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■ ORIGINAL ARTICLE ■

The Expression of CD10 and CD15 Is Progressively Increased during Colorectal Cancer Development

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Background: The aim of this study was to examine the expression of CD10 and CD15 in tumor cells, stromal cells and infiltrating inflammatory cells during colorectal carcinoma (CRC) development and to investigate their expression levels between the tumor center and invasive front and compare them to clinicopathological parameters in invasive CRC. **Methods:** We performed immunohistochemical staining for CD10, CD15, and E-cadherin in 42 cases of CRC, 49 of tubular adenoma, 15 of hyperplastic polyp, and 17 of non-neoplastic colon. **Results:** CD10 was expressed in tumor cells (tCD10), stromal cells (sCD10) and infiltrating inflammatory cells (iCD10), and CD15 was expressed in tumor cells (tCD15) and infiltrating inflammatory cells (iCD15). Their expressions were progressively increased during CRC development and the iCD10 expression level was significantly correlated with the iCD15 expression level in invasive CRC. Invasive front revealed a higher expression level of iCD10 and iCD15 than the tumor center. Moreover, the iCD15 expression level of invasive front was significantly correlated with the degree of tumor budding and tCD15 in whole tissue sections was closely associated with tumor depth. **Conclusions:** The present study suggests that the expression of CD10 and CD15 is associated with the development and progression of CRC.

Key Words: Neprilysin; Antigens, CD15; Colorectal neoplasms

Matrix metallopeptidases (MMPs) are involved in cancer development via the release of bioactive molecules that inhibit apoptosis and stimulate invasion, degradation of extracellular matrix (ECM) components, promotion of angiogenesis and modulation of the immune response.[1] CD10 is a membrane-bound zinc-dependent metalloprotease that has been denominated neutral endopeptidase, enkephalinase, neprilysin, and common acute lymphoblastic leukemia antigens.[2] CD10 is normally expressed in such tissues as epithelial cells of the kidney, breast, lung, intestine, and prostate and the derangement of CD10 expression has been linked to the development in many kinds of tumors.[2] Previous studies have reported that CD10 suppressed the growth of pancreas and lung cancer cells.[3,4] Another recent study demonstrated a higher degree of CD10 expression in primary bladder tumors and tumor center compared to nodal metastatic foci and invasion front.[5] In that study, primary tumors with high CD10 expression were also correlated with a good prognosis. In addition, CD10 expression was associated with a favorable outcome in uterine cervical cancer and non-small cell lung cancer.[2] Moreover, CD10-expressing fibroblasts induced by interleukin-1 produced by squamous cell carcinoma inhibited the invasion of cancer cells through the degradation of substance P.[6] CD10 expression in colorectal cancer (CRC) tissue has been observed in tumor cells, tumor-associated fibroblasts and infiltrating inflammatory cells.[7-11] Contrary to the biologic effects of CD10 in other tumors, its expression in CRC cells was closely correlated with liver metastasis and a high clinical stage in CRC.[7,8] CD10 expression in stromal cells was increased during CRC development and was also associated with liver metastasis.[9] In addition, recent studies have reported that CD10 expression in infiltrating myeloid cells of CRC tissue is associated with enhanced tumor budding grade and a poor prognostic for recurrence-free and overall survival in stage I-III CRC.[10,11]

CD15, also called sialyl Lewis x (sLex), is expressed on the surface of human leukocytes and interacts with activated endothelial cells.[12] CD15 on tumor cells mediated the adhesion of tumor cells to endothelial cells and its high expression was cor-

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related with invasive potential.[13,14] CD15 expression of tumor cells in CRC was related to CRC development and poor clinical outcomes.[15,16] Recently, CD15 expression in both tumor cells and infiltrating leukocytes was strongly correlated with CD10 expression in CRC.[10] The tumor microenvironment at the invasive front of CRC may reflect the biologic behavior of the tumor since tumor progression and tumor cell dissemination take place in that area. For example, tumor budding is frequently observed at the invasive front of CRC and is widely considered to be a tumor dissemination phenotype and is related to aggressiveness in CRC.[17]

The aim of this study was to examine the expression of CD10 and CD15 in tumor cells, stromal cells, and infiltrating inflammatory cells during CRC development, investigate their expression levels between the tumor center and the invasive front and compare them to clinicopathological parameters in invasive CRC.

