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Original Article

CD103 expression in normal epithelium is associated with poor prognosis of colorectal cancer patients within defined subgroups

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Abstract: The immune system plays an important role in the development and progression of colorectal cancer (CRC). CD103 can be detected mainly on T cell populations within mucosal epithelium of the gut. However, clinical significance of $CD103^+$ cells in normal epithelium of CRC patients remain poorly understood. A collection of normal colonic mucosal specimens from 239 CRC patients was analyzed by immunohistochemistry for the presence of $CD103^+$ immune cells. Immunofluorescence was used to determine the cellular source of CD103. Prognostic capabilities were evaluated by Cox's proportional hazard model. Our results showed that $CD103^+$ cells were equally located in normal epithelium and lamina propria. $CD8^+$ T cells but not natural killer cells accounted for most CD103 expressing cells in normal colonic mucosa. Among the whole cohort, intraepithelial $CD103^+$ cell density did not show any association with overall survival (OS) and disease-free survival (DFS). On stratified univariate analysis, intraepithelial $CD103^+$ cell density was associated with poor DFS in colon cancer. Likewise, high density of $CD103^+$ cells was found to be a negative factor for both of OS and DFS in the subgroup with KRAS wild type tumors. These observations were confirmed by multivariate cox regression models. Therefore, intraepithelial $CD103^+$ cell density may specifically define a subgroup of patients with unfavorable outcome in colon cancer or KRAS wild type CRC patients.

Keywords: Colorectal cancer, normal mucosa, CD103, prognosis

Introduction

Colorectal cancer (CRC) is the one of the most common cause of cancer deaths worldwide [1]. Despite surgery with curative intent and advanced treatment approaches for tumor, the prognosis of CRC is still not ideal [2]. A large body of evidence has been reported supporting the notion that CRC is both clinically and biologically a heterogeneous disease [3]. At each tumor stage, the incidence of tumor recurrence varies. Furthermore, molecular characteristics, prognosis and corresponding drug targets in colon tumor are quite different from rectal tumor [4, 5]. Thus, identification of patients with a high-risk of disease recurrence remains a major clinical issue.

Series data over the last several years has provided evidence strongly supports the hypothesis that the immune system can affect colorectal cancer growth and metastatic dissemination [6, 7]. Biomarkers of cellular immune activation may act as potential mediators linking immunity, inflammation, and CRC. Immune infiltrates in colorectal cancer are being acknowledged as increasingly important predictors for survival [8-10]. High level infiltrates of $CD3^+$, $CD8^+$ and $CD45RO^+$ cells are becoming increasingly recognized to be associated with better clinical outcomes [11-14]. Despite the roles of intratumoral immune cells in predicting prognosis have been well documented, a number of $CD8^+$ T cells, B cells, dendritic cells (DCs) have also been found in normal mucosa, suggesting that

cell-mediated immunity also depends on the localization and functional role of immune cells in corresponding non-tumoral mucosa [6, 15]. A number of reports have shown that regulatory T cells (Tregs) density within tumor was associated with clinical outcomes for CRC [16, 17]. Likewise, FOXP3+ Treg density in histologically normal colonic mucosa from the surgical margin could predict prognosis as well [18, 19]. Thus, defining the potential significance of immune cells normal mucosa is essential to understanding their roles and mechanisms in tumor immunopathogenesis.

CD103 is the α_E subunit of the dimeric $\alpha_E\beta_7$ integrin, which is expressed mainly by cells of the T lymphocyte lineage within mucosal tissues and binds to the epithelial cell surface molecule E-cadherin [20, 21], can mediate cell adhesion, migration and signaling, and is also important for T-cell localization [15, 22]. Accordingly, CD103 can be detected in most of intra-epithelial lymphocytes (IELs) of the gut mucosal epithelium and skin, only 0.5-3% of peripheral blood lymphocytes (PBL) [23]. It is also clear that a proportion of DCs and T regulatory cells in intestinal mucosa express CD103, and CD103+ DCs can influence the balance between effector and T regulatory cells in the intestine [24]. Thus it has been suggested that CD103 is a critical component of intestinal immune homeostasis. In tumor tissues with colorectal cancer, approximately 90% CD103+ IELs co-expressed CD8, and CD8+CD103+ IELS were more prominent in microsatellite instability (MSI) colorectal cancer [25]. CD103+ can also mark a particular subtype of Treg with more strongly suppressive function compare to the CD103- subtype in colorectal cancer [26]. Recent evidence suggests that CD103 also plays an important role in immunity against cancer, and in particular, cancers of epithelial origin. CD103: E-cadherin interactions have been implicated in the recognition of tumor cells by cytotoxic T cells in colon [27]. Together, these findings lead us to hypothesize that CD103+ cells might demarcate colorectal patients with distinct prognosis. However, the clinical significance of CD103+ cells in tumor and corresponding normal mucosa are largely unknown. In the other hand, recent reports showed that high density of intratumoral CD103+ cells was were correlated with improved survival in lung [28], breast [29] and ovarian cancer [30]. In addition, we recently reported that Intratumoral CD103+ TILs could potentially serve as a prognostic marker in patients with bladder urothelial cell carcinoma [31]. Given the striking significance of CD103 in the epithelial cancers and expression of CD103 in colorectal cancer and colonic epithelium, we hypothesize that intraepithelial (IE) CD103+ cells might confer prognostic significance in colorectal cancer.

