ORIGINAL ARTICLE

Expression of FoxP3+ Tregs and its Association with Stage and Grade of Colorectal Cancer Patients

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ABSTRACT

Introduction: Forkhead-box protein P3 regulatory T cells (FoxP3+ Tregs) play an important role in tumour progression by evading the immune escape mechanism. However, the prognostic significance of these FoxP3+ Tregs in colorectal cancer (CRC) remains to be elucidated. This study aims to evaluate the association between the FoxP3+ Tregs immunohistochemistry expression with clinicopathological features of CRC. **Materials and methods:** This study is a cross-sectional study assessing immunohistochemistry expression of FoxP3+ Tregs in 202 CRC cases from two tertiary hospitals: Hospital Sultanah Bahiyah (HSB), Kedah and Hospital Universiti Sains Malaysia (HUSM), Kelantan, from January 2017 to December 2019. FoxP3+ Tregs immunoexpression was divided into a low and high group expression using the median value (19.7) as a separating point. Pearson's Chi-square and Fisher's exact tests were used to analyze the association. P value of less than 0.05 was considered statistically significant. **Results:** 102 (50.5%) cases showed high expression of FoxP3+ Tregs within the intra- and peritumoral stroma of CRC, while 100 (49.5%) showed low expression. There is a significant association between FoxP3+ Tregs immunoexpression with pathologic T stage (p=0.001), lymph node involvement (p=0.025), and Modified Dukes staging (p=0.032). **Conclusion:** This study suggests that FoxP3+ Tregs immunoexpression could serve as a novel marker for classifying CRC and predicting its prognosis in the future.
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Keywords: FoxP3+ Tregs, Colorectal cancer, Tumour stage, Tumour grade, Tumour microenvironment

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INTRODUCTION

Colorectal cancer (CRC) is a highly heterogeneous malignancy of intestinal origin, with conventional adenocarcinoma being the most common histological type (1,2) Worldwide, CRC is the third most common cancer, representing about 10.0% of all new cancers in 2020. It comprised of more than 1.9 million new cases after lung and breast cancers (3) and was responsible for second place in cancer death (9.4 %) worldwide (3). According to the GLOBOCAN report, Malaysia had the third-highest overall incidence of CRC (19.6 per 100,000) across Southeast Asia. Based on human development projections, the number of new CRC cases is predicted to hit 3.2 million globally by 2040 (4).

A two-tiered system for tumour grading is recommended in view of its proven prognostic value, simplicity, and reproducibility (1,4). The low-grade is for well and moderately differentiated tumours, and the high-grade tumour is for the poorly and undifferentiated tumour (1,4). Various multivariate analyses demonstrated that histologic grade is an independent prognostic factor in CRC. More precisely, a high tumour grade indicates a poor prognosis (4). Similarly, the staging system of TNM, developed by the American Joint Committee on Cancer (AJCC) / International Union against Cancer (UICC) is also widely used for prognostic indicator for the outcome of CRC patient (4).

Another commonly used staging system is the Dukes staging that was initially developed by Cuthbert E. Dukes in the 1930s (5). It was later modified by adding stage D for distant metastases) and now is routinely used as it is reflecting the prognosis very well (5,6). Interestingly, the expression of FoxP3+ Treg cells has been discovered by

Liu et al. to have a positive correlation with the Dukes staging system (7).

Apart from the established carcinogenesis pathway, tumour development is also contributed by the interaction of a complex tissue network into which many different cell types are recruited, forming tumour microenvironment (TME)(8). The major components of the TME are usually the tumour-infiltrating lymphocytes (TILs), composed of CD3+CD4+ and CD3+CD8+ T-cells (9). In peripheral blood, regulatory T-cells (Tregs) account for 5% of circulating CD4+ T-cells. Tregs were found in greater numbers in the blood, tumour mass, and lymph nodes of patients with various solid tumours (10). Furthermore, a subpopulation of CD4+ T-cells which is the CD4+CD25high FoxP3+ T-cells or known as Tregs is increased in TIL at a rate of approximately 5 to 15% of CD3+CD4+ T-cells (11).

