Immunohistochemical Study of the CD44 Expression in Benign, Borderline and Malignant Surface Epithelial Ovarian Tumors and Metastatic Ovarian Tumors

Gamze YURDAKAN[1], Nilüfer ONAK KANDEMİR[1], Sacide ÇOLAK[1], Aylin EGE GÜL[2], Nimet KARADAYI[2] Şükrü Oğuz ÖZDAMAR[1]

Zonguldak, Turkey

OBJECTIVE: Carcinogenesis and metastasis are multistep processes involving complex interactions between tumor cells and the environment. The main cause of tumor cell movement in invasive carcinomas may be the loss of the intercellular adherence junction. One adhesion receptor, CD44, binds to hyaluronan, an extracellular matrix component. This study investigated a series of benign, borderline, and malignant ovarian serous neoplasms to elucidate the role of CD44.

STUDY DESIGN: Paraffin-embedded formalin-fixed blocks from benign serous tumors (n=11), benign mucinous tumors (n= 8), borderline serous tumors (n=6), borderline mucinous tumors (n=1), primary malignant ovarian serous tumors (n=9), and metastatic ovarian tumors (n=12) were stained immunohistochemically for CD44. The percentages of reactive tumor cells and stromal cells with CD44 were scored. The staining intensity was graded from 1+ to 3+. CD44 protein was preferentially expressed along the basolateral domain of the plasma membrane of tumor cells.

RESULTS: CD44 was not detected in only 1 (5.2%) benign tumor. The remaining tumors were reactive for CD44 to different degrees and in different locations. CD44 staining was observed in 4 (8.33%), 21 (43.75%), 16 (33.33 %), and 7 (14.58%) of all grade 0, 1, 2, and 3 tumors, respectively. Statistically significant associations were found between serous and mucinous benign tumors (p=0.0001), borderline and malignant serous tumors (p=0.001), malignant serous tumors and metastatic carcinomas (p=0.054), and primary malignant ovarian tumors and metastatic carcinomas (p=0.002) based on the CD44 staining grade. Grade 0,1,2, and 3 stromal staining was seen in 14 (29.16%), 31 (64.58%), 1 (2.08%), and 1 (2.08%) ovarian tumors, respectively, although there was no statistical difference in the CD44 reaction in stromal cells. In primary malignant tumors, CD44 was detected significantly more often than in primary benign ovarian tumors (p=0.0001).

CONCLUSION: These results suggest that CD44 expression is important in differentiating between borderline and malignant serous tumors, primary malignant ovarian tumors, and metastatic carcinomas. In addition, CD44 expression is a characteristic factor in the stromal invasion of ovarian serous carcinomas. Additional studies are necessary to verify the prognostic significance of CD44 expression in tumor progression.

(Gynecol Obstet Reprod Med;13:3 160-163)

Key Words: Serous epithelial tumors, Ovary, CD44, Immunohistochemistry

Introduction

Surface epithelial tumors of the ovary are the most common tumors of the ovary. These tumors have a wide proliferation spectrum and are classified as benign, borderline, (intermediate, low malignancy potential, possibly malignant), and malignant according to the amount of nuclear atypia, mitotic activity, stratification, and stromal invasion. Although benign and malignant tumors are easily differentiated using definitive criteria, borderline tumors pose diagnostic difficulties because they are named according to their nuclear appearance, even if they have focal proliferative characteristics along with a stromal micro-invasion focus and distant metastasis. Since the determination of stromal invasion is difficult due to the complex interaction between glands and stroma, compared with serous tumors, it is more difficult to distinguish borderline mucinous tumors from malignant mucinous tumors. One of the difficulties affecting the differential diagnosis of tumors located in the ovary is that primary tumors can be confused with some metastatic tumors histopathologically. The most common metastases to the ovary are from the stomach, colon, appendix, breasts, lungs, and skin. Metastases to the ovary from endometrial carcinoma in particular, and differentiating

[1]*Zonguldak Karaelmas University, Faculty of Medicine, Department of Pathology, Zonguldak, Turkey*

[2]*Dr. Lütfi Kırdar Kartal Researches and Training Hospital, Department of Pathology, İstanbul, Turkey*

