Research Article

Immune Condition of Colorectal Cancer Patients Featured by Serum Chemokines and Gene Expressions of CD4+ Cells in Blood

Takuya Komura,1 Masaaki Yano,2 Akimitsu Miyake,3 Hisashi Takabatake,2 Masaki Miyazawa,2 Norihiko Ogawa,1 Akihiro Seki,1 Masao Honda,4 Takashi Wada,5 Shigeyuki Matsui,3 Shuichi Kaneko,1,4 and Yoshio Sakai4

1System Biology, Graduate School of Advanced Preventive Medical Sciences, Kanazawa University, Japan
2Disease Control and Homeostasis, College of Medical Pharmaceutical and Health Sciences, Kanazawa University, Japan
3Department of Biostatistics, Nagoya University Graduate School of Medicine, Japan
4Department of Gastroenterology, Kanazawa University Hospital, Kanazawa, Japan
5Department of Nephrology, Kanazawa University Hospital, Kanazawa, Japan

Correspondence should be addressed to Yoshio Sakai; yoshios@m-kanazawa.jp

Received 22 January 2018; Revised 20 April 2018; Accepted 9 May 2018; Published 11 June 2018

Academic Editor: Maria Gavriatopoulou

Copyright © 2018 Takuya Komura et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Colorectal cancer (CRC), the most common malignancy worldwide, causes inflammation. We explored the inflammatory pathophysiology of CRC by assessing the peripheral blood parameters. Methods. The differences in gene expression profiles of whole blood cells and cell subpopulations between CRC patients and healthy controls were analyzed using DNA microarray. Serum cytokine/chemokine concentrations in CRC patients and healthy controls were measured via multiplex detection immunoassays. In addition, we explored correlations between the expression levels of certain genes of peripheral CD4+ cells and serum chemokine concentrations. Results. The gene expression profiles of peripheral CD4+ cells of CRC patients differed from those of healthy controls, but this was not true of CD8+ cells, CD14+ cells, CD15+ cells, or CD19+ cells. Serum IL-8 and eotaxin-1 levels were significantly elevated in CRC patients, and the levels substantially correlated with the expression levels of certain genes of CD4+ cells. Interestingly, the relationships between gene expression levels in peripheral CD4+ cells and serum IL-8 and eotaxin-1 levels resembled those of monocytes/macrophages, not T cells. Conclusions. Serum IL-8 and eotaxin-1 concentrations increased and were associated with changes in the gene expression of peripheral CD4+ cells in CRC patients.

1. Introduction

Colorectal cancer (CRC) is the third most common fatal malignancy worldwide [1]. It is important to detect CRC early to improve prognosis [2, 3]. Currently, the fecal occult blood test (FOBT) for in vitro diagnostic use is used to screen for CRC; however, the positive predictive rate is poor [3]. Colonoscopy is superior, but this is invasive and associated with multiple complications including perforation, pain, and discomfort [4]. Thus, alternative noninvasive diagnostic tests are required. To this end, it is necessary to understand the pathological features, including the immune status, of CRC patients.

We previously reported that the gene expression profiles of peripheral blood cells from patients with cancers of the digestive system differed from those of noncancerous controls [5]. Peripheral blood contains many types of immune cells including neutrophils, monocytes, and macrophages [6]. Changes in gene expression are hypothesized to reflect the reactions of the immune system to cancer, because cancer is frequently associated with the appearance of various types of inflammatory cells [7]. These include helper T cells and cytotoxic T lymphocytes [8], which inhibit cancer progression, and myeloid-derived suppressor cells [9], regulatory T cells [10], and programmed cell death 1 (PD-1) expressing T cells [11], which promote cancer development. We previously
reported that immune response-eliciting or immunosuppressive molecules mediated interactions between circulating peripheral blood cells and local cancer tissues in patients with pancreatic ductal adenocarcinomas [12] and hepatocellular carcinomas [13, 14]. In contrast, the features of immune pathophysiology reflected in peripheral blood of colorectal cancer have yet to be investigated.

Here, we observed that the gene expression profiles of peripheral CD4+ cells and whole blood cells of CRC patients differed from those of healthy controls. The serum concentrations of IL-8 and eotaxin-1 were elevated in CRC patients compared to healthy controls.

2. Methods

2.1. CRC Patients and Healthy Controls. Blood was drawn from CRC patients prior to treatment and from healthy controls. A total of 30 CRC patients and 28 healthy controls (Supplemental Table 1) provided serum samples for cytokine and chemokine analyses. CRC was clinically staged using the tumor, node, and metastasis staging system of the Union of International Cancer Control (8th edition). Five CRC patients and seven healthy volunteers donated peripheral blood for gene expression analyses (Supplemental Table 2). Serum cytokine/chemokine levels were measured in four CRC patients and five healthy volunteers (Supplemental Table 2). Written informed consent was obtained from all participants. This study was approved by our Institutional Review Board and was performed in accordance with all relevant tenets of the Declaration of Helsinki.

