The Korean Journal of Pathology
2006; 40: 103-11

Enhanced CD24 Expression in Colorectal Cancer Correlates with Prognostic Factors

Yoon-La Choi[5] · Yan Hua Xuan[1,7] Sang-Jeon Lee[2] · Seon Mee Park[3] Wun Jae Kim[4] · Hee-Jin Kim[6] Seok-Hyung Kim[1]

[1]Department of Pathology, [2]Department of Surgery, [3]Department of Internal Medicine, [4]Department of Urology, Chungbuk National University College of Medicine, Cheongju; [5]Department of Pathology, [6]Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [7]Department of Pathology, Yanbian University College of Medicine, Yanji, China

Received : January 3, 2006
Accepted : March 14, 2006

Corresponding Author
Seok-Hyung Kim, M.D.
Department of Pathology, College of Medicine, Chungbuk National University, 62 Gaesin-dong, Heungduk-gu, Cheongju 361-763, Korea
Tel: 043-269-6276
Fax: 043-269-6269
E-mail: platoshkim@chungbuk.ac.kr

Background : CD24 was originally described as a B cell-specific marker, however its aberrant expression in various solid tumors has recently been reported. Our objective was to determine the pattern and extent of the CD24 expression in colorectal cancer and its related lesions, and to clarify its correlation with clinico-pathological parameters and especially those associated with patients' prognoses. **Methods :** A total of 307 colorectal cancers and the related lesions (150 carcinomas, 30 high-grade adenomas, 49 low-grade adenomas, 41 hyperplastic polyps, and 37 normal colorectal epithelia) were immunohistochemically analyzed by treating CD24 monoclonal antibody onto tissue embedded paraffin blocks. **Results :** CD24 expression was very rarely observed in the normal epithelia, hyperplastic polyps, and low-grade adenomas; however, in high-grade adenomas, the CD24 expression was shown to be mildly increased in the cytoplasm (13.3%). In carcinomas, the CD24 expression was increased substantially in both the membrane (38.0%) and the cytoplasm (44.7%). The expression of CD24 in the membrane was positively correlated with tumor size ($p<0.01$). The CD24 expression in the cytoplasm was positively correlated with several unfavorable parameters, including a larger tumor size ($p<0.01$), a higher tumor grade ($p<0.01$), a higher rate of tumor invasion ($p<0.05$), and a higher pTNM stage ($p<0.05$). **Conclusion :** High levels of CD24 expression in the membrane and cytoplasm were characteristic in colorectal cancer, and the cytoplasmic CD24 expression was correlated with several unfavorable clinical parameters.

Key Words : CD24; Colorectal neoplasm; Immunohistochemistry

CD24 consists of a small protein core that contains 27 amino acids. It is extensively glycosylated and then bound to the membrane via a phosphatidylinositol anchor.[1,2] CD24 was originally described as a B cell-specific marker that was expressed at the early stages of B cell development. Subsequent studies have demonstrated a high degree of CD24 expression on neutrophils and renal tubular epithelial cells, and several studies have also shown that CD24 can be expressed on several solid (non-hematopoietic) tumors including small cell carcinoma,[3] neuroblastoma,[4] renal cell carcinoma,[5] ovary serous adenocarcinoma,[6] and prostate adenocarcinoma.[7,8]

Colorectal carcinoma ranks the second most common cause of cancer deaths in Westernized countries. With an estimated 134,000 new cases per year and about 55,000 deaths per year occurring in association with this condition, the disease is responsible for 10% of all-cancer related deaths in the United States.[9] There are only a limited number of reports on the CD24 expression in colorectal cancer, and there have been no comprehensive studies designed to determine the clinical significance of the CD24 expression in the context of colorectal cancer. Akashi *et al*. have demonstrated the CD24 expression in a few colorectal cancer cell lines with using RT-PCR.[10] Nestl *et al*. have demonstrated the expression of CD24 mRNA in 10 cases of colorectal carcinoma via *in situ* hybridization.[11] Saito *et al*. have revealed an increased CD24 expression in two colon cancer specimens, with using suppression subtractive hybridization (SSH).[12] However, these findings were not confirmed at the protein level. In addition, all of these studies were conducted on an extremely small scale,

and the results were not correlated with the clinico-pathological findings. On the other hand, the significance of CD24 expression has recently been investigated in several hematological and non-hematological malignancies other than colon cancer, in terms of both tumor progression and the ultimate prognoses.[13] In cases of ovarian cancer, high levels of cytoplasmic CD24 expression were correlated with unfavorable clinical parameters and poor prognoses.[6,14] In addition, the upregulation of CD24 is considered to be an independent prognostic marker in the cases of lung,[15] breast,[16,17] and prostate cancer.[7,8] In this regard, we conducted a comprehensive study of CD24 expression at the protein level via performing immunohistochemistry in cases of colorectal cancer and its precancerous lesions to determine the correlation between the CD24 expression and a set of clinico-pathologic parameters including the patient survival rates and survival durations. Our results demonstrated that aberrant and inappropriate CD24 expression in cases of colorectal cancer was significantly associated with the progression of cancer.

