



CD70 expression in tumor-associated fibroblasts predicts worse survival in colorectal cancer patients

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Abstract

The anticancer effects of immune checkpoint inhibitors against CTLA4 and CD274-PDCD1 axes are evident. However, these immunotherapies for colorectal cancers (CRCs) are now limited to a small subset of patients with microsatellite unstable tumors. Thus, therapeutics targeting other types of CRCs is desired. The CD70–CD27 axis plays a co-stimulatory role in promoting the expansion and differentiation of T-lymphocytes through the activation of NFκB pathway. Aberrant activation of the CD70–CD27 axis accelerates tumor cell proliferation, survival, and immune evasion of tumor cells. Based on these observations, drugs modulating the CD70–CD27 axis have been developed with expectation of anticancer effects. In the present study, 269 primary CRCs were evaluated immunohistochemically for CD70, CD27, and FOXP3 expression to assess their clinical usage and the application of CD70–CD27 axis modulating drugs. CRC tumor cells rarely (2.2%) expressed CD70. In contrast, tumor-surrounding fibroblasts showed various CD70 expressions (fCD70) in 14.9%. The logistic regression analysis revealed significant association of fCD70 expression with incomplete resection status (OR, 2.60; 95% CI, 1.10–6.13; $P=0.029$). Overall survival was significantly decreased in the cohort of the patients with fCD70-positive tumor ($P=0.0078$). Furthermore, significantly more CD27+ tumor-associated lymphocytes were detected within the primary CRCs without metastases ($P=0.024$). Thus, the CD70–CD27 axis may have several roles in CRCs independent from their mismatch repair (MMR) system status. CD70–CD27 pathway-modulating therapies may be applied to CRC patients regardless of their tumor MMR status.

Keywords Colorectal cancer (CRC) · Immunohistochemistry · CD70 · CD27 · FOXP3

Introduction

The discovery of immune checkpoint signaling axes, including the CTLA4 and CD274 (PD-L1)/PDCD1 (PD-1) axes, introduced a new era in cancer therapy. Moreover, the evidence for the anticancer effects of immune checkpoint inhibitors is accumulating [1, 2]. However, in colorectal cancers

(CRCs), the application of these therapies is currently limited to a small subset of patients with microsatellite unstable tumors [3].

CD70 is a type II transmembrane surface antigen belonging to the tumor necrosis factor (TNF) super family (TNFSF). This protein is comprised of 193 amino acids with an extracellular stalk region, transmembrane domain, and C-terminal TNF homology domain which identifies CD70 as a member of the TNFSF [4, 5]. In normal tissues, the expression of CD70 is tightly regulated, and it is only transiently expressed on activated T and B lymphocytes, and mature dendritic cells [6].

CD27 is a member of the tumor necrosis factor receptor superfamily (TNFRSF), and it is composed of two complete cysteine rich domains and one incomplete cysteine rich domain, which is characteristic of the TNFRSF [4, 5]. CD27 is a co-stimulatory immune-checkpoint receptor, and it is constitutively expressed on a broad range of T cells (naïve, $\alpha\beta$, $\gamma\delta$, and memory T cells), B cells, and NK cells. The CD70–CD27 signaling pathway plays a co-stimulatory role in promoting T

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cell expansion and differentiation through the activation of the NF κ B pathway under normal physiological conditions [7, 8].

In contrast to limited CD70 expression in normal tissues, aberrant CD70 expression has been documented in hematological tumors [8] and solid malignancies such as renal cell carcinoma [9] and glioblastoma [10]. In hematological malignancies, the aberrant CD70 expression has been implicated to accelerate tumor cell proliferation and survival through its interaction with co-expressed CD27 on the tumor cells [11]. Furthermore, CD70 expression predicts poor clinical outcome in B cell lymphoma [12]. In ovarian cancer, the association between tumor CD70 expression and cisplatin resistance has been reported [13]. However, in colorectal cancers, the expressions of CD70 and CD27 as well as their correlation to patient survival have not been fully analyzed.

FOXP3-positive regulatory T cells (Tregs) suppress aberrant immune response against self-antigens in physiological conditions. In many types of cancer, Tregs also suppresses the antitumor immune response and their presence within the tumor microenvironment enhances the immune evasion of tumor cells [14]. Tumor CD70 expression has been suggested to help tumor immune evasion and accelerate tumor growth through several distinct mechanisms including Treg expansion, reduction of tumor-specific T cell responses, and increased angiogenesis [8, 10, 15–20].

The present study examined the expression status of CD70, CD27 and FOXP3 in colorectal cancer (CRC) cells, tumor-associated fibroblasts (TAFs), and tumor-associated lymphocytes (TALs). The association of these protein expressions to clinicopathological features and clinical outcome were analyzed to assess their potential for clinical use. Through those, we propose a prognostication method and evaluation scheme of the CRC patients for CD70–CD27 axis-modulating therapy.