2.2. Serum Cytokine and Chemokine Analyses. Serum cytokine and chemokine levels were measured using a Bio-Plex human cytokine 27-plex panel (Bio-Rad, Tokyo, Japan) according to the manufacturer's instructions. This kit was used to detect interleukin- (IL-) 1β, IL-6, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, fibroblast growth factor-basic, eotaxin-1, G-CSF, granulocyte colony stimulating factor, IFN-γ, IP-10, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein- (MIP-) 1α, MIP-1β, platelet-derived growth factor-BB, tumor necrosis factor- (TNF-) α, and vascular endothelial growth factor.

2.3. Isolation of Peripheral Blood Mononuclear Cells. Peripheral blood mononuclear cells were isolated from heparinized venous blood via Ficoll-Hypaque (Sigma-Aldrich, St. Louis, MO, USA) density gradient centrifugation, as previously described [13]. Then the cells were incubated with bead-labeled anti-CD4, anti-CD8, anti-CD14, or anti-CD15 antibodies (Miltenyi, Cologne, Germany) and isolated using a magnet.

2.4. DNA Microarray and Data Analysis. PAXgene® Blood RNA Tubes (PreAnalytiX GmbH, Germany) were used to collect samples for mRNA extraction. Total RNA was isolated from subfractionated peripheral blood cells using a microRNA isolation kit (Stratagene, La Jolla, CA, USA). Isolated RNA was labeled with Cy3 using the Quick-Amp Labeling Kit (Agilent Technologies, Palo Alto, CA, USA) and hybridized to the Whole Human Genome Microarray kit, 4x44K (Agilent Technologies). The slides were scanned using a microarray scanner (Model G2505B; Agilent Technologies), and gene expression analyses were performed using the BRB array tools (NCI, http://linus.nci.nih.gov/BRB-ArrayTools.html). Hierarchical clustering of gene expression data was used to identify differentially expressed genes. Biological processes and networks were analyzed with the aid of the MetaCore® software suite (GeneGo, Carlsbad, CA, USA).

2.5. Statistical Analysis. The unpaired Student's t-test was used to assess differences between groups, and a p < 0.05 was considered statistically significant. Pearson correlations between IL-8 and eotaxin-1 levels and clinical parameters were calculated. Spearman correlations were derived to explore associations between changes in chemokine concentrations and genes that were differentially expressed in peripheral CD4+ cells of CRC patients and healthy volunteers.

3. Results

3.1. Serum IL-8 and Eotaxin-1 Levels in CRC Patients. First, we measured cytokine and chemokine levels in 30 CRC patients and 28 healthy controls using a bead-based multiplex immunoassay (Supplemental Table 1). The serum concentrations of IL-8 and eotaxin-1 were significantly elevated in CRC patients (n=30) compared to healthy controls (n=28) (Figures 1(a) and 1(b)). However, we did not observe an increase of the other proinflammatory cytokines such as TNF-α, IFN-γ, and IL-12, of a decrease of anti-inflammatory cytokines such as IL-10 (Supplemental Fig. 2).

IL-8 concentrations were only elevated in patients of advanced clinical stage (Stage IV; Figure 1(c); eotaxin-1 levels did not differ by clinical stage (Figure 1(d)). IL-8 concentrations correlated with those of CEA (Figure 1(e)) and CA19-9 (Figure 1(f)) (r=0.577273 and r=0.591704, respectively), whereas eotaxin-1 concentrations did not (r=0.008045 and r=-0.06421, respectively) (data not shown). No correlation between the level of any other serum cytokine/chemokine and stage or level of tumor marker CEA or CA19-9 was apparent (data not shown).

3.2. Gene Expression Profiling of Peripheral Blood Cells from CRC Patients. Next, we used DNA microarray to determine if gene expression was altered in peripheral blood cells of five CRC patients and seven healthy volunteers (Supplemental Table 2). Unsupervised clustering analyses revealed a difference in the gene expression patterns of whole blood (Figure 2(a)) and CD4+ cells (Figure 2(b)), but not of CD8+ (Figure 2(c)), CD14+ (Figure 2(d)), or CD15+ cells (Figure 2(e)).

