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Significance of E-cadherin and CD44 expression in patients with unresectable metastatic colorectal cancer

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Abstract. The loss of adhesion molecules is reported to be associated with tumor invasion and metastasis in numerous types of cancer. Epithelial (E)-cadherin is an important molecule for cell-to-cell adhesion, while cluster of differentiation (CD)44 is an important molecule for cell-to-extracellular matrix adhesion. The focus of the present study was to evaluate the significance of the expression of E-cadherin and CD44 in patients with the unresectable metastatic colorectal cancer (CRC) who are undergoing palliative chemotherapy. Formalin-fixed, paraffin-embedded samples were obtained from 49 patients who underwent primary tumor resection and who were receiving palliative chemotherapy for unresectable metastatic CRC. The expression of E-cadherin and CD44 was evaluated using immunohistochemistry. The expression of E-cadherin was not significantly associated with progression-free survival (PFS; P=0.2825) or overall survival (OS; P=0.6617). The expression of CD44 was not associated with PFS (P=0.4365), but it did exhibit a certain level of association with OS (P=0.0699). However, the combined low expression of E-cadherin and CD44 demonstrated a significant association with decreased PFS (P=0.0101) and OS (P=0.0009). The combined loss of E-cadherin and CD44 expression also led to a reduction in the objective response rate and disease control rate (P=0.0076 and P=0.0294, respectively). A univariate analysis indicated that the combined low expression of E-cadherin and CD44 (P=0.0474) and sex (P=0.0330) were significantly associated with decreased PFS, and multivariate analysis confirmed combined low expression of E-cadherin and CD44 as an independent risk factor for decreased PFS [hazard ratio (HR), 8.276; 95% confidence interval (CI), 1.383-43.311; P=0.0227]. Univariate and multivariate analyses also indicated that the combined low expression of E-cadherin and CD44 expression was a significant prognostic factor for poor OS (HR, 15.118; 95% CI, 2.645-77.490; P=0.0039). Therefore the current study suggests that the combined low expression of E-cadherin and CD44 is an effective independent predictor of decreased chemotherapeutic outcome and survival in patients with unresectable metastatic CRC.

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Abbreviations: CRC, colorectal cancer; ECM, extra cellular matrix; HA, hyaluronan; OX, oxaliplatin; IRI, irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin; FOLFOX, 5-FU/LV+OX; FOLFIRI, 5-FU/LV+IRI; CapeOX, capecitabine+OX; SOX, S-1+OX; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval

Key words: E-cadherin, cluster of differentiation 44, unresectable metastatic colorectal cancer, prognosis, chemotherapeutic outcome, cell-to-cell adhesion, cell-to-extracellular matrix adhesion

Introduction

Colorectal cancer (CRC) is the third leading cause of cancer-associated mortality in Japan (1), and the clinical outcomes of patients with unresectable metastatic CRC are particularly poor. Although novel anti-cancer agents, molecularly targeted drugs and surgical procedures have improved the prognosis of unresectable metastatic CRC, the clinical outcomes associated with unresectable metastatic CRC remain unfavorable, with a median survival time of only ~30 months (2,3).

Adhesion molecules are involved in cell-to-cell adhesion and cell-to-extracellular matrix (ECM) adhesion (4,5). The loss of adhesion molecules in CRC is reported to have an important role in the metastasis and invasion of tumors (4,6) and to be associated with a poor clinical outcome (5-8). In addition, the loss of adhesion molecules is reported to be associated with resistance to chemotherapy (9).

E-cadherin serves a pivotal role in cell-to-cell adhesion (10,11), and the loss of E-cadherin is associated with tumor de-differentiation and metastasis, and, therefore, poor clinical outcome (6). Additionally, the loss of E-cadherin is associated with chemotherapy resistance via numerous pathways, including the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway and the Wingless type (Wnt)/β-catenin pathway (12-15).

Cluster of differentiation (CD) 44, a type 1 transmembrane glycoprotein, is a receptor for hyaluronan (HA) and has pathological and physiological roles in the homing of lymphocytes, cell-to-ECM adhesion, tumor growth, angiogenesis, and

