Altered expression of cytokeratin 7 and CD117 in transitional mucosa adjacent to human colorectal cancer

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Abstract. The multi-step progression of colorectal cancer through precancerous lesions (adenoma and dysplasia) is associated with cumulative molecular alterations, a number of which have also been demonstrated to be present in morphologically normal transitional mucosa adjacent to colorectal cancer. The cytoskeletal protein cytokeratin 7 (CK7) and the receptor tyrosine kinase, KIT proto-oncogene receptor tyrosine kinase (CD117), encoded by the proto-oncogene c-Kit, are lacking in normal colorectal crypt epithelium and are aberrantly expressed in a subset of colorectal cancer. The aim of the present study was to evaluate the expression of CK7 and CD117 in morphologically normal transitional mucosa adjacent to colorectal cancer. Immunohistochemical staining for CK7 and CD117 was performed in the mucosa adjacent to five groups of surgically resected colorectal tumors [low-grade adenoma, high-grade adenoma, mucosal adenocarcinoma, small-sized invasive adenocarcinoma ($\leq$2 cm) and large-sized invasive adenocarcinoma (>2 cm)]. CK7 was expressed in the mucosa adjacent to a subset of colorectal tumors, and the positivity ratio increased according to tumor grade from low-grade adenoma up to small-sized invasive adenocarcinoma (61.2%). However, the positivity ratio of CK7 in the mucosa adjacent to the large-sized invasive adenocarcinoma (25.0%) was significantly lower compared with that of the next lower grade. CD117 was also expressed in the mucosa adjacent to a subset of colorectal tumors. In contrast to CK7, the positivity ratio of CD117 increased according to tumor grade from low-grade adenoma all the way through to the large-sized invasive adenocarcinoma (45.0%). Based on these results, the mechanism of CK7 and CD117 expression in the transitional mucosa adjacent to colorectal cancer may be different, and analysis of their individual expression may provide novel insights into the development and progression of colorectal cancer.

Introduction

Human colorectal cancer has multiple steps of progression, and neoplastic conversion of the colorectal crypt epithelium is associated with cumulative alterations of numerous genes, proteins and molecular processes (1). Molecular changes associated with the earlier steps may be demonstrated in precancerous lesions, including adenoma and dysplasia, which occasionally coexist with overt cancer (1-3). Increasing data indicates that a number of these molecular alterations also occur in morphologically normal transitional mucosa adjacent to colorectal cancer, under the basic concept of either field cancerization (areas of preneoplastic status) (4-7) or molecular crosstalk between cancer and surrounding non-neoplastic tissues (8).

Cytokeratin (CK)7 is one of the intermediate filament proteins that constitute the cytoskeleton of numerous types of epithelial cells. Colorectal crypt epithelia and tumors derived from it are representative of tissue that is negative for CK7, and the coordinate expression pattern of CK7 negativity with CK20-positivity (CK7-/CK20+) is used for confirmation of the colorectal origin of metastatic tumors (9). A number of studies have noted aberrant expression of CK7 in a subset of colorectal cancer (10,11).

The type III receptor tyrosine kinase, KIT proto-oncogene receptor tyrosine kinase (CD117), which is encoded by the c-kit proto-oncogene, is normally expressed in a variety of human tissues, including hematopoietic stem cells, melanocytes, mast cells, germ cells and interstitial cells of Cajal (12-24). CD117 is also expressed in human tumors, including myeloid leukemia, melanoma, glioblastoma, germ cell tumors, breast cancer, small cell lung cancer and gastrointestinal stromal tumors (GISTs) (24). CD117 is not expressed in the normal crypt epithelium in the colon and the rectum, but aberrant expression of CD117 has been reported in a subset of cancers (24).

CK7 and CD117 expression has been demonstrated not only in developed colorectal cancer, but also in adenoma and dysplasia (2,3,24). To the best of our knowledge, however, the expression of CK7 and CD117 in morphologically normal

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transitional mucosa adjacent to colorectal cancer has not been investigated to date. The lack of this information prompted the authors of the present study to assess the expression of CK7 and CD117 in morphologically normal transitional mucosa adjacent to colorectal cancer.

