Decreased CD44 Standard Form Expression Correlates With Prognostic Variables in Ovarian Carcinomas

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Abstract

Expression of CD44 standard form (CD44s) was evaluated by automated immunohistochemical analysis using the anti-CD44 A3D8 clone in 101 ovarian epithelial neoplasms including 82 primary tumors (64 carcinomas and 18 tumors of low malignant potential [LMP]), 9 lymph node metastases, 8 malignant ascites, and 2 peritoneal implants. Immunostaining was scored semiquantitatively. Tumors were graded according to the FIGO (International Federation of Gynecology and Obstetrics) classification system. Tumor stage and patient survival were determined from the patient records. While 9 of 18 LMP tumors expressed CD44s, only 15 of 64 carcinomas expressed it. In the carcinomas, univariate analysis revealed that decreased CD44s expression correlated with high tumor grade, advanced stage, and shortened survival. Loss of CD44s expression also was noted in the tumor cells in 8 of 9 lymph node metastases, 7 of 8 malignant ascites, and 1 of 2 implants. Multivariate analysis revealed that only tumor stage independently correlated with patient survival.

Loss of CD44s expression determined by immunohistochemical analysis is more common in ovarian carcinomas than in LMP tumors; correlates with prognostic variables including tumor grade, stage, and survival; and may have an important role in the dissemination of ovarian cancer.

CD44 is a cell surface hyaluronate receptor¹ and cell adhesion molecule that is widely expressed in a variety of cells of hematopoietic,² epithelial,³ and mesothelial⁴ origins. The CD44 molecule has been associated with cell-cell adhesion,⁵ cell-matrix interaction,⁶ and lymphocyte homing and circulation.²,⁴,⁷ The CD44 cell surface receptor includes a family of immunologically related integral membrane glycoproteins with molecular weights ranging from 80 to 250 kd.⁸,⁹ The CD44 gene is a single gene containing 20 exons¹⁰ localized to chromosome 11.¹¹ Alternative splicing of the 10 exons located between exons 5 and 16 (exon v1 to exon v10) of the CD44 gene may produce variant isoforms (CD44v).¹⁰,¹²-¹⁴ The most abundant form of CD44 protein in tissues is the standard form (CD44s) or hematopoietic form (85-95 kd) in which there is no expression of any of the 10 variant isoforms.⁷,¹⁵

A variety of clinical and experimental studies have linked perturbations of the CD44 molecule with cancer progression, invasion, and metastasis.¹⁶ Enhanced expression of CD44s and/or its isoforms has been associated with aggressive disease in non-Hodgkin lymphoma,¹⁷ breast carcinoma,¹⁸,¹⁹ colorectal cancer,²⁰ and high-grade renal cell carcinomas.²¹ CD44s immunoreactivity has been associated with the presence of thyroid cancer, especially the papillary subtype.²²,²³ Alternatively, down-regulation of CD44 has been reported in the progression of dysplasia to carcinoma in esophageal Barrett epithelium,²⁴ metastasis of endometrial cancer cells through lymph-vascular spaces,²⁵ high-grade bladder transitional cell carcinoma,²⁶ prostatic adenocarcinoma,²⁷-²⁹ and high-grade brain tumors.³⁰ CD44s immunostaining seems to be more intense in squamous cell carcinomas compared with adenocarcinomas of the lung.³¹ Recently, much attention has been drawn to the potential role of CD44 variant isoforms in tumor metastasis. In animal...
models, transfection of a nonmetastatic carcinoma cell line with a variant isoform containing the v6 exon has been shown to induce metastatic behavior in tumor cells.\textsuperscript{32} In human malignant neoplasms, carcinomas of the stomach,\textsuperscript{33,34} colon,\textsuperscript{35,36} breast,\textsuperscript{37,38} and uterine cervix\textsuperscript{39} have been associated with a variety of CD44v expression and aggressive tumor behavior.

CD44s expression has not been studied extensively in ovarian epithelial neoplasms. Studies of ovarian cancer cell lines and xenografts have suggested a potential role of CD44 in ovarian cancer progression.\textsuperscript{40-43} Studies on human primary and metastatic ovarian carcinomas using clinical tissue samples have yielded variable and conflicting results with regard to the prognostic significance of CD44s and variant isoforms.\textsuperscript{44-48} We studied the immunohistochemical expression of CD44s in ovarian tumors, including primary and disseminated forms, and evaluated its prognostic significance.

