Colorectal micropapillary carcinomas are associated with poor prognosis and enriched in markers of stem cells

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Colorectal micropapillary carcinoma has recently been reported as an aggressive variant of adenocarcinoma with a high incidence of lymph node metastasis, but has not been well investigated in terms of survival analysis. This study analyzed the clinicopathological characteristics, including survival data, of the patients with micropapillary carcinoma. We hypothesized that the aggressive features of micropapillary carcinoma might be related to the presence of more tumor cells with stem cell phenotype in colorectal cancer. Fifty-five (10%) micropapillary carcinoma cases were identified among 561 cases of colorectal cancer. We compared the clinicopathological characteristics, including survival data and immunohistochemical profiles of stem cell markers (SOX2, NOTCH3, CD44v6, CD166, ALDH1) of micropapillary carcinomas with those of randomly selected 112 conventional adenocarcinomas lacking micropapillary carcinoma components (non-micropapillary carcinoma) in the colorectum. To exclude the possibility of dilution of control group by patients with microsatellite instability-high carcinomas, we divided non-micropapillary carcinomas into microsatellite instability-high carcinoma and microsatellite stable tumors. Micropapillary carcinomas were characterized by more frequent lymphovascular invasion ($P < 0.0001$) and lymph node metastasis ($P < 0.0001$), higher pathological T and tumor node metastasis stages ($P = 0.047$ and $P = 0.001$), and more frequent SOX2 ($P = 0.038$) and NOTCH3 expressions ($P = 0.005$). Overall 5-year survival rate for patients with micropapillary carcinoma (37%) was significantly lower than for microsatellite instability-high carcinoma and microsatellite stable carcinoma patients (92 and 72%, $P < 0.0001$). The presence of the micropapillary carcinoma component was shown to be associated with a significantly worse survival rate in univariate ($P < 0.0001$) and multivariate ($P = 0.003$, Cox hazard ratio 2.402) analyses. In conclusion, recognition of the micropapillary carcinoma component in colonic adenocarcinoma is very important, because the micropapillary carcinoma has been associated with a significantly worse prognosis. We also found a higher expression rate of cancer stem cell markers in micropapillary carcinomas, suggesting their potential contribution to the survival disadvantage of micropapillary carcinoma.

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Colorectal cancer is one of the leading causes of cancer deaths worldwide, and both the prevalence

and the incidence rate in South Korea are rapidly rising.¹ For metastatic colorectal cancer, the 5-year survival rate is only about 10%.² Micropapillary carcinoma is a relatively uncommon and distinctive tumor characterized by small clusters of tumor cells in the clear lacunar spaces, mimicking lymphatic or vascular channels.³ Micropapillary carcinoma has high potential to metastasize to the regional lymph node, and has been described in carcinomas from many organs, including the breast, urinary bladder,

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lung, ovary, gall bladder, and stomach.^{3–7} In colorectal carcinoma, micropapillary carcinoma was first reported by Kim *et al*⁸ in 2006. Colorectal micropapillary carcinoma is considered an aggressive variant of adenocarcinoma with a high incidence of lymph node metastasis, but it has not been well investigated in terms of overall and disease-free survivals. Previous published studies found that the proportion of micropapillary carcinoma to the entire tumor ranged from 5 to 80%, and defined micropapillary carcinoma as carcinoma with at least 5% of micropapillary components.^{8–11}

On the other hand, as the identification of tumor-initiating cells, also referred to as cancer stem cells, in acute myeloid leukemia, their presence has also been identified in a number of solid tumor types, such as breast, lung, ovarian, brain, and colon cancer.^{12–17} Emerging evidence suggests that stem cells have a crucial role not only in the embryonic development and maintenance of mature normal tissues, but also in the development of malignancies and disease progression associated with poor prognosis. In fact, the expression of stem cell markers and their prognostic significance have been reported in several solid tumors, including colorectal cancer.^{16,18–23} Several cell surface markers, including CD44, CD133, and CD166, have been used for identification of colorectal cancer stem cells.^{24–26} Activity of aldehyde dehydrogenase 1 (ALDH1), a detoxifying enzyme that oxidizes intracellular aldehydes, has also been used as a colorectal cancer stem cell marker.²⁷ We hypothesized that the aggressive features of micropapillary carcinoma might be related to the presence of more tumor cells with stem cell phenotype in colorectal cancer.

