Immunological Differences Between Right-Sided and Left-Sided Colorectal Cancers: A Comparison of Embryologic Midgut and Hindgut

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Purpose: There are known differences in embryology, clinical symptoms, incidences, molecular pathways involved, and oncologic outcomes of right-sided and left-sided colorectal cancers. However, immunologic study has only been characterized for healthy adults. The present study was designed to identify differences in immune cell populations in patients with right-sided and left-sided colorectal cancers.

Methods: A total of 35 patients who underwent colorectal resection for cancer between November 2016 and August 2017 at a tertiary teaching hospital were enrolled in this study. Patients were excluded if they had a disease affecting their immune system. Populations of immune cells, including mucosal-associated invariant T (MAIT), gamma delta T, invariant natural killer T, T, natural killer, and B cells, were measured in the peripheral blood and cancer tissues using flow cytometry, and then assessed based on the origin of the colorectal cancer.

Results: Fifteen had right-side and 20 had left-side colorectal cancer. There were no significant differences between the 2 cohorts for patient characteristics including pathologic stage. Peripheral blood from patients with right-side colon cancers contained fewer MAIT (0.87% right-side vs. 1.74% left-side, P = 0.028) and gamma delta T cells (1.10% right-side vs. 3.05% left-side, P = 0.002). Although the group with right-side colorectal cancer had more MAIT cells in cancer tissues (1.71% vs. 1.00%), this difference was not statistically significant.

Conclusion: There is a difference in population sizes of immune cells in blood between patients with right-sided and left-sided colon cancers. The immune cell composition was determined to be distinct based on embryologic origin.

Keywords: Colorectal cancer; Embryology; Sidedness; Immune cell

INTRODUCTION

During embryonic development, the colon originates from the midgut and hindgut, and becomes differentiated into the right-sided and left-sided colon based on the location. Tumors in the cecum, ascending colon, and proximal part of the transverse colon are defined as midgut tumors, while those in the distal transverse, descending, sigmoid colon, and rectum are considered hindgut tumors. Differences have been reported between right-sided and left-sided colon cancers for clinical symptoms [1], incidences [1], molecular pathways involved [2], and oncologic outcomes [1, 3-5], as well as embryologic origins. However, immunological differences in the right-sided and left-sided colons have only been reported in healthy adults [6]. Studies have shown the short- and long-term outcomes of colorectal cancers depend on the location of the cancer and, therefore, it is argued the treatment plans for these cancers should be distinct. Meanwhile, cells involved in mucosal immunity have been recently discovered [7]. Therefore, the present study aimed to identify immunological differences between patients with right-sided and left-sided colon cancers.
METHODS

The patients enrolled in this study were aged at least 18 years and diagnosed with colorectal cancer. Patients with diseases that could directly affect or that require treatments that affect immune cell populations, such as unregulated diabetes, tuberculosis, autoimmune diseases, and treatments involving immunosuppressants, were excluded. The embryologic boundary of sidedness has not been clearly defined. Therefore, patients with transverse colon, mid- and lower rectal cancers were also excluded. A total of 35 patients were included in this study and were enrolled between November 2016 and August 2017.

Patients’ clinical data, including age, sex, body mass index, American Society of Anesthesiologists physical status classification, previous medical history, surgical history, operation duration, and volume of estimated blood loss were analyzed and compared between cohorts. Pathologic outcomes assessed were tumor size and stage, including lymph node status. Postoperative outcomes, including days to bowel movement and diet resumption, length of hospital stay, and postoperative complications, were also compared between the groups.

The immune cell populations were measured based on the presence of cell surface antigens using a cell analyzer and the Kaluza program by separating peripheral blood mononuclear cells (PBMCs) from peripheral blood and cancer tissue samples, staining for 20 minutes with various monoclonal antibodies, and then analyzing by flow cytometry. The immune cells in the colonic mucosa included mucosal-associated invariant T (MAIT), gamma delta (γδ) T, invariant natural killer T (iNKT), T, natural killer (NK), and B cells. Immune cells are known to be difficult to store because their phenotype may change when exposed to changes in temperature. Therefore, peripheral blood collected just before surgery was immediately transferred aseptically to the laboratory for analysis.

