CD24 expression is a poor prognostic marker in endometrial carcinoma

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Summary

Objective: CD24 is a cell adhesion molecule that has been implicated in metastatic tumor progression of various solid tumors. Its expression is known to be related to the prognosis of several kinds of tumors. This study was designed to examine the prognostic significance of CD24 in endometrial cancer patients.

Methods: Forty-four endometrial carcinoma tissues were immunostained for CD24 antibody (Ab2, clone 24 C02). Cytoplasmic and membranous immunoreactivity were scored semiquantitatively by Fisher's exact test.

Results: CD24 expression was detected in 34 (77.3%) out of 44 cases. Membranous and cytoplasmic staining of CD24 was significantly associated with the International Federation of Gynecology and Obstetrics (FIGO) grade ($p = 0.011$ and $p = 0.002$, respectively) and nodal status ($p = 0.002$ and $p = 0.000$, respectively).

Conclusion: Our data suggests that CD24 expression in endometrial carcinoma as detected by immunohistochemistry might be a new marker for a more aggressive endometrial cancer biology. CD24 is commonly up-regulated in endometrial cancer and this corroborates the importance of CD24 in tumor progression among these cases.

Key words: CD24; Endometrial carcinoma; Immunohistochemistry.

Introduction

Endometrial carcinoma is the most common invasive neoplasm of the female genital tract and the fourth most frequently diagnosed cancer in women in the United States [1]. It is only the third most common cause of gynecologic cancer deaths following ovarian and cervical cancer [2]. Worldwide, approximately 150,000 cases are diagnosed each year, making endometrial carcinoma the fourth in incidence among invasive tumors in women following breast lung, and colon cancer. Endometrial cancer occurs in both premenopausal (25%) and postmenopausal women (75%) [2]. The most commonly affected age group is between 50 and 59 years of age. Most endometrial carcinomas are diagnosed in early stages and are associated with favorable outcome. However, the prognosis of advanced diseases is poor [1-3].

Well established conventional prognostic markers in endometrial cancer according to FIGO are stage, grade, patient age, and lymph node metastasis. In addition to these clinicopathological parameters, molecular markers are being sought and established for a wide variety of tumors [4]. A recently identified novel prognostic marker gene CD24 is showing great promise. It is a small, heavy glycosylated protein core that consists of 27 amino acids and is attached to the cell membrane by a phosphoatidylinositol anchor [5]. It was first identified as the marker of B cells [6]. Although CD24 is not present in adult human tissues, it is expressed in many human carcinomas [7]. CD24 expression was noted in various hematologic malignancies and solid tumors such as non-small cell lung cancers, breast cancers, prostate cancers, ovarian cancers and colorectal carcinomas and was related closely with tumor metastasis and survival rate of patients [6, 8-12]. If functions as an alternative ligand of P-selectin, an adhesion receptor expressed on activated endothelial cells and platelets, and could thus enhance the metastatic potential of CD24 expressing tumor cells [13].

In the present study, we aimed to evaluate the expression of the CD24 in endometrial carcinomas by immunohistochemistry and its relationship with clinicopathologic parameters and disease stage.

Materials and Methods

Patients and samples

Tissue samples from 44 patients with endometrial carcinoma which were diagnosed between 1998 and 2005 at the Department of Pathology, Suleyman Demirel University School of Medicine, were included in this study. The age, tumor type, myometrial and vascular invasion, lymph node metastasis, peritoneal cytology, FIGO grade and stage were evaluated by reviewing the medical charts and pathological records. Glass slides were reviewed for histological classification according to the FIGO criteria [4]. Two independent observers did the histopathological evaluations twice.

