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CD44s Expression, in Benign, Borderline and Malignant Tumors of Ovarian Surface Epithelium. Correlation with p53, Steroid Receptor Status, Proliferative Indices (PCNA, MIB1) and Survival

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Abstract. *Aim: To investigate the role of CD44s in the biological behavior of surface epithelial ovarian tumors and its correlation with clinicopathological parameters, prognosis, p53, steroid receptor status and proliferative indices (PCNA, MIB1). Materials and Methods: We analyzed a total of 83 patients with ovarian surface epithelial tumors, for the immunohistochemical expression of CD44s and the possible correlation with clinicopathological factors and patients' outcome. Results: A statistically significant correlation was found between the expression of CD44s, which was higher in cancer cases than in benign cystadenomas ($p<0.0001$) and, between cancer cases, which was lower in borderline tumors, ($p=0.05$). No statistical correlation was found between CD44s expression and the examined markers. In overall survival analysis we did not detect a statistically significant correlation with the expression of CD44s. Conclusion: The current study demonstrates that CD44s may be functionally involved in the pathogenesis of epithelial ovarian lesions.*

CD44 characterizes a polymorphic family of cell-surface glycoproteins, which are involved in cell-to-cell and cell to extracellular matrix adhesive interactions. They have been found to participate in several biological processes, such as cell trafficking, lymphocyte homing, haematopoiesis, wound healing and inflammation (1, 2). The biological aptitudes of these molecules have brought the CD44 family under intense investigation. Published data associate CD44 with cancer as a potential tumor marker (3, 4) and with chronic inflammation as a therapeutic intervention (5, 6).

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Genomic analysis revealed that the CD44 gene is located on the short arm of human chromosome 11, covering 50 to 60 kb of genomic DNA. It contains at least 20 exons, half of which are constitutively expressed, encoding the standard form of the molecules of CD44s. Alternative slicing of the remaining 10 exons gives rise to multiple variant isoforms of CD44 (CD44v), which add additional sequence to the extracellular membrane-proximal region of CD44 (7, 8). Overexpression of CD44 variable isoforms has been associated with tumor aggressiveness and poor prognosis in human solid tumors and also non-Hodgkin's lymphoma (9). Opposing results have appeared considering breast, endometrial and cervical cancer in which the molecule is associated with poor prognosis (10-12), and other studies which failed to detect any prognostic relationship (13-15). Also no prognostic association was found in colorectal cancer (16).

CD44s, which represent the major type of CD44, found on leucocytes and fibroblasts but also in epithelial cells, is the most studied type in the ovary (17-19). This has been found expressed in normal ovarian epithelium but also in ovarian malignant cell lines and seems to play an important role in ovarian metastasis (20). In the metastatic process, CD44 is the primary receptor for hyaluronan and published data reported that the attachment of tumor ovarian cells to the peritoneal mesothelium is mediated through the interaction between CD44 expressed in malignant cells and hyaluronic acid expressed on the mesothelial surface (21, 22).

p53 is the most frequently mutated gene (23). It is believed to play an important role in the control of cell proliferation and loss of its normal function may lead to uncontrolled cell proliferation and subsequently tumor progression. p53 has been associated with CD44s, as markers of tumor progression in renal carcinomas (24), but we found no association between p53 and CD44s in endometrial carcinoma (25).

Table I. *Histological type of the examined cases.*

Histology		Patients	%
Benign	serous	11	13.3
tumors	mucinous	10	12.0
Borderline	serous	6	7.2
	mucinous	8	9.6
Malignant	serous		
tumors	cystadenocarcinomas	31	37.3
	mucinous cystadenocarcinomas	4	4.8
	mixed	3	3.6
	clear cell	3	3.6
	poorly-differentiated	7	8.4
Total		83	100

Table II. *Antibodies used.*

Antibodies	Supplier	Dilution	Incubation time
CD44 (DF 1485)	Dako	1:40	overnight*
ER (M7047)	Dako	1:50	1 hour
PR (M3569)	Dako	1:75	1 hour
MIB1 *	Dako	1:50	1 hour
PCNA (PC-10)	Dako	1:50	1 hour
P53 (DO7, IgG2b)	Ylem	1:200	1 hour*

* with microwave oven antigen retrieval

The purpose of this study was to investigate the significance of the immunohistochemical expression of CD44s in the full spectrum of ovarian tumors that originate from the ovarian surface epithelium, from benign cystadenomas to cancer. We also investigated the correlations with p53, steroid receptor status, proliferative associated indices (MIB1, PCNA) and survival.

