March 12, 2023

# Difference of protumor immunological factors between the tumor site and the tumor-free site is associated with colorectal cancer initiation and invasion

*Running head: Immunological factors and colorectal neoplasms*

**Guanglin Cui[1,2], Aping Yuan[1], Zhigang Pang[1], Jon Florholmen[3]**

[1] Research Group of Gastrointestinal Diseases, the Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan province, China

[2] Faculty of Health Science, Nord University, Campus Levanger, Levanger, Norway

[3] Department of Gastroenterology, University Hospital of North Norway, University of Tromsø, Tromsø, Norway

Guanglin Cui: Guanglin.cui@nord.no
Aping Yuan: aping_yuan@yahoo.com
Zhigang Pang: pzg63726@sina.com
Jon Florholmen: jon.florholmen@unn.no

**All authors have read and approved this manuscript**

*Address correspondence to: Guanglin Cui, Faculty of Health Science, Nord University, Campus Levanger, Norway or Research Group of Gastrointestinal Diseases, the Second Affiliated Hospital of Zhengzhou University, Zhengzhou, China.

E-mail: guanglin.cui@nord.no

Telephone: +47 74022557

.

**Abbreviations used in this paper:**

COX2: cyclooxygenase 2

CRC: colorectal cancer

HGD: high grade dysplasia

IL: interleukin

LGD: low grade dysplasia

MDSCs: myeloid-derived suppressor cells

q-PCR: quantitative real-time polymerase chain reaction

SEM: standard error of the mean

TGFβ: transforming growth factor beta

*Highlights*

- Monitoring changes of host immunosurveillance provides the key information to understand the mechanisms of cancer invasion and metastasis.
- Different levels of immunological factor expression between the adenoma/CRC site and adjacent tumor-free site were observed.
- Such difference of immunological factors reflects the protumor/antitumor force balance, and is associated with CRC initiation and invasion.

## Abstract

To study the role of host immune surveillance in the initiation and progression of colorectal cancer (CRC), a set of protumor immunological factors was determined by quantitative real-time PCR (q-PCR) between the primary tumor and the adjacent tumor-free site tissues in 63 patients with colorectal neoplasms. Their clinicopathological and prognostic significance was investigated. Results showed that levels of a set of immunological factors e.g., interleukin (IL)-1β, IL-6, IL-8, IL-17A, IL-23, and cyclooxygenase 2 (COX2) mRNAs, except transforming growth factor beta (TGFβ), in adenoma tissues were significantly higher than that in relative adjacent tissues. Difference of immunological factor level between adenoma and adjacent tissues (Δ values) was in an order of ΔIL-8>ΔIL-6>ΔIL-17A>ΔIL-1β>ΔCOX2>ΔIL-23; Further analysis showed that the value of ΔCOX2 correlated to the grade of dysplastic degree in patients with adenoma. Notably, levels of all these immunological factors in CRC tissues were significantly increased than that in adenoma tissues and relative tumor-free tissues adjacent to CRC, the order of values of Δ immunological factors was ΔIL-8>ΔCOX2>ΔIL-6>ΔIL-1β>ΔIL-17A>ΔIL-23>ΔTGFβ. Clinicopathological analysis revealed that increased value of Δ IL-1β was associated with advanced TNM stage, a higher value of Δ COX2 tended to predicate a deeper degree of tumor invasion; and higher values of Δ IL-1β, IL-6 and COX2 closely correlated to lymph node metastasis in patients with CRC. Therefore, we concluded that the difference of protumor immunological factors between the primary tumor site and tumor-free site along the adenoma-carcinoma sequence reflects the protumor/antitumor force balance, which is associated with CRC initiation and invasion.

**Key words:** Immunological factors; adjacent mucosa; colorectum; adenoma; cancer

## 1. Introduction

Colorectal cancer (CRC) is one of the most common cancers with an estimated 1.8 million new diagnosed cases and an estimated 0.8 million death according to the statistical data in 185 countries in 2020 [1]. Extensive evidence suggests that human immunity is the most principal factor in determining the development, progression, and metastasis of CRC. Therefore, changes in immunological features may provide a critical crude for the understanding of pathogenesis of CRC [2-4]. Pathologically, CRC invasion and metastasis is a multistep process, cancer cells invade and penetrate firstly from primary site into lymphatics and microvessels after escaping immunosurveillance control, and then transport to the near and distant tumor-free sites with suitable growing environment [4, 5]. Studies showed that antitumor force within the primary tumor core is significantly inhibited, but protumor force is enhanced by factors released by cancer cells. In the tumor-free site, host immunosurveillance force will inevitably attempt to fight with and kill metastatic cancer cells and block their survival as the “seeds” settling down in local tissue (so called “soil” ) [4, 6, 7], which leads to a significant immunological difference between the tumor site and the tumor-free site adjacent to CRC. Therefore, the balance between protumor and antitumor force will determine whether cancer cells can finally survival in the tumor-free or metastatic site.

Clinical observational studies have reported that significant immunological alterations occurred between the tumor center and the tumor-free site adjacent to the CRC, in addition to histological and genetic factor changes [8-22]. For example, studies have observed significant phenotypic and functional differences of immune cells and cytokines between the primary tumor site and the tumor-free site in patients with CRC [17, 23]. In

addition, these cytokines have been shown to play an important role in modulating the biological behaviors of cancer stem-like cells and stromal cells and are involved in the tumor invasion and metastasis in the CRC [24-26]. All these findings in turn result in increasing attempt to get an improved understanding of immunological features that can specifically affect the development and progression of CRC and may offer novel potential to improve the efficacy of current immunotherapies [27, 28].