Tregs function as suppressors of immune cells directed towards self or foreign antigens, hence ensuring immunological homeostasis. Treg cells are now largely regarded as playing an important role in tumour immunity. Treatments targeting Treg cells are now being extensively studied (12). The effector Tregs exhibit their suppressive activity by means of antigen-specific and antigen-nonspecific mechanisms. In antigen specific mechanism, they inhibit the maturation of antigen-presenting cells (APCs) such as dendritic cells (DCs) through direct interaction. In antigen nonspecific manner, they show their suppressive activity through IL-10, TGF-β, IL-35; inhibitory cytokines, and ATP degradation (13).

A recent meta-analysis found that high frequencies of FOXP3+ tumour-infiltrating cells in solid tumours like those in the cervix, kidney, breast, and melanomas are linked to poorer outcomes. However, the impact of increased Treg cells on colorectal cancer (CRC) prognosis is debated. Conflicting results may stem from treating diverse FOXP3+ cells, including Treg and non-Treg cells, as a single group. A recent study classified CRC patients into two groups: one with tumours dominated by suppression-competent Treg cells and another with a mix of FOXP3+ non-Treg cells and Treg cells. This highlights the need to assess FOXP3+ T cell heterogeneity to accurately evaluate their role in cancer prognosis (14).

Forkhead box P3 (FoxP3) is a transcription factor that belongs to the forkhead/winged-helix family. This gene plays a role in immunological responses, as well as the growth and function of Tregs. The nuclear expression of FoxP3 is considered to be the most specific marker for Tregs and is a key element in their immunosuppressive functions in autoimmune processes or inflammatory responses (10). In contrast to the alleged protumorigenic effect, several researches indicate that FoxP3+ Tregs are in favour of good clinical outcomes in colorectal and head and neck cancers. Even though FoxP3+ Tregs may encourage cancer progression, they may also help to reduce inflammation. Since chronic inflammation is a crucial factor in carcinogenesis and tumour formation, FoxP3+ Tregs are generally able to suppress the c inflammatory responses in protumorigenic process (10,15).

CRC cases are increasing in trend annually both in Malaysia and worldwide. FoxP3+ Tregs expression and its role in carcinogenesis has been clearly depicted on various cancers, however the data particularly on CRC is still controversial. Currently, there is yet any study published on FoxP3+ Tregs expression in CRC in the local settings. This study was aimed to bridge the knowledge gap and understand the characteristics of FoxP3+ Tregs and its relationship with the clinicopathological features of CRC patients, especially its tumor stage and grade.

MATERIALS AND METHODS

Study design and population

This is a cross-sectional study in two tertiary centers; HSB, Kedah and HUSM, Kelantan between January 2017 and December 2019. All patients who have undergone primary colorectal resection with histopathological diagnosis of CRC were selected. Cases that have incomplete clinical information, inadequate or missing tissue block, secondary malignancies metastasized to colorectal, and patients who had prior neoadjuvant chemotherapy were excluded from the samples recruited.

Sample size

The sample size was determined based on two proportion formula using Power and Sample Size Calculation Software with type 1 error of 0.05 and power of 80%. Dropout was considered as 10%. After calculation, the required sample size was 202.

Data collection

Data was collected from the electronic Hospital Information System (eHIS) at HSB and the Laboratory Information System (LIS) at HUSM. The patients' clinicopathological data obtained was assigned with individual identification number and was filled in the generated proforma form. Subsequently, the data from the assessment of the FoxP3+ Tregs expression was completed in the proforma form, accordingly.

The corresponding formalin-fixed paraffin-embedded (FFPE) CRC tissue blocks were retrieved from Department of Pathology, HSB and HUSM archives. The best representative tissue block comprising >80% tumor tissue was chosen from each case for the immunohistochemistry (IHC) study.

