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Subsite heterogeneity in the profiles of circulating cytokines in colorectal cancer

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ABSTRACT

Colorectal cancers (CRCs) are treated as one entity but are in fact a heterogeneous group of diseases. If not addressed, subsite-associated variability may interfere with mechanism-targeted therapies and accuracy of potential CRC biomarkers. Little is known about the contribution of systemic inflammatory and immune mediators to subsite heterogeneity in CRC. Our purpose was to compare the profiles of key cytokines between right and left colonic and rectal CRCs. Using Luminex xMAP® technology, serum concentrations of eotaxin, IL-1β, IL-1ra, 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, IFNγ, IP-10, FGF-2, G-CSF, GM-CSF, MCP-1, MIP-1α and β, PDGF-BB, RANTES, TNFα, and VEGF-A were determined in 104 CRC patients. We found the concentrations of IL-12(p70), IL-10, IL-1ra, IL-4, IL-6, IL-7, IL-8, G-CSF and TNFα to be significantly higher in right-sided and GM-CSF in left-sided than rectal CRCs. The concentrations of IFNγ and MIP-1α were significantly higher in right-sided CRCs as compared to cancers of other locations combined. In turn, MIP-1β was higher in rectal CRCs as compared to colon cancers. Taken together, our results show subsite heterogeneity of CRC cancers in terms of systemic inflammatory and immune responses that ought to be taken into account when attempting immunotherapy or developing biomarkers. Additionally, more pronounced T_H2 response accompanied by T_H1 immunity and more prominent tumor-promoting inflammation in CRC patients with primary tumors originating from right-sided colon may constitute a molecular background of unfavorable prognosis associated with this location.

1. Introduction

Colorectal cancer (CRC) remains one of the commonest cancers worldwide and the major cause of cancer-related deaths [1,2]. Recently, a distributional shift towards right-sided cancers [3,4] and adenomas [5] has been observed with left-sided and rectal cancers becoming relatively less frequent. Historically treated as one entity; CRCs are in fact a heterogeneous group of diseases varying by their risk and protective factors, presentation, and outcome, with right-sided cancers having the least and rectal cancers the most favorable characteristics [6,7].

At molecular level, tumors arising from different segments of large bowel display distinct patterns of alterations, as exemplified by differences in prevalence of microsatellite (MSI) or chromosomal instability (CIN), CpG island methylator phenotype (CIMP), and the mutations of *KRAS*, *BRAF*, or *APC* [7–9]. Differences in a molecular profile of cancer cells stem from differences in the bowel content along the large bowel, both in terms of exposure to carcinogens and gut microbiota. They further translate into differences in clinical behavior of cancer arising from different subsites, affecting treatment efficacy and patients'

Abbreviations: CIMP, CpG island methylator phenotype; CIN, chromosomal instability; CRC, colorectal cancer; FGF-2, fibroblast growth factor 2 (basic); G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocytemacrophage colony-stimulating factor; IFNγ, interferon γ; IP-10, IFNγ-induced protein 10; MCP-1, monocyte chemotactic protein; MDSCs, myeloid-derived suppressive cells; MIP-1, macrophage inflammatory protein 1; MSI, microsatellite instability; PDGF-BB, platelet-derived growth factor BB; RANTES, Regulated on Activation, Normal T-cell Expressed and Secreted; TNFα, tumor necrosis factor α; VEGF-A, vascular endothelial growth factor A

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prognosis [7]. If not addressed, subsite-associated variability may contribute to the failure of mechanism-targeted therapies for CRC as well as poor accuracy of potential CRC biomarkers.

Immunotherapy is one of the relatively novel approaches for CRC treatment, considered promising particularly for metastatic and chemo-/radio-therapy resistant cancers as well as for preventing relapse of the disease [10]. Immune system plays a dual role in cancer. Cytotoxic $CD8^+$T lymphocytes, helper $CD4^+T_H1$ lymphocytes and natural killer (NK) cells play a protective role, being involved in surveillance against and removal of nascent tumor cells. On the other hand, the role of suppressors of anti-tumor responses and of promoters of cancer development is traditionally attributed to helper $CD4^+T_H2$ and regulatory (Treg) lymphocytes, lymphocytes B, tumor-associated macrophages (M2 macrophages), and myeloid-derived suppressive cells (MDSCs), key regulators of tumor growth and metastasis in the tumor microenvironment [11,12]. Cancer immunotherapy is designed to boost natural antitumor immunity and/or to dampen tumor-promoting immunosuppressive responses [10].

Although molecular variability associated with anatomic subsites of CRC has recently gained attention, its focus is mostly on genetic and epigenetic markers. Little is known about the contribution of local or systemic mediators of immune responses like cytokines and growth factors to subsite heterogeneity in CRC. Previously, our group reported variability in the expression of pre-B-cell colony-enhancing factor 1 and immunomodulatory midkine in CRC tumors with different subsite location [13–15]. We also observed more pronounced elevation of circulating IL-7 and VEGF-A in patients with CRCs originating from right-sided colon [16,17]. This study was designed to comprehensively analyze the systemic profiles of key cytokines, chemokines and growth factors mediating inflammatory, immune and angiogenic responses in CRC patients with reference to anatomical subsite of primary tumor.