Immunohistochemistry

Immunohistochemical analysis for CD24 was performed on formalin-fixed, parafin-embedded archival tissue using the streptvavidin-biotin-peroxidase technique. For all cases, 4 μm histologic section was deparaffinized in xylene and dehydrated in descending dilution of ethanol. For the antigen retrieval, slides were treated by microwave heating in citrate buffer (pH 6.0) for 20 min. Endogenous peroxidase activity was blocked by 20 min of incubation with 0.3% hydrogen peroxidase. Slides

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were tested with mouse monoclonal anti CD24 antibody (1:100, Clone 24C02, Neomarkers). Sections were tested with the streptavidin-biotin-peroxidase kit (Ultra Vision Large Volume Detection System Anti-polyvalent, HRP, LabVision, USA), and after incubation the reaction product was detected using diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer's hematoxylin, and mounted with mounting medium. The positive control for CD24 was inflamed granulation tissue.

The membranous and the cytoplasmic staining intensity of CD24 were evaluated separately and scored semiquantitatively as CD24 negative, weak, moderate, or strongly positive. Negative cases had to show definitely no CD24 immunoreactivity in any part of the tumor. Weak staining was defined by positive immunoreactivity in up to 10% of the tumor. Moderate staining was defined by positive immunoreactivity in 11-50% of the tumor, and strong staining was defined by positive immunoreactivity in > 50% of the tumor.

Statistical Analysis

For statistical evaluation, the SPSS software version 12 was used. We used Fisher's exact test to assess the statistical significance of the correlation between expression of CD24 and clinicopathological parameters. A p value < 0.05 was considered as significant.

Results

Our group consisted of 44 patients. Ages of the patients ranged from 37 to 80 years (median 57 years). There were five patients under 46 years (11.4%), and 39 patients over 46 years (88.6%). Among 44 endometrial carcinomas, two (4.5%) were serous papillary carcinomas and 42 (95.5%) were endometrioid carcinomas. Among 44 endometrial carcinomas studied, 12 (27.3%) were diagnosed as nuclear grade 1, 25 (56.8%) were nuclear grade 2 and seven (15.9%) were nuclear grade 3. There were 27 patients (61.4%) with histological grade 1, ten patients (22.7%) with histological grade 2, and seven patients (15.9%) with histological grade 3, according to FIGO. There was invasion in less than half of the myometrium in 22 patients (50%). Twenty-five patients (56.8%) were Stage 1, five (11.4%) were Stage 2, and 14 (31.8%) were Stage 3. Eleven cases (25%) had lymph node metastases and 14 cases (31.8%) had vascular invasion. The clinicopathologic characteristics of the endometrial cancer patients are shown in Table 1.

Table 1. — *Clinicopathologic characteristics of endometrial carcinoma.*

Characteristics		N = 44 (%)
Age	> 45	39 (88.6%)
	≤ 45	5 (11.4%)
Nuclear Grade	I	12 (27.3%)
	II	25 (56.8%)
	III	7 (15.9%)
FIGO Grade	I	27 (61.4%)
	II	10 (22.7%)
	III	7 (15.9%)
Myometrial	< 1/2	22 (50%)
invasion	> 1/2	22 (50%)
FIGO Stage	I	25 (56.8%)
	II	5 (11.4%)
	III	14 (31.8%)
Nodal status	(+)	11 (25%)
	(–)	33 (75%)
Vascular	(+)	14 (31.8%)
invasion	(–)	30 (68.2%)
Peritoneal	(+)	5 (11.4%)
cytology	(–)	39 (88.6%)