MATERIALS AND METHODS

Patients, tissue samples, and reagents

We investigated 307 cases of colorectal carcinoma and its related lesions, and these were obtained from the surgical pathology files kept by the Department of Pathology of the Chungbuk National University Hospital. The criteria for inclusion were as follows: the histopathologic diagnosis of colorectal lesions, the availability of clinical follow-up data, and the availability of paraffin-embedded tissue specimens. The selected cases included 150 cases of adenocarcinoma, 30 cases of high-grade adenoma, 49 cases of low-grade adenoma, 41 cases of hyperplastic polyps, and 37 cases of normal colorectal epithelium. All of the cancer patients had undergone surgical operations, and none had received either chemotherapy or radiotherapy before their surgical resections.

The pathology slides were reviewed in order to analyze the pathologic parameters, including tumor size, the histological grade, depth of invasion, and the presence of nodal metastasis. The 150 colorectal carcinomas [patient age range=27-87 years; average age=60.54 years; 75 female and 75 male cases] encompassed 90 early cases (pTNM stage I=24, pTNM stage II=66), and 60 advanced cases (pTNM stage III=44, pTNM stage IV=16). Of these cases, 28 cases (18.8%) were classified as well-differentiated adenocarcinomas, 107 cases (72.5%) as moderately-differentiated, and 13 cases (8.7%) as poorly-differentiated adenocarcinomas. TNM staging was assessed according to the staging system established by the American Joint Committee on Cancer (AJCC).[18] The tissue samples of benign lesions including the adenomas and hyperplastic polyps, were mostly obtained from the independent patients and partly from the tissues in the vicinity of the main malignant tumor. The tissue samples of normal colorectal epithelium were obtained from tissues in the vicinity of main malignant tumor.

We employed tissue microarray slides for the purpose of performing effective detection. For preparation of these slides, we punched tissue cores (3.0 mm in diameter) from the original blocks and then inserted them into the new paraffin blocks (each of which contained 30 holes) that were prepared to accept the tissue cores. The serially-sectioned slides were then produced. Each tissue microarray slide (1×3 inches) held 30 specimens, which allowed us to analyze 30 specimens simultaneously with a minimum of variation during the staining process. Each specimen was round in shape, and it was 3.0 mm in diameter, this provided a sufficient amount of tissue for the histopathologic analysis.

All the archival materials had been routinely fixed in 10% neutral-buffered formalin and then embedded in paraffin. Four micrometer-sections were prepared on silane-coated slides (Sigma, St Louis, MO, USA). The immunostaining kits were purchased from DAKO, Inc. (Glostrup, Denmark).

The immunohistochemical staining procedure

The tissue sections in the microslides were deparaffinized with xylene, hydrated in series of graded alcohol solutions and they were immersed in 3% H_2O_2 in order to quench the endogenous peroxidase activity. The sections were then microwaved in 40 mM Borate buffer (pH 8.3) supplemented with 1 mM EDTA and 1 mM NaCl for 20 min for enhancing antigen retrieval.[19,20] After antigen retrieval, avidin and biotin were consecutively applied to the slides in order to prevent any endogenous biotin-related background staining.[21] The sections were then incubated with 1:100 diluted anti-CD24 monoclonal antibody (Ab-2, clone 24C02, Neomarkers, Fremont, CA) for 60 min; this was followed by three successive rinses with washing buffer and then further incubation with biotinylated goat anti-mouse Abs (DAKO) for an additional 20 min. After rinsing, the tissue sections were incubated with HRP-conjugated streptavidin (DAKO) for 20 min at room temperature. The slides were washed and the chromogen was developed for 5 min with liquid 3,3'-diaminoben-

zidine (DiNonA, Seoul, Korea). The slides were counter-stained with Meyer's hematoxylin, dehydrated, and then mounted with Canada balsam for examination. We used distilled water with 0.1% tween 20 as a rinsing solution.[22]

Evaluation of results of immunohistochemical staining

The scoring method of Sinicrope *et al*. (1995)[23] was applied for the evaluation of both the intensity of the immunohistochemical staining and the proportion of the stained epithelial cells. The membranous and nuclear stainings were independently analyzed. The staining intensity was subclassified as follows: 1, weak; 2, moderate; or 3, strong. The positive cells were quantified as a percentage of the total number of epithelial cells, and the results were assigned to one of five categories: 0, <5%; 1, 5-25%; 2, 26-50%; 3, 51-75%; and 4, >75%. The percentage of positivity of the tumor cells and the staining intensity were then multiplied in order to generate the immunoreactive score (IS) for each of the tumor specimens. The positiviy of the immunostaining was determined when the IS was one or more. Each lesion was separately examined and scored by two pathologists (Y.L.C & S.H.K), and those cases with discrepant scores were discussed to obtain a consensus.

