facilitate the cyst dissection. A 0.5-cm wide cut that was made to the cyst wall was sutured. The cyst was completely released with a sharp and blunt dissection and was completely removed (Figure 3). The specimen was send to frozen section. Although the examination of the frozen section had no macroscopic or microscopic findings suggesting malignancy; intraperitoneal staging with total abdominal hysterectomy, bilateral salpingo-oophorectomy, and infracolic omentectomy was performed as the final surgery because of risk of misdiagnosis at frozen section. Intraoperative total blood loss was 800 cc, while the urine output was 2,600 cc. The total weight of the aspirated cyst content plus excised mass was calculated to be 42.5 kg. Reconstruction of the abdominal wall was not performed for the residual abdominal skin after the excision of the mass. On the postoperative first day, the patient’s bowel sounds were detected to be normoactive, so the nasogastric tube was removed and the patient was started on a liquid diet. The patient was given an intermittent supply of oxygen due to difficulties in deep breathing and coughing. Moreover, she was fitted with an elastic abdominal corset, and started breathing exercises. On the fifth postoperative day those findings resolved completely. The patient did not have any problems and was discharged on the seventh postoperative day. The evaluation on the 15th postoperative day showed that sagging of the abdominal skin was reduced, and serum CA 19.9 levels were measured to be 60 IU/ml. Final pathology indicated the presence of an intestinal type borderline mucinous tumor in the right ovary and there was no evidence of intraperitoneal spread.

Discussion

There is lack of information about management of giant ovarian tumors because of their rare incidence and experiences are limited with case series. Dotters et al. [3] stated that only 20 cases with tumors exceeding 23 kg have been reported during the period of 1946-1988.

The literature on the management of massive ovarian tumors indicated that serious and even fatal complications may occur up to 30%. According to reports from Symmonds et al. [1] in 11 cases before 1905, a total of six patients died; one before the operation, one during the operation, and four in the postoperative period. The majority of publications in the literature on how to remove large ovarian tumors recommend showing maximum effort to remove the mass intact in order to prevent the spread of the malignant tumor [3-5]. However, most of the complications during treatment are associated with sudden changes in abdominal pressure during excision of the masses. Also general condition of many of these patients are not suitable for long and invasive surgeries. Kincey et al. had removed a pelvic mass weigh-
A successful management of a giant mucinous ovarian tumor with intraoperative controlled fluid aspiration

ing 107 kg via initial aspiration technic in lateral decubitus position and turning to supine position for removing the mass and adding abdominoplasty, subsequently. They reported no complications and the patient was satisfied with her postoperative appearance [6]. In the present case the authors also aspirated the cyst but they performed aspiration, only a slight head up supine position. They believe that, changing the position during the operation may cause a sudden decrease in blood pressure due to a decrease in caval pressure. They did not add any reconstructive surgery for abdominal sagging. It was not necessary to repositioning the patient during the operation and the patient is also satisfied with her postoperative appearance. The authors may speculate that, the size of the masses are the major determiners of the operation techniques.

One of the challenges for these patients is the structure of the abdominal and diaphragm muscles. They are thin and weakened due to constant stretching, and with the removal of the mass serious insufficiency in respiratory function may occur [6]. Deep breathing and coughing cannot be done effective. Pulmonary edema may develop immediately as well as during the later period [7]. Another difficulty is the sudden drop of abdominal pressure that may cause severe hypotension during and after the operation [6]. To reduce these risks; tumours had been removed, without circulatory or respiratory complications, by the gradual drainage of 44 - 48.4 L of cyst fluid over four or five days before definitive surgeries in the literature [7]. However, this slow technique limits patients activity and prolonges hospital admission before removal of the tumour. For these reasons the present authors decided to drain the cyst in a controlled manner during the operation. They did not experience any problems during the cyst aspiration, except for a slight decrease in blood pressure and this was managed by anesthesiologist by intravenous fluid replacement. The ovarian cyst weighing 42.5 kg was removed via controlled aspiration technic slowly without spreading into the peritoneal cavity. With this method, surgical morbidity, intraoperative, and postoperative complications were decreased and no serious complications were observed.