CD24 expression was detected in 34 (77.3%) out of 44 cases. CD24 immunostainings showed separable staining qualities in endometrial carcinoma tissues such as membranous, cytoplasmic and membranous-cytoplasmic immunoreactivity. While there was no staining with CD24 in the superficial epithelium and stromal gland epithelium of normal endometrial mucosa, strong CD24 expression was observed in the neoplastic glands with a distinct interface between normal endometrial mucosa and tumor tissue (Figures 1a and b). A moderate to strong membranous, cytoplasmic, and membranous+cytoplasmic CD24 staining was observed in 17 (38.6%), ten (22.7%), and seven (15.9%) cases, respectively. Ten cases (22.7%) had no staining. Eight of the membranously stained endometrial tumors were nuclear grade 1 (47.0%), eight nuclear grade 2 (47%), and one was nuclear grade 3 (6%) (p = 0.017). One of ten cases (10%) which had cytoplasmic stainging was nuclear grade 1, five were nuclear grade 2 (50%), and four were nuclear grade 3 (40%) (p = 0.023). Six of seven cases which had cytoplasmic-membranous staining were nuclear grade 2 (85.7%) and one was nuclear grade 3 (14.3%) (p = 0.258). Fifteen of 17 cases which had membranous staining were histologic grade 1 (88.2%), one was grade 2 (5.9%), and the remaining one was histologic grade 3 (5.9%) (p = 0.011). One of ten cases (10%) which had cytoplasmic staining was histologic grade 1, six (60%) were histological grade 2, and three were histologic grade 3 (30%) (p = 0.02) (Figures 2, 3, 4). Three of seven cases (42.8%) which had cytoplasmic-membranous staining were histologic grade 1, three were histologic grade 2 (42.8%), and one was histologic grade 3 (14.4%) (p = 0.522).

Seventeen of 22 cases which had myometrial invasion of greater than one-half showed CD24 expression (77.3%) (p = 0.640). Seven of these 17 cases were membranous (p = 0.268), seven were cytoplasmic (p = 0.140), and three were cytoplasmic-membranous (p = 0.500).

Eighteen of 25 cases (72%) with Stage I had CD24 reactivity and 13 (72%) of them had membranous, one (5.5%) had cytoplasmic and four (22.5%) had cytoplasmic-membranous staining. Four of the five cases (80%) with FIGO Stage II showed CD24 reactivity and three (75%) of these four cases had membranous and one (25%) had cytoplasmic-membranous staining. Twelve of the 14 cases (85.7%) with FIGO Stage III showed CD24 reactivity and one (8.3%) of these 12 cases had membranous, nine (75%) had cytoplasmic, and two (17.7%) had cytoplasmic-membranous staining (p = 0.009). Ten of 14 (71.4%) cases which had vascular invasion and ten of 11 (99%) cases with lymph node metastasis showed CD24 reactivity. Nine of the ten cases with lymph node metastasis had cytoplasmic and one had cytoplasmic-membranous staining while there was no membranous staining (p > 0.05) (Table 2).

Table 2. — *Relation of cytoplasmic CD24 and membranous CD24 expression with various clinicopathologic factors in patients with endometrial carcinoma.*

		mCD24(–)	mCD24 (+)	cCD24(–)	cCD24 (+)	p mCD24	p cCD24
Nuclear grade	1 (n = 12)	4 (33.3%)	8 (66.7%)	11 (91.7%)	1 (8.3%)	0.017	0.023
	2 (n = 25)	17 (68.0%)	8 (32.0%)	20 (80.0%)	5 (20.0%)		
	3 (n = 7)	6 (85.7%)	1 (14.3%)	3 (42.9%)	4 (57.1%)		
FIGO grade	1 (n = 27)	12 (44.4%)	15 (55.6%)	26 (96.3%)	1 (3.7%)	0.011	0.002
	2 (n = 10)	9 (90.0%)	1 (10.0%)	4 (40.0%)	6 (60.0%)		
	3 (n = 7)	6 (85.7%)	1 (14.3%)	4 (57.1%)	3 (42.9%)		
Myometrial invasion	> 1/2 (n = 22)	12 (54.5%)	10 (45.5%)	19 (86.4%)	3 (13.6%)	0.268	0.140
	< 1/2 (n = 10)	15 (68.2%)	7 (31.8%)	15 (68.2%)	7 (31.8%)		
FIGO stage	I (n = 25)	12 (48.0%)	13 (52.0%)	24 (96.0%)	1 (4.0%)	0.009	0.000
	II (n = 5)	2 (40.0%)	3 (60.0%)	5 (100.0%)	0 (0.0%)		
	III (n = 14)	13 (92.9%)	1 (7.1%)	5 (35.7%)	9 (64.3%)		
Vascular invasion	(+) (n = 14)	12 (85.7%)	2 (14.3%)	6 (42.9%)	8 (57.1%)	0.024	0.001
	(–) (n = 30)	15 (50.0%)	15 (50.0%)	28 (93.3%)	2 (6.7%)		
Nodal status	(+) (n = 11)	11 (100.0%)	0 (00.0%)	2 (18.2%)	9 (81.8%)	0.002	0.000
	(–) (n = 33)	16 (48.5%)	17 (51.5%)	32 (97.0%)	1 (3.0%)		