Material and methods

Tissue samples

This project was approved by the Institutional Ethical Review Board of Aichi Medical University Hospital. Two hundred and sixty-nine formalin-fixed paraffin-embedded (FFPE) samples of primary colorectal tumors resected at the Aichi Medical University Hospital from 2009 to 2012 were collected depending on the availability of tissue samples and clinical information. After surgery, patients were followed up for up to 90 months. All of the tumors were diagnosed to be invasive and naïve to chemotherapy or radiotherapy. Tumors with glandular formation (> 50%) or mucus production (> 50% of area) were defined as differentiated or mucus-producing histology. A single 4.5 mm core tumor tissue sample derived from FFPE specimens was assembled into multitumor blocks containing

up to 30 samples. The size of tumor tissue samples was estimated to exceed the size of a single 0.6 mm² core by a factor of 8–9.

Fifty-two normal colonic mucosae adjacent to the tumor, 11 hyperplastic polyps, 2 sessile serrated adenomas/polyps, 5 traditional serrated adenomas, and 12 tubular adenomas were also immunohistochemically analyzed.

Cultured cells

The origin of the cultured cells, the protocols for siRNA transfection, and immunoblot analyses have been reported previously [21, 22]. SW48 and ACC-MESO-1 cells were kindly provided by Dr. Yutaka Kondo (Nagoya University) and Dr. Yoshitaka Sekido (Aichi Cancer Centre Research Institute), respectively. FFPE sections of ACC-MESO-1 cells with CD70 knockdown were used as negative controls for immunohistochemistry and those without it as positive ones.

Immunohistochemistry and immunoblot analyses

The antibodies used in the present study are summarized in Table 1. Immunohistochemistry was performed using the Ventana BenchMark XT automated immunostainer (Roche Diagnostics, Basel, Switzerland). Signals were visualized by 3,3'-diaminobenzidine (DAB). CD70 immunoreactive areas were evaluated by using ImageJ software (NIH, Bethesda, MD) (Supplementary Figure S1). In the present study, tumors containing more than 4.5×10^{-2} mm² (1000 pixels) of CD70 immunoreactive area in TAFs were defined as fCD70-positive. The numbers of CD27-positive and FOXP3-positive TALs were counted in high-power field (HPF, $\times 400$).

Statistical analyses

All statistical analyses were performed with EZR software version 1.32 [23]. Chi-square, Fisher's exact, Cochran–Armitage Trend, Mann–Whitney *U*, or Kruskal–Wallis tests were performed to analyze the statistical correlation between categorical data. Simple Bonferroni correction for multiple hypothesis testing was applied to adjusted two-sided alpha level at 0.0036 (= 0.05/14).

Multivariate logistic regression analysis was performed to analyze the association of fCD70 expression (dependent variable) and other factors (independent variables). Variables with *P* value < 0.50 with univariate analyses, such as age (< 70 years old vs. ≥ 70 years old), pT stage (pT2 vs. pT3 vs. pT4), tumor differentiation (well to moderately vs. poorly differentiation including signet ring cell-like feature), operation status (complete vs. incomplete resection), CD27+ TALs (< 32/HPF vs. ≥ 32 /HPF), and mismatch repair (MMR) system status (preserved vs. deficient), were included in the initial multivariable logistic regression analysis model. For CD27+

Table 1 Antibodies and conditions for immunohistochemistry

Genes	Reagent	Dilution	Antibodies
CD27	OV	100	Clone EPR8569, Abcam (Cambridge, UK)
CD70	OV	400	Clone #301731, R&D systems/Thermo Fisher Scientific (Waltham, MA,)
FOXP3	OV	100	Clone SP97, Abcam (Cambridge, UK)
MLH1	OV	200	Clone G168-728, BD Biosciences, (Franklin Lakes, NJ)
MSH2	OV	200	Clone G219-1129, BD Biosciences, (Franklin Lakes, NJ)
MSH6	OV	400	Clone 44/MSH6, BD Biosciences, (Franklin Lakes, NJ)
PMS2	OV + Linker	50	Clone A16-4, BD Biosciences, (Franklin Lakes, NJ)

Antigen retrieval was performed with heat activation in high pH buffer

OV OptiView reagent

TAL categorization, patients were divided into two groups at the median value and for FOXP3+ TALs at 25 percentile value. A backward elimination with a threshold of $P=0.05$ was used to select variables in the final model.

For survival analyses, Kaplan–Meier survival estimates with log-rank test were performed. Cox proportional hazards regression analysis was used to analyze the association of survival and other factors. The initial model included variables with P value < 0.50 in log-rank test, gender (male vs. female), age (< 70 years old vs. ≥ 70 years old), tumor size (< 5 cm vs. ≥ 5 cm), primary tumor location (right-sided colon vs. left-sided colon vs. rectum), pT stage (pT2 vs. pT3 vs. pT4), tumor histology (well to moderately vs. poorly differentiated), mucus production (positive vs. negative), lymph node metastasis (positive vs. negative), peritoneal metastasis (positive vs. negative), distant organ metastasis (positive vs. negative), operation status (complete vs. incomplete resection), and immunohistochemistry data (fCD70-positive vs. CD70-negative and FOXP3+ TALs (< 22 /HPF vs. ≥ 22 /HPF)). A backward

elimination with a threshold of $P=0.05$ was used to select variables in the final model.