MATERIALS AND METHODS

Patients and tissue samples

A total of 123 patients with non-neoplastic colon (n=17), hyperplastic polyp (n=15), low grade tubular adenoma (n=22), high grade tubular adenoma (n=27), intramucosal CRC (n=10), and invasive CRC (n=32; 5 with T2 CRC and 27 with T3 CRC) treated at Dongguk University Gyeongju Hospital between 2009 and 2012 were enrolled in this study. High grade tubular adenoma is defined as a mucosal change with cytological and architectural features of malignancy but without evidence of invasion into the stroma.[18] We selected patients whose paraffin embedded tissues were relatively well preserved and whose medical records were complete. We excluded patients who underwent preoperative chemotherapy and emergency surgery, and patients who were diagnosed with mucinous adenocarcinoma. The characteristics of the study subjects are summarized in Table 1. Specimens were fixed in 10% formalin for 12-24 hours and embedded in paraffin blocks. Tissue sections were sampled along the maximum tumor diameter and we included the deepest site of cancer invasion.

Microscopic examination, immunohistochemistry and assessment

Differentiation, tumor depth and status of lymph node metastasis were assessed after reviewing each tumor slide. The stage was defined according to the TNM staging system of the American Joint Committee on Cancer. The presence of budding was determined according to the criteria proposed by Ueno *et al*.[17] The authors defined an isolated single cancer cell and a cluster composed of fewer than five cancer cells as tumor budding. In invasive CRC, tumor budding foci was examined in tissue sections immunohistochemically stained using the anti-E-cadherin antibody and the number of tumor budding (NTB) was counted in a field in which budding intensity was considered maximal at high power magnification. To identify the correlation between NTB and the expression level of CD10 and CD15 in invasive CRC, our cases were divided into two categories based on the NTB: low NTB (0-10) and high NTB (>10).

Tissue sections of 4 μm thickness were made and were spread on poly-L-lysine coated slides. The paraffin sections were immersed in three changes of xylene and hydrated using a graded series of alcohol solutions. Antigen retrieval was performed routinely by immersing the sections in 0.01 M citrate buffer (pH 6.0) in an autoclave for 15 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 minutes, followed by incubation of the sections with primary antibody for two hours at room temperature; the primary antibodies included anti-E-cadherin (1:1,000, Transduction Laboratories, Lexington, KY, USA), anti-CD10 (1:100, Novocastra Laboratories Ltd., Newcastle, UK), and anti-CD15 (1:250, Dako, Santa Barbara, CA, USA). Immunohistochemical staining was performed using an EnVision kit (Dako) and the color was developed with 3,3'-diaminobenzidine tetrahydrochloride (Zymed Laboratories, Inc., South San Francisco, CA, USA) as a chromogen. The sections were counterstained with Meyer's hematoxylin for three minutes and then mounted. Mouse and rabbit IgG isotypes, rather than the primary antibody, were used as negative controls.

The immunoreactivity of CD10 was evaluated based on the extensity of tumor cells (tCD10), stromal cells (sCD10) and infiltrating inflammatory cells (iCD10). The immunoreactivity of CD15 was also determined on the extensity of tumor cells or epithelial cells (tCD15) and infiltrating inflammatory cells (iCD15). tCD10 and tCD15 were expressed at the apical membrane or cytoplasm. Positive tCD10 and tCD15 were defined as the presence of 10% or more positive cells in tumor cells or epithelial cells.[10] In whole tissue section, the extensity of sCD10, iCD10 and iCD15 was graded according to a 4-point scale based on the percentage of stained area: 0 (stained area, 0-10%), 1 (stained area, 11-20%), 2 (stained area, 21-50%), and 3 (stained area, >50%). In invasive CRC, the extent of sCD10, iCD10, and iCD15 was evaluated in the tumor center and invasive front. For statistical analysis, our cases were divided into two groups: the

negative group (0, 1) and the positive group (2, 3).

Statistical analysis

The chi-square test, Fisher exact test and Pearson correlation were used. Statistical significance was determined as p-value less than 0.05.

