Expression of Cell Adhesion Molecules, CD44s and E-Cadherin, and Microvessel Density in Carcinoid Tumors

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Although all carcinoids are potentially malignant, their biologic behavior is quite variable. Currently there are no reliable morphological criteria to predict metastatic potential. Cell adhesion molecules, such as CD44 and E-cadherin, are considered important in regulating invasion and metastasis of tumors. Also, angiogenesis has been shown to be associated with tumor growth and progression. In this study, we examined 51 carcinoids, including 13 carcinoids with known lymph node and/or visceral metastasis, for expression of CD44s (the standard form of CD44) and E-cadherin by immunohistochemistry. We found that 55% and 37% of carcinoids were negative for CD44s and E-cadherin, respectively. Carcinoids with lymph node and/or visceral metastasis were significantly more frequently negative for CD44s than were those without demonstrated metastasis ($P = .030$). Ten of 11 tumors with lymph node metastasis lacked CD44s ($P = .022$), whereas E-cadherin was negative in only 3 ($P = .975$). Additionally, we analyzed microvessel density to evaluate the role of tumor angiogenesis in the tumor behavior. Carcinoid tumors in general demonstrated high microvessel density (160 ± 82/five 200× fields), irrespective of location and with and without metastasis. These results suggest that loss of CD44s, but not E-cadherin, may be a useful predictor of metastatic potential of carcinoid tumors.

KEY WORDS: Angiogenesis, Carcinoid tumor, CD44, Cell adhesion molecules, E-cadherin, Metastasis, Microvessel density.

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Although most carcinoid tumors exhibit benign behavior, a significant proportion of them act in a malignant fashion and are difficult to manage (1, 2). Evaluation of the malignant potential of a carcinoid tumor remains problematic. Primary tumor site, tumor size, level of urinary 5-hydroxyindoleacetic acid, and specific histologic growth patterns have been touted as useful prognostic factors. However, information regarding the utility of these data in predicting behavior is controversial (3, 4). Recently, several other tumor markers such as Ki-67, Bcl-2, and p53; cell adhesion molecules; and tumor angiogenesis have been explored as possible prognostic markers (5–10).

Cell adhesion molecules play a critical role in a variety of processes such as embryonic development, extracellular matrix binding, hematopoiesis, lymphocyte homing, cell migration, and tumor metastasis (11–14). CD44 and E-cadherin (ECAD) are two important adhesion molecules that have been extensively studied. CD44 is a large family of cell surface transmembrane glycoproteins whose members differ in their extracellular domains as a result of alternative splicing (12, 15). The data regarding the role of CD44 in neoplasia are controversial in regard to whether CD44 acts as a growth/invasion- promoting molecule or a tumor suppression cofactor (16, 17). The standard or hematopoietic form of CD44 (CD44s) is a receptor for hyaluronan and is highly expressed on human lymphocytes. Expression of CD44s has been shown to be associated with good prognosis in patients with bronchial carcinoids (8, 9). ECAD is a calcium-dependent transmembrane protein. Decreased expression of ECAD has been correlated with regional lymph node metastasis in squamous cell carcinomas (18) and with poor prognosis in gastric and colorectal cancers (19–21). Expression of ECAD in carcinoid tumors has not been reported previously. In this study, we have investigated the relationship of CD44s expression, ECAD expression, and microvessel density to the biological behavior of carcinoid tumors.

MATERIALS AND METHODS

Archival materials of 51 carcinoid tumors from the lung ($n = 20$), large bowel ($n = 11$), appendix ($n = 7$), small bowel ($n = 6$), stomach ($n = 5$), ovary ($n = 1$), and kidney ($n = 1$) that were accessioned in the surgical pathology files at our institution from 1996 to 2000 were used in this study. In 26 cases, regional lymph nodes were available for evaluation. Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue with monoclonal antibodies directed against the standard form of CD44 (CD44s, DAKO), ECAD (Zymed), and CD34 (Immunotech) using a standard avidin-biotin-peroxidase method (22). Briefly, 4-μm-thick paraffin sections were dewaxed and treated with 1% H_2O_2 in methanol for 30 minutes to block endogenous peroxidase activity. Antigen retrieval was performed in a microwave oven (pressure cooker) with Antigen Retrieval Citra microwave solution (BioGenex). The sections were incubated for 30 minutes with normal nonimmune serum to eliminate nonspecific staining. The antibody (1:200 dilution for anti-CD44s and 1:500 dilution for anti-ECAD) was then applied for 2 hours at room temperature. For anti-CD34, manufacturer-prediluted antibody solution was used and reaction was carried out in a Ventana machine (Tucson, AZ) for 32 minutes at 37° C. Antigen was detected with a biotin-labeled secondary antibody and avidin-biotin peroxidase technique using 3',3'-diaminobenzidine as the chromogen. Sections were counterstained with hematoxylin. Negative controls were performed by omitting the primary antibodies. For CD44s and ECAD, immunohistochemical reactions were graded as positive or negative based on the staining intensity of the membrane as well as on the number of cells stained (23). Tumors with no reactivity, weak reactivity, or moderate to strong reactivity in $<10\%$ of tumor cells were graded as negative, whereas those with a moderate to strong degree of reactivity in $\geq 10\%$ tumor cells were considered positive. For microvessel density, microvessels highlighted by anti-CD34 were counted in each case from the five most vascular, nonoverlapping fields (20× objective and 10× ocular, Nikon, 0.74 mm^2 per field as measured with an ocular micrometer) in a section. The averages of the five counts were used for statistical analyses.