3.3. Elevated Serum IL-8 and Eotaxin-1 Concentrations Were Significantly Correlated with Genes Expression, the Levels of...
Which Were Altered in the Peripheral CD4+ Cells of CRC Patients. The data described above suggested that expression of the humoral chemokines, eotaxin-1 and IL-8, played a role in the inflammation of CRC patients. In addition, both peripheral CD4+ cells and whole blood cells were affected. Therefore, we derived Spearman’s correlations between the serum concentrations of IL-8 and eotaxin-1 and the expression levels of 8,061 genes, the levels of which were altered (FDR < 0.05) in CD4+ cells of four CRC patients compared to five healthy volunteers (Supplemental Table 2). We also confirmed that the serum concentrations of IL-8 (Supplemental Fig. 1A), eotaxin-1 (Supplemental Fig. 1B), MIP-1a (Supplemental Fig. 1C), and MCP-1 (Supplemental Fig. 1D) were significantly increased in the sera of CRC patients compared to healthy controls. The distribution frequencies of the 8,061 genes in terms of their Spearman correlations with eotaxin-1 levels are shown in Figure 3(a). Notably, the expression levels of all 8,061 genes correlated with serum eotaxin-1 concentrations. A total of 1,063 of these genes were involved in cell adhesion, inflammation, and the immune response (e.g., MHC, CD1d, TLR4, IL-15, Fc gamma, and Hck; Table 1). A total of 974 genes, the expression levels of which were negatively correlated with eotaxin-1 concentrations, were involved in proteolysis, development, and reproduction (Table 2). These biological processes are characteristics of monocytes and macrophages rather than T cells. The distribution frequencies of the 8,061 genes in terms of their Spearman correlations with serum IL-8 concentrations are shown in Figure 3(b). Almost all genes were so correlated. A total of 250 genes expressed in peripheral CD4+ cells were positively correlated with the serum IL-8 concentration, the genes played roles in cell adhesion, inflammation, the immune response, cytoskeletal processes, and development (Table 3). The expression levels of 586 genes were negatively correlated with serum IL-8 concentration; these genes were involved in cell proliferation, development, and reproduction (Table 4). These biological processes were also characteristic of monocytes and macrophages, rather than T cells. Overall, the serum levels of eotaxin-1 and IL-8 in CRC patients substantially correlated with the expression levels of certain genes in peripheral CD4+ cells compared to healthy controls.

4. Discussion

Based on our previous findings that the immune pathophysiology of digestive system cancers is reflected in peripheral
blood, we investigated the inflammatory conditions of CRC patients by assessing cytokine/chemokine and performing gene expression analyses of peripheral blood using bead-based multiplex immunoassay and DNA microarray, respectively. Gene expression in peripheral CD4+ and whole blood cells differed between CRC patients and healthy controls [5]. The serum levels of eotaxin-1 and IL-8 were significantly elevated in CRC patients, and the levels significantly correlated with changes in the gene expression levels in CD4+ cells.
Table 1: Biological process networks for 1063 genes whose expression in peripheral CD4+ cells was positively correlated with serum eotaxin concentration.

| Networks                              | Total | P value     | False discovery rate | In data | Network objects from active data                                                                 |
|---------------------------------------|-------|-------------|-----------------------|---------|--------------------------------------------------------------------------------------------------|
| **Immune response**                   |       |             |                       |         |                                                                                                  |
| Phagocytosis                           | 222   | 2.27E-08    | 1.81E-06              | 33      | ITGB2, Syk, IL-15, RhoA, Myosin I, C/EBP, Dectin-1, Fc gamma RII beta, IIT4, MSN (moesin), ERM proteins, SHPS-1, Fc epsilon RI gamma, MSRI, MANR, Lyn, PLC-gamma 2, IL-15RA, Btk, IIT2, Hck, TLR4, MARCO, MARCKS, MLCK, PLC-gamma, gp91-phox, p40-phox, PAK1, p67-phox, FGR, Fc alpha receptor, Fc gamma RII alpha |
| **Cell adhesion**                      |       |             |                       |         |                                                                                                  |
| Platelet aggregation                   | 158   | 2.34E-08    | 1.81E-06              | 27      | ITGB2, Syk, GAB2, PLA2, RhoA, Thrombospondin 1, G-protein beta/gamma, COX-1 (PTGS1), Fc epsilon RI gamma, c-Src, ENPI, THAS, cPLA2, PTAfVR, Lyn, PLC-gamma 2, PKA-reg (cAMP-dependent), GP-IX, P2Y12, Gab, G-protein alpha-i family, G-protein alpha-12, P2X1, MLCK, PLC-gamma, CD36, VAV-2 |
| **Cell adhesion**                      |       |             |                       |         |                                                                                                  |
| Amyloid proteins                       | 195   | 6.02E-06    | 3.09E-04              | 26      | RhoA, FZD1, Nicastrin, NOTCH2, APLP2 active fragment, G-protein beta/gamma, jagged1, Nidogen, p120-catenin, Tcf(Lef), c-Src, Presenilin 2, FZD2, FZD5, Notch, Presenilin 1, Alpha-catenin, APLP2 precursor, Cathepsin D, MALS, Frizzled, ADAM9, PKC, PAK1, Plakoglobin, Presenilin |
| **Immune response**                   |       |             |                       |         |                                                                                                  |
| Antigen presentation                   | 197   | 6.23E-05    | 2.40E-03              | 24      | CIITA, ICAM1, MHC class II beta chain, CD1b, HLADPAI, CD1d, HLA-DQA1, JAK2, HA2Z, Fc epsilon RI gamma, IP-30, HLA-DM, HLA-DRB1, CD1a, Cathepsin S, LFA-3, MHC class II, HLA-DQB1, HLA-DRD1, HLA-DRB4, CD86, CD74, RING6 |
| **Proliferation**                      |       |             |                       |         |                                                                                                  |
| Positive regulation cell proliferation | 221   | 1.48E-04    | 4.56E-03              | 25      | p21, HGF, RhoG, Beta-arrestin1, GAB2, Fra-1, RhoA, Galphai-specific peptide GPCRs, JAK2, MTG16 (CBFA2T3), c-Src, VEGF-A, RasGAP4, PKA-reg (cAMP-dependent), G-protein alpha-15, TCIRGI (Atp6i), CCR1, G-protein alpha-i family, M-CSF receptor, G-protein alpha-12, CSDA, MLCK, c-Fes, FLT3, PAK1 |
| **Chemotaxis**                         | 137   | 2.00E-04    | 5.14E-03              | 18      |                                                                                                  |
| **Inflammation**                       |       |             |                       |         |                                                                                                  |
| IFN-gamma signaling                    | 109   | 4.08E-04    | 8.97E-03              | 15      | CIITA, p21, ITGB2, IL-15, IL-18, ICAM1, PKC-delta, KL2, JAK2, MIG, c-Src, PLC-gamma 2, TLR4, PLC-gamma, Fc alpha receptor |
| **Apoptosis**                          | 111   | 4.97E-04    | 9.57E-03              | 15      | IL-15, MyD88, G-protein beta/gamma, TNF-R2, VEGF-A, CD30(TNFSF8), CSF2RA, PKA-reg (cAMP-dependent), IL-15RA, G-protein alpha-i family, TLR4, Bcl-3, TL1A(TNFSF15), APRIL(TNFSF13), BAFF(TNFSF13B) |
| Anti-apoptosis mediated by external signals via NF-kB | 215 | 6.08E-04 | 1.02E-02 | 23 | ITGB2, CSaR, PLA2, ICAM1, GRO-2, RhoA, PKC-delta, G-protein beta/gamma, TNF-R2, Galphai-specific peptide GPCRs, Syntaxin 7, cPLA2, Btk, G-protein alpha-i5, G-protein alpha-i family, G-protein alpha-12, PA24A, gp91-phox, ALOX5, p40-phox, PAK1, p67-phox, PLD1 |
| Neutrophil activation                  | 115   | 7.27E-04    | 1.02E-02              | 15      | HLADPAI1, HLA-DQAI1, JAK2, MHC class II, Bax, HLA-DQB1, HLA-DRAI1, HLA-DBP1, HLA-DRB4, CD86, CD74, c-Fes, CD13, IL13RA1, Fc gamma RII alpha |
Table 2: Biological process networks for 974 genes whose expression was negatively correlated with CD4+ peripheral blood cells and Eotaxin.