Materials and Methods

Tissue Samples

We randomly selected 101 ovarian epithelial neoplasms (accessioned between August 1991 and September 1995) from the files of the pathology department at the Albany Medical Center Hospital, Albany, NY. The cases included 82 tumors at the primary site, 9 lymph node metastases, 8 malignant ascites, and 2 peritoneal implants. All H&E stained slides, where applicable, were reviewed to confirm the original diagnoses. The median age of the patients was 59.8 years (range, 30-85 years). A gynecologic oncologist (J.H.M.) performed exploratory laparotomy on 93 patients. The remaining 8 cases were aspirated fluid from malignant ascites in patients with documented ovarian cancer. For patients with tumors diagnosed at frozen section with histologic features indicative of low malignant potential, hysterectomy and bilateral salpingo-oophorectomy were performed in 15 of 18 cases; 3 patients underwent unilateral salpingo-oophorectomy to preserve reproductive capabilities. All 75 women with carcinoma underwent hysterectomy with bilateral salpingo-oophorectomy to preserve reproductive capabilities. All 75 women with carcinoma underwent hysterectomy with bilateral salpingo-oophorectomy and received postoperative multidrug chemotherapy including cisplatin. The 18 patients with tumors of low malignant potential did not receive additional chemotherapy. The clinical charts were reviewed, and information on tumor type, stage, recurrence, and survival rates was recorded for all patients. The follow-up period for the study ranged from 1 to 70 months (mean, 32 months).

There were 18 tumors of low malignant potential (serous, 13; mucinous-gastrointestinal type, 4; endometrioid, 1) and 64 carcinomas (serous, 45; endometrioid, 9; clear cell, 5; mucinous, 5). Tumors were graded as well, moderate, and poorly differentiated and staged according to the FIGO (International Federation of Gynecology and Obstetrics) classification system. In the carcinoma group, there were 8 grade I (12%), 17 grade 2 (27%), and 39 grade 3 (61%) tumors. While 19 primary site carcinomas (30%) were stage I or II tumors, the remaining 45 cases (70%) were stage III or IV tumors. Surgical pathology blocks were stored in ambient conditions for 2 to 6 years before immunohistochemical staining.

Immunohistochemical Staining

After endogenous peroxidase activity was blocked, unstained 5-µm sections from a representative block of formalin-fixed, paraffin-embedded tissue in each case were stained for a 12-hour period with the A3D8 clone of the mouse monoclonal IgG1 class anti-human antibody against CD44s (Sigma Immunochemicals, St Louis, MO) at a concentration of 28 µg/mL. The A3D8 monoclonal antibody immunoprecipitates the 80-kd form of CD44.\textsuperscript{46} A papillary carcinoma of the thyroid known to be positive for CD44s served as the positive control.\textsuperscript{22,23} Negative control slides also were processed in parallel using a nonspecific IgG (Sigma) at the same concentration as the primary antibody. Immunohistochemical staining was performed on the Ventana ES Automated Immunohistochemistry System (Ventana Medical Systems, Tucson, AZ). After exposure to the primary antibody, the slides were incubated sequentially with the universal biotinylated immunoglobulin secondary antibody, avidin horseradish conjugate, and diaminobenzidine (DAB) followed by copper sulfate enhancement and counterstained with hematoxylin. Antigen retrieval procedures were not used in the immunostaining protocol.

Staining Interpretation

The staining was assessed semiquantitatively based on staining intensity and distribution as previously described.\textsuperscript{27,49} Tumors that showed intense diffuse, intense regional, and moderate diffuse patterns were considered positive for CD44s expression. All cases with other staining patterns or with no membranous staining were considered as down-regulated for this protein.

Statistical Analysis

For statistical comparison of CD44s expression with histologic types and tumor stage, the chi-square test was used. Univariate survival and recurrence analysis were based on the Kaplan-Meier and the Breslow-Gehan-Wilcoxon test. The criterion for significance was \( P = .05 \).

Results

Nine (50%) of 18 tumors of low malignant potential were considered positive for CD44s immunoreactivity in...
comparison with 15 (23%) of 64 carcinomas ($P = .03$).

**Histologic Type**

The loss of CD44s immunostaining in the tumors with serous differentiation was statistically significant ($P = .013$). Of 58 serous tumors, 40 (69%) showed CD44 down-regulation as opposed to 10 (42%) of 24 nonserous tumors. There were no significant differences in the comparative staining in the individual subtypes of nonserous tumors.

**Tumor Grade and Stage**

Four (50%) of 8 grade 1 carcinomas demonstrated loss of CD44s expression in comparison with 45 (80%) of 56 grade 2 and 3 tumors ($P = .05$). CD44s down-regulation correlated with tumor stage, with 9 (47%) of 19 stage I and II carcinomas showing loss of CD44s compared with 40 (89%) of 45 stage III and IV tumors ($P = .006$). In addition, tumor cells in 8 (89%) of 9 lymph node metastases Image 3, 7 (88%) of 8 malignant ascites Image 4, and 1 (50%) of 2 implants Image 5 demonstrated CD44s down-regulation.

**Survival Analysis**

At the time of completion of follow-up analysis, all patients with tumors of low malignant potential were alive and free of clinical evidence of disease. While 28 (44%) of 64 patients with carcinomas died of disease, 8 (22%) of 36 patients who were alive had either clinical or surgically proven evidence of recurrent disease. Univariate analysis revealed that loss of CD44s was associated with shortened patient survival ($P = .04$) Figure 1. However, multivariate analysis revealed that only tumor stage correlated independently with survival ($P = .006$).