Materials and methods

Patients and tissue specimens. The tissue specimens analyzed in the present study consisted of tissue from 76 lesions of low-grade adenoma (from 65 patients), 18 lesions of high-grade adenoma (from 17 patients), 12 lesions of mucosal adenocarcinoma (from 12 patients), 67 lesions of small-sized invasive adenocarcinoma (≤2 cm in maximum diameter extending to the submucosa or deeper; from 66 patients) and 20 lesions of large-sized invasive adenocarcinoma (>2 cm in maximum diameter extending to the submucosa or deeper; from 20 patients). The main clinical and pathological characteristics of the patients and the colorectal tumors are listed in Table I. The archival samples were retrieved from the database of Kyorin University Hospital (Tokyo, Japan). The cases were diagnosed and treated by surgical resection (colectomy) between January 2007 and December 2011. To simplify the interpretation of obtained results, patients with either preceding inflammatory bowel disease or hereditary backgrounds, including familial adenomatous polyposis, or who had received preoperative chemo- or radiotherapy, were excluded from the present study. Serrated adenomas and adenocarcinomas developed through serrated pathways were also excluded. The present study was approved by the Ethics Committee of Kyorin University, School of Medicine. The need for written, informed consent was waived due to the retrospective, non-interventional nature of the present study.

Tissue preparation and immunohistochemistry. Formalin-fixed and paraffin-embedded tissues from the aforementioned samples were used for immunohistochemical analysis. The samples were originally fixed in 4% phosphate-buffered formaldehyde at room temperature for 24 to 72 h and embedded in paraffin, according to routine procedures. From each sample, 4 μm sections were cut, dried, dewaxed in xylene and rehydrated in descending alcohol series (100, 90 and 70%, twice in each concentration). The subsequent immunohistochemical staining procedure was conducted using a semiautomatic staining machine (Ventana ES; Ventana Medical Systems, Inc., Oro Valley, AZ, USA) according to the manufacturer's protocol. The following primary antibodies were used for immunohistochemistry: Anti-human CK7 mouse monoclonal antibody (clone OV-TL 12/30; cat. no. M7018; dilution, 1:200; Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) and anti-human CD117 rabbit polyclonal antibody (cat. no. A4502; dilution, 1:400; Dako; Agilent Technologies, Inc). Samples were incubated with these antibodies at 37˚C for 16 min. For reference, hematoxylin and eosin staining was performed on the same samples (Carrazzi's hematoxylin solution and 1% eosin Y).

Evaluation of CK7 and CD117 expression in colorectal tumors, transitional mucosa adjacent to the tumor and distant normal mucosa. For each patient, the expression of CK7 and CD117 in tumor lesions, in transitional mucosa adjacent to the tumor and in distant normal mucosa was immunohistochemically evaluated under light microscope. For each area, 4 fields of view were assessed at magnification, x100.

The expression levels of the proteins were scored between 0 and 3+ according to the extent of the immunoreactive glands. The scores 1+ to 3+ were defined as follows: 1+, 1-2 consecutive glands; 2+, 3-5 glands; and 3+, ≥6 glands. In solid or diffusely infiltrating tumors without gland formation, each 100 μm-size area was counted as one gland. Scores of 1+ to 3+ were considered as positive. Subjects without or with a few scattered immunoreactive cells were scored as 0 (negative). In cases of heterogeneous staining, areas of the highest reactivity were selected for scoring. All immunohistochemical staining was independently evaluated by two pathologists, and then consensus scores were adopted.

Statistical analysis. Staining data were analyzed using Statcel v.3 software (OMS, Tokorozawa, Japan; http://www.oms-publ.co.jp). The Mann-Whitney U-test was applied to determine the difference in the positivity rate for expression of CK7 and CD117. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of CK7 in colorectal tumors, in transitional mucosa adjacent to the tumor and in distant normal mucosa. The immunohistochemical analysis of the expression of CK7 in the tumor, in transitional mucosa adjacent to the tumor and in distant normal mucosa is summarized in Table II. Regarding CK7 expression in the transitional mucosa adjacent to the tumor, a positive result (score of 1+ to 3+) was obtained in 7.9% (6/76) of low-grade adenomas, 11.1% (2/18) of high-grade adenomas, 50.0% (6/12) of mucosal adenocarcinomas, 61.2% (41/67) of small-sized invasive adenocarcinomas and 25.0% (5/20) of large-sized invasive adenocarcinomas, irrespective of CK7 expression in the corresponding tumors. The CK7 positivity rate in the transitional mucosa adjacent to the tumor exhibited a stepwise increase according to the grade of the corresponding tumor, increasing from a low-grade adenoma through to a small-sized invasive adenocarcinoma (Fig. 1). However, in the large-sized invasive adenocarcinoma cases, the positivity rate for CK7 in the transitional mucosa adjacent to the tumor was significantly lower compared with the next lower tumor grades (Fig. 1). Representative images of the transitional areas revealed that expression of CK7 in the mucosa adjacent to the small-sized invasive adenocarcinoma (score 3+; positive) differed from that in the mucosa adjacent to the large-sized invasive adenocarcinoma (score 0; negative; Fig. 2).