Results

Expression of CD70, CD27, and FOXP3 in normal colonic mucosae and CRCs

Representative images for immunohistochemistry are shown in Figs. 1 and 2. All of the normal colonic mucosae and benign polypoid lesions were negative for CD70. In normal colonic mucosae, abundant CD27+ and few FOXP3+ lymphoid cells were observed. The CD70 expression in tumor cells (tCD70) was detected on the apical side cytomembrane and/or Golgi area of the tumor cells in only 2.2% of the cases (6/269). Similar to this result, no colorectal cancer cell line (0/7) expressed CD70 (Supplementary Figure S2). In contrast, CD70 expression in tumor-associated fibroblasts (fCD70) was detected in 14.9%

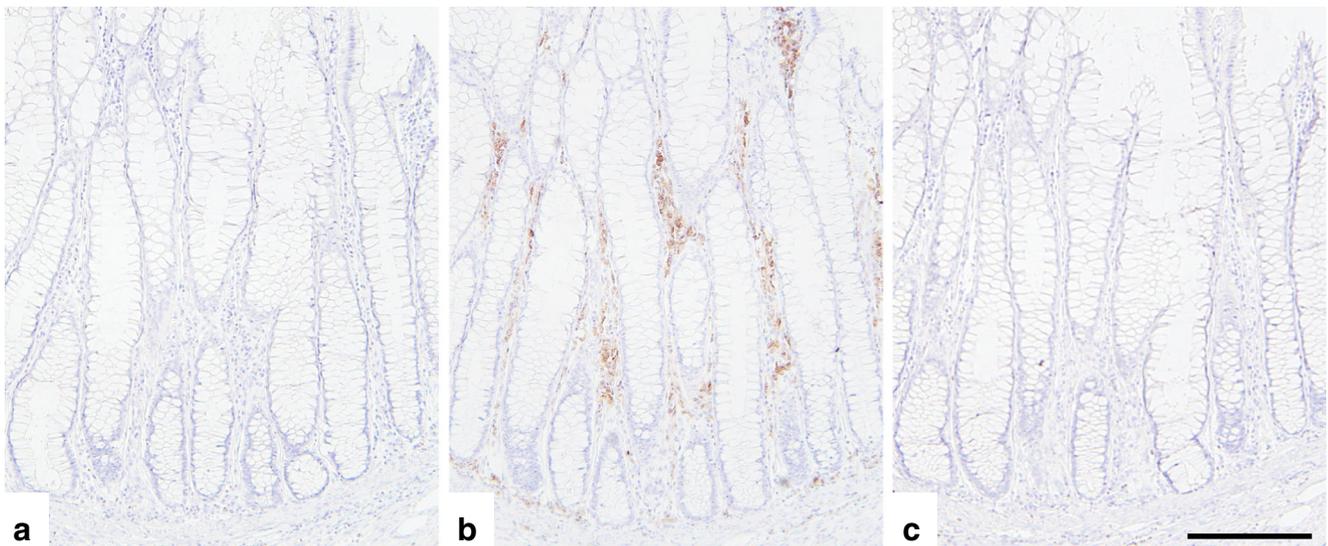


Fig. 1 Representative images of CD70, CD27, and FOXP3 immunostaining in normal colonic mucosae. **a** CD70 was expressed at under detectable levels in normal colonic mucosae. **b** CD27+ lymphoid

cells were abundant in the lamina propria. **c** FOXP3+ lymphoid cells were rarely observed. Bar, 200 μ m