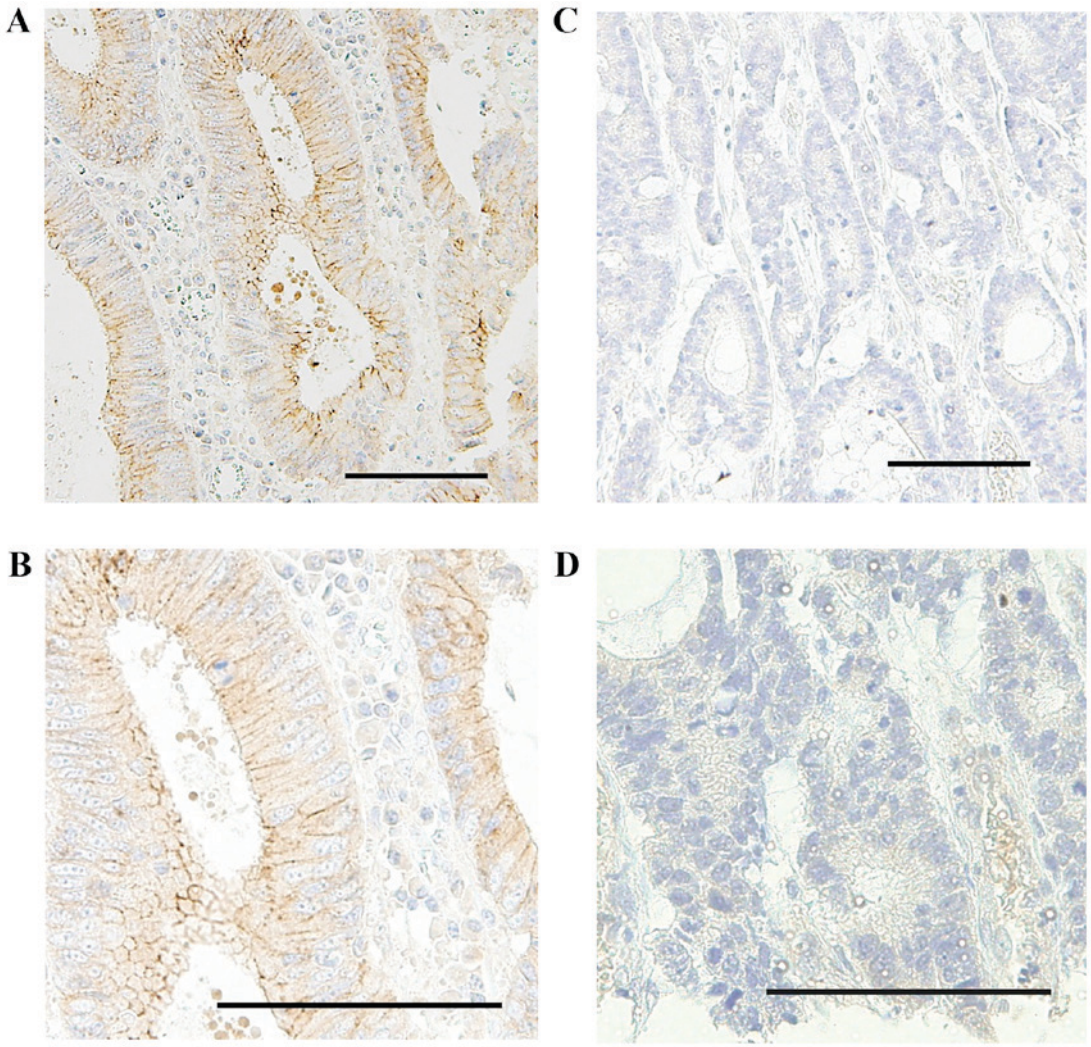

Figure 1. Expression of epithelial cadherin. (A) Positive sample (magnification, x200); (B) positive sample (magnification, x400); (C) negative sample (magnification, x200); and (D) negative sample (magnification, x400). Membranous staining was the focus for analysis. Sections were counterstained with hematoxylin. Scale bar, 100 μm.

inflammation (5,16-21). The decrease of cell-to-ECM adhesion caused by the loss of CD44 induces tumor cell detachment from the basal membrane and the invasion of cancer cells (8). Furthermore, loss of CD44 expression is reported to be associated with chemotherapy resistance and with a poor clinical outcome (6).

The focus of the present study was to evaluate the significance of E-cadherin and CD44 expression, which have separate roles in cellular adhesion, in unresectable metastatic CRC.

Materials and methods

Patient characteristics and therapy. The characteristics of 49 patients with unresectable metastatic CRC, who underwent surgery for the primary tumor at the Department of Surgical Oncology, Osaka City University (Osaka, Japan) between April 2005 and December 2013, were retrospectively reviewed. The median follow-up period was 26.7 months (range, 5.8-63.2 months). All of the patients underwent first-line combination chemotherapy with oxaliplatin (OX) or irinotecan (IRI) + 5-fluorouracil (5-FU)/leucovorin (LV), or a prodrug of 5-FU, which is converted to 5-fluorouracil (5-FU) *in vivo* to exert antitumor activity, such as S-1 and capecitabine. The chemotherapy regimens that were administered were as follows: 5-FU/LV+OX (FOLFOX; n=30), 5-FU/LV+IRI (FOLFIRI; n=6), capecitabine+OX (CapeOX; n=12), and S-1+OX (SOX; n=1). In total, 21 patients underwent chemotherapy combined with a molecularly targeted agent. Written informed consent was obtained from the patients for participation, and the Ethics Committee of Osaka City University approved the current study protocol. The investigation was conducted according to the principles expressed in the Declaration of Helsinki. All patients were followed up until May 2015 or until the date of their mortality.

Antibodies. Commercially available monoclonal antibodies were selected. Mouse anti-human E-cadherin (catalog no., M106; 2 μg/ml) was purchased from Takara Bio, Inc. (Otsu, Japan), and mouse anti-human CD44 (catalog no., M708201-2; dilution, 1:50) was purchased from Dako (Agilent Technologies, Inc., Santa Clara, CA, USA).

Immunohistochemistry. All tissue specimens were fixed in 10% buffered formalin and embedded in paraffin.

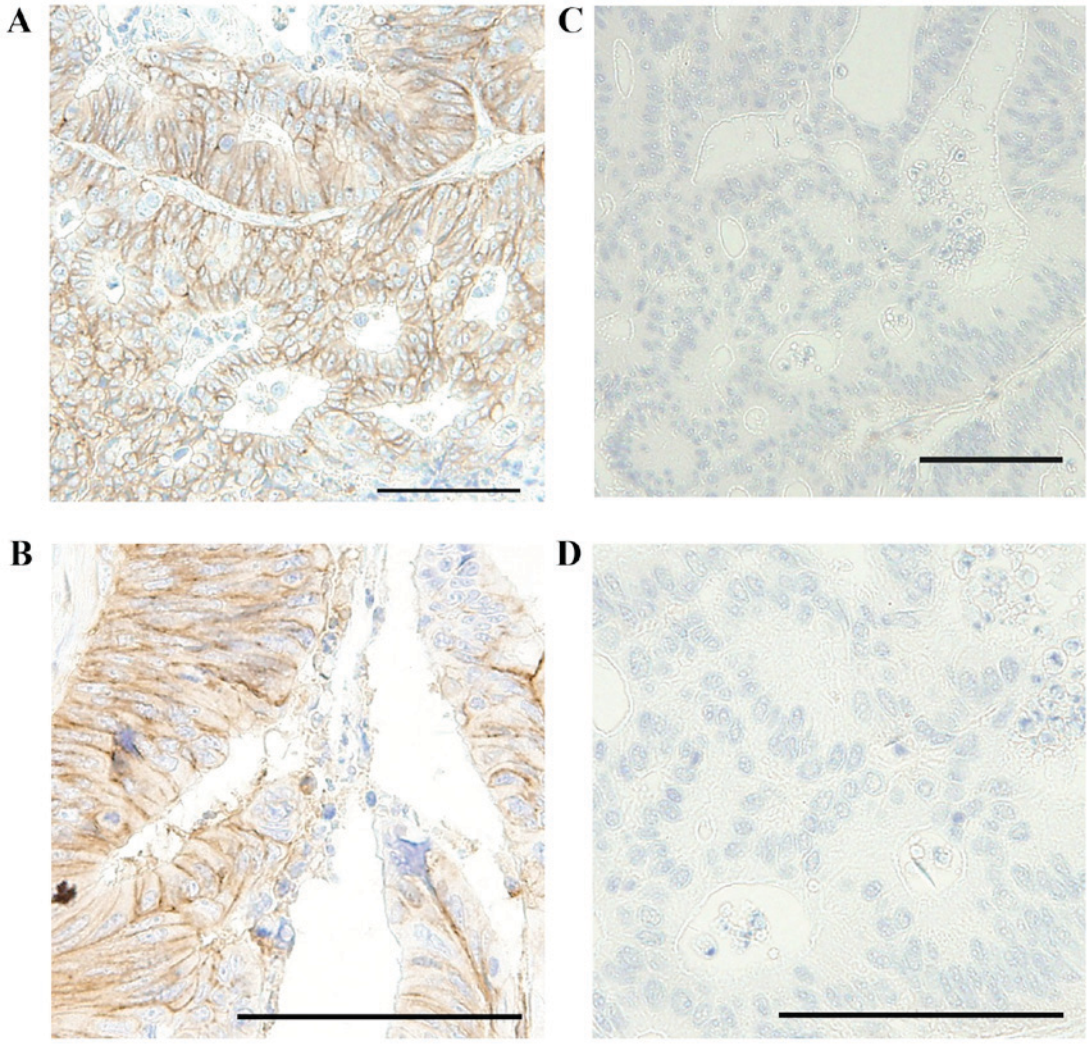

Figure 2. Expression of CD44. (A) Positive sample (magnification, x200); (B) positive sample (magnification, x400); (C) negative sample (magnification, x200); and (D) negative sample (magnification, x400). Membranous staining was the focus for analysis. Sections were counterstained with hematoxylin. Scale bar, 100 μm.