RESULTS

As shown in Fig. 1, CD10 was expressed in tumor cells, stromal cells and infiltrating immune cells. tCD10 was expressed at the apical membrane or cytoplasm. sCD10 was mainly localized in subepithelial stromal cells in the periluminal region of the lamina propria in tubular adenoma and intramucosal CRC. On the contrary, sCD10 expression in invasive CRC was diffusely scattered around tumor cells. Inflammatory cells with CD10 expression (iCD10) were predominantly neutrophils. We examined the immunoreactivity of CD10 expression during CRC development. As shown in Fig. 2A, the positive rate of tCD10 expression was 0% (0 out of 17) in non-neoplastic colon, 0% (0 out of 15) in hyperplastic polyp, 14% (3 out of 22) in low grade tubular adenoma, 22% (6 out of 27) in high grade tubular adenoma, 40% (4 out of 10) in intramucosal CRC and 44% (14 out of 32) in invasive CRC. The positive rate of sCD10 expression was 0% in non-neoplatic colon and hyperplastic polyp, 14% (3 out of 22) in low grade tubular adenoma, 41% (11 out of 27) in high grade tubular adenoma, 70% (7 out of 10) in intramucosal CRC and 88% (28 out of 32) in invasive CRC. The positive rate of iCD10 expression was 47% (15 out of 32) in invasive CRC and nearly 0% in other diseases. Therefore, tCD10 expression level in invasive CRC was significantly higher than that of non-neoplastic diseases and low grade tubular adenomas ($p < 0.05$), and there was an insignificant difference in its expression level among high grade tubular adenoma, intramucosal and invasive CRC. sCD10 expression level was significantly higher in invasive CRC compared to other diseases, except for intramucosal CRC ($p < 0.05$) and there was an insignificant difference in its expression level between high grade tubular adenoma and intramucosal CRC. In addition, both tCD10 and sCD10 in adenoma were not related to the degree of dysplasia. iCD10 expression level was the highest in invasive CRC compared to all other diseases ($p < 0.05$).

As shown in Fig. 1, CD15 was expressed in tumor cells and infiltrating immune cells. The expression pattern of tCD15 was similar to that of tCD10. Inflammatory cells with CD15 expression (iCD15) were also predominantly neutrophils. We also examined the immunoreactivity of CD15 expression during CRC development. As shown in Fig. 2B, the positive rate of tCD15 was 0% in non-neoplastic colon, 13% (2 out of 15) in hyperplastic polyp, 23% (5 out of 22) in low grade tubular adenoma,