Materials and Methods

A total of 83 cases with diagnosis of ovarian surface epithelial tumors were retrieved from the archives of the Surgical Pathology Department of the University Hospital of Ioannina, Greece, for the period 1979 to 2003. All patients were surgically treated at the University Hospital of Ioannina. The surgical treatment for benign tumors was simple cystectomy, for borderline tumors conservative or radical surgery, but for malignant tumors radical surgery, which included hysterectomy with bilateral salpingo-oophorectomy and omenctomy. All patients with malignancy in this series received chemotherapy at the Medical Oncology Department of the same hospital according to running treatment protocols. Two pathologists reviewed the histological diagnosis and two representative blocks from each case were selected for immunohistochemistry. Each specimen was examined histologically on H&E-stained slides. All of the cases were analyzed by age, histological type, tumor grade and FIGO stage. Histological typing was performed according to the WHO criteria. Histological grade of malignancy ranged from GI (well-differentiated) to GIII (poorly-differentiated) (26).

Overall, 21 cases of benign cystadenomas (11 serous and 10 mucinous), 14 borderline (low malignant potential tumors, 8 mucinous and 6 serous) and 48 cases of ovarian cancer were analyzed (Table I). In 21 of the 48 investigated cases of malignant tumors a complete follow-up of patients was available, including chemotherapy administered, response to the therapy, time to recurrence and survival. Regarding chemotherapy administered, 18 patients received the carboplatin-plaxitel combination, while in 3 cases single-platinum chemotherapy was administered.

Immunohistochemistry. On two selected paraffin blocks, from each case, we performed immunohistochemistry on 4μm tissue sections placed on poly-L-lysine-coated glass slides. Consequently, the sections were deparaffinised in xylene and dehydrated. All sections were treated for 30 min with 0.3% hydrogen peroxide (in methanol) to quench endogenous peroxidase activity and then were incubated with primary antibodies. We used the method involving the avidin-biotin-peroxidase complex and developed the chromogen with immersion of the slides in a diamino-benzidine-H_2O_2 substrate for 5 min. The slides were counterstained in Harris' haematoxylin, dehydrated and mounted. To assess the specificity of the reaction, negative controls were included, and tumor sections subjected to the procedure except for incubation without the primary antibody. The antibody sources and dilutions are shown in Table II.

Immunohistochemical evaluation. Anti-CD44s reactivity was evaluated as positive when cytoplasmic membrane staining was observed and was reported as percentage of positive epithelial cells in relation to the total number of cells.

Anti-ER and *anti-PR* reactivity was evaluated by the observation of the positive epithelial cell nuclei. The scoring system was as follows: 0=negative, (1+) for positive cells below 10%, (2+) 11-50% and (3+) for more than 51% stained cells. Only cases with score 2+ and 3+ were considered as positive.

Anti-p53 reactivity was evaluated only when brown nuclear staining was detected, and was scored as follows: 0 when less than 10% reactive cells, (1+) when the reactivity was between 10% and 25%, (2+) for 26% to 50% and (3+) when more than 51% cells were positive. Any case scored at least as (1+) was considered positive. The selection of this scoring system was based on the observation that, when more than 10% of the tumor nuclei are stained with anti-p53, the highest correlation with the presence of structural mutations in the p53 gene is observed (27).

Anti-Mib1 and *anti-PCNA* reactivity was evaluated as positive, only when epithelial nuclear staining was observed. For the statistical analysis the cases were divided for Mib1 into two groups, (<10% and >10%) and for PCNA into three groups (<10%, 11-50% and >50%).

Statistical analysis. All data were statistically analyzed by SPSS ver10 statistical programme. Non-parametric test, Mann-Whitney *U*-test type, was used for the association of continuous variables. For relapse-free and overall survival analyzed by expression of CD44 we used Kaplan-Meier plots and the log-rank test, while the Graph pad Prism 4 programme analyzed the results. p values less than 0.05 were considered statistically significant.

