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ORIGINAL ARTICLE

Loss of Interleukin-17RA Expression is Associated with Tumour Progression in Colorectal Carcinoma

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Abstract

Interleukin-17 (IL-17) is a pro-inflammatory cytokine found in various cancers. Current evidence indicates that IL-17 plays a vital role in tumour initiation and progression in colorectal carcinoma (CRC) via binding with its receptor, IL-17RA. However, the association between clinicopathological features and presence of IL-17 and IL-17RA protein in primary CRC tissues remains unclear. This study also investigates the difference between the presence of IL-17 and IL-17RA in the paired tumour tissues versus adjacent normal tissues. The presence of IL-17RA and IL-17 protein in primary CRC tissues was determined by immunohistochemistry. Associations between clinicopathological features and IL-17RA and IL-17 immunoreactivity, were analyzed by $\chi 2$ tests. We found that both IL-17RA ($p = 0.001$) and IL-17 ($p = 0.025$) in tumour cells of primary CRC tissues was significantly lower as compared to adjacent normal tissue. Positive immunoreactivity for IL-17RA and IL-17 were detected in 51.0% and 16.8% of tumour tissues, respectively. Furthermore, negative immunoreactivity of IL-17R was significantly associated with advanced stage according to TNM classifier ($p = 0.027$), high grade of tumour ($p = 0.019$), increased depth of tumour invasion ($p = 0.023$) and vascular invasion ($p = 0.039$). Positive IL-17 immunoreactivity was associated with advanced stage ($p = 0.008$) and lymph node metastasis ($p = 0.008$). Thus, this study suggests that the loss of IL-17RA expression occurs as tumour progresses and this may predict the aggressiveness of tumour whilst expression of IL-17 promotes tumour progression and lymph node metastasis. Thus, loss of IL-17RA could be a useful prognostic biomarker for tumour progression in CRC patients.

Keywords IL-17 · IL-17RA · Colorectal carcinoma · Prognostic biomarker · Tumour progression

Introduction

Colorectal carcinoma (CRC) is the third most common human cancer [1] with approximately one million new cases and more than 500,000 deaths every year [2]. Some patients respond well with conventional therapies while others do not respond [3]. Although CRC detection is possible through screening strategies with the advances in technology, CRC development and mortality persist. Roughly, 50% of colorectal carcinoma patients develop liver metastasis during their lifetime and rarely survive longer than three years despite the current improvement of therapies [4]. A better understanding on the pathogenesis of the disease would greatly enhance development of effective therapies for CRC.

Interleukin-17 (IL-17) is a pro-inflammatory cytokine and mediates its function via its receptor, IL-17R, to promote the generation of pro-inflammatory cytokines and chemokines [5] which recruit monocytes and neutrophils into the inflammatory site [6, 7]. Albeit IL-17 is the hallmark of T helper 17 cells (Th17 cells), innate immune cells including γδ T cells, invariant natural killer T cells, neutrophils, macrophages, and mast cells also serve as sources of IL-17 [6]. In CRC, infiltrating IL-

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17^+ cells facilitate angiogenesis via stimulating vascular endothelial growth factor (VEGF) production from tumour cells [8].

IL-17 links innate and adaptive immunity [9] by participating in both innate and adaptive immunity during the physiological or pathological processes in autoimmunity, allergy, host defence and cancer [10, 11]. The presence of IL-17 has been found in various cancers including breast cancer, lung cancer, prostate cancer, glioma, skin cancer, hepatocarcinoma and sarcoma [12]. Elevated IL-17 was reported in glioma [13], lung cancer [14], colon cancer [15] and prostate cancer [16]. Pan *et al*. reported that IL-17 is associated with enhanced angiogenesis and poorer survival in lung cancer [14] while Cui and colleagues reported that IL-17 level correlated with the severity of dysplasia in colon cancer [15].