It has been hypothesized that the “soil” will be changed and skew to a favorable milieu to adapt the arrival of cancer cells to the tumor-free or metastatic site when invasion or metastasis occurs. To create a supportive condition that allows the “seeds” to settle down and grow up in the tumor-free or metastatic site, host immunity, as a main force against tumor, will inevitably be altered in the “soil”, reflecting in changed immune cell populations, phenotypes, and functions in the local environment [4, 6, 7]. These changes will induce an enhanced production of protumor immune factors in both the primary tumor core and the near adjacent site [17, 23], even in the distant site [29-31]. For example, Zeng et al. [32] have recently shown that an supportive microenvironment formed by immunosuppressive cells is observed in the pre-metastatic liver in patients with CRC, which is regulated by myeloid-derived suppressor cells (MDSCs). Furthermore, Liu et al. [30] have observed that immune phenotypes between primary and metastatic CRCs are distinctly different, such phenotypic change is associated with the process of CRC metastasis from the primary site to the liver. Previously, we have revealed that certain protumor immunological factors e.g., IL-6, IL-17A and IL-33 can promote the progression and metastasis of CRC by stimulating the growth of CRC cells and enhancing tumor angiogenesis [16, 24, 33, 34]. Other studies have also demonstrated

that immunological features are significantly different between CRC tissues and tumor-free mucosa adjacent to CRC, in which changed immunological factors are associated with enhanced angiogenesis and immune function defect [17, 22, 23]. For example, IL-8 is an proinflammatory cytokine with multifunction [35, 36], and plays an important role in tracking immune cells i.e. neutrophils, monocytes, dendritic cells to the primary tumor site. Moreover, research evidence suggested that IL-8 is both a potent proangiogenic factor and growth factor for CRC cells that stimulates induction of angiogenesis, cancer invasion and metastasis [37-40]. We and others have demonstrated that increased IL-8 expression in the adenoma tissues is early event occurred in the adenoma stage and contributed to the transformation of adenomas to CRCs [41, 42], indicating IL-8 as an important protumor immunological factor involving in the pathological procedure of CRC. Based on above evidence, we hypothesize that a supportive “soil” created by protumor features is a key step for the process of CRC invasion and metastasis from a primary site to a tumor-free site.

We have therefore designed this study to evaluate what and how difference of protumor immunological factors that related to tumor expansion and invasion between the primary tumor site and the tumor-free site, and their influence on the features of tumor severity/invasion and prognosis in patients with adenoma and CRC.

## 2. Materials & Methods

### 2.1. Biopsies

Both adenoma and adjacent tumor-free biopsies were prospectively collected from 31 patients with colorectal adenoma (22 males, 9 females, rang of age 49-92 years, and average age 65 years) by colonoscopy, CRC and tumor-free surgical biopsies (~10 cm far from the tumor site) from 32 patients with CRC (male 29, female 3, range of ages from 42 to 85 years, and average age 67 year) admitted to the Department of Gastroenterology and Surgery, University Hospital of North Norway according to standardized diagnostic criteria respectively. Control colorectal biopsies from eighteen subjects without pathological evidence (10 males, 8 females, range of ages from 33 to 80 year, and average age 55 year) by colonoscopy were used as the controls. All those biopsies were confirmed to have "normal" histology characterized by conventional histological examination with hematoxylin and eosin (*H&E*) staining. Detailed information for each group is presented in Table 1. The Norwegian Regional Ethical Committee of North Norway approved the study and the Norwegian Health Department approved the storage of human biological materials. Informed consent was obtained from the patients.

### 2.2. Tissue total RNA extraction and cDNA synthesis

To avoid RNA degradation, biopsies were collected in *RNA later* solution (Invitrogen Life Tech., Carlsbad, MA, USA) and total RNA was extracted by the *Trizol* method (Invitrogen Life Tech., Carlsbad, MA, USA) [43]. Total RNA quality control was done by the by the measurement of RNA integrity with an Agilent 2100 Bioanalyzer with RNA 6000 Nano chips (Agilent Technology, Inc., Böblingen, Germany) according to the manufacturer's instructions. Reverse transcription for cDNA synthesis was performed

with *SuperScript II* (Invitrogen Life Tech., Carlsbad, MA, USA) according to our previous report [43].

### 2.3. Protumor immunological factors quantified by quantitative real-time PCR (q-PCR)

The expression levels of protumor immunological factors IL-1β, IL-6, IL-8, IL-17A, IL-23, transforming growth factor beta (TGFβ) and cyclooxygenase 2 (COX2) transcripts were quantified. Primers and probes for these factors and the housekeeping gene beta-actin (Table 2) were listed in Table 2 and the mRNAs of immunological factors in the tumor-free mucosa adjacent to adenomas, CRCs and the controls were quantified with an *ABI-prism 7900* sequence detector (Applied Biosystems/Roche, Branchburg, NJ, USA) in 25μL format and the expression levels of these target genes were calculated as relative fold changes ($2^{-\Delta\Delta CT}$ method) according to our previous published method [42, 44].

### 2.4. Analysis of value difference (Δ) of immunological factors between the primary site of adenoma/CRC and the tumor-free adjacent site against clinicopathological variables in patients with adenoma or CRC

Values of Δ immunological factor levels between the primary site of adenoma/CRC and the tumor-free adjacent site against histological types and dysplastic degrees in patients with adenoma or CRC, and against TNM stages, invasion degrees and lymph node involvement in patients with CRC were analyzed individually.

### 2.5. Statistics

Results were expressed as mean ± standard error of the mean (SEM) unless otherwise stated. Mann-Whitney tests were used to compare differences between groups and one-way ANOV (non-parametric Kruskal-Wallis test) was used to compare differences among three groups. $P<0.05$ was considered as statistic significant.

## 3. Results

### 3.1. The expression levels of protumor immunological factors in adenoma and adjacent pair tissues

As shown in Table 3, the expression levels of IL-1β, IL-6, IL-8, IL-17A, IL-23 and COX2 transcripts in the mucosa adjacent to colorectal adenomas were remarkably lower than that in the adenoma tissues (all $P<0.01$). In which, values of Δ immunological factors were in an order of ΔIL-8>ΔIL-6>ΔIL-17A>ΔIL-1β>ΔCOX2>ΔIL-23; However, the level of TGFβ in the adjacent mucosa was significantly higher than that in adenoma tissues ($P<0.01$).

### 3.2. Values of Δ protumor immunological factors between adenoma core and adjacent tissues were analyzed against clinical pathological parameters in patients with adenoma

Since data showed a remarkable difference of immunological factors between adenoma tissues and adjacent tissues in patient cohort of adenomas, values of Δ immunological factors between adenoma core and adjacent tissues were analyzed against clinical pathological parameters.