Address of Correspondence: *Gamze Yurdakan*
Zonguldak Karaelmas Üniversitesi
Tıp Fakültesi Patoloji Anabilim Dalı
Esenköy Kozlu, Zonguldak, Turkey
gamzenu@yahoo.com

Submitted for Publication: *08.11.2007*
Accepted for Publication: *24.12.2007*

independent tumors in both the ovary and endometrium, pose some difficulties. Therefore, the identification of cellular characteristics that determine tumor behavior may help in the differentiation of tumors with low malignant potential (borderline) and malignant tumors or primary and metastatic tumors.[1]

CD44 is a transmembrane protein encoded by a single gene located on chromosome 11 in humans and is the main surface receptor for the extracellular polysaccharide hyaluronan. CD44 regulates cell-cell and cell-matrix interactions. In addition, reports indicate that CD44 is a potential tumor marker.[2,3] The expression of CD44 in ovarian cellular cultures displaying malignant transformation indicates that it plays an important role in ovary metastasis.[4]

This study investigated the importance of the immunohistochemical expression of CD44 in ovarian tumors of surface epithelial origin ranging from benign cystadenoma to cancer and in tumors metastasizing to the ovary.

Material and Methods

Forty-seven cases with a diagnosis of ovarian surface epithelial tumor or metastatic tumor were retrieved from the archives of the Pathology Department of the Zonguldak Karaelmas University School of Medicine and Pathology Department of the Dr. Lütfi Kirdar Kartal Research and Training Hospital for the period 2001 to 2006. These included 19 benign cystic adenomas (11 serous, 8 mucinous), 7 borderline tumors (tumors with low malignant potential: 6 serous, 1 mucinous), 9 primary malignant serous tumors, and 12 metastatic ovarian tumors.

All cases were analyzed by age, histological type, and tumor grade. Histological typing was performed according to WHO criteria. The histological grade of malignancy ranged from GI (well-differentiated) to GIII (poorly-differentiated).

Two pathologists reviewed the histological diagnosis and two representative blocks from each case were chosen for the immunohistochemical study. Paraffin sections (5 μm) were deparaffinized and hydrated through graded ethanol solutions. Endogenous peroxidase was blocked with 3% hydrogen peroxide (methanol and hydrogen peroxide) and then the specimens were rinsed in phosphate-buffered saline (PBS) for 10 min. Antigen retrieval was achieved using 10 mM citrate buffer (pH 6.0) for CD44. Sections and buffers were heated to the boiling point in a 750–850-W microwave oven for 10 min. The specimens were cooled for 20 min and then washed twice in PBS. Blocking was carried out with Ultra-V-Block (LabVision TA-125-UB) for 5 to 10 min at room temperature. The sections were incubated with a mouse monoclonal antibody to CD44v (variant 3, clone VFF-327v3, Neomarkers/Lab Vision, Fremont, CA, USA, 1:10-1:20 dilution) for 90 min. The slides were washed in two changes of PBS, incubated for 30 min with a biotin-linked antibody, washed in two changes of PBS, and finally incubated for 30 min with streptavidin peroxidase complex. The peroxidase reaction was conducted for 10 min in diaminobenzidine (DAB) solution. The slides were counterstained with Mayer's hematoxylin for 30 sec and then washed in water for 3 min before dehydration through graded ethanol solutions; they were then cleared in xylene before being mounted in Entellan.

Positive staining in at least 10% of the cells and high staining intensity was required to consider a case positive. The percentages of reactive tumor cells and stromal cells with CD44 were scored. Staining intensity was graded from 1+ to 3+.

Statistical analyses were conducted using SPSS for Windows (version 13.0; Chicago, IL, USA). Variables are reported as the mean ± standard deviation (SD). The unpaired t-test and Mann-Whitney U-test were used in the statistical analysis. A p value less than 0.05 was deemed to be statistically significant.

Results

At the time of diagnosis the patients' ages ranged from 19 to 81 years and averaged 49.44±13.70.

Histologically, there were 25 (52.08%) serous, 9 (18.75%) mucinous, and 12 (25%) metastatic ovarian tumors. There were no malignant mucinous tumors. On examination of the metastatic tumors, four (33.33%) were endometrial carcinoma, two (16.66%) were signet ring cell tumors, one (8.33%) was adenocarcinoma of the colon, one (8.33%) was metastatic serous papillary carcinoma, and two (16.66%) were tumor metastases of unknown primary source.

