CD24 Expression in Colorectal Carcinoma

Kolorektal Karsinomda CD24 Ekspresyonu

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Abstract
Purpose: 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 is designed to examine the prognostic significance of CD24 in colorectal cancer.
Material and Methods: Forty two colorectal carcinoma tissues were immunostained for CD24 antibody. Cytoplasmic and membranous immunoreactivity semi quantitatively scored. Chi-Square test was used to assess the statistical significance of the correlation between expression of CD24 and clinicopathological parameters.
Results: Thirty eight cases (90.5%) showed CD24 expression. Membranous and cytoplasmic staining of CD24 was significantly associated with the histological grade (p=0.011, p=0.023). In well differentiated colorectal tumors membranous staining was diffuse and in less differentiated tumors cytoplasmic CD24 expression was prominent. There was no statistically significant relation with CD24 expression and clinical stage, size and location of tumor, lymph node metastasis, perineural invasion and vascular invasion.
Conclusion: In the light of our findings in colorectal carcinomas, CD24 expression was evaluated as a new prognostic marker that could be used immunohistochemically.

Key Words: **CD24 protein, human; Colorectal Neoplasms; Immunohistochemistry.**

Özet
Amaç: CD24, çeşitli solid tümörlerin metastatik tümör gelişiminde rol oynayan bir hücre adezyon molekülüdür. Ekspresyonunun çeşitli tümörlerin prognozu ile ilişkili olduğu bilinmektedir. Bu çalışma CD24'ün kolorektal kanserde prognostik anlamlılığını incelemek için planlandı.
Gereç ve Yöntemler: Kırkiki kolorektal karsinom dokusu immunohistokimyasal olarak CD24 antikoru ile boyandı. Sitoplazmik ve membranöz immunoreaktivite semikantitativ olarak puanlandırıldı. CD24 ekspresyonu ve klinikopatolojik parametreler arasındaki ilişkinin istatistiksel anlamlılığını değerlendirmek için Ki-kare testi kullanıldı.
Bulgular: Otuzsekiz olguda (%90.5) CD24 ekspresyonu izlendi. Membranöz ve sitoplazmik CD24 boyanması histolojik grade ile anlamlı şekilde ilişkili bulundu (p=0,011; p=0,023). İyi diferansiye kolorektal kanserde membranöz boyanma diffüz iken, kötü diferansiye tümörlerde sitoplazmik CD24 ekspresyonu belirgindi. CD24 ekspresyonu ile klinik evre, tümör boyutu ve yerleşimi, lenf nodu metastazı, perinöral invazyon ve damar invazyonu arasında istatistiksel olarak anlamlı bir ilişki bulunamadı.
Sonuç: Bulgularımız ışığında CD24 ekspesyonu, kolorektal karsinomlarda immunohistokimyasal yöntemle kullanılabilecek yeni bir prognostik belirleyici olarak değerlendirildi.

Anahtar kelimeler: **İnsan CD24 Proteini; İmmunohistokimya; Kolorektal Meoplazmlar.**

Submitted : February 19, 2008
Revised : July 16, 2008
Accepted : August 06, 2008

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Introduction

Colorectal cancer is the third most common cause of death from cancer in both sexes in the western world (1). Although this disease is surgically curable in early stages, the tumor frequently does not become symptomatic before the metastatic stage, which is associated with high mortality (2). It is of general importance to predict the biology of the tumor and, thus, the course of the disease in the individual patient to ensure adequate therapy and surveillance (1,2). Currently, the most important conventional prognostic factors for patient survival are histological tumor grade and tumor stage at time of diagnosis, including depth of tumor invasion, involvement of regional lymph nodes, and metastatic spread to distant organs (2).

Additionally, molecular markers are being sought and established to allow for a refined classification of prognosis (3). The molecular biology of colorectal carcinoma is under intense international investigation in order to characterize new molecular marker genes which might be helpful in diagnosis or therapy. A gene that recently has gained new interest is CD24 (3,4).

CD24 is a small, heavily glycosylated, mucin-like cell surface protein. It 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 (5-7). To date, only limited data on CD24 RNA expression in colonic cancer have been published (4).

