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The Stromal Overexpression of Decay Accelerating Factor (DAF/CD55) Correlates with Poor Clinical Outcome in Colorectal Cancer Patients

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Background: Decay accelerating factor (DAF/CD55), regulates the complement system by accelerating decay of the C3 convertase, has been described in several malignancies, however, the clinicopathologic significance of CD55 and its receptor CD97 has not been fully investigated. We examined the expression patterns of both CD55 and CD97 and their association with clinicopathologic parameters in colorectal cancers (CRCs). **Methods:** Expression patterns of CD55 and CD97 in the stroma and tumor cells at tumor center and invasive front were examined in 130 CRCs, and their significance was statistically evaluated. **Results:** CD55-high stroma was correlated with tumor border ($p = 0.006$) and invasion depth ($p = 0.013$). CD55-high tumor cells at tumor center and invasive front were correlated with histologic grade, and CD55-high tumor cells at invasive front with tumor, node and metastasis (TNM) stage ($p < 0.05$). CD97-high stroma was correlated with lymph node metastasis ($p = 0.016$) and TNM stage ($p = 0.030$). CD97-high tumor cells at tumor center and invasive front were correlated with tumor size and CD97-high tumor cells at tumor center with tumor border ($p < 0.05$). Patients with CD55-high stroma showed poor overall and recurrence-free survival ($p < 0.05$) in univariate analysis, and were independently associated with short recurrence-free survival ($p = 0.025$) in multivariate analysis. **Conclusions:** Stromal CD55 overexpression would be an indicator of adverse clinical outcome and a useful prognostic factor.

Key Words: Decay accelerating factor (DAF/CD55); CD97; Colorectal cancers; Immunohistochemistry; Prognostic factor

Active inflammation at the invasive front of the malignant tumor has been postulated as a key component in tumor dissemination and progression, as well as epithelial-mesenchymal transition (EMT).[1,2] Either excessive activation or inappropriate control in the complement cascade system may contribute to amplification of inflammation.[3] Because the complement system is nonselective and unable to distinguish between pathogen and host, host cells have to form a defense mechanism consisting of complement regulatory proteins (CRPs), which are normally expressed on the cell surface and regulate the activity of the complement cascades to maintain homeostasis.[2,4]

Decay accelerating factor (DAF/CD55) is a 70 kDa protein and a member of CRPs.[4,5] CD55 accelerates the decay of the C3 convertase and interferes with the assembly of C3/C5 convertases, therefore it controls the C3 convertase activity in both classic and alternative pathways of the complement cascade system.[1,4-7] Three CD55 isoforms have been described, i.e., glycosylphosphatidylinositol-linked, transmembrane- and soluble-CD55.[4] CD55 is also defined as a ligand for CD97, which is a family member of class II G-protein-coupled transmembrane receptors and is normally expressed on cell surfaces of leukocytes, erythrocytes, and several epithelial cancer cells.[8-10]

Recently, the interests in the activities of hypoxia-related EMT markers, as well as the roles of microenvironment in cancer dissemination, have grown in the field of cancer progression.[2,11-15] In particular, the roles of the CD55 in tumor microenvironment and the functional relationship between CD55 and CD97 are presently under intense investigation.[7,16,17] Although high expressions of CD55 and CD97 have been described in several malignancies,[1,5,8] the clinicopathologic significance of CD55 and CD97 in colorectal cancer (CRC) has not been fully understood and there have been limited studies on clinical outcomes.[8,9]

We hypothesized that CD55 protects both tumor and host cells against complement-mediated attack in the invasive front of cancer progression. The aim of the present study was to de-

termine the expression patterns of CD55 and CD97 in the tumor cells and stroma of the CRCs, in relation to tumor dissemination and prognosis. We evaluated the correlation between the clinicopathologic characteristics and the expression of CD55 and CD97.

MATERIALS AND METHODS

Case selection and specimens

The study comprised a series of 130 cases of CRC undergoing curative surgery in the Eulji University Hospital, from January 2001 to December 2004. Specimens obtained from patients who received preoperative chemoradiotherapy (CRT) were excluded in this study. Postoperative CRT was recommended in patients with completely resected stage II or III rectal cancers. All cases were confirmed histologically as primary adenocarcinomas of the colorectum. We obtained clinical and pathologic data from the patients' records, pathology reports and review of the hematoxylin and eosin slides, which were examined independently by two pathologists. The tumor grade was classified into two categories: low grade (well to moderately differentiated) and high grade (poorly to undifferentiated), as described previously.[18] Signet ring cell carcinomas and mucinous carcinomas with less than 50% gland forming were also defined as high grade tumors. We defined the tumor budding as a single or a group of less than 5 detached tumor cells[19] and classified into three grades, according to previously described criteria,[14,20] as follows: mild, tumor cells present at $<1/3$ of the entire invasive margin; moderate, 1/3-2/3 of the entire invasive margin; and marked, $>2/3$ of the entire invasive margin. Recurrence was defined as tumor occurring at the anastomosis site, in the regional lymph nodes, perineum or pelvic wall, and diagnosed by radiologic image, histological examination and colonoscopy or surgical exploration. Distant metastasis was defined as tumor outside the area of resection, including the lung, liver, bone or peritoneum.