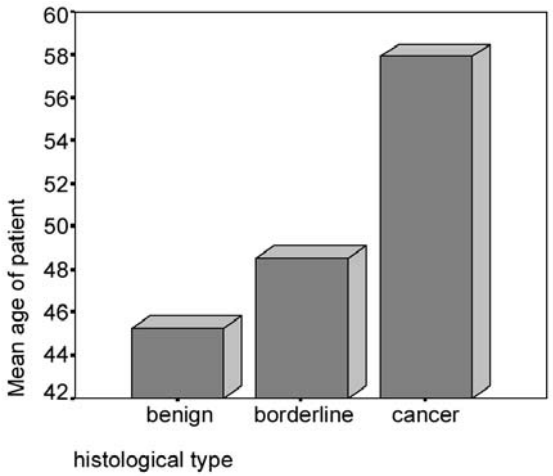

Figure 1. *Histological type by age.*

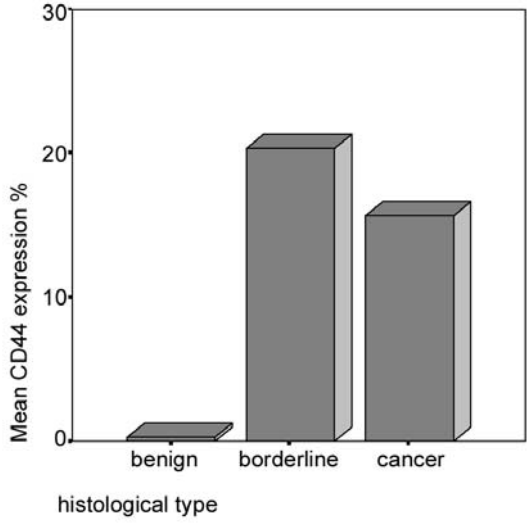

Figure 2. *CD44 expression by tumor type.*

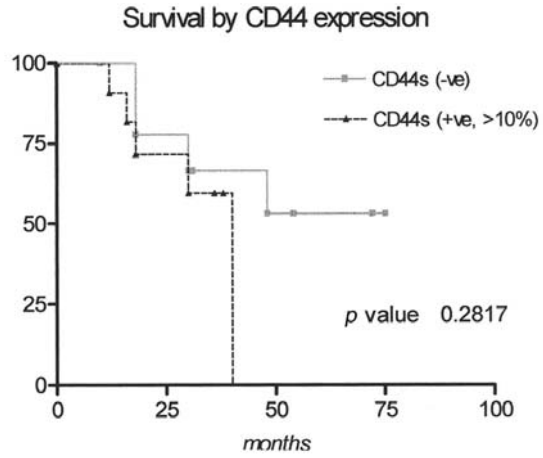

Figure 3. *Survival graph of 21 ovarian cancer patients by CD44s expression.*

Results

Patients' age at the time of the diagnosis ranged from 17 to 85 years old (median+ 58, mean=53) and was clearly related to the presence of malignancy: age was lower for patients diagnosed with benign and higher for those with malignant tumors (Figure1). Regarding the 48 carcinomas, 10 were well-differentiated (20.8%), 14 moderately-differentiated (29.2%) and 24 poorly-differentiated (50%); by histology, 38/48 (79.2%) were serous cystadenocarcinomas, 4/48 (8.3%) mucinous cystadenocarcinomas, 3/48 (6.3%) mixed carcinomas and 3/48 (6.3%) clear cell carcinomas.

All the positive tumor cells were characterized by intense membrane staining. In the positive tumor cases small foci, single or multiple, of intense membrane staining were noticed, but the rest of the tumor cells were negative (Figures 4, 5). The mean value of CD44s expression in cancer cases was 17.4%. Fifty percent of the cases were negative (24/48), in 29.2% (14/48) of the cases the expression was between 0 to 50% and in 10 out of 48 cases (20.8%) the expression was higher than 50%. A statistically significant correlation was found between the expression of CD44s, which was higher in cancer cases than in benign cystadenomas ($p<0.0001$) and between cancer cases, which was lower than in borderline tumors, ($p=0.05$) (Figure 2).

The mean value of CD44s expression was 12 in grade I (well-differentiated), 18.2 in grade II (moderately-differentiated) and 19.1 in grade III (poorly-differentiated), but no statistical correlation was reached between grade or histological type and CD44s expression. ER expression was in 33/48 (68.8%) cases lower than 10% and in 15 (31.3%) higher than 10%. PR expression was in 39/48 (81.3%) lower than 10% and in 9/48 (18.8%) higher than 10%. No statistical correlation was found between CD44s expression and the examined markers (Table III).

