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ORIGINAL ARTICLE

Expression of CDCP1 and ADAM12 in the ovarian cancer microenvironment

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Summary

Purpose: *The tumor microenvironment in ovarian cancer (OC) seems to play an important role, and besides tumor cells, biomarkers can derive from endothelial cells. We investigated CDCP1 and ADAM12 expression in relation with other clinical and pathological characteristics in OC patients.*

Methods: *We retrospectively evaluated patient files between 2006-2011. A histochemical score was developed to evaluate tumor staining, the microvessel density (MVD), and stromal expression patterns for both ADAM12 and CDCP1. A CD34 antibody was used to assess tumor MVD.*

Results: *102 patients were selected and 83% had FIGO stage III/IV. A high CDCP1 tumor score correlated significantly with a shorter overall survival (OS) (p<0.01). Cases with positive CDCP1 had an elevated CD34 MVD (p<0.01). An absent/low ADAM12 tumor score correlated with significantly improved OS (p<0.01). Mean CD34 MVD was higher in cases with positive ADAM12 MVD (p=0.012).*

Conclusions: *Our results indicate that both tumor markers are negative prognostic factors for overall survival and additional studies are required to validate their future potential.*

Key words: *ADAM12, CDCP1, ovarian cancer, prognosis, tumor microenvironment*

Introduction

OC is characterized by nonspecific symptoms with a protracted period before diagnosis that is usually made in advanced stage, with bulky intra-abdominal tumor dissemination [1], and despite advancements in the biology and treatment of OC survival rates have remained practically unchanged [2]. Evidence suggests that OC is a heterogeneous disease and might also include primary tumors originating in the distal part of the fallopian tube [3]. Even within the most common histological presentation, i.e. high grade serous OC, a series of distinct molecular profiles have been identified [4]. Furthermore, the tumor microenvironment seems to play an important role concerning tumor progression, and besides tumor cells, biomarkers can derive from the endothelial cells of the microvessels-surrounding network that have a distinct molecular expression [5,6].

CUB domain–containing protein 1 (CDCP1) is a 836-amino acids type I transmembrane protein [7] and its cytoplasmic domain has been found to interact with the Src family protein kinase C δ, indicating a possible pro-metastatic function [8,9]. Overexpression of CDCP1 has also been linked with a shorter OS and recurrence free survival in several malignancies [10-12]. In an experimental model of high grade serous OC, targeting CDCP1

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reduced migration and tumor burden in a patient-derived xenograft [13].

A disintegrin and metalloproteinase 12 (ADAM12) is also a type I transmembrane protein with functions in cell signaling and adhesion [14]. Existing evidence suggests that elevated levels of ADAM12 correlated with the stage of disease in breast and OC [15,16]. Furthermore, upregulation of ADAM12 has also been found in breast and ovarian tumor vasculature in comparison with its normal counterpart [17,18].

Following our previously published review [19], we investigated the expression of CDCP1 and ADAM12 in the OC microenvironment, with an emphasis on the immunohistochemical expression of tumor cells, microvessels and stroma in relation with other clinical and pathological characteristics.

Methods

Patients diagnosed and treated for OC were retrospectively selected from the Institute of Oncology "Prof. Dr. Ion Chiricuta" database between 2006 and 2011. The inclusion criteria were: 1) histologicaly confirmed serous OC; 2) first line treatment consisting in optimal surgery and platinum-based chemotherapy; 3) regular follow-up intervals; and 4) available parrafin embedded OC tissue. Patients with suboptimal surgical treatment, neoadjuvant chemotherapy, sinchronous or metachronous malignancies, missing data or tumor tissue were excluded from the study. Written informed consent was obtained from all patients and ethical approval was granted by the Ethics Committee of the Medical Study Board.