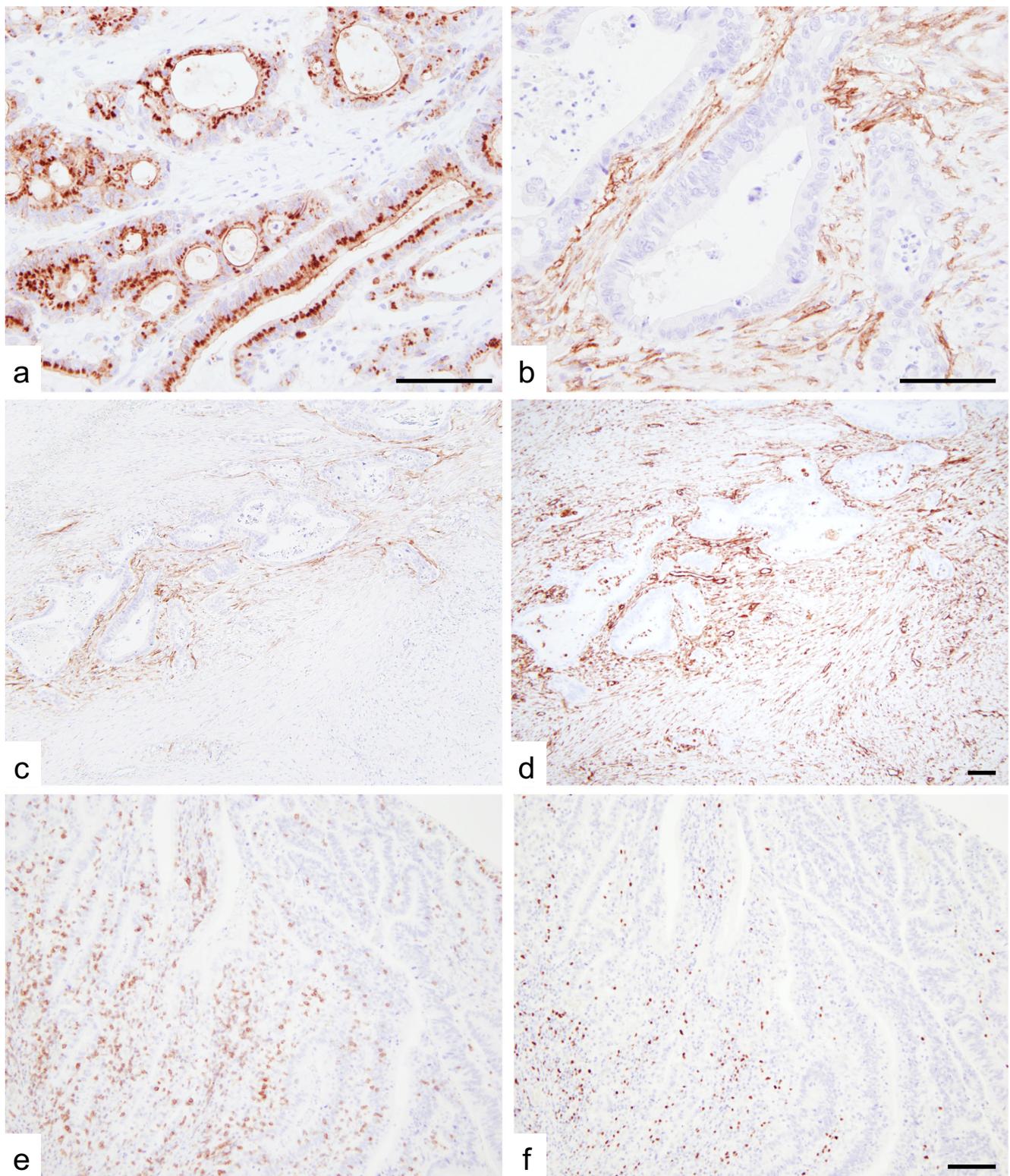


Fig. 2 Representative images of CD70, CD27, and FOXP3 immunostaining in colorectal cancers. **a** Tumor CD70 (tCD70) was detected on the cell membrane and in the cytoplasm. **b** Tumor-surrounding fibroblasts expressed CD70 (fCD70) in the cytoplasm. **c**, **d** fCD70

expression (**c**) was detected mainly in vimentin (**d**)-positive fibroblasts surrounding tumor foci. **e**, **f** Tumor-associated lymphocytes (TALs) showed CD27 (**e**) and FOXP3 expression (**f**). Bar, 100 μ m

Table 2 Characteristics of colorectal carcinomas with or without CD70-positive fibroblasts

	fCD70						<i>P</i> value
	Total no.		Positive		Negative		
	269	(100%)	40	(15%)	229	(85%)	
Gender							0.55 ^a
Male	143	[53%]	19	[48%]	124	[54%]	
Female	126	[47%]	21	[53%]	105	[46%]	
Age (years) (mean ± S.D.)	68.6 ± 12.6		66.1 ± 14.2		69.1 ± 12.3		0.17 ^b
Size (cm) (mean ± S.D.)	4.99 ± 2.6		5.2 ± 2.7		5.0 ± 2.5		0.68 ^b
Tumor location							0.86 ^a
Right-sided colon	124	[46%]	20	[50%]	104	[45%]	
Left-sided colon	86	[32%]	12	[30%]	74	[32%]	
Rectum	59	[22%]	8	[20%]	51	[22%]	
pT stage							0.23 ^c
pT2	36	[13%]	1	[3%]	35	[15%]	
pT3	189	[70%]	33	[83%]	156	[68%]	
pT4	44	[16%]	6	[15%]	38	[17%]	
Histological differentiation							0.39 ^d
Well to moderately	242	[90%]	38	[95%]	204	[89%]	
Poorly	27	[10%]	2	[5%]	25	[11%]	
Mucus production							0.24 ^d
Positive	14	[5%]	0	[0%]	14	[6%]	
Negative	255	[95%]	40	[100%]	215	[94%]	
Lymph node metastasis							0.59 ^a
Positive	124	[49%]	20	[54%]	104	[48%]	
Negative	129	[51%]	17	[46%]	112	[52%]	
Peritoneal metastasis							1 ^a
Positive	50	[19%]	7	[18%]	43	[19%]	
Negative	219	[81%]	33	[83%]	186	[81%]	
Distant organ metastasis							0.82 ^a
Positive	44	[16%]	7	[18%]	37	[16%]	
Negative	225	[84%]	33	[83%]	192	[84%]	
Operation status							0.034 ^a
Complete resection	237	[88%]	31	[78%]	206	[90%]	
Incomplete resection	32	[12%]	9	[23%]	23	[10%]	
CD27+ TAL median (min, max) /HPF	32 (0, 816)		32.5 (0, 361)		32 (0, 816)		0.47 ^e
FOXP3+ TAL median (min, max) /HPF	38 (0, 213)		38.5 (3, 105)		38 (0, 213)		0.63 ^e
MMR system status							0.060 ^a
Deficient	31	[12%]	1	[3%]	30	[13%]	
Preserved	238	[88%]	39	[98%]	199	[87%]	