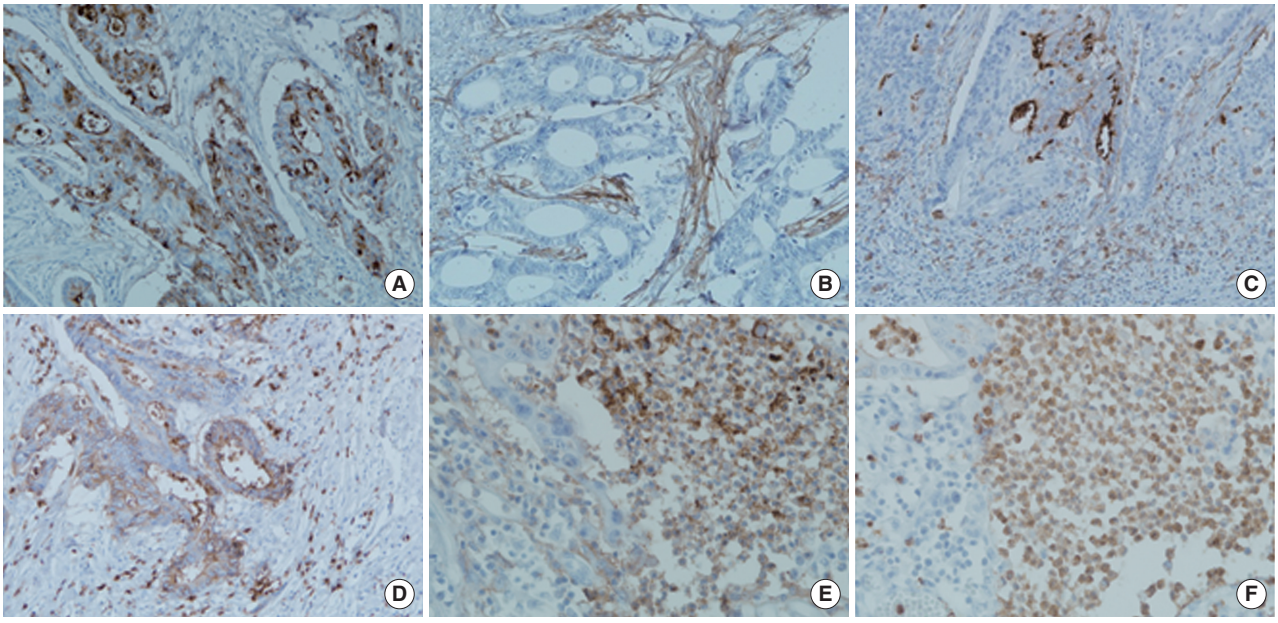

Fig. 1. Immunohistochemical staining of CD10 (A-C, E) and CD15 (D, F) in representative invasive colorectal carcinoma. CD10 is expressed in tumor cells (A), stromal cells (B), and infiltrating inflammatory cells (C). (D) CD15 is also expressed in tumor cells and infiltrating inflammatory cells. The corresponding area is examined for CD10 (E) and CD15 (F) in inflammatory cells. CD10-expressing inflammatory cells (E) are mainly neutrophils and also show CD15 expression (F).

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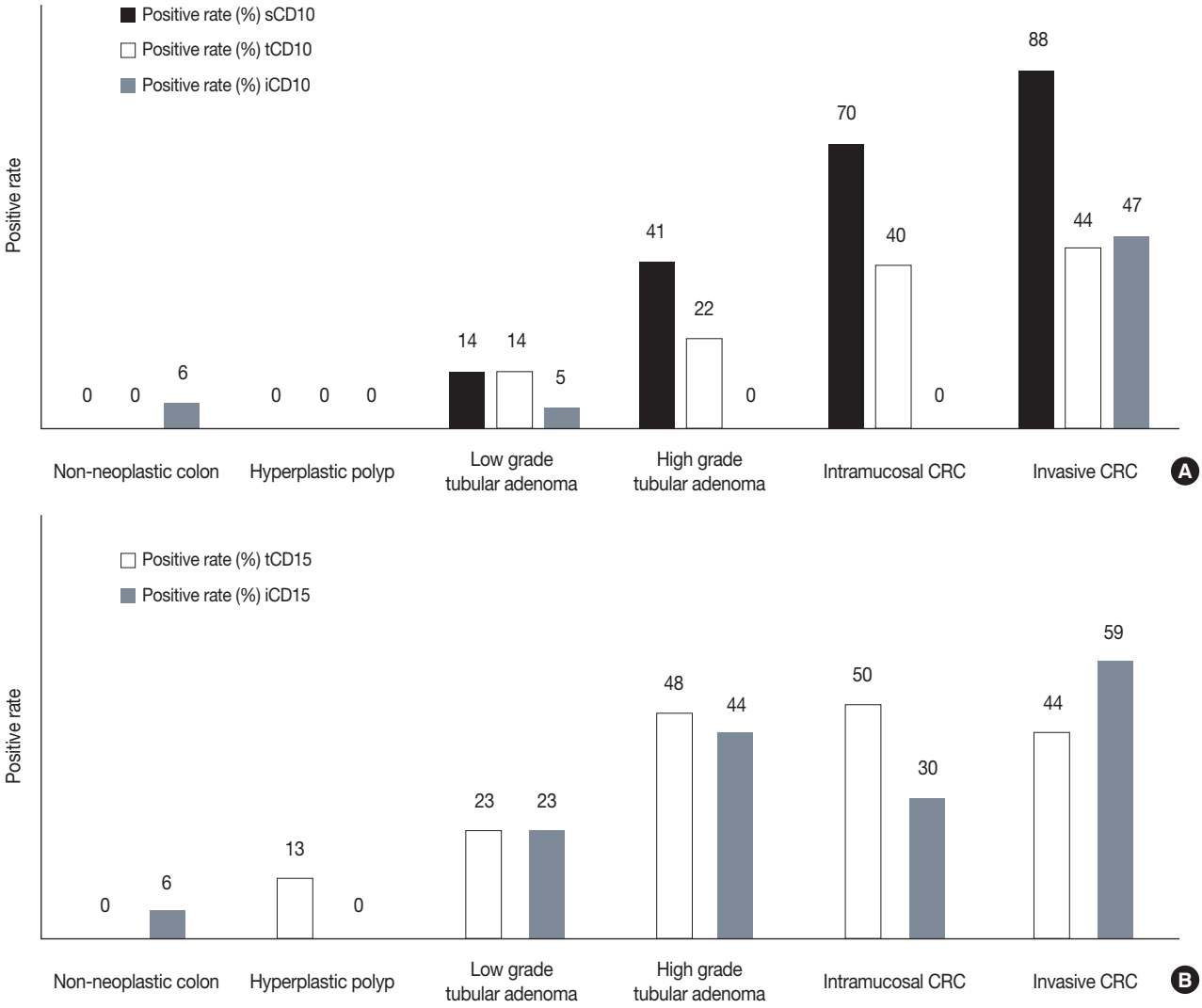