IL-17 displays either pro-tumour or anti-tumour role in various cancers [17]. Cumulative evidence points to IL-17 being involved in tumor initiation and progression in cancer through suppression of anti-tumour immunity and promoting tumour growth via AKT- dependent IL-6/JAK2/STAT3 [7]. Recent studies have shown that IL-17 plays a critical role in modulating angiogenesis and production of a variety of pro-angiogenic factors in cancer [18]. In CRC, IL-17 induces secretion of angiogenic molecules including VEGF, matrix metalloproteinase-9 (MMP-9) which promote tumour proliferation and metastasis [19, 20]. In addition, IL-17 promotes tumour initiation and progression through activation of STAT3 pathway and ERK1/2 pathway. Another mechanism of tumorigenesis facilitated by IL-17 is through suppression of anti-tumour immunity such as recruiting myeloid-derived suppressor cells (MDSCs) and regulatory T (Treg) cells but inhibiting $CD8^+$ T cells infiltration [7, 20]. However, IL-17 can play a role in anti-tumour immunity by recruiting the infiltration of IFN-γ^+ effector T-cells, NK cells [21] and, cytotoxic $CD8^+$ T cells into the tumour nests [22].

IL-17RA (130 kDa) is a type I transmembrane protein that is widely expressed in various tissue such as brain, lung and spleen [23] as well as myeloid cells and fibroblasts [24]. Ligation of IL-17RA by IL-17 activates the phosphorylation of Mitogen Activated Protein Kinases (MAPK) and Nuclear Factor-κB (NF-κB) pathway to induce pro-inflammatory cytokines in tissue homeostasis [25] and tumour cell proliferation, tumour growth and progression in cancer [12]. Various tumour tissues including lung, osteosarcoma, prostate [26] and stomach cancer showed high expression level of IL-17RA [23]. However, to the best of our knowledge, there have been no previous studies evaluating whether the presence of both IL-17RA and IL-17 in primary CRC tissues is associated with disease progression and other clinicopathological factors.

This study examines the presence of IL-17 and IL-17RA in tumour and adjacent normal tissues of primary colorectal carcinoma by immunohistochemistry and their relationship with clinicopathological features. This will provide information on the role of IL-17 and IL-17RA in CRC pathogenesis and their possibility as potential prognostic biomarkers for tumour progression in CRC patients.

Materials and Methods

Patients and Specimens

A total of 147 formalin fixed paraffin embedded (FFPE) primary colorectal carcinoma tissues between 2006 and 2015 were obtained from Hospital Kuala Lumpur. Of these tissue blocks, only 113 contained adjacent normal tissue. Adjacent normal tissues in this study are the apparently non-cancerous epithelium. The total number of stage I, II, III and IV cases were 22, 45, 77 and 3, respectively. The clinical stage of the tumour was determined according to the TNM (for tumours/nodes/metastasis) system from the American Joint Committee on Cancer (AJCC). This study was approved by the Ethics and Research Committee of the Malaysian Ministry of Health and registered with National Medical Research Register (NMRR-12-435-11565). Clinicopathological data of these patients were reported by qualified histopathologists and retrieved from the electronic medical records of Hospital Kuala Lumpur.

Immunohistochemistry

FFPE tissue sections of 4 μm thickness were dewaxed with xylene and rehydrated with decreased concentrations of ethanol. Heat-induced epitope retrieval (HIER) step was performed for 20 min using a microwave in boiled Tris EDTA buffer (1 mM pH 9) for IL-17RA or boiled sodium citrate buffer (1 mM pH 7.6) for IL-17. The samples were then left to cool to room temperature. Next, endogenous peroxidase activity were blocked using 3% H_2O_2 and then 3% of bovine serum albumin (BSA) containing 0.1% of sodium azide for 10 min and 1 h, respectively, followed by incubation with IL-17RA antibody (1:200 dilution) for 1 h and IL-17 antibody (1:50 dilution) for 2 h. Anti-IL-17R (sc-30175) and anti-IL-17 (sc-7927) polyclonal antibody were purchased from Santa Cruz Biotechnology, Inc. The peroxidase-labeled polymer detection system was used as according to the manufacturer's instructions. Negative control sections which are those without incubation with primary antibodies were processed in parallel. Positive immunoreactivity was visualized as yellow-brown colour by using 3'-diaminobenzidine (DAB) chromogen solution in the final step and the cell nuclei were counterstained with hematoxylin. Evaluation of immunoreactivity for IL-17 [27] and IL-17RA [18] was performed as described previously.