Immunohistochemistry and interpretation

Immunohistochemical stainings were performed on formalin-fixed, paraffin embedded blocks, with LSAB detection kit (Dako, Carpentaria, CA, USA), according to the manufacturer's instructions. The primary antibodies were anti-CD55 (1:100, sc-9156, Santa Cruz, CA, USA) and anti-CD97 (1:100, EPR-4427, Abcam, Cambridge, UK). Tissue sections of 4 μm thickness were cut and mounted on the ProbeOn slides (Fisher Scientific, Pittsburgh, PA, USA). The sections were deparaffinized and rehydrated through a series of xylene and graded alcohols. After deparaffinization, slides were treated in 10 mM/L sodium citrate buffer (pH 7.0) for 20 minutes, with autoclave at 120°C for antigen retrieval. All sections for immunohistochemistry were incubated in 3% H_2O_2 for 10 minutes, to inactivate internal peroxidase, washed with 10 mM/L phosphate buffered saline buffer (pH 7.4) and incubated with normal bovine serum to reduced nonspecific antibody binding. The sections were then incubated with a primary antibody for 60 minutes, at room temperature. Detection of the immunohistochemical staining was performed with 3-amino-9-etyle carbazole, as chromogen. The sections were counterstained for 1 minute with Mayer's hematoxylin and then mounted.

Immunohistochemistry results were analyzed in a semi-quantitative manner by two independent pathologists who were blinded to outcome, using the immunoreactive score (IRS), according to previous study,[21] as the product of staining intensity (graded 0 to 3; 0, negative; 1, weak; 2, moderate; and 3, strong staining) and the percentage of positive cells (graded 0 to 4; 0, 0%; 1, $<10\%$; 2, 10-50%; 3, 51-80%; and 4, $>80\%$), resulting in a score from 0 to 12. We considered the positive if the IRS was more than 2. To determine the statistical significance of the association between CD55 and CD97 expression and clinicopathologic parameters, and to compare with results from a previous report in CRC patients,[9] all cases were categorized into two groups, based on the expression levels, i.e., a low expression group (IRS, 0 to 6) and a high expression group (IRS, 7 to 12). We separately analyzed the immunoreactivity of CD55 and CD97 in the tumor cells at the invasive front and tumor center, as well as stroma around the invasive front, respectively. Cases with conflicting results were reviewed on a multi-headed microscope and then consensus was reached.

Statistical analysis

Statistical analyses were performed using SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA). Correlations between CD55 or CD97 expression and the various clinicopathologic parameters were analyzed by Pearson's chi-square test or Fisher's exact test. To analyze overall survival and recurrence-free survival, Kaplan-Meier curves were plotted and log rank tests were used to calculate p-values. Multivariate analysis for overall survival and recurrence-free survival was performed using the Cox's propor-

tional hazard model. In all statistical analyses, p-values < 0.05 were considered as statistically significant.

RESULTS

Clinicopathologic features

The patients included 130 patients (64 men and 66 women), with a mean age of 62.4 years (range, 28 to 86 years). The mean tumor size was 5.3 cm (range, 0.8 to 15.0 cm) in maximum diameter. The majority of specimens were moderately differentiated adenocarcinoma and 101 (77.7%) cases were classified as low grade (5 well differentiated and 96 moderately differentiated) and 29 (22.3%) cases were high grade tumors (17 poorly to undifferentiated carcinomas, 3 signet ring cell carcinomas and 9 high grade mucinous carcinomas). Ninety-one (70.0%) cases had lymphovascular tumor invasion. Among the 130 patients, mild tumor budding was found in 33 (25.4%), moderate in 31 (23.8%) and marked in 66 (50.8%) cases, respectively. According to the 7th edition of the tumor, node and metastasis (TNM) classification of the American Joint Committee on Cancer (AJCC)/Union Internationale Contre le Cancer (UICC),[18] 5 (3.8%) cases were at pT1, 19 (14.5%) were at pT2, 93 (71.5%) were at pT3 and 13 (10.0%) were pT4. Sixty eight (52.3%) cases showed regional lymph node metastasis and 19 (14.6%) cases revealed distant metastasis. Nineteen (14.6%) cases were at pathologic stage I, 42 (32.3%) were at stage II, 51 (39.2%) were at stage III and 18 (13.8%) were at stage IV, respectively. Twenty seven (20.8%) cases were treated with postoperative CRT. The mean follow-up period was 52.1 months (range, 1 to 111 months). Table 1 lists the clinicopathologic characteristics of 130 CRC patients.