Paraffin blocks were retrieved from our histopathological archive and assessed using tissue microarray (TMA), as previously described [20]. Tissue microarray blocks were assembled from representative areas of hematoxylin-eosin stained OC slides areas as indicated by our pathologist. Sections 4 μm thick were mounted on silane coated slides and incubated for 24 hrs at 37°C. After dewaxing in xylene, rehydration with ethanol, and washing, heat-induced antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) for 20 min. After cooling at 22°C, rinsing in tris-buffered saline slides were treated with 3% H_2O_2 for 5 min to block the endogenous peroxidase. The NovoLink Polymer Detection System (RE7140-CE; Leica Biosystems, Nussloch, DE) was used. To enhance the final result, slides were incubated with Protein Block (Leica Biosystems) for 5 min to reduce nonspecific binding.

To evaluate ADAM12 expression, a primary rabbit polyclonal IgG antibody (SC-25579; Santa Cruz Biotechnology, Inc., Dallas, Texas, USA) was used at a dilution of 1:100 for 30 min. Positive control for ADAM12 showed positive cytoplasmic and nuclear staining of intraductal cells in seminiferous tubes and Leydig cells of human testis. For CDCP1 detection, a primary rabbit polyclonal IgG antibody (ab188818; Abcam, Cambridge, UK) was used at a dilution of 1:150 for 30 min. Positive control for CDCP1 showed positive cytoplasmic, membranous and nuclear staining of human prostatic glandular epithelium. The primary antibody used for the assesment of the MVD was a monoclonal mouse antibody to human CD34 (M7165; Dako, Glostrup, Denmark) at 1:350 dilution for 30 min.

After washing in TBS, slides were incubated with Post Primary Block (Leica Biosystems) for 30 min and with the mouse/rabbit IgG-Poly-HRP secondary antibody (Leica Biosystems). Peroxidase activity was developed with DAB working solution (Leica Biosystems). After counterstaining with Mayer's hematoxylin, slides were rinsed in saturated solution of lithium carbonate, dehydrated, cleared in xylene and mounted in Faramount Mounting Medium (S3025; Dako, Glostrup, Denmark). Negative controls were created by omitting the primary antibodies.

A semi-quantitatively histochemical score was developed to evaluate tumor staining for CDCP1 and ADAM12. Evaluation was performed by an experienced pathologist that took into account both the staining intensity (absent=0, weak=1, moderate=2, strong=3) and the percentage of positive cells (≤ 50%=0, > 50%=1). The final score was the sum of the staining intensity and the percentage of positive cells.

To determine MVD, samples were immunohistochemically stained with a monoclonal antibody against CD34 which is known as a useful tool to determine MVD within tumors [21]. All microvessels that stained for CD34 and had a clearly visible lumen at 400 × magnification were counted together with clusters of positive endothelial cells [22]. The MVD represented the total number vascular spots on the 3.14 mm^2 surface of the TMA and for ADAM12 and CDCP1 it was determined in the same way, by replacing the primary CD34 antibody with the corresponding ADAM12 or CDCP1 antibody.

Statistics

Clinical and survival data was collected from patient files and the Regional Cancer Registry. For data analysis MedCalc v.12.7 and R v.3.03 have been used. For the general characterization, descriptive statistics were first calculated. Student's t-test, chi-square test and Fisher's F test were used. Kaplan-Meier method was used for survival analysis and log-rank test was performed to compare differences between groups. P values were two-sided and were considered significant at the level <0.05.

Results

In total, 102 patients were included in this study. All patients had serous OC and underwent

Table 1. Baseline patient therapy and disease characteristics

Characteristics	*N*	*%*
FIGO stage		
I	11	10.78
II	6	5.88
III	75	73.52
IV	10	9.80
Ascites	72	70.58
Peritoneal carcinomatosis	74	72.54
ECOG performance status		
0	40	39.21
1	53	51.96
2	9	8.82
Secondary cytoreductive surgery	18	17.64
Chemotherapy lines after relapse		
1	73	71.56
≥1	50	49.01
Relapse	78	76.47
Death	55	(53.92)