Fig. 2. The analysis of CD10 (A) and CD15 (B) expression during colorectal carcinoma (CRC) development. tCD10 expression level in invasive CRC is significantly higher than that of non-neoplastic diseases and low grade tubular adenomas ($p < 0.05$), and there is an insignificant difference in its expression level among high grade tubular adenoma, intramucosal and invasive CRC. sCD10 expression level is significantly higher in invasive CRC compared to other diseases except for intramucosal CRC ($p < 0.05$) and there is an insignificant difference in its expression level between high grade tubular adenoma and intramucosal CRC. In addition, both tCD10 and sCD10 in adenoma is not related to the degree of dysplasia. iCD10 expression is the highest in invasive CRC ($p < 0.05$). tCD15 expression level is significantly higher in high grade tubular adenoma, intramucosal and invasive CRC than in non-neoplastic colon ($p < 0.05$), and there is an insignificant difference in its expression level among neoplastic diseases. iCD15 expression level is significantly higher in invasive CRC than in non-neoplastic diseases and low grade tubular adenoma ($p < 0.05$), there is an insignificant difference in its expression level among high grade tubular adenoma, intramucosal and invasive CRC. The numbers on the bar graphs indicate percentage.

48% (13 out of 27) in high grade tubular adenoma, 50% (5 out of 10) in intramucosal CRC and 44% (14 out of 32) in invasive CRC. Therefore, tCD15 expression level was significantly higher in high grade tubular adenoma, intramucosal and invasive CRC compared to non-neoplastic colon ($p < 0.05$), and there was an insignificant difference in its expression level among neoplastic diseases. The positive rate of iCD15 was 6% (1 out of 17) in non-neoplastic colon, 0% in hyperplastic polyp, 23% (5 out of 22) in low grade tubular adenoma, 44% (12 out of 27) in high grade tubular adenoma, 30% (3 out of 10) in intramucosal CRC and 59% (19 out of 32) in invasive CRC. Therefore, the iCD15 expression level was significantly higher in invasive CRC than in non-neoplastic diseases and low grade tubular adenoma ($p < 0.05$) and there was an insignificant difference in its expression level among high grade tubular adenoma, intramucosal and invasive CRC.

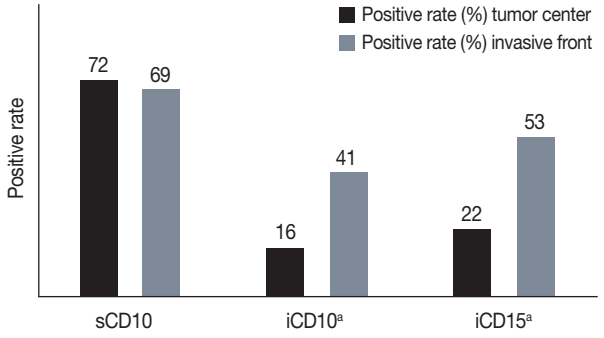

Fig. 3. An analysis for the expression of sCD10, iCD10, and iCD15 in the tumor center and invasive front. The expression level of iCD10 and iCD15 is significantly higher in the invasive front than in the tumor center ($^{a}p < 0.05$), however there is no significant difference in sCD10. Numbers on the bar graphs indicate percentage.