Using a new anti-CD103 antibody that works with formalin-fixed, paraffin-embedded tissues, we performed the analysis of the CD103+ cells in normal colonic mucosa with colorectal cancer. We show herein that CD103 is widely expressed by CD8+ lymphocytes in both colonic epithelium and lamina propria from patients with CRC. Importantly, subgroup prognostic analysis revealed that the density of CD103+ cells within epithelium is associated with a poor outcome in patients with colon cancer or CRC patients with KRAS wild type tumors.

Materials and methods

Patient cohort

A total of 239 patients treated for CRC between January 2009 and December 2012 in the Colorectal Surgery Department, the Sixth Affiliated Hospital of Sun Yat-sen University was included in this study. All patients underwent curative resection, defined as removal of all macroscopic tumor masses, absence of microscopic residual tumor, histologically confirmed negative resection margins, and extension of lymphadenectomy beyond involved nodes. No patient had distant metastasis and none received anticancer therapy preoperatively. Written informed consent was obtained from all of the patients, and the study was approvedby the Clinical Research Ethics Committee of the Sixth Affiliated Hospital of Sun Yat-sen University. Tumors were graded according to the World Health Organization criteria and staged according to the 7th edition tumor-node-metastasis (TNM) classification of the American Joint Committee on Cancer. Tumor-location was classified as colon and rectum. Resected tumors from 198 CRC patients were evaluated for the presence of the most common mutations of the KRAS (codons 12 and 13) and/or BRAF (codon 600) genes.