The Bonferroni-corrected *P* value for significance was $P \approx 0.0036$ (0.05/14)

^a *P* values were calculated by the chi-square test for fCD70 expression

^b *t* test

^c Cochran-Armitage trend test

^d Fisher's exact was used to calculate *P* values

^e Mann-Whitney *U* test was used to calculate *P* values

(40/269), mainly in tumor-surrounding areas. Clinical, pathological, and immunohistochemical features of analyzed tumors are

summarized in Table 2 according to fCD70 expression. fCD70 positivity showed tendency to associate with incomplete

Table 3 Logistic regression analysis of colorectal cancer patients

	Odds ratio	95% CI		P value
		Min	Max	
Operation status (incomplete resection)	2.60	1.10	6.13	0.029

The logistic regression analysis model initially included age, pT stage, tumor histological differentiation, mucus production, operation status, CD27+ TALs, and MMR system status. A backward elimination with a threshold of $P=0.05$ was used to select variables in the final model

resection operation status ($P=0.034$) or MMR-preserved phenotype ($P=0.060$). No significant association was detected between fCD70 positivity and the numbers of CD27+ TALs ($P=0.47$) or FOXP3+ TALs ($P=0.63$). The logistic regression analysis revealed significant associations of fCD70 expression with incomplete resection status (OR, 2.60; 95% CI, 1.10–6.13; $P=0.029$) (Table 3).

Various numbers of CD27+ TALs or FOXP3+ TALs were detected in CRCs. The primary CRCs with metastatic lesion (vs. without metastases, $P=0.024$) or mucus production (vs. without mucus production, $P=0.0016$) contained significantly fewer CD27+ TALs (Fig. 3). In contrast, CRCs of 5 cm or larger tumors (vs. < 5 cm, $P=0.0029$), in advanced pT stage ($P<0.001$) or with mucus production (vs. without mucus production, $P=0.034$) contained significantly fewer FOXP3+ TALs (Fig. 4). There was no significant correlation between MMR status and the numbers of CD27+ TALs ($P=0.75$) or FOXP3+ TALs ($P=0.23$).

Survival analyses of CRC patients

Patients with fCD70-positive CRCs had a significantly worse 5-year survival rate (55% vs. 77% in fCD70-negative CRC

patients; $P<0.0078$). No significant difference was found between the overall survival and CD27+ TALs ($P=0.69$). CRC patients with fewer FOXP3+ TALs tended to show a worse 5-year survival rate (64.1% vs. 76.4% in higher FOXP3+ TAL patients; $P=0.10$) (Fig. 5). The multivariable Cox hazards regression analysis revealed tubular-forming histology (HR, 0.16; 95% CI, 0.08–0.31; $P<0.0001$) and younger age (< 70 years old; HR, 0.45; 95% CI, 0.25–0.79; $P=0.0054$) as potential favorable factors. It was also revealed the presence of lymph node metastasis (HR, 1.88; 95% CI, 1.08–3.28; $P=0.026$), mucus production (HR, 2.67; 95% CI, 1.02–7.01; $P=0.045$), fCD70 expression (HR, 3.92; 95% CI, 2.03–7.58; $P<0.0001$), and peritoneal metastasis (HR, 8.03; 95% CI, 4.37–14.7; $P<0.0001$) as potential independent risk factors for CRC patients (Table 4).

Discussion

Aberrant CD70 expression has been documented in hematological and solid malignancies, and it has been associated with poor clinical outcome [8–10, 12]. The tumor CD70 expression has been suggested to contribute to the immune evasion of tumor