In borderline tumors the mean value of CD44s expression was 20.3. The expression of CD44s in 42.9% (6/14) of the cases was between 10 and 50%. No statistical correlation was found between CD44s expression and the examined markers (Table III).

In overall survival analysis we did not detect a statistical difference in this small number of patients when categorized by expression of CD44s (Figure 3).

Discussion

Ovarian epithelial cancer is a leading cause of death among gynecological malignancies due to advanced stage at presentation and the development of resistance to

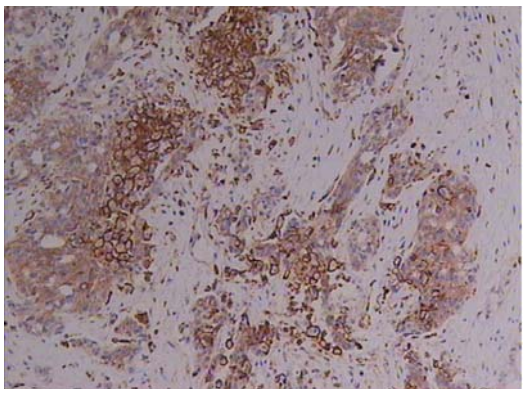

Figure 4. *Immunohistochemical expression of CD44s in ovarian cancer (x200).*

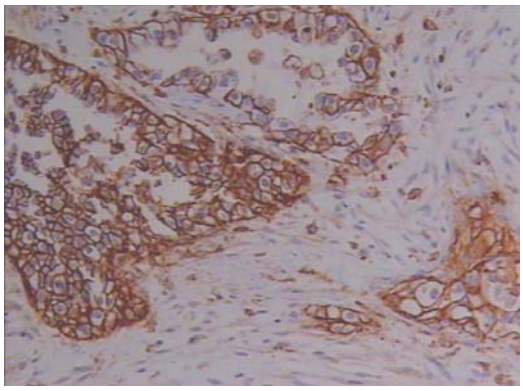

Figure 5. *Immunohistochemical expression of CD44s in ovarian cancer (x400).*

Table III. *Immunohistochemical expression of ER, PR, p53, MIB1, PCNA and correlation with CD44s in cancer cases.*

	Frequency	%	CD44s +ves	CD44s -ves
ER				
Less than 10%	31	64.6%	15 (31.3%)	16 (33.3%)
Greater than 10%	17	35.4%	7 (14.6%)	10 (20.8%)
PR				
Less than 10%	39	81.3%	18 (37.5%)	21 (43.8%)
Greater than 10%	9	18.8%	6 (12.5%)	3 (6.3%)
p53				
Less than 10%	25	52.1%	16 (33.3%)	9 (18.8%)
Greater than 10%	23	47.9%	8 (16.7%)	15 (31.3%)
MIB1				
Less than 10%	5	10.4%	-	5 (10.4%)
Greater than 10%	43	89.6%	24 (50%)	19 (39.6%)
PCNA				
Less than 10%	3	6.3%	-	3 (6.3%)
10-50%				
Greater than 50%	7	14.6%	2 (4.2%)	5 (10.4%)
	38	79.2%	22 (45.8%)	16 (33.3%)

Table IV. *Immunohistochemical expression of ER, PR, p53, MIB1, PCNA and correlation with CD44s in LMP tumor.*

	Frequency	%	CD44s +ves	CD44s -ves
ER				
Less than 10%	13	92.9%	12(85.7%)	1 (7.1%)
Greater than 10%	1	7.1%	1 (7.1%)	-
PR				
Less than 10%	14	100.0%	13 (92.9%)	1 (7.1%)
Greater than 10%	-	-	-	-
p53				
Less than 10%	14	100.0%	13 (92.9%)	1 (7.1%)
Greater than 10%	-	-	-	-
MIB1				
Less than 10%	3	21.4%	2(14.3%)	1(7.1%)
Greater than 10%	11	78.6%	11 (78.6%)	-
PCNA				
Less than 10%	3	21.4%	2 (14.3%)	1(7.1%)
10-50%				
Greater than 50%	3	21.4%	3 (21.4%)	-
	8	57.1%	8 (57.1%)	-

chemotherapy. The majority of women, after an initial response to first-line chemotherapy, will eventually relapse to a more resistant status (28). It is noticeable that, despite improvements in the treatment of ovarian cancer, incidence and mortality rate steadily remain high (29, 30). The five-year survival for women diagnosed with ovarian cancer is only 50% (31). These clinical data strongly support the need for establishing tumor markers, which could offer predictive information with regard to biological behavior of a tumor and expected response to chemotherapy and thus facilitate the decision about the initial clinical management.