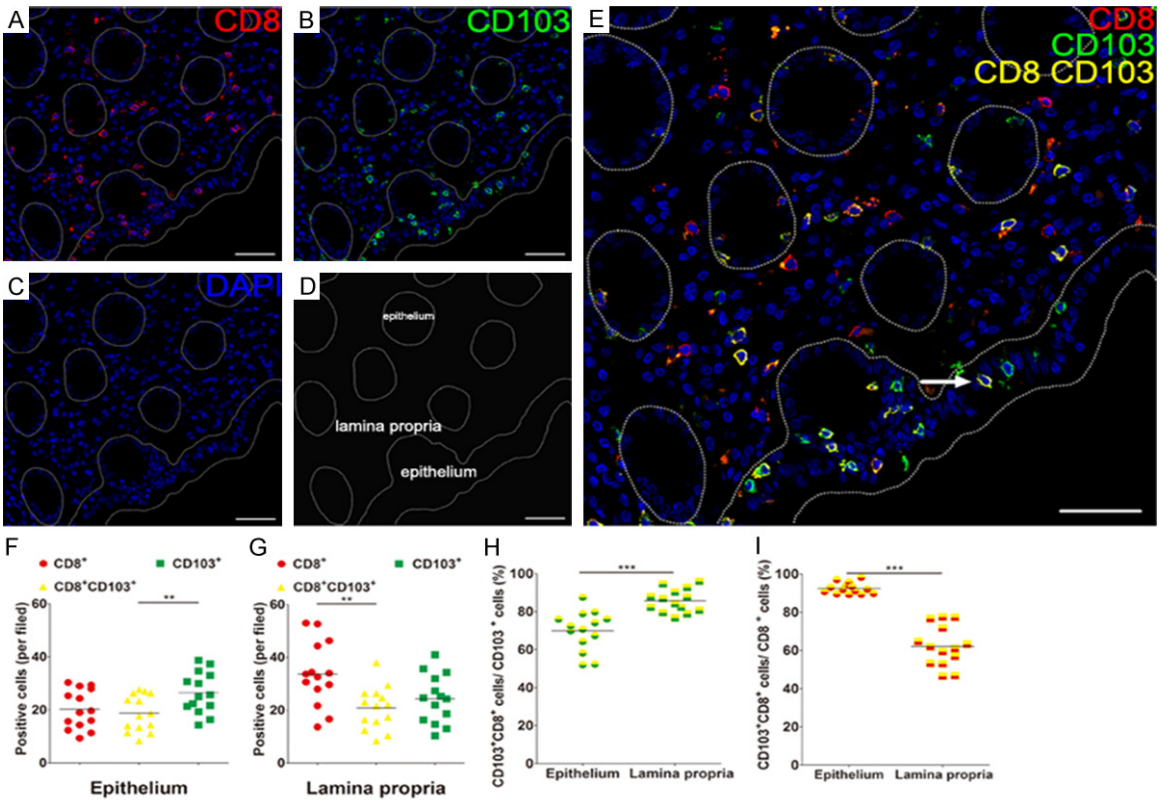

Figure 1. CD103+CD8+ lymphocytes were enriched in normal colonic mucosa. Representative examples of high power views of 14 paraffin embedded normal colonic tissue sections from colorectal patients show 2-color immunofluorescence staining for CD103 (green areas) and cytotoxic cell marker CD8 (red areas) (A-E). Note co-localization of CD103 with markers (yellow areas and white arrows). DAPI counter stain (blue areas). Scale bars indicate 40 μm. Mean ± SEM number and proportion of CD103+CD8+ cells among CD103+ and CD8+ cells in colonic epithelium and lamina propria calculated per × 400 field (F-I). $^{**}P < 0.01$, $^{***}P < 0.001$.

Follow-up was carried out until August 2016. The median follow-up was 51 months (range 1.4 to 88.8 months). Overall survival (OS) was defined as the interval between tumor resection and death; patients alive at the end of follow-up were censored. Disease-free survival was measured from the date of surgery to that of recurrent of metastatic disease occurrence. There were 34 cancer specific deaths (mean time 27.51 months from treatment) and 205 survivors (mean time to last follow-up date 57.28 months).

Immunohistochemistry and immunofluorescence

Histologically normal colonic mucosa from the surgical margin was retrieved for immunohistochemical analysis of CD103+ cell density. Formalin-fixed, paraffin-embedded samples were cut into 4 μm sections and used for immunohistochemical staining. The tissue sections were deparaffinized, and rehydrated through graded alcohol to water, and endogenous peroxidase activity was blocked by incubating the slides in 0.3% H_2O_2. Antigen retrieval was performed by microwave treatment in Tris-HCL (pH 9.2). The sections were blocked with goat serum. Next, the sections were then incubated with a monoclonal anti-CD103 primary antibody (1:5000 dilution, Abcam) overnight at 4°C. Subsequently, the sections were incubated with the secondary antibodies conjugated with horseradish peroxidase (Envision+ Dual Link Kit, DAKO, for mouse/rabbit antibodies) for 30 min at 37°C and then detected with 3,3'-diaminobenzidin for 1 minutes and counterstained with hematoxylin.

Fordouble-color immunofluorescence, preparation and antigen retrieval of paraffin-embedded tissue sections were performed as described in immunohistochemistry. The sections were first incubated with rabbit anti-human