For statistical analyses, χ^2 and Fisher exact tests using the Prophet 5.0 statistics program (BBN System & Technology) were applied, and a P value of $<.05$ was considered significant.

RESULTS

Fifty-one carcinoid tumors included in this study were obtained from 30 females and 21 males, ranging in age from 17 to 80 years (median, 53 y). Except

TABLE 1. Immunohistochemical Detection of CD44s and ECAD in Carcinoid Tumors

Substance	Lung	S. Bowel	L. Bowel	Appendix	Stomach	Ovary	Kidney	Total, *n* (%)
CD44s+	11	1	6	2	2	1	0	23 (45)
CD44s−	9	5	5	5	3	0	1	28 (55)
ECAD+	11	4	8	5	2	1	1	32 (63)
ECAD−	9	2	3	2	3	0	0	19 (37)
Total	20	6	11	7	5	1	1	51 (100)

S. Bowel, small bowel; L. Bowel, large bowel.

TABLE 2. Expression of CD44s and ECAD *versus* Tumor Size and Patients' Age

Substance	Tumor Size (cm)[a]		Age (y)		Metastasis	
	>2	<2	≥50	<50	+	−
CD44s+	8	12	14	9	2	21
CD44s−	12	12	19	9	11	17
P value	0.507		0.603		0.030	
ECAD+	12	14	19	13	10	22
ECAD−	8	10	14	5	3	16
P value	0.909		0.301		0.220	
Total	20	24	33	18	13	38

[a] Data on tumor size were available in 44 of the 51 cases.

TABLE 3. Expression of CD44s and ECAD *versus* Tumor Lymph Node Status

Substance	Lung		Small Bowel		Large Bowel		Appendix		Total	
	LN+	LN−	LN+	LN−	LN+	LN−	LN+	LN−	LN+	LN−
CD44s+	1	8	0	1	0	0	0	0	1	9
CD44s−	2	3	3	1	3	2	2	0	10	6
P value									0.022	
ECAD+	2	8	2	2	2	1	2	0	8	11
ECAD−	1	3	1	0	1	1	0	0	3	4
P value									0.975	

Lymph nodes were available for evaluation in 26 of the 51 cases.

for two carcinoids, all the other tumors were located either in the lung or gastrointestinal tract (Table 1). Of the 20 pulmonary carcinoids, 18 were typical and the other 2 were classified as atypical carcinoids according to the WHO classification (24). Of the 29 carcinoids from gastrointestinal tract, 25 were classified as classical type, showing trabecular and insular morphological features, and the other 4 (from appendix) were classified as goblet cell type. A total of 13 cases demonstrated either lymph node (10 patients) or visceral (2 patients) or both lymph node and visceral (1 patient) metastasis (Tables 2 and 3). No difference in the morphological features of carcinoids with and without metastasis was observed.

CD44s and ECAD expression were detected in 45% (23/51) and 63% (32/51) of carcinoid tumors, respectively (Table 1). Coexpression of the two adhesion molecules was seen in 19 tumors, and both markers were negative in 15 tumors, with only 4 tumors expressing only CD44s, and 13 tumors, only ECAD ($P = .01$). There was no significant difference in the expression of CD44s or ECAD in carcinoids derived from different sites. However, 85% of carcinoids (11 of 13) with known metastasis lacked CD44s expression compared with 45% of tumors (17 of 38) without demonstrated metastasis ($P = .030$; Table 2). Similarly, in the subgroup of patients who had lymph nodes available for evaluation, CD44s negativity was more frequently observed in tumors with lymph node metastasis (10/11) than in those with negative lymph nodes (6/15; $P = .022$; Table 3 and Fig. 1A–1D). No difference was observed with ECAD expression in tumors with and without metastasis. Furthermore, no significant difference in the expression of the two adhesion molecules was observed between tumors of different sizes or with respect to age of the patients (Table 2). All four goblet cell carcinoid tumors were positive for ECAD, but only two were positive for CD44s.