As for CK7 expression in the tumor itself, CK7 was expressed in 1.3% (1/76) of low-grade adenomas, 5.6% (1/18) of high-grade adenomas, 8.3% (1/12) of mucosal adenocarcinomas, 10.4% (7/67) of small-sized invasive adenocarcinomas and 25.0% (5/20) of large-sized invasive adenocarcinomas. Distant normal mucosa was virtually negative for CK7 except for 3.0% (2/66) of small-sized invasive adenocarcinoma cases.

Expression of CD117 in colorectal tumors, transitional mucosa adjacent to the tumor and distant normal mucosa. The

Table I. Characteristics of patients with colorectal adenoma or adenocarcinoma.

Diagnosis	Total number of neoplasms, n	Age, years (mean, range)	Male: Female	Left: Right	Size, mm (mean, range)
Low-grade adenoma	76	72 (47-91)	42:23	41:35	8.2 (3-36)
High-grade adenoma	18	68 (47-91)	12:5	9:9	16.2 (8-25)
Mucosal adenocarcinoma	12	70 (59-89)	11:1	8:4	19.6 (6-60)
Small-sized invasive adenocarcinoma	67	70 (37-88)	40:26	41:26	16.3 (7-20)
Large-sized invasive adenocarcinoma	20	70 (44-86)	12:8	8:12	49.9 (24-110)

Table II. Summary of cytokeratin 7 expression in adjacent mucosa, tumor and distant mucosa.

| Diagnosis	Region	n	Score 0	1+	2+	3+
Low-grade adenoma	Adjacent mucosa	76[a]	70	4	2	0
	Tumor	76[a]	75	1	0	0
	Distant normal mucosa	65	65	0	0	0
High-grade adenoma	Adjacent mucosa	18[a]	16	2	0	0
	Tumor	18[a]	17	1	0	0
	Distant normal mucosa	17	17	0	0	0
Mucosal adenocarcinoma	Adjacent mucosa	12	6	3	3	0
	Tumor	12	11	0	1	0
	Distant normal mucosa	12	12	0	0	0
Small-sized invasive adenocarcinoma	Adjacent mucosa	67[a]	26	24	10	7
	Tumor	67[a]	60	1	2	4
	Distant normal mucosa	66	64	2	0	0
Large-sized invasive adenocarcinoma	Adjacent mucosa	20	15	4	1	0
	Tumor	20	15	1	1	3
	Distant normal mucosa	20	20	0	0	0

[a]Including multiple tumors.
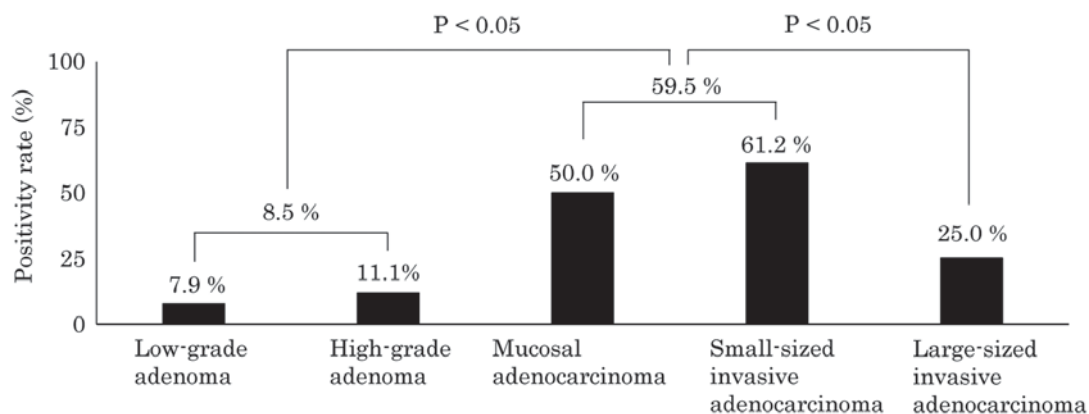
Figure 1. Schematic presentation of the cytokeratin 7 positivity rate in transitional mucosa adjacent to colorectal tumors.