| Networks                                      | Total | P value       | False discovery rate | In data | Network objects from active data                                                                 |
|-----------------------------------------------|-------|---------------|-----------------------|---------|--------------------------------------------------------------------------------------------------|
| Proteolysis, ECM remodeling                   | 85    | 5.38E-05      | 7.21E-03              | 10      | Collagen XIV, Tenasin-C, NEPH2, MMP-16, Protein C inhibitor, Serpin B12, COL18A1, Kallikrein 2, Trypsin II, Aggrecanase-1 |
| Neurophysiological process, Transmission of nerve impulse | 212   | 3.13E-04      | 2.10E-02              | 15      | L-type Ca(II) channel, alpha IC subunit, GABA-A receptor gamma-2 subunit, KCC2, mGluR3, Galphai-specific peptide GPCRs, mGluR1, Galphaiq-specific metabotropic glutamate GPCRs, Ionotropic glutamate receptor, Galphai-specific metabotropic glutamate GPCRs, GluR6, Galphai-specific amine GPCRs, CHT1, RIN, G-protein alpha-s, Kainate receptor |
| Reproduction, Gonadotropin regulation         | 199   | 1.64E-03      | 5.29E-02              | 13      | L-type Ca(II) channel, alpha IC subunit, GABA-A receptor gamma-2 subunit, mGluR3, mGluR1, Galphaiq-specific metabotropic glutamate GPCRs, Ionotropic glutamate receptor, Galphai-specific metabotropic glutamate GPCRs, Secretogranin 1, Protein kinase G1, Adenylate cyclase, G-protein alpha-s, Protein kinase G, Kainate receptor |
| Development, Blood vessel morphogenesis       | 228   | 1.97E-03      | 5.29E-02              | 14      | PDE, Galphai-specific peptide GPCRs, PDE7A, Endomucin, Galphaiq-specific amine GPCRs, Galphai-specific amine GPCRs, Protein kinase G1, Galphaiq-specific peptide GPCRs, COL18A1, Tissue kallikreins, Neurepilin-1, G-protein alpha-s, Protein kinase G, Transferrin |
| Reproduction, Spermatogenesis, motility and copulation | 228   | 1.97E-03      | 5.29E-02              | 14      | PDGF receptor, MFGE8, IGF-1 receptor, Ropporin, MSK1, S5AR2, BBS2, Tissue kallikreins, BMP2, Kallikrein 2, SOX5, CREM (activators), ZFP37, PDGF-R-alpha |
| Proteolysis, Connective tissue degradation     | 119   | 3.23E-03      | 6.80E-02              | 9       | Trypsin, Tenasin-C, MMP-16, Protein C inhibitor, Serpin B12, Tissue kallikreins, Kallikrein 2, Trypsin II, Aggrecanase-1 |
| Development, Neurogenesis in general          | 192   | 3.55E-03      | 6.80E-02              | 12      | WNT4, RET, CHRM, Neuremodulin, WNT7A, WNT, Galphaiq-specific amine GPCRs, Galphai-specific amine GPCRs, HDAC7, ACM3, SOX8, SOX14 |
| Development, Cartilage development            | 66    | 6.45E-03      | 1.08E-01              | 6       | TR-alpha, Noggin, COL1A2, BMP2, SOX5, Aggrecanase-1 |
| Reproduction, Male sex differentiation        | 243   | 8.98E-03      | 1.30E-01              | 13      | AP-2A, PDGF receptor, Olfactory receptor, RET, IGF-1 receptor, MSK1, S5AR2, HSF2, BMP2, SOX5, CREM (activators), ZFP37, PDGF-R-alpha |
| Reproduction, GnRH signaling pathway          | 166   | 9.70E-03      | 1.30E-01              | 10      | GABA-A receptor gamma-2 subunit, mGluR3, mGluR1, Galphaiq-specific metabotropic glutamate GPCRs, Ionotropic glutamate receptor, Galphai-specific metabotropic glutamate GPCRs, Protein kinase G1, G-protein alpha-s, Protein kinase G, Kainate receptor |