Table 1. Clinicopathologic characteristics of the 130 cases of colorectal cancer

Factors	No. of patients (%)
Age (yr)	62.4 ± 12.3 (28.0-86.0)[a]
< 50	22 (16.9)
≥ 50	108 (83.1)
Sex	
Male	64 (49.2)
Female	66 (50.8)
Tumor size (cm)	5.3 ± 2.2 (0.8-15.0)[a]
< 5.0	57 (43.8)
≥ 5.0	73 (56.2)
Histologic grade	
Low (well to moderate)	101 (77.7)
High (poorly to undifferentiated)	29 (22.3)
Lymphovascular invasion	
Absent	39 (30.0)
Present	91 (70.0)
Tumor border	
Pushing	21 (16.2)
Infiltrating	109 (83.8)
Tumor budding	
Mild	33 (25.4)
Moderate	31 (23.8)
Marked	66 (50.8)
Invasion depth (pT)	
pT1	5 (3.9)
pT2	19 (14.6)
pT3	93 (71.5)
pT4	13 (10.0)
Lymph node metastasis (pN)	
pN0	62 (47.7)
pN1	21 (16.1)
pN2	47 (36.2)
Distant metastasis	
Absent	111 (85.4)
Present	19 (14.6)
TNM stage	
I	19 (14.6)
II	42 (32.3)
III	51 (39.2)
IV	18 (13.9)

[a]Data shown as mean ± standard deviation (range).
TNM, tumor, node and metastasis.

Tumor cells and stroma around the invasive front overexpress CD55 protein in colorectal cancer

While CD55 expression was not detectable or only occasionally seen at low level in normal colorectal mucosae, the tumor cells showed positive immunostaining in 92 (70.8%) cases in the cytoplasm or cytoplasmic membrane of the luminal border, with or without luminal secretion (Fig. 1). Stroma around the invasive front expressed CD55 immunoreactivity in 74 (56.9%) cases and highly expressed it in 36 (27.7%) cases however, 56 (43.0%) cases were negative.

The relationship between CD55 expression and clinicopathologic characteristics was analyzed and summarized in Table 2. In brief, the stromal CD55 expression was significantly correlated with tumor border ($p = 0.006$) and advanced invasion depth ($p = 0.013$), respectively. However, other variables, such as tumor size, tumor grade, lymphovascular invasion, tumor budding, lymph node metastasis, distant metastasis and TNM stage, did not show significant correlation with stromal CD55 expression. In contrast, overexpression of CD55 in tumor cells at the tumor center showed a significant correlation with histologic grade ($p < 0.001$). Additionally, high expression of CD55 in tumor cells at the invasive front were correlated with histo-

