

Colorectal Cancer Progression Is Associated with Accumulation of Th17 Lymphocytes in Tumor Tissues and Increased Serum Levels of Interleukin-6

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Colorectal cancer is a significant worldwide health problem, and an altered immunoresponse plays an important role in colorectal tumorigenesis and cancer progression. T helper 17 (Th17) lymphocytes (a subgroup of CD4⁺ T cells), together with the related cytokines, such as interleukin (IL)-6 and IL-17A, participate in cancer-related immunity. In this study, we measured the percentage of Th17 cells in peripheral blood mononuclear cells (PBMCs) and the levels of IL-17A and IL-6 in serum samples or tumor tissues, prepared from 40 colorectal cancer patients (a median age of 75 years), 32 age-matched healthy controls (a median age of 74 years), and 30 young healthy controls (a median age of 26 years). The percentage of Th17 cells in PBMCs and the serum IL-6 levels were higher in cancer patients than those in elderly controls, and they were associated with tumor progression. Moreover, the percentage of Th17 cells and the serum IL-6 levels were higher in elderly healthy controls than those in young healthy controls. In contrast, serum IL-17A levels were similar in the cancer patients and the elderly controls, although its serum levels were higher than those in young controls. Importantly, Th17 cells were accumulated in the intratumor and peritumor regions. In conclusion, the percentage of Th17 cells and the serum IL-6 level are significantly increased with aging, and their higher levels are correlated with colorectal cancer progression. The percentage of Th17 cells in PBMCs and the serum IL-6 level are potential biomarkers for predicting disease progression.

Keywords: aging; colorectal cancer; interleukin-17; interleukin-6; T helper 17 cells

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Introduction

Inflammation is a part of the complex biological response of the host tissues to harmful stimuli, such as pathogens, damaged cells, and irritants, and is also closely associated with tumorigenesis because many human cancers arise from sites of infection, chronic irritation, and inflammation (Coussens and Werb 2002). Inflammation creates a microenvironment around a tumor lesion to facilitate proliferation, survival, migration, and metastasis of tumor cells (Coussens and Werb 2002), whereas antitumor immunity also occurs with the tumor-related inflammatory response (Monteleone et al. 2012). For example, patients with inflammatory bowel disease are at increased risk of developing colorectal cancer (Terzić et al. 2010), while cancer occurs most often in elderly individuals, including colorectal cancer patients (Jemal et al. 2011). The term “inflamm-aging”, defined by chronic low-grade upregulated serum levels of inflammatory cytokines, acute-phase

proteins, and coagulation factors, has been recently proposed (Cevenini et al. 2013). Immunosenescence-induced altered immunosurveillance against cancer may also contribute to tumorigenesis and cancer progression (Pawelec et al. 2010). However, inflammation and immunity are involved in different B- and T-lymphocytes and macrophages. Thus, studying these cells in cancer patients may sort out their role in tumor immunity.

T helper (Th) 17 cells, a fourth arm of effector CD4⁺ T cells in addition to Th1, Th2, and T regulatory cells, are responsible for immunopathological responses based on their pattern of cytokine secretion, transcription factor expression, and other functions (Annunziato et al. 2013). However, previous studies also have shown that Th17 cells may attack cancer cells, although this is debatable (Muranski et al. 2008; Wu et al. 2009; Martin-Orozco et al. 2009). Moreover, human Th17 cells produce interleukin (IL)-17, which has been reported to be abnormally elevated in several types of infectious diseases and autoimmune dis-

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eases (Tesmer et al. 2008; Miossec et al. 2009). IL-17 is a member of the cytokine family and is released by various cell types, including Th17 cells, monocyte-macrophages, and neutrophils. IL-17A is a member of the IL-17 family of proteins and is the main factor of Th17 cells. IL-17A levels are increased in many types of cancer, including lung cancer (Chen et al. 2010), hepatocellular carcinoma (Zhang et al. 2009), and colorectal cancer (Liu et al. 2011). Normally, Th17 cells are CD4-positive and IL-17A-positive. In addition, IL-6 is a multifunctional cytokine, which is mainly secreted by lymphocytes and monocyte-macrophages and plays an essential role in autoimmunity, chronic inflammation, and inflammation-associated cancer (Neurath and Finotto 2011). As an indispensable regulatory signaling molecule, IL-6 mediates Th17 immunity (Miossec et al. 2009). Emerging lines of evidence have demonstrated that the serum IL-6 level is a reliable marker for functional disability and a predictor of disability and mortality among older individuals (De Martinis et al. 2005). Nevertheless, the roles of Th17 cells and IL-17A and IL-6 proteins in colorectal cancer remain to be clarified; thus, in the present study, we measured the percentage of Th17 cells in peripheral blood mononuclear cells (PBMCs) and their accumulation in cancer tissues as well as the serum IL-6 level from colorectal cancer patients, age-matched elderly healthy controls, and young healthy controls. In addition, we determined the alteration of these indices in young and elderly healthy subjects. The association among Th17 cells, IL-6, and IL-17A may provide valuable insights for aging and colorectal tumorigenesis.