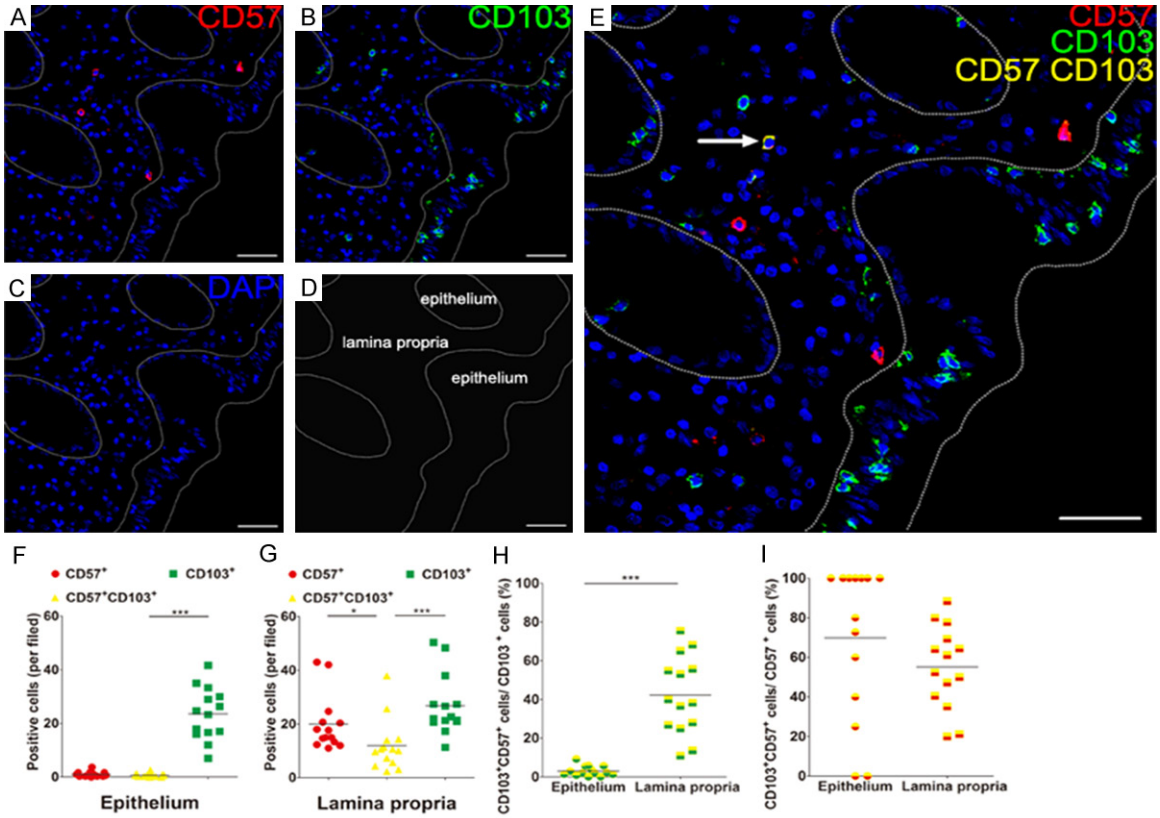

Figure 2. CD103+CD57+ NK cells were rare in normal epithelium. Representative examples of high power views of 14 paraffin embedded normal colonic tissue sections from colorectal patients show 2-color immunofluorescence staining for CD103 (green areas) and NK cell marker CD57 (red areas) (A-E). Note co-localization of CD103 with markers (yellow areas and white arrows). DAPI counter stain (blue areas). Scale bars indicate 40 μm. Mean ± SEM number and proportion of CD103+CD57+ cells among CD103+ and CD8+ cells in colonic epithelium and lamina propria calculated per × 400 field (F-I). *P < 0.05, ***P < 0.001.

CD103 (1:5000 dilution, Abcam) and mouse anti-human CD8 (1:100 dilution, Thermo Fisher), mouse anti-human CD57 (1:100 dilution, ZSGB-BIO) overnight at 4°C. Sections were subsequently incubated with goat-anti-mouse Alexa Fluor 555 (1:4000 dilution, Life Technologies), goat-anti-rabbit Alexa Fluor 488 (1:4000 dilution, Life Technologies) and DAPI (Life Technologies).

Immunohistology evaluation

Analysis was performed by two independent observers who were blinded to patient outcomes and other clinical findings. To evaluate the density of CD103+ cells, areas of normal epithelium were screened at low power (100 × magnification) and the five most representative high-power fields were selected at 400 × magnification (0.1255 mm^2 per field) for each area of every specimen. CD103+ cells in each area were counted manually. Data are shown as the mean ± SE number of cells per field.

Immunofluorescence quantification

Positively stained lymphocytes were counted based on localization in the normal epithelium and lamina propria. Single or double positive lymphocytes of interest in each region of the three most representative high-power fields at 400 × magnification (0.0845 mm^2 per field) were counted manually by 2 independent blinded observers. Data are shown as the mean ± SE number of lymphocytes per field.

Statistical analysis

Statistical analyses were performed with the SPSS, version 20. Associations between CD103+ cell density and clinicopathological variables were evaluated using t test. We used a cutoff value at the median frequency of IE

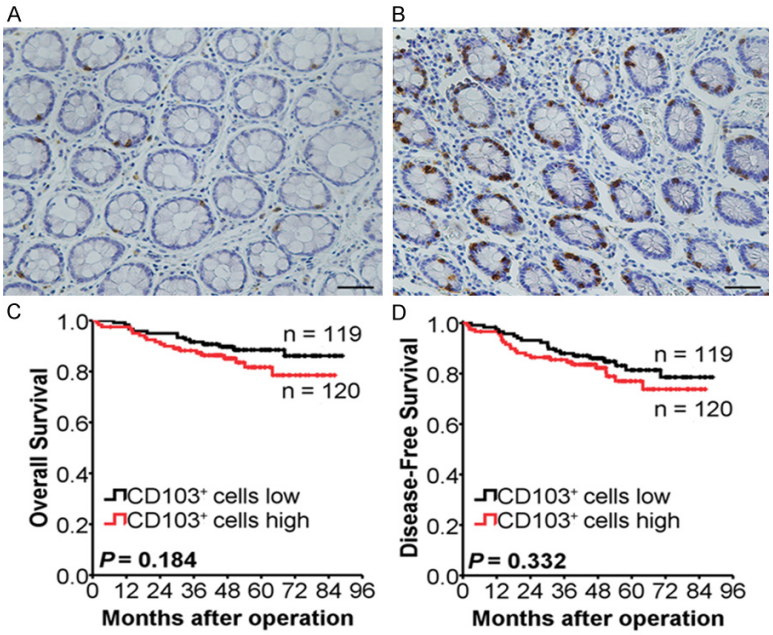

Figure 3. No prognostic effect of IE CD103+ cells was found in the whole cohort. Representative immunohistochemistry images demonstrated high (A) and low (B) number of IE CD103+ cells. Brown areas indicated positively stained cells. Scale bars represent 40 μm. In the whole cohort, IE CD103+ cell density showed a trend toward poorer prognosis for OS (C) and DFS (D), even with no statistical significance.