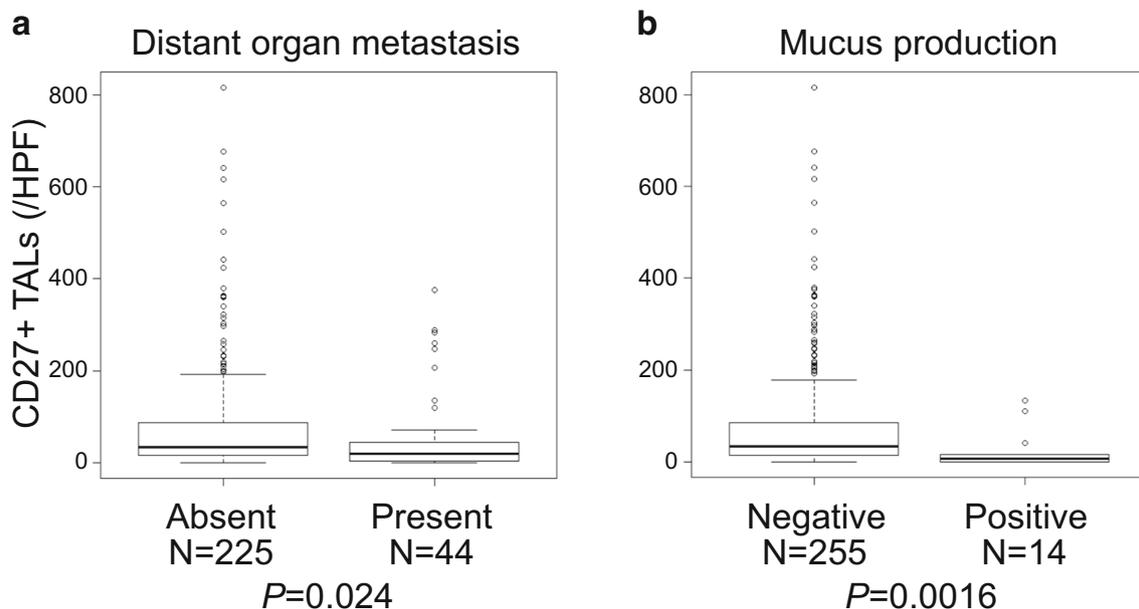


Fig. 3 Primary CRCs with distant organ metastases or mucus production contained significantly fewer CD27+ TALs. **a** Primary tumors without distant organ metastases contained significantly more CD27+ TALs ($P=0.024$). **b** Mucus-producing tumors contained significantly fewer CD27+ TALs ($P=0.0016$)

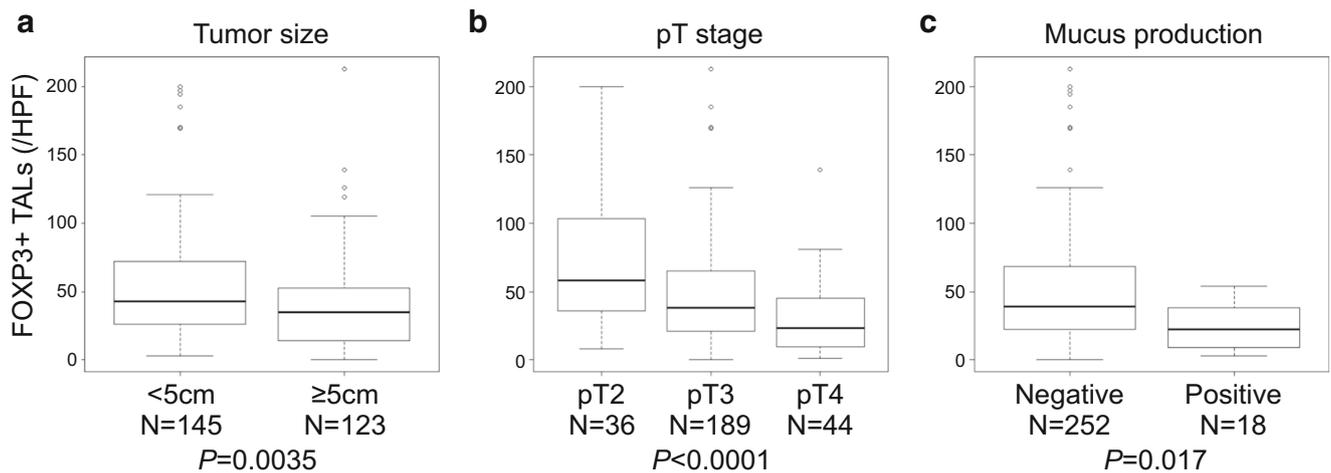


Fig. 4 Larger tumors, advanced tumors, or mucus producing tumors contained significantly fewer FOXP3+ TALs. **a** Smaller tumors contained significantly more FOXP3+ TALs ($P=0.0035$). **b** An inverse correlation was detected between FOXP3+ TALs and pT stages

($P<0.001$, T2 vs. T3; $P<0.001$, T2 vs. T3; $P=0.0058$, T3 vs. T4). Note that P values were calculated by Holm method. **c** Mucus-producing tumors contained significantly fewer FOXP3+ TALs ($P=0.017$)

cells through the following three distinct mechanisms: (1) T cell apoptosis [10, 15, 17, 19], (2) regulatory T cell (Treg) expansion [8, 16, 18], and (3) T cell exhaustion [20]. In the present study, 269 advanced CRCs were immunohistochemically evaluated for the expression of CD70, CD27, and FOXP3 in colorectal cancer (CRC) cells, tumor-associated fibroblasts (TAFs), and tumor-associated lymphocytes (TALs). Moreover, the association of CD70, CD27, and FOXP3 expressions to clinicopathological parameters and clinical outcome was analyzed to assess their potential for prognostication and the application of CD70–CD27-modulating therapy.