Table 2. Histopathological tumor details

Tumor details	*N*	*(%)*
Tumor grade		
G1	18	17.65
G2-G3	84	82.35
Mean CD34 MVD**	46.77 ± 22.3*	
CDCP1		
Tumor score		
1-2	37	36.27
3-4	65	63.72
CDCP1 positive microvessels	43	42.15
Mean CDCP1 MVD**, N=43	25.9 ± 14.28*	
Stromal positive expression***	33	32.35
ADAM12		
Tumor score		
0	34	33.33
1-2	50	49.01
3-4	18	17.64
ADAM12 positive microvessels	27	26.47
Mean ADAM12 MVD**, N=27	19.07 ± 13.08*	
Stromal positive expression***	20	19.60

* mean ± standard deviation, ** microvessel density, *** >10% positive stromal cells

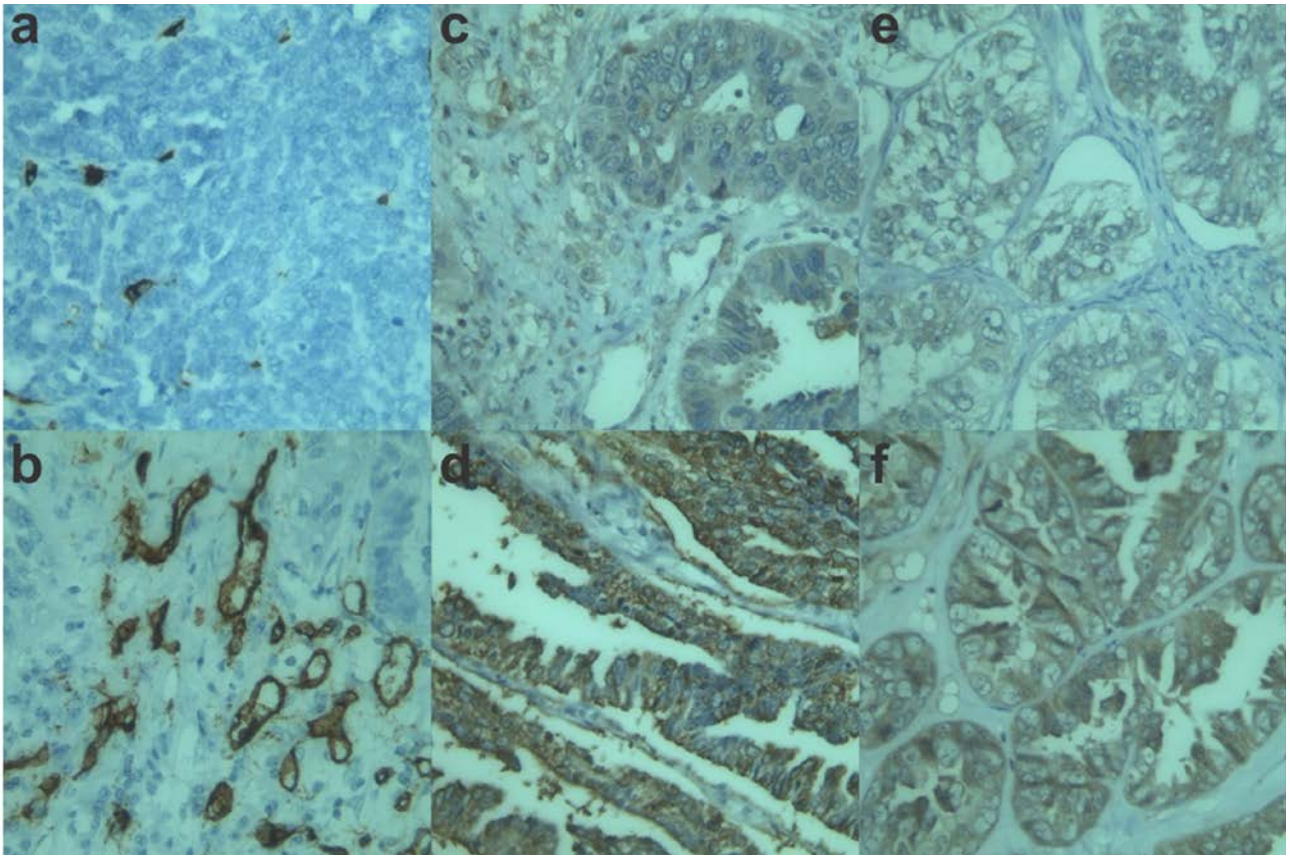

Figure 1. a: Immunohistochemical expression of CD34, CDCP1 and ADAM12
a: CD34 expression, sample of ovarian cancer with low MVD; **b:** CD34 expression, sample of ovarian cancer with high MVD; **c:** CDCP1 expression, sample of ovarian cancer with low expression; **d:** CDCP1 expression, sample of ovarian cancer with high expression; **e:** ADAM12 expression, sample of ovarian cancer with low expression; **f:** ADAM12 expression, sample of ovarian cancer with high expression. Original magnification×400.

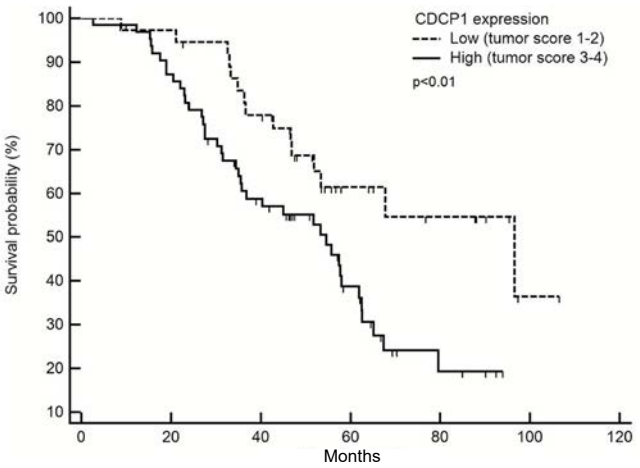

Figure 2. Kaplan-Meier overall survival for low and high CDCP1 expression.