Immunohistochemical staining for E-cadherin and CD44 was performed on 4-μm-thick sections of each of the CRC tissue samples. The slides were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. Immunohistochemical staining was performed as previously described (22). Briefly, the sections were subjected to endogenous peroxidase blocking in 1% H_2O_2 solution in methanol for 15 min. Antigen retrieval was performed by autoclaving the sections at 105˚C for 15 min in Dako Target Retrieval solution (Dako; Agilent Technologies, Inc. Santa Clara, CA, USA). Serum blocking was performed with 10% normal rabbit serum for 10 min. Following H_2O_2 and serum blocking, the slides were incubated with the primary antibody at 4˚C overnight. The secondary antibody was a biotin-labeled rabbit anti-mouse IgG + IgA + IgM (Nichirei Biosciences, Inc., Tokyo, Japan; dilution, 1:500). Normal rabbit serum, a biotin-labeled rabbit anti-mouse antibody and peroxidase-labeled streptavidin, were used, which are included in the Histfine SAB-PO(M) kit (catalog no., 424032; Nichirei Biosciences, Inc., Tokyo, Japan.) according to the manufacturer's protocol. Detection was performed with a 3,3'-diaminobenzidine tetrahydrochloride kit (Histofine Simple Stain kit; catalog no., 415174; Nichirei Biosciences, Inc., Tokyo, Japan). The sections were counterstained with hematoxylin, dehydrated, cleared and mounted on glass coverslips. Sections in which the primary antibodies were absent were used as negative controls.

Evaluation. First, to determine the tumor area, the entire section was surveyed with a low-magnification objective lens. Subsequently, three locations within the selected tumor area were evaluated with a x200 lens with BX43 (Olympus Coporation, Tokyo, Japan); the three microscopic fields were randomly selected to calculate the mean number of positively stained cells. The membranous staining was focused on to assess the expression levels of E-cadherin and CD44. With regard to E-cadherin expression, tissues in which <25% of the cells were stained or in which there was an absence of staining were assigned to the low expression group, whilst tissues in which ≥25% of the cells were stained were assigned to the high expression group (Fig. 1) (6).

With regard to CD44 expression, tissues in which <10% of the cells were stained or in which there was an absence

of staining were assigned to the low expression group, whilst tissues in which ≥10% of the cells were assigned to the expression group (Fig. 2) (23).

The staining intensity was disregarded. Two pathologists who were blinded to the clinicopathological or survival data of the patients at the time of the analysis, evaluated the data. If the observers reported different results, they reviewed the slides by microscope until a consensus was reached.

Statistical analysis. The statistical differences between the groups were analyzed using the χ^2 test, Fisher's exact test and Student's t-test. The duration of survival was calculated using the Kaplan-Meier method. Differences in the survival curves were assessed using the log-rank test. A multivariate analysis of the associations between clinicopathological characteristics and survival was performed using a Cox proportional hazards model. JMP software version 12 (SAS Institute, Inc., Cary, NC, USA) was used for all of the statistical analyses. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics. The clinicopathological characteristics of the patients are presented in Table I. The study population consisted of 26 male and 23 female patients with a median age of 63 years (range, 40-80 years). In total, 32 patients had colon cancer and 17 patients had rectal cancer. There were 44 patients with low-grade tumors (including well-differentiated or moderately differentiated adenocarcinomas), and the 5 remaining patients had high-grade tumors (including poorly-differentiated or mucinous adenocarcinomas). With regard to metastases, 38 patients had liver metastases, 14 had lung metastases, 14 had peritoneal disseminations and 14 had distant lymph node metastases. The site of metastasis was a single organ in 28 patients, two organs in 16 patients and three organs in 5 patients.

Associations between the expression of E-cadherin/CD44 and clinicopathological characteristics. The expression of E-cadherin alone was not significantly associated with any of the clinicopathological factors (Table II). The expression of CD44 was only significantly associated with sex (P=0.0202; Table II).

Associations between the expression of E-cadherin/CD44 and the efficacy of chemotherapy. The low expression of E-cadherin was significantly associated with a lower objective response rate (ORR; P=0.0491), but it did not correlate with a lower disease control rate (DCR) to a significant extent (P=0.3438; Table III). CD44 expression did not correlate with the efficacy of chemotherapy (Table III). The patients were categorized into four groups according to combination of E-cadherin and CD44 expression: Group 1, high expression of E-cadherin and CD44 (n=29); Group 2, low expression of E-cadherin and high expression of CD44 (n=5); Group 3, high expression of E-cadherin and low expression of CD44 (n=12); and Group 4, low expression of E-cadherin and CD44 (n=3). Patients were further categorized into two groups: Group A consisted of all of the patients in Groups 1, 2 and 3, and Group B consisted of the patients in Group 4. The ORRs of Groups A and B were 71.7 and 0%, respectively. Additionally, the DCRs of Groups A and B were 89.1 and 33.3%, respectively (Table III). The ORRs and DCRs of the patients in Group A were significantly higher compared with those in Group B (P=0.0076 and P=0.0294, respectively; Table III).

Table I. Clinicopathological characteristics of the patients.

Clinicopathological characteristics	n=49
Sex, male:female	26:23
Age, years, median (range)	63 (40-80)
Location, colon:rectum	32:17
Differentiation, well + moderately: mucinous + poorly	44:5
Tumor depth, T1-3:T4	24:25
Lymph node metastasis, negative:positive	6:43
Lymph vessel invasion, negative: positive: unknown	4:41:4
Venous invasion, negative:positive:unknown	23:22:4
Liver metastasis, negative:positive	11:38
Lung metastasis, negative:positive	35:14
Peritoneal dissemination, negative:positive	35:14
No. of organs affected by metastasis, 1:≥2	28:21
CD44, negative:positive	14:35
E-cadherin, negative:positive	8:41
Chemotherapy, FOLFOX+CapeOX+ SOX:FOLFIRI	43:6
Molecular targeted agent, negative:positive	18:31

FOLFOX, 5-Fluorouracil (FU)+Leucovorin (LV)+Oxaliplatin (OX); CapeOX, Capecitabine+OX; FOLFIRI, 5-FU+LV+irinotecan; SOX, S-1+OX; CD44, cluster of differentiation 44; E-cadherin, epithelial cadherin.