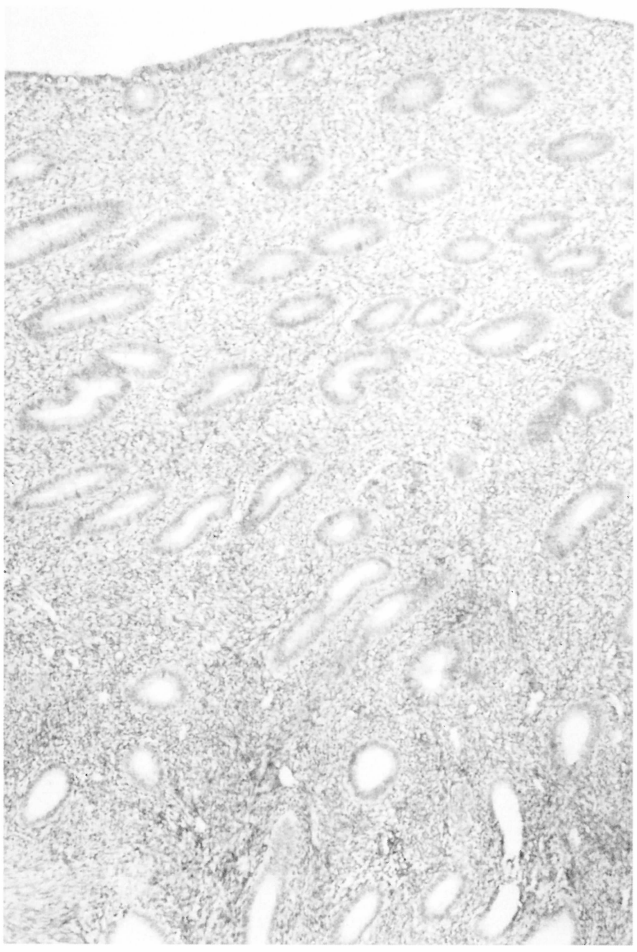


Fig. 1a

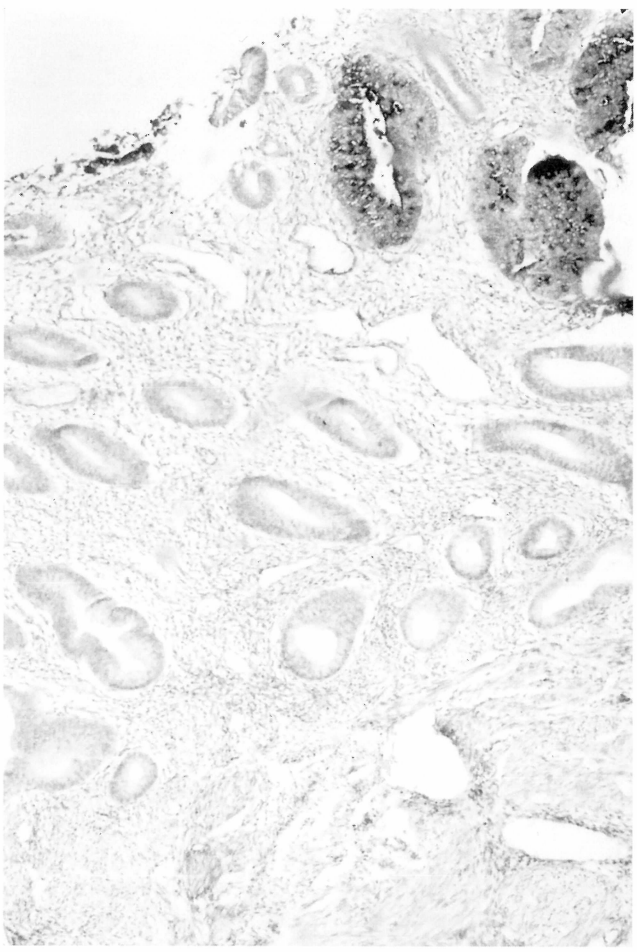


Fig.