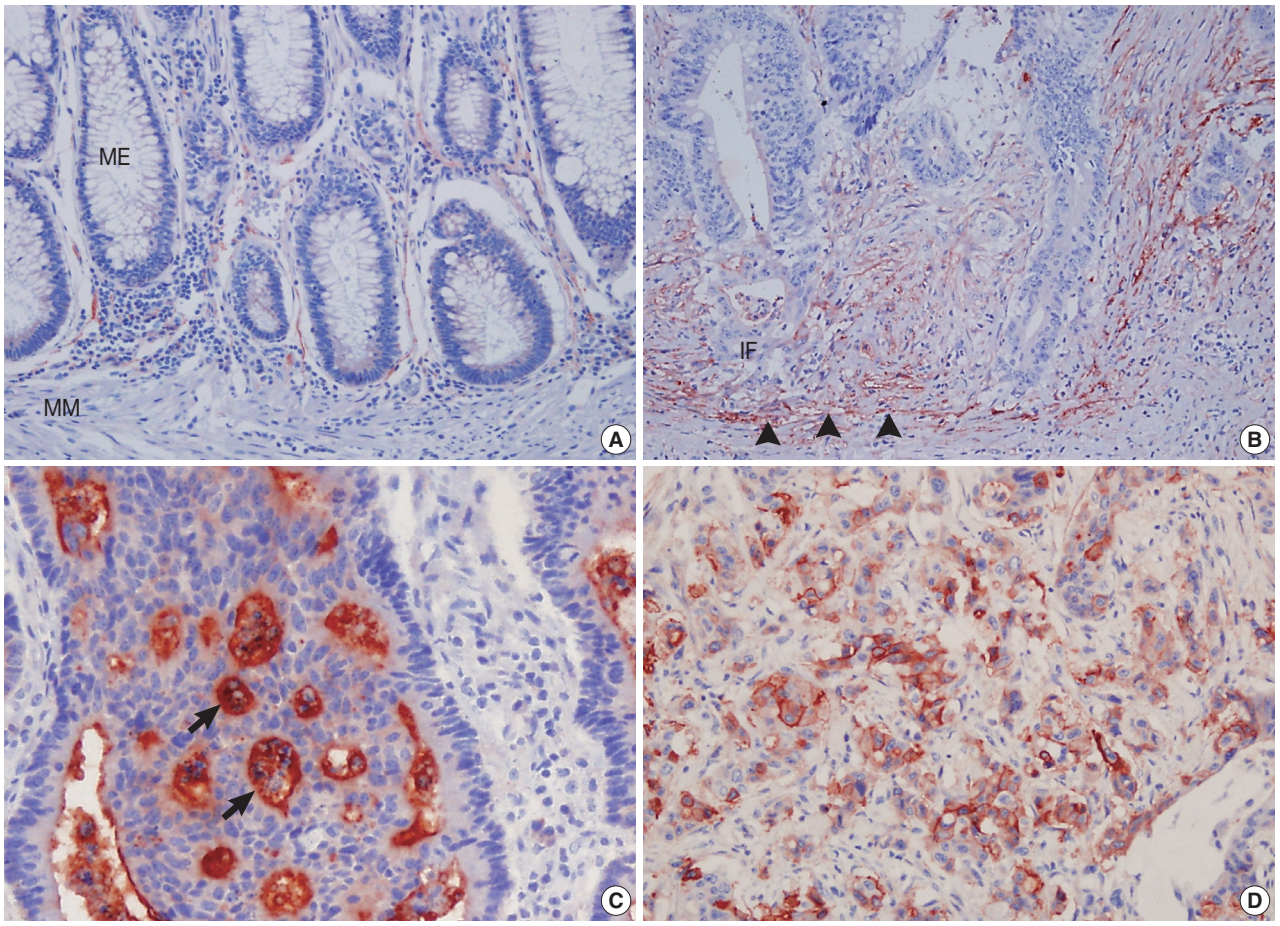

Fig. 1. CD55 immunoreactivity in colorectal carcinoma. (A) In normal glands and adjacent stroma, weak or no immunoreactivity for CD55 is identified. (B) Stroma reveals positive immunostaining for CD55 and arrowheads indicate strong immunoreactivity, especially at the invasive front. (C) Tumor cells show CD55 positive immunostining and arrows reveal strong immunostaining along the luminal borders, with secretion. (D) Tumor cells show strong CD55 immunoreactivity in the tumor cell membrane and cytoplasm. ME, mucosal epithelium; MM, muscularis mucosae; IF, invasive front.

logic grade ($p = 0.001$) and TNM stage ($p = 0.044$), respectively.

CD97 expression in colorectal cancer correlates with lymph node metastasis, tumor border and tumor size

In normal colorectal tissue, immunoreactivity for CD97 was mostly weak in the epithelia and lamina propria (IRS, 0 to 6), but strong in the smooth muscle and various inflammatory cells, including leukocytes and macrophages (Fig. 2). The expression pattern of CD97 was different from that of CD55 and readily detectable in the cell membrane and/or cytoplasm of tumor cells, without luminal secretion (Fig. 2). In tumor cells, CD97 was uniformly and highly expressed in 70 (53.9%) cases, however, 57 (43.8%) cases showed a heterogeneous expression pattern, with strong expression at the invasive front, but weak expression in the tumor center (data not shown). The tumor cells at invasive front showed higher expression of both CD55 and CD97 than those at the center ($p < 0.001$) (Fig. 3).

The correlation of CD97 expression with the clinicopathologic features was evaluated and summarized in Table 3. A significant correlation was observed between stromal CD97 expression and both lymph node metastasis ($p = 0.016$) and advanced TNM stage ($p = 0.030$). Interestingly, CD97 expression in tumor was correlated with tumor size ($p < 0.05$) and tumor border ($p = 0.043$) (Table 3). The immunohistochemical relationship between CD55 and CD97 expression in tumor cells and stroma was summarized in Table 4. There were 48 (36.9%) CD55 cases and 114 (97.7%) CD97 cases showing positive in both tumor cells and stroma. There was significant correlation between CD97 positive stroma and CD97 positive tumor cells ($p < 0.001$) (Table 4).