Analysis revealed that majority of values of Δ immunological factor expression levels were neither associated with histological types of adenomas nor dysplastic degree (refer to Table 4), except the value of Δ COX2 level was associated with dysplastic degree, patients with a higher dysplastic degree tended to have a higher value of Δ COX2.

### 3.3. The expression level of protumor immunological factors in CRC and adjacent tumor-free tissues

The expression levels of examined immunological factors in the tumor-free mucosa adjacent to CRC were significantly lower than that in the CRC tissues (all $P<0.01$, refer to Table 5). Values of Δ immunological factors were in an order of ΔIL-8>ΔCOX2>ΔIL-6>ΔIL-17A>ΔIL-1β>ΔIL-23>ΔTGFβ.

### 3.4. Values of Δ protumor immunological factors between CRC and adjacent tissues were analyzed against clinical pathological parameters in patients with CRC

Data analyses showed that the value of Δ IL-1β was associated with TNM stages, patients with an advanced stage (TNM III) tended to have a higher value of Δ IL-1β. In addition, the value of Δ COX2 was associated the invasion depth (refer to Table 6).

Analyses further revealed that values of Δ IL-1β, ΔIL-6 and ΔCOX2 were positively associated with lymph node metastasis, patients with lymph node involvement have higher values of Δ IL-1β, IL-6 and COX2 than those without lymph node involvement (all $P<0.01$, refer to Table 7). In addition, the value of Δ IL-17A was associated with differentiation degree of CRC ($P<0.05$, Table 7). Analyses showed that values of Δ IL-8, IL-23A and TGFβ did not impact these variables in patients with CRC.

Finally, we analyzed the impact of values of Δ immunological factors on the survival rates after surgery in patients with CRC. The analysis showed that only values of Δ IL-1β had a predicative significance on the survival rate after surgery (Fig. 1A), values of Δ IL-6 (Fig. 1B), ΔIL-8 (fig. 1C), ΔIL-17A (Fig. 1D), ΔIL-23A (Fig. 1E), ΔTGFβ (Fig. 1F) and ΔCOX2 (Fig. 1G) did not affect the survival rate after surgery in patients with CRC.

## 4. Discussion

In the present study, we evaluated the difference of protumor factors in both the primary tumor site and the adjacent site and have observed an distinct profile of protumor immunological factors between the adenoma/CRC and the tumor-free adjacent tissues. Such difference of protumor immunological factors were associated with tumor invasion and metastasis in the CRC. Therefore, such difference of protumor immunological factors between the primary tumor site and adjacent site may reflect the changed “soil” condition in different histopathological sites along the colorectal adenoma-carcinoma sequence and be a driving force for the development and progression of human CRC.

Based on the analysis of present data, IL-8 was shown to be the most changed cytokine level between adenoma/CRC center and adjacent mucosa. Values of Δ IL-8 were over ~94-fold increase in patients with adenoma and ~956-fold increase in patients with CRC. Furthermore, values of Δ IL-6, IL-1β, COX2 and IL-23 between the adenoma core and the adjacent site, following Δ value of IL-8, were also shown to be remarkably increased. Since all these factors have been demonstrated to be the driving force for CRC development and progression [45-51], increased values of Δ these factors between the adenoma/CRC tissues and the adjacent tissues may reflect the fact that immunosurveillance in the adenoma/CRC center is suppressed by these factors produced by tumor cells or surrounding cells in the tumor microenvironment and is not strong enough to eradicate adenomatous/CRC cells. TGFβ is a immunosuppressive factor produced by both tumor cell and infiltrating T lymphocytes and contributes to the formation of an supportive TME by inducing polarization of immunosuppressive cells [52]. Interestingly, an switch of TGFβ profile from the adenomatous stage to the CRC

stage was observed in this study, in which it was lower in the adenoma tissues than that in adjacent site. However, it was significantly increased at the CRC stage, and the value of Δ TGFβ at the CRC stage was higher than that at the adenoma stage. These findings suggest that TGFβ plays an weak role in the formation of immunosuppressive milieu at the precancerous adenoma stage, however it plays a strong role in the establishment of immunosuppressive milieu at the CRC stage [53]. Enhanced production of TGFβ by CRC cells may add an additional effect [53]. Moreover, the analysis of values of Δ these immunological factors again histological types and dysplastic degree in patients with adenomas revealed that only values of Δ COX2 were associated with dysplastic degree, patients with a higher value of Δ COX2 tended to have a high degree of dysplasia. Furthermore, analysis showed that all values of examined immunological factors were not associated with histological types of adenomas. Since the cohort size in this study was relatively small, their predicative significance is still waiting for the validation in large cohort of adenoma patents in the future.

At the CRC stage, we found that increased values of Δ protumor immunological factors between CRC tissues and adjacent tissues were even higher than that at the adenoma stage. The increasing trend of values of immunological factors was ΔIL-8>ΔCOX2>ΔIL-6>ΔIL-17A>ΔIL-1β>ΔIL-23>ΔTGFβ, in which values of Δ IL-8 and COX2 were the most increased two factors. Levels of these protumor immunological factors in the tumor-free tissues adjacent to CRCs were higher than that in the tissues adjacent to adenomas, suggesting that established CRCs result in an stronger immunosuppressive milieu in the adjacent site, which may represent a skew to a permissive immunological microenvironment that can promote CRC invasion and

metastasis [45-49, 53]. Moreover, this study also showed that levels of immunological factors in CRC tissues were significantly higher than that in adenoma tissues. This finding may suggest that immunological factors produced by CRC cells add an additional promotive effect.