Figure 1a. — No staining in proliferative endometrial glands.

Figure 1b. — CD24 immunohistochemistry, interface between endometrial mucosa and endometrial carcinoma. Strong CD24 expression was observed in the neoplastic glands with a distinct interface between proliferative endometrial glands and endometrial cancer (DAB x 100).

ig. 2

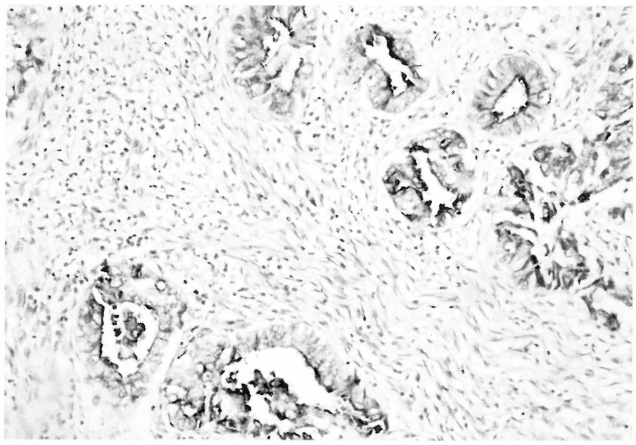
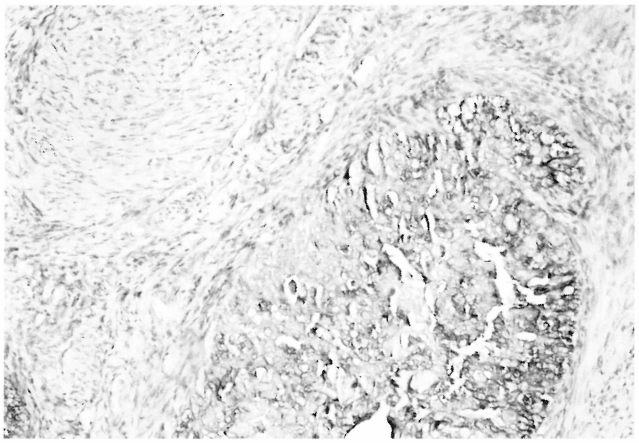


Fig. 3



g. 4

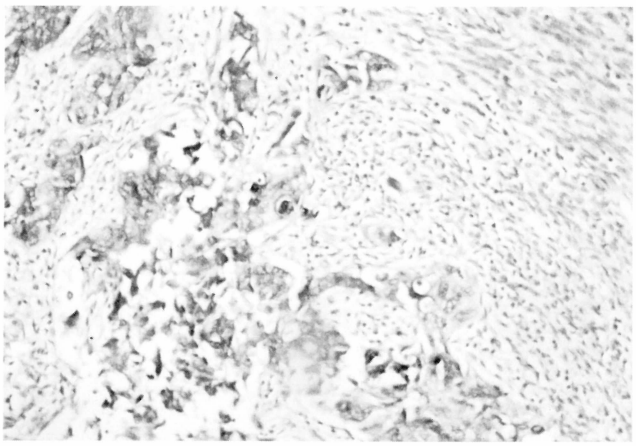


Figure 2. — Representative immunohistochemical analyses for membranous CD24 in FIGO grade 1 endometrial carcinoma (DAB x 200).

Figure 3. — Representative immunohistochemical analyses for cytoplasmic CD24 in FIGO grade 2 endometrial carcinoma (DAB x 200).

Figure 4. — Representative immunohistochemical analyses for cytoplasmic CD24 in FIGO grade 3 endometrial carcinoma (DAB x 200).