Evaluation of IHC Staining Results with IL-17 and IL-17RA

IL-17 and IL-17RA positivity is defined as yellow-brown colour mainly distributed in the tumour cells and adjacent normal tissue cells. A semi-quantitative evaluation of positive immunostaining for IL-17 and IL-17RA was performed by scoring 10 fields with the Olympus BX51 upright microscope under 100x magnification and scores were based on two variable factors: the abundance of the positively stained cells and the intensity of the staining. The abundance of stained cells was graded from 0 to 4 ($0 \leq 5\%$; 1 = 6%-25%; 2 = 26%-50%; 3 = 51%-75%; 4 = 76%-100%) and 0–4 (0 = 0%; 1 = < 25%; 2 = 26%-50%; and $3 \geq 50\%$) for IL-17 and IL-17RA, respectively. The intensity of stained cells was graded from 0 to 3 (0 = absent; 1 = weak; 2 = moderate; 3 = strong) for both IL-17 and IL-17RA. The final score for IL-17 was obtained by multiplying the score of the two variables and the score varied from 0 to 12. A score greater than 8 was categorized as positive whereas a score of 8 or less as negative [27]. In contrast, the score for IL-17RA was obtained by addition of the score of the two variables and thus, the total immunostaining score ranged from 0 to 6. A score greater than 2 was categorized as positive whereas a score of 2 or less as negative immunoreactivity [18].

Statistical Analysis

Statistical analysis was performed using the SPSS statistical software package (version 20.0; SPSS, Inc. Chicago, Illinois, USA). The significant difference between expression of tumour tissues and adjacent normal tissue of primary colorectal carcinoma were determined by the Wilcoxon signed-rank test which is a non-parametric test. The positive rate of the IL-17 and IL-17RA in tumour and adjacent normal tissue of primary colorectal carcinoma were analyzed by χ^2 tests. The correlation between expression of IL-17 or IL-17RA and clinicopathological parameters were also analyzed by χ^2 tests. Differences were considered significant at p-values less than 0.05.

Results

Expression of IL-17RA and IL-17 in Primary Colorectal Carcinoma

In this study, immunohistochemical staining was used to evaluate the expression of IL-17RA and IL-17 in 147 and 143 primary tumour tissues of colorectal carcinoma, respectively. For IL-17RA immunostaining, only 113 out of 147 tumour tissues contained adjacent normal tissue. For IL-17, data for immunostaining was only available for 143 tumour tissues and 105 adjacent normal tissues due to poor staining in 4 of the tumour tissues and 8 of the normal adjacent tissue. To compare the expression of IL-17RA and IL-17 in primary tumour tissue and adjacent normal tissue in primary human CRC, the final scores of IL-17 and IL-17RA expression, which were obtained from both the percentage of positive cells and staining intensity in paired tumor and adjacent normal tissues were used to perform the Wilcoxon signed-rank test. Data from this study showed that both IL-17 ($p = 0.025$) and IL-17RA ($p = 0.001$) expression in the primary tumour tissue was significantly lower compared to adjacent normal tissue (Table 1). Both the expression of IL-17RA (Fig. 1) and IL-17 (Fig. 2) were observed in the tumour cells. No staining was observed in negative controls. χ^2test showed that the positive expression rate of IL-17 ($p = 0.40$) and IL-17RA ($p = 0.30$) in primary tumour tissue was significantly lower than adjacent normal tissue (Table 2).

Downregulation of IL-17RA Expression is Associated with Progression of Tumour in Colorectal Carcinoma Patients

The χ^2 test was used in this study to evaluate the association between clinicopathological features of CRC and IL-17RA or IL-17 expression. The negative expression of IL-17RA was significantly associated with advanced stage ($p = 0.027$), high grade of tumour ($p = 0.019$), increased depth of tumour invasion ($p = 0.023$) and vascular invasion ($p = 0.018$) (Table 3). The positive expression of IL-17 was significantly associated with clinicopathological features of CRC with advanced stage ($p = 0.008$) and lymph nodes metastasis ($p = 0.008$) (Table 4).