Table 2. Correlation between CD55 expression levels and clinicopathologic parameters

Characteristics	Stroma			Tumor					
				Center			Invasive front		
	L	H	p-value	L	H	p-value	L	H	p-value
Tumor size (cm)			0.481			0.715			0.987
<5	43	14		46	11		36	21	
≥5	51	22		57	16		46	27	
Histologic grade			0.339			**<0.001**			**0.001**
Low	71	30		90	11		71	30	
High	23	6		13	16		11	18	
LV invasion			0.231			0.332			0.178
Absent	31	8		33	6		28	11	
Present	63	28		70	21		54	37	
Tumor border			**0.006**[a]			0.135[a]			0.174
Pushing	20	1		19	2		16	5	
Infiltrating	74	35		84	25		66	43	
Tumor budding			0.064[a]			0.374			0.401
Mild	29	4		26	7		24	9	
Moderate	20	11		22	9		18	13	
Marked	45	21		55	11		40	26	
Invasion depth			**0.013**[a]			0.203[a]			0.180
pT1+pT2	22	2		21	3		18	6	
pT3+pT4	72	34		82	24		64	42	
LN metastasis			0.395			0.704			0.075
pN0	47	15		50	12		44	18	
pN1+pN2	47	21		53	15		38	30	
Distant metastasis			0.129			0.591[a]			0.307
Absent	83	28		88	23		72	39	
Present	11	8		15	4		10	9	
TNM stage			0.457			0.772			**0.044**
I+II	46	15		49	12		44	17	
III+IV	48	21		54	15		38	31	

$p < 0.05$ are highlighted in bold.
[a]Fisher's exact test.
L, low expression (IRS 0-6); H, high expression (IRS 7-12); LV, lymphovascular; LN, lymph node; TNM, tumor, node and metastasis; IRS, immunoreactive score.

Overexpression of CD55 in stroma is correlated with patients' survival rates

After an average follow-up of 52.1 months (range, 1 to 111 months), we found that 47 (36.1%) patients died of CRC with or without metastasis, 7 (5.4%) patients died of unrelated causes, 14 (10.8%) patients were still alive with local recurrence and/or distant metastasis and 62 (47.7%) patients remained alive and recurrence-free. Analysis of Kaplan-Meier survival curves showed significant difference in adverse overall survival rates ($p = 0.046$) and recurrence-free survival rates ($p = 0.001$) between patents with high and low stromal CD55 expression (Fig. 4). Other risk factors, including tumor size, grade, lymphovascular tumor invasion, lymph node metastasis, invasion depth and TNM stage, were also significantly correlated with survival rates (data not shown).

Multivariate analysis, including stromal CD55 expression, histologic grade, TNM stage and invasion depth, was performed for the overall survival and recurrence-free survival. Stromal CD-55 overexpression was independently associated with shorter recurrence-free survival ($p = 0.025$), but not with overall survival ($p = 0.171$). TNM stage was significantly correlated with both of overall survival and recurrence-free survival ($p < 0.05$). However, CD97 expression in stroma and tumor cells were not significantly correlated with survival rates (data not shown). Multivariate analysis of clinicopathologic parameters was listed in Table 5.

DISCUSSION

In the areas showing tumor budding, there was rapid deposition of leukocytes and cytokines, and up-regulation of pro-inflammatory gene expression,[22] as well as increased expression of