results of immunohistochemical analysis of CD117 expression are summarized in Table III. Concerning CD117 expression in the transitional mucosa adjacent to the tumor, positive results (a score of 1+ to 3+) were obtained in 10.5% (8/76) of low-grade adenomas, 16.7% (3/18) of high-grade adenomas, 25.0% (3/12) of mucosal adenocarcinomas, 38.8% (26/67) of small-sized
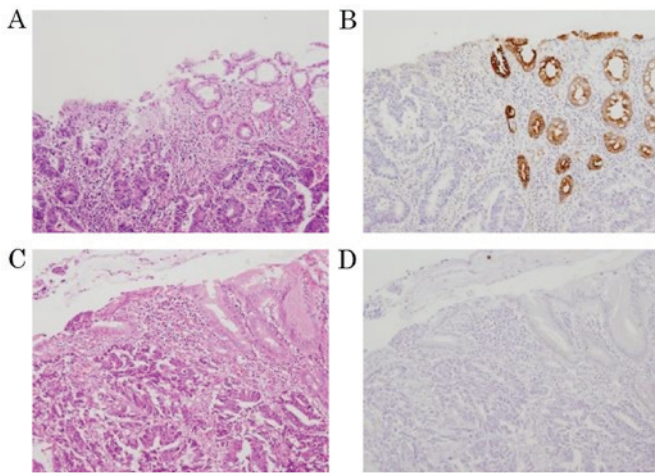
Figure 2. Representative images of the transitional area of small-sized and large-sized invasive adenocarcinoma. (A) Histological and (B) immunohistochemical staining for CK7 of small-sized invasive adenocarcinoma. (C) Histological and (D) immunohistochemical staining for CK7 of large-sized invasive adenocarcinoma (original magnification, x100). CK7, cytokeratin 7.

invasive adenocarcinomas and 45.0% (9/20) of large-sized invasive adenocarcinomas, irrespective of CD117 expression in the corresponding tumors. In contrast to CK7, the positivity rate for CD117 in the transitional mucosa adjacent to the tumor increased stepwise according to tumor grade, from low grade adenoma all the way through to large-sized invasive adenocarcinoma cases, and there was a significant difference in CD117 expression in this location between the adenoma group and the adenocarcinoma group (P<0.05; Fig. 3). Representative images of the transitional areas demonstrated expression of CD117 in the adjacent mucosa of small-sized invasive adenocarcinoma (score 3+; positive) and large-sized invasive adenocarcinoma (score 3+; positive; Fig. 4).

As for CD117 expression in the tumor itself, CD117 was expressed in 25.0% (19/76) of low-grade adenoma, 33.3% (6/18) of high-grade adenoma, 8.3% (1/12) of mucosal adenocarcinoma, 14.9% (10/67) of small-sized invasive adenocarcinoma and 5.0% (1/20) of large-sized invasive adenocarcinoma. Notably, the tumor positivity rate for CD117 was significantly increased in the adenoma group compared with the adenocarcinoma group (P<0.05). In distant normal mucosa, CD117 expression was detected only in a small percentage of low-grade adenoma cases [6.2% (4/65)] and small-sized invasive adenocarcinoma cases [7.6% (5/66)].

Discussion

In the present study, CK7 and CD117 were demonstrated to be expressed in the transitional mucosa adjacent to colorectal tumors. In adenoma, mucosal adenocarcinoma and small-sized invasive adenocarcinoma cases, the positivity ratio of CK7 and CD117 increased in a stepwise manner according to tumor grade. Regarding CK7 and CD117 expression in large-sized invasive adenocarcinoma cases, there was a difference between whether expression was increased (CD117) or decreased (CK7) compared with the expression in small-sized invasive carcinoma cases. The results of the present study indicated that the expression mechanisms of

CK7 and CD117 were different in the transitional mucosa adjacent to colorectal cancer.