Unexpectedly, only a few CRC cases (6/269) showed the tumor CD70 expression (tCD70). In contrast, CD70 expression in TAFs (fCD70) was detected in 14.9% (40/269) with association to incomplete resection ($P=0.029$). Furthermore, the patients with fCD70-positive tumor showed significantly worse overall survival ($P=0.0078$). Recently, CD70 expression in

colorectal cancer-associated fibroblasts (CAFs) has been reported in a small cohort (total of 51 cases, including 6 pTis and 9 pT1 tumors) with association to worse prognosis [24]. These authors also claimed significant associations of CD70+ CAFs with the higher pT stage and increased numbers of Tregs [24]. However, neither of the associations was detected in the present study. Although these discrepancies may be due to the total number of cases, immunohistochemical detection system, or measurement methods (in the past report, the authors did not use measurement machinery system), the critical difference can be fCD70 positivity in pT3 and pT4 tumors (77% and 85%), which was approximately 4.5- and 6.0-fold higher than the present cohort [24].

Evidence for tumor supporting capacities of the CAFs has been accumulating. It has been reported that not only cancer cell proliferation, invasion, and metastasis but also tumor immunity can be modulated by CAFs [25, 26]. Generally, CAFs are considered to promote an immunosuppressive tumor

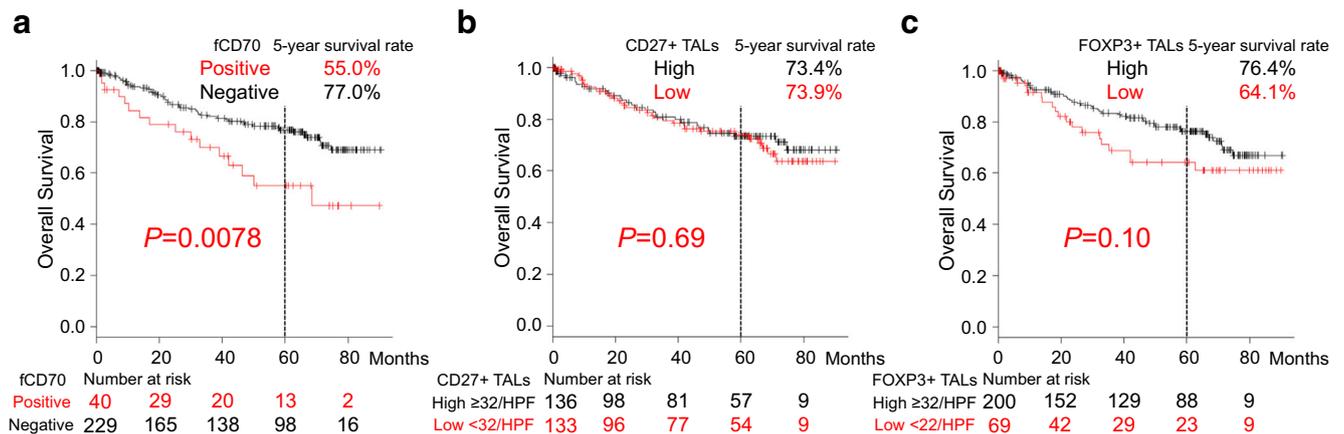


Fig. 5 Overall survival of colorectal cancer cases classified with fCD70, CD27, and FOXP3. Kaplan–Meier curves for the patients classified by fCD70 (**a**), CD27 (**b**), and FOXP3 (**c**) positivity. **a** Patients with CRCs containing higher fraction of fCD70 showed worse overall survival ($P=$

0.0078). **b** No significant correlations were detected between patient overall survival and CD27+ TALs ($P=0.69$). **c** Patients with CRC containing increased numbers of FOXP3+ TALs tended to show a better clinical outcome ($P=0.10$)

Table 4 Multivariable Cox hazards analysis of colorectal cancer patients

	Hazard ratio	95% CI		<i>P</i> -value
		Min	Max	
Well to moderately differentiated histology	0.16	0.08	0.31	< 0.0001
Age (< 70)	0.45	0.25	0.79	0.0054
Lymph node metastasis	1.88	1.08	3.28	0.026
Mucus production	2.67	1.02	7.01	0.045
fCD70 expression	3.92	2.03	7.58	< 0.0001
Peritoneal metastasis	8.03	4.37	14.7	< 0.0001

The multivariable Cox hazards analysis model initially included gender, age, primary tumor location, tumor size, pT stage, operation status, tumor histology, mucus production, lymph node metastasis, distant organ metastasis, peritoneal metastasis, fCD70 expression, and CD27+ TALs. A backward elimination with a threshold of $P = 0.05$ was used to select variables in the final model

micro environment through secretion of cytokines, chemokines, and pro-angiogenic factors. It has also been reported that CD70-positive CAFs induce Tregs in in vitro experimental conditions [24]. In the present study, the possibility for tumor immune evasion through aberrant CD70 expression on TAFs was assessed immunohistochemically. On one hand, no correlation between fCD70 positivity and CD27+ TALs ($P = 0.47$) or FOXP3+ TALs ($P = 0.63$) was found. These results might indicate that CD70-positive TAFs have limited capacities regarding the T cell apoptosis, Treg expansion, and T cell exhaustion in colorectal cancer microenvironment. On the other, the significant association between fCD70 positivity and incomplete resection status was elucidated ($P = 0.0029$). Based on the enhanced migration activity of CD70-expressing CAFs [24], our results may indicate that CD70-positive TAFs help the invasion rather than the immune evasion of colorectal cancer cells in the tumor microenvironment.