Discussion

In the present study, we found that the immunoreactivity of IL-17RA was lower in CRC tissues compared to adjacent normal tissue. Furthermore, we observed the loss of IL-17RA protein in CRC is associated with several clinicopathological features, such as stage and grade of tumour, depth of tumour invasion and vascular invasion, indicating the clinical significance of IL-17RA in CRC. Thus, negative expression of IL-17RA is associated with advanced stage according to TNM classifier, poorly differentiated tumour, and increased depth of tumour invasion.

Interestingly, our findings with IL-17RA in CRC are not in accordance with most other cancers including, gastric cancer, non-small cell lung cancer (NSCLC) and osteosarcoma [23, 28, 29] where IL-17RA was overexpressed in these cancer. Overexpression of IL-17RA was found to mediate tumour invasion via p38 MAPK signaling pathway *in vitro* in NSCLC [23]. In gastric cancer, high expression of IL-17RA

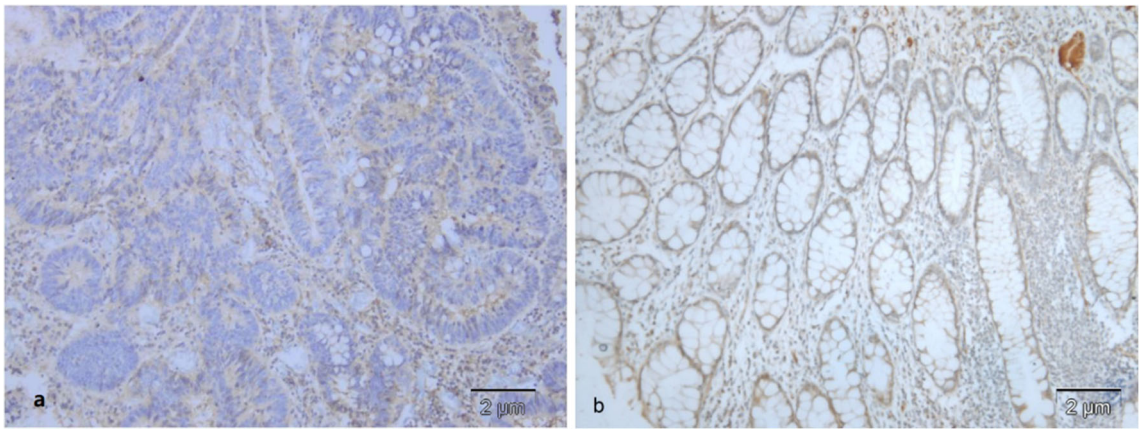

Fig. 1 Representative immunohistochemical staining of the IL-17RA protein in colorectal carcinoma. (a) Tumour tissue. (b) Adjacent normal tissue. (100x magnification)

associated with increased depth of tumour invasion as well as lymph nodes and distant metastasis [28]. Furthermore, high expression of IL-17RA is associated with poor overall survivor in both NSCLC and gastric cancer.

However, a cross cancer analysis by Yan *et al*. [30] revealed that IL-17RA deletion is a common event in CRC patients and is associated with deletion of A20, a negative regulator of NF-κB, WNT and JNK-c-Jun signaling pathways. Various genes associated with tumour proliferation, invasion and metastasis including VEGFA were significantly upregulated in IL-17RA deleted tumours. Furthermore, the expression of IL-17RA in immunohistochemistry staining decreases with advanced stage [30]. Thus, our finding is consistent with the report by Yan *et al*. [30].

In addition, this study demonstrated that negative expression of IL-17RA in tumour tissue of colorectal carcinoma was associated with vascular invasion. A previous study by Fujii *et al*. demonstrated that vascular invasion is a strong indicator for aggressiveness of CRC [31]. Thus, this indicates that down-regulation of expression of IL-17RA in CRC probably aggravates tumour progression and aberration of IL-17 signaling pathway. Furthermore, it was reported that loss of IL-17RA expression triggers aberrant cellular responses and intracellular signaling pathways which consequently, promotes tumour progression and thus aggravates the disease [30]. Therefore, loss of expression IL-17RA in CRC has an important impact on the pathogenesis of CRC and reflects tumour progression in CRC.