CD103+ cells to divided patients into high and low groups. Cox's proportional hazard model was used for univariate and multivariate analyses of prognostic factors. Survival curves were compared with the log-rank test. All statistical tests were two-sided, with significance defined as a *P*-value of < 0.05.

Results

Expression of CD103 in normal colonic mucosa

To identify the cellular source of CD103 in normal colonic mucosa, we performed co-localization studies and calculated the frequency of CD103+ cells among total CD8+ T and CD57+ NK cells. CD103+ cells were found to be equally detectable in epithelium and lamina propria, (18.79 ± 1.82 vs. 20.83 ± 2.16 per field, P = 0.475). We first investigated CD8 co-localization with CD103 (**Figure 1**). In 14 preparations CD8+ T cells represented most CD103+ cells in lamina propria (**Figure 1H**), but CD8+ T cells represented less CD103+ cells in epithelium (85.70% vs. 69.94%. P < 0.001). However, in 14 preparations CD57+ NK cells represented only less than half of all CD103+ cells in lamina propria (42.28%) (**Figure 2**). In epithelium, CD57+ NK cells are rare (1.02 ± 0.25 per field), and only 3.04% CD103+ cells expressed CD57 (**Figure 2**). These findings indicated that most CD103 expressing cells were CD8+ T cells in colonic mucosa. To assess the frequency and distribution of CD103+ cells within normal mucosa, we used a recently developed anti-CD103 antibody to stain the normal colonic tissues from a cohort of 239 CRC specimens. The median frequency of IE CD103+ cells was 43 cells per field, ranged 5 to 173 (**Figure 3**).

Correlation of intraepithelial CD103+ cell density with clinicopathological variables

To better understand the clinical relevance of IE CD103 within normal mucosa, we next examined the association between CD103+ cell density and clinicopathological variables. Among cases in the cohort, patients with colon cancer harbored a higher mean number of IE CD103+ cells compared with rectal cancer patients (45.11 ± 16.64 vs. 54.88 ± 32.19. P = 0.004) (**Table 1**). However, no significant correlation was seen between CD103+ cell density and age (P = 0.276), gender (P = 0.287), histological grade (P = 0.402), histological subtype (P = 0.816), tumor status (P = 0.486), nodal status (P = 0.174), vessel invasion (P = 0.557), preineural invasion (P = 0.139), KRAS status (P = 0.580) and BRAF status (P = 0.384) (**Table 1**).

Prognostic value of intraepithelial CD103+ cell density

For the entire study population, the OS and DFS rates were 97.06% and 95.55% at 1 year, 85.13% and 76.18% at 5 years, respectively. To confirm that IE CD103+ cell density correlates with CRC prognosis, OS and DFS rates were compared between CD103+ cell high and CD-

Table 1. The association of CD103 cells density with clinicopathological variables inCRC patients

Variables	N = 239	CD103 cells density	
		Mean ± SD	*P*-value
Age (years)			0.276
>60	132 (55.2%)	48.39 ± 25.11	
≤60	107 (44.8%)	52.11 ± 27.31	
Gender			0.287
Female	111 (46.4%)	48.12 ± 28.09	
Male	128 (53.6%)	51.73 ± 24.28	
Histological grade			0.402
Poor	46 (19.2%)	52.97 ± 24.23	
Moderate, High	193 (80.8%)	49.36 ± 26.57	
Mucinous			0.816
Yes	31 (13.0%)	51.08 ± 25.09	
No	208 (87.0%)	49.90 ± 26.33	
Location			0.004
Colon	121 (50.6%)	54.88 ± 32.19	
Rectum	118 (49.4%)	45.11 ± 16.64	
Tumor status			0.486
T3-4	184 (77.0%)	50.70 ± 26.54	
T1-2	55 (23.0%)	47.90 ± 24.78	
Nodal status			0.174
N1-N2	85 (35.6%)	46.96 ± 23.16	
N0	154 (64.4%)	51.76 ± 27.55	
Vessel invasion			0.557
Yes	18 (7.5%)	53.54 ± 30.49	
No	221 (92.5%)	49.77 ± 25.80	
Perineural invasion			0.139
Yes	20 (8.4%)	58.34 ± 33.93	
No	219 (91.6%)	49.30 ± 25.26	
KRAS status (n = 197)			0.580
Mutant	72 (36.5%)	49.78 ± 20.88	
Wild type	125 (63.5%)	51.97 ± 29.57	
BRAF status (n = 198)			0.384
Mutant	8 (4.0%)	43.08 ± 21.35	
Wild type	190 (95.6%)	51.47 ± 26.81	

CRC, colorectal cancer.

