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# Cell adhesion molecule profiles, proliferation activity and p53 expression in advanced epithelial ovarian cancer induced malignant ascites—Correlation of tissue microarray and cytology microarray

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ABSTRACT

*Introduction:* Peritoneal dissemination accompanied by ascites formation is common in epithelial ovarian cancer (EOC). Adhesion molecules are crucial in metastatic spread and the latter involves epithelial-mesenchymal transition (EMT). This study aimed at: (1) clarifying whether E-cadherin and β-catenin expression and proliferative activity in metastatic ovarian cancer are inter-related; (2) Identifying possible correlations between cell adhesion molecular expression profiles, the proliferative activity and p53 expression of tumor cells and tumor grade and stage; (3) testing the cytology microarray (CMA) technique in analyzing metastasis formation.

*Material and methods:* Both tumorous and ascitic samples from 27 EOC patients were examined by using tissue microarray (TMA) and cytology microarray (CMA), respectively. CMA blocks were constructed using cores from each cell block of the ascitic specimens. Expression of E-cadherin, β-catenin, Ki-67 and p53 was immunohistochemically detected both in TMA and CMA blocks.

*Results:* E-cadherin expression was higher in ascitic cells than in primary tumor cells ($p = .294$). β-catenin expression was significantly lower in ascitic cells than in primary tumor cells ($p = .006$). Expression of Ki-67 was lower and expression of p53 was higher in primary tumors than in ascitic cells, for p53 the difference was significant ($p = .001$). Both Ki-67 and p53 expression elevated significantly in high-grade primary tumor cells and in ascites cells ($p = .039$, and $p = .004$, respectively).

*Conclusion:* Epithelial-mesenchymal transition- mesenchymal-epithelial transition is suggested as the best descriptive term for our IHC observations which accompany increased proliferative activity of ascitic cells.

The CMA method is an adequate and reliable method for the analysis of ascitic tumor cells disseminating from ovarian malignancies.

## 1. Introduction

Ovarian cancer is one of the most frequent cause of females' cancer-related-deaths. Close to 239.000 new cases of ovarian cancer (OC) are diagnosed annually world-wide and this malignancy carries a higher mortality rate (approximately 152.000 each year) than all other female genital cancers combined [1]. Striking geographical differences in its occurrence are noted. OC ranked 5th among the most common lethal cancers in the US in 2014 [2], and its prevalence is known to be unusually high in Eastern- and Central Europe [3]. According to the American Cancer Society in 2017 approximately 22,440 women will be diagnosed with ovarian cancer, and 14,080 women will die of this disease [2]. Epithelial ovarian cancer (EOC) accounts for 90% of all malignant ovarian tumours. The overall prognosis is poor, rate of remission sets only 40–50% of all cases [4]. Ascites and peritoneal dissemination is a typical and prospective phenomenon is EOCs. Tumor cells detach from the primary neoplasms and easily survive whilst floating in ascites fluid and eventually anchor onto the visceral or parietal peritoneum [5]. As a result of the Darwinian selection of cancer cell subtypes, only those cells which are capable of avoiding detachment-induced anoikis will survive [6].

Role of β-catenin and E-cadherin is well known and studied

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**Table 1**
Primary antibodies, dilutions and antigen retrieval methods.

| antibody | clone | manufacturer | dilution | antigen retrieval | instrument |
|---|---|---|---|---|---|
| E-cadherin | NCH 38 | Dako | 1:100 | * | Bond™ Max autostainer |
| β-catenin | 14 | BioCare Medical | 1:200 | citrate buffer ph 6.0 for 12 min 120–125 °C | manual |
| Ki-67 | MIB-I | Dako | 1:200 | * | Bond™ Max autostainer |
| p53 | DO-7 | Dako | 1:200 | ** | Bond™ Max autostainer |

*Novocastra Bond Epitope Retrieval Solution 2, Leica, Code: AR9640.
**Novocastra Bond Epitope Retrieval Solution 1, Leica, Code: AR9961.

phenomenon in cell-cell adhesion. Transmembrane glycoprotein E-cadherin (120 kDa) coded by CDH1 gene on chromosome 16q22 plays important role in intercellular adhesions by Ca+ dependent extracellular domain to the surrounding cells cadherin molecules [7]. Intracellular domain forms complexes with α-catenin, β-catenin and γ-catenin to assure actins' connection to cytoskeleton [8,9]. β-catenin forms complexes in the cell membrane while binding E-cadherin. This complex is responsible for adherent junctions' formation called zonula adherens.