Immunohistochemistry procedures

The FFPE tissue blocks that fulfilled the inclusion

and exclusion criteria were sectioned into 2 to 4µm thickness onto a polylysine glass slide. Then, the next step is incubation of the slides in the Dako PT Link pre-treatment module for deparaffinization, rehydration, and retrieval of the target antigen for 20 minutes at 97°C using EnVision Flex Target Retrieval Solution, High pH (9.0). Next, the slides were incubated with the primary antibody monoclonal anti-FoxP3 antibody (Abcam [236A/E7]) with a 1:500 dilution for one hour at room temperature. The slides were rinsed with distilled water for five minutes and then washed in Tris Buffer Saline (TBS) for five minutes.

Subsequently, the slides were treated using EnVision Flex Peroxidase Blocking reagent for 5 minutes to suppress endogenous peroxidases. The slides were rinsed with distilled water for five minutes and then washed in TBS for five minutes. Next, the slides were incubated with a mouse linker reagent for 15 minutes to amplify the signal of the primary antibodies. The slides were rinsed with distilled water for five minutes and then washed in TBS for five minutes. At this point, the slides were allowed to react with the corresponding secondary antibody solution for 20 minutes at room temperature. After two washes with TBS for five minutes each, the slides were treated with 0.05% diaminobenzidine tetrahydrochloride (DAB), washed in running tap water and rinsed with distilled water for five minutes. Following that, the slides undergone a counterstaining process with haematoxylin. Finally, the slides were mounted using a coverslip and dibutylphthalate polystyrene xylene (DPX) mounting solution.

Immunohistochemistry assessment

A total of 102 (50.5%) cases has high expression of FoxP3+ Tregs and 100 (49.5%) cases have 23 low expressions of FoxP3+ Tregs. The anti-FoxP3 antibody positive T-cell staining pattern is characterized by distinct brown stained complete nuclear staining. For positive control, tonsil tissue is used, whereas the primary antibody was omitted but all the other steps were followed for negative control.

The FoxP3+ Tregs expression was assessed according to the Sinicrope method, involving manual calculation to determine the number of positive FoxP3+ Treg cells in the intra and peritumoral areas. Tumour tissue sections were examined under light microscopy at a low magnification (x100) to identify areas with high numbers of immunopositive FoxP3+ Treg cells. To quantify the number of FoxP3+ Tregs within the intra and peritumoural areas, a quantitative scoring approach according to the Sinicrope method was applied. The average values (number) of FoxP3+ Tregs were calculated from 10 high power fields (x400 magnification) within the most representative areas within the intra- and peritumoural areas where the density of immunopositive FoxP3+ Tregs cells was highest (16).

Thus, the FoxP3 For the expression FoxP3+ Tregs in CRC, the value was categorized using median as a separating point, in view of the data presented was not normally distributed, as recommended by Hanke (2015) and Hu (2017) (17,18). High expression of FoxP3+ Tregs was defined as a value of more than 19.7 (the median value), while low expression of FoxP3+ Tregs was defined as a value of less than 19.7. All the IHC sections are evaluated blindly without the knowledge of the corresponding clinicopathological information, and two pathologists verified them.

Statistical analysis

IBM SPSS Statistics for Windows 26.0 2019 (Armonk, NY: IBM Corp) was used for data entry and analysis. Descriptive analysis was done for selected variables. Results were presented as frequency (n) and percentage (%) for categorical variables. For numerical variables, mean with standard deviation (SD) or median with interquartile range (IQR) were presented based on the distribution.

The association between the expression of FoxP3+ Tregs and clinicopathological characteristics was identified using Pearson's Chi-square test for Independence, while Fischer's exact test was used if the assumptions of Pearson's Chi-square test for Independence are violated. All probability values are two-sided, and a significance level of less than 0.05 (p-value <0.05) was considered statistically significant.