A number of previous studies have shown that immunological factors released from diverse cell types, such as cancer cells, stromal cells and recruited immune cells in the CRC microenvironment, contribute to the cancer cell growth and invasion [54, 55], and finally impact the disease stage and prognosis in patients with CRC [56, 57]. We have therefore analyzed the correlation between values of Δ immunological factors and clinicopathological features in patients with CRC. Data analysis revealed that the value of Δ IL-1β was associated with TNM stages, CRC patients with a higher value of Δ IL-1β level tended to have an advanced TNM stage. Furthermore, the values of Δ IL-17A was associated with differentiation degree of CRC, and the value of Δ of COX2 was associated with invasion depth. Analyses further showed that values of Δ IL-1β, IL-6 and COX2 levels were associated with positive lymph node involvement, indicating an involvement of these factors on lymph node metastasis in patients with CRC. These findings suggest that values of Δ protumor immunological factors between CRC tissues and adjacent tissues may represent as a driving force that are associated with the invasion and metastasis in patients with CRC. Since previous studies have shown that the levels of immunological factors have a significance in predicating survival of CRC patents, we therefore analyzed the predicative significance of values of Δ immunological factors in patients with CRC. Our data revealed that only the value of Δ IL-1β showed a predictive significance for the survival rate after surgery, others did not show such predictive

significance. Our data suggest that the survival rate after surgery is perhaps determined by many factors and a multiple analysis that combined with other variables, such as genetical background, immune function, and disease stages, is needed.

## 5. Study limitations

Limitations for this study should be addressed and discussed. First, this cohort of CRC patients contained more male subjects than female subjects (male/female ratio 29/3), the ration of male/female was lower than the clinical epidemiological data reported, particularly in patients with colon cancer [58]. This may be due to the small sample size included in this study. Second, tissues of adenomas were sampled from colonoscopic resected polyps, tissue sizes for immunological quantitative work were varied that depended on polyp size. Tissues of CRC were obtained from surgical resection samples, which were much bigger than adenomatous tissues and contained varying proportions of tumor/stromal tissue. To date, the quantitative differentiation of immunological factor amounts between adenoma/CRC cells and stromal cells is difficult. However, the influence of these features on the expression of immunological factors must be considered. Therefore, future research is necessary to replicate the results by projects with larger sample size and differentiate the production of immunological factors from different cellular sources.

## 6. Conclusion

The present study revealed a gradient change of values of Δ protumor immunological factors between the tumor center site and the adjacent tumor-free site along the human colorectal adenoma-carcinoma sequence. values of Δ protumor immunological factors between the adenoma/CRC and the adjacent tumor-free tissues

may reflect gradually changed pro/antitumor force from adenoma/CRC site to the adjacent tumor-free site (Fig. 2), which may be associated with the formation of a permissive local milieu that facilitates adenoma progression and CRC invasion.

## Statements & Declarations

### Author Contributions:

**GC:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Draft manuscript. **AY:** Data curation; Formal analysis; Investigation; Visualization and review draft. **ZP & JF:** writing-review and editing. All authors read and agreed to the submitted version of the manuscript.

**Funding:** This project was supported by the grants from Medical Research Program, Northern Norway Regional Health Authority (SFP-44-04).

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Ethical Approval:** The studies involving human participants were reviewed and approved by **t**he Norwegian Regional Ethical Committee of North Norway and the storage of human biological materials was approved by the Norwegian Health Department. Informed consent was obtained from human subjects. Consent to publish is not applicable.

**Data availability:** The data that support the findings of this study are available from our hospital but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of our hospital.

References


[1] Sung, H. Ferlay, J. Siegel, RL. Laversanne, M. Soerjomataram, I. Jemal, A, Bray, F, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin, 71 (2021):209-249. https://doi:10.3322/caac.21660

[2] Evans, CF. Galustian, C. Bodman-Smith, M. Dalgleish, AG, Kumar, D, The effect of colorectal cancer upon host peripheral immune cell function. Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland, 12 (2010):561-569. https://doi:10.1111/j.1463-1318.2009.01819.x

[3] Pancione, M. Giordano, G. Remo, A. Febbraro, A. Sabatino, L. Manfrin, E. Ceccarelli, M, Colantuoni, V, Immune escape mechanisms in colorectal cancer pathogenesis and liver metastasis. J Immunol Res, 2014 (2014):686879. https://doi:10.1155/2014/686879

[4] Pretzsch, E. Bosch, F. Neumann, J. Ganschow, P. Bazhin, A. Guba, M. Werner, J, Angele, M, Mechanisms of Metastasis in Colorectal Cancer and Metastatic Organotropism: Hematogenous versus Peritoneal Spread. J Oncol, 2019 (2019):7407190. https://doi:10.1155/2019/7407190

[5] Li, N. Wang, J. Shen, S. Bu, X. Tian, X, Huang, P, Expression of p53, Ki-67 and c-Myc proteins is predictive of the surgical molecular margin in colorectal carcinoma. Pathol Oncol Res, 17 (2011):479-487. https://doi:10.1007/s12253-010-9323-1

[6] Fidler, IJ, The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer, 3 (2003):453-458. https://doi:10.1038/nrc1098

[7] Yu, X, Li, B, Seed or soil: Tracing the immune subsets in metastatic tumors. Cancer cell, 40 (2022):353-355. https://doi:10.1016/j.ccell.2022.03.001

[8] Kuniyasu, H. Yasui, W. Shinohara, H. Yano, S. Ellis, LM. Wilson, MR. Bucana, CD. Rikita, T. Tahara, E, Fidler, IJ, Induction of angiogenesis by hyperplastic colonic mucosa adjacent to colon cancer. Am J Pathol, 157 (2000):1523-1535

[9] Salama, P. Stewart, C. Forrest, C. Platell, C, Iacopetta, B, FOXP3+ cell density in lymphoid follicles from histologically normal mucosa is a strong prognostic factor in early stage colon cancer. Cancer Immunol Immunother, 61 (2012):1183-1190. https://doi:10.1007/s00262-011-1191-3

[10] Filipe, MI. Mughal, S, Bussey, HJ, Patterns of mucus secretion in the colonic epithelium in familial polyposis. Invest Cell Pathol, 3 (1980):329-343

[11] Hao, CY. Moore, DH. Wong, P. Bennington, JL. Lee, NM, Chen, LC, Alteration of gene expression in macroscopically normal colonic mucosa from individuals

with a family history of sporadic colon cancer. Clin Cancer Res, 11 (2005):1400-1407