Monoclonal antibodies (mAb) blocking immune checkpoint pathways, such as CTLA-4 or PDCD1 axes, have been introduced to cancer therapy and have displayed significant anticancer effects [1, 2]. Based on the limited expression in normal tissues and aberrant expression of CD70 on the tumor cells, anti-CD70 mAbs, such as SGN-75 (vorsetuzumab mafodotin, a humanized monoclonal antibody conjugated with a cytotoxic agent noncleavable monomethyl auristatin F) and ARGX-110 (a blocking antibody causing antibody-dependent cellular cytotoxicity), have been developed and subjected to clinical trials [27, 28]. In the present study, fCD70 expression was detected in 14.9% of cases with the association to worse clinical outcome. Based on the tumor-promoting and tumor-supporting effects of the CAFs [25, 26], CRC patients with fCD70 expression might be targeted with these drugs. However, the efficacy of these drugs should be carefully assessed by using animal models before treating patients.

CD27 is expressed on a broad range of immune cells and plays a key role in the survival, activation, and the effector functions of T cells as well as the proliferation and cytotoxic

activity of NK cells [7, 8]. To the best of our knowledge, there is no study examining CD27 expression in CRCs. The present study demonstrated no correlation between CD27+ TALs and fCD70 positivity ($P = 0.47$) or MMR status ($P = 0.75$). However, an interesting association between CD27+ TALs and the primary CRCs without metastases ($P = 0.026$) was uncovered. This result may indicate the metastasis-inhibiting potential of CD27+ TALs in CRC patients. The antitumor effects of agonistic anti-CD27 mAb (Varlilumab) have been demonstrated in mouse models through Treg depletion and effector T cell co-stimulation [29, 30]. Recently, varlilumab has been used in clinical trials in combination with CD274/PDCD1 pathway blockers (NCT02335918) [30]. Based on the present observation of no significant correlation between CD27+ TALs and MMR status, the appropriate dose of CD27 agonists may be applied to other types of CRCs.

FOXP3, a hallmark of Treg, has been shown to be crucial in the development and immunosuppressive function of Tregs [31]. In many types of cancer, FOXP3+ Tregs suppress the antitumor immune response and accelerates tumor immune evasion within tumor microenvironment [14]. In contrast, the tumor-suppressive effects of Tregs and prolonged survival of patients with Tregs-containing tumors have been reported in several types of cancer including CRCs [32, 33]. In CRCs, the inflammatory antimicrobial response involves Th17 cells contributing to tumor promotion through angiogenesis and inflammatory reaction. A potential explanation for the favorable role of Tregs in CRC prognosis is due to the attenuated pro-inflammatory and tumor-enhancing response induced by Tregs [32]. In the present study, whose results were similar to a previous larger cohort study, [34] FOXP3+ Tregs within the tumor micro environment were inversely correlated with tumor diameter ($P = 0.0028$) and pT stage ($P < 0.0001$). Also, favorable survival was found in the patients carrying CRC with higher numbers of FOXP3+ Tregs [34]. Furthermore, no correlation between fCD70 positivity and FOXP3+ Tregs was found in the present study. Based on these findings, unlike

in other tumors [8, 16, 18], the Treg-induction through aberrant CD70 may not be a major mechanism for CRC cells immune evasion.

Conclusion

The present study demonstrated significant expressions of fCD70, CD27, and FOXP3 in patients with CRCs. Among them, immunohistochemistry for fCD70 could be used for the prognostication of CRC patients. Different from CD274 (PD-L1), all of the markers were independent from MMR-deficient phenotype [35]. CD70–CD27 axis-modulating therapies may be candidate therapies for CRC patients of all molecular subtypes.

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Author's contributions Shingo Inaguma: conceived, designed, and supervised the overall study; Satoshi Inoue, Shingo Inaguma: performed molecular experiments, histological and statistical analyses, made the figures and tables, and wrote the manuscript; Hideaki Ito, Takumi Tsunoda, Hideki Murakami: performed immunohistochemical staining; Satoshi Inoue, Masahide Ebi, Naotaka Ogasawara: collected and analyzed the clinical data; Kunio Kasugai, Kenji Kasai, Hiroshi Ikeda: critically reviewed the manuscript. All authors have read and gave final approval to the submitted version.

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Compliance with ethical standards This project was approved by the Institutional Ethical Review Board of Aichi Medical University Hospital.

Conflict of interest The authors declare that they have no conflict of interest.

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