**Discussion**

CD44 is a widely expressed cell surface antigen that serves as an adhesion molecule in cell-cell and cell-matrix interactions. Expression of the CD44 gene becomes disorderly in the early stages of carcinogenesis, and excessive quantities of many inappropriate alternatively spliced CD44 variants accumulate in cancer cells. The role of the CD44 adhesion molecule has been reported to be variable in different carcinomas. Increased expression of CD44 has been postulated to be associated with aggressive tumor behavior as demonstrated in animal studies and in human carcinomas of breast, stomach, and non-Hodgkin lymphoma. In contrast, correlation has been reported of down-regulation of CD44s with prognostic variables in other solid cancers including bladder, prostate, endometrium, and neuroblastoma. Although the exact basis for this variation seems unclear, we hypothesize that tumor invasion and metastasis may be enhanced by either increased cell-matrix interaction (associated with increased CD44) or, alternatively, decreased cell-cell adhesion (associated with decreased CD44). In bladder carcinomas, the strongest reaction to CD44s was seen in the basal epithelial region next to the basement membrane, suggesting an important role in the attachment between basal cell and basal lamina. It also is
Possible that the increased CD44 protein content reported in some malignant neoplasms may in fact represent the isoform variant rather than the standard form. Expression of CD44 splice variants has been linked to tumor dedifferentiation and progression in carcinomas of stomach, lung, breast, colon, and cervix.19,31,34-39

Studies on CD44 in ovarian tumors have been limited and yield conflicting data. Kayastha et al,48 in their study of 56 primary epithelial ovarian tumors, found CD44s down-regulation in 61% but with no correlation with histologic type, tumor grade, or stage. However, a significant correlation between CD44s down-regulation and survival revealed by both univariate and multivariate analysis was reported.48 In contrast, in another study of 115 ovarian carcinomas,46 the authors reported correlation between loss of CD44s and carcinomas in comparison with tumors of low malignant potential; and in the carcinomas, with histologic type and advanced stage. There was no correlation between CD44s loss and poor survival in the study.46 The results of the present study concur with these previously published data in
that CD44s down-regulation correlated with prognostic variables in ovarian carcinomas, including shortened survival, albeit on univariate analysis.

Although we did not evaluate the expression of CD44 variant isoforms that are produced due to alternatively spliced exons (v1-10), others have reported a site-dependent prognostic significance for these proteins. CD44v3 expression was reported to be an independent predictive factor for poor survival in ovarian carcinomas. In contrast, Schroder et al. evaluated the expression of CD44v5-10 in ovarian carcinomas and reported no correlation with survival. Although the prognostic role of CD44 variant isoforms in ovarian carcinoma is unclear from these limited data, the isoform CD44v6 has been widely reported to confer aggressiveness and metastatic potential in numerous other human malignant neoplasms, including carcinomas of lung, liver, stomach, breast, and non-Hodgkin lymphoma. In the colon, while normal mucosa was negative for CD44v6, this protein was demonstrated to progressively increase with increasing grades of dysplasia and transformation to carcinoma.

Studies on other müllerian tumors, notably endometrial carcinomas, have been scarce and report variable findings. Fujita et al. using both immunohistochemical and molecular analysis, demonstrated progressive loss of CD44 variants in endometrial hyperplasia and carcinoma in comparison with normal endometrium. In addition, these authors found a propensity for vascular space involvement by cancer cells in the CD44+ group compared with the CD44- group, suggesting an important role for CD44 and variant forms in the evolution and spread of endometrial cancers. In contrast, Saegusa et al. reported increased expression of CD44s in endometrial carcinomas compared with proliferative phase endometrium and hyperplasia and found no application for CD44s and variants v3 and v6 as prognostic markers.

The polysaccharide hyaluronic acid is ubiquitously present in the extracellular matrix and modifies cell–extracellular matrix interactions, thus regulating cell migration. It has been shown previously that interactions between hyaluronic acid and CD44 are directly implicated in the development and progression of tumors. Ovarian carcinomas frequently exfoliate into body cavities and induce accumulation of ascites. In vitro studies have shown that a significant subpopulation of human ovarian tumor cells attach to peritoneum via CD44. Yeo et al. evaluated this phenomenon using freshly harvested mouse ovarian tumor cells from peritoneal cavities and demonstrated that the majority of the tumor cells that attached to the mesentery during the early stages of invasion expressed CD44. Interestingly, these authors reported that in the later stages, approximately 90% of the tumor cells showed loss of CD44 and exfoliated into the ascitic fluid. While our study is not designed to evaluate the role of CD44 in mediating tumor cell–peritoneum attachment, our finding of loss of this protein in tumor cells of ascitic fluid and metastatic lymph nodes concurs with the data reported for animal models.

The loss of CD44s in ovarian carcinomas compared with tumors of low malignant potential and tumor cells at disseminated sites supports the role of this protein in the evolution and spread of ovarian cancer.

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References