Cytokines/chemokines (humoral immunomodulators) regulate cancer-associated immune responses [15]. Eotaxin-1 (CCL11) controls both the eosinophil-mediated immune response [16] and other immune responses of Th2 cells [17]. We found that eotaxin-1 levels were elevated in the sera of CRC patients. The expression levels of certain other genes that significantly differed between peripheral CD4+ cells of CRC patients and those of healthy controls also correlated with serum eotaxin-1 concentrations. The upregulated genes, including those encoding MHC, CD1d, TLR4, IL-15, Fc gamma, and Hck [18], are normally expressed in monocytes/macrophages. In humans, both T cells and monocytes express CD4; however, the cell functions differ [19].

Serum IL-8 (CXCL8) levels were significantly elevated in CRC patients and those with other cancers [20–22]. IL-8 fosters CRC tumor growth, invasion, and metastasis [23, 24], promoting in vitro cell proliferation of human colon carcinoma cells via metalloproteinase-mediated cleavage [25].
Table 3: Biological process networks for 250 genes whose expression in peripheral CD4+ cells was positively correlated with serum IL-8 concentration.

| Networks                                      | Total | P value   | False discovery rate | In data                      | Network objects from active data                                                                 |
|-----------------------------------------------|-------|-----------|----------------------|------------------------------|--------------------------------------------------------------------------------------------------|
| Cytoskeleton_Actin filaments                  | 176   | 2.12E-05  | 2.31E-03             | 10                           | Actin muscle, Talin, Tropomyosin, RhoA, Myosin I, CAPZA, MELC, TARA, Actin, CAPZAI                 |
| Cytoskeleton_Regulation of cytoskeleton rearrangement | 183   | 8.79E-04  | 3.67E-02             | 8                            | Actin muscle, Talin, RhoA, CAPZA, MELC, TARA, Actin, CAPZAI                                      |
| Development_Skeletal muscle development       | 144   | 1.01E-03  | 3.67E-02             | 7                            | ACTA2, Smooth muscle myosin, Actin muscle, Tropomyosin, RhoA, MELC, Actin                        |
| Muscle contraction                             | 173   | 2.90E-03  | 6.54E-02             | 7                            | ACTA2, Syndephrin B, Smooth muscle myosin, Actin muscle, Tropomyosin, MELC, Actin                |
| Immune response_Phagocytosis                  | 222   | 3.00E-03  | 6.54E-02             | 8                            | ILT2, Talin, CD63, RhoA, Myosin I, MR1, MELC, Actin                                             |
| Cell adhesion_Integrin priming                | 110   | 7.22E-03  | 1.17E-01             | 5                            | ACTA2, ITGA2B, Talin, Integrin, Actin                                                          |
| Cell adhesion_Platelet aggregation            | 158   | 7.84E-03  | 1.17E-01             | 6                            | COX-1 (PTGS1), Talin, RhoA, ENP1, MELC, GP-IB beta                                              |
| Cell adhesion_Integrin-mediated cell-matrix adhesion | 214   | 9.17E-03  | 1.17E-01             | 7                            | ITGA2B, ITGB5, Talin, Integrin, RhoA, MELC, Actin                                              |
| Inflammation_Amphoterin signaling             | 118   | 9.64E-03  | 1.17E-01             | 5                            | ITGAM, MyD88, RhoA, MELC, Actin                                                                |
| Proteolysis_Proteolysis in cell cycle and apoptosis | 125   | 1.22E-02  | 1.33E-01             | 5                            | Presenilin 2, Cathepsin C, FBX6, Pseudo-ICE, Presenilin                                        |