Ethical Clearance

The study was ethically approved by Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM/JEPeM/20020080) and Medical Research and Ethics Committee (MREC) Ministry of Health, Malaysia (NMRR-20-125-52666).

RESULTS

Clinicopathological characteristics

The result for clinicopathological analysis for CRC cases are summarised in Table I. The patients were between 19 and 94 years old, with a mean age and standard deviation of 63.11 and 12.20 years at the time of presentation and diagnosis was made. Most cases were Malay (74.8%) and male (53.5%). Most CRC patients had tumours located in left colon (72.3%) and low-grade tumours (89.6%), with moderately differentiated adenocarcinoma being the most common subtype of CRC cases, constituting 83.7% of all CRC cases.

Table I: The distribution of colorectal cancer patients according to clinicopathological characteristics (n=202)

Variables		Frequency (%)	Mean (SD)
Age (years)			63.11 (12.20)
Race	Malay	151 (74.8)	
	Chinese	42 (20.8)	
	Indian	6 (3.0)	
	Others	3 (1.5)	
Gender	Male	108 (53.5)	
	Female	94 (46.5)	
Tumour location	Right colon	56 (27.7)	
	Left colon	146 (72.3)	
Tumour grade	Low-grade	181 (89.6)	
	High-grade	21 (10.4)	
Histological subtypes	Well differentiated adenocarcinoma	12 (5.9)	
	Moderately differentiated adenocarcinoma	169 (83.7)	
	Poorly differentiated adenocarcinoma	6 (3.0)	
	Mucinous adenocarcinoma	14 (6.9)	
	Signet-ring cell carcinoma	1 (0.5)	
Pathologic T stage	pT1	3 (1.5)	
	pT2	35 (17.3)	
	pT3	109 (54.0)	
	pT4	55 (27.2)	
Pathologic N stage	pN0	105 (52.0)	
	pN1	70 (34.7)	
	pN2	27 (13.4)	
Nodal metastasis	Negative	105 (52.0)	
	Positive	97 (48.0)	
Modified Dukes Stage	A	19 (9.4)	
	B	91 (45.0)	
	C	82 (40.6)	
	D	10 (5.0)	
Resection margin involvement	No	192 (95.0)	
	Yes	10 (5.0)	
Diabetes Mellitus	No	95 (62.9)	
	Yes	56 (37.1)	
Hypertension	No	71 (47.0)	
	Yes	80 (53.0)	
Smoking status	No	107 (70.9)	
	Yes	44 (29.1)	
FoxP3+ Treg average value per 10 h.p.f.			Median = 19.7 (17.8)
FoxP3+ Treg expression	Low expression	100 (49.5)	
	High expression	102 (50.5)	

Most patients were diagnosed with pathologic stage pT3 and stage B (45.0%) at diagnosis based on the Modified Dukes staging system. Ninety-seven cases (48.0%) were noted to have nodal metastasis and only ten (5.0%) cases showed resection margin involvement. With regard to risk factors for CRC, 37.1 % of the cases had coexisting diabetes mellitus type 2, 53.0 % had hypertension, and 29.1% were smokers.

The association between the expression of FoxP3+ Tregs with the clinicopathological characteristics of CRC

The expression of FoxP3+ Tregs within the CRC intratumoral and peritumoural stroma using median (IQR) is 19.7 (17.8) cells per high power field, as they are not normally distributed. The authors use this value to classify the expression of FoxP3+ Tregs into two groups; low (< 19.7 positive cells) and high (≥ 19.7 positive cells) expression, as demonstrated by Figure 1. A total of 102 (50.5%) cases have high expression of FoxP3+ Tregs and 100 (49.5%) cases have low expression of FoxP3+ Tregs. Tumor cells did not display any positivity for the FoxP3 IHC in this study.