Previously, Xie *et al*. reported that marked elevated expression IL-17RA was found in CRC tissue as compared to normal colon tissue [32] while our finding showed otherwise. The discrepancy between our study and others was because the presence of IL-17RA in tumour cells in our study was detected by using immunohistochemistry method while the previous study [32] detected total IL-17RA expression in CRC tissue using Western Blotting which would likely include stromal components. In conclusion, our studies and that by Yan *et al*. 2019 suggest that the role of IL-17RA in CRC appears to be different from other cancers such

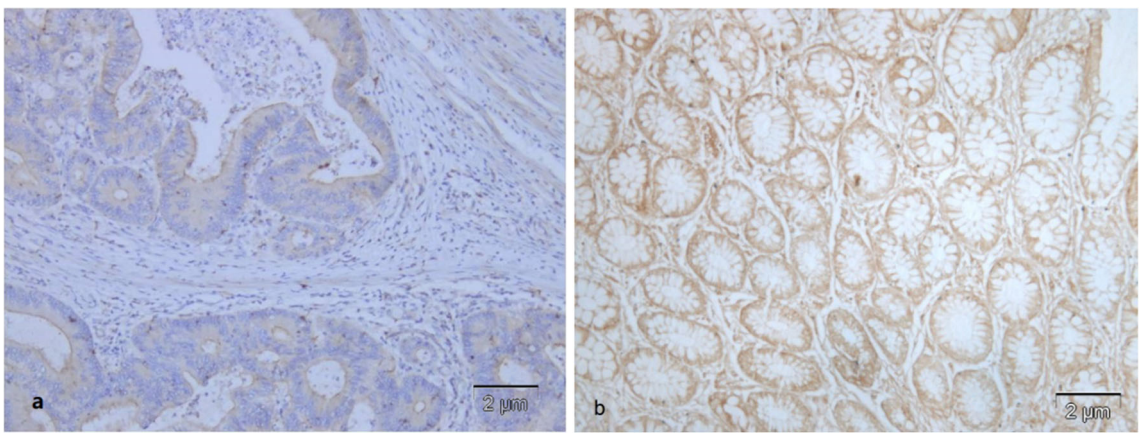

Fig. 2 Representative immunohistochemical staining of the IL-17 protein in colorectal carcinoma. (a) Tumour tissue. (b) Adjacent normal tissue. (100x magnification)

Table 1 Comparison of expression of IL-17RA and IL-17 in primary tumour tissue versus adjacent normal tissue in primary human colorectal carcinoma

	N	Tumour Tissue Mean± SD	Adjacent Normal Tissue Mean± SD	P value
IL-17RA	113	1.76±1.41	2.31±1.53	**0.001***
IL-17	113	7.60±1.62	8.16±2.04	**0.025***

Abbreviations: SD, standard deviation

* *p*–values were estimated by Wilcoxon signed–rank test

* Statistically significant, $p<0.05$

as gastric cancer, non-small cell lung cancer (NSCLC) and osteosarcoma [23, 28, 29]. Therefore, considerable caution is needed in designing potential therapy targeting IL-17RA in various cancers.

Expression of IL-17 is elevated in various cancer including breast, hepatocellular carcinoma, esophageal cancer, ovarian cancer and cervical cancer [33]. In CRC, IL-17 is highly associated with tumour aggressiveness and progression. Elevated expression of IL-17 was reported significantly increased in CRC tumour tissue compared with non-tumour tissue [8] and adenoma tissue [15, 32]. In these studies, IL-17 in CRC was mainly expressed by $CD4^+$ cells [8, 15] and macrophages [8]. Furthermore, IL-17 was reported to facilitate metastasis in CRC via enhanced circulating tumour cells motility [34]. In addition, IL-17 expression correlated with angiogenesis in CRC via cancer cell-mediated VEGF production [8]. On the other hand, Lin and colleagues found that positive IL-17 expression was associated with early stage of CRC and well-differentiated tumour tissue. Their immunohistochemistry staining finding includes tumour cell, stromal cells and epithelial cells [35].