Materials and Methods

Study populations

In this study, we first recruited 40 elderly colorectal cancer patients (24 males and 16 females with a median age of 75 years old, ranging between 65 and 93 years old) from The First Affiliated Hospital of Dalian Medical University, Dalian, China, between March 2012 and May 2013. The patients were histologically diagnosed using biopsies or surgical tissue specimens. Of these 40 patients, 12 were classified as early stage (Stage I/II) and 28 were at the advanced stage (Stage III/IV) according to the American Joint Committee on Cancer (AJCC) tumor, lymph node, and metastasis staging system (Stephen 2010). None of these patients received radiotherapy, chemotherapy, or immunotherapy or had a history of cancer. Both tumor and nontumor tissues (more than 5 cm away from the tumor lesion) were collected postoperatively.

In addition, the study recruited 32 age-matched healthy subjects (17 males and 15 females with a median age of 74 years old, ranging between 60 and 90 years), and 30 young healthy subjects (13 males and 17 females with a median age of 26 years old, ranging between 24 and 34 years old) from the same hospital during the same time period. These individuals all received a routine physical examination in our hospital. Blood samples were obtained from all subjects. This study was approved by the Ethics Committee of The First Affiliated Hospital of Dalian Medical University, and informed consent was provided by the recruited patients.

Isolation and culture of peripheral blood mononuclear cells (PBMCs)

Peripheral blood was collected from all studied subjects using sodium heparin as an anticoagulant. PBMCs were separated in Ficoll (Tianjin Haoyang Biology Manufacture Co., Ltd., Tianjin, China) by gradient centrifugation at 2,000 rpm for 20 min, which provided 95% surviving cells. The cell density was then adjusted to 2×10^6 cells/mL, and the cells were subsequently cultured in the presence of phorbolmyristate acetate (12.5 μ g/mL), ionomycin (0.25 mg/mL), monensin (0.35 mg/mL), and brefeldin A (0.75 mg/mL); all reagents were from MultiSciences Biotech Co., Ltd., Hangzhou, China. Cell cultures were maintained in a 5% CO₂ incubator at 37°C for 4 h and then subjected to different assays.

Flow cytometric analysis

The expression of cell surface markers was detected using flow cytometry. Briefly, PBMCs were fixed, permeabilized using FIX and PERM Reagent (MultiSciences Biotech Co., Ltd.), and then incubated with FITC-conjugated anti-human CD3, APC-conjugated anti-human CD8, or PE-conjugated anti-human IL-17A antibody (all from eBioscience, USA) at 4°C for 30 min. An isotype control was also prepared and used. Because the phorbolmyristate acetate could induce CD4 endocytosis during PBMC isolation, CD4-positive T cells were analyzed using a gate to exclude CD3-positive and CD8-negative cells instead of simply using the CD4 antibody results. Th17 cells, defined as CD3-positive, IL-17A-positive and CD8-negative cells, were analyzed using a gate to exclude IL-17A-positive cells.

Enzyme-linked immunosorbent assay (ELISA)

The serum was collected from peripheral blood via centrifugation. Serum IL-17A and IL-6 levels were analyzed using an ELISA kit (MultiSciences Biotech Co., Ltd.), according to the manufacturer's instructions.

Immunohistochemistry

Paraffin sections from tissue specimens were deparaffinized, rehydrated, and then heated in citrate buffer (10 mM, pH 6.0) at 100°C. After blocking, the sections were stained with anti-IL-17A (1:75 dilution) or anti-CD4 (1:100 dilution) (from BioVision, USA and Boster Biotech, China, respectively) at room temperature for 1 h, followed by incubation with anti-rabbit IgG secondary antibody (Boster Biotech) at 37°C for 20 min. After three washes with phosphate-buffered saline, the sections were subjected to a colorimetric reaction by incubation with 3,3'-diaminobenzidine (Beyotime Institute of Biotechnology, Jiangsu, China). Subsequently, all sections were counterstained with hematoxylin and reviewed and scored under a light microscope. To score the immunostained sections, we randomly selected ten fields at 40 \times magnification and scored the staining intensity (0, no staining; 1, weakly positive; 2, positive; and 3, strongly positive) and the staining percentage (0, < 5% of cells stained positively; 1, 5-25%; 2, 26-50%; and 3, > 50%). If the sum of these two scores was ≤ 2 , the case was considered as negatively stained for IL-17A; and if it was > 2 , the case was considered as positively stained. The consecutive sections obtained from the same tissue were stained with anti-IL-17A or anti-CD4 antibody. Cells positive for both IL-17A and CD4 represent Th17 cells in tumor tissues.

Statistical analysis

Data were analyzed by using SPSS 19.0 software (SPSS, Chicago, IL, USA) and plotted as mean \pm standard deviation.

Analysis of statistically significant differences of quantitative data between two groups was carried out by the Student's *t*-test, while the quality of the data was analyzed using the χ^2 test. Comparison among the three different experimental groups was achieved by one-way analysis of variance. Simple correlation analysis was adopted for two variables. A *P* value < 0.05 was considered as statistically significant.