103+ cell low groups using a cutoff value at the median frequency of IE CD103+ cells. Next, we determine whether CD103+ density in addition to other clinicopathological variables was related to patient prognosis. On univariate analysis, age, tumor status, nodal status, vessel invasion and BRAF status were prognostic factors for OS and/or DFS (**Table 2**). However, CD103+ cell density did not show any relevant prognostic effect in the entire study cohort (**Figure 3**; **Table 2**).

Furthermore, subgroup analyses were performed for the clinicopathological variables mentioned above. Here, stratified analysis identified IE CD103+ cell density to be associated with DFS (*P* = 0.023) but not OS (*P* = 0.076) for colon cancer patients (**Figure 4**; **Table 3**). In KRAS wild type patients, high density of CD103+ cells correlated with significantly shorter OS (*P* = 0.029) and DFS (*P* = 0.026) (**Figure 4**; **Table 4**). Nevertheless, OS and DFS were not significantly between CD103+ cell high and low groups within patients with rectal cancer (*P* = 0.881 and *P* = 0.469) (**Figure 4**; **Table 3**) or KRAS mutant tumors (*P* = 0.956 and *P* = 0.619) (**Figure 4**; **Table 3**).

A stratified Cox proportional-hazards model was constructed according to the subgroups with colon cancer and KRAS wild type tumors to determine whether CD103+ cell density was an independent prognostic factor of survival. Multivariate analysis confirmed the observed negative DFS (*P* = 0.018) effect of CD103+ cell density for colon cancer (**Table 3**). Likewise, the present data showed that high density of CD103+ cells was found to be an independent negative factor of OS (*P* = 0.024) and DFS (*P* = 0.020) in the subgroup with KRAS wild type tumors (**Table 4**).

Discussion

CD103 expression is a common feature of IE CD8+ TILs and associated with patient survival in a broad range of epithelial cancers [28-31]. However, prognostic value of CD103+ cells in nontumoral tissues has long been unclear. Mucosa immediately adjacent or even longer to colorectal cancer shares biological features with the corresponding tumor and is referred to as transitional mucosa [32]. CD103 is increasingly recognized as a definitive marker of lymphocytes, especially CD8+ T cells in vari-

Table 2. UnivariateAnalysis of Factors Associated with CRC patient outcomes (OS and DFS, n = 239)

Variable	OS			DFS		
	Univariate			Univariate		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age, years (>60/≤60)	3.415	1.741-6.696	0.0004	1.421	0.7858-2.569	0.2451
Gender (female/male)	1.491	0.7593-2.927	0.2460	0.7204	0.3984-1.303	0.2778
Histological grade (Poor/Moderate, High)	2.140	0.8937-5.123	0.0877	0.9546	0.4483-2.033	0.9041
Mucinous (Yes/No)	1.155	0.4259-3.132	0.7771	0.8314	0.3501-1.974	0.6756
Location (Colon/Rectum)	0.6970	0.3558-1.365	0.2927	0.5692	0.3151-1.028	0.0617
Tumor status (T3-4/T1-2)	2.253	1.017-4.989	0.0453	1.495	0.7409-3.018	0.2615
Nodal status (N1-2/N0)	1.905	0.9417-3.852	0.0730	2.605	1.402-4.843	0.0025
Vessel invasion (Yes/No)	9.941	2.552-38.72	0.0009	3.144	0.9548-10.35	0.0596
Perineural invasion (Yes/No)	1.707	0.4899-5.946	0.4012	2.549	0.8176-7.947	0.1068
KRAS status (Mutant/Wild type, n = 197)	0.7077	0.3123-1.604	0.4074	1.069	0.5218-2.189	0.8558
BRAF status (Mutant/Wild type, n = 198)	416.6	40.56-4280	< 0.0001	24.32	3.246-182.2	0.0019
CD103+ cells (high/low)	1.580	0.8047-3.103	0.1839	1.341	0.712-2.427	0.3320

HR greater and less than 1 indicate increased and decreased death risk, respectively. HR, hazard ratio. CRC, colorectal cancer. OS, overall survival. DFS, disease free survival.

ous tissues, including gut and skin [33]. Therefore, we examined the hypothesis that normal IE CD103+ cells might involve in determining the prognosis of colorectal cancer patients. To the best of our knowledge this is the first demonstration of prognostic significance of IE CD103+ cell density. Our data indicates that high IE CD103+ cell density is independently associated with worse survival of the patients within the subgroup of colon cancer or KRAS wild type tumors.