Surface epithelial tumors are categorized by histopathological criteria for grading, as benign, borderline or low malignant potential (LMP) and malignant tumors. It is unknown if this classification denotes a sequence to malignant transformation or whether it simply represents a spectrum of diseases. In this respect great interest has been raised in studying LMP tumors. In a large study of Kurman and Trimble, which included 953 serous LMP tumors, only less than 1% demonstrated a malignant transformation. This was the reason that this pathological entity was renamed as proliferative tumor (32).

Surface epithelial tumors originate from the celomic epithelium that forms epithelial glands and cysts (33). The epithelium is continuous with the mesothelium that covers the peritoneal cavity. This anatomical consideration explains the metastatic behavior of these types of tumors, which is actually unique. The dissemination occurs principally within the abdominal cavity, by intraperitoneal adhesion of the tumor cells to the mesothelium and penetration to the

submesothelial matrix. Haematogenous dissemination is rare and lymphatic spread also occurs at low frequency (34).

The significance of the immunohistochemical expression of the CD44 family in ovarian cancer has been investigated during recent years. Cannistra and collaborators (35) investigated CD44s and CD44v9 in 31 cases of ovarian cancer and found no prognostic significance. In contrast Uhl-Steidl *et al.* (36), also in 1995, published a study, which included 44 patients and reported that CD44-positive cancer cases were associated with shortened overall survival. In 1999 three studies were published, which associated CD44s and variants with poor prognosis. Schroder *et al.*, examined CD44s and CD44v5-v8 and v10 in 50 cases and reported that CD44v5 and v6 were associated with intraperitoneal implantation. Kayastha *et al.* (37) investigated the immunohistochemical expression of CD44s in a series of 56 cases, and reported that CD44s was associated with poorer overall survival. Saegusa *et al.*, (38) in 115 cases, using immunohistochemistry and RT-PCR, examined CD44s, v3 and v6, and reported that CD44v3 loss of expression is associated with poor prognosis. In 2001, Ross *et al.* (19) in a study, which included 101 cases, investigated CD44s by immunohistochemistry and reported that decreased expression of CD44s was associated with shortened survival. In the present study, with a small number of patients with complete follow-up, we did not reveal any statistically significant correlation between the expression of CD44s and the examined markers, the clinicopathological parameters or survival. The benign lesions, as a group, showed minimal or even no reactivity for CD44s. On the other hand the expression of CD44s in the LMP group was higher when compared to malignant lesions.

The expression of ER, PR in cancer cases was lower than 10% in 64.6% and 81.3%, respectively, and we did not reveal a statistically significant correlation between these two markers and CD44s. It remains to be found if there is a relationship between them.

Despite that, the immunohistochemical expression of PCNA and MIB1, which are markers of proliferation, was higher in cancer cases than in the two other examined groups (LMP, benign tumors) (Tables III, IV) and could be used as a method of distinguishing them. We are in agreement with Anreder *et al.*, that light microscopy remains the method of choice to evaluate proliferative from malignant lesions (39). p53 has been correlated with CD44 in renal carcinoma (23). In ovarian cancer no association was found between CD44v6 and p53 (40). In the current study we did not reveal any statistically significant correlation between p53 and CD44s. So, it remains to be discovered whether these two markers are independent or correlated in epithelial ovarian cancer. In LMP tumors the expression of p53 was in all the examined cases lower than 10% or even absent. We agree with the results published by Wertheim *et al.* (41), who investigated the p53 gene mutation in borderline tumors and concluded that the gene mutation is a rare event and is not important in the pathogenesis of these lesions, which represent a distinct biological entity. No correlation was found between p53 and CD44s. The existing published data do not concur. This could be related to differences in technical methods or in the interpretation of the immunohistochemical results.

In conclusion, the current study demonstrates that CD44s may be functionally involved in the pathogenesis of epithelial ovarian lesions.

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References

1 Ponta H, Wainright D and Herrlich P: Molecules in focus: the CD44 protein family. Inter J Biochem Cell Biol *30*: 299-305, 1998.

2 Bajorath J: Molecular organization, structural features, and ligand binding characteristics of CD44, a highly variable cell surface glycoprotein with multiple functions. Prot Struct Funct Genet *39*: 103-111, 2000.