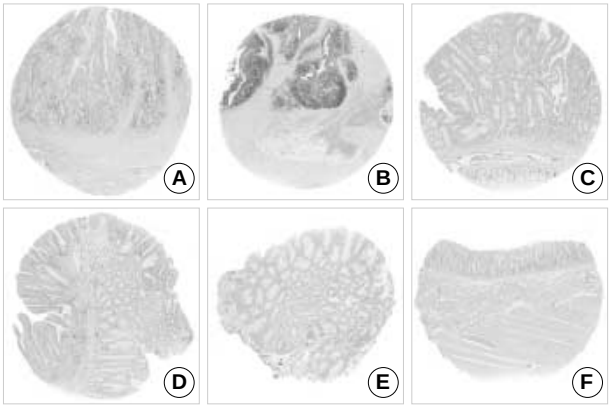

Fig. 1. Immunohistochemical staining of the microarray punches from the samples of colorectal adenocarcinoma and its related lesions. a, early stage adenocarcinoma; b, advanced stage adenocarcinoma; c, high grade adenoma; d, low grade adenoma; e, hyperplastic polyp; f, normal colorectal mucosa. Diffuse and strong expression of CD24 was observed in adenocarcinoma (A & B). Focal and weak staining was observed in high-grade adenoma (C). No expression was noted in low-grade adenoma (D), hyperplastic polyp (E), and normal colorectal mucosa (F).

Statistical analysis

Statistical analyses were conducted with Fisher's exact tests, Pearson's χ^2 tests, ANOVA, Mann-Whitney tests, Tukey's HSD, and Duncan's test as a *post hoc* test. With regard to the survival analysis, we analyzed 150 colorectal carcinoma patients via Kaplan-Meier analyses. We used log rank tests in order to compare the different survival curves. Univariate and multivariate survival analyses were then conducted with using the Cox regression model. The models were adjusted for age and gender of the patients, the location, grade, and stage of the tumors, the chemotherapy, and the membranous and cytoplasmic expression levels of CD24. p values less than 0.05 were regarded to be statistically significant. All statistical analyses were performed using the SPSS software (SPSS, Chicago, USA).

RESULTS

CD24 expression patterns in colorectal carcinoma and its related lesions

In the normal colorectal mucosa and precancerous lesions, we found that the CD24 expression was either completely absent or very low (Table 1). The CD24 membranous staining mostly showed apical localization rather than basolateral or circumferential pattern, and no significant differences were evident for the membranous staining among the benign lesions (Table 1, Fig. 1, 3). No significant difference was also observed in the

Table 1. Expression of CD24 in the colorectal carcinoma and its related lesions

Diagnosis	No.	Mean of IS of CD24 Immunostaining		P	Percentage of CD24 positive cases		P
		Membrane staining	Cytoplasmic staining		Membrane staining	Cytoplasmic staining	
Carcinoma	150	1.23±2.09	1.27±2.15	<0.001	57/150 (38.0%)	67/150 (44.7%)	<0.001
Adenoma High grade	30	0.03±0.18	0.17±0.46		1/30 (3.3%)	4/30 (13.3%)	
Adenoma Low grade	49	0.02±0.14	0.02±0.14		1/49 (2.0%)	1/49 (2.0%)	
Hyperplasia	41	0.00	0.00		0/41 (0%)	0/41 (0%)	
Normal	37	0.02±0.16	0.05±0.23		1/36 (2.7%)	2/36 (5.4%)	

IS, Immunostaining Score; P, probability.

CD24 cytoplasmic staining among the benign lesions except for the high grade adenoma. The expression rate was significantly higher in the high-grade adenoma than in the other benign lesions ($p<0.05$) (Table 1, Fig. 1). Both the membranous and cytoplasmic CD24 levels were significantly higher in the carcinoma than were those in any of the other benign lesions, including the precancerous lesions, in term s of both the average staining intensity (mean of the IS) and the expression rate ($p<0.001$) (Table 1).

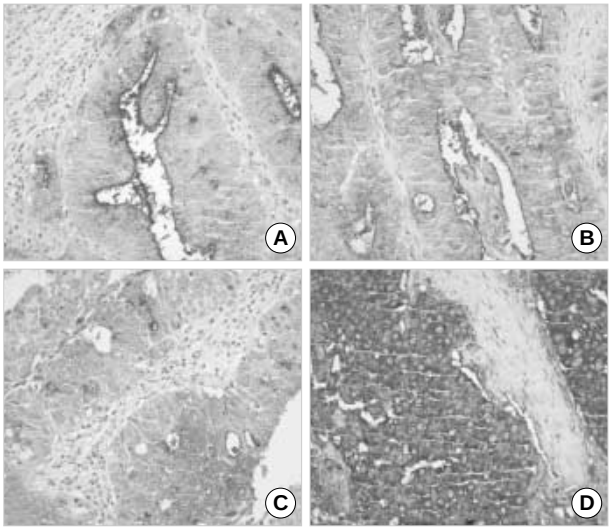

Fig. 2. Immunohistochemical staining of colorectal adenocarcinoma (high-power view). a, adenocarcinoma pTNM stage I; b, adenocarcinoma pTNM stage II; c, adenocarcinoma pTNM stage III; d, adenocarcinoma pTNM stage IV. Diffuse and strong CD24 expression is seen in all cases of adenocarcinomas (A-D). Strong membranous CD24 staining along the apical surface is observed in earlier stages of adenocarcinoma (A & B). However, in advanced stage, membranous staining was determined to be much weaker than in earlier stage (C & D).