Results

Altered percentage of Th17 cells in PBMCs from colorectal cancer patients

The percentage of Th17 cells, defined as CD3-positive and IL-17A-positive but CD8-negative cells, was assessed by flow cytometry. The data showed that the percentage of Th17 cells was higher in PBMCs from elderly healthy subjects compared to the young subjects ($0.54 \pm 0.22\%$ vs. $0.33 \pm 0.23\%$, *P* = 0.002; Fig. 1A-C). Importantly, the percentage of Th17 cells was significantly higher in colorectal cancer patients ($1.19 \pm 0.31\%$) than in the elderly healthy subjects (*P* < 0.001).

Moreover, the percentage of Th17 cells was higher in PBMCs from patients with a more advanced stage of disease than those at an early stage ($1.26 \pm 0.3\%$ vs. $1.03 \pm$

0.26% , *P* < 0.05; Fig. 1D). These findings suggest that the number of Th17 cells in PBMCs might be associated with both aging and the progression of colorectal cancer.

Increased serum levels of IL-17A and IL-6 in cancer patients

Next, we determined serum levels of IL-17A and IL-6 proteins in these three groups of subjects. As shown in Fig. 2A, the IL-17A level was significantly higher in the colorectal cancer patients (2.23 ± 0.99 pg/mL) and the elderly healthy subjects (2.12 ± 1.08 pg/mL), compared to young healthy subjects (1.16 ± 0.85 pg/mL, *P* < 0.001); however, the IL-17A level observed between the cancer patients and elderly controls was not significantly different (*P* = 0.64). Furthermore, the serum IL-17A level was not significantly different between early and advanced stages of disease (2.01 ± 1.13 pg/mL vs. 2.33 ± 0.94 pg/mL, *P* > 0.05; Fig. 2B).

The serum IL-6 level was significantly different among these three groups of subjects: 32.83 ± 5.23 pg/mL in cancer patients, 13.89 ± 4.32 pg/mL in elderly controls, and 5.62 ± 3.89 pg/mL in young controls (*P* < 0.001; Fig. 2C).

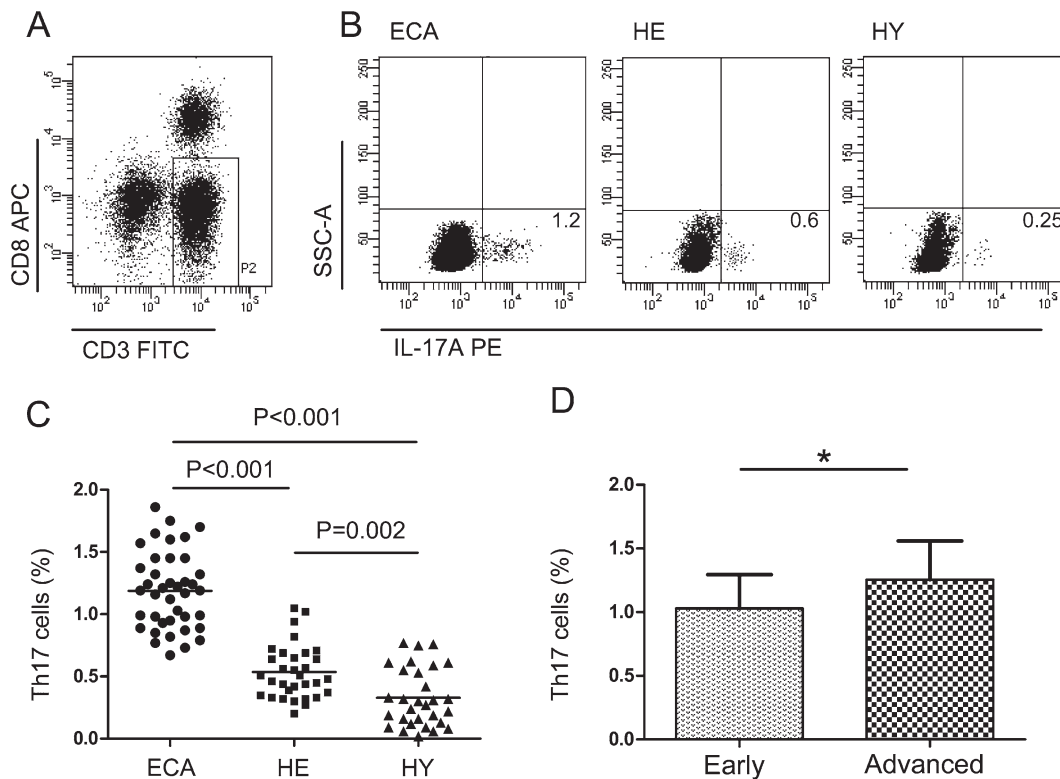


Fig. 1. Altered percentage of Th17 cells in PBMCs from cancer patients.