In one case of mucinous cystadenoma, there was a mature cystic teratoma in the ipsilateral ovary, and in one case of serous papillary cystadenoma with micro-invasion there was a serous papillary cystadenoma in the contralateral ovary. One (11.11%) of the primary ovarian carcinomas was a well-differentiated tumor, seven (77.77%) were moderately differentiated tumors, and one (11.11%) was a poorly differentiated tumor.

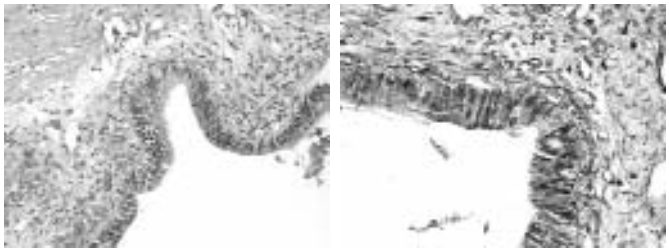
Homogenous CD44v3 expression was observed in the basolateral membranes of the tumor cells in most (89.37%) of the cases. Cytoplasmic staining was observed in two primary and four metastatic ovarian tumors.

The average staining percentage of CD44 expression was 48.5±27.9% and the average degree of staining was 1.5±0.8. A reaction of 0,+1, +2, and+3 was observed in 8.33, 43.75, 33.33, and 14.58% of the cases, respectively.

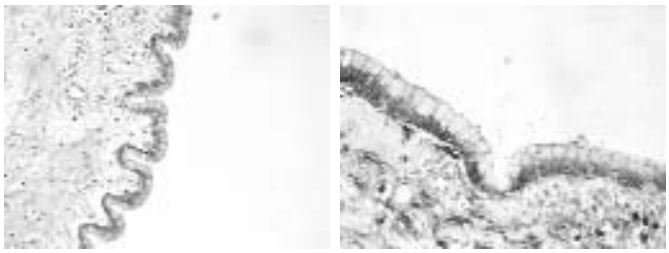
Regarding the degree of staining, the score was higher in malignant tumors. Statistically significant differences were found between benign and malignant serous tumors

(p=0.0001), borderline and malignant serous tumors (p=0.01), malignant serous and metastatic tumors (p=0.054), and primary malignant and metastatic tumors (p=0.002) (Figures 1-5). The percentage staining averaged 31.75% in benign tumors, 46.42% in borderline tumors, and 62.62% in malignant tumors. When all of the benign and malignant tumors were compared, there was a significant difference in the percentage of staining (p=0.0001). There were significant differences between benign/malignant serous (p=0.01), borderline/malignant serous (p=0.03), and primary malignant/-metastatic tumors (p=0.083).

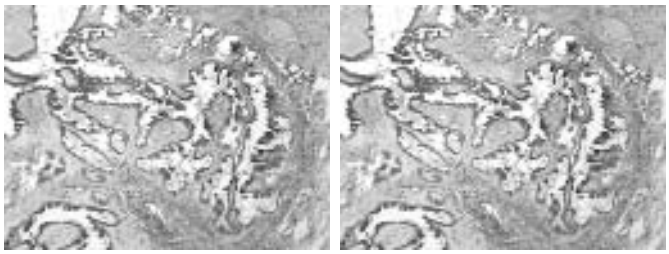
While CD44 expression according to the degree of staining in stromal cells was observed in the order 29.16% (score 0), 64.58% (+1), 2.08% (+2), and 2.08% (+3), no differences between groups were observed.