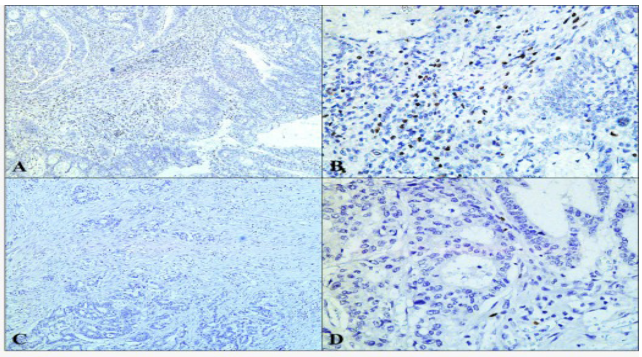

Figure 1: Immunohistochemistry (IHC) evaluation of FoxP3+ Tregs in CRC. (A) Low and (B) high power views showing high expression of intra- and peritumoural Tregs stained by FoxP3 IHC (x100 and x400 magnifications). (C) Low and (D) high power views showing low expression of intra- and peritumoural Tregs stained by FoxP3 IHC (x100 and x400 magnifications).

As summarised in Table II, the FoxP3+ Tregs expression of is significantly associated with the pathologic T stage (p=0.001), lymph nodes involvement (p=0.025), and the Modified Dukes staging (p=0.032).

Table II: The association of FoxP3+ Treg expression with clinicopathological characteristics among colorectal cancer patients (n=202)

Variables		FoxP3+		X^2 statistic (df)	*p*-value
		Low *n* (%)	High *n* (%)		
Age (years)		100 (49.5)	102 (50.5)	1.97(200)	0.775
Race	Malay	77 (51.0)	74 (49.0)		0.596[b]
	Chinese	18 (42.9)	24 (57.1)		
	Indian	4 (66.7)	2 (33.3)		
	Others	1 (33.3)	2 (66.7)		
Gender	Male	51 (47.2)	57 (52.8)		0.487[a]
	Female	49 (52.1)	45 (47.9)		
Tumour location	Right colon	24 (42.9)	32 (57.1)		0.242[a]
	Left colon	76 (52.1)	70 (47.9)		
Tumour grade	Low-grade	88 (48.6)	93 (51.4)		0.460[a]
	High-grade	12 (57.1)	9 (42.9)		
Histologic subtypes	Well-differentiated adenocarcinoma	7 (58.3)	5 (41.7)		0.847[b]
	Moderately differentiated adenocarcinoma	81 (47.9)	88 (52.1)		
	Poorly differentiated adenocarcinoma	3 (50.0)	3 (50.0)		
	Mucinous adenocarcinoma	8 (57.1)	6 (42.9)		
	Signet ring cell carcinoma	1 (100.0)	0 (0.0)		
Pathologic T stage	pT1	1 (33.3)	2 (66.7)		0.001[b]
	pT2	16 (45.7)	19 (54.3)		
	pT3	44 (40.4)	65 (59.6)		
	pT4	39 (70.9)	16 (29.1)		
Pathologic N stage	pN0	44 (41.9)	61 (58.1)		0.065[a]
	pN1	39 (55.7)	31 (44.3)		
	pN2	17 (63.0)	10 (37.0)		
Nodal metastasis	Negative	44 (41.9)	61 (58.1)		0.025[a]
	Positive	56 (57.7)	41 (42.3)		
Modified Dukes Stage	A	7 (36.8)	12 (63.2)		0.032[a]
	B	41 (45.1)	50 (54.9)		
	C	43 (52.4)	39 (47.6)		
	D	9 (90.0)	1 (10.0)		
Resection margin involvement	No	94 (49.0)	98 (51.0)		0.535[b]
	Yes	6 (60.0)	4 (40.0)		
Diabetes Mellitus	No	43 (45.3)	52 (54.7)		0.941[a]
	Yes	25 (44.6)	31 (55.4)		
Hypertension	No	30 (42.3)	41 (57.7)		0.518[a]
	Yes	38 (47.5)	42 (52.5)		
Smoking	No	51 (47.7)	56 (52.3)		0.311[a]
	Yes	17 (38.6)	27 (61.4)		