In this study, we analyzed the clinicopathological characteristics, including survival of the patients and immunohistochemical profiles of stem cell markers, of 55 cases of micropapillary carcinoma, compared with non-micropapillary carcinoma cases in the colorectum. To exclude the possibility of dilution of control group by patients with microsatellite instability-high carcinomas, which intrinsically have better prognosis, we divided non-micropapillary carcinomas into microsatellite instability-high carcinomas and microsatellite stable tumors.²⁸

Materials and methods

Case Selection

We selected 561 cases of colorectal adenocarcinoma from the surgical pathology files of the Gangneung Asan Hospital (Gangneung, South Korea), occurring between June 2004 and December 2009. None of the patients had received chemotherapy or radiotherapy before surgery. Hematoxylin and eosin-stained slides from each tumor block were reviewed, and we estimated the percentage of micropapillary carcinoma component in 5% increments. Fifty-five cases

of adenocarcinoma with micropapillary carcinoma characteristics were identified. In particular, we defined micropapillary carcinoma as the occurrence of recognizable, obvious focus or foci of small papillary cell clusters (at least 5%) lacking true fibrovascular cores, surrounded by empty lacunar spaces.

For comparison, 112 cases of conventional non-micropapillary colorectal adenocarcinoma with moderately (50–95% of tumor composed of glands) or poorly differentiated (49% or less composed of glands) histology were randomly selected and studied. Patients were staged based on the seventh edition of the AJCC tumor node metastasis (TNM) staging system.²⁹ The patients' medical records were reviewed for clinical information, and histological parameters were evaluated on the hematoxylin and eosin-stained slides. The clinicopathological parameters were evaluated in each case, including the patient's age at diagnosis, gender, the primary tumor location, tumor size, grade, invasion depth (pathological (p)T stage), lymphovascular invasion, lymph node metastasis, overall tumor stage, occurrence of other malignancies, operation date, presence or absence of recurrence, most recent follow-up date, and survival status.

Tissue Microarray Construction

Formalin-fixed, paraffin-embedded tissue samples of micropapillary carcinomas ($n=55$) and non-micropapillary carcinomas ($n=112$) were obtained and arrayed using a tissue-arraying instrument (Quick-Ray, Unitma, Seoul, Korea). Representative areas of each tumor were selected and marked on the hematoxylin and eosin-slide, and its corresponding tissue block was sampled. The designated area of each donor block was punched with a 2-mm-diameter tissue cylinder, and the sample was transferred to a recipient block. Each sample was arrayed to the duplicated blocks to minimize tissue loss.

Immunohistochemistry

Immunohistochemical staining for SOX2, NOTCH3, CD166, CD44v6, ALDH1, MLH1, MSH2, MSH6, and PMS2 was performed on the arrayed blocks containing micropapillary carcinoma and non-micropapillary carcinoma samples. In equivocal cases, we also stained for D2–40 and factor VIII antigen in the whole tumor section blocks. All immunostainings were performed in the Bond-Max automatic immunostaining device (Leica Biosystem, Newcastle, UK) using a bond polymer intensity detection kit (Leica Biosystem) for formalin-fixed, paraffin-embedded tissue sections. Five-micrometer-thick sections were obtained by microtome, transferred onto adhesive slides, and dried at 62°C for 30 min. Antigen retrieval was carried out in all cases (Table 1). Slides were counterstained with Harris hematoxylin. As positive controls, we used squamous cell carcinoma of the lung for SOX2,

Table 1 Antibodies used for this study

<i>Antibody</i>	<i>Source</i>	<i>Location</i>	<i>Dilution</i>	<i>Antigen retrieval</i>
SOX2	Abcam	Cambridge, UK	1:500	Heat (Citrate), 15 min
NOTCH3	Abcam	Cambridge, UK	1:500	Heat (Citrate), 15 min
CD166	Leica Biosystem	Newcastle, UK	1:100	Heat (Citrate), 15 min
CD44v6	Leica Biosystem	Newcastle, UK	1:200	Heat (Citrate), 15 min
ALDH1	Abcam	Cambridge, UK	1:200	Heat (EDTA), 15 min
D2-40	DakoCytomation	Glostrup, Denmark	1:100	Heat (EDTA), 15 min
Factor VIII Antigen	Leica Biosystem	Newcastle, UK	1:200	Heat (Citrate), 15 min
MLH1	Leica Biosystem	Newcastle, UK	1:200	Heat (EDTA), 20 min
MSH2	Leica Biosystem	Newcastle, UK	1:400	Heat (EDTA), 20 min
MSH6	Cell Marque	Hotsprings, USA	1:100	Heat (EDTA), 30 min
PMS2	Cell Marque	Hotsprings, USA	Predilution	Heat (EDTA), 60 min