[12] Hao, CY. Moore, DH. Chiu, YS. Wong, P. Bennington, JL. Smith, AP. Chen, LC, Lee, NM, Altered gene expression in normal colonic mucosa of individuals with polyps of the colon. Dis Colon Rectum, 48 (2005):2329-2335

[13] Chen, LC. Hao, CY. Chiu, YS. Wong, P. Melnick, JS. Brotman, M. Moretto, J. Mendes, F. Smith, AP. Bennington, JL *et al*, Alteration of gene expression in normal-appearing colon mucosa of APC(min) mice and human cancer patients. Cancer Res, 64 (2004):3694-3700

[14] Kuniyasu, H. Ohmori, H. Sasaki, T. Sasahira, T. Yoshida, K. Kitadai, Y, Fidler, IJ, Production of interleukin 15 by human colon cancer cells is associated with induction of mucosal hyperplasia, angiogenesis, and metastasis. Clin Cancer Res, 9 (2003):4802-4810

[15] Fox, SH. Whalen, GF. Sanders, MM. Burleson, JA. Jennings, K. Kurtzman, S, Kreutzer, D, Angiogenesis in normal tissue adjacent to colon cancer. J Surg Oncol, 69 (1998):230-234

[16] Cui, G. Goll, G. Olsen, T. Steigen, S. Husebekk, A. Vonen, B, Florholmen, J, Reduced expression of microenvironmental Th1 cytokines accompanies adenomas-carcinomas sequence of colorectum. Cancer Immunology & Immunotherapy, 56 (2007):985-995

[17] Cui, G. Yuan, A. Goll, R. Olsen, T. Husebekk, A. Vonen, B, Florholmen, J, Distinct changes of dendritic cell number and IL-12 mRNA level in adjacent mucosa throughout the colorectal adenoma-carcinoma sequence. Cancer Immunol Immunother, 56 (2007):1993-2001. https://doi:10.1007/s00262-007-0345-9

[18] Fantini, MC, Pallone, F, Cytokines: from gut inflammation to colorectal cancer. Curr Drug Targets, 9 (2008):375-380

[19] Sanz-Pamplona, R. Berenguer, A. Cordero, D. Mollevi, DG. Crous-Bou, M. Sole, X. Pare-Brunet, L. Guino, E. Salazar, R. Santos, C *et al*, Aberrant gene expression in mucosa adjacent to tumor reveals a molecular crosstalk in colon cancer. Mol Cancer, 13 (2014):46. https://doi:10.1186/1476-4598-13-46

[20] Pandey, S. Gordon, PH, Wang, E, Expression of proliferation-specific genes in the mucosa adjacent to colon carcinoma. Dis Colon Rectum, 38 (1995):462-467

[21] Barrier, A. Boelle, PY. Lemoine, A. Tse, C. Brault, D. Chiappini, F. Lacaine, F. Houry, S. Huguier, M. Flahault, A *et al*, Gene expression profiling of nonneoplastic mucosa may predict clinical outcome of colon cancer patients. Dis Colon Rectum, 48 (2005):2238-2248. https://doi:10.1007/s10350-005-0175-9

[22] Strasser, K. Birnleitner, H. Beer, A. Pils, D. Gerner, MC. Schmetterer, KG. Bachleitner-Hofmann, T. Stift, A. Bergmann, M, Oehler, R, Immunological differences between colorectal cancer and normal mucosa uncover a prognostically relevant immune cell profile. Oncoimmunology, 8 (2019):e1537693. https://doi:10.1080/2162402X.2018.1537693

[23] Cui, G. Yang, H. Zhao, J. Yuan, A, Florholmen, J, Elevated proinflammatory cytokine IL-17A in the adjacent tissues along the adenoma-carcinoma sequence. Pathol Oncol Res, 21 (2015):139-146. https://doi:10.1007/s12253-014-9799-1

[24] Cui, G. Li, Z. Florholmen, J, Goll, R, Dynamic stromal cellular reaction throughout human colorectal adenoma-carcinoma sequence: A role of TH17/IL-17A. Biomed Pharmacother, 140 (2021):111761. https://doi:10.1016/j.biopha.2021.111761

[25] Cui, G. Yuan, A. Goll, R. Vonen, B, Florholmen, J, Dynamic changes of interleukin-8 network along the colorectal adenoma-carcinoma sequence. Cancer Immunol Immunother, 58 (2009):1897-1905. https://doi:10.1007/s00262-009-0702-y

[26] Conciatori, F. Bazzichetto, C. Falcone, I. Ferretti, G. Cognetti, F. Milella, M, Ciuffreda, L, Colorectal cancer stem cells properties and features: evidence of interleukin-8 involvement. Cancer Drug Resistance, 2 (2019):968-979. https://doi:10.20517/cdr.2019.56

[27] Cui, G, The Mechanisms Leading to Distinct Responses to PD-1/PD-L1 Blockades in Colorectal Cancers With Different MSI Statuses. Front Oncol, 11 (2021):573547. https://doi:10.3389/fonc.2021.573547

[28] Cui, G, Towards a precision immune checkpoint blockade immunotherapy in patients with colorectal cancer: Strategies and perspectives. Biomed Pharmacother, 149 (2022):112923. https://doi:10.1016/j.biopha.2022.112923

[29] Christian, LS. Wang, L. Lim, B. Deng, D. Wu, H. Wang, XF, Li, QJ, Resident memory T cells in tumor-distant tissues fortify against metastasis formation. Cell Rep, 35 (2021):109118. https://doi:10.1016/j.celrep.2021.109118

[30] Liu, Y. Zhang, Q. Xing, B. Luo, N. Gao, R. Yu, K. Hu, X. Bu, Z. Peng, J. Ren, X *et al*, Immune phenotypic linkage between colorectal cancer and liver metastasis. Cancer cell, 40 (2022):424-437 e425. https://doi:10.1016/j.ccell.2022.02.013

[31] Lazarus, J. Maj, T. Smith, JJ. Perusina Lanfranca, M. Rao, A. D'Angelica, MI. Delrosario, L. Girgis, A. Schukow, C. Shia, J *et al*, Spatial and phenotypic immune profiling of metastatic colon cancer. JCI Insight, 3 (2018). https://doi:10.1172/jci.insight.121932