3 Gunthert U, Hofmann M, Rudy W, Reber S, Zoller M, Haussmann I, Matzku S, Wenzel A, Ponta H and Herlich P: A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. Cell *65*: 13-24, 1991.

4 Ponta H, Sleeman J, Dall P, Moll J, Sherman L and Herlich P: CD44 isoforms in metastatic cancer. Inv Metast *14*: 82-86, 1995.

5 Johnson P, Maiti A, Brown KL and Li R: A role for the cell adhesion molecule CD44 and sulfation in leukocyte-endothelial cell adhesion during an inflammatory response? Biochem Pharmacol *59*: 455-465, 2000.

6 Pure E and Cuff CA: A crucial role for CD44 in inflammation. Trends Mol Med *7*: 213-221, 2001.

7 Gunthert U: CD44: a multitude of isoforms with diverse functions. Curr Top Microbiol Immunol *184*: 47-63, 1993.

8 Screaton GR, Bell MV, Jackson DG, Cornelis FB, Gerth U and Bell JI: Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. Proc Natl Acad Sci USA *89*: 12160-12164, 1992.

9 Jalkanen S, Joensuu H, Soderstrom KO *et al*: Lymphocyte homing and clinical behavior of non-Hodgkin's lymphoma. J Clin Invest *87*: 1835- 1840, 1991.

10 Tempfer C, Lösch A, Heinzl H, Häusler G, Hanzal E, Kölbl H, Breitenecker G and Kainz Ch: Prognostic value of immunohistochemically detected CD44 isoforms CD44v5, CD44v6, and CD44v7-8 in human breast cancer. Eur J Cancer *32(11)*: 2023-2025, 1996.

11 Stokes GN, Shelton JB, Zahn CM and Kendall BS: Association of CD44 isoform immunohistochemical expression with myometrial and vascular invasion in endometrioid endometrial carcinoma. Gynecol Oncol *84*: 58-61, 2002.

12 Speiser P, Wanner C, Tempfer C, Mittelbock M, Hanzal E, Bancher-Todesca D, Gitch G, Reinthaller A and Kainz C: CD44 is an independent prognostic factor in early-stage cervical cancer. Int J Cancer *74*: 185-188, 1997.