Abbreviation: ALDH1, aldehyde dehydrogenase 1.

normal liver tissue for ALDH1, peripheral nerve fiber in skin for CD166, breast carcinoma for NOTCH3, and urothelial carcinoma for CD44v6. Negative controls were performed by omitting the primary antibodies.

The SOX2 stains appeared in the tumor nuclei, NOTCH3 appeared in the nuclei and cytoplasm, CD44v6 and CD166 appeared in the cytoplasm and cell membrane, and ALDH1 appeared in the cytoplasm of the neoplastic cells. The results of immunohistochemical staining for each marker were scored based on the intensity of staining as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong), and the percentage of positive epithelial cells in 5% increments. Receiver-operating characteristic curve analysis against 5-year survival was performed to set the cutoff scores for each marker.^{30,31} Scores above the cutoff were considered positive.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences software (version 12.0, SPSS Inc, Chicago, IL, USA). A comparison of means was performed using an unpaired Student's *t*-test. The linear-by-association χ^2 -test and χ^2 -test were performed to examine associations among categorical variables. Survival analysis was plotted using the Kaplan-Meier method, and differences were evaluated using the log-rank test. A multivariate regression analysis (Cox proportional hazards model) with enter method was used to define specific risk factors for overall survival. All *P* values were two-sided, and statistical significance was accepted when *P* < 0.05.

Results

Clinicopathological Characteristics of Cases

Of the initial 561 colorectal carcinomas considered, 55 (10%) cases contained >5% of micropapillary carcinoma components (Figure 1a). The clinicopathological features of the cases with micropapillary carcinoma (55 cases) and non-micropapillary carcinoma

(112 cases) are summarized in Table 2. Microsatellite instability-high carcinoma was defined as loss of expression for at least one of MLH1, MSH2, MSH6, and PMS2, and detected in 15 cases (five with loss of MLH1 expression, eight with loss of MSH2 expression, eight with loss of MSH6 expression, and six with loss of PMS2 expression).³² Three of microsatellite instability-high carcinoma cases were micropapillary carcinomas. Postoperative chemotherapy was performed for 87 (52%) out of 167 patients (stage I, 0%; stage II, 34%; stage III, 72%; and stage IV, 70%). No primary cancers of other sites were recorded in any cases, except for in two patients, who had concurrent early gastric cancer and thyroid papillary carcinoma, respectively. Twenty-four patients with micropapillary carcinoma were alive at the time of last follow-up. The age of patients with micropapillary carcinoma and non-micropapillary carcinoma ranged from 35 to 88 years (median age, 68 years) and 33 to 93 years (median age, 67 years), respectively. The proportion of the micropapillary carcinoma component in patients with micropapillary carcinoma ranged from 5 to 90% of total tumor volumes. There was no case that contained pure micropapillary carcinoma component. The micropapillary carcinoma component was frequently mixed with non-micropapillary carcinoma cells and identified in the center, deeper, and peripheral portions of the tumor.

Micropapillary carcinoma samples exhibited a higher level of positive lymphovascular invasion (micropapillary carcinoma, 62%; microsatellite stable carcinoma, 30%; microsatellite instability-high carcinoma, 8%; *P* < 0.0001), more lymph node metastasis (micropapillary carcinoma, 71%; microsatellite stable carcinoma, 40%; microsatellite instability-high carcinoma, 33%; *P* < 0.0001), and higher stage grouping (stage III–IV; micropapillary carcinoma, 73%; microsatellite stable carcinoma, 40%; microsatellite instability-high carcinoma, 33%; *P* = 0.001) (Table 3). The mean number of involved nodes was four in micropapillary carcinomas and two in non-micropapillary carcinomas (*P* = 0.003), whereas the mean number of all nodes examined for micropapillary carcinomas (*N* = 15)

Figure 1 Micropapillary carcinoma histology and immunohistochemical staining. (a) Micropapillary carcinoma components in colorectal carcinoma. (b) Prominent nuclear expression of SOX2 is noted in micropapillary carcinoma. (c) Tumor cells show strong cytoplasmic immunoreactivity for NOTCH3. (d and e) Cytoplasmic and/or membranous expression pattern is identified in CD166 (d) and CD44v6 (e) staining. (f) ALDH1 immunostaining reveals diffuse cytoplasmic pattern.