Correlation of the CD24 expression with the clinico-pathological parameters

Neither the membranous nor the cytoplasmic CD24 expression was correlated with the patients' age, gender, lymph node metastasis, or the location of main tumor (Table 2). The average staining intensity in both the membrane and the cytoplasm was determined to be significantly higher in the cases with large tumor size ($p<0.05$). Both the membranous and cytoplasmic CD24 expressions were clearly associated with the grades of the tumors ($p<0.005$); however, the details of their patterns were different. The membranous CD24 staining was significantly higher in the grade 2 (moderately differentiated) tumors than in grade 1 (well differentiated) tumors and in the grade 3 (poorly differentiated) tumors. By contrast, the cytoplasmic staining increased proportionally with the grade of the tumor. Membranous CD24 expression was not found to be significantly correlated with direct tumor invasion or the pTNM stage (clinical stage), whereas the cytoplasmic expression was found to be increased in the cases with a higher degree of tumor invasion and

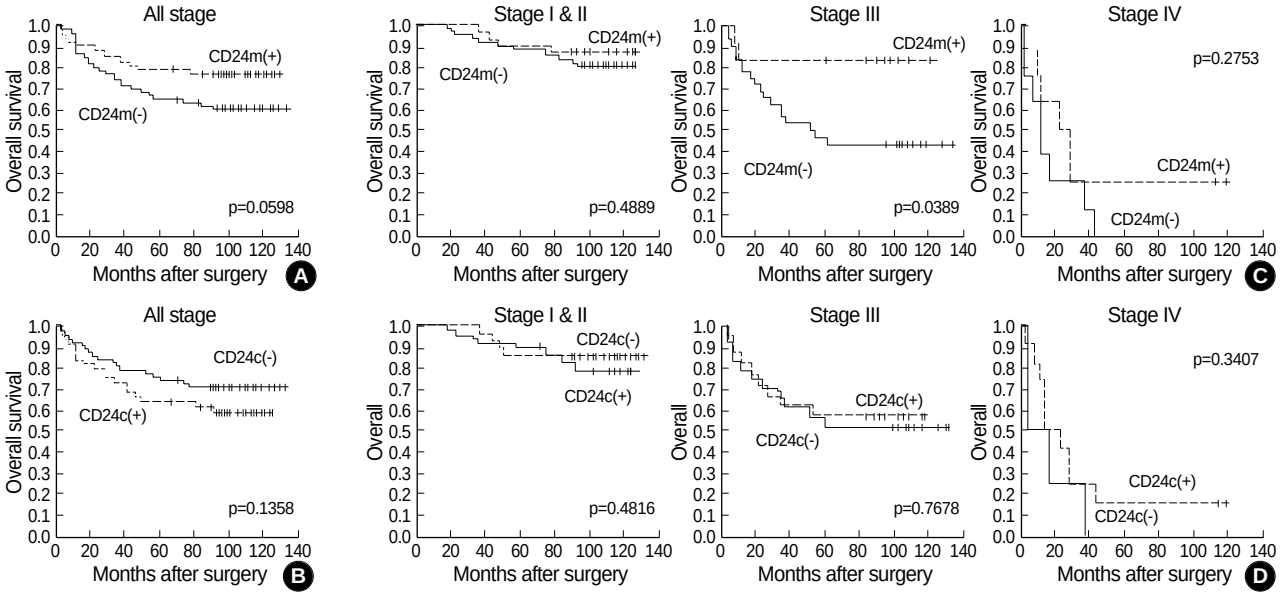

Fig. 3. Univariate survival analysis of CD24 expression in 150 cases of colorectal adenocarcinoma. Neither cytoplasmic (A) nor membranous staining (B) showed a statistically significant correlation with prognosis. However, when membranous expression was analyzed separately in each group of cancer stage (C), membranous staining was significantly associated with better prognosis in patients with stage III. On the other hand, cytoplasmic staining was not associated with patients' survival when analyzed separately by cancer stage (D).

Table 2. Correlation of CD24 membranous and cytoplasmic expression with clinicopathological features in colorectal carcinomas