Carcinoid tumors in general demonstrated a high microvessel density (mean 160 ± 82; Table 4 and Fig. 1E). Microvessel density of carcinoid tumors of the lung (190 ± 106) was higher than that of tumors from other sites (142 ± 59), although the difference was not statistically significant. The goblet cell carcinoids were histologically less cellular and had a more myxoid background. These tumors had lower microvessel density than did other carcinoids ($P = .0195$; Fig. 1F). Microvessel density was not significantly different in tumors with or without metastasis, whether they were compared in the whole series or in the subgroup with lymph node dissection.

DISCUSSION

The biological behavior of carcinoids, in general, is unpredictable, although size, location, and morphological features may provide some clues regarding behavior. Recently, various other parameters have been evaluated as potential prognostic indicators for carcinoid tumors. Some parameters, such as proliferative activity and expression of growth factors have proved somewhat useful (6). Because cell adhesion molecules are considered important in tumor growth, invasion, and metastasis, we evaluated the prognostic value of these molecules in carcinoids arising from different sites and carcinoids with known metastasis. In this study, we found reduced expression of CD44s in tumors with metastasis. In contrast, the expression of ECAD showed no correlation with metastatic spread. Similarly, microvessel density also failed to distinguish carcinoid tumors by site or potential for metastatic spread. Although our clinical follow-up interval of 2 to 6 years is relatively short, outcome for our patients was good with 47 of the 51 patients alive and only 2 succumbing to the disease. Longer follow-up is required to more firmly establish the correlation between clinical outcome and the expression of the cell adhesion molecules and the effect of angiogenesis.

The role of CD44s and its various isoforms in tumorigenesis and tumor progression is very controversial. For example, loss of CD44s expression was reported to be associated with unfavorable outcome in patients with neuroblastoma (25), prostate carcinoma (26), transitional cell carcinoma of the bladder (27) and differentiated thyroid carcinoma (28).

However, opposite results have been reported in primary lung adenocarcinoma (29), colorectal cancer (30), and pancreatic endocrine tumors (5). The complexity of the biological function of the molecule, the small sample size in some studies, and the methods used to evaluate significance of these molecules may have all contributed to this controversy.

Reports on CD44 expression in carcinoid tumors are limited. Coppola and coworkers (6) showed decreased expression of CD44s and CD44v6 in atypical carcinoids and small cell carcinomas of the lung compared with the case of typical carcinoids. These investigators suggested that loss of CD44 expression correlated with more aggressive phenotypes. More recently, Granberg and coworkers (8, 9) demonstrated that expression of CD44s as well as CD44v7–8 and v9 was associated with decreased risk for distant metastases and with a more favorable outcome in patients who had typical carcinoids of the lung. In our series, we included not only carcinoid tumors from the lung but also those from other organs such as gastrointestinal tract. Our results showed that CD44s expression was more frequently negative in tumors with lymph node and/or visceral metastasis than in those without demonstrated metastasis ($P = .030$). A statisti-