[a] Chi-square test for independence, [b] Fisher's exact test

DISCUSSION

In the current study, mean age for CRC patients in these two tertiary hospitals is 63.11 years old similar to study done by Saizul et al (19). The incidence of CRC remarkably increases after the fifth decade of life, generally due to prolonged exposure to carcinogens, and other risk factors such as sedentary lifestyle, unhealthy diet, diabetes mellitus, hypertension, and smoking (20,21). The youngest patient in this study was diagnosed at the age of 19 years old with moderately differentiated adenocarcinoma. Further investigation revealed that this patient has Familial Adenomatous Polyposis (FAP) which is responsible for about one percent of CRC cases in young age group (22). In this disease entity, the major manifestation is the presence of multiple colonic adenomas, which eventually become malignant before the age of 40 (22).

The rate of CRC varies between different ethnic groups. Malaysian National Cancer Registry (2017-2021) reported that the incidence rate of CRC was highest among the Chinese for both sexes, with an age-standardized rate (ASR) of 19.6 per 100000, followed by the Malay (12.2) and the Indian (11.0) (23). However, in this current study, majority of the CRC cases are among the Malay (74.8%), followed by Chinese (20.8%) and Indian (3.0%). This is due to the large proportion of Malay patients involved in the study, which is consistent with the major ethnic group in Kedah and Kelantan. According to the National Department of Statistic, the Malay accounts for 79.8% and 96.0% of the population in Kedah and Kelantan, respectively, followed by Chinese, with 12.4% and 3.1%, Indian, with 6.7% and

0.3%, and others, with 1.1% and 0.6%, respectively (24,25).

Multiple studies have discovered that the high FoxP3+ Tregs expression in CRC is associated with the early stage of the tumour (Stage I and II) (16,18,26–29). This study consistently shows a significant association of FoxP3+ Tregs expression with the tumour stage (pT) (p=0.001). High expression of FoxP3+ Treg is associated with a higher proportion of patients with an early stage, pT1 (66.7%). However, the results might be biased due to the uneven distribution of the number of T1 stage cases. Furthermore, the current data suggested that higher FoxP3+ Treg expression within the tumour stroma is correlated with an early stage of CRC and thus imparts a favourable prognosis. This is supported by various research which showed that high expression of FoxP3+ Tregs infiltration in the CRC associated with better prognosis (17,18,26–28,30–36).

A meta-analysis by Hu (2017) involving 17 studies found that the expression of tumor-infiltrating FoxP3+ Tregs within the tumor stroma is linked to favorable clinical outcomes in CRC patients (18). Moreover, high FoxP3+ Treg infiltration may contribute to a better prognosis in early-stage CRC (17,26). Current evidence suggests that evaluating the expression of FoxP3+ tumor-infiltrating T-cells could improve the prognostic classification of stage II and III CRC (26). FoxP3+ Treg expression levels are inversely correlated with CRC progression. In stage II CRC, higher FoxP3+ Treg expression was observed in patients with favourable outcomes compared to those with lower levels. FoxP3+ Tregs were also associated with reduced rates of disease recurrence and improved long-term survival (26). This indicates that FoxP3+ Treg cells expression level in the primary tumour of CRC may serve as a crucial predictor for identifying stage II tumors that would benefit from adjuvant chemotherapy, as opposed to those that would not. Such predictive insights could lead to the development of personalized treatment regimens tailored to each patient in the future (26). However, this study also demonstrated reduced expression of FoxP3+ Tregs in advanced stage pT4 which similar pattern was found in a previous study by Loddenkemper et al (37). The study showed Treg cell levels were higher in stages II and III but not in stage IV (37). This may be attributed to the dynamic process of Treg infiltration in which Treg levels increase at the primary tumour site during early stages of CRC and decreases in more advanced stages of disease. Additionally, it suggests that each type of malignancy may have unique features concerning the impact of Treg infiltration on survival (37). This concept could also help to explain the findings of this current study.