Table 4: Biological process networks for 586 genes whose expression in peripheral CD4+ cells was negatively correlated with serum IL-8 concentration.

| Networks                                      | Total | P value   | False discovery rate | In data                      | Network objects from active data                                                                 |
|-----------------------------------------------|-------|-----------|----------------------|------------------------------|--------------------------------------------------------------------------------------------------|
| Muscle contraction                             | 173   | 2.69E-03  | 1.78E-01             | 7                            | K(+) channel, subfamily J, Dystrophin, PKC-alpha, MyHC, PKC, Titin, cPKC (conventional)          |
| Development_Blood vessel morphogenesis        | 228   | 3.26E-03  | 1.78E-01             | 8                            | PDE3B, PKC-alpha, COL18A1, PDE, PDE9A, TERT, PDE7A, Endomucin                                    |
| Cardiac development_Wnt_beta-catenin, Notch, VEGF, IF3 and integrin signaling | 150   | 5.75E-03  | 2.09E-01             | 6                            | PKC-alpha, Polycystin, CHIBBY, MyHC, Titin, LRP6                                                 |
| Cardiac development_FGF_ErbB signaling        | 124   | 1.12E-02  | 3.05E-01             | 5                            | PKC-alpha, Polycystin, MyHC, Titin, gpl30                                                       |
| Development_Skeletal muscle development       | 144   | 2.02E-02  | 4.41E-01             | 5                            | Dystrophin, HDAC7, Histone deacetylase class II, MyHC, Titin                                    |
| Proliferation_Positive regulation cell proliferation | 221   | 3.32E-02  | 5.15E-01             | 6                            | PUR-alpha, RASGRF1, COL18A1, IGF-I receptor, IL-11 receptor, gp130                               |
| Development_Cartilage development             | 66    | 3.51E-02  | 5.15E-01             | 3                            | TR-alpha, Noggin, Aggrecanase-1                                                                 |
| Reproduction_Spermatogenesis, motility and copulation | 228   | 3.78E-02  | 5.15E-01             | 6                            | MSK1, MPGE8, Oct-3/4, IGF-1 receptor, PKC, ZFP37                                               |
| Reproduction_Male sex differentiation          | 243   | 4.90E-02  | 5.41E-01             | 6                            | MSK1, Oct-3/4, IGF-1 receptor, PKC, ZFP37, PMEPA1                                               |
| Transcription_Chromatin modification          | 127   | 4.97E-02  | 5.41E-01             | 4                            | SATB1, MSK1, HDAC7, Histone deacetylase class II                                                |

Additionally, tumor-derived IL-8 induces the formation of immunosuppressive neutrophils and myeloid-derived suppressor cells in tumor microenvironments [26, 27]. Thus, elevated serum IL-8 levels in CRC patients may play an important role in cancer progression; indeed, attainment of an advanced clinical stage was associated with an increase in serum IL-8 concentration. Serum IL-8 levels correlated with changes in the expression levels of CD4+ cell genes compared to healthy controls; these changes also suggested that phagocytosis was in play.
Because immune-mediating cells are miscellaneous, including myeloid-derived cells such as neutrophils, monocytes, and lymphocytes, the interaction of these immune-mediating cells in CRC should be studied to further understand the immune pathophysiological features of CRC. The most frequent subpopulation of whole blood cells is neutrophils. We observed that the gene expression profile of whole blood cells and CD4+ cells was discernible between CRC patients and healthy volunteers; thus, the interaction between these two populations should particularly be investigated.

Collectively, this study showed that transcriptional alteration of peripheral blood, especially CD4+ cells, and elevation of humoral mediators were possibly reflection of immune pathophysiology of CRC, which are compatible to the recent other reports showing gene expression profile alteration [28–30] as well as alteration of concentration of humoral immune mediators [31] in peripheral blood. Humoral immune mediators and cellular immunity are interactive [32,33]. As the immune system and its reaction are extremely complex, especially in cancers [34], each humoral mediator and cellular fraction should be further investigated to understand immune pathophysiology in detail. Despite these possible immune pathophysiological features being reflected by serum chemokines and peripheral CD4+ cells, further analysis in a larger cohort than that used in the current study should be performed to explore interactive features between chemokines, eotaxin-1 and IL-8, and CD4+ cells in peripheral blood of CRC patients.

In conclusion, we showed that CRC featured systemic inflammation, changes in the serum concentrations of eotaxin-1 and IL-8, and correlated changes in gene expression in peripheral blood CD4+ cells. Further studies exploring the roles played by chemokines and peripheral CD4+ cells in CRC patients are required. In addition, it should be explored how eotaxin-1 and IL-8 elevation is correlated with clinical outcome of CRC in terms of overall survival, therapeutic response after curative treatment with endoscopy or surgery, and relapse rate after complete cure.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

This study was approved by the Institutional Review Board and was performed in accordance with the Declaration of Helsinki.