The positivity ratio for CK7 in the transitional mucosa adjacent to the tumor was lower in large-sized invasive adenocarcinoma cases compared with in mucosal and small-sized invasive adenocarcinoma cases. This lower CK7 positivity ratio in the large-sized invasive adenocarcinoma cases may be partially explained by the encroachment of cancer cells into morphologically normal, CK7-positive mucosa, which predisposes the tissue to cancerous development.

Cytokeratins are a family of cytoplasmic structural proteins that have been described in human epithelium, which demonstrate variable expression depending on the type of tissue and its differentiation status (25,26). CK7 is expressed in several simple ductal and glandular epithelia, including the lung, breast, bile and pancreatic ducts, urinary tracts, ovary and endometrium (27). In contrast, CK7 expression is generally lacking in normal mucosa of the colon, rectum and, correspondingly, in colorectal adenoma and adenocarcinoma (11,28). The biological significance of CK7 expression remains largely unknown. Kirchner *et al* (29) reported that CK7 is aberrantly expressed in boundary mucosa adjacent to gastric cancer, and proposed that this expression may reflect transient dedifferentiation, which is associated with metaplastic and dysplastic change in the gastritis-cancer sequence. The same transient dedifferentiation step may occur in the transitional mucosa adjacent to colorectal cancer.

Within the concept of field cancerization, also termed the field effect or the field defect, morphologically normal mucosa surrounding the cancer is considered to be biologically different compared with the normal mucosa of subjects with no malignant or premalignant lesions. This concept was first introduced by Slaughter *et al* (30) to explain the presence of multifocal oral squamous cell carcinomas that developed out of a field of precancerous change, and the concept has now been applied to a variety of organs, including the lung, breast, esophagus, stomach and colon (31). In the colon and rectum, field cancerization has been described in cancer associated with chronic inflammatory bowel disease, including ulcerative colitis and Crohn's disease (32-35), and also in sporadic cases (5,6,31). Field cancerization has not been well defined in molecular terms; however, previous studies have proposed mutated genes, including tumor protein p53 and DNA methylation genes, as candidate mediators of the field cancerization in colorectal cancer (4,6,31). Therefore, aberrant expression of CK7 is considered to be another candidate marker for field cancerization in colorectal cancer.

The areas of CK7-positive crypts observed in the present study were localized close to the tumor, and the positive areas were smaller than the fields demonstrated by other studies that used different markers (5,7,36). Facista *et al* (37) hypothesized that the molecular alterations associated with field cancerization progress outwards, and that the most extensive regions reflect the earliest event in carcinogenesis. If this is the case, then aberrant expression of CK7 would be the latest event for tumorigenesis in the colon and rectum.

With respect to CK7 expression in the tumors, colorectal cancers commonly lack CK7 expression, and positive immunostaining for CK7 in metastatic tumors supports the exclusion of a colorectal origin for these tumors (10,11,26,28).

Table III. Summary of KIT proto-oncogene receptor tyrosine kinase expression in adjacent mucosa, tumor and distant mucosa.

| Diagnosis	Region	n	Score 0	1+	2+	3+
Low-grade adenoma	Adjacent mucosa	76[a]	68	2	2	4
	Tumor	76[a]	57	5	7	7
	Distant normal mucosa	65	61	0	0	4
High-grade adenoma	Adjacent mucosa	18[a]	15	0	0	3
	Tumor	18[a]	12	0	2	4
	Distant normal mucosa	17	17	0	0	0
Mucosal adenocarcinoma	Adjacent mucosa	12	9	0	0	3
	Tumor	12	11	0	1	0
	Distant normal mucosa	12	12	0	0	0
Small-sized invasive adenocarcinoma	Adjacent mucosa	67[a]	41	6	4	16
	Tumor	67[a]	57	0	3	7
	Distant normal mucosa	66	61	0	0	5
Large-sized invasive adenocarcinoma	Adjacent mucosa	20	11	3	2	4
	Tumor	20	19	0	0	1
	Distant normal mucosa	20	20	0	0	0