Table 2 Comparison of clinicopathologic data between micropapillary carcinoma and non- micropapillary carcinoma

Variable	Characteristics	Micropapillary carcinoma (%)	Non-micropapillary carcinoma		P
			Microsatellite stable tumor (%)	Microsatellite instability/high tumor (%)	
Age (years)	< 67	23 (42)	46 (47)	7 (58)	0.615
	≥ 67	32 (58)	52 (53)	5 (42)	
Gender	Male	30 (55)	63 (64)	10 (83)	0.300
	Female	25 (45)	35 (36)	2 (17)	
Differentiation	MD	54 (98)	96 (98)	11 (92)	1.000
	PD	1 (2)	2 (2)	1 (8)	
Location	Right	11 (20)	11 (11)	6 (50)	0.085
	Left	18 (33)	30 (31)	3 (25)	
	Rectum	26 (47)	57 (58)	3 (25)	
Lymphovascular invasion	Positive	34 (62)	29 (30)	1 (8)	<0.0001
	Negative	21 (38)	69 (70)	11 (92)	
pT stage	T1	2 (4)	2 (2)	0 (0)	0.047
	T2	5 (9)	11 (11)	0 (0)	
	T3	35 (63)	81 (83)	11 (92)	
	T4	13 (24)	4 (4)	1 (8)	
Lymph node metastasis	Positive	39 (71)	39 (40)	4 (33)	<0.0001
	Negative	16 (29)	59 (60)	8 (67)	
Distant metastasis	Positive	7 (13)	4 (4)	0 (0)	0.067
	Negative	48 (87)	94 (96)	12 (100)	
TNM stage	Stage I	4 (7)	11 (11)	0 (0)	0.001
	Stage II	11 (20)	48 (49)	8 (67)	
	Stage III	34 (62)	35 (36)	4 (33)	
	Stage IV	6 (11)	4 (4)	0 (0)	
Adjuvant chemotherapy	Positive	35 (64)	45 (46)	5 (42)	0.044
	Negative	20 (36)	53 (54)	7 (58)	

Abbreviations: MD, moderately differentiated; PD, poorly differentiated; TNM, tumor node metastasis.

and non-micropapillary carcinomas ($N = 16$) did not significantly differ. There were no significant differences between micropapillary carcinoma and non-micropapillary carcinoma samples with respect to gender or anatomical location.

Immunohistochemical Evaluation

Tumors with no staining or weak staining intensity were considered negative. Receiver-operating characteristic curve analysis against 5-year survival was

Table 3 Relationship between the amount of micropapillary component and clinicopathological factors

Micropapillary carcinoma component	Lymphovascular invasion (%)	P	Nodal metastasis (%)	P	Distant metastasis (%)	P
<5% (N=112)	30 (27)	<0.0001	44 (40)	<0.0001	4 (4)	0.055
5–9% (N=15)	7 (45)		8 (53)		2 (13)	
10–29% (N=26)	16 (62)		18 (69)		4 (15)	
≥30% (N=14)	11 (79)		13 (93)		1 (7)	

Table 4 Cutoff scores derived from the area under the receiver-operating characteristic curve and frequency of positive expression for immunohistochemistry

Marker	Area under the receiver-operating curve	Cutoff score (%)	Positive expression (%)
SOX2	0.558	5	36 (22)
NOTCH3	0.485	20	81 (50)
CD166	0.509	20	38 (23)
CD44v6	0.569	5	46 (28)
ALDH1	0.532	20	47 (30)

Abbreviation: ALDH1, aldehyde dehydrogenase 1.

performed, and the results are summarized in Table 4. SOX2 expression was more frequently observed in micropapillary carcinoma (17/53, 32%) than in microsatellite stable carcinoma (16/98, 16%) samples ($P=0.038$; Figure 1b and Table 5). NOTCH3 expression was also more frequently observed in micropapillary carcinoma (35/51, 69%) than in microsatellite stable carcinoma (42/97, 43%) samples ($P=0.005$; Figure 1c). Although CD166 was more frequently expressed in micropapillary carcinoma (16/52, 31%) than in microsatellite stable carcinoma (17/98, 17%) samples, this difference was not statistically significant (Figure 1d). The difference in CD44v6 expression between micropapillary carcinoma (18/53, 34%) and microsatellite stable carcinoma (25/98, 26%) was also not statistically significant (Figure 1e). ALDH1 expression was not different in micropapillary carcinoma and non-micropapillary carcinoma groups (Figure 1f).