PBMCs derived from elderly cancer patients (ECA; *n* = 40), elderly healthy controls (HE; *n* = 32), and young healthy controls (HY; *n* = 30) were probed with FITC-conjugated anti-human CD3, APC-conjugated anti-human CD8, and PE-conjugated anti-human IL-17A, followed by flow cytometric analysis. (A) CD4-positive T cells were CD3-positive and CD8-negative (because CD4 endocytosis may occur during PBMC isolation, the test results would be affected if the CD4 antibody was used). (B) Representative flow cytometric analysis data of Th17 cells. Th17 cells are defined as CD3-positive, IL-17A-positive, but CD8-negative cells. SSC-A (side scatter A) shows the physical parameters of the cells. (C) The percentage of Th17 cells in these three groups of subjects was plotted. (D) The percentage of Th17 cells in PBMCs derived from patients with early (*n* = 12) or advanced (*n* = 28) colorectal cancer was presented. **P* < 0.05.

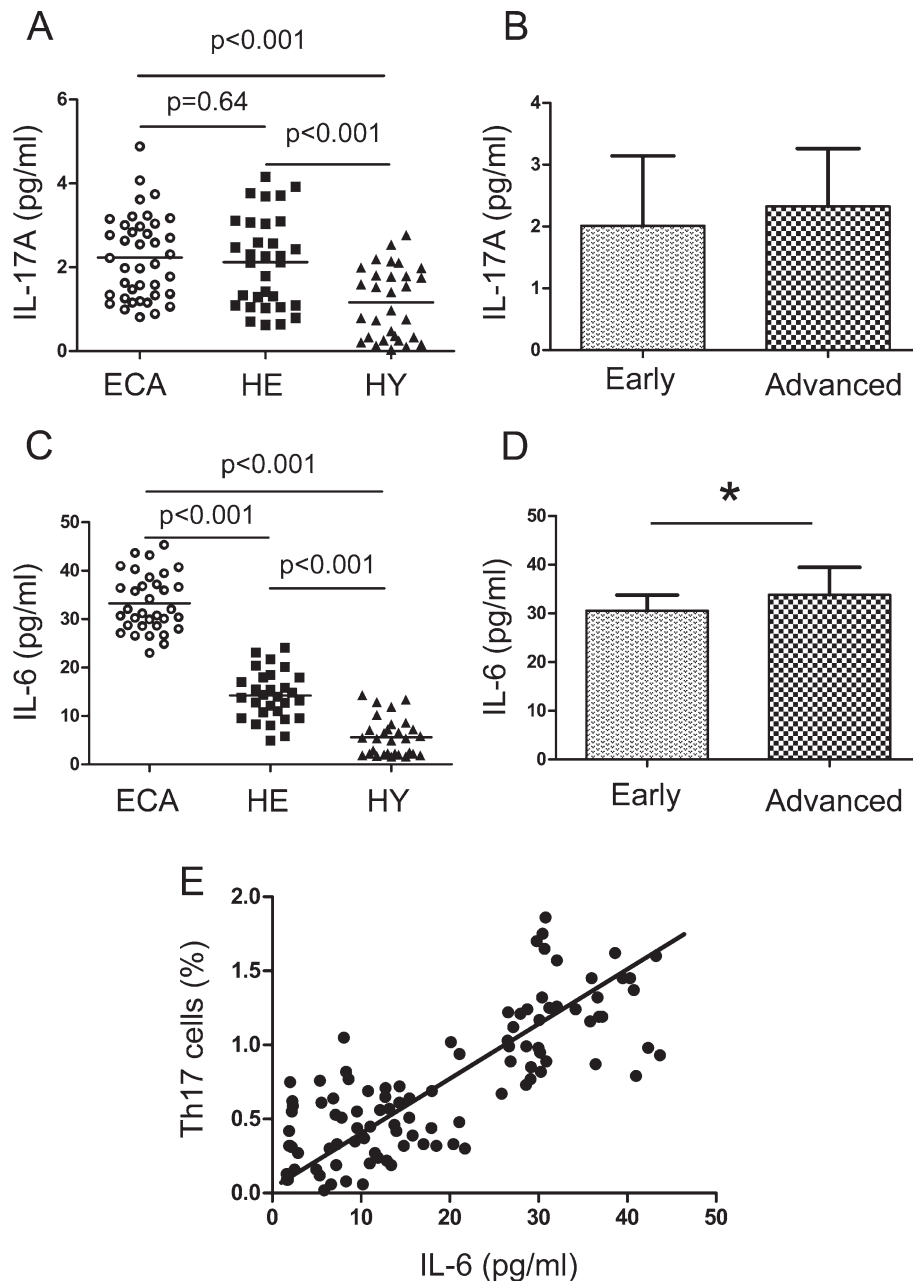


Fig. 2. Serum levels of IL-17A and IL-6 proteins in the three groups of subjects.