Table 1. Patient characteristics

	No. of cases	Age distribution (yr)	Gender (male:female)
Non-neoplastic colon	17	43-76	11:6
Hyperplasitc polyp	15	22-67	9:6
Low grade tubular adenoma	22	35-78	12:10
High grade tubular adenoma	27	42-82	20:7
Intramucosal CRC	10	52-78	5:5
Invasive CRC	32	57-87	20:12

CRC, colorectal carcinoma.

As shown in Fig. 1E and F, CD10-expressing inflammatory cells showed CD15 expression. There was a positive correlation between the expression level of iCD10 and iCD15 in whole tissue sections, the tumor center and invasive front of invasive CRC ($r = 0.815$, $r = 0.730$, and $r = 0.776$, respectively; $p < 0.05$). In invasive CRC, we compared the expression of sCD10, iCD10 and iCD15 in the tumor center to that in the invasive front and examined the relationship between their expression levels in the invasive front and NTB. As shown in Fig. 3, the positive rate of sCD10 was 72% (23 out of 32) in the tumor center and 69% (22 out of 32) in the invasive front, that of iCD10 was 16% (5 out of 32) in the tumor center and 41% (13 out of 32) in the invasive front, and that of iCD15 was 22% (7 out of 32) in the tumor center and 53% (17 out of 32) in the invasive front. Therefore, the expression level of iCD10 and iCD15 was significantly higher in the invasive front compared to the tumor center ($p < 0.05$). In invasive CRC, there were 11 tumors with low NTB and 21 tumors with high NTB. As shown in Fig. 4, the positive rate of iCD15 was 18% (2 out of 11) in invasive CRC with low NTB and 71% (15 out of 21) in invasive CRC with high NTB. There was a significant correlation between NTB and iCD15 expression of the invasive front ($p < 0.05$).

We compared the expression of CD10 and CD15 to clinicopathologic parameters of invasive CRC. As shown in Table 2, the positive rate of tD15 in whole tissue sections was 0% (0 out of 5) in T2 CRC and 52% (14 out of 27) in T3 CRC. The expression level of tCD15 was significantly associated with tumor depth ($p < 0.05$). However, any other clinicopathologic parameters did not show significant association with the expression of CD10 and CD15. In addition, the expression of CD10 and CD15 in the tumor center and invasive front, respectively, was not significantly associated with any clinicopathologic parameters (data not shown).

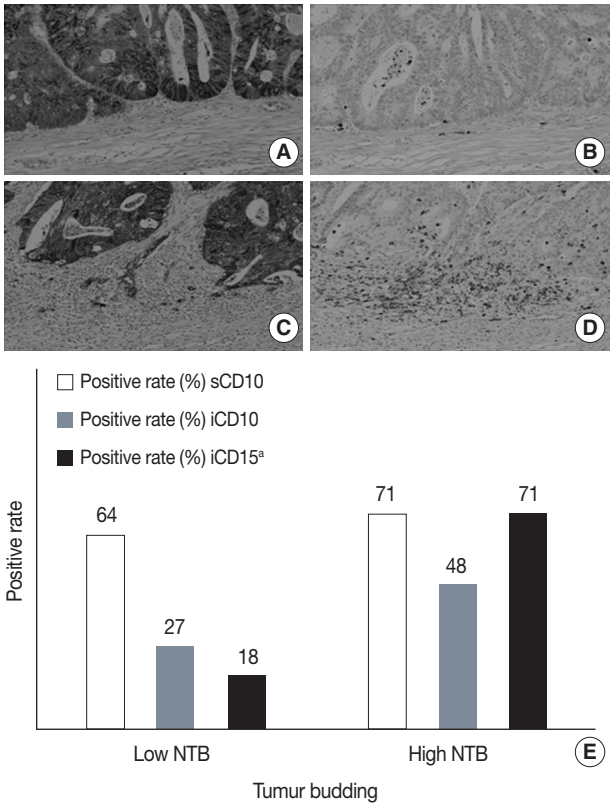

Fig. 4. Immunohistochemical staining of E-cadherin (A, C) and CD15 (B, D) in corresponding areas of invasive front in invasive colorectal cancer (CRC) and the relationship between tumor budding and the expression of sCD10, iCD10, and iCD15 in invasive CRC (E). Tumor infiltrating inflammatory cells with CD15 expression are more frequently seen in the invasive front with a high degree of tumor budding. There is a significant correlation between the number of tumor budding (NTB) and iCD15 expression of the invasive front ($^{a}p < 0.05$). Numbers on the bar graphs indicate percentage.