Survival Analysis

The median follow-up period for the 167 patients was 54.5 months (range 2–94 months), and 60 patients died of the disease and 66 cases recurred by the end of follow-up. The overall 1, 3, and 5-year survival rates of patients with micropapillary carcinoma were 80, 50, and 37%, respectively, were 94, 84, and 72%, respectively, for those with microsatellite stable carcinoma, and were 100, 92, and 92%, respectively, for those with microsatellite instability-high carcinoma. Patients with micropapillary carcinoma had a significantly shorter overall survival than non-micropapillary carcinoma patients ($P<0.0001$; Figure 2a).

The 1, 3, and 5-year disease-free survival rates of patients with micropapillary carcinoma were 69, 38,

Table 5 Summary of immunohistochemical staining

	Micropapillary carcinoma (%)	Microsatellite stable tumor (%)	P
SOX2			0.038
Positive	17 (32)	16 (16)	
Negative	36 (68)	82 (84)	
NOTCH3			0.005
Positive	35 (69)	42 (43)	
Negative	16 (31)	55 (57)	
CD166			0.066
Positive	16 (31)	17 (17)	
Negative	36 (69)	81 (83)	
CD44v6			0.345
Positive	18 (34)	25 (26)	
Negative	35 (66)	73 (74)	
ALDH1			0.576
Positive	14 (28)	32 (33)	
Negative	37 (72)	65 (67)	

Abbreviation: ALDH1, aldehyde dehydrogenase 1.

and 33%, respectively; were 92, 80, and 70%, respectively, for microsatellite stable carcinoma patients; and were 100, 92, and 92%, respectively, for microsatellite instability-high carcinoma patients. Patients with micropapillary carcinoma had a significantly shorter disease-free survival than those with non-micropapillary carcinoma ($P<0.0001$; Figure 2b).

When patient survival was further analyzed by TNM stage stratification, the overall 5-year survival rates of stage III colorectal carcinoma patients with micropapillary carcinoma (36%) were significantly lower than those of microsatellite stable carcinoma patients (72%, $P<0.0001$). Although the overall 5-year survival rates of stage I–II colorectal carcinoma patients with micropapillary carcinoma (59%) were worse than those of microsatellite stable carcinoma patients (76%); the difference was not statistically significant ($P=0.554$). The survival outcome of patients with stage IV carcinoma was not different between micropapillary carcinoma and microsatellite stable carcinoma groups ($P=0.817$). In stage III, patient survival was further analyzed by application of adjuvant chemotherapy. The overall survival of micropapillary carcinoma patients was not affected by adjuvant chemotherapy ($P=0.719$). Although statistical significance was not found, microsatellite stable carcinoma patients with adjuvant chemotherapy showed better survival outcome than those without adjuvant therapy ($P=0.101$, Figure 2c).

Figure 2 Kaplan–Meier survival analysis (log rank test). (a and b) Non-micropapillary carcinoma patients experience significantly better overall survival rates ($P < 0.0001$, a) and disease-free survival rates ($P < 0.0001$, b) than patients with micropapillary carcinoma. (c) In stage III, the overall survival of micropapillary carcinoma patients is not affected by adjuvant chemotherapy ($P = 0.719$). However, microsatellite stable patients with adjuvant chemotherapy show better survival outcome than those without adjuvant therapy ($P = 0.101$).

In addition, the disease-free 5-year survival rates of stage III–IV colorectal carcinoma patients with micropapillary carcinoma (22%) were significantly lower than those of microsatellite stable carcinoma patients (63%, $P = 0.001$). The disease-free 5-year survival rates of stage I–II colorectal carcinoma patients with micropapillary carcinoma (51%) were worse than those of microsatellite stable carcinoma patients (75%, $P = 0.122$).