It has been previously demonstrated that molecularly targeted agents may improve the survival of these patients (2,3). The number of patients who underwent chemotherapy combined with a molecularly targeted agent in Group A was 20 (43.5%), while in Group B it was 1 (33.3%). However, this difference was not statistically significant (P=0.7277).

Survival analysis according to the expression of E-cadherin and CD44. The expression of E-cadherin was not significantly associated with progression-free survival (PFS; P=0.2825; Fig. 3A), or overall survival (OS; P=0.6617; Fig. 3B). The expression of CD44 was not significantly associated with PFS (P=0.4365; Fig. 4A), however, it tended (non-significantly) to correlate with OS (P=0.0699; Fig. 4B).

Survival analysis according to the combination of E-cadherin and CD44 expression. Group 4 was associated with decreased PFS in comparison with Group 1 (P=0.0126) and Group 3 (P=0.0317; Fig. 5A). Group 4 was associated with significantly reduced OS compared with Groups 1 (P=0.0011), 2 (P=0.0279) and 3 (P=0.0352; Fig. 5B). The PFS and OS rates of the patients in Group B were significantly reduced compared with those of

Table II. Associations between the adhesion molecules and the clinical backgrounds of the patients.

	CD44 expression			E-cadherin expression		
Characteristics	High n=35	Low n=14	P-value	High n=41	Low n=8	P-value
Sex			0.0202			0.5587
Male	20	3		21	5	
Female	15	11		20	3	
Age, years, median (range)	63.0 (40-80)	64.5 (48-80)	0.3105	64.0 (40-80)	61.0 (53-72)	0.8939
Location			0.2055			0.1494
Colon	21	11		25	7	
Rectum	14	3		16	1	
Tumor invasion			0.0707			0.4777
T1-3	20	4		21	3	
T4	15	10		20	5	
Histology			0.5505			0.8146
Well + moderately	32	12		37	7	
Poorly + mucinous	3	2		4	1	
Lymphatic vessel invasion			0.1817			0.3299
Negative	4	0		4	0	
Positive	28	13		33	8	
Blood vessel invasion			0.2793			0.3957
Negative	18	5		20	3	
Positive	14	8		17	5	
Lymph node metastasis			0.7127			0.2416
Negative	4	2		4	2	
Positive	31	11		36	6	
Liver metastasis			0.9135			0.8501
Negative	8	3		9	2	
Positive	27	11		32	6	
Lung metastasis			0.4839			0.5411
Negative	24	11		30	5	
Positive	11	3		11	3	
Peritoneal dissemination			0.1615			0.2713
Negative	27	8		28	7	
Positive	8	6		13	1	
Number of organs affected by metastasis			1.0000			0.7378
1	20	8		23	5	
≥2	15	6		18	3	

CD44, cluster of differentiation 44; E-cadherin, epithelial cadherin.

the patients in Group A (P=0.0101 and P=0.0009, respectively; Fig. 6).

The correlations between the clinicopathological characteristics and prognosis were then examined. In the univariate analysis, sex (P=0.0333) and the combination of E-cadherin and CD44 expression (P=0.0474) were identified to be significantly associated with PFS (Table IV). When multivariate analysis was performed, the peritoneal dissemination and the number of organs affected by metastasis, which are established prognostic factors, were added as covariates (24,25). A multivariate analysis demonstrated that the combination of E-cadherin and CD44 expression was the only independent risk factor for PFS [hazard ratio (HR), 8.276, 95% confidence interval (CI), 1.383-43.311; P=0.0227; Table IV].

According to a univariate analysis, the combination of E-cadherin and CD44 expression was significantly associated with the OS (P=0.0177; Table V). When multivariate analysis was performed, the peritoneal dissemination and the number

Table III. Effects of chemotherapy and CD44 and E-cadherin expression levels.

	CD44 expression			E-cadherin expression			Combination of E-cadherin and CD44 expression		
Response	High n=35	Low n=14	P-value	High n=41	Low n=8	P-value	Other n=46	E-cadherin(-)/CD44(-) n=3	P-value
Complete response, n	2	0		2	0		2	0	
Partial response, n	23	8		28	3		31	0	
Stable disease, n	6	3		6	3		8	1	
Progressive disease, n	4	3		5	2		5	2	
ORR, %	71.4	57.1	0.3412	73.2	37.5	0.0491	71.7	0.0	0.0076
DCR, %	88.6	78.6	0.3813	87.8	75.0	0.3438	89.1	33.3	0.0294

ORR, objective response rate; DCR, disease control rate; CD44, cluster of differentiation 44; E-cadherin, epithelial cadherin.

of organs affected by metastases, which are established prognostic factors, were added as covariates. The multivariate analysis demonstrated that the combination of E-cadherin and CD44 expression was an independent risk factor for OS (HR, 15.118; 95% CI, 2.645-77.490; P=0.0039; Table V).

Discussion

The current study has demonstrated that the combined loss of E-cadherin and CD44 expression is associated with the reduced efficacy of chemotherapy and also decreased survival rate. The cadherin family is one comprised of cell-to-cell adhesion molecules, whilst CD44 is a cell-to-ECM adhesion molecule (21). The loss of cell adhesion molecules is reported to be associated with the metastasis and invasion of CRC, since the activation of cell motility and detachment from other cells, the stroma and the ECM represent the biological basis of metastasis and invasion (26-29). The downregulation of cell-to-cell adhesion reduces the maintenance of cell shape and polarity (30) and increases cellular motility and migration (30). The loss of cell-to-ECM adhesion induces cellular detachment from the basal membrane, the ECM and connective tissue (8,5). This is the hypothesis as to why the loss of the adhesion molecules is associated with tumor progression (6,7). Additionally, the loss of adhesion molecules is also reported to be associated with resistance to chemotherapy (9).

E-cadherin is a transmembrane glycoprotein that is required for calcium-dependent cell-to-cell adhesion in the formation of adherens junctions (4,31,32). The hypothesized underlying mechanism for the association between E-cadherin loss and chemotherapy resistance is as follows: Cadherin switching [the alteration from E-cadherin to neural (N)-cadherin] occurs in tumors; N-cadherin subsequently activates the PI3K/Akt pathway (12); and the PI3K/Akt pathway induces chemotherapy resistance by decreasing apoptosis and increasing proliferation (13,14). In addition, the loss of E-cadherin induces an increase in the levels of cytoplasmic β-catenin (15), which is then relocated into the nuclei, where it activates the Wnt/β-catenin pathway (15); the Wnt/β-catenin pathway is associated with chemotherapy resistance through the maintenance and proliferation of cancer stem cells (15).