Serum IL-17A (A) and IL-6 (C) levels from elderly cancer patients (ECA; $n = 40$), elderly healthy controls (HE; $n = 32$), and young healthy controls (HY; $n = 30$) were evaluated by ELISA. IL-17A (B) and IL-6 (D) levels in patients with early ($n = 12$) or advanced stages of the disease ($n = 28$) were also determined. (E) The association between the serum IL-6 level and the percentage of Th17 cells in PBMCs of all studied subjects. * $P < 0.05$.

Increased IL-6 levels occurred in cancer patients with an advanced stage of disease (30.57 ± 3.18 pg/mL vs. 33.79 ± 5.67 pg/mL, $P < 0.05$; Fig. 2D). In addition, the serum IL-6 level was closely associated with the percentage of Th17 cells in the peripheral blood of all three groups of subjects ($r = 0.786$, $P < 0.001$; Fig. 2E).

Accumulation of Th17 cells in tumor tissues

Tumor tissues were collected from 39 colorectal cancer patients out of the 40 patients. These 39 patients were

diagnosed pathologically as having adenocarcinoma. The clinicopathological data are shown in Table 1. In order to determine whether Th17 cells gathered in tumor tissues, tumor sections were subjected to immunohistochemical analysis of IL-17A and CD4 expression, both of which are expressed in Th17 cells. Normal tissues contained no or a low number of IL-17A-positive cells (Fig. 3A), whereas a dramatically increased number of IL-17A-positive cells were detected in the intratumor and peritumor regions (Fig. 3B). Serial sections obtained from the same tissue sample

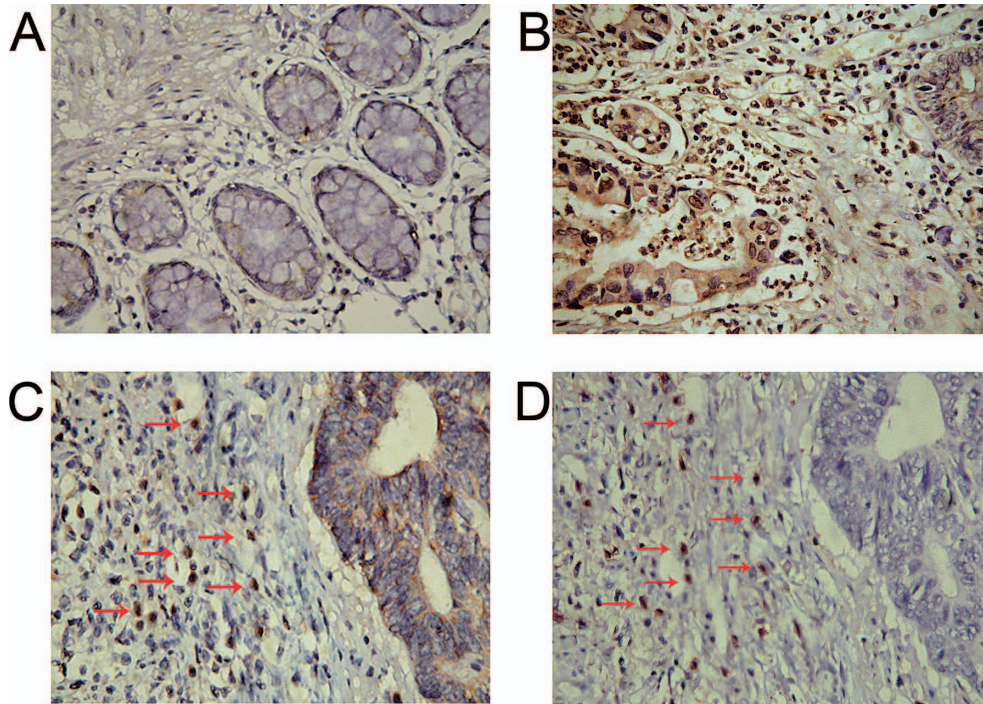


Fig. 3. Accumulation of Th17 cells in tissue specimens from colorectal cancer patients.

(A) Nontumor tissues (more than 5 cm away from the tumor lesion) and (B) tumor and peritumor tissues were derived from a single patient. These tissues were stained with the anti-IL-17A antibody. The data showed that IL-17A was mainly distributed in the intratumor and peritumor regions. In order to identify Th17 cells in tumor tissues, tumor and peritumor tissue samples derived from another patient were used for IL-17A staining (C) and CD4 staining (D). The arrows show both IL-17A- and CD4-positively stained cells, indicating Th17 cells. All images were taken at 400 \times magnification. The scores of the tissues were as follows: A, 0; B, 5; and C, 3.

Table 1. Association of IL-17A expression with clinicopathological data from elderly colorectal cancer patients.