DISCUSSION

CD10 is an important molecule capable of integrating signals from either the cell environment or the intracellular compartment by cleaving peptides through enzymatic activity and

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Table 2. Relationship between clinicopathological parameters and the expression of CD10 and CD15 in whole tissue sections of invasive colorectal carcinoma

	No. of cases	Positive, n (%)				
		sCD10	tCD10	iCD10	iCD15	tCD15
Gender						
Male	20	18 (90)	10 (50)	9 (45)	10 (50)	10 (50)
Female	12	10 (83)	4 (33)	6 (50)	9 (75)	4 (12)
Differentiation						
Well	5	4 (80)	2 (40)	4 (80)	4 (80)	1 (20)
Moderate to poor	27	24 (89)	12 (44)	11 (41)	15 (56)	13 (48)
Location						
Right and transverse	13	11 (85)	6 (46)	7 (54)	8 (62)	6 (46)
Left	19	17 (89)	8 (42)	8 (42)	11 (58)	8 (42)
Depth						
T2	5	5 (100)	3 (60)	4 (80)	4 (80)	0 (0)
T3	27	23 (85)	11 (41)	11 (41)	15 (56)	14 (52)[a]
Node metastasis						
Absence	21	17 (81)	8 (38)	10 (21)	14 (67)	8 (38)
Presence	11	11 (100)	6 (55)	5 (11)	5 (45)	6 (55)

[a]$p < 0.05$.

through intracellular signaling pathways that interfere with other major signaling pathways.[2] Therefore, the deregulation of CD10 expression leads to the accumulation or loss of peptides, disturbing the regulation of cellular proliferation and differentiation, and altering intracellular signaling pathways.[2] It is thus obvious that the derangement of CD10 expression is associated with the development or progression of a variety of tumors. In the current study, CD10 was expressed in stromal cells, inflammatory cells, and tumor cells during CRC development, which is consistent with previous publications.[7-11] Furthermore, its expression level in each cell was progressively increased from non-neoplastic colon to CRC. These findings suggest that CD10 expression in the tumor microenvironment is involved in CRC development.

In the current study, tCD10 expression was significantly higher in invasive CRC than in non-neoplastic diseases and low grade tubular adenoma, and did not show significant difference among high grade tubular adenoma, intramucosal and invasive CRC. sCD10 expression was significantly higher in invasive CRC than other diseases except for intramucosal CRC and there was an insignificant difference in its expression level between high grade tubular adenoma and intramucosal CRC. These findings indicate that sCD10 expression is more important in cancer cell invasion than tCD10 expression and the biologic behavior of tCD10 and sCD10 is similar between high grade tubular adenoma and intramucosal CRC. Inflammatory and healing processes accompany tumor cell invasion of the surrounding tissue. A previous study suggested that sCD10 expression may be induced by the local inflammatory process in malignant tumors.[19] CD10 can create a tumor microenvironment to facilitate cancer cell invasion and metastasis due to its structural similarity to MMPs.[2] In addition, a recent in vitro study has demonstrated that sCD10 strongly interacted with CD133-positive colon cancer cells and enhances its invasion.[20] Moreover, a previous study suggested that sCD10 had an important role in invasion and metastasis in the tissues of CRC.[9] On the other hand, some reports have shown that tCD10 expression in CRC was closely related to liver metastasis and high clinical stages.[7,8] Mucosal high grade neoplasia in gastrointestinal epithelial neoplasia is composed of four diagnostic terms such as high grade adenoma, carcinoma in situ, suspicious for invasive carcinoma and intramucosal carcinoma.[21] The rationale for putting together these diagnoses under one category is that their clinical behavior is nearly identical and they cannot be reproducibly diagnosed. Based on the studies that sCD10 and tCD10 are involved in cancer cell invasion and metastasis, the current result that the expression of sCD10 and tCD10 is similar between high grade adenoma and intramucosal CRC supports the notion that the clinical behavior is the same among the four diagnoses categorized under mucosal high grade neoplasia. Our study showed that there was no significant correlation between expression of tCD10 and sCD10 and prognostic factors such as nodal metastasis and tumor depth in invasive CRC. This may be due to so the low number of CRC cases analyzed.