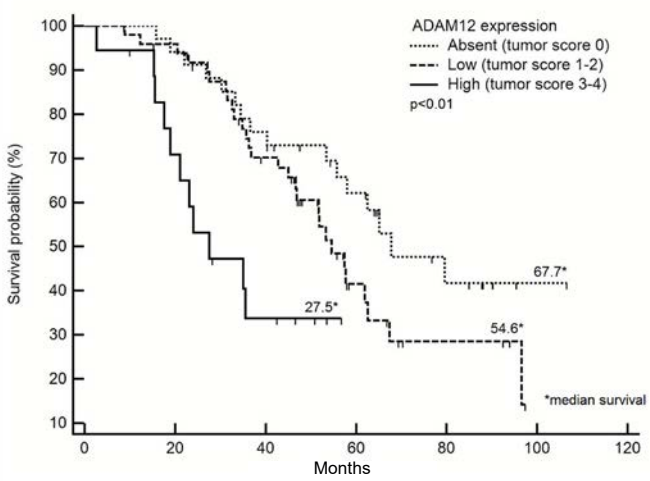

Figure 3. Kaplan-Meier overall survival for low and high ADAM12 expression.

optimal surgical debulking followed by platinum-based chemotherapy. The mean age at diagnosis was 56 years (range 35-79) and most patients (83%) were diagnosed with advanced stage disease (FIGO stage III/IV). Ascites (70.58%) and peritoneal carcinomatosis (72.54%) were common findings. After disease relapse in 78 (76.47%) patients, 18 of them underwent secondary cytoreductive surgery, followed by at least one or more lines of chemotherapy in 73 (71.56%) and 50 (49.01%) patients, respectively. Median follow-up time was 57.4 months (range 33-96). The baseline clinical characteristics of all patients are presented in Table 1.

Within the tumor microenvironment, 63.72% of cases expressed high tumor levels of CDCP1 (tumor score 3-4), 42.15% had endothelial cells positive for CDCP1 and 32.35% had positive stromal expression of CDCP1. Regarding ADAM12, 66.65% of samples exhibited positive tumor islets and 26.47% of the cases had positive endothelial cells for ADAM12 while 19.6% of the cases displayed positive stromal expression for ADAM12. Histopathological characteristics are detailed in Figure 1 and Table 2.