The extent of micropapillary carcinoma (the proportion of the micropapillary carcinoma component) in the primary tumors did not affect patient survival (data not shown).

Univariate survival analysis (log rank test) indicated that the factors micropapillary carcinoma, lymphovascular invasion, pT stage, lymph node metastasis, and distant metastasis were associated with a worse prognosis in colorectal carcinomas (Table 6).

Multivariate Cox proportional hazards analyses were performed to identify factors that were

independent prognostic predictors of cancer-related death. Micropapillary carcinoma ($P = 0.003$) was an independent prognostic factor for survival (Table 7).

Discussion

Micropapillary carcinoma showed a distinct histology, characterized by small clusters of tumor cells in the clear lacunar spaces, which has been attributed to reversion of cell polarization.⁷ Several studies showed the prevalence of micropapillary carcinoma was 9–19% in colorectal carcinoma, and it was associated with aggressive histological features, including higher levels of lymphovascular invasion and perineural invasion, more frequent lymph node and distant metastases, and higher TNM stages (III/IV).^{8–11} In concordance with previous studies, we identified 10% of colorectal carcinoma with micropapillary carcinoma component from our cohort (55/561 cases), and micropapillary carcinoma was significantly associated with higher

Table 6 Univariate analysis for overall survival in colorectal adenocarcinoma

Variable	Characteristics	Overall 5YSR (%)	s.e.	P
Micropapillary carcinoma	Positive	37	0.079	<0.0001
	Negative	74	0.043	
Age	<67	65	0.059	0.324
	≥67	62	0.064	
Gender	Male	68	0.050	0.071
	Female	56	0.065	
Differentiation	MD	63	0.040	0.577
	PD	75	0.217	
Location	Right	66	0.094	0.635
	Left	68	0.073	
	Rectum	60	0.055	
Lymphovascular invasion	Positive	50	0.067	0.001
	Negative	71	0.048	
Microsatellite instability	Positive	87	0.088	0.055
	Negative	62	0.043	
pT stage	T1	50	0.250	<0.0001
	T2	73	0.138	
	T3	68	0.043	
	T4	22	0.098	
Lymph node metastasis	Positive	51	0.059	<0.0001
	Negative	73	0.050	
Distant metastasis	Positive	0	0	<0.0001
	Negative	68	0.040	
Adjuvant chemotherapy	Positive	58	0.057	0.355
	Negative	69	0.055	
SOX2	Positive	60	0.084	0.292
	Negative	65	0.045	
NOTCH3	Positive	66	0.056	0.880
	Negative	63	0.057	
CD166	Positive	62	0.087	0.708
	Negative	65	0.045	
CD44v6	Positive	55	0.079	0.174
	Negative	68	0.046	
ALDH1	Positive	58	0.078	0.347
	Negative	67	0.048	

Abbreviations: ALDH1, aldehyde dehydrogenase 1; MD, moderately differentiated; PD, poorly differentiated; 5YSR, 5 year survival rate; s.e., standard error.

Table 7 Multivariate analysis for overall survival in colorectal adenocarcinoma

Variable	Cox hazard ratio	95% confidence interval	P
Micropapillary carcinoma; positive	2.402	1.347–4.282	0.003
Gender; female	1.213	0.707–2.080	0.483
Lymphovascular invasion; present	1.047	0.548–2.001	0.888
Microsatellite instability; present	0.167	0.035–0.791	0.024
T stage; III–IV	1.220	0.474–3.136	0.680
Lymph node metastasis; present	1.705	0.883–3.292	0.112
Distant metastasis; present	29.680	10.682–82.462	<0.0001

levels of lymphovascular invasion, more frequent lymph node metastasis, and higher stage.