13 Morris SF, O'Hanlon DM, Mclaughlin R, Mchale T, Connoly GE and Given HF: The prognostic significance of CD44s and CD44v6 expression in stage two breast carcinoma: an immunohistochemical study. EJSO *27*: 527-531, 2001.
14 Tokumo K, Kodama J, Seki N, Miyagi Y, Yoshinuchi M and Kudo T: CD44 exon v6 is not implicated in the progression and metastasis of endometrial cancer. Cancer Letter *125(1-2)*: 221, 1999.
15 Saegusa M, Hashimura M, Machida D and Okayasu I: Down-regulation of CD44 standard and variants isoforms during the progression of uterine cervical tumors. J Pathol *187*: 173-183, 1999.
16 Givehchian M, Wörner S, Sträter J, Zöller M, Heuschen U, Lehnert T, Herfarth C and von Knebel Doeberitz M: No evidence for cancer-related CD44 splice variants in primary and metastatic colorectal cancer. Eur J Cancer *34*: 1099-1104, 1998.
17 Cannistra SA, Kansas GS, Niloff J, De Franzo B, Kim Y and Ottensmeir C: Binding of ovarian cancer cells to peritoneal mesothelium *in vitro* is partly mediated by CD44H. Cancer Res *53*: 3830-3838, 1993.
18 Kayastha S, Freedman AN, Piver MS, Mukamalla J, Romero-Guittirez M and Werness BA: Expression of the Hyaluronan receptor, CD44s, in epithelial ovarian cancer is an independent predictor of survival. Clin Cancer Res *5*: 1073-1076, 1999.
19 Ross JS, Sheehan CE, Williams SS, Malfetano JH, Szyfelbein WM and Kallakury BV: Decreased CD44 standard form expression correlates with prognostic variables in ovarian carcinomas. Am J Clin Pathol *116(1)*: 122-8, 2001.
20 Lesley J, Hyman R and Kincade PW: CD44 and its interactions with extracellular matrix. Adv Immunol *54*: 271-335, 1993.
21 Kokenyesi R: Ovarian carcinoma cells synthesize both condroitin sulfate and heparan sulfate cell surface proteoglycans that mediate cell adhesion to interstizial matrix. J Cellular Biochem *83*: 259-270, 2001.
22 Gardner MJ, Jones LMH, Catterall JB and Turner GA: Expression of cell adhesion molecules on ovarian tumor cell lines and mesothelial cells, in relation to ovarian cancer metastasis. Cancer Letters *91*: 229-234, 1995.
23 Levine AJ, Momand J and Finlay CA: The p53 tumor suppressor gene. Nature *351*: 453-6, 1991.
24 Zolota V, Tsamandas A, Melachrinou M, Batistatou A and Scopa C: Expression of CD44 protein in renal carcinomas: association with p53 expression. Urol Oncol *7*: 13-17, 2002.
25 Zagorianakou N, Ioachim E, Mitselou A, Kitsou E, Zagorianakou P, Makrydimas G and Agnantis NJ: Glycoprotein CD44s expression in normal, hyperplastic and neoplastic endometrium. An immunohistochemical study including correlation with p53, steroid receptor status and proliferative indices (PCNA, MIB1). Eur J Gynecol Cancer *4(6)*: 500-4, 2003.
26 Kurman RJ: Blaunstain's Pathology of the Female Genital Tract, 4th ed. Springer, New York, 1994.
27 Levine AJ: The tumor suppressor genes. Annu Rev Biochem *62*: 623-651, 1993.
28 Johnston SRD and Gore ME: Caelyx®: Phase II studies in ovarian cancer. Eur J Cancer *37*: S8-S14, 2001.
29 National Cancer Institute. SEER Cancer Statistics Review 1973-1997, 2001.
30 Statistisches Bundesamt. Fascherie 12 (Gesundheitswesen), Reihe 4: Todesursachenstatistik in Dtl. Stuttgart, Metzler Poeschel, 1996.
31 Greenlee RT, Hill-Harmon MB, Murray T and Thun M: Cancer statistics, 2001. CA: A Cancer J Clin *51*: 15-36, 2001.
32 Kurman RJ and Trimble CL: The behaviour of serous tumors of low malignant potential: are they ever malignant? Int J Gynecol Pathol *12*: 120-127, 1993.
33 Auersperg N, Edelson MI, Mok SC, Johnson SW and Hamilton TC: The biology of ovarian cancer. Semin Oncol *25*: 281-304, 1998.
34 Cannistra SA: Cancer of the ovary. N Engl J Med *329*: 1550-1559, 1993.
35 Cannistra SA, Abu-Jawdeh G, Niloff J, Strobel T, Swanson L, Anderson J and Ottensmeir C: CD44 variant expression is a common feature of epithelial ovarian cancer: lack of association with standard prognostic factors. J Clin Oncol *13(8)*: 1912-21, 1995.
36 Uhl-Steidl M, Muller-Holzner E, Zeimet AG, Adolf GR, Daxenbichl, Marth C and Dapunt O: Prognostic value of CD44v splice variant expression in ovarian cancer. Oncology *52(5)*: 400-6, 1995.
37 Schroder W, Rudlowski C, Biesterfeld S, Knobloch C, Hauptmann S and Rath W: Expression of CD44 (v5-v10) splicing variants in primary ovarian cancer and lymph node metastasis. Anticancer Res *19 (5B)*: 3901-6, 1999.
38 Saegusa M, Machida D, Hashimura M and Okayasu I: CD44 Expression in benign, premalignant and malignant ovarian neoplasms: relation to tumor development and progression. J Pathol *189*: 326-337, 1999.
39 Anreder M, Freeman S, Megori A, Halabi S and Marrogi A: p53, c-erbB2, and PCNA status in benign, proliferative and malignant ovarian surface epithelial neoplasms. Arch Pathol Lab Med *123*: 310-316, 1999.
40 Kamura T, Sakai K, Kaku T, Kobayashi H, Mitsumoto M, Tsueyoshi M and Nakao H: Comparison of p53 and CD44 variant 6 expression between paired primary and recurrent ovarian cancer: an immunohistochemical analysis. Oncol Rep *6(1)*: 97-101, 1999.
41 Werteim I, Muto M, Welch W, Bell D, Berkowitz R and Mok C: p53 gene mutation in human borderline epithelial ovarian tumors. J Nat Cancer Inst *86*: 1549-1551, 1994.

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