In contrast, our study explores the association between clinicopathological features and tumour cells and found that positive IL-17 expression is associated with advanced stage of CRC. Our study reveals that IL-17 is positively associated with lymph nodes metastasis. This finding suggested that the autocrine activity of IL-17 produced by tumour cells may enhance the production of MMP-9 to facilitate tumour metastasis [34, 36]. This implies IL-17 expressed by tumour cells contributes to tumour progression. The difference between our results and those in other studies is likely due to the type of cells that were examined. The expression of IL-17 in tumour cells was our focus while other studies examined the IL-17 produced by immune cells and total IL-17 production by tumour tissue including the stroma.

Although IL-17 expression was associated with advanced stage, IL-17 was under-expressed in tumour cells as compared to the adjacent normal tissues. This observation is in contrast to the typical prediction pattern of IL-17 expression. This could be attributed to chronic inflammation and cancer immunoediting in tumorigenesis of CRC. During chronic inflammation, intestinal epithelial cells increase IL-17 expression to recruit immune cells as a host defense mechanism inducing DNA modification and subsequent development of tumour cells [37]. Subsequently, cancer immunoediting takes place. The cancer Immunoediting hypothesis states that the three components, elimination, equilibrium, and escape, control growth of the tumour and shape tumour immunogenicity. In the elimination phase, host immunity detects and destroys abnormal expressing IL-17-tumour cells. However, rare tumour cells survive and escape from this phase and proceed to equilibrium and later in the escape phase, there is an increase in tumour cells and expression of IL-17 also increases. Therefore, as chronic inflammation precedes tumourigenesis and later immunoediting takes place during tumourigenesis, a decreased-then-increased pattern of IL-17 expression occurs as seen in this study [38]. Taken together, these results suggest that IL-17 expression in CRC by tumour and non-tumour cells may have distinctive role in contributing to tumour progression.

The observation that expression of IL-17RA is lost but expression IL-17 increases in CRC tumour cells as the tumour progresses is interesting. It is likely that even though loss of IL-17RA occurs in the tumour cells, thus inhibiting the direct stimulation of release of a variety of proangiogenic factors by IL-17, tumour-secreted-IL-17

Table 2 Expression of IL-17RA and IL-17 in Tumour Tissue and Adjacent Normal Tissues in primary human CRC

Clinicopathological Features	IL-17RA			IL-17		
	Positive (%)	Negative (%)	*p*-value	Positive (%)	Negative (%)	*p*-value
Tumour Tissue	75(51.0)	72(49.0)	**0.030***	24(16.8)	119(83.2)	**0.040***
Adjacent Normal Tissue	78(69.0)	35(31.0)		29(27.6)	76(72.4)	

* *p*–values were estimated by $\chi 2$ test

Statistically significant, $p<0.05$

Table 3 Correlation between IL-17RA in colorectal carcinoma tissue and clinicopathological features

Clinicopathological Features	n[a]	n[b]	Negative	Positive	*p*-value
Age at Diagnosis	144	3			0.594
< 60			22(46.8)	25(53.2)	
≥ 60			50(51.5)	47(48.5)	
TNM staging	147	0			**0.027***
Stage 1			6(27.3)	16(72.7)	
Stage 2- Stage 4			66(52.8)	59(47.2)	
Grade	147	0			**0.019***
Well			2(20.0)	8(80.0)	
Moderate			67(50.0)	67(50.0)	
Poor			3(80.0)	0(0)	
Tumour Invasion	145	2			**0.023***
T.0			6(27.3)	16(72.7)	
T > 2			66(53.7)	57(46.3)	
Lymph Nodes Metastasis	144	3			0.065
N0			28(41.2)	40(58.8)	
N ≥ 01			43(56.6)	33(43.4)	
Distant Metastasis	142	5			0.556
M0			70(50.4)	69(49.6)	
M1			1(33.3)	2(66.7)	
Vascular Invasion	115	32			**0.018***
No			28(40.6)	41(59.4)	
Yes			29(63.0)	17(37.0)	
ILI	94	53			0.141
No			7(63.6)	4(36.4)	
Mild			27(44.3)	34(55.7)	
Moderate			0(0)	3(100.0)	
Marked			10(52.6)	9(47.4)	
PLA	96	51			0.231
No			11(55.0)	9(45.0)	
Mild			27(42.2)	37(57.8)	
Marked			8(66.7)	4(33.3)	
Site of tumour	143	4			0.067
Right			34(58.6)	24(41.4)	
Left			35(41.7)	49(58.3)	
Mix			1(100.0)	0(0)	