	N	IL-17A		χ^2	P
		Negative	Positive		
Sex				0.742	0.494
Male	22	8	14		
Female	17	4	13		
Depth of invasion				3.374	0.102
T ₁ +T ₂	9	5	4		
T ₃ +T ₄	30	7	23		
Differentiation				1.140	0.446
Well-Moderate	28	10	18		
Poor	11	2	9		
Lymph node metastasis				0.577	0.498
Negative	16	6	10		
Positive	23	6	17		
AJCC cancer stage				6.182	0.023*
I/II	12	7	5		
III/IV	27	5	22		

All data were analyzed by the χ^2 test to compare sex (male vs. female), depth of invasion (T₁+T₂ vs. T₃+T₄), differentiation (well-moderate vs. poor), lymph node metastasis (negative vs. positive), and AJCC cancer stage (I/II vs. III/IV). * $P < 0.05$.

were immunostained with anti-IL-17A or anti-CD4 antibody. Cells positive for both IL-17A and CD-4 were defined as Th17 cells (Fig. 3C and D, arrows). The data showed that the Th17 cells were mainly distributed in the intratumor and peritumor regions rather than the non-tumor regions. The presence of IL-17A-positive cells was associated with the tumor clinical stage ($P < 0.05$), but not with gender, tumor invasion, differentiation, or lymph node metastasis (Table 1).

Discussion

Chronic inflammation, caused by a variety of factors, is associated with a high risk of carcinogenesis (Shacter and Weitzman 2002). The close correlation between inflammatory bowel disease and the increased risk of colorectal cancer has now been widely confirmed (Lakatos and Lakatos 2008). The elderly population is a special cohort with immunosenescence (Pawelec et al. 2010) and “inflammageing” (Cevenini et al. 2013). Chronic low-grade inflammation, a fundamental characteristic of aging, might be associated with an increased risk of tumorigenesis in older populations. Th17 cells have been implicated to be associated with the carcinogenic inflammatory process of colorectal cancer (Erdman and Poutahidis 2010). These cells may persist or promote the disease by generating inflammatory cytokines, such as IL-17A. However, the involvement of Th17 cells and the related cytokines in elderly patients with colorectal cancer remains unclear. The altered percentage of Th17 cells as well as IL-17A and IL-6 levels in serum or tumor tissues during aging and colorectal tumorigenesis, in addition to their potential correlation, are poorly understood. Our current study demonstrated that during aging and colorectal cancer development, the percentage of Th17 cells and the IL-17A and IL-6 levels were all increased.

Indeed, an increased percentage of Th17 cells has been associated with several types of cancer, including colon cancer (Wu et al. 2009), gastric cancer (Zhang et al. 2008), prostate cancer (Sfanos et al. 2008), and ovarian cancer (Miyahara et al. 2008). In the present study, we found that the percentage of Th17 cells in the PBMCs derived from elderly healthy control subjects was greater than that from young subjects, suggesting that the number of Th17 cells might be positively associated with aging. Notably, the percentage of Th17 cells was significantly greater in elderly colorectal cancer patients compared to elderly healthy control subjects. Moreover, the level of Th17 cells was increased in advanced stages of colorectal cancer. These findings indicate that Th17 cells may play a role in colorectal cancer progression in the elderly population, which is consistent with previous reports on lung cancer (Chen et al. 2010) and gastric cancer (Zhang et al. 2008).

Th17 cells secrete the signature cytokine IL-17A as well as other cytokines, such as IL-17F, IL-21, IL-22, granulocyte-macrophage colony-stimulating factor, and potentially tumor necrosis factor (Korn et al. 2009). The increased number of Th17 cells in elderly cancer patients

was hypothesized to correlate with an elevated level of serum IL-17A. However, we found that serum IL-17A was higher in the elderly healthy controls compared to the young subjects, whereas there was no significant difference between the cancer patients and elderly controls. These data allowed us to speculate that the serum IL-17A level increases with age; thus, detection of the serum IL-17A level may not be a useful biomarker for predicting colorectal tumor progression. In order to understand the potential involvement of IL-17A-mediated tumorigenesis in colorectal cancer, immunohistochemical analysis was conducted. Semi-quantitative results showed that the number of IL-17A-positive cells was significantly increased in intratumor and peritumor tissues, whereas IL-17A-positive cells were rarely detected in normal tissues, suggesting that IL-17A does promote colorectal tumorigenesis, which is consistent with the data reported previously showing that IL-17A expression was mainly restricted to the cancer area of colorectal cancer but not in nontumor regions (Liu et al. 2011). It is possible that IL-17A may promote angiogenesis by upregulating a variety of proangiogenic factors produced by fibroblasts and tumor cells, thereby enhancing tumor cell growth (Numasaki et al. 2003).