As the loss of E-cadherin induces an increase in cellular motility and loss of cell-to-cell adhesion, E-cadherin is involved in tumor budding and facilitates invasion (33). Therefore, it is associated with a poor clinical outcome (34). Consistently, the present study identified that E-cadherin expression was associated with the objective response to chemotherapy. Therefore, although E-cadherin expression was not correlated with survival (possibly due to the influence of numerous clinical factors on the survival of patients with unresectable metastatic CRC), the current study demonstrated that E-cadherin expression is associated with the response to chemotherapy.

CD44, which is a class 1 transmembrane glycoprotein, has important roles in lymphocyte homing, cell proliferation, angiogenesis, inflammation and motility (5,16-21), and also serves an vital role in cell-to-ECM adhesion in association with HA and glycosaminoglycans (35,36). As cancer cells are connected with the stroma or basal membrane by CD44, which is a receptor for HA, the loss of CD44 induces detachment from

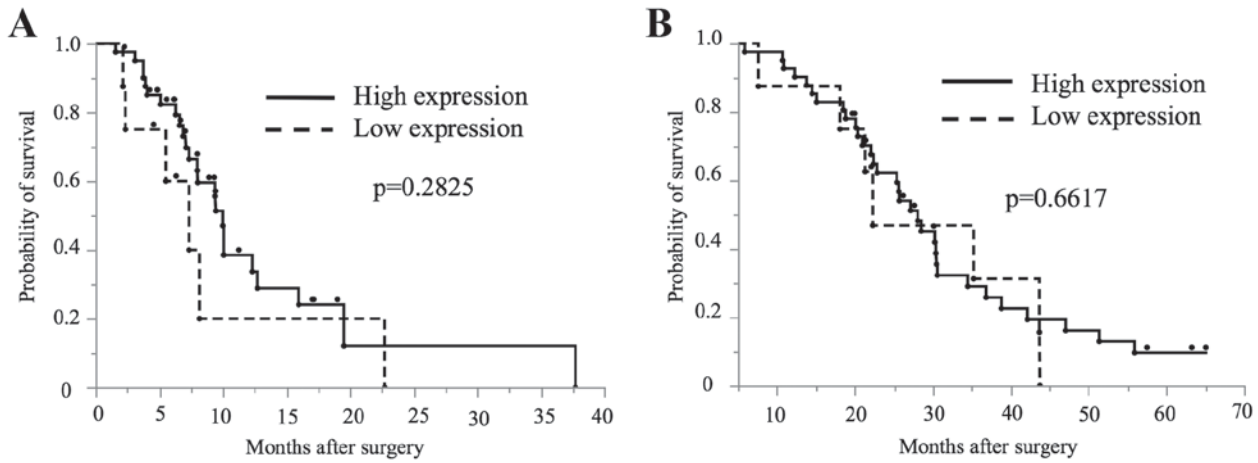

Figure 3. Survival curves for E-cadherin expression. (A) The survival curve for E-cadherin expression and progression-free survival. E-cadherin expression was not associated with survival. (B) The survival curve for E-cadherin expression and overall survival. E-cadherin expression was not associated with survival. E-cadherin, epithelial cadherin.

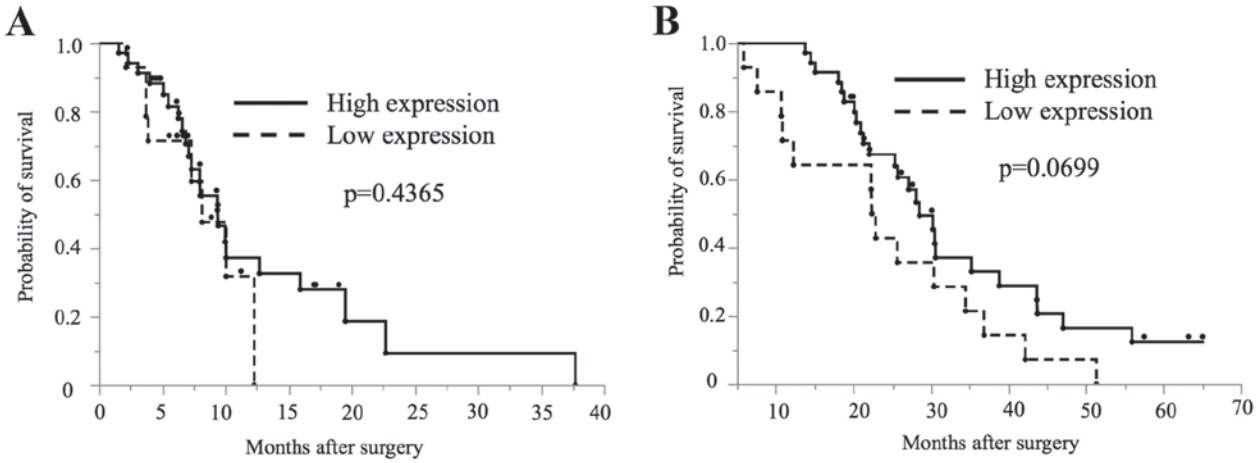

Figure 4. Survival curves for CD44 expression. (A) The survival curve for CD44 expression and progression-free survival. CD44 expression was not associated with survival. (B) The survival curve for the expression of CD44 and overall survival. Low CD44 expression was associated with reduced survival rate, but did not reach statistical significance. CD44, cluster of differentiation 44.