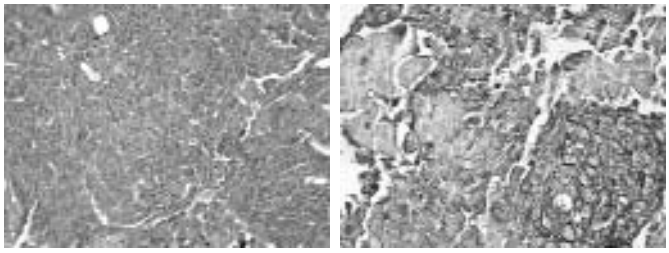
Figür 1a-b: Serous cystadenoma



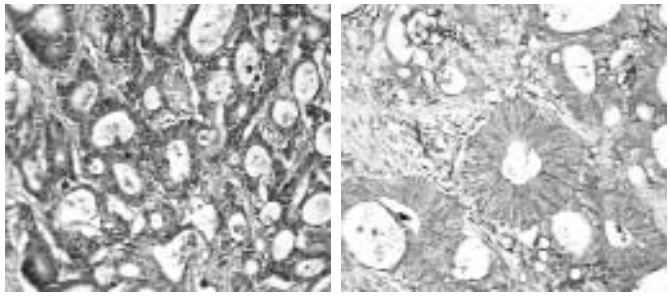
Figür 2a-b: Mucinous cystadenoma



Figür 3a-b: Borderline papillary serous tumor



Figür 4a-b: Primary malign serous tumor



Figür 5a-b: Metastatic ovarian tumor

Figures 1-5: A; H&E,X60,B; CD44v3 expression in the tumor cells, (B-SA,DAB,X120).

Discussion

The most important step in the carcinogenetic and metastatic processes of tumors is the binding of tumor cells to the nearest microenvironment area, combined with the loss of intercellular connections. Numerous adhesion molecules (E-cadherin, catenin, integrins, CD44, etc.) play roles in this process.

The role of CD44 in human malignancies is complex. There are associations between a reduction in the expression of CD44 and its variants and poor clinical prognosis in colorectal carcinoma, prostate cancer, and melanomas. However, failure to find a similar association in some tumors like stomach cancer suggests that CD44 exerts different influences on disease progression, depending on the type of tumor.[5]

Studies have investigated the prognostic importance of the immunohistochemical expression of the CD44 family in cancers of the ovary. While some studies found associations between CD44 overexpression and high tumor grade, advanced stage, and low overall survival, others did not detect any statistical correlation with survival or clinicopathological parameters.[6,7]

Expression of the tumor cell receptor CD44, which enables binding to stromal hyaluronan, and the assessment of stromal invasion may have some importance as diagnostic criteria in serosal surface-derived tumors of the ovary. Comparing benign and malignant tumors, Cho et al. detected higher ratios of reactive tumor cells with CD44 in borderline tumors. In that study, CD44 expression was found in 60-65% of borderline cases with peritoneal implantation and serosal involvement. They explained this as indicating the carcinomatous progression in borderline tumors.[6] In our study, CD44 expression increased gradually from benign to malignant tumors and statistically significant differences were found between benign/malignant and borderline/malignant tumors. This finding was interpreted as indicating that molecules regulating the relationship of the cell population that has metastasizing potential with the microenvironment are important in determining tumor behavior. Whether it starts in a de novo

tumor initially or develops in an originally benign or borderline tumor, increased CD44 expression in malignant tumors implies that tumor cells have lost their cohesiveness, separated from the main tumor population, and progressed along the stroma. The observation of increased CD44 overexpression in borderline tumors compared with benign tumors suggests that the abnormal expression of CD44 is a pre-invasive condition, seen in squamous metaplasia in the lungs, colonic adenoma, esophageal dysplasia, and squamous intraepithelial lesions of the cervix.[8-11] This finding supports the idea that in the carcinogenesis process of the ovary, borderline tumors reflect the pre-invasive stage.[12] In our study, the presence of differences among benign, borderline, and malignant tumors supports the diagnostic value of stromal invasion in surface epithelial tumors of the ovary.

The detection of abnormal expression of CD44 and its variants in tumors with metastatic spread and local invasion ability is not a surprise. Although no studies have examined CD44 expression in tumors metastasizing to the ovary, in tumors of the stomach, colon, endometrium, and lungs, which metastasize to the ovary most frequently, there is a significant relationship between serosal infiltration, adnexal invasion, metastatic potential, and tumor progression in early stage carcinomas and CD44 expression.[13-16] In our cases, the difference between primary tumors of the ovary and metastatic tumors was interpreted as a behavioral difference due to a tumor-specific ability to metastasize and invade locally. It is not appropriate to differentiate between primary and metastatic tumors by looking at the degree of CD44 expression. One should remember that in binding of the tumor cells to the stroma, similar to the relationship between CD44 and hyaluronan, integrin-independent mechanisms are active as well as integrin-dependent mechanisms.[17]

In conclusion, we are of the opinion that in tumors of the ovary derived from surface epithelium, CD44 expression is a valuable indicator of stromal invasion and, just as their name suggests, borderline tumors are tumors with low malignant potential that have completed their carcinomatous progression.