In the current study, CD10 was also expressed in inflammatory cells, which revealed CD15 expression and showed morphology similar to neutrophils. Recent studies have demonstrated that most CD10-positive immune cells are derived from

myeloid cells and are neutrophil granulocytes based on the results showing the coexpression of CD11b and CD15, and morphology similar to neutrophils.[10,11] The present study showed that iCD15 expression level was higher than the iCD10 expression level during CRC development. This indicates that tumor-infiltrating neutrophils were divided into a high CD10-expressing group and a low CD10-expressing group. Granulocyte-macrophage colony-stimulating factor (GM-CSF) can enhance CD10 expression in normal neutrophils *in vitro*.[22] Its serum level was significantly higher in CRC than in normal persons.[23] The infiltration of neutrophils with high CD10 expression was correlated with a poor prognosis, while low expressing neutrophils showed a favorable prognosis in CRC.[11] However, the present study did not show any correlation between the iCD10 expression level and prognostic factors such as nodal metastasis and tumor depth. This may be caused by the low number of CRC cases analyzed. The possibility that other pathophysiological functions of neutrophils may be closely associated with the prognosis of CRC also cannot be excluded.

Recently, accumulating evidence indicates that neutrophils play an important role in malignant transformation, tumor progression, angiogenesis and the modulation of the antitumor immunity in several tumors.[24] Neutrophils regulate the invasion at multiple levels. Neutrophils can facilitate tumor invasion by directly degrading ECM through the release of MMP-9.[25] Tumor-derived cytokines such as TNF-alpha and GM-CSF induce the release of hepatocyte growth factor and oncostatin M by neutrophils, which promote the invasion and migration of tumor cells.[26] Neutrophils may prepare tumor cells to degrade ECM and enhance the motility of tumor cells through the activation of MMP-2 and Rho kinase, and by phosphorylating focal adhesion kinase and paxillin.[27] In the current study, iCD15 and iCD10 were expressed more in the invasive front than in the tumor center. In addition, the iCD15 expression level in the invasive front was closely associated with the degree of tumor. These findings suggest that tumor-infiltrating neutrophils are involved in tumor invasion and its mechanism can be explained by the above mentioned pathophysiological function as well as CD10 function.

CD15 is expressed in neutrophils and it binds to the E-selectin of endothelial cells, which leads to neutrophil migration into tissue.[12] CD15 expression in tumor cells was also involved in the adhesion of tumor cells to endothelial cells and its expression level in colon cancer cells was correlated with the capacity of invasion and metastasis.[13,14] Therefore, previous publications have reported that high tCD15 expression was related to liver metastasis tumor depth and disease recurrence in CRC.[16,28] At this time, the current study could not confirm the role of tCD15 expression on disease recurrence and metastasis. However, our result that tCD15 expression was associated with tumor depth could verify the biologic function of tCD15 expression related to tumor invasion. In addition, a recent publication has reported that CD15 expression in tumor or epithelial cells was observed in 7% of healthy tissue, 27% of adenomas and 75% of CRC.[15] In that report, its expression level in adenoma was significantly associated with the degree of dysplasia. However, the current study showed that tCD15 expression was higher in high grade tubular adenoma and CRC than in non-neoplastic colon, but there was an insignificant difference in its expression level among neoplastic diseases. This discrepancy in the role of tCD15 during CRC development is to be confirmed by more extensive studies.

In summary, the present study showed that CD10 was expressed in tumor cells, stromal cells and inflammatory cells, and CD15 was also expressed in tumor cells and inflammatory cells. Their expressions progressively increased during CRC development. iCD10 expression level was significantly correlated with iCD15 expression level in invasive CRC. iCD10 and iCD15 were expressed more in the invasive front than in the tumor center. Moreover, the iCD15 expression level of the invasive front was significantly correlated with the degree of tumor budding. tCD15 expression level was closely associated with the tumor depth of invasive CRC. In conclusion, the expression of CD10 and CD15 are associated with the development and progression of CRC.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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