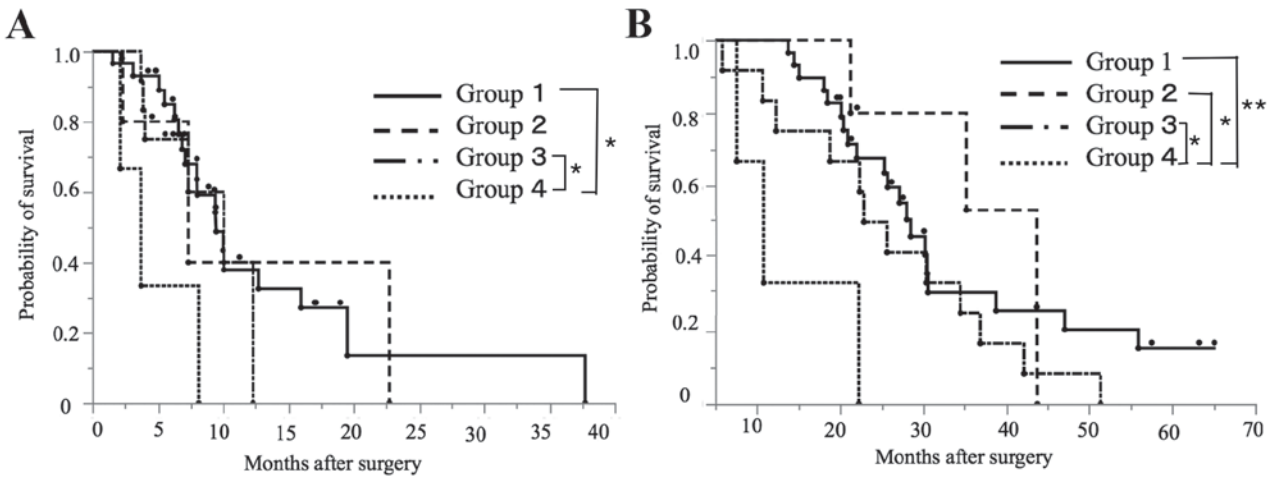

Figure 5. Survival curves for the combination of E-cadherin and CD44 expression. The patients were categorized into four groups according to combination of E-cadherin expression and CD44 expression: Group 1, high expression of both E-cadherin and CD44 (n=29); Group 2, low expression of E-cadherin and high expression of CD44 (n=5); Group 3, high expression of E-cadherin and low expression of CD44 (n=12); and Group 4, low expression of both E-cadherin and CD44 (n=3). (A) Group 4 was associated with reduced survival rate compared with Groups 1 (P=0.0126) and 3 (P=0.0317) and was not associated with poorer progression-free survival compared with Group 2 (P=0.2706). (B) Group 4 was associated with poorer overall survival compared with Groups 1 (P=0.0011), 2 (P=0.0279) and 3 (P=0.0352). *P<0.05, **P<0.01. E-cadherin, epithelial cadherin; CD44, cluster of differentiation 44.

Table IV. Results of the univariate and multivariate analyses of prognostic factors for progression-free survival.

	Univariate			Multivariate		
Factor	HR	95% CI	P-value	HR	95% CI	P-value
Sex, male vs. female	2.336	1.069-5.265	0.0333	1.952	0.817-4.715	0.1309
Age, ≥65 vs. <65 years	0.826	0.382-1.730	0.6139			
Location, rectum vs. colon	0.739	0.306-1.620	0.4626			
Tumor invasion, T4 vs. T1-3	1.464	0.704-3.195	0.3111			
Histology, mucinous + poorly vs. well + moderately	0.670	0.156-1.966	0.5010			
Lymphatic vessel invasion, positive vs. negative	3.094	0.603-57.148	0.2082			
Blood vessel invasion, positive vs. negative	0.677	0.677-1.508	0.3368			
Lymph node metastasis, ≥N2 vs. N0+1	0.755	0.366-1.560	0.4446			
Liver metastasis, positive vs. negative	1.228	0.531-3.341	0.6492			
Lung metastasis, positive vs. negative	1.210	0.540-2.542	0.6285			
Peritoneal dissemination, positive vs. negative	0.990	0.438-2.099	0.9800	1.507	0.540-4.283	0.4329
CD44/E-cadherin expression, Group 4 vs. Groups 1-3	4.405	1.020-13.324	0.0474	8.276	1.383-43.311	0.0227
No. of organs affected by metastasis, ≥2 vs. 1	1.074	0.497-2.313	0.8542	0.929	0.326-2.500	0.8860

CD44, cluster of differentiation 44; E-cadherin, epithelial-cadherin; HR, hazard ratio; CI, confidence interval; Group 1, high expression of E-cadherin and CD44; Group 2, low expression of E-cadherin and high expression of CD44; Group 3, high expression of E-cadherin and low expression of CD44; Group 4, low expression of E-cadherin and CD44.

Table V. Results of the univariate and multivariate analyses of prognostic factors affecting the overall survival.

	Univariate			Multivariate		
Factor	HR	95% CI	P-value	HR	95% CI	P-value
Sex, male vs. female	1.621	0.327-1.159	0.1325			
Age, ≥65 vs. <65 years	1.120	0.622-0.253	0.5787			
Location, rectum vs. colon	0.887	0.446-1.691	0.7210			
Tumor invasion, T4 vs. T1-3	1.794	0.947-3.463	0.0730			
Histology, mucinous + poorly vs. well + moderately	0.742	0.178-2.070	0.6065			
Lymphatic vessel invasion, positive vs. negative	2.414	0.729-14.946	0.2118			
Blood vessel invasion, positive vs. negative	0.968	0.506-1.840	0.9210			
Lymph node metastasis, ≥N2 vs. N0+1	1.376	0.732-2.625	0.3215			
Liver metastasis, positive vs. negative	1.142	0.548-2.684	0.7365			
Lung metastasis, positive vs. negative	1.220	0.582-2.377	0.0581			
Peritoneal dissemination, positive vs. negative	0.939	0.458-1.811	0.8546	1.055	0.469-2.265	0.8939
CD44/E-cadherin expression, Group 4 vs. Groups 1-3	6.423	1.468-19.953	0.0177	15.118	2.645-77.490	0.0039
No. of organs affected by metastasis, ≥2 vs. 1	1.281	0.677-2.410	0.4422	1.367	0.659-2.805	0.3971

CD44, cluster of differentiation 44; E-cadherin, epithelial-cadherin; HR, hazard ratio; CI, confidence interval; Group 1, high expression of E-cadherin and CD44; Group 2, low expression of E-cadherin and high expression of CD44; Group 3, high expression of E-cadherin and low expression of CD44; Group 4, low expression of E-cadherin and CD44.

the basal membrane and allows tumor cell invasion (8). Since the loss of CD44 leads to metastasis and invasion, it is associated with decreased survival time (6). In a previous study, the loss of CD44 in the tumor cells and clusters at the invasive periphery of tumor tissue was associated with chemotherapy resistance (9). The current study failed to confirm the initial hypothesis that the loss of CD44 expression may be associated with the efficacy of chemotherapy. Thus, it is possible that the influence of CD44 on chemotherapy resistance is minor, and that it contributes to the decrease in OS via the aforementioned mechanism of promoting invasion and metastasis.