In the postoperative period, a fatal intestinal distension may be observed due to sudden decompression and formation of the gas diffusion in the bowel [2]. The present authors applied an abdominal bandage to their patient at the first postoperative day to increase abdominal pressure and observed that the patient was able to cough and discard phlegm more easily.

Although removal of excess abdominal wall and various reconstructive techniques were proposed in the literature [2, 6], the present authors did not performed abdominoplasty because of recovery potential of the abdominal muscles and skin in expanding conditions such as pregnancy and obesity. In addition, they also observed that sagging of the abdominal wall was greatly reduced within 15 days.

Conclusions

Giant ovarian cysts can be managed with controlled aspiration of the cyst fluid in supine position followed by staging procedure via midline laparotomy incision. The present authors suggest that this procedure can be performed safely without increasing the risk of spreading malignancy and can prevent sudden decreases of intraoperative abdominal pressure.

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Carcinosarcoma of the fallopian tube with disappearance of carcinoma cells by neoadjuvant chemotherapy: case study

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Summary
The authors report a case of carcinosarcoma (CS) of the fimbria of the fallopian tube in which carcinoma cells disappeared with neoadjuvant chemotherapy (NAC). A 74-year-old woman visited the present hospital with a large pelvic mass and pleural effusion. A magnetic resonance image of the tumor was highly suggestive of ovarian carcinoma. Due to the presence of both serous adenocarcinoma cells in pleural effusion and pulmonary thrombosis, the patient was given NAC consisting of carboplatin plus paclitaxel (TC) and anti-coagulant therapy with warfarin potassium. With six courses of NAC, the pleural effusion and pulmonary thrombosis disappeared, and the tumor decreased 36.2% in greatest diameter. Maximum debulking surgery was then performed. The tumor was found to be located in the fimbria of the right fallopian tube. Hysterectomy and bilateral salpingo-oophorectomy were performed, and histologic examination revealed chondrosarcoma with the presence of necrotic epithelial cells. The necrotic areas were interspersed with papillary structures, and immunohistochemical study showed positivity for CK7 and negativity for CK20, p53, and estrogen receptor (ER), indicating serous adenocarcinoma. Thus, heterologous CS with disappearance of viable carcinoma cells by NAC was diagnosed. The patient was given adjuvant chemotherapy consisting of three courses of TC, and there has been no evidence of disease for 20 months. The authors’ experience in this case of gynecologic CS indicates that a serous adenocarcinomatous component of tubal CS can be well cured by TC-based NAC.

Key words: Carcinosarcoma; Fallopian tube; Neoadjuvant chemotherapy.

Introduction
Carcinosarcoma (CS) of the female genital tract, though uncommon, occurs most often in the uterus, followed by the ovary and fallopian tube [1]. Although several kinds of chemotherapy and operative procedures have been performed in patients with gynecological CS, survival rates are poor. Herein, the authors report a case of CS of the fallopian tube in which the carcinoma cells disappeared with neoadjuvant chemotherapy (NAC).

Case Report
Clinical course
A 74-year-old multiparous woman visited the present hospital complaining of lower abdominal pain. Thirty years prior, she had undergone gastrectomy with omentectomy for gastric cancer, and there had been no recurrence. Upon admission to this hospital, a large solid pelvic mass was detected by abdominal ultrasonography. Magnetic resonance imaging revealed a 17.1 cm solid tumor of variable signal intensity on T2-weighted images (Figure 1). On contrast-enhanced images, the tumor was of heterogeneous signal intensity, with the polypoid portion of the tumor showing intense enhancement. Computed tomography showed massive pleural effusion and revealed filling defects in both pulmonary arteries indicative of pulmonary thrombosis. Blood tests revealed serum CA125 elevation (3,470 U/ml), whereas CA19-9 and CEA levels were within normal ranges. Cytologic examination of effusion cell blocks showed atypical epithelioid cells with a high nuclear/cytoplasmic ratio. Immunohistochemical study of effusion cell blocks showed positivity for CK7 (Figure 2a). Accordingly, the tumor was diagnosed as an FIGO Stage IV ovarian serous adenocarcinoma.