Variables	No.	Mean of IS				CD24(+) No. (%)			
		Membrane staining	p	Cytoplasmic staining	p	Membrane staining	p	Cytoplasmic staining	p
Age									
≥60 years	96	1.29±2.12	0.614	1.41±2.24	0.292	38 (39.6%)	0.486	47 (49.0%)	0.124
<60 years	54	1.11±2.07		1.02±2.00		18 (33.3%)		19 (35.2%)	
Sex									
Female	75	1.00±1.82	0.186	1.11±1.77	0.365	30 (40.0%)	0.306	35 (46.7%)	0.311
Male	75	1.45±2.33		1.43±2.49		26 (34.7%)		31 (41.3%)	
Tumor size									
<6.0 cm	96	0.97±1.88	<0.05	0.91±1.44	<0.01	30 (31.3%)	0.077	38 (39.6%)	0.230
≥6.0 cm	52	1.69±2.43		1.87±3.00		24 (46.2%)		26 (50.0%)	
Node metastasis									
Negative	95	1.42±2.21	0.163	1.29±2.37	0.835	39 (41.1%)	0.227	37 (38.9%)	0.125
Positive	55	0.89±1.85		1.22±1.75		17 (30.9%)		29 (52.7%)	
Location									
Rectum	93	1.06±1.98	0.289	1.10±1.91	0.238	31 (33.3%)	0.299	39 (41.9%)	0.735
Non-rectum	57	1.44±2.26		1.53±2.51		24 (42.1%)		26 (45.6%)	
Grade									
I	28	0.25±0.84	<0.005	0.21±0.57	<0.005	3 (10.7%)	<0.005	4 (14.3%)	<0.005
II	107	1.54±2.32		1.50±2.20		48 (44.9%)		55 (51.4%)	
III	13	0.92±1.32		1.85±3.29		5 (38.5%)		7 (53.8%)	
Tumor invasion									
I	6	0.17±0.41	0.63	0.00±0.00	<0.05	1 (16.7%)	0.68	0 (0 %)	<0.05
II	19	1.53±2.93		0.89±1.59		6 (31.6%)		7 (36.8%)	
III	121	1.22±2.00		1.35±2.27		47 (38.8%)		55 (45.5%)	
IV	3	1.00±1.73		3.00±1.73		1 (33.3%)		3 (100%)	
pTNM staging									
I	24	1.25±2.66	0.253	0.54±1.28	<0.05	7 (29.2%)	0.18	6 (25.0%)	<0.05
II	66	1.42±2.06		1.29±2.09		29 (43.9%)		27 (40.9%)	
III	44	0.77±1.49		1.09±1.65		12 (27.3%)		21 (47.7%)	
IV	16	1.63±2.66		2.75±3.70		8 (50.0%)		12 (75.0%)	

an advanced pTNM stage; this finding was statistically significant ($p<0.05$) (Table 2, Fig. 2).

Univariate and multivariate survival analyses for the colorectal cancer patients

The univariate analysis using the Kaplan-Meier method revealed that gender, pTNM stage, tumor grade, and chemotherapy were associated with the patients' survival (Table 3). The correlation of the membranous and cytoplasmic CD24 expressions with the overall survival rate of the colorectal cancer patients was also analyzed by using the Kaplan-Meier method (Table 3, Fig. 3A, B). However, neither the membranous nor cytoplasmic CD24 expression were found to be associated with the patients' survival with any statistical significance. We further analyzed the effect of the CD24 expression on the overall survival rate according to the pTNM stage. The CD24 expression in the membrane (CD24m) was significantly correlated with a better prognosis for the patients with stage III but not for the patients with other stages (Fig. 3C), whereas the CD24 expression in the cytoplasm (CD24c) was not associated with the patients' survival in any stages (Fig. 3D).

We also performed univariate and multivariate analyses in order to assess the relative contribution of the important clinicopathologic parameters, as well as the CD24 expression, on the survival of the patients with using the Cox-proportional hazards regression model (Table 4). Specifically, gender, age, tumor location, chemotherapy, tumor grade, and pTNM stage were included as the clinico-pathologic parameters. Among these, tumor grade, pTNM stage, and the membranous CD24 expression were selected as independent prognostic factors. The membranous CD24 expression (CD24(m)) was significantly associated with a better prognoses ($p=0.01$) (The ratio of risk=0.367), while the cytoplasmic CD24 expression (CD24(c)) was not an independent prognostic factor (Table 4). We also separately performed the univariate and multivariate analyses for each clinical stage separately (Table 5). Specifically, gender and chemotherapy were clearly associated with the patients' prognosis in stage III (Table 5). The

Table 3. Univariate analysis of the overall survival of colorectal cancer patients using Kaplan-Meier survival analysis

Variables	No. of patients	Overall survival (%)		p-value
		1-year	3-year	
Sex				
Male	75	86.16	70.38	
Female	74	90.41	83.37	0.0487
Age				
<60	53	92.38	76.98	
≥60	96	86.05	77.08	0.8427
pTNM stage				
I-II	90	100.00	93.93	
III	44	79.55	63.64	
IV	16	50.00	25.00	<.0001
Tumor size				
<6.0	95	90.17	77.67	
≥6.0	52	84.62	75.00	0.5528
Location				
Colon	57	83.73	78.11	
Rectum	92	91.21	76.58	0.1829
Tumor grade				
I	28	92.86	89.14	
II	107	92.35	82.36	
III	13	46.15	7.69	<.0001
Chemotherapy				
No	44	90.58	87.83	
Yes	104	90.38	73.08	0.0460
CD24 (m)				
<1	94	87.05	73.68	
≥1	56	90.69	83.01	0.0598
CD24 (c)				
<1	84	91.53	81.38	
≥1	66	84.48	71.81	0.1358

membranous CD24 expression (CD24(m)) was not associated with the patients' prognosis in any of the stages (Table 5). The cytoplasmic CD24 expression (CD24(c)) was correlated with the patients' better prognosis in stage IV (RR=0.048, p=0.017) but not in other stages (Table 5).