CD24 gene over expression may serve as a potential early tumor biomarker. In the gastric cancer (NUGC-2) and colonic cancer (COLO-2001) cell lines, gene expression of CD24 core polypeptide was detected in RNA blot analysis. Since both gastric and colonic cancers are supposed to originate from the cells in the proliferative zone located in the neck region of gastric mucosa or in the lower region of intestinal crypts, it is reasonable to assume that carcinoma cells of gastrointestinal origin express CD24 core polypeptide as a phenotypic marker of their origin (3).

In this study, we aimed to investigate the expression of CD24 in colorectal carcinomas by immunohistochemistry and its relationship with clinicopathological features.

Materials and Methods

Patients and samples. Tissue samples from 42 patients with colorectal carcinoma which were diagnosed at the Department of Pathology, Suleyman Demirel University School of Medicine, between 1998 and 2005 were included in this study. The age, gender, tumor location, vascular and perineural invasion and pTNM stage were evaluated by reviewing the medical charts and pathological records. Glass slides were reviewed for histological classification according to the World Health Organization (WHO) classification system (8). According to differentiation, the histological grades of tumors were subdivided into well-differentiated (grade 1), moderately differentiated (grade 2), and less differentiated (grade 3). Tumors were staged according to the TNM Staging and Astler and Coller Classification Systems (9).

Immunohistochemistry. Immunohistochemical analysis for CD24 was performed on formalin fixed, parafin-embedded archival tissue using the streptavidin-biotin-peroxidase technique. For all cases, 4µm-thick histological sections were 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 were incubated with mouse monoclonal anti CD24 antibody (1:100, Clone 24C02, Neomarkers). Sections were than treated with 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, cytoplasmic and membranous-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 11 was used. We used Chi-Square test to assess the statistical significance of the correlation between expression of CD24 and clinicopathological parameters.

Results

Our group was consisted of 26 (61.9%) men and 16 (38.1%) women. Ages of the patients ranged from 27 to 86 years (median 55.8 ± 14.39 years). In 42 cases, minimal tumor diameter was 2.5, maximally 11 cm and mean was 5.9 cm. The distribution of tumors according to the anatomic location was as follows: colon 15 (35.7%), rectum 20 (47.6%), rectosigmoid 5 (11.9%) and the cecum 2 (4.8%) cases. Among 42 colorectal carcinomas studied, 26 (61.9%) were diagnosed as well differentiated, 10 (23.8%) moderately differentiated, and 6 (14.3%) poorly differentiated. Twenty four (57.1%) cases had lymph node metastases. Fifteen (35.7%) cases had perineural invasion. Twenty one (50%) cases had vascular invasion and 3 (7.1%) cases had distant metastases. Clinical stage distribution at time of diagnosis was as follows: stage I 2 (4.8%), stage II 15 (35.7%), stage III 20 (47.6%), stage IV 5 (11.9%). According to Astler and Coller grading system cases were reported as following: 2 cases (4.8%) A, 15 cases (35.7%) B, 20 cases (47.6%) C, 5 cases (11.9%) The clinicopathological characteristics of the colorectal cancers are shown in Table I.

CD24 expression was detected in 38 (90.5%) out of 42 cases. CD24 immunostainings showed separable staining qualities in colorectal carcinoma tissues, a membranous, cytoplasmic and membranous-cytoplasmic immunoreactivity. Normal colon mucosa was virtually CD24 negative, although some cases exhibited a weak membranous staining at the brush border. In general colorectal carcinomas showed higher levels of CD24 expression, often with a relatively abrupt up-regulatin in the transition zone of normal mucosa to neoplastic epithelium. (Figure 1). A moderate to strong membranous, cytoplasmic, membranous and cytoplasmic CD24 staining was observed in 16(38.1%), 11(26.2%), 11 (26.2%) cases respectively. Four cases (9.5%) had no staining. Membranous staining of CD24 showed negative correlation (p=0.011), and cytoplasmic CD24 expression showed positive correlation (p=0.023) with the histological grade (Table 2). In well differentiated colorectal tumors membranous staining was diffuse and when differentiation was getting decreased, membranous CD24 expression was vanished but cytoplasmic CD24 expression was prominent (Figure 2, 3, 4). There was no statistically significant relation with CD24 expression and clinical stage, size and location of tumor, lymph node metastasis, perineural invasion, and vascular invasion.