Consent

Written informed consent was obtained from all participants.

Conflicts of Interest

The authors declare that they have no conflicts of interest in this paper.

Authors’ Contributions

Takuya Komura and Masaaki Yano contributed to write a manuscript and analysis and interpretation of data. Akimitsu Miyake contributed to acquisition of data and interpretation of data. Hisashi Takabatake, Masaki Miyazawa, Norihiko Ogawa, and Akihiro Seki contributed to acquisition of data and analysis of data. Masao Honda and Takashi Wada contributed to analysis of data and interpretation of data. Shigeyuki Matsui and Shuichi Kaneko contributed to interpretation of data and supervision of the research. Yoshio Sakai contributed to interpretation of data and supervision of the research and wrote a manuscript. Takuya Komura and Masaaki Yano contributed equally to this study.

Acknowledgments

This study was supported, in part, by a grant-in-aid from the Japanese Agency for Medical Research and Development.

Supplementary Materials

Supplementary 1. Supplemental Table 1. Characteristics of serum chemokine study subjects.

Supplementary 2. Supplemental Table 2. Characteristics of study subjects.

Supplementary 3. Supplemental Fig. 1. Serum concentrations of cytokines and chemokines.

Supplementary 4. Supplemental Fig. 2. Serum concentrations of cytokines and chemokines.

References

[1] R. A. Smith, D. Manassaram-Baptiste, D. Brooks et al., “Cancer screening in the United States, 2014: A review of current American Cancer Society guidelines and current issues in cancer screening,” CA: A Cancer Journal for Clinicians, vol. 64, no. 1, pp. 30–31, 2014.

[2] J. H. Bond, “Colorectal cancer screening: The potential role of virtual colonoscopy,” Journal of Gastroenterology, vol. 37, no. 13, pp. 92–96, 2002.

[3] J. H. Bond, “Fecal occult blood test screening for colorectal cancer,” Gastrointestinal Endoscopy Clinics of North America, vol. 12, no. 1, pp. 11–21, 2002.

[4] M. Ramos, M. Llagostera, M. Esteve et al., "Knowledge and attitudes of primary healthcare patients regarding population-based screening for colorectal cancer," BMC Cancer, vol. 11, 2011.

[5] M. Honda, Y. Sakai, T. Yamashita et al., “Differential gene expression profiling in blood from patients with digestive system cancers,” Biochemical and Biophysical Research Communications, vol. 400, no. 1, pp. 7–15, 2010.

[6] T. Pecht, A. Gutman-Tirosh, N. Bashan, and A. Rudich, “Peripheral blood leucocyte subclasses as potential biomarkers of adipose tissue inflammation and obesity subphenotypes in humans,” Obesity Reviews, vol. 15, no. 4, pp. 322–337, 2014.

[7] W. H. Fridman, F. Pagès, C. Sautès-Fridman, and J. Galon, “The immune contexture in human tumours: impact on clinical outcome,” Nature Reviews Cancer, vol. 12, no. 4, pp. 298–306, 2012.
[8] N. Zhang and M. I. Bevan, "CD8+ T cells: foot soldiers of the immune system," *Immunity*, vol. 35, no. 2, pp. 161–168, 2011.

[9] S. Solito, I. Marigo, L. Pinton, V. Damuzzo, S. Mandruzzato, and V. Bronte, "Myeloid-derived suppressor cell heterogeneity in human cancers," *Annals of the New York Academy of Sciences*, vol. 1319, no. 1, pp. 47–65, 2014.

[10] A. Facciabene, G. T. Motz, and G. Coukos, "T-Regulatory cells: key players in tumor immune escape and angiogenesis," *Cancer Research*, vol. 72, no. 9, pp. 2662–2671, 2012.

[11] T. Okazaki, S. Chikuma, Y. Iwai, S. Fagarasan, and T. Honjo, "A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application," *Nature Immunology*, vol. 14, no. 12, pp. 1212–1218, 2013.

[12] T. Komura, Y. Sakai, K. Harada et al., "Inflammatory features of pancreatic cancer highlighted by monocytes/macrophages and CD4+ T cells with clinical impact," *Cancer Science*, vol. 106, no. 6, pp. 672–686, 2015.

[13] Y. Sakai, M. Honda, H. Fujinaga et al., "Common transcriptional signature of tumor-infiltrating mononuclear inflammatory cells and peripheral blood mononuclear cells in hepatocellular carcinoma patients," *Cancer Research*, vol. 68, no. 24, pp. 10267–10279, 2008.

[14] Y. Sakai, I. Tatsumi, M. Higashimoto et al., "Association of changes in the gene expression profile of blood cells with the local tumor inflammatory response in a murine tumor model," *Biochemical and Biophysical Research Communications*, vol. 428, no. 1, pp. 36–43, 2012.

[15] Z. Pengjun, W. Xinyu, G. Feng et al., "Multiplexed cytokine profiling of serum for detection of colorectal cancer," *Future Oncology*, vol. 9, no. 7, pp. 1017–1027, 2013.