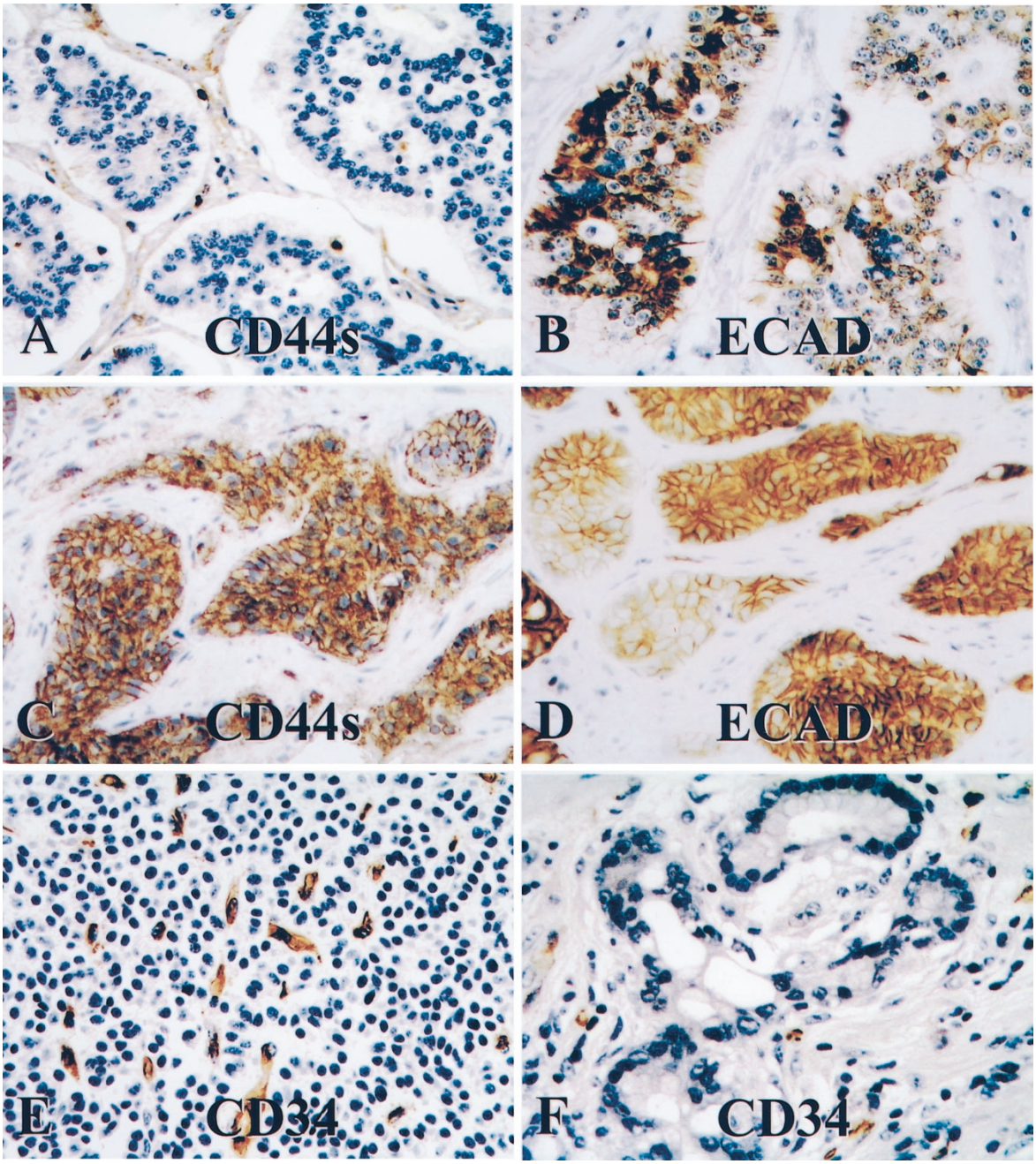

FIGURE 1. **A** and **B,** primary carcinoid tumor of colon from a 49-year-old male with known metastasis to two of seven lymph nodes. Cells lack CD44s expression but show membranous expression of ECAD. **C** and **D,** diffuse membranous CD44s and ECAD expression in carcinoid tumor of the colon from a 50-year-old female with no tumor metastasis. **E** and **F,** Demonstration of MVD in carcinoid of colon (**E**) and goblet cell carcinoid of the appendix (**F**). In the goblet cell carcinoid, MVD (counted in five 200× fields) was low compared with the case of the typical carcinoid tumor (43 *versus* 256).

cally significant difference was also noted in the subgroup of 26 patients whose lymph node status was known ($P = .022$). These findings further support the concept that loss of the adhesion molecule plays a crucial role in tumor progression and metastases in carcinoids.

TABLE 4. Microvessel Density (MVD) in Carcinoid Tumors (per Five 200× Fields)

Case No.	L	SB	LB	A	S	O	LN+	LN−	Met+	Met−	Gob
	19	6	10	6	5	2	11	15	13	38	4
MVD ± SD	190 ± 106	163 ± 59	132 ± 55	110 ± 61	147 ± 41	210 ± 50	152 ± 70	198 ± 96	131 ± 84	163 ± 82	66 ± 20

L, lung; SB, small bowel; LB, large bowel; A, appendix; S, stomach; O, other organs; LN+ or LN−, lymph node status; Met+ or Met−, with or without known metastasis.

ECAD and the associated catenin complex play an integral role in epithelial cell adhesion. Reduced expression of ECAD has been associated with lymph node metastasis in various carcinomas (18, 21). In our series, ECAD expression correlated with CD44s expression, although more tumors were positive for ECAD than for CD44s. Unlike CD44s, ECAD expression was not significantly different in lymph node–positive and –negative groups. Although a larger series is needed to confirm this finding, different cell adhesion molecules may be involved in the regulation of metastatic spread of different tumors.

Clinical and experimental studies have shown that angiogenesis is a prerequisite for tumor growth and progression and has been correlated with metastasis in breast, prostate, and bladder carcinomas and in melanomas (31–33). Previous studies, however, failed to demonstrate correlation of angiogenesis with the metastatic potential of pulmonary carcinoid tumors (10). Our data also showed no statistically significant difference in MVD between tumor groups with or without lymph node metastasis or among tumors of various origins. Although the reason for this lack of correlation in carcinoid tumors is not known, factors other than MVD, which is normally high in carcinoid tumors, may be more important in controlling the metastatic process.

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