DISCUSSION

CD24 as a marker of progressions in colorectal neoplasm

In this study, we showed that the cytoplasmic and membranous expressions of CD24 were rare in the normal, benign, and premalignant lesions, but we also noted an abrupt rise in the rates of both expressions in cases of colorectal cancer. In addition, cytoplasmic CD24 staining was positively correlated with the cancer stage. Considering the adenoma-carcinoma sequence in colorectal carcinogenesis and the restriction of the CD24 expression to the carcinoma, CD24 can be considered to be a marker of progression during the colorectal carcinogenesis, and CD24 can be used as a diagnostic marker to differentiate the carcinoma from benign precursor lesions including high grade adenoma. The development of carcinoma from adenomatous lesions is referred to as the adenoma-carcinoma sequence and this has been well documented for colorectal carcinoma.[24] The functional implication of the CD24 expression in terms of carcinogenesis needs to be elucidated.

Is the CD24 expression a prognostic factor in colorectal cancer?

Considering the results of the univariate and multivariate survival analyses, it is unlikely that the cytoplasmic CD24 expression is a prognostic factor for colorectal cancer despite its clear correlation with several prognosis-related clinico-pathological parameters. The positive correlation of the cytoplasmic CD24 expression with a better prognosis for stage IV cancer patients is shown in Table 5, but its significance remains to be determined. However, the prognostic significance of membranous CD24 is more complicated. According to the result of the Kaplan-Meier survival analysis, the membranous CD24 expression seemed to be clearly correlated with better survival for the tage III patients (Fig. 4C). Multivariate analysis using the Cox-proportional hazards regression model including almost all the prognostic factor as covariates revealed that the membranous CD24 expression was an independent prognostic factor (Table 4). However, when the survival analysis was done separately for each group of cancer stages and adjusted with several covariates via the Cox-proportional hazards regression model, the CD24 membranous expression was not associated with the patients' prognosis in any of the cancer stages (Table 5). Collectively, it is still questionable whether the membranous CD24 expression is an independent prognostic factor for colorectal cancer.

Why was the CD24 cytoplasmic expression not associated with patients' survival despite its clear correlation with prognosis-related clinico-pathological parameters?

The cytoplasmic CD24 expression was obviously correlated with a high tumor grade, aggressive tumor invasion, and an advanced stage (Table 2). The question then is: why was the cytoplasmic CD24 expression was not associated with the patients' survival?. One of the possible reasons for this discrepancy can be a confounding factor such as the different treatments after surgery, such as chemotherapy. However, since almost all covari-

Table 4. Univariate and multivariate analyses for prognostic variables of overall survival of colorectal cancer patients using Cox-proportional hazards regression

Predictors	Univariate		Multivariate	
	Ratio of risk (95% CI)	p value	Ratio of risk (95% CI)	p value
Sex		0.052		0.323
Male	1.00		1.00	
Female	0.559 (0.310-1.006)		0.725 (0.384-1.371)	
Age		0.843		0.636
0-59	1.00		1.00	
60+	1.062 (0.586-1.925)		1.167 (0.616-2.213)	
Location		0.187		0.152
Rectum	1		1	
Colon	0.650 (0.343-1.233)		0.613 (0.314-1.197)	
Chemotherapy		0.052		0.984
Not done	1		1	
Done	2.214 (0.992-4.942)		1.010 (0.372-2.740)	
Tumor grade		0.001		0.001
I	1.00		1.00	
II	1.112 (0.486-2.546)		1.612 (0.625-4.155)	
III	9.803 (3.755-25.598)		7.047 (2.313-21.475)	
pTNM staging		0.000		0.000
I	1.00		1.00	
II	4.187 (0.544-32.202)		3.727 (0.461-30.143)	
III	13.212 (1.772-98.500)		12.707 (1.529-105.578)	
IV	48.054 (6.268-368.393)		36.635 (4.269-314.395)	
CD24(m)		0.065		0.010
Negative	1.00		1.00	
Positive	0.539 (0.280-1.038)		0.367 (0.171-0.788)	
CD24(c)		0.140		0.537
Negative	1.00		1.00	
Positive	1.540 (0.868-2.732)		1.246 (0.620-2.502)	

$h(t/x)=h_0(t)\exp(\beta_0+\beta_1 sex+\beta_2 age+\beta_2 location+\beta_4 grade+\beta_5 chemoTx+\beta_6 stage+\beta_7 CD24)$.

ates, including chemotherapy, that can affect the patients' survival were controlled and adjusted for by using Cox-proportional hazard regression model, it is unlikely that this discrepancy was caused by the confounding factors such as chemotherapy. The essential reason for this discrepancy may be the absence of correlation with lymph nodal metastasis rather than the confounding factors. The cytoplasmic CD24 expression was correlated with direct tumor invasion and differentiation of tumor cells rather than lymph nodal metastasis (Table 2). Considering that most of the patients in this study underwent complete excision, nodal metastasis might have exerted a greater influence on patients' prognosis rather than that of direct tumor invasion.