Furthermore, the expression of FoxP3+ Tregs in CRC was found to correlated with the Modified Dukes Stage (p=0.032). A higher proportion of Modified Dukes stage A (63.2%) was observed in the group with high FoxP3+ Tregs expression compared to 36.8 % in the group with low FoxP3+ Tregs expression. These findings suggest that high expression of FoxP3+ Treg is associated with earlier Dukes stage. In Similar to the study by Liu (2014), the expression of FoxP3+ Treg was inversely correlated with the Dukes staging (p<0.05) (7). Our results reinforce the idea that immune cell infiltration could serve as novel independent prognostic factor in colorectal cancer. Interestingly, this new system may even surpass the traditional Dukes staging system in prognostic value (38).

Involvement of the lymph node is one of the crucial prognostic indicators in CRC, and cases with cancer cells infiltrated into the lymph node had a considerably poorer survival outcome (39,40). In other studies, high expression of FoxP3+ Treg infiltration in CRC showed a significant correlation with negative nodal metastasis (29,40). This is in agreement with our study, which showed the expression of FoxP3+ Tregs in CRC is associated with negative nodal metastasis (p=0.025). A higher proportion of negative nodal metastasis (58.1%) is seen within the high expression of the FoxP3+ Treg group. In contrast, a higher proportion of positive nodal metastasis (57.7%) is seen within the low expression of FoxP3+ Treg.

High tumour grade is generally considered a poor prognostic indicator for CRC (4). A meta-analysis by Xu (2017) found that high level of tumour infiltrating FoxP3+ Tregs were significantly associated with well or moderately differentiated CRC (OR=0.77, 95% CI=0.61, 0.98, p=0.032) (35). However, our study did not show any association between FoxP3+ Tregs expression in CRC and tumour grade or histologic subtypes (p=0.460, p=0.847). This was likely attributed by the large proportion of low-grade tumours (89.6%) in our study sample. Nonetheless, our findings are consistent with those of Hanke (2015), who also observed no correlation between FoxP3+ Treg expression and tumour grade in CRC (17).

Numerous microorganisms have been identified in the human colon, and many bacterial species have been reported to be enriched in CRC samples. Various colonic pathogenic bacteria are known to trigger inflammatory responses. Compared to other types of cancer with a sterile environment, CRC provides a unique septic environment conducive to the growth of many bacterial species (15). A previous study proposed that a T-helper cell, Th17-mediated inflammatory response in colonic mucosa, triggered by pathogens, could have a protumorigenic effect, and potentially accelerate the progression of CRC (14). However, this activity is believed to be inhibited by T Reg cells which

help to prevent tumour progression which consistent with our study's findings. Nonetheless, further studies are required to assess and validate these findings.

Some limitations had been addressed in the current study. Some of the clinical information was not available due to several factors, such as a long period of inactivation of the cases or defaulted follow-up cases. A few of the available tissue blocks were exhausted and not feasible for IHC staining.

This study was only a dual-centre retrospective study with univariate analysis. Therefore, a prospective study with a larger sample size involving a multicentre survey as well as correlation with patient outcome is warranted to validate the findings of the expression of FoxP3+ Tregs within CRC. Thus, the authors hope to expand the study by collaborating with other centres and proceeding with the multivariate regression and survival analysis of the outcome. In addition, molecular and genetic testing of FoxP3 expression might also help establish a deep understanding of the role of FoxP3 expression in CRC.

CONCLUSION

This study found significant inverse associations between the expression of FoxP3+ Tregs in T lymphocytes within the peritumoral and intratumoral microenvironments of CRC with tumour stage, lymph node involvement, and Modified Dukes Staging. These findings suggest that FoxP3+ Tregs could serve as a reliable immunomarker for classifying early-stage CRC and predicting its prognosis. However, due to sample limitation, the study needs to be confirmed by covering broad range of sample that include equal distribution of stage.

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