An elevated serum IL-6 level has been detected in several types of human cancer, including hepatocarcinoma (Jang et al. 2012) and colorectal cancer (Schneider et al. 2000). The serum IL-6 level has been shown to have a prognostic value for hepatocarcinoma patients (Jang et al. 2012). In addition, a previous study has demonstrated that an increased serum IL-6 level is associated with an advanced stage of disease and a poor prognosis for colorectal cancer patients (Knupfer and Preiss 2010). Moreover, an increased serum level of IL-6 protein is associated with poor physical performance and muscle strength in older individuals (Cesari et al. 2004). In the current study, we found that the increased serum IL-6 level was not only associated with aging but also with colorectal cancer progression. IL-6 is an indispensable cytokine in triggering Th17 cell differentiation (Acosta-Rodriguez et al. 2007). IL-6 evokes lymphocyte differentiation to Th17 cells but promotes tumor growth by activation of the signal transducer and activator of transcription signaling pathway (Wang et al. 2009). Indeed, we confirmed that the serum IL-6 level was positively correlated with the percentage of Th17 cells in PBMCs in all three groups of subjects. Therefore, it is possible that disrupting the balance of the pro- and anti-inflammatory systems in the elderly population may promote the secretion of pro-inflammatory cytokines, such as IL-6, which benefits Th17 differentiation. The increased number of Th17 cells may aggravate inflammation and promote tumor growth by increasing IL-6 secretion (Gaffen 2008). On the other hand, IL-6 itself also has the capability to promote tumorigenesis (Becker et al. 2005). Thus, we concluded that the serum IL-6 level might be a useful biomarker for predicting colorectal tumorigenesis and disease progression.

Collectively, our current study demonstrated that the percentage of Th17 cells and the serum IL-6 level are closely correlated and are significantly increased with aging and colorectal cancer progression. Previous studies have shown that IL-17A possesses both anti- and pro-tumor functions, depending on the tumor types and organ sites (Wilke et al. 2011). Thus, future studies are needed to determine their functions in colorectal cancer development and progression.

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Conflict of Interest

The authors declare no conflict of interest.