The relationship between nodal metastasis and the patients' survival has been suggested in other studies. In breast cancer, the CD24 expression did not correlate with direct tumor invasion or tumor cell differentiation; however, it was associated with nodal metastasis and shortened overall survival for the patients suffering with breast cancer.[13]

Comparison of our results with those in previous studies

Significant rates of CD24 positivity have been reported for many common human tumors. Furthermore, for several types of tumor entities, higher rates of CD24 expression or CD24 positivity have been significantly associated with a shorter survival of patients.[13] And CD24 expression has been reported in colorectal cancer,[25-27] however, the clinical significance of the CD24 expression varies considerably depending on the types of tissues. According to Weichert *et al.*, CD24 correlates with patients survival only when the CD24 is highly expressed. In cases of pancreatic cancer and hepatocellular carcinoma, there was no correlation between the expression of CD24 and the attenuation of the overall survival of the patients,[28,29] and our results were in line with these findings.

There have been few reports that have evaluated the membranous and cytoplasmic CD24 expressions separately to determine their clinical significance, and these limited studies reported no

Table 5. Univariate and multivariate analysis of the overall survival of patients with stage I-II, III, and IV colorectal cancer using Cox-proportional hazards regression

Predictors	Stage I & II				Stage III				Stage IV			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	RR (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p
Sex		0.646		0.911		0.064		0.019		0.832		0.788
Male	1		1		1		1		1		1	
Female	0.774 (0.260-2.305)		0.939 (0.312-2.822)		0.418 (0.167-1.050)		0.307 (0.114-0.825)		0.832 (0.271-2.563)		1.229 (0.274-5.506)	
Age		0.370		0.754		0.769		0.653		0.277		0.144
0-59	1		1		1		1		1		1	
60+	0.608 (0.204-1.808)		0.839 (0.280-2.513)		1.141 (0.473-2.754)		1.247 (0.476-3.268)		2.301 (0.511-10.356)		3.566 (0.648-19.610)	
Location		0.079		0.085		0.268		0.470		0.963		0.899
Rectum	1		1		1		1		1		1	
Colon	0.161 (0.021-1.235)		0.166 (0.021-1.282)		0.582 (0.223-1.517)		0.686 (0.246-1.911)		0.975 (0.331-2.867)		0.932 (0.311-2.787)	
Chemotherapy		0.061		0.069		0.014		0.008		0.725		0.322
Not done	1		1		1		1		1		1	
Done	7.026 (0.913-54.046)		6.762 (0.863-52.976)		0.050 (0.004-0.547)		0.031 (0.002-0.402)		1.229 (0.390-3.876)		2.371 (0.429-13.106)	
CD24(m)		0.492		0.449		0.058		0.236		0.295		0.163
Negative	1		1		1		1		1		1	
Positive	0.662 (0.204-2.149)		0.630 (0.190-2.085)		0.243 (0.056-1.049)		0.393 (0.084-1.838)		0.563 (0.192-1.648)		0.334 (0.072-1.557)	
CD24(c)		0.484		0.545		0.769		0.835		0.361		0.017
Negative	1		1		1		1		1		1	
Positive	1.476 (0.496-4.392)		1.410 (0.463-4.298)		0.876 (0.363-2.115)		1.106 (0.429-2.854)		0.575 (0.175-1.884)		0.048 (0.004-0.578)	

$h(t/x)=h_0(t)\exp(\beta_0+\beta_1 sex+\beta_2 age+\beta_3 location+\beta_4 chemoTx+\beta_5 CD24)$.

apparent relationship between the membranous CD24 expression and favorable prognoses. Kristiansen *et al*.[14] have shown that performing immunostaining of ovarian cancer revealed two distinct staining modalities; membranous immunoreactivity and an additional cytoplasmic staining. In this study, membranous CD24 expression was found to be absent in the normal ovarian surface epithelium, but it was frequently observed in the cases of invasive carcinoma, with no correlation to the clinicopathological parameters. However, the cytoplasmic CD24 expression was correlated with an attenuation of patient survival on both univariate and multivariate analyses, although there was no correlation with tumor grade, the pT stage, or the pM status.[14] Our results were generally in accordance with these findings with regard to the significance of the cytoplasmic and membranous CD24 expression.

In summary, we showed that CD24 is abundantly expressed in colorectal cancer tissues, and that the cytoplasmic and membranous overexpression of CD24 can be a specific marker of disease progression from the premalignant tumor phase to the development of carcinoma. The cytoplasmic CD24 expression was associated with a variety of clinicopathological parameters related to poor prognoses. Although the biological function of CD24 and its clinical significance are not completely understood, our results clearly demonstrated the diagnostic and therapeutic usefulness of the CD24 protein in the context of colorectal cancer.

ACKNOWLEDGEMENT

This work was supported by a Chungbuk National University Research grant.

We wish to thank Jung Sun Lee and Mee-Young Shim for their technical assistance in the preparation of the manuscript.