CD103+ defines CD8+ cells that are localized in the epithelial compartment of epithelium-derived tumors in many types of cancer. It has been shown that in human colon cancer samples CD8+ IELs were mainly CD103 and CD49d/β7 positive, and expression of CD103 was restricted to a proportion of CD8+ lymphocytes [27]. To investigate the correlation between CD103+ cells and CD8+ cells in normal colonic mucosa, Two-color immunofluorescence staining was performed. In normal colonic mucosa, we found that CD103+ cells distributed equally within epithelial and lamina propria compartments. In epithelium, most of the CD8+ cells co-expressed CD103, but only 69.94% of CD103+ cells were CD8+, rare CD103+ cells were CD57+. It indicated that IE CD103+ cells were not restricted to CD8+ cells and CD57+ natural killer (NK) cells. Previous reports have shown that CD103 can be also detected on DC population [34], but whether IE CD103+ cells in normal epithelium include DCs warrants future investigation.

In our analysis of 239 CRC patients, the density of IE CD103+ cells ranged widely, from 5 to 175 cells per field. The underlying cause of this variability in the apparently normal tissue is unclear. Previous studies have demonstrated that TGF-β dominantly promote expression CD103 in many tissues, including intestinal epithelium [35]. This variability seemed to reflect differences in the levels of the TGF-β. Of course, we couldn't exclude the effect of tumor cells, which may cause the variability. Therefore, in the present study, we paid attention to the interaction of CD103 expression in normal epithelium and clinicopathological features. IE CD103+ cell density was found to be merely correlated with primary tumor location. This finding is consistent with the recent notion that colon cancer is quite different from rectal cancer.

As first described in CRC, the so-called Immunoscore was considered to be an important component of cancer classification [36]. The ultimate goal of this effort is to bring Immunoscore into standard clinical practice as a prognostic biomarker. Our results suggest that immunohistochemical detection of CD103 expression in normal epithelium provide an objective surrogate to score IE immune cells. Indeed, IE CD103+ cell density could predict poor outcomes in patients with colon cancer or KRAS

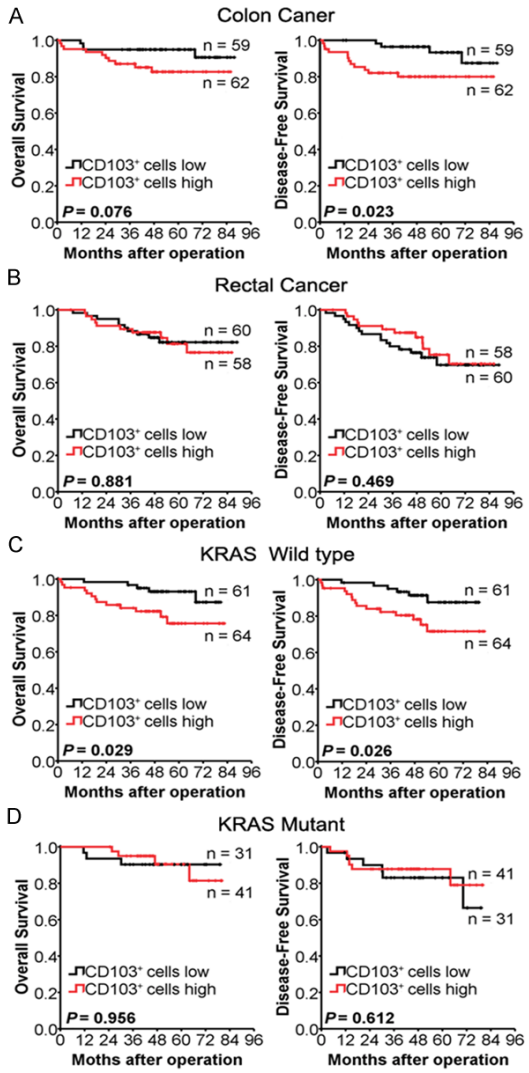

Figure 4. Prognostic impact of IE CD103 cell density depended on primary tumor location and KRAS status. Univariate analysis demonstrated a significant negative effect of IE CD103 cell density on OS and/or DFS for patients with colon cancer (A) or KRAS wild type tumors (C). No prognostic effect of IE CD103+ cells was found in patients with rectal cancer (B) or with KRAS mutant tumors (D).

wild type tumors. However, IE CD103+ cell density could not significantly stratify patients' outcomes in the subgroups with rectal cancer or KRAS mutant tumors. One explanation for this might be that IE CD103+ cells in patients with rectal cancer or KRAS mutant tumors are not as functionally relevant and/or include a higher proportion of irrelevant 'bystanders'.

With respect to the recent literature, previous studies regarding the prognostic role of CD103 for patients with cancer were mainly according to the expression of CD103 in tumor tissues. These studies mostly suggest that high density of intratumoral CD103+ cells was responsible for improved prognosis. In contrast to the favorable prognosis associated with CD103 expression in tumor epithelium, our data revealed that high levels of CD103 in normal epithelium tended to be associated with poor prognosis. The biology of an association between IE CD103+ cells and poor outcome in patients with colon cancer or KRAS wild type tumors is not well understood. A potential explanation is that the negative prognostic impact of IE CD103+ cells in these conditions may indicate the presence of a mixed immune cell infiltrate containing cytotoxic and regulatory T cells and reflect a partially imbalanced local cellular immune response, which still contributes to immunosuppressive functions.