Cancer stem cells are defined by their ability to self-renew and generate a progeny of both tumorigenic and non-tumorigenic cancer cells.³³ With this property, cancer stem cells are thought to be more

resistant to chemotherapeutic agents or radiotherapy.^{34,35} Expression of stem cell markers in some tumors has been associated with adverse clinical outcomes.^{16,18–23} Cancer stem cells are also known to have an important role in the process of metastasis.³⁶ We found higher expression rates of SOX2, NOTCH3, CD166, and CD44v6 in micropapillary carcinoma than in non-micropapillary carcinoma samples. Fu and colleagues also found that invasive micropapillary carcinoma of the breast showed chemo-resistance and higher prevalence of CD44⁺/CD24^{-/low} tumor cells, which have been known to have tumor-initiating properties in breast cancer.^{37,38}

SOX2 belongs to group B of the SOX family, which consists of transcription factors containing a high-mobility group of DNA-binding domains that is expressed in a wide variety of tissues, and it has been suggested that it has critical roles in organogenesis, differentiation, and proliferation.^{39,40} Although SOX2 is expressed in embryonal stem cells, a growing body of evidence has revealed the association between SOX2 expression and cancer stem cell properties. Several kinds of tumors, including lung, stomach, breast cancers and gliomas, showed SOX2 overexpression.^{41–44} Recently, two studies demonstrated that SOX2 expression was correlated with lymph node metastasis, distant spread, and poor prognosis in colorectal carcinoma patients.^{31,45}

The NOTCH signaling pathway is an evolutionarily conserved intercellular signal transfer system regulating cell differentiation, proliferation, and apoptosis.⁴⁶ This pathway has been identified as a critical system for intrinsic maintenance of colorectal cancer initiating cell self-renewal.⁴⁷ Among the various components of the NOTCH pathway, the activation of NOTCH3 was reported in epithelial ovarian cancer, extrahepatic cholangiocarcinoma, gallbladder cancer, and T-acute lymphoblastic leukemia.^{23,48,49} We found 50% of colorectal cancer expressed NOTCH3. Compared with non-micropapillary carcinoma, cases with micropapillary carcinoma showed significantly higher levels of NOTCH3 expression (42 vs 69%).

To the best of our knowledge, only one previous study has investigated the significance of micropapillary carcinoma for survival in colorectal carcinoma.¹⁰ Xu *et al*¹⁰ analyzed 30 colorectal carcinomas with micropapillary component from 1990 to 2000. This study revealed the presence of micropapillary components had an independent importance for survival of colorectal cancer patients, especially in TNM stage I and II. Although patients with micropapillary carcinoma showed worse disease-free and overall survival rates than those without micropapillary carcinoma in our data, the difference was significant only in stage III cancers.⁵⁰ This discrepancy might be derived from the differences in archived time periods and postoperative treatment. Stage III colorectal cancers are defined as tumors that have metastasized to the lymph node without distant

metastasis.²⁹ The incidence of stage III at presentation ranges between 20 and 30%.⁵¹ The 3-year disease-free survival in stage III colon cancer without any postoperative chemotherapy ranges between 44 and 52%.^{52,53} As adjuvant chemotherapy with 5-fluorouracil and levamisole improved disease-free survival and overall survival in stage III colon cancer, adjuvant chemotherapy has been considered as a standard therapeutic modality for this disease.^{52,54} Although postoperative adjuvant chemotherapy was applied to 54 of 75 (72%) stage III cases, our data showed significantly lower 3-year disease-free survival rates in patients with micropapillary carcinoma than in those without micropapillary carcinoma (50 vs 84%, $P < 0.0001$). Poor survival outcomes in stage III patients with micropapillary carcinoma and higher expression of stem cell markers in micropapillary carcinoma might suggest that the presence of more tumor cells with stem-like features in micropapillary carcinoma may be related with chemotherapy resistance.

The limitations of this study include the retrospective nature of the study design and relatively small sample size of the test participants. In addition, fundamental problems of IHC study including subjective scoring system were not completely resolved.

In summary, we examined 561 colorectal carcinomas and identified 55 cases of micropapillary carcinoma. The proportion of micropapillary carcinoma in colorectal carcinoma was small (10%). Colorectal carcinomas with micropapillary carcinoma showed higher levels of lymphovascular invasion, more frequent lymph node metastasis, and higher stage. The presence of micropapillary carcinoma was associated with lower rates of patient survival through univariate and multivariate analyses. Therefore, we recommend that the presence or absence of micropapillary carcinoma component in colorectal carcinoma should be marked on the pathological report to provide better prognostic information on patients with colorectal carcinoma. We also found a higher expression rate of cancer stem cell markers in micropapillary carcinomas than non-micropapillary carcinomas. Further studies addressing the relationship between chemo- and/or radiotherapy resistance and the presence of micropapillary carcinoma in colorectal carcinoma are warranted.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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