Table 1: Clinicopathological characteristics of the colorectal cancers

Variable		N (%)
All cases		42 (100)
Age	>60	21 (50)
	<60	21(50)
Gender	Male	26 (61.9)
	Female	16(38.1)
Grade	I	26(61.9)
	II	10(23.8)
	III	6(14.3)
Stage	I	2 (4.8)
	II	15(35.7)
	III	20(47.6)
	IV	5(11.9)
Dukes stage	A	2(4.8)
	B	15(35.7)
	C	20(47.6)
	D	5(11.9)
Nodal status	(+)	24 (57.1)
	(-)	18 (42.9)
Distant metastasis	Mx	39 (92.9)
	pM1	3(7.1)
Tumor location	colon	15(35.7)
	rectum	20(47.6)
	rectosigmoid	5(11.9)
	cecum	2(4.8)
Perineural invasion	(+)	15(35.7)
	(-)	27(64.3)
Vascular invasion	(+)	21(50%)
	(-)	21(50)

Table 2: Relation of cytoplasmic CD24 and membranous CD24 expression and various clinicopathological factors in all patients with colorectal carcinoma.

		mCD24(-)	mCD24 (+)	p:	cCD24(-)	cCD24 (+)	p:
Age	>60	13(61.9%)	8(38.1%)	0.63	17(81%)	4(19%)	0.24
	<60	13(61.9%)	8(38.1%)		14(66.7%)	7(33.3%)	
Dukes stage	A	1(50%)	1(50%)	0.48	1(50%)	1(50%)	0.28
	B	9(60%)	6(40%)		10(66.7%)	5(33.3%)	
	C	12((60%)	8(40%)		16(80%)	4(20%)	
	D	4(80%)	1(20%)		4(80%)	1(20%)	
Stage	I	1(50%)	1(50%)	0.48	1(50%)	1(50%)	0.28
	II	9(60%)	6(40%)		10(66.7%)	5(33.3%)	
	III	12(60%)	8(40%)		16(80%)	4(20%)	
	IV	4(80%)	1(20%)		4(80%)	1(20%)	
Grade	1	12(46.2%)	14(53.8%)	0.01	23(88.5%)	3(11.5%)	0.02
	2	10(100%)	0(0%)		5(50%)	5(50%)	
	3	4(66.7%)	2(33.3%)		3(50%)	3(50%)	
Distant metastasis	Mx	23(59%)	16(41%)	0.23	29(74.4%)	10(25.6%)	0.61
	pM1	3(100%)	0(0%)		2(66.7%)	1(33.3%)	
Vascular invasion	(+)	13 (61.9%)	8(38.1%)	0,62	16(7 6.2%)	5(23.8%)	0.50
	(-)	13 (61.9%)	8(38.1%)		15(71.4%)	6(28.6%)	
Nodal Status	(+)	15(62.5%)	9(37.5%)	0.50	18(75%)	6(25%)	0.56
	(-)	11(61.1%)	7(38.9%)		13(72.2%)	5(27.8%)	

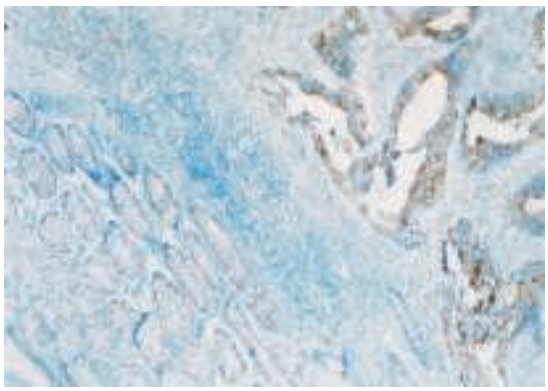

Figure 1. CD24 immunhistochemistry, interface between colon mucosa and colon carcinoma. Strong CD24 expression was observed in the neoplastic glands with a distinct interface between colon mucosa and colon cancer (DABX100).

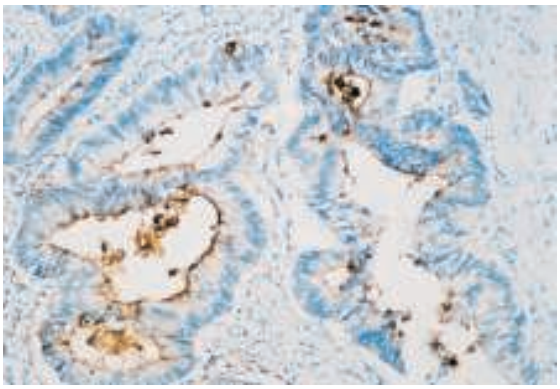

Figure 2. Representative immunohistochemical analyses for CD24 in well-differentiated colorectal carcinomas (DABX200).