Discussion

The human CD24 antigen is a glycoprotein composed of a short peptide core of only 31 to 35 amino acids with a very high carbohydrate content [5, 6]. CD24 has been identified as a ligand to P-selectin, and it is conceivable that this function contributes to a more aggressive metastatic behavior of CD24-positive tumor cells, as in vitro evidence suggests [7]. Physiologically P-selectin is expressed by activated endothelial cells and platelets and plays an important role in marginal adhesion and migration of cells under shear forces in the bloodstream. Its primary ligand is P-selectin glycoprotein ligand-1 (PSGL-1), which is expressed by neutrophils [6]. It is conceivable that CD24-expressing tumor cells can spread more easily due to their capacity to form thrombi with activated platelets or to adhere to endothelia in the bloodstream, which has been shown for CD24 expressing breast cancer cells [14]. This mechanism of metastasis does not only require expression of CD24 in the tumor, but also expression of P-selectin in platelets and endothelia of the vasculature of the target organs [12, 14, 15]. MEDLINE and PubMed were searched to identify peer-reviewed English-language studies published between January 1965 and January 2006 reporting on CD24 expression in endometrial cancer. To our knowledge this is the first study evaluating CD24 expression in endometrial cancer. In our study, 77.3% of the cases with endometrial carcinoma showed immunohistologically positive staining for CD24.

It is well known that lymph node and distant metastases are determinants of poor prognosis for patients with advanced stages in endometrial carcinoma [3, 4]. By the subtractive technique, CD24 was selected as one of the metastasis-associated genes, and CD24 was confirmed to be over-expressed in the metastatic phenotype [13]. In our study, ten of 11 cases (90.1%) that had lymph node metastasis had CD24 positive reactivity. There were statistically significant relationships detected between CD24 expression and lymph node metastasis and advanced stage.

In the study of Kristiansen *et al.*, among 56 cases of ovarian carcinomas four had metastatic disease when diagnosed [11]. In our study, there were 14 FIGO Stage 3 cases. In 12 there was CD24 positive staining. Nine of the 12 cases that had positive staining showed cytoplasmic staining. A significant relationship was detected between FIGO stage and CD24 expression. In the present study we revealed a significant relationship between cytoplasmic CD24 expression, advanced FIGO stage, grade and nodal status. There was no significant relationship between CD24 expression and myometrial depth of invasion by the tumor. When histologic grade and stage increased, cytoplasmic reactivity superseded membranous immunoreactivity. Lim *et al.* reported a correlation between cytoplasmic expression of CD24 and lymph

node metastasis [20]. Our results were in line with the literature findings in that we showed a positive correlation between cytoplasmic expression of CD24 and lymph node status. Three of the five cases that had positive peritoneal cytology showed cytoplasmic CD24 immunoreactivity. Although a significance was not reached due to the low number of cases with positive peritoneal cytology, this finding substantiated a relationship between the advanced stage of the tumor and CD24 expression.

Conclusions

Significant rates of CD24 positivity have been reported for a variety of the most common human tumors [16-20]. Moreover, in several tumor entities higher rates of CD24 expression or CD24 positivity were significantly associated with shorter patient survival [10-12]. We feel that these observations are consistent with in vitro data supporting a P-selectin dependent pro-metastatic function, and thus qualify to underscore the importance of CD24 in the metastatic progression of human carcinomas. Our findings demonstrate that positive CD24 expression occurs in a subset of endometrial carcinomas with advanced stage, advanced grade and positive lymph nodes, and the positive expression of cytoplasmic CD24 characterizes endometrial carcinomas with a more aggressive phenotype. However, the emphasis on the adhesive function of CD24 does not exclude an additional role for the growth regulation of tumor cells. It is expected that the description of CD24 as a prognostic marker in human tumors may open up new diagnostic options and possibly CD24 represents a target for therapeutic purposes. This study establishes CD24 expression as a poor prognostic tumor marker in endometrial carcinomas. CD24 expression in endometrial carcinomas can define an aggressive phenotype and take part in tumor progression.

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