In the present study, the individual losses of E-cadherin and CD44 expression, which are reported to be poor prognostic factors for patients who undergo curative surgery, were not prognostic factors for patients with unresectable metastatic CRC. However, there was a significant association between

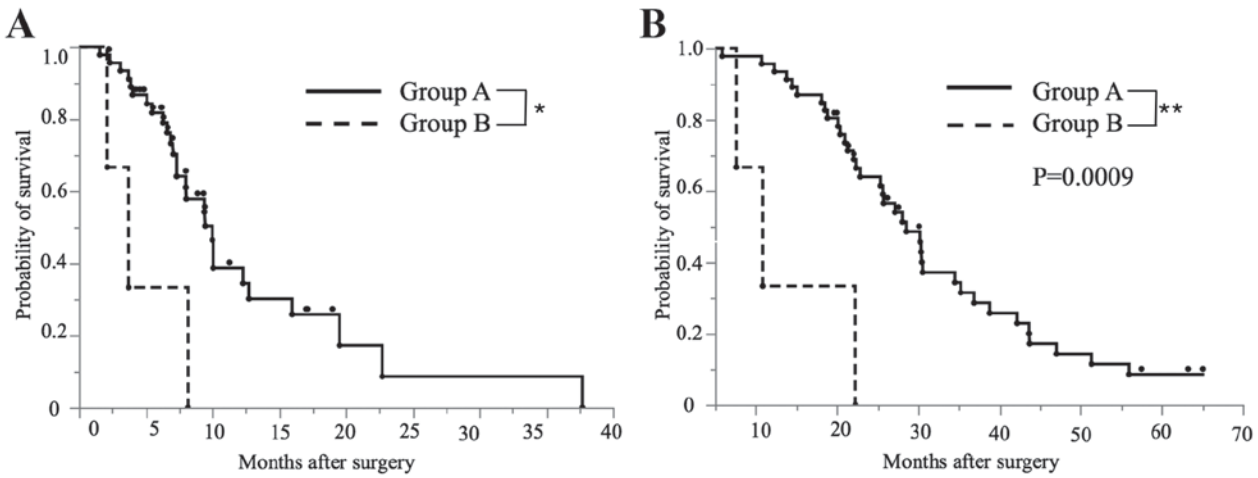

Figure 6. Survival curves for the combination of CD44 and E-cadherin expression. Group A consisted of the patients with high E-cadherin/high CD44, low E-cadherin/high CD44, and high E-cadherin/low CD44 expression. Group B consisted of the patients with low expression of both E-cadherin and CD44. (A) Group B was associated with a poorer progression-free survival rate compared with Group A (P=0.0101). (B) Group B was associated with poorer overall survival rate compared with Group A (P=0.0009). *P<0.05, **P<0.01. E-cadherin, epithelial cadherin; CD44, cluster of differentiation 44.

the combined loss of E-cadherin and CD44 expression and a poor clinical outcome. Although the mechanism remains to be elucidated, Ngan *et al* (6) reported that E-cadherin and CD44 may have an interdependent role in sustaining the adhesive function, inhibiting invasion and metastasis, and promoting chemotherapy resistance.

The current study has certain limitations as it was retrospective in nature and included a small number of patients. Furthermore, certain previous studies on E-cadherin and CD44 identified that neither E-cadherin nor CD44 correlated with survival (37-40). The inconsistent results regarding the importance of E-cadherin and CD44 expression as prognostic factors may be the result of differing experimental methods, threshold values for the expression of E-cadherin or CD44, or the evaluation of immunoexpression. Additionally, the diversity in patient backgrounds and the chemotherapies that are administered in patients with unresectable metastatic CRC, may have led to results that differed from the original hypothesis of the current study.

Although these data are based on an analysis of 3 patients in whom the expression of E-cadherin and CD44 was low, the prognosis of Group B was identified to be poorer compared with that of Group A in the present study. As the present study included patients who had distant metastasis and who underwent surgical resection of their primary tumor, the number of eligible patients was small. In addition to this, as the significance of the E-cadherin and CD44 expression were evaluated with regard to OS and also the efficacy of chemotherapy, only patients who underwent combination chemotherapy as first-line chemotherapy were selected. As a consequence, the sample size was decreased. Further studies, including prospective studies with a large study population, are required to confirm whether the combined low expression of E-cadherin and CD44 may be an independent predictor of prognosis in patients with unresectable metastatic CRC, as indicated by the results of the current study.

In conclusion, the present study demonstrated that the combination of E-cadherin and CD44 expression may be an effective prognostic factor for estimating the survival and chemotherapeutic outcome of patients with unresectable metastatic CRC. Further studies are required to confirm these results.

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References

1. Matsuda A, Matsuda T, Shibata A, Katanoda K, Sobue T and Nishimoto H; Japan Cancer Surveillance Research Group: Cancer incidence and incidence rates in Japan in 2008: A study of 25 population-based cancer registries for the monitoring of cancer incidence in Japan (MCIJ) project. Jpn J Clin Oncol 44: 388-396, 2014.
2. Heinemann V, von Weikersthal LF, Decker T, Kiani A, Vehling-Kaiser U, Al-Batran SE, Heintges T, Lerchenmüller C, Kahl C, Seipelt G *et al*: FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): A randomised, open-label, phase 3 trial. Lancet Oncol 15: 1065-1075, 2014.
3. Loupakis F, Cremolini C, Masi G, Lonardi S, Zagonel V, Salvatore L, Cortesi E, Tomasello G, Ronzoni M, Spadi R, *et al*: Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. N Engl J Med 371: 1609-1618, 2014.
4. Beavon IR: The E-cadherin-catenin complex in tumour metastasis: Structure, function and reglation. Eur J Cancer 36: 1607-1620, 2000.
5. Ponta H, Sherman L and Herrlich PA: CD44: From adhesion molecules to signalling regulators. Nat Rev Mol Cell Biol 4: 33-45, 2003.
6. Ngan CY, Yamamoto H, Seshimo I, Ezumi K, Terayama M, Hemmi H, Takemasa I, Ikeda M, Sekimoto M and Monden M: A Multivariate analysis of adhesion molecules expression in assessment of colorectal cancer. J Surg Oncol 95: 652-662, 2007.
7. Lugli A, Lezzi G, Hostettler I, Murano MG, Mele V, Tornillo L, Carafa V, Spagnoli G, Terracciano L and Zlobec I: Prognostic impact of the expression of putative cancer stem cell markers CD133, CD166, CD44s, EpCAM, and ALDH1 in colorectal cancer. Br J Cancer 103: 382-390, 2010.
8. Sugino T, Gorham H, Yoshida K, Bolodeoku J, Nargund V, Cranston D, Goodison S and Tarin D: Progressive loss of CD44 gene expression in invasive bladder cancer. Am J Pathol 149: 873-882, 1996.
9. Bhangu A, Wood G, Beown G, Darzi A, Tekkis P and Goldin R: The role of epithelial mesenchymeal transition and resistance to neoadjuvant therapy in localy advanced rectal cancer. Colorectal Dis 16: O133-O143, 2014.