References

- Acosta-Rodriguez, E.V., Napolitani, G., Lanzavecchia, A. & Sallusto, F. (2007) Interleukins 1 β and 6 but not transforming growth factor- β are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat. Immunol.*, **8**, 942-949.
- Annunziato, F., Cosmi, L., Liotta, F., Maggi, E. & Romagnani, S. (2013) Main features of human T helper 17 cells. *Ann. NY Acad. Sci.*, **1284**, 66-70.
- Becker, C., Fantini, M.C., Wirtz, S., Nikolaev, A., Lehr, H.A., Galle, P.R., Rose-John, S. & Neurath, M.F. (2005) IL-6 signaling promotes tumor growth in colorectal cancer. *Cell Cycle*, **4**, 217-220.
- Cesari, M., Penninx, B.W., Pahor, M., Lauretani, F., Corsi, A.M., Rhys Williams, G., Guralnik, J.M. & Ferrucci, L. (2004) Inflammatory markers and physical performance in older persons: the InCHIANTI study. *J. Gerontol. A Biol. Sci. Med. Sci.*, **59**, 242-248.
- Cevenini, E., Monti, D. & Franceschi, C. (2013) Inflamm-aging. *Curr. Opin. Clin. Nutr. Metab. Care*, **16**, 14-20.
- Chen, X., Wan, J., Liu, J., Xie, W., Diao, X., Xu, J., Zhu, B. & Chen, Z. (2010) Increased IL-17-producing cells correlate with poor survival and lymphangiogenesis in NSCLC patients. *Lung Cancer*, **69**, 348-354.
- Coussens, L.M. & Werb, Z. (2002) Inflammation and cancer. *Nature*, **420**, 860-867.
- De Martinis, M., Franceschi, C., Monti, D. & Ginaldi, L. (2005) Inflamm-aging and lifelong antigenic load as major determinants of ageing rate and longevity. *FEBS Lett.*, **579**, 2035-2039.
- Erdman, S.E. & Poutahidis, T. (2010) Roles for inflammation and regulatory T cells in colon cancer. *Toxicol. Pathol.*, **38**, 76-87.
- Gaffen, S.L. (2008) An overview of IL-17 function and signaling. *Cytokine*, **43**, 402-407.
- Jang, J.W., Oh, B.S., Kwon, J.H., You, C.R., Chung, K.W., Kay, C.S. & Jung, H.S. (2012) Serum interleukin-6 and C-reactive protein as a prognostic indicator in hepatocellular carcinoma. *Cytokine*, **60**, 686-693.
- Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E. & Forman, D. (2011) Global cancer statistics. *CA Cancer J. Clin.*, **61**, 69-90.
- Knupfer, H. & Preiss, R. (2010) Serum interleukin-6 levels in colorectal cancer patients: a summary of published results. *Int. J. Colorectal Dis.*, **25**, 135-140.
- Korn, T., Bettelli, E., Oukka, M. & Kuchroo, V.K. (2009) IL-17 and Th17 Cells. *Annu. Rev. Immunol.*, **27**, 485-517.
- Lakatos, P.L. & Lakatos, L. (2008) Risk for colorectal cancer in ulcerative colitis: changes, causes and management strategies. *World J. Gastroenterol.*, **14**, 3937-3947.
- Liu, J., Duan, Y., Cheng, X., Chen, X., Xie, W., Long, H., Lin, Z. & Zhu, B. (2011) IL-17 is associated with poor prognosis and promotes angiogenesis via stimulating VEGF production of cancer cells in colorectal carcinoma. *Biochem. Biophys. Res. Commun.*, **407**, 348-354.
- Martin-Orozco, N., Muranski, P., Chung, Y., Yang, X.O., Yamazaki, T., Lu, S., Hwu, P., Restifo, N.P., Overwijk, W.W. & Dong, C. (2009) T helper 17 cells promote cytotoxic T cell activation in tumor immunity. *Immunity*, **31**, 787-798.
- Miossec, P., Korn, T. & Kuchroo, V.K. (2009) Interleukin-17 and type 17 helper T cells. *N. Engl. J. Med.*, **361**, 888-898.
- Miyahara, Y., Odunsi, K., Chen, W., Peng, G., Matsuzaki, J. & Wang, R.F. (2008) Generation and regulation of human CD4⁺ IL-17-producing T cells in ovarian cancer. *Proc. Natl. Acad. Sci. USA*, **105**, 15505-15510.
- Monteleone, G., Pallone, F. & Stolfi, C. (2012) The dual role of inflammation in colon carcinogenesis. *Int. J. Mol. Sci.*, **13**, 11071-11084.
- Muranski, P., Boni, A., Antony, P.A., Cassard, L., Irvine, K.R., Kaiser, A., Paulos, C.M., Palmer, D.C., Touloukian, C.E., Ptak, K., Gattinoni, L., Wrzesinski, C., Hinrichs, C.S., Kerstann, K.W., Feigenbaum, L., et al. (2008) Tumor-specific Th17-polarized cells eradicate large established melanoma. *Blood*, **112**, 362-373.
- Neurath, M.F. & Finotto, S. (2011) IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer. *Cytokine Growth Factor Rev.*, **22**, 83-89.
- Numasaki, M., Fukushi, J., Ono, M., Narula, S.K., Zavodny, P.J., Kudo, T., Robbins, P.D., Tahara, H. & Lotze, M.T. (2003) Interleukin-17 promotes angiogenesis and tumor growth. *Blood*, **101**, 2620-2627.
- Pawelec, G., Derhovanessian, E. & Larbi, A. (2010) Immunosenescence and cancer. *Crit. Rev. Oncol. Hematol.*, **75**, 165-172.
- Schneider, M.R., Hoeflich, A., Fischer, J.R., Wolf, E., Sordat, B. & Lahm, H. (2000) Interleukin-6 stimulates clonogenic growth of primary and metastatic human colon carcinoma cells. *Cancer Lett.*, **151**, 31-38.
- Sfanos, K.S., Bruno, T.C., Maris, C.H., Xu, L., Thoburn, C.J., DeMarzo, A.M., Meeker, A.K., Isaacs, W.B. & Drake, C.G. (2008) Phenotypic analysis of prostate-infiltrating lymphocytes reveals TH17 and Treg skewing. *Clin. Cancer Res.*, **14**, 3254-3261.
- Shacter, E. & Weitzman, S.A. (2002) Chronic inflammation and cancer. *Oncology (Williston Park)*, **16**, 217-226, 229; discussion 230-232.
- Stephen, B.E. (2010) Colon and rectum AJCC Cancer Staging Manual. In *AJCC Cancer Staging Manual*, 7th ed., edited by Edge, S.B., Bird, D.R., Compton, C.C., Fritz, A.G., Greene, F.L. & Trotti, A. Springer, Berlin, pp. 143-164.
- Terzić, J., Grivennikov, S., Karin, E. & Karin, M. (2010) Inflammation and colon cancer. *Gastroenterology*, **138**, 2101-2114.
- Tesmer, L.A., Lundy, S.K., Sarkar, S. & Fox, D.A. (2008) Th17 cells in human disease. *Immunol. Rev.*, **223**, 87-113.
- Wang, L., Yi, T., Kortylewski, M., Pardoll, D.M., Zeng, D. & Yu, H. (2009) IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. *J. Exp. Med.*, **206**, 1457-1464.
- Wilke, C.M., Kryczek, I., Wei, S., Zhao, E., Wu, K., Wang, G. & Zou, W. (2011) Th17 cells in cancer: help or hindrance? *Carcinogenesis*, **32**, 643-649.
- Wu, S., Rhee, K.J., Albesiano, E., Rabizadeh, S., Wu, X., Yen, H.R., Huso, D.L., Brancati, F.L., Wick, E., McAllister, F., Housseau, F., Pardoll, D.M. & Sears, C.L. (2009) A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat. Med.*, **15**, 1016-1022.
- Zhang, B., Rong, G., Wei, H., Zhang, M., Bi, J., Ma, L., Xue, X.,

Wei, G., Liu, X. & Fang, G. (2008) The prevalence of Th17 cells in patients with gastric cancer. *Biochem. Biophys. Res. Commun.*, **374**, 533-537.

Zhang, J.P., Yan, J., Xu, J., Pang, X.H., Chen, M.S., Li, L., Wu, C.,

Li, S.P. & Zheng, L. (2009) Increased intratumoral IL-17-producing cells correlate with poor survival in hepatocellular carcinoma patients. *J. Hepatol.*, **50**, 980-989.