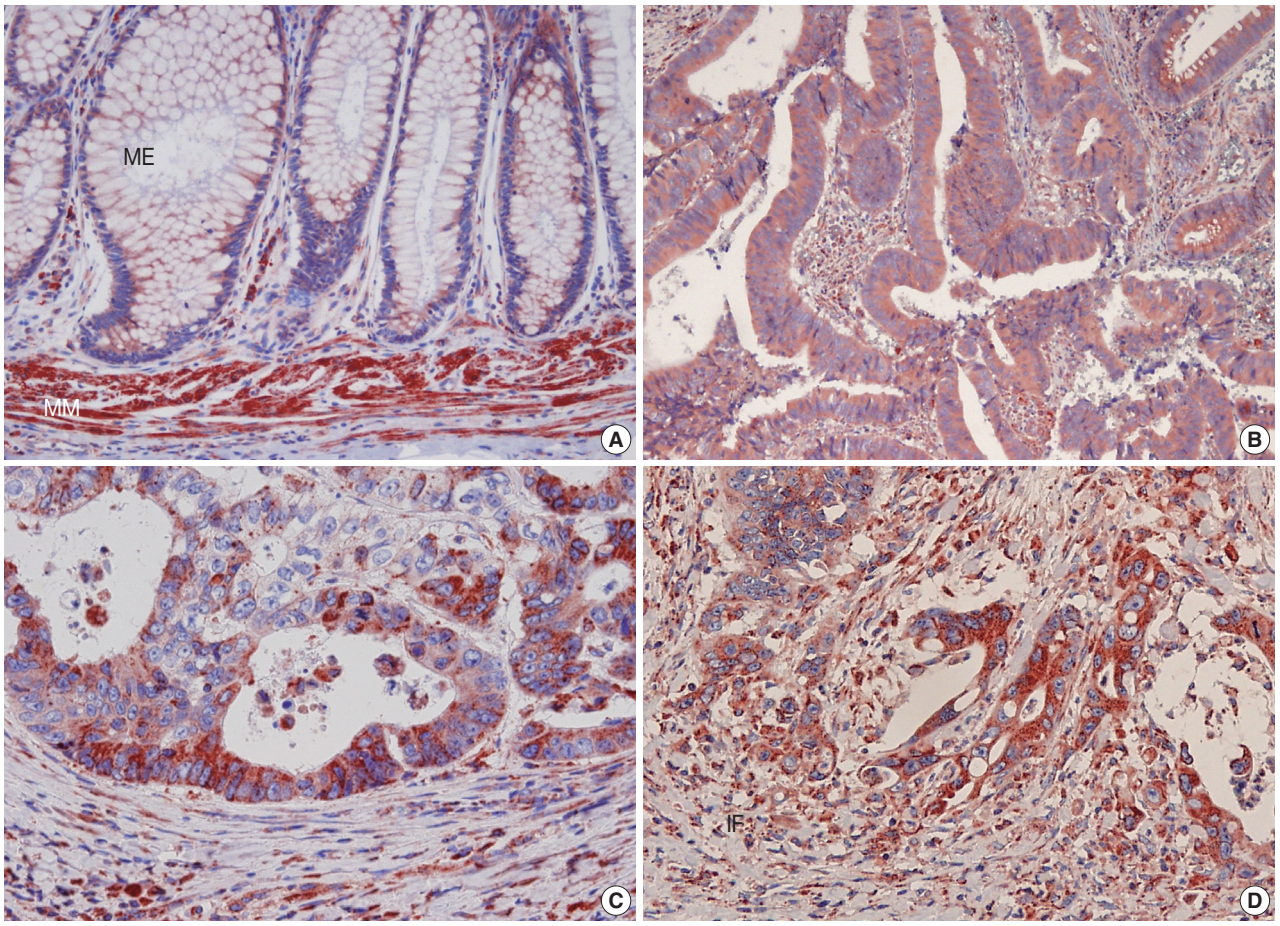

Fig. 2. CD97 immunoreactivity in colorectal carcinoma. (A) In normal glands and adjacent stroma, weak or strong CD97 expression is identified. Note that leukocytes, macrophages and smooth muscle cells show strong CD97 expression. (B) Tumor cells at tumor center show uniformly positive staining for CD97. (C) Tumor cells show heterogeneously expression for CD97 immunostining. (D) Strong positive immunostaining for CD97 is noted in tumor cells at the invasive front and in stroma around the invasive front. ME, mucosal epithelium; MM, muscularis mucosae; IF, invasive front.

proteolytic matrix metalloproteinases,[23] all of which being regarded as indication of the existence of active inflammation in the area of tumor invasion.[11,24] In active inflammation, CRPs may provide the first line of defense mechanism against complement-mediated damage to the host.[1,4-6]

Many reports suggested that CD55 provides a protective barrier to the host cells from the complement-mediated damage, in places where tumor invasion occurs.[16,17,25] Therefore, it has been postulated that tumor cells may express CD55 to protect them against complement attack. Previous studies reported CD55 expression in CRC,[7,9] however there have been conflicting results regarding the expression pattern. Niehans *et al.*[7] showed that CD55 was expressed in the stroma and tumor cells with localization to the luminal surface and basement membrane in CRCs. Han *et al.*[9] demonstrated that stroma expressed the CD-55 in rectal adenocarcinoma, but did not mention whether tumor cells express the CD55. In the present study, we show a unique expression pattern that its expression in stroma and tumor cells is particularly higher at the invasive front, than in the inner part of the tumor. Tumor cells showed CD55 immunoreactivity in cell membrane and/or cytoplasm and along the luminal border of the tumor glands, with or without secretion. Strong deposition of CD55 in the stroma and luminal border of tumor glands is consistent with the results of other investigators, who have suggested that tumor glands may release CD55 in soluble form into the adjacent extracellular matrix.[7]

It has been well established that tumor cells at the invasive front are closely related to EMT and associated with the hypoxia/hypoxia inducible factor-1α/vascular endothelial growth factor (VEGF) signaling pathway.[15,26] Moreover, the fact that VEGF up-regulated CD55 at the cell surface and within the extracellular matrix was reported in previous study.[15] Our results sug-