[32] Zeng, D. Wang, M. Wu, J. Lin, S. Ye, Z. Zhou, R. Wang, G. Wu, J. Sun, H. Bin, J *et al*, Immunosuppressive Microenvironment Revealed by Immune Cell Landscape in Pre-metastatic Liver of Colorectal Cancer. Front Oncol, 11 (2021):620688. https://doi:10.3389/fonc.2021.620688

[33] Shi, Y. Lin, H. Cui, J. Qi, H. Florholmen, J. Liu, Z, Cui, G, The Role of Interleukin-17A in Colorectal Tumorigenesis. Cancer Biother Radiopharm, 28 (2013):429-432. https://doi:10.1089/cbr.2012.1396

[34] Cui, G. Yuan, A. Pang, Z. Zheng, W. Li, Z, Goll, R, Contribution of IL-33 to the Pathogenesis of Colorectal Cancer. Front Oncol, 8 (2018):561. https://doi:10.3389/fonc.2018.00561

[35] Yuan, A. Chen, JJ. Yao, PL, Yang, PC, The role of interleukin-8 in cancer cells and microenvironment interaction. Front Biosci, 10 (2005):853-865

[36] Xie, K, Interleukin-8 and human cancer biology. Cytokine Growth Factor Rev, 12 (2001):375-391

[37] Itoh, Y. Joh, T. Tanida, S. Sasaki, M. Kataoka, H. Itoh, K. Oshima, T. Ogasawara, N. Togawa, S. Wada, T *et al*, IL-8 promotes cell proliferation and migration through metalloproteinase-cleavage proHB-EGF in human colon carcinoma cells. Cytokine, 29 (2005):275-282

[38] Brew, R. Erikson, JS. West, DC. Kinsella, AR. Slavin, J, Christmas, SE, Interleukin-8 as an autocrine growth factor for human colon carcinoma cells in vitro. Cytokine, 12 (2000):78-85. https://doi:10.1006/cyto.1999.0518

[39] Brew, R. Southern, SA. Flanagan, BF. McDicken, IW, Christmas, SE, Detection of interleukin-8 mRNA and protein in human colorectal carcinoma cells. Eur J Cancer, 32A (1996):2142-2147

[40] Li, A. Varney, ML, Singh, RK, Expression of interleukin 8 and its receptors in human colon carcinoma cells with different metastatic potentials. Clin Cancer Res, 7 (2001):3298-3304

[41] Rubie, C. Frick, VO. Pfeil, S. Wagner, M. Kollmar, O. Kopp, B. Graber, S. Rau, BM, Schilling, MK, Correlation of IL-8 with induction, progression and metastatic potential of colorectal cancer. World J Gastroenterol, 13 (2007):4996-5002

[42] Cui, G. Yuan, A. Goll, R. Vonen, B, Florholmen, J, Dynamic changes of interleukin-8 network along the colorectal adenoma-carcinoma sequence. Cancer Immunol Immunother, 58 (2009):1897-1905. https://doi:10.1007/s00262-009-0702-y

[43] Cui, G. Olsen, T. Christiansen, I. Vonen, B. Florholmen, J, Rasmus, G, Improvement of Real-time PCR for quantifying TNF-a mRNA expression in inflamed colorectal mucosa-An approach to optimize procedures for clinical use. The Scandinavian Journal of Clinical and Laboratory Investigation, 66 (2006):249-259

[44] Yuan, A. Steigen, SE. Goll, R. Vonen, B. Husbekk, A. Cui, G, Florholmen, J, Dendritic cell infiltration pattern along the colorectal adenoma-carcinoma sequence. Apmis, 116 (2008):445-456

[45] Waldner, MJ. Foersch, S, Neurath, MF, Interleukin-6--a key regulator of colorectal cancer development. International journal of biological sciences, 8 (2012):1248-1253. https://doi:10.7150/ijbs.4614

[46] Sheng, J. Sun, H. Yu, FB. Li, B. Zhang, Y, Zhu, YT, The Role of Cyclooxygenase-2 in Colorectal Cancer. Int J Med Sci, 17 (2020):1095-1101. https://doi:10.7150/ijms.44439

[47] Lee, YS. Choi, I. Ning, Y. Kim, NY. Khatchadourian, V. Yang, D. Chung, HK. Choi, D. LaBonte, MJ. Ladner, RD *et al*, Interleukin-8 and its receptor CXCR2 in the tumour microenvironment promote colon cancer growth, progression and metastasis. Br J Cancer, 106 (2012):1833-1841. https://doi:10.1038/bjc.2012.177

[48] Najdaghi, S. Razi, S, Rezaei, N, An overview of the role of interleukin-8 in colorectal cancer. Cytokine, 135 (2020):155205. https://doi:https://doi.org/10.1016/j.cyto.2020.155205

[49] Gelfo, V. Romaniello, D. Mazzeschi, M. Sgarzi, M. Grilli, G. Morselli, A. Manzan, B. Rihawi, K, Lauriola, M, Roles of IL-1 in Cancer: From Tumor Progression to Resistance to Targeted Therapies. Int J Mol Sci, 21 (2020). https://doi:10.3390/ijms21176009

[50] Almand, B. Resser, JR. Lindman, B. Nadaf, S. Clark, JI. Kwon, ED. Carbone, DP, Gabrilovich, DI, Clinical significance of defective dendritic cell differentiation in cancer. Clin Cancer Res, 6 (2000):1755-1766

[51] Mager, LF. Wasmer, MH. Rau, TT, Krebs, P, Cytokine-Induced Modulation of Colorectal Cancer. Front Oncol, 6 (2016):96. https://doi:10.3389/fonc.2016.00096

[52] Flavell, RA. Sanjabi, S. Wrzesinski, SH, Licona-Limon, P, The polarization of immune cells in the tumour environment by TGFbeta. Nat Rev Immunol, 10 (2010):554-567. https://doi:10.1038/nri2808