PBMCs were isolated by density-gradient centrifugation with Ficoll-Paque Plus solution (Amersham Bioscience, Uppsala, Sweden) using peripheral venous blood samples collected in heparin-containing tubes. Total lymphocyte numbers were measured by Coulter LH750 automatic hematology analyzer (Beckman Coulter, Miami, FL, USA). MAIT cells were phenotypically identified by flow cytometry using CD3+TCRγδ-Vα7.2+CD161\textsuperscript{high}. The absolute number of MAIT cells was obtained by multiplying the total lymphocyte number per microliter and the percentage of the CD3+γδ-T cells in MAIT cell. PBMCs were stained with PE-conjugated anti-CD3, FITC-conjugated anti-TCR γδ, APC-conjugated anti-TCR Va7.2, and PE-Cy5-conjugated anti-CD161 to sort the MAIT cells. Then, CD3+TCRγδ-Va7.2+CD161\textsuperscript{high} MAIT cells were obtained with FACS Aria I sorter (BD Biosciences, Mountain View, CA, USA). The MAIT cells were separated over 98% purity with this process. The cancer tissues were taken from the central lesion such as fungating or ulcero-fungating area with a size of 1 cm × 1 cm without including the deep area for verification of stage T. Single-cell suspension was prepared on 40-μm cell strainers within 30 minutes after resection from patients for flow cytometry. The specimen was fixed with neutral buffered formalin, then was embedded in paraffin for immunofluorescence staining. Immune cells were analyzed and compared in the peripheral venous blood, however, cancer tissues were only analyzed those being showed difference in blood samples.

All patients were fully informed and consent was obtained prior to enrollment in this study. This study was approved by the Institutional Review Board (CNUH-2017-066) at Chonnam National University Hospital.

Statistical analysis

Differences between groups were assessed using Student t-test or the Mann-Whitney U-test for continuous data and the chi-square or Fisher exact test for categorical data. Statistical analyses were performed using IBM SPSS Statistics ver. 20.0 (IBM Co., Armonk, NY, USA). A P-value ≤ 0.05 was considered statistically significant.

RESULTS

Patient characteristics and perioperative results

Of the 35 patients enrolled in this study, 15 (42.9%) had right- and 20 had left-sided colon cancer. The following parameters were analyzed, but did not differ significantly between the groups: mean age, sex, body mass index, American Society of Anesthesiologists physical status classification, past medical history, previous abdominal operative history, and preoperative carcinoembryonic antigen level (Table 1). Surgical factors, such as operative methods and average operating time, did not differ between the 2 groups. The average length of hospital stay (overall average, 8.4 days; 9 days right-side vs. 8.2 days left-side, P = 0.771), complications during hospitalization, and pathologic stage (P = 0.379) of the patients were also not statistically different (Table 2).

Mucosal immune cell populations

Peripheral blood from patients with right-side colon cancers contained fewer MAIT (0.87% right-side vs. 1.74% left-side, P = 0.028) and γδ T cells (1.10% right-side vs. 3.05% left-side, P = 0.002). No significant differences were observed in the other immunologic cell types assessed: iNKT, T, NK, and B cells. While the patients with right-side cancer had more MAIT cells in cancer tissues (1.71% right-side vs. 1.00% left-side, P = 0.256), this difference was not statistically significant (Table 3).

DISCUSSION

In the present study, it was found mucosal-associated immune cells, such as MAIT and γδ T cells, had differences in distribution based on whether patients had right- or left-side tumors. A 2015 review [2] reported they had differences such as frequency, histol-
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Immunology, oncology, and immunology depending on the location of the cancer. However, immune factors have only been compared in the right-sided and left-sided colon in healthy adults [6], not in patients with colon cancer. Therefore, to the best of our knowledge, this is the first report to assess distribution differences in immune cells in patients based on the side of colon cancer location. Studies of sidedness have only identified differences in outcomes and reactions to chemotherapy. In addition, these findings are controversial because bias may have been introduced through range of resection. The present study may reveal fundamental differences between right-sided and left-sided colorectal cancers because it was carried out preoperatively or before administration of chemotherapy. It is expected this study will serve as the basis for further studies into the antitumor effect of immune cells and ongoing research on functional differences. In addition, future work may include identifying biologic markers for disease prediction, evaluating response to adjuvant treatments, and determining prognosis of patients with colorectal cancer.

The outcomes on sidedness of colorectal cancer are still debatable. The American College of Surgeons National Surgical Quality Improvement Program database showed no differences in comparison of short-term outcomes between the groups only with the exception of the incidence of superficial surgical site infection, which was more prevalent in left-side colon cancer [8]. In 2011, the Surveillance, Epidemiology, and End Results Program