Due to the pulmonary thrombosis, the patient was first given anti-coagulant therapy with heparin and warfarin. She then underwent NAC consisting of paclitaxel (175 mg/m² on day 1) and carboplatin (area under curve = 5 on day 1) at three-week intervals. Her serum CA125 level decreased to 340 U/ml after four courses of chemotherapy and to 183 U/ml after six courses. The pleural effusion disappeared, the tumor shrank from 17.1 cm to 10.9 cm (36.2%), and the result was classified as a partial response according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Maximum debulking surgery was performed after NAC. During surgery, the tumor was found to be located in the fimbria of the right fallopian tube, with no extension into the pelvic and upper abdominal cavities. Hysterectomy and bilateral salpingo-oophorectomy were performed, and the patient was given three courses of postoperative adjuvant chemotherapy consisting of TC. Now, 20 months after the initial chemotherapy, the patient remains well without recurrence.

Pathological findings
Cytologic examination of pleural effusion specimens revealed atypical epithelioid cells with a high nuclear/cytoplasmic ratio. Cells in the papillary structures were clear with vacuolated cytoplasm (Figure 2a). Immunohistochemical study of effusion cell blocks showed positivity for CK7 and negative for CK20 (Figure 2b), p53, and estrogen receptor (ER), and TTF-1. Thus, the atypical cells were identified as serous adenocarcinoma cells, and the ovary was thought to be the origin of the carcinoma.
Examination of the surgical specimens, including the uterus and bilateral adnexa, showed the tumor to be 15 x 10 x 10 cm in size and located in the fimbria of the right fallopian tube (Figure 3a). The tumor was a solid mass, markedly hemonecrotic, with fibrous and myxoid areas (Figure 3b). Histologically, chondromatous islands with atypical chondrocytes having bizarre or multiple nuclei were noted at many sites (Figures 4a and b), and this element was diagnosed as chondrosarcoma on the basis of the marked cellular atypia. Massive necrosis was observed in many areas of the tumor, and no viable carcinoma cells were found in the tumor tissue, even by examination of many histologic sections. However, in some areas, necrotic epithelioid cells and papillary structures were noted (Figures 5a and b). Immunohistochemically, the necrotic cells were positive for CK7 (Figure 5c) and negative for CK20 (Figure 5d), p53, and ER. Cells in the pleural effusion and the necrotic cells had the same immunoprofile, positivity for CK7 and negativity for CK20 in particular. Thus, the necrotic cells were thought to have derived from the serous adenocarcinoma, with necrosis resulting from the NAC. In addition, in many areas, fibrosis and inflammatory cell infiltration were noted.

**Discussion**

CS of the fallopian tube is rare, with fewer than 100 cases reported in the literature. The present authors previously
Carcinosarcoma of the fallopian tube with disappearance of carcinoma cells by neoadjuvant chemotherapy: case study

Figure 3. — Gross photograph of the carcinosarcoma in the right fallopian tube. (a) The bulky solid tumor originates from the fimbria of right fallopian tube. (b) Marked hemonecrosis with fibrous and myxoid areas is seen in cut sections of the tumor.

Figure 4. — Histologic images of the chondrosarcomatous element in the carcinosarcoma. (a) Chondromatous islands surround and intermingle with fibrous elements and necrotic areas. (b) High-power view of a chondromatous island. Atypical chondrocytes with multinucleated and bizarre nuclei are seen.

Figure 5. — Histological images of the necrotic carcinomatous element in the carcinosarcoma. (a) Necrotic atypical cells with epithelioid structure. HE stain. (b) Confirmation of the papillary formation of necrotic atypical cells by reticulum staining. (c) Necrotic atypical cells are positive for CK7. (d) Necrotic atypical cells are negative for CK20.
encountered two cases of CS of the fallopian tube [1], and, based on their previous experience, they diagnosed the mass described herein as that of a heterologous CS with disappearance of viable carcinoma cells by NAC. Moreover, in the present case, positivity for CK7 and negativity for CK20, which are key findings for a diagnosis of serous adenocarcinoma, were detected in the necrotic atypical cells. Immunohistochemical detection of necrotic tumor cells in cases of malignant diffuse large B-cell type lymphoma is similar. Carcinomas of the fallopian tube have the same immunoprofile as ovarian carcinomas because the two carcinomas are of the same origin. The characteristic findings of serous adenocarcinoma in both organs are positivity for CK7 and negativity for CK20 and ER. Therefore, in the present case, the authors considered the disappearing carcinosarcomatous element as serous adenocarcinoma.