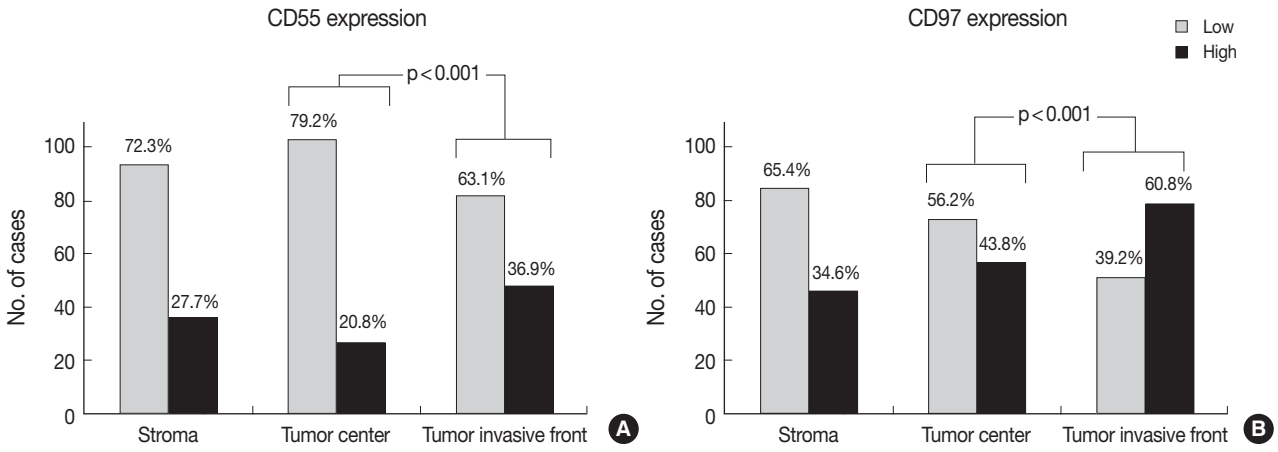

Fig. 3. Relative expression of CD55 and CD97. There are significant differences in the proportion of CD55 (A) and CD97 (B) expressions in tumor center and invasive front.

Table 3. Correlation between CD97 expression levels and clinicopathologic parameters

Characteristics	Stroma			Tumor					
				Center			Invasive front		
	L	H	p-value	L	H	p-value	L	H	p-value
Tumor size (cm)			0.310			**0.033**			**0.016**
<5	40	17		38	19		29	28	
≥5	45	28		35	38		22	51	
Histologic grade			0.986			0.069			0.145
Low	66	35		61	40		43	58	
High	19	10		12	17		8	21	
LV invasion			0.070			0.133			0.610
Absent	21	18		18	21		14	25	
Present	64	27		55	36		37	54	
Tumor border			0.714			**0.043**			0.907
Pushing	13	8		16	5		8	13	
Infiltrating	72	37		57	52		43	66	
Tumor budding			0.517			0.515			0.878
Mild	21	12		18	15		13	20	
Moderate	18	13		15	16		11	20	
Marked	46	20		40	26		27	39	
Invasion depth			0.201			0.250			0.463
pT1+pT2	13	11		16	8		11	13	
pT3+pT4	72	34		57	49		40	66	
LN metastasis			**0.016**			0.319			0.056
pN0	34	28		32	30		19	43	
pN1+pN2	51	17		41	27		32	36	
Distant metastasis			0.825			0.869			0.817
Absent	73	38		62	49		44	67	
Present	12	7		11	8		7	12	
TNM stage			**0.030**			0.425			0.076
I+II	34	27		32	29		19	42	
III+IV	51	18		41	28		32	37	

p<0.05 are highlighted in bold.
L, low expression (IRS, 0-6); H, high expression (IRS, 7-12); LV, lymphovascular; LN, lymph node; TNM, tumor, node and metastasis; IRS, immunoreactive score.

gest that CD55 provides cancer cells with protection against complement-mediated attack, and therefore contributes to survival of tumor cells. In support of this proposition, both membranous and cytoplasmic expression patterns of CD55 were more

strongly stained in tumor cells at the invasive front than those in solid tumor center. CD55 overexpression in tumor cells at the invasive front could be transient, as previously demonstrated for alteration in β-catenin localization.[27-29]

Table 4. Immunohistochemical stainings of CD55 and CD97 between stroma and tumor cells

	CD55 stroma			CD97 stroma		
	Negative (n=56)	Positive (n=74)	p-value	Negative (n=16)	Positive (n=114)	p-value
CD55 tumor cells						
Negative (n=38)	12	26	0.089	3	35	0.251[a]
Positive (n=92)	44	48		13	79	
CD97 tumor cells						
Negative (n=3)	2	1	0.396[a]	3	0	**<0.001**[a]
Positive (n=127)	54	73		13	114	

p<0.05 are highlighted in bold.
[a]Fisher's exact test.