10. Dorudi S, Sheffield JP, Pouulsom R, Northover JM and Hart IR: E-cadherin expression in colorectal cancer: An immunetochemical and *in situ* hybridization study. Am J Pathol 142: 981-986, 1993.
11. Ghadimi BM, Behrens J, Hoffman I, Haensch W, Birchmeier W and Schlag PM: Immunohistological analysis of E-cadherin, alpha-, beta- and gamma-catenin expression in colorectal cancer: Implications for cell adhesion and signaling. Eur J Cancer 35: 60-65, 1999.
12. Wheelock MJ, Shintani Y, Maeda M, Fukumoto Y and Johnson KR: Cadherin switching. J Cell Sci 121: 727-735, 2008.
13. Shintani Y, Maeda M, Chaika N, Johnson KR and Wheelock MJ: Collagen I promotes epithelial-to-mesenchymal transition in lung cancer cells via transforming growth-factor signaling. Am J Respir Cell Mol Biol 38: 95-104, 2008.
14. Suyama K, Shapiro I, Guttman M and Hazan RB: A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor. Cancer Cell 2: 301-314, 2002.
15. Kanwar SS, Yu Y, Nautiyal J, Patel BB and Majumdar AP: The Wnt/beta-catenin pathway regulates growth and maintenance of colonospheres. Mol Cancer 9: 212, 2010.
16. Miyake K, Underhill CB, Lesley J and Kincade PW: Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. J Exp Ned 172: 69-75, 1990.
17. Aruffo A, Stamenkovic I, Melnick M, Underhill CB and Seed B: CD44 is the principal cell surface receptor for hyaluronate. Cell 61: 1303-1313, 1990.
18. Dougherty GJ, Lansdorp PM, Cooper DL and Humphries RK: Molecular cloning of CD44R1 and CD44R2, two novel isoforms of the human CD44 lymphocyte 'homing' receptor expressed by hemopoietic cells. J Exp Med 174: 1-5, 1991.
19. Goldstein LA, Zhou DF, Picker LJ, Minty CN, Bargatze RF, Ding JF and Butcher EC: A human lymphocyte homin receptor, the hermes antigen, is related to cartel proteoglycan core anf link proteins. Cell 56: 1063-1072, 1989.
20. Stamenkovic I, Amiot M, Pesando JM and Seed B: A lymphocyte molecule implicated in lymph node homing is a member of the cartel link protein family. Cell 56: 1057-1062, 1989.
21. Marhaba R and Zöller M: CD44 and in cancer progression: Adhesion, migration and growth regulation. J Mol Histol 35: 211-231, 2004.
22. Sugano K, Maeda K, Ohtani H, Nagahara H, Shibutani M and Hirakawa K: Expression of xCT as a predictor of disease recurrence in patients with colorectal cancer. Anticancer Res 35: 677-682, 2015.
23. Al-Maghrabi J, Gomaa W, Buhmeida A, Al-Qahtani M and Al-Ahwal M: Decreased immunoexpression of standard form of CD44 is an independent favourable predictor of nodal metastasis in colorectal carcinoma. Anticancer Res 32: 3455-3461, 2012.
24. Jayne DG, Fook S, Loi C and Seow-Choen F: Peritoneal carcinomatosis from colorectal cancer. Br J Surg 89: 1545-1550, 2002.
25. Kobayashi H, Kotake K and Sugihara K: Prognostic scoring system for stage IV colorectal cancer: Is the AJCC sub-classification of stage IV colorectal cancer appropriate? Int J Clin Oncol 18: 696-703, 2013.
26. Barker N and Clevers H: Tumor environment: A potent driving force in colorectal cancer? Trends Mol Med 7: 535-537, 2001.
27. Hur K, Toiyama Y, Takahashi M, Balaguer F, Nagasaka T, Koike J, Hemmi H, Koi M, Boland CR and Goel A: MicroRNA-200c modulates epithelial-to-mesenchymal transition (EMT) in human colorectal cancer metastasis. Gut 62: 1315-1326, 2013.
28. Bates RC: Colorectal cancer progression: Integrin alphavbeta6 and the epithelial-mesenchymal transition (EMT). Cell Cycle 4: 1350-1352, 2005.
29. Brabletz T, Hlubek F, Spaderna S, Schmalhofer O, Hiendlmeyer E, Jung A and Kirchner T: Invasion and metastasis in colorectal cancer: Epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and beta-catenin. Cells Tissues Organs 179: 56-65, 2005.
30. Arias AM: Epithelial mesenchymal interactions in cancer and development. Cell 105: 425-431, 2001.
31. Polyak K and Weinberg RA: Transitions between epithelial and mesenchymal state: Acquisition of malignant and stem cell traits. Nat Rev Cancer 9: 265-273, 2009.
32. Wijnhoven BP, Dinjens WN and Pignatelli M: E-cadherin-catenin cell-cell adhesion complex and human cancer. Br J Surg 87: 992-1005, 2000.
33. Zlobec I, Lugli A, Baker K, Roth S, Minoo P, Hayashi S, Terracciano L and Jass JR: Role of APAF-1, E-cadherin and peritumoral lymphocytic infiltration in tumour budding in colorectal cancer. J Pathol 212: 260-268, 2007.
34. He X, Chen Z, Jia M and Zhao X: Downregulated E-cadherin expression indicates worse prognosis in Asian patients with colorectal cancer: Evidence from meta-analysis. PLoS One 8: e70858, 2013.
35. Sleeman J, Kondo K, Moll J, Ponta H and Herrlich P: Variant exon v6 and v7 together expand the repertoire of glycolsaminoglycans bound by CD44. J Biol Chem 272: 31837-31844, 1997.
36. Yang AD, Fan F, Camp ER, van Buren G, Liu W, Somcio R, Gray MJ, Cheng H, Hoff PM and Ellis LM: Chronic oxaliplatin resistance induces epithelial-to-mesenchymal transition in colorectal cancer cell lines. Clin Cancer Res 12: 4147-4153, 2006.
37. Mulder JW, Kruyt PM, Sewnath M, Oosting J, Seldenrijk CA, Weidema WF, Offerhaus GJ and Pals ST: Colorectal cancer prognosis and expression of exon-v6-containing CD44 proteins. Lancet 344: 1470-1472, 1994.
38. Morrin M and Delaney PV: CD44v6 is not relevant in colorectal tumour progression. Int J Colorectal Dis 17: 30-36, 2002.
39. Stanczak A, Stec R, Bodnar L, Olszewski W, Cichowicz M, Kozlowski W, Szczylik C, Pietrucha T, Wieczorek M and Lamparska-Przybysz M: Prognostic significance of Wnt-1, β-catenin and E-cadherin expression in advanced colorectal carcinoma. Pathol Oncol Res 17: 955-963, 2011.
40. Ilyas M, Novelli M, Wilkinson K, Tomlinson IP, Abbasi AM, Forbes A and Talbot IC: Tumour recurrence is associated with Jass grouping but not with differences in E-cadherin expression in moderately differentiated Dukes' B colorectal cancers. J Clin Pathol 50: 218-222, 1997.