With increasing FIGO stage (IV vs III vs I-II), patients had a significant shorter progression free (PFS) and OS (p=0.002). Analyzing the presence of peritoneal carcinomatosis and ascites, both of them were associated with significantly decreased PFS and OS (p<0.05).

A significantly increased number of cases with high tumor grade (G2-G3) were associated with advanced FIGO stage (IIIC-IV), presence of peritoneal carcinomatosis, ascites (p<0.05), and a shorter OS (p=0.026) in comparison with cases with low tumor grade (G1).

The mean MVD assessed by CD34 antigen in advanced FIGO stage (III-IV) was higher in comparison with FIGO stage I-II (47.7±2.45 vs 41.7±5.01), although not statistically significant.

Patients with a high tumor score of CDCP1 (3-4 vs 1-2) had a significantly shorter OS (p<0.01) as seen in Figure 2, and a significantly higher number of positive CDCP1 microvessels (odds ratio 2.75, 95% CI 1.16-6.85, p=0.02). Among cases that exhibited positive CDCP1 microvessels, the mean MVD assessed by CD34 antigen was significantly higher in comparison with cases that had negative CDCP1 microvessels (mean, 55 vs 40.77, p<0.01).

Analyzing ADAM12 expression, an absent or low tumor score significantly correlated with improved OS in comparison with samples that exhibited high tumor score (p<0.01; Figure 3). Tissue samples with positive tumor score for ADAM12 had a significantly higher number of positive ADAM12 microvessels (OR 3.78, 95%CI 1.25-13.94, p=0.017) in comparison with samples that had an absent tumor score. The mean MVD assessed by CD34 antigen was higher in cases with positive ADAM12 microvessels in comparison with samples with negative ADAM12 microvessels (mean 55.92 vs 43.48, p=0.012).

Discussion

Although a number of studies addressing the role of CDCP1 and ADAM12 in cancer progression have been published, there are only a few published studies that have investigated CDCP1 [13] and ADAM12 [16,18] expression within the ovarian cancer microenvironment.

CDCP1 was found to be overexpressed in 74%

of high grade serous ovarian cancer cases using an immunohistochemical approach according to our results. Furthermore, this study demonstrated in a proof of concept approach that silencing CDCP1 with a lentiviral construct significantly reduced cell migration *in vitro* and tumor burden in vivo. Additionally, anti-CDCP1 antibody treatment reduced tumor burden in a patient-derived xenograft model [13].

Tanaka et al. [16] using quantitative RT-PCR have shown that the expression index of ADAM12 is higher in stage III-IV OC in comparison to early-stage disease. Similarly, in our study we could observe a gradual overexpression of ADAM12 with increasing FIGO stage although it did not reach a statistically significant difference. Other reports have focused on the tumor associated endothelial cells from invasive ovarian [17] or breast [18] carcinomas. In these studies there was a significant increase of ADAM12 expression in the tumor vasculature in comparison with the normal tissue. In the present study we could observe ADAM12 positive microvessels in only 26.47% of the cases. However, for ADAM12 we observed a stromal positive expression in 19.6% of the cases. These differences are currently difficult to interpret due to the limited number of cases, regional variations and different assessment methods.

Besides the negative prognostic factors such as advanced FIGO stage, ascites, peritoneal carcinomatosis or high tumor grade, our results regarding ADAM12 and CDCP1 elevated expression suggest a negative outcome concerning overall survival. For both markers, high tumor scores significantly correlated with a decreased OS and cases with positive endothelial staining for ADAM12 and CDCP1 had a significantly increased MVD. Our study has limitations regarding its retrospective design, limited number of cases and a semi-quantitatively immunohistochemical evaluation of protein expression, therefore additional studies are required to validate our results and better characterize the role of CDCP1 and ADAM12 in the OC microenvironment as prognostic biomarkers and evaluate their possible therapeutic potential.

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Conflict of interests

The authors declare no confict of interests.

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