Table 1. Patients’ characteristics

| Characteristic                  | Right          | Left           | P-value |
|--------------------------------|----------------|----------------|---------|
| Age (yr)                       | 66.73 ± 2.27   | 64.43 ± 2.05   | 0.493   |
| Sex                            | 0.828          |                |         |
| Male                           | 9 (60)         | 19 (63.3)      |         |
| Female                         | 6 (40)         | 11 (36.7)      |         |
| Body mass index (kg/m²)        | 22.55 ± 0.95   | 23.31 ± 0.59   | 0.483   |
| ASA PS classification          |                |                | 0.659   |
| I                              | 3 (20)         | 5 (16.5)       |         |
| II                             | 11 (73.3)      | 20 (67)        |         |
| III                            | 1 (6.7)        | 5 (16.5)       |         |
| Medical history                |                |                | 0.783   |
| Yes                            |                |                |         |
| Endocrine disease (DM)         | 3 (20)         | 5 (16.7)       |         |
| Cardiac disease (HTN)          | 8 (53)         | 14 (46.7)      |         |
| Pulmonary disease              | 1 (6.7)        | 16 (53.3)      |         |
| No                             | 3 (20)         | 5 (16.7)       |         |
| Previous abdominal operative history | 6 (40)       | 15 (50)        | 0.526   |
| Substance use                  |                |                |         |
| Smoking                        | 2 (18.2)       | 5 (23.8)       | 0.715   |
| Alcohol                        | 3 (27.3)       | 6 (28.6)       | 0.938   |
| Preop CEA (ng/mL)              | 13.7 ± 5.9     | 9.48 ± 3.6     | 0.515   |

Values are presented as mean ± standard deviation or number (%).

Table 2. Operative and postoperative variables

| Diagnosis                        | Right          | Left           | P-value |
|----------------------------------|----------------|----------------|---------|
| Ascending colon                  |                |                |         |
| Hepatic flexure colon            |                |                |         |
| Proximal transverse colon        |                |                |         |
| Splenic flexure colon            |                |                |         |
| Descending colon                 |                |                |         |
| Sigmoid colon                    |                |                |         |
| Rectosigmoid junction            |                |                |         |
| Upper rectum                     |                |                |         |
| Operative methods                |                |                | 0.053   |
| Open                             | 12 (80)        | 15 (50)        |         |
| Laparoscopy                      | 3 (20)         | 15 (50)        |         |
| Operating time (min)             | 104.4 ± 13.7   | 125.7 ± 8.0    | 0.161   |
| Staging                          |                |                | 0.379   |
| 0                                | 3 (20)         | 1 (3.3)        |         |
| I                                | 4 (26.7)       | 6 (20)         |         |
| II                               | 4 (26.7)       | 10 (33.3)      |         |
| III                              | 3 (20)         | 10 (33.3)      |         |
| IV                               | 1 (6.7)        | 3 (10)         |         |
| Discharge (day)                  | 9.0 ± 2.1      | 8.2 ± 1.6      | 0.771   |
| Postop complications             |                |                |         |
| Postoperative ileus              | 3 (20)         | 6 (20)         | 1       |
| Urinary incontinence             | 3 (20)         | 4 (13.3)       | 0.561   |
| Surgical site infection          | 2 (13.3)       | 3 (10)         | 0.737   |

Values are presented as number (%) or mean ± standard deviation.

Table 3. Immunologic cell analysis

| Variable                          | Right          | Left           | P-value |
|-----------------------------------|----------------|----------------|---------|
| MAIT cell, blood (%)              | 0.87 ± 0.18    | 1.74 ± 0.34    | 0.028   |
| γδ T cell, blood (%)              | 1.10 ± 0.24    | 3.05 ± 0.52    | 0.002   |
| iNKT cell, blood (%)              | 0.15 ± 0.29    | 0.16 ± 0.20    | 0.915   |
| T cell, blood (%)                 | 59.01 ± 4.98   | 56.78 ± 2.94   | 0.683   |
| NK cell, blood (%)                | 27.95 ± 5.76   | 33.90 ± 3.33   | 0.346   |
| B cell, blood (%)                 | 11.08 ± 2.32   | 8.25 ± 1.92    | 0.370   |
| MAIT cell, tissue (%)             | 1.71 ± 0.56    | 1.00 ± 0.11    | 0.256   |

Values are presented as mean ± standard deviation.

MAIT, mucosal-associated invariant T; γδ T, gamma delta T; iNKT, invariant natural killer; NK, natural killer.
MAIT cells play a crucial role in colon cancer, with a study in 2010 showing that right-side colon cancers are more likely to be at a higher stage (P < 0.001) than left-side colon cancers. This study also found that left-side colon cancers had more frequent metastasis to the abdominal cavity and were associated with a lower survival rate (P < 0.01). It was also found that left-side colon cancers had a higher distribution of circulating MAIT cells compared to right-side colon cancers.

Studies on the roles of immune cells in colon cancer are frequently being published. According to a study published in 2012 using the mouse colitis-associated colon cancer model, iNKT cells inhibit tumor progression [11]. A study aiming to decrease colorectal metastasis in mice found more tumor-infiltration of activated CD8+ T cells and fewer regulatory T cells in the spleen than in patients with colorectal cancers with distinct embryologic origins. In conclusion, the proportion of specific immune cells present in patients with colorectal cancers with distinct embryologic origins was confirmed to be different.

CONFLICT OF INTEREST

There were no potential conflicts of interest relevant to this article.

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