Abbreviation: ILI, Intratumoral Lymphocytic Infiltration; PLA, Peritumoral Lymphocytes Aggregates;

[a] Number of cases with the relevant clinicopathological data. Percentages are calculated per cases with available data

[b] Number of missing cases as information was not available

Table 4 Correlation between IL-17 in colorectal carcinoma tissue and clinicopathological features

Clinicopathological Features	n[a]	n[b]	Negative	Positive	*p*-value
Age at Diagnosis	140	3			0.303
< 60			40(88.9)	5(11.1)	
≥ 60			78(82.1)	17(17.9)	
TNM staging	143	0			**0.008***
Stage 1- Stage 2			60(92.3)	5(7.7)	
Stage 3- Stage 4			59(75.6)	19(24.4)	
Grade	143	0			0.648
Well			8(88.9)	1(11.1)	
Moderate			108(82.4)	23(17.6)	
Poor			3(100.0)	0(0)	
Tumour Invasion	141	2			0.281
T ≤ 2			20(90.9)	2(9.1)	
T > 2			97(81.5)	22(18.5)	
Lymph Nodes Metastasis	140	3			**0.008***
N0			61(92.4)	5(7.6)	
N ≥ 01			56(75.7)	18(24.3)	
Distant Metastasis	138	5			0.446
M0			113(83.7)	22(16.3)	
M1			3(100.0)	0(0)	
Vascular Invasion	113	30			0.548
No			56(82.4)	12(17.6)	
Yes			35(77.8)	10(22.2)	
ILI	91	52			0.141
No			10(90.9)	1(9.1)	
Mild			46(78.0)	13(22.0)	
Moderate			2(100.0)	0(0.0)	
Marked			14(73.7)	5(26.3)	
PLA	93	50			0.101
No			18(90.0)	2(10.0)	
Mild			47(77.0)	14(23.0)	
Marked			9(75.0)	3(25.0)	
Site of tumour	139	4			0.870
Right			46(83.6)	9(16.4)	
Left			68(81.9)	15(18.1)	
Mix			1(100.0)	0(0)	

Abbreviation: ILI, Intratumoral Lymphocytic Infiltration; PLA, Peritumoral Lymphocytes Aggregates;

[a] Number of cases with the relevant clinicopathological data. Percentages are calculated per cases with available data

[b] Number of missing cases as clinicopathological data was not available

* Statistically significant, $p < 0.05$.Statistical analyses by χ^2 test

* For 1 L-17, data for immunostaining was only available for 143 tumour tissues and 105 adjacent normal tissues due to poor staining in 4 of the tumour tissues and 8 of the normal adjacent tissue

* Statistically significant, $p < 0.05$.Statistical analyses by $\chi 2$ test

*For 1 L-17RA immunostaining, only 113 out of 147 tumour tissues contained adjacent normal tissue

indirectly promote metastasis and angiogenesis in tumour via inducing stromal cells including fibroblasts [17] or recruit myeloid-derived suppressive cells (MDSCs) [19] and macrophages [25, 39] to produce a wide range of molecules including VEGF and MMP-9.

Conclusions

In conclusion, the present study demonstrates that loss of IL-17RA expression in CRC is associated with advanced stage, high grade of tumour, increased depth of tumour invasion and vascular invasion. This differs from previous studies in gastric and non-small cell lung cancer. Our results suggest that the loss of IL-17RA expression occurs as tumour progresses and may predict the aggressiveness of the tumour. Therefore, loss of IL-17RA expression may potentially serve as a prognostic biomarker for tumour progression in CRC patients. On the other hand, the presence of IL-17 in tumour cells is associated with lymph node metastasis. This finding is consistent with previous reports on the ability of IL-17 to induce secretion of angiogenic molecules including VEGF, MMP-9 which promote tumour proliferation and metastasis [19, 20]. It is likely that IL-17 secreted by tumour cells affects cells in the tumour microenvironment including stromal cells to promote tumour proliferation and metastasis.

Compliance with Ethical Standards

Conflict of Interest These authors disclose no conflicts of interests.

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