[a]Including multiple tumors.
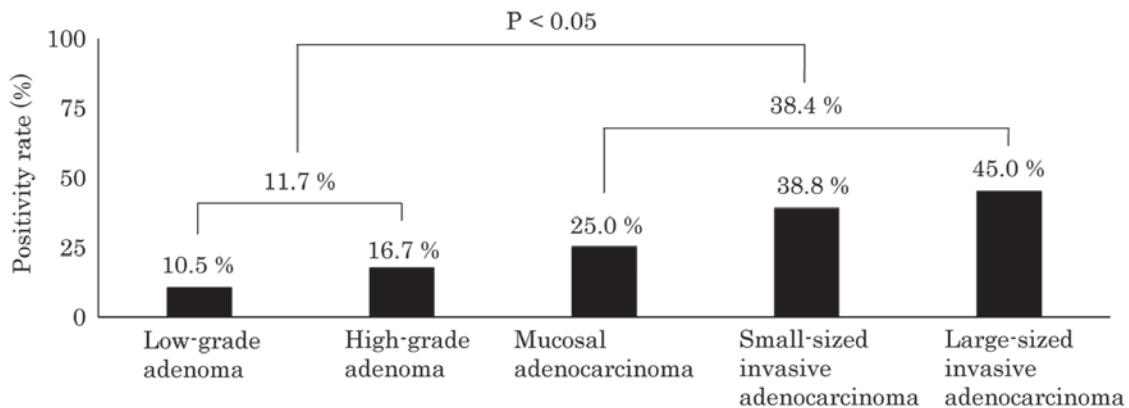
Figure 3. Schematic presentation of the KIT proto-oncogene receptor tyrosine kinase positivity rate in transitional mucosa adjacent to colorectal tumors.

However, not all colorectal cancers lack CK7 expression, and CK7 positivity in colorectal cancers has been reported to range from 0-16% (reviewed by Harbaum) (38). CK7 expression in colorectal cancer was reported to be associated with high-grade, right-sided tumors (39). In the present study, CK7 expression was observed in 2.1% of colorectal adenomas and 13.1% of carcinomas, and the frequency of expression increased according to the tumor grade. These results are in line with those of other previous studies (9,38,39). However, no association was observed between CK-7-positivity and the location of the tumor (data not shown).

In contrast to CK7, the positivity rate for CD117 in transitional mucosa adjacent to the large-sized invasive adeno-carcinoma cases was increased compared with its expression in the mucosa adjacent to preinvasive and small-sized invasive tumors. Based on this result, expression of CD117

in transitional mucosa adjacent to colorectal cancer may be secondarily induced by the corresponding cancer rather than predisposing the tissue to cancerous development.

The proto-oncogene c-kit encodes a transmembrane tyrosine kinase receptor CD117, which is associated with the platelet-derived growth factor PDGF/colony stimulating factor 1 (c-fms) receptor subfamily (40,41). The ligand for CD117 is stem cell factor (SCF), which activates the tyrosine kinase activity of CD117 via homodimerisation and auto-phosphorylation of the receptor at specific tyrosine residues in the intracellular domain of the receptor (42,43). CD117 is normally expressed in a variety of human tissues, including hematopoietic stem cells, melanocytes, mast cells, germ cells and interstitial cells of Cajal (44). Immunohistochemical analyses indicated that the localization of CD117 protein in the normal colon was restricted to interstitial cells of Cajal
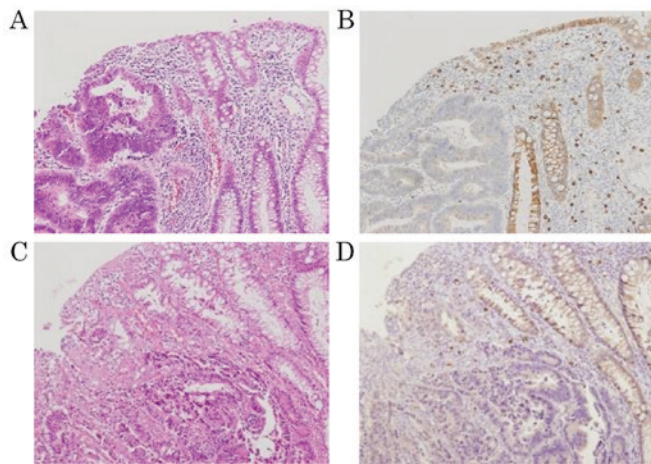
Figure 4. Representative images of the transitional area of small-sized and large-sized invasive adenocarcinoma. (A) Histological and (B) immuno-histochemical staining for CD117 of small-sized invasive adenocarcinoma. (C) Histological and (D) immunohistochemical staining for CD117 of large-sized invasive adenocarcinoma (original magnification, x100). CD117, KIT proto-oncogene receptor tyrosine kinase.