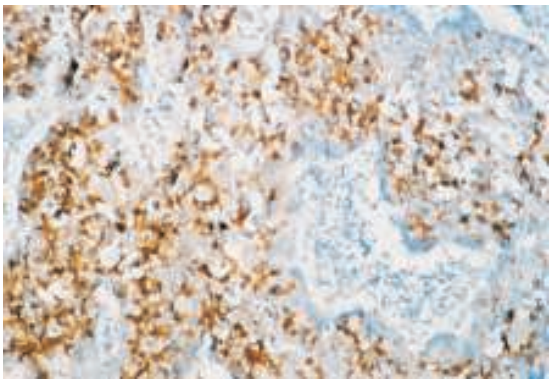

Figure 3. Representative immunohistochemical analyses for CD24 in moderately-differentiated colorectal carcinomas (DABX200). Cytoplasmic and membranous staining.

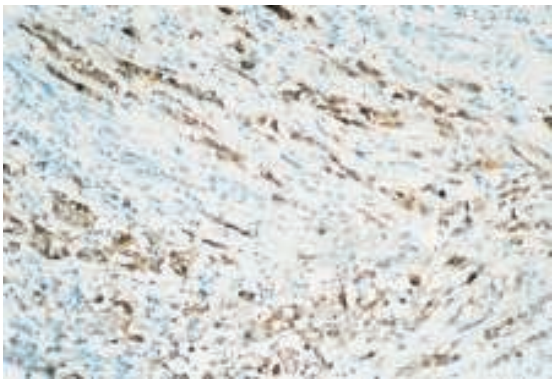

Figure 4. Representative immunohistochemical analyses for CD24 in less-differentiated colorectal carcinomas, (DABX200).

Discussion

The human CD24 antigen is a glycoprotein composed of a short peptide core of only 31 to 35 amino acids with very high carbohydrate content (10). 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 (10,11). 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. 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 (11). 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 (10).

In our study, tissue of 42 colorectal carcinoma cases were immunohistologically examined for the expression of CD24 protein. We found CD24 expression in 90.5% of cases.

CD24 positivity has been reported for variety of the most common human tumors. CD24 has been described in B-cell neoplasia, renal cell carcinoma, small cell lung cancer, nasopharyngeal carcinoma, hepatocellular carcinoma, bladder carcinoma, glioma, breast cancer and ovarian cancer (5, 12-19). Moreover, in several tumor entities higher rates of CD24 expression or CD24 positivity were significantly associated with shorter patient survival times (20). 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. In this study we found CD24 over expression in neoplastic colonic epithelia of carcinomas. Moreover, staining of CD24 was significantly associated with the histological grade.

It is well known that the depth of tumor invasion, distant metastasis and lymph node metastasis are the major prognostic factors in colorectal carcinoma (2). Recently, the expression of different cell surface antigens in various malignant tumors has been reported to be one of the biologic markers of malignant potential. In our study, 87.5 % of cases with lymph node metastasisÊstained with CD24Êpositively. Cytoplasmic staining of CD24 was more apparent in less differentiated tumors. In study of Kristiansen et al (21), which included 56 cases of invasive ovarian carcinoma, 4 cases had distant metastasis. Three of these casesÊwere stained positively with CD24 (21). In our study only three cases had known distant metastasis at the time of diagnosis. There was cytoplasmic CD24 expression in all these cases.

Weichert et al. (22) revealed a significant relationship between cytoplasmic CD24 expression and tumor grade, lymph node status in colorectal carcinomas. Higher rates of membranous CD24 immunoreactivity were associated with systemic metastasis but showed no significant correlation to other clinicopathological variables (22). In our study membranous and cytoplasmic staining of CD24

was significantly associated with the histological grade but there was no significant relation with other clinicopathological variables. In well-differentiated colorectal tumors, membranous staining was diffuse but, less differentiated tumors showed prominent cytoplasmic CD24 expression.

Significant rates of CD24 positivity have been reported for a variety of the most common human tumors. Moreover, in several tumor entities higher rates of CD24 expression or CD24 positivity were significantly associated with shorter patient survival times (20). 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. In this study we found CD24 overexpression in neoplastic colonic epithelia of carcinomas. Moreover, CD24 expression was significantly associated with the histological grade.

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