References

1. Rosai J. Female Reproductive System, Ovary. In: Rosai J Rosai and Ackerman's Surgical Pathology, Vol 2 Ninth Ed, Newyork: Mosby 2004; pp:1649-1736.
2. Gunthert U, Hofmann M, Rudy W, et al.: A new variant of glycoprotein CD44 confers metastatic potential tor at carcinoma cells. Cell 1991, 65:13-24,
3. Ponta H, Sleeman J, Dallp, Moll J, Sherman L and Herlich P: cd44 isoforms in metastatic cancer. Inv Metast 1995, 14:82-86,
4. Lesley J, Hyman R and Kincade PW: CD44 and its interactions with extracellular matrix. Adv Immunol 1993,54: 271-335,
5. Sillanpää S, Anttila A, Voutilainen K, et al.CD44 expression indicates favorable prognosis in epithelial ovarian cancer. Clin Cancer Res 9:5318-5324,2003
6. Cho EY, Choi YL, Chae SW, Sohn JH, Ahn GH. Immuno histochemical study of the expression of adhesion molecu les in ovarian serous neoplasms. Path Int 56:62-70, 2006
7. Zagorianakou N,Stefanou D,Makrydimas G,et al.NJ. CD 44s expression, in benign, borderline and malignant tu mors of ovarian surface epithelium. Correlation with p53, steroid receptor status, proliferative indices (PCNA, MIB 1) and survival. Anticancer Research 24:1665-1670, 2004
8. Penno MB, August JT, Baylin SB et al. Expression of CD44 in human lung tumors. Cancer Res. 1994;54;1381-1387
9. CastellÃ E, Ariza A, FernÃandez-Vasalo A, Roca X, Ojanguren I. Expression of CD44H and CD44v3 in normal oesophagus, Barrett mucosa and oesophageal carcinoma. J Clin Pathol 1996;49(6):489-92
10. FernÃandez JC, Vizoso FJ, Corte MD, et al.CD44s expression in respectable colorectal carcinomas and surrounding mucosa Cancer Inves. 2004;22(6):878-885
11. Callagy G, O'Grady A, Butler D, Leader M, Kay E. Expression of CD44 in uterine cervical squamous neoclassical: a predictor of microinvasion? Gynecol Oncol. 2000;76(1):73-79
12. Darai E, Walker-Combrouze F, Fauconnier A, Madelenat P, Potet F, Scoazec JY. Analysis of CD44 expression in serous and mucinous borderline tumors of the ovary: comparison with cystadenomas and overt carcinomas. Histopathology 1998; 32:151-159.
13. Xin Y, Yi XL, Wang XP, et al.Relationship between phenotypes of cell function differentiation and pathobiological behavior of gastric carcinomas. World J Gastroenterol 2001; 7(1); 53-59
14. Hoshimoto K, Yamanchi N, Takazawa Y, Onda T, Taketani Y, Fukayama M. CD44 variant 6 in endometrioid carcinoma of the uterus: Its expression in the adenocarcinoma component is an independent prognostic marker. Pathol Res Prac. 2003; 199: 71-77.
15. Visca P, Del Nonno F, Botti C, Marandino F, Sebatiani V et al. Pole and prognostic significance of CD44s expression in colorectal cancer. Anticancer Res. 2002;22:2671-2676.
16. Kerr KM, MacKenzie SJ, Ramasami S, et al.Expression of Fhit, cell adhesion molecules and matrix metalloproteinases in atypical adenomatous hyperplasia and pulmonary adenocarcinoma. J Pathol 2004; 203(2): 638-644
17. Casey RC, Skubitz AP. CD44 and beta 1 integrins mediate ovarian carcinoma cell migration toward extracellular matrix proteins. Clin Exp Metastasis 2000; 18 (1):67-75.