[53] Itatani, Y. Kawada, K, Sakai, Y, Transforming Growth Factor-beta Signaling Pathway in Colorectal Cancer and Its Tumor Microenvironment. Int J Mol Sci, 20 (2019). https://doi:10.3390/ijms20235822

[54] West, NR. McCuaig, S. Franchini, F, Powrie, F, Emerging cytokine networks in colorectal cancer. Nat Rev Immunol, 15 (2015):615-629. https://doi:10.1038/nri3896

[55] Li, J. Huang, L. Zhao, H. Yan, Y, Lu, J, The Role of Interleukins in Colorectal Cancer. International journal of biological sciences, 16 (2020):2323-2339. https://doi:10.7150/ijbs.46651

[56] Gunawardene, A. Dennett, E, Larsen, P, Prognostic value of multiple cytokine analysis in colorectal cancer: a systematic review. J Gastrointest Oncol, 10 (2019):134-143. https://doi:10.21037/jgo.2018.07.11

[57] Akhmaltdinova, L. Sirota, V. Babenko, D. Zhumaliyeva, V. Kadyrova, I. Maratkyzy, M. Ibrayeva, A, Avdienko, O, Proinflammatory cytokines and colorectal cancer - the impact of the stage. Contemp Oncol (Pozn), 24 (2020):207-210. https://doi:10.5114/wo.2020.102551

[58] Abancens, M. Bustos, V. Harvey, H. McBryan, J, Harvey, BJ, Sexual Dimorphism in Colon Cancer. Front Oncol, 10 (2020):607909. https://doi:10.3389/fonc.2020.607909

Table 1. Basic histological information of specimens from patients with adenoma

| | Pathology | | Dysplasia | |
|---|---|---|---|---|
| | Tubular | tubule-villous | LGD | HGD |
| Adenoma | 18 | 13 | 13 | 18 |

| | Differentiation | | | TNM classification | | | Lymph Node | |
|---|---|---|---|---|---|---|---|---|
| | Low | Moderate | High | I | II | III | Positive | Negative |
| CRC | 6 | 10 | 16 | 3 | 14 | 15 | 14 | 18 |

LGD: Low grade dysplasia.

HGD: High grade dysplasia.

Table 2. Real-time PCR primer sequences for cytokine quantification

| Assay | | Primer | Sequence |
|---|---|---|---|
| β-actin | TaqMan | Forward | 5’ TGCCGACAGGATGCAGAAG 3’ |
| | | Reverse | 5’ GCCGATCCACACGGAGTACT 3’ |
| | | Probe | FAM 5’ AGATCAAGATCATTGCTCCTCCTGAGCGC 3’ TAMRA |
| IL1β | TaqMan | Forward | 5’ CCTGAGCTCGCCAGTGAAA 3’ |
| | | Reverse | 5’ TTTAGGGCCATCAGCTTCAAA 3’ |
| | | Probe | FAM 5’ ATGGCTTATTACAGTGGCAATGAGGATGACTTG 3’ TAMRA |
| IL-6 | TaqMan | Forward | 5’ CCAGGAGCCCAGCTATGAAC 3’ |
| | | Reverse | 5’ CCCAGGGAGAAGGCAACTG 3’ |
| | | Probe | FAM 5’ CCTTCTCCACAAGCGCCTTCGGT 3’ TAMRA |
| IL-8 | TagMan | Forward | 5’ TCTTGGCAGCCTTCCTGATT 3’ |
| | | Reverse | 5’ TTTCTGTGTTGGCGCAGTGT 3’ |
| | | Probe | FAM 5’ CTGCAGCTCTGTGTGAAGGTGCAGT 3’ TAMRA |
| IL-17A | TagMan | Forward | 5’ TGATTGGAAGAAACAACGATGACT 3’ |
| | | Reverse | 5’ ATTGTGATTCCTGCCTTCACTATG 3’ |
| | | Probe | FAM 5’ TGGTGTCACTGCTACTGCTGCTGAGC3’ BHG |
| IL-23A | TagMan | Forward | 5’ CCCAAGGACTCAGGGACAAC 3’ |
| | | Reverse | 5’ TCCTAGCAGCTTCTCATAAAAAATCA 3’ |
| | | Probe | FAM 5’ TCAGTTCTGCTTGCAAAGGATCCACCAG 3’ BHQ |
| TGFβ | TagMan | Forward | 5’ CTGCTGAGGCTCAAGTTAAAAGTG 3’ |
| | | Reverse | 5’ TGAGGTATCGCCAGGAATTGT 3’ |
| | | Probe | FAM 5’ CAGCACGTGGAGCTGTACCAGAAATACAGC3’ BHQ |
| COX2 | TagMan | Forward | 5’ GAATCATTCACCAGGCAAATTG 3’ |
| | | Reverse | 5’ TTTCTGTACTGCGGGTGGAAC 3’ |
| | | Probe | FAM 5’ TTCCTACCACCAGCATCCCTGCCA 3’ TAMRA |

Table 3. Immunological factor difference (Δ) between adenoma and adjacent tissues

| Factors | Control | Adenoma tissues | Adjacent tissues | *P* | Δ(Adenoma-adjacent) |
|---|---|---|---|---|---|
| IL-1β | 1.23±0.25 | 23.93±10.01 | 3.88±0.93 | <0.01 | 20.05±9.974 |
| IL-6 | 5.73±2.78 | 63.08±23.44 | 5.34±1.07 | <0.01 | 57.73±22.8 |
| IL-8 | 2.25±0.64 | 111±40.81 | 17.04±6.20 | <0.01 | 93.95±41.4 |
| IL-17A | 1.53±0.39 | 42.62±23.14 | 3.14±0.84 | <0.01 | 39.48±22.69 |
| IL-23A | 1.92±0.45 | 6.37±2.17 | 0.95±0.43 | <0.01 | 5.43±2.18 |
| TGFβ | 1.24±0.20 | 1.66±0.30 | 11.49±4.94 | <0.01 | -9.84±4.95 |
| COX2 | 4.61±2.36 | 22.58±3.97 | 5.85±1.70 | <0.01 | 16.12±4.71 |