A major task of the mucosal immune system is to provide protection against intestinal pathogens; however, mucosal IELs are extremely heterogeneous. As a results, there was evidence showing direct correlation of IELs activation and disease progression [37]. Our present results show the dark side of CD103+ cells in colorectal cancer.

Although the markers of dendritic cells were not detected in our study, combined previous reports and only 69.94% CD8+CD103+/CD103+ cells in mucosal epithelium revealed that CD103+ DCs may exist in mucosa. It has been shown that the presence of intratumoral CD103+ DCs correlated with cancer control [38]. Whether CD103+ DCs in adjacent colonic mucosa prohibit cancer progression was unknown. If there area proportion of CD103+ DCs in colonic mucosa, maybe the immune balance has been broken or there may be CD103+ DC subsets with promotion value on caner progression.

Another mechanism by which IE CD103 cells contributes to poor clinical outcomes in CRC may be through the CD103 inducer TGFβ. The TGFβ signaling pathway has been considered as both a tumor suppressor and a cancer promoter [39]. It's widely accepted that TGFβ1 upregulates expression of CD103 on T cells and DCs. In contrast, TGFβ1 down-regulates expression of CD103's only known ligand E-cadherin, an integral adherens junction component [40]. Furthermore, TGFβ could suppress T cell func-

Table 3. Univariate and Multivariate Analysis of Factors Associated with colon cancer patient outcomes (OS and DFS, n = 121)

Variable	OS				DFS			
	Univariate	Multivariate			Univariate	Multivariate		
		HR	95% CI	*P*-value		HR	95% CI	*P*-value
Age, years (>60/≤60)	0.003	13.808	1.763-108.136	0.012	0.442			NA
Gender (female/male)	0.500			NA	0.056			NA
Histological grade (Poor/Moderate, High)	0.042	3.247	1.051-10.029	0.041	0.657			NA
Mucinous (Yes/No)	0.709			NA	0.494			NA
Tumor status (T3-4/T1-2)	0.148			NA	0.117			NA
Nodal status (N1-2/N0)	0.629			NA	0.031	3.345	1.204-9.297	0.021
Vessel invasion (Yes/No)	< 0.001	5.824	1.912-17.740	0.002	0.158			NA
Perineural invasion (Yes/No)	0.501			NA	0.579			NA
CD103+ cells (high/low)	0.076			NA	0.023	3.938	1.259-12.319	0.018

HR greater and less than 1 indicate increased and decreased death risk, respectively. HR, hazard ratio. OS, overall survival. DFS, disease free survival.

Table 4. Univariate and Multivariate Analysis of Factors Associated with KRAS Wild type patient outcomes (OS and DFS, n = 125)

Variable	OS				DFS			
	Univariate	Multivariate			Univariate	Multivariate		
		HR	95% CI	*P*-value		HR	95% CI	*P*-value
Age, years (>60/≤60)	0.017	5.414	1.655-17.706	0.005	0.265			NA
Gender (female/male)	0.967			NA	0.569			NA
Histological grade (Poor/Moderate, High)	0.317			NA	0.187			NA
Mucinous (Yes/No)	0.256			NA	0.412			NA
Location (Colon/Rectum)	0.257			NA	0.179			NA
Tumor status (T3-4/T1-2)	0.125			NA	0.224			NA
Nodal status (N1-2/N0)	0.033	3.348	1.258-8.915	0.016	0.001	4.915	1.877-12.869	0.001
Vessel invasion (Yes/No)	< 0.001	6.678	2.383-18.716	< 0.001	0.002	2.743	0.981-7.674	0.054
Perineural invasion (Yes/No)	0.184			NA	0.278			NA
CD103+ cells (high/low)	0.029	3.387	1.172-9.784	0.024	0.026	3.150	1.199-8.274	0.020

HR greater and less than 1 indicate increased and decreased death risk, respectively. HR, hazard ratio. OS, overall survival. DFS, disease free survival.

tion, promoting tumor immune escape [41]. Thus, high CD103+ density in normal epithelium may reflect an aggressive subtype. This possibility merits further investigation.

In conclusion, our study demonstrated that CD103 cell density in normal colonic epithelium is an independent negative prognostic factor of OS and/or DFS in colon cancer or colorectal cancer with wild type KRAS status. We propose that CD103 expression in the colonic epithelium suppresses immunosurveillances. Future studies are required to better define the impact of CD103 subsets on CRC outcomes.

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Disclosure of conflict of interest

None.

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