2. Materials and methods

2.1. Patients

One hundred four patients admitted to the Regional Specialist Hospital, Wroclaw (2013–2015) or to the First Department of Oncological Surgery of Lower Silesian Oncology Center, Wroclaw (2011–2012) for the curative resection of histologically confirmed adenocarcinoma of colon or rectum who did not undergo radio- or chemotherapy prior surgery were enrolled for present study. Inclusion criteria were age > 18 yrs. and willingness to participate. Patients with poor overall physical status (ASA physical status classification system > 3), gross metastatic disease or locally advanced cancers not amenable to curative resection or requiring emergency surgery were excluded. Resected tumors were staged pathologically according to UICC TNM7th system. CRC patients were stratified by tumor subsite: with tumors located in the cecum, ascending colon, hepatic flexure or proximal half of transverse colon were categorized as having right-sided colonic cancers (n = 29) and with tumors located in the distal half of transverse colon, splenic flexure, descending colon, sigmoid colon or rectosigmoid junction were categorized as having left-sided colonic cancers (n = 22). In remaining 53 patients tumors were located in the rectum.

Control group consisted of 20 individuals: eight apparently healthy blood donors (inclusion criterion: age > 60 yrs.) from Lower Silesian Center of Blood Donation and Therapy, Wroclaw, Poland and 12 patients from Research, Science, and Educational Center of Dementia Diseases, Scinawa, Poland, admitted for the diagnostics (also using neuroimaging) of headaches, dizziness and/or complaints on memory in whom dementia, mild cognitive impairment or other diseases of the brain were excluded and who had otherwise no significant health history. There was no age difference between study and control groups (66.3 ± 10.6 and 67.2 ± 8.5, p = 0.761) as well as no significant difference in sex distribution (38% of females in CRC and 55% in controls, p = 0.214).

2.2. Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from study participants.

2.3. Analytical methods

Blood was drawn in a fasting state prior to any procedure by venipuncture and allowed to clot for 30 min and centrifuged (15 min, 720*g*). Collected sera were aliquoted and kept frozen at −80 °C until examination. Circulating cytokines and growth factors were evaluated in duplicates by means of flow cytometry-based method utilizing magnetic microspheres conjugated with monoclonal antibodies using the BioPlex 200 platform with HRF (BioRad, CA, USA), incorporating Luminex xMAP® technology, and validated 27-plexes for simultaneous measurement of eotaxin, interleukin (IL)-1β, IL-1ra, 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, interferon (IFN)-γ, IFNγ-induced protein (IP)-10, fibroblast growth factor (FGF)-2, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1α and β, platelet-derived growth factor (PDGF)-BB, Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES), tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF)-A (BioRad). The bioassays were conducted according to manufacturer's instructions. Standard curves were drawn using 4- or 5-PL logistic regression and the data were analyzed using BioPlex Manager 6.0 software (BioRad).

2.4. Statistical analysis

Data distribution and homogeneity of variances was tested using, respectively, Kolmogorov-Smirnov and Levene's tests. Data were log-transformed if necessary. Data are presented as means or medians with 95%*CI* and analyzed using one-way ANOVA with Bonferroni correction for multiple testing and Student-Newman-Keuls post-hoc test and *t*-test for independent samples or Kruskal-Wallis *H* test and Mann-Whitney *U* test. Distribution of demographic and clinical categorical data was analyzed using χ^2 test.

All calculated probabilities were two-tailed and p-values ≤0.05 were considered statistically significant. The statistical analysis was conducted using MedCalc Statistical Software version 16.8 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016).

3. Results

Study groups based on tumor location were well balanced with respect to the distribution of age, sex and disease advancement (Table 1).

Out of 27 cytokines and growth factors measured, the levels of IL-2, IL-15 and IL-17 were below the limit of detection of the assay in a number of patients. Therefore, these cytokines were excluded from further analysis.

As compared to controls, CRC patients had higher concentrations of all measured cytokines except for eotaxin and PDGF-BB. In case of IFNγ and IL-7, the difference was significant exclusively for right colon cancer patients and in case of GM-CSF – for right and left colon but not rectum cancer patients (Table 2).

The serum levels of G-CSF, GM-CSF, IL-10, IL-12(p70), IL-1ra, IL-4, IL-6, IL-7, IL-8, and TNFα in CRC patients differed significantly with respect to tumor location and IFNγ, IL-1β, IL-13, MIP-1α and MIP-1β showed a similar tendency. Most of examined cytokines were higher in right-sided colonic cancers as compared to rectal ones with IL-12(p70)

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