Table 4. Immunological factor difference (values of Δ) between adenoma and adjacent tissues against adenoma pathological features

| Δ factors | Histology | | *P1* | Dysplastic degree | | *P2* |
|---|---|---|---|---|---|---|
| | Tubular | Tubulovillous | | LGD | HGD | |
| IL-1β | 21.61±12.83 | 16.26±13.34 | >0.05 | 11.69±8.32 | 28.40±17.75 | >0.05 |
| IL-6 | 54.66±22.39 | 66.63±61.31 | >0.05 | 59.37±38.72 | 55.97±24.30 | >0.05 |
| IL-8 | 82.12±51.12 | 130.5±82.56 | >0.05 | 81.95±52.61 | 105.9±65.95 | >0.05 |
| IL-17A | 3.08±1.09 | 3.28±1.37 | >0.05 | 2.46±0.96 | 3.75±1.37 | >0.05 |
| IL-23A | 1.18±0.62 | 0.44±0.22 | >0.05 | 1.63±1.18 | 0.56±0.13 | >0.05 |
| TGFβ | -9.84±6.43 | -9.84±6.52 | >0.05 | -12.14±6.66 | -3.40±1.09 | >0.05 |
| COX2 | 18.35±5.51 | 10.33±9.57 | >0.05 | 5.03±5.52 | 27.22±5.75 | <0.05 |

Table 5. Immunological factor difference (Δ) between CRC and tumor-free adjacent tissues

| Factors | Control | CRC tissues | Adjacent tissues | *P* | Δ (CRC-adjacent) |
|---|---|---|---|---|---|
| IL-1β | 1.23±0.25 | 68.83±24.69 | 10±3.17 | <0.01 | 58.84±25.27 |
| IL-6 | 5.73±2.78 | 1066±463 | 94.59±46.52 | <0.01 | 958.8±486.4 |
| IL-8 | 2.25±0.64 | 2842±1247 | 378±227.4 | <0.01 | 2464±1291 |
| IL-17A | 1.53±0.39 | 33.96±19.28 | 6.52±2.46 | <0.01 | 27.44±19.72 |
| IL-23 | 1.92±0.45 | 15.97±4.27 | 0.53±0.11 | <0.01 | 15.44±4.26 |
| TGFβ | 1.24±0.20 | 5.53±2.43 | 1.83±0.46 | <0.01 | 3.74±2.42 |
| COX2 | 4.61±2.36 | 1405±1031 | 23.87±9.56 | <0.01 | 1381±1032 |

Table 6. Immunological factor difference (values of Δ) against CRC TNM stages and invasion degree

| Δ factors | TNM | | | *P1* | Invasion | | *P2* |
|---|---|---|---|---|---|---|---|
| | I | II | III | | T1+T2 | T3+T4 | |
| IL-1β | 12.88±10.86 | 1.50±7.42 | 151.6±61.32 | <0.05 | 80.97±76.69 | 52.88±25.51 | >0.05 |
| IL-6 | -11.7±72.49 | 169±213.2 | 2374±1219 | >0.05 | 1355±1368 | 875±541.4 | >0.05 |
| IL-8 | 1089±1050 | 1385±1483 | 4710±3142 | >0.05 | 4873±4450 | 1699±902.4 | >0.05 |
| IL-17A | 3.55±2.27 | 10.05±7.96 | 8.65±10.25 | >0.05 | 3.55±2.27 | 9.36±6.23 | >0.05 |
| IL-23 | 6.53±2.50 | 12.01±3.98 | 22.55±9.48 | >0.05 | 0.36±0.09 | 3.95±2.86 | >0.05 |
| TGFβ | 1.13±0.46 | 1.86±1.35 | 7.12±6.24 | >0.05 | 1.13±0.46 | 4.35±3.04 | >0.05 |
| COX2 | -10.89±38.45 | 114.7±117.2 | 3327±2511 | >0.05 | -21.08±29.04 | 1731±1283 | <0.05 |

Table 7. Immunological factor difference (Δ) between CRC and adjacent tissues against CRC differentiation degree and lymph node metastasis

| Δ factors | Differentiation | | | *P1* | Lymph node | | *P2* |
|---|---|---|---|---|---|---|---|
| | Low | Moderate | High | | Positive | Negative | |
| IL-1β | 171.1±86.76 | 7.24±9.18 | 39.94±29.33 | >0.05 | 142.2±57.18 | 4.624±6.35 | <0.01 |
| IL-6 | 3422±2182 | 79.44±183.8 | 472.7±266.6 | >0.05 | 2374±1219 | 118.9±154.6 | <0.05 |
| IL-8 | 9490±8623 | 3327±2398 | 654.2±470.1 | >0.05 | 4249±2708 | 1242±1107 | >0.05 |
| IL-17A | -13.18±12.53 | -1.81±2.52 | 19.96±6.67 | <0.01 | 8.49±8.88 | 8.19±6.21 | >0.05 |
| IL-23A | 25.48±20.93 | 18.50±6.47 | 11.40±4.63 | >0.05 | 20.06±8.43 | 11.64±3.20 | >0.05 |
| TGFβ | 2.02±1.01 | 11.99±9.81 | 0.96±0.45 | >0.05 | 6.21±5.28 | 1.67±1.0 | >0.05 |
| COX2 | 1112±984.7 | 189.4±136.2 | 152.9±156.6 | >0.05 | 3327±2511 | 83.27±88.50 | <0.05 |

**Figure legends**

**Fig. 1. The impact of Δ values of these immunological factors on the survival rate after surgery in patients with CRC**

Kaplan–Meier analysis showed that Δ values of IL-1β between the tumor tissue and tumor-free tissue were associated with the survival rate after surgery (Fig. 1A), but Δ values of other factors (Fig. 1B-G) did not affect the survival rate after surgery in patients with CRC (*P* values were obtained from the log-rank test).

**Fig. 2. Schematic summarized the difference of protumor force between tumor center and adjacent tumor-free tissues**

Present data suggests that protumor force is strong inside the tumor center and antitumor force is present in the tumor-free adjacent tissues, they combat in the invading front and determine the progression and metastasis in the CRC.