In general, the treatment strategy for tubal carcinoma follows that of ovarian carcinoma. Primary debulking surgery is the cornerstone of treatment in patients with ovarian carcinoma. It is performed to establish the diagnosis, and the tumor reduction improves the response to chemotherapy. Recently, however, NAC followed by interval surgical debulking has been proposed for patients with established bulky disease [2]. Possible advantages of NAC include resolution of the pleural effusion and ascites, an increased rate of optimal residual disease, a need for less extensive surgery, reduced blood loss, decreased morbidity, shortened hospital stay, and improved quality of life [2]. Kuhn et al., in their nonrandomized phase II study of FIGO Stage III patients, compared three cycles of NAC followed by debulking surgery and three cycles of additional chemotherapy to conventional tumor debulking surgery followed by six cycles of adjuvant chemotherapy, and the resulting tumor resection rate (84%) and median survival (42 months) were significantly superior to those (63% and 23 months, respectively) in the conventionally treated group [3]. Vergote et al. compared primary debulking surgery performed for patients with advanced ovarian carcinoma during the period 1980-1988 vs. the period 1989-1997 (285 total patients). Patients treated during the latter period were surgically evaluated to receive primary NAC or primary debulking surgery. The resulting three-year crude survival rate was significantly higher in the latter period (26% vs. 42%) [4]. These results suggest that NAC is a good alternative to primary debulking surgery especially for patients with advanced-stage ovarian carcinoma and/or poor performance status. In the present case, the patient was given NAC because of the pulmonary thrombosis, and the neoadjuvant therapy resulted in a cure for the serous adenocarcinoma component of the CS.

To the present authors’ knowledge, there is no report regarding the optimal chemotherapy regimen for tubal CS, and most reports detailing therapy and outcomes in cases of ovarian CS describe small numbers of patients treated with various regimens over extended periods of time [5]. The recently published Gynecologic Oncology Group phase II study of single-agent cisplatin as initial chemotherapy for ovarian CS had 136 eligible patients enrolled between 1977 and 1996, underscoring the rarity of these tumors and the difficulty of adequate enrollment in prospective clinical trials [6]. The study showed an overall response rate of 20% with single-agent cisplatin, providing the first prospective evidence that platinum is active as an initial therapy for patients with ovarian CS [6]. Because of the high response rates in this series, agreement exists regarding cisplatin as the main component of first-line therapy, but the optimal combination regimen remains undetermined [6, 7]. Duska et al. reported that 16 of 26 patients who were treated with paclitaxel and platinum as first-line therapy achieved a high complete clinical response of 55% with a total response rate of 72% [8]. Leiser et al. reported 40% complete and 23% partial response in patients treated with platinum-paclitaxel combinations, which included particularly carboplatin-paclitaxel [9]. In view of the established role of the doublet carboplatin-paclitaxel chemotherapy in patients with epithelial ovarian carcinoma and of the lack of data regarding the best agent for use in combination with a platinum compound in patients with ovarian CS, the platinum-taxane combination is considered a reasonable and certainly better tolerated option [10]. In the case described herein, the authors selected paclitaxel-carboplatin combination chemotherapy because they initially suspected a serous adenocarcinoma of the ovary. With the tumor being a serous adenocarcinoma, albeit of the fallopian tube, the carcinosarcomatous element disappeared.

The main carcinosarcomatous elements of tubal and ovarian CSs are serous and endometrioid adenocarcinomas. The authors’ limited experience indicates that a serous carcinosarcomatous component of tubal CS can be cured by NAC with TC. The authors believe that further studies of NAC for gynecologic CS will lead to fully effective treatment strategies.

References

tion of gynecological carcinosarcoma and its influence on phenotypic diver-
delshausen B., et al.: “Neoadjuvant chemotherapy followed by tumor debulking prolongs survival for patients with poor prognosis in Interna-
tional Federation of Gynecology and Obstetrics Stage IIIC ovarian car-


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