scattered in the stroma, whereas the non-neoplastic epithelium was always negative (45).

Abnormal levels of CD117 expression have been observed in a variety of human tumors, including GISTs, myeloid leukemia, melanoma, glioblastoma, germ cell cancer, breast cancer, prostatic cancer and small cell lung cancer (24,44,45). Two general mechanisms of CD117 activation in these tumors have been described: Acquisition of activating mutations that result in constitutive ligand-independent CD117 phosphoryla-tion, and autocrine or paracrine stimulation of the receptor by its ligand SCF (24).

Data concerning the expression of CD117 in colorectal cancer are controversial (24,44-46). Overall, as determined by immunohistochemistry, the expression of CD117 is rare, and CD117 expression has been detected in only 1.6-25% of colorectal carcinomas examined (24,44-48). Colorectal cancer with overexpression of CD117 did not demonstrate c-kit gene mutations in hot spot regions (44). The majority of CD117-positive carcinomas co-expressed SCF, and the existence of autocrine/paracrine mechanisms has been proposed (24,49,50).

There is increasing data to support a central function for the tumor microenvironment in the mechanisms for the progression of colorectal cancer (8,51,52). The tumor microen-vironment is composed not only of a heterogeneous population of stromal cells, including fibroblasts and immune cells, extra-cellular matrix and secreted factors within the tumor bulk, but also of the adjacent non-tumor mucosa, which interacts and communicates with the cancer. This molecular crosstalk is mediated thorough cytokines and other factors secreted by the tumor, which activate receptors in the adjacent colonic tissue; and vice versa, providing novel insights into the micro-ecology of colorectal tumorigenesis.

In the present study, CD117 expression in transitional mucosa adjacent to colorectal cancer was considered to be the result of paracrine stimulation by SCF secreted by the cancer. CD117 was not included in genes activated in the mucosa adjacent to colorectal cancer in an extensive transcriptomic

analysis performed by Sanz-Pamplona *et al* (8). The results of the present study indicated that molecular crosstalk through SCF and CD117 may be rather weak, and be restricted to the mucosa just beside the edge of the colorectal tumor.

In the colorectal tumors of the present study, the expres-sion of CD117 in carcinoma was less frequent than in adenoma (12.1 and 26.6%, respectively). This result concurred with that reported by Bellone *et al* (24), who also described lower CD117 expression in carcinoma (10.6%) compared with adenoma (12.5%). Similarly, in the uterus, CD117 was reported to be expressed less frequently in endometrial carcinoma than in endometrial hyperplasia (53). Mutational switches for tumor growth other than the SCF-CD117 autocrine/paracrine system may be turned on in the course of progression of these tumors.

Although antibodies for CK7 and CD117 are convention-ally used in daily diagnostic practice, they may provide novel insights into the development and progression of colorectal cancer (9-24). A potential weakness of the present study was that it was conducted retrospectively and dealt with archival histopathological specimens. Further prospective studies, including those using cancer-grafted model animals, may provide more evidence to support the results of the present study.

In conclusion, the present study demonstrated that CK7 and CD117 were expressed in the transitional mucosa adjacent to colorectal tumors. However, their expression mechanisms were considered to be different. From the prac-tical point of view, staining for CK7, as a marker for field cancerization, may be used for evaluation of the surgical margin and for prediction of the risk of local recurrence. An approach for analysis of the molecular crosstalk between receptors, including CD117 and signaling proteins secreted by the tumor, may be useful in order to gain insight into the maintenance of the microenvironment of colorectal cancer. Disruption of this intricate molecular network of cell-cell communication may be a novel therapeutic strategy for the treatment of colorectal cancer.

Acknowledgements

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