CD97 interacts with CD55 via its N terminal epidermal growth factor like domains, and is broadly expressed in both leukocytes and lymphoid cells.[8-10] Immunohistochemical studies for CD97 in CRC and other malignancy have suggested that CD97 expression correlates with the aggressiveness, recurrence and lymph node metastasis.[8-10] Han *et al.*[9] noted a significant correlation between strong CD97 expression at the invasive front of the tumor and poor survival. In contrast, we did not find significant correlation between CD97 overexpression of the tumor cells at the invasive front and overall or recurrence-free survival rates (p>0.05).

In conclusion, CD55 was clearly overexpressed in both stroma and tumor cells, especially at the invasive front in CRCs. We first demonstrated significant correlations between stromal CD55 overexpression and adverse overall and recurrence-free survival rates in patients of CRC. Additionally, our results demonstrate that overexpression of both CD55 and CD97 may in-

Table 5. Multivariate analysis of various prognostic parameters

Clinico-pathologic parameters	Overall survival		Recurrence-free survival	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Stromal CD55 overexpression	1.51 (0.84-2.723)	0.171	1.80 (1.08-3.02)	**0.025**
Histologic grade	2.28 (1.22-4.26)	**0.010**	1.47 (0.82-2.61)	0.194
TNM stage	2.43 (1.27-4.64)	**0.007**	1.85 (1.07-3.20)	**0.027**
Invasion depth (pT)	7.20 (0.96-53.94)	0.055	6.09 (1.44-25.77)	**0.014**

p<0.05 are highlighted in bold.
CI, confidence interval; TNM, tumor, node and metastasis.

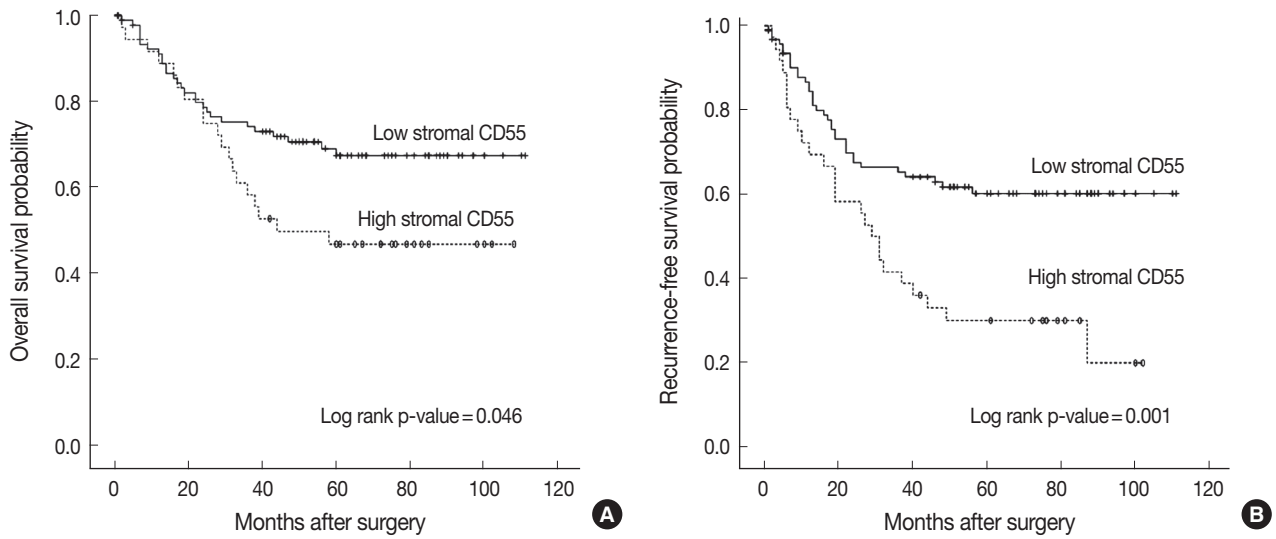

Fig. 4. Comparison of Kaplan-Meier's survival rates between colorectal cancer patients with high and low levels of stromal CD55 expression. (A) Overall survival rates. (B) Recurrence-free survival rates. High stromal CD55 expression (immunoreactive score [IRS], 7-12) is represented by the dotted line and low stromal CD55 expression (IRS, 0-6) is designed by the dashed line. There are significant differences in adverse overall survival rates and recurrence-free survival rates with stromal CD55 overexpression.

dicate poor clinical outcomes. Taken together, our findings suggest that stromal CD55 overexpression may play the important roles in tumor progression and is an independent prognostic factor for predicting short recurrence-free survival in CRC patients.

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