The main goals of the current study are listed as (1) To clarify whether there is any possible significance of altered expression of E-cadherin and β-catenin in ovarian cancer cell propagation in ascites fluid; (2) To correlate these alterations with changes of proliferation potential of the same tumor cells; (3) To identify possible correlations between cell adhesion molecular expression profiles, the proliferative activity and p53 expression of tumor cells and tumor grade and stage; (4) To test the applicability of cytology microarray (CMA) techniques in analyses of tumor progression and metastasis formation [10].

## 2. Materials and methods

The archives of the Department of Pathology at the University of Debrecen Clinical Centre were searched for cases of malignant epithelial ovarian tumors for which tissue samples of the primary tumor or that of a peritoneal deposit (omentum, parietal pelvic peritoneum, abdominal viscera) together with cell blocks of tumorous ascites samples from the same patient were both available. A total of 27 patients met the inclusion criteria. These EOC cases were collected covering the time period of 5 years (2009–2014).

### 2.1. Tissue microarray (TMA) preparation

All histological sections were reviewed by two pathologists (LT and BN) for confirmation of the initial diagnoses. Three individual tissue cores, each of 1 mm in diameter, from representative areas of either primary or metastatic tumor samples were used to construct TMA blocks using the TMA master (3DHistech Budapest, Hungary). Sections for HE and IHC staining were cut at 4 μm thickness and processed routinely.

### 2.2. Cell microarray (CMA) preparation

Ascites samples were handled as follows. 50 mL aliquots of ascites fluid were fixed in 10% buffered formalin for 12–24 h at 4 °C, followed by 10 min centrifugation at 2500 rpm. The sediment was mixed into 3% agar gel at 56 °C. After solidification at room temperature was complete, the cell-containing agar gel was transferred into routinely used tissue cassettes which allowed further fixation, dehydration and routine tissue processing. The samples were categorized as either low tumor cell density (LTCD) or high tumor cell density (HTCD) type. The LTCD type was defined as a sample which contained < 6 tumor cells/HPF (high power field: 40x objective; 0.5 mm field diameter), those which contained more than 6 tumor cells/HPF belonged to the HTCD group. Only the "high tumor cell density" samples (HTCD group N = 22) were used to obtain 2 mm thick cores (3 cores from each) which were adequate for CMA block construction using again the TMA master (3DHistech Budapest, Hungary). Since any given 2 mm core represented 16 HPF area a HTCD core, on average, included more than 100 tumor cells. The remaining 5 low cellularity samples (low tumour cell density (LTCD) group) were used for IHC analysis as conventional "whole-surface" sections.

### 2.3. Immunohistochemical reactions

4 μm sections were deparaffinated with xylol and 96% etanol, endogen peroxidase activity was blocked using 0,5% methanol containing hydrogen-peroxide for 30 min, washed with distillated water and stained for p53, Ki-67 and E-cadherin in Bond™ Max autostainer. Sections were pre-treated in Novocastra Bond Epitope Retrieval Solution 2 (Leica, Code: AR9640) for Ki-67, E-cadherin reaction or Novocastra Bond Epitope Retrieval Solution 1 (Leica, Code: AR9961) for p53 reaction. DS9800 Leica detection system was used for visualization. β-catenin reaction was performed manually: sections were deparaffinated in xylol, rehydrated with 96% ethanol, endogen peroxidase activity was blocked using 0.5% methanol containing hydrogen-peroxide for 10 min, sections were then washed with distillated water and pretreated in citrate buffer pH 6.0 for 12 min (120–125 °C). Sections were then incubated with the primary antibody for 60 min at room temperature, and for another 30 min with the secondary antibody: detection reagent of EnVision™ FLEX K8000 detection system (EnVision™/HRP). EnVision™ FLEX DAB+ was used for detection: mixture of 50 μL chromogen reagent (1–5% 3,3′-diaminobenzidine tetrahydrochloride) and 1 mL EnVision™ FLEX Substrate Buffer. After washing sections were counterstained with hematoxylin, dehydrated, and mounted for light microscopy observations. Table 1. summarizes details of immunohistochemical reactions.

IHC reactions were recorded and digitalized by Mirax slidescanner. Evaluation of slides were made on digitalized slides. LTCD groups slides were evaluated in usual analogue system.

### 2.4. IHC scoring

Nuclear and cell membrane decoration for β-catenin were assessed separately. For E-cadherin only membrane-localized decoration was valued as positive, while for p53 and Ki-67 only nuclear reactivity meant a positive score. In each positive case the actual percentage of positively decorated tumor cells was determined.

### 2.5. Statistical analysis

The IBM SPSS 24 software (specifically Mann-Whitney-Wilcoxon test) was utilized for comparative evaluation of the results which have been collected from primary tumors and ascites samples, and also Spearman correlations were calculated. Statistical significance was indicated by $p < 0.05$.

## 3. Results

The relevant clinical-pathological patient-information is presented in Table 2. The total number of cases included in the study was 27, out