REFERENCES

1. Pirruccello SJ, LeBien TW. The human B cell-associated antigen CD24

is a single chain sialoglycoprotein. J Immunol 1986; 136: 3779-84.
2. Fischer GF, Majdic O, Gadd S, Knapp W. Signal transduction in lymphocytic and myeloid cells via CD24, a new member of phosphoinositol-anchored membrane molecules. J Immunol 1990; 144: 638-41.
3. Jackson D, Waibel R, Weber E, Bell J, Stahel RA. CD24, a signal-transducing molecules expressed on human B cells, is a major surface antigen on small cell lung carcinomas. Cancer Res 1992; 52: 5264-70.
4. Mechtersheimer G, Barth T, Ludwig R, Staudter M, Moller P. Differential expression of leukocyte differentiation antigens in small round blue cell sarcomas. Cancer 1993; 71: 237-48.
5. Droz D, Zachar D, Charbit L, Gogusev J, Chretein Y, Iris L. Expression of the human nephron differentiation molecules in renal cell carcinomas. Am J Pathol 1990; 137: 895-905.
6. Choi YL, Kim SH, Shin YK, *et al*. Cytoplasmic CD24 expression in advanced ovarian serous borderline tumors. Gynecol Oncol 2005; 97: 379-86.
7. Kristiansen G, Pilarsky C, Pervan J, *et al*. CD24 expression is a significant predictor of PSA relapse and poor prognosis in low grade or organ confined prostate cancer. Prostate 2004; 58: 183-92.
8. Kristiansen G, Pilarsky C, Wissmann C, *et al*. Expression profiling of microdissected matched prostate cancer samples reveals CD1666/MEMD and CD24 as new prognostic markers for patient survival. J Pathol 2005; 205: 359-76.
9. Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1998. CA Cancer J Clin 1998; 48: 6-29.
10. Akashi T, Shirasawa T, Hirokawa K. Gene expression of CD24 core polypeptide molecule in normal rat tissues and human tumor cell lines. Virchows Arch 1994; 425: 399-406.
11. Nestl A, Von Stein OD, Zatloukal K, *et al*. Gene expression patterns associated with the metastatic phenotype in rodent and human tumors. Cancer Res 2001; 61: 1569-77.
12. Saito A, Fujii G, Sato Y, Gotoh M, Sakamoto M, Toda G, Hirohashi S. Detection of genes expressed in primary colon cancers by in situ hybridization: overexpression of RACK1. J Clin Pathol 2002; 55: 34-9.
13. Kristiansen G, Sammar M, Altevogt P. Tumour biological aspects of CD24, a mucin-like adhesion molecule. J Mol Histol 2004; 35: 255-62.
14. Kristiansen G, Denkert C, Schluns K, Dahl E, Pilarsky C, Hauptmann S. CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival. Am J Pathol 2002; 161: 1215-21.
15. Kristiansen G, Schluns K, Yongwei Y, Denkert C, Dietel M, Petersen I. CD24 is an independent prognostic marker of survival in nonsmall cell lung cancer patients. Br J Cancer 2003; 88: 231-6.
16. Fogel M, Friederichs J, Zeller Y, *et al*. CD24 is a marker for human breast carcinoma. Cancer Lett 1999; 143: 87-94.
17. Kristiansen G, Winzer KJ, Mayordomo E, *et al*. CD24 expression is a new prognostic marker in breast cancer. Clin Cancer Res 2003; 9: 4906-13.
18. Greene FL, Page DL, Fleming ID, *et al*. American Joint Committee on Cancer Staging Manual 6th ed. Springer 2002; 139-44.
19. Kim SH, Kook MC, Song HG. Optimal conditions for the retrieval of CD4 and CD8 antigens in formalin-fixed, paraffin-embedded tissues. J Mol Histol 2004; 35: 403-8.
20. Kim SH, Kook MC, Shin YK, Park SH, Song HG. Evaluation of antigen retrieval buffer systems. J Mol Histol 2004; 35: 409-16.
21. Kim SH, Jung KC, Shin YK, *et al*. The enhanced reactivity of endogenous biotin-like molecules by antigen retrieval procedures and signal amplification with tyramine. Histochem J 2002; 34: 97-103.
22. Kim SH, Shin YK, Lee KM, Lee JS, Yun JH, Lee SM. An improved protocol of biotinylated tyramine-based immunohistochemistry minimizing nonspecific background staining. J Histochem Cytochem 2003; 51: 129-32.
23. Sinicrope FA, Ruan SB, Cleary KR, Stephens LC, Lee JJ, Levin B. bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res 1995; 55: 237-41.
24. Cho KR, Vogelstein B. Genetic alterations in adenoma-carcinoma sequence. Cancer 1992; 70: 1727-31.
25. Lim SC, Oh SH. The role of CD24 in various human epithelial neoplasias. Pathol Res Pract 2005; 201: 479-86.
26. Lee SK, Lim SC, Kim KJ. The role of CD24 in colonic adenocarcinoma. J Korean Soc Coloproctal 2005; 21: 13-8.
27. Weichert W, Denkert C, Burkhardt M, *et al*. Cytoplasimic CD24 expression in colorectal cancer independently correlates with shortened patient survival. Clin Cancer Res 2005; 11: 6574-81.
28. Jacob J, Bellach J, Grutzmann R, *et al*. Expression of CD24 in adenocarcinomas of the pancreas correlates wit higher tumor grades. Pancreatology 2004; 4: 454-60.
29. Huang LR, Hsu HC. Cloning and expression of CD24 gene in human hepatocellular carcinoma: potential early tumor marker gene correlates with p53 mutaion and tumor differentiation. Cancer Res 1995; 55: 4717-21.