[16] M. Kitaura, T. Nakajima, T. Imai et al., "Molecular cloning of human eotaxin, an eosinophil-selective CC chemokine, and identification of a specific eosinophil eotaxin receptor, CC chemokine receptor 3," *The Journal of Biological Chemistry*, vol. 271, no. 13, pp. 7725–7730, 1996.

[17] L. M. Teran, M. Mochizuki, J. Bartels et al., "Th1- and Th2-Type Cytokines Regulate the Expression and Production of Eotaxin and RANTES by Human Lung Fibroblasts," *American Journal of Respiratory Cell and Molecular Biology*, vol. 20, no. 4, pp. 777–786, 1999.

[18] G. W. Lynch, S. Turville, B. Carter et al., "Marked differences in the structures and protein associations of lymphocyte and monocyte CD4: Resolution of a novel CD4 isoform," *Immunology & Cell Biology*, vol. 84, no. 2, pp. 154–165, 2006.

[19] G. Szabo, C. L. Miller, and K. Kody, "Antigen presentation by the CD4 positive monocyte subset," *Journal of Leukocyte Biology*, vol. 47, no. 2, pp. 111–120, 1990.

[20] K. Xie, "Interleukin-8 and human cancer biology," *Cytokine & Growth Factor Reviews*, vol. 12, no. 4, pp. 375–391, 2001.

[21] D. J. Waugh and C. Wilson, "The interleukin-8 pathway in cancer," *Clinical Cancer Research*, vol. 14, no. 21, pp. 6735–6741, 2008.

[22] W.-J. Jin, J.-M. Xu, W.-L. Xu, D.-H. Gu, and P.-W. Li, "Diagnostic value of interleukin-8 in colorectal cancer: a case-control study and meta-Analysis," *World Journal of Gastroenterology*, vol. 20, no. 43, pp. 16334–16342, 2014.

[23] M. Baggiolini, B. Dewald, and B. Moser, "Human chemokines: an update," *Annual Review of Immunology*, vol. 15, pp. 675–705, 1997.

[24] Y. Ning and H.-J. Lenz, "Targeting IL-8 in colorectal cancer," *Expert Opinion on Therapeutic Targets*, vol. 16, no. 5, pp. 491–497, 2012.

[25] Y. Itoh, T. Joh, S. Tanida et al., "IL-8 promotes cell proliferation and migration through metalloproteinase- cleavage proHB-EGF in human colon carcinoma cells," *Cytokine*, vol. 29, no. 6, pp. 275–282, 2005.

[26] Z. G. Fridlender, J. Sun, I. Mishalian et al., "Transcriptomic analysis comparing tumor-associated neutrophils with granulocytic myeloid-derived suppressor cells and normal neutrophils," *PLoS ONE*, vol. 7, no. 2, Article ID e31524, 2012.

[27] H.-L. Rao, J.-W. Chen, M. Li et al., "Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients' adverse prognosis," *PLoS ONE*, vol. 7, no. 1, Article ID e30806, 2012.

[28] L. Ciarloni, S. Hosseinion, S. Monnien-Benoit, N. Imaizumi, G. Dorita, and C. Ruegg, "Discovery of a 29-gene panel in peripheral blood mononuclear cells for the detection of colorectal cancer and adenomas using high throughput real-time PCR," *PLoS ONE*, vol. 10, no. 4, Article ID e0123904, 2015.

[29] Y.-T. Chang, C.-S. Huang, C.-T. Yao et al., "Gene expression profile of peripheral blood in colorectal cancer," *World Journal of Gastroenterology*, vol. 20, no. 39, pp. 14463–14471, 2014.

[30] C. Nichita, L. Ciarloni, S. Monnien-Benoit, S. Hosseinion, G. Dorita, and C. Ruegg, "A novel gene expression signature in peripheral blood mononuclear cells for early detection of colorectal cancer," *Alimentary Pharmacology & Therapeutics*, vol. 39, no. 5, pp. 507–517, 2014.

[31] N. A. Johdi, L. Mazlan, I. Sagap, and R. Jamal, "Profiling of cytokines, chemokines and other soluble proteins as a potential biomarker in colorectal cancer and polyps," *Cytokine*, vol. 99, pp. 35–42, 2017.

[32] T. Komura, H. Takabatake, K. Harada et al., "Clinical features of cystatin A expression in patients with pancreatic ductal adenocarcinoma," *Cancer Science*, vol. 108, no. 11, pp. 2122–2129, 2017.

[33] G. Bernardini, F. Antonangeli, V. Bonanni, and A. Santoni, "Dysregulation of chemokine/chemokine receptor axes and NK cell tissue localization during diseases," *Frontiers in Immunology*, vol. 7, no. 402, 2016.

[34] P. H. Pandya, M. E. Murray, K. E. Pollok, and J. L. Renbarger, "The Immune System in Cancer Pathogenesis: Potential Therapeutic Approaches," *Journal of Immunology Research*, vol. 2016, Article ID 4273943, 13 pages, 2016.