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Expression of B7-H2 on CD8$^+$ T cells in colorectal cancer microenvironment and its clinical significance

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ABSTRACT

The knowledge about B7-H2 expression in tumor is growing, but many questions remain unresolved. Especially in human tumor microenvironment, little studies were done. To explore the expression and clinical significance of B7-H2 on T cells in colorectal cancer microenvironment, fresh tumor tissues and paired non-tumor tissues collected from 25 patients with colorectal cancer were made to research B7-H2 expression on the infiltrating T cells including CD8$^+$ T cells and CD4$^+$ T cells. Also, tumor bearing mice were sacrificed on day 5, day 10, day 15, day 20, day 25 and flow cytometry was used to analyze B7-H2 expression on CD8$^+$ T cells and CD4$^+$ T cells in mouse tumors and spleens. Then, it was found that B7-H2 expression on CD8$^+$ T cells in patients' tumor tissues was significantly higher than in non-tumor tissues. The expression of B7-H2 on CD8$^+$ T cells in tumor microenvironment was significantly higher in patients with age ≤60 years old and the stage I-II. The expression level of B7-H2 on CD8$^+$ T cells in mouse tumors and spleens both reached the highest level at the early stage of inoculation (on day 5), decreased to the lowest level on day 10 and day 15 separately, and then gradually increased. In mouse spleens, B7-H2 expression on CD8$^+$ T cells was all significantly higher than on CD4$^+$ T cells in five time periods. So, in this study, it was found that B7-H2 expression on CD8$^+$ T cells in tumor microenvironment was closely related to the progression of colorectal cancer.

1. Introduction

Colorectal cancer is a serious threat to human health, which is one of the common malignant tumors. Its morbidity and mortality are very high [1]. Its occurrence and development are the results of a multi-gene participation and multi-stage accumulation. Early colorectal cancer prognosis is good and has long-term survival after surgery, but advanced metastatic colorectal cancer prognosis is poor and patients often die in a very short time. In clinical diagnosis and treatment, patients diagnosed with colorectal cancer often have liver metastases. And, traditional surgery, chemotherapy, radiotherapy and others to extend the survival time of patients is not ideal. Therefore, it is necessary to find new and effective treatments. In recent years, immunotherapy has become a hot topic in the field of cancer therapy, and in the late palliative treatment of cancer patients has achieved remarkable results [2].

The anti-tumor immunity of the body plays a role mainly through the activation of T lymphocytes. And T lymphocyte activation requires two signals simultaneously: 1) MHC-antigen and antigen receptor complex with TCR to provide the first stimulus signal; 2) B7-CD28 and other costimulatory molecules to provide the key second signal; Costimulatory molecules mainly divided into two major families: B7-CD28 family and TNF-TNFR family, and B7 family plays more attention [3]. In recent years, the three main members of B7 family: CTLA-4, PD-1 and PD-L1 (B7-H1) as the representative of the immune card point control treatment in clinical cancer treatment have made a breakthrough [4,5]. For example, B7-H1/PD-1 pathway in the treatment of lung cancer, malignant melanoma achieved significant efficacy [6].

Abbreviations: MHC, major histocompatibility complex; TCR, T cell receptor; CTLA-4, cytotoxic T lymphocyte associated antigen-4; PD-1, programmed deth-1; PD-L1, programmed deth-1 ligand; TILs, tumor infiltrating lymphocytes; SPF, specific pathogen free

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So far, B7-1, B7-2, B7-H1, B7-H2, B7-H3, B7-DC and B7-H4 have been found in B7 family. B7-H2 (also known as B7RP-1, CD275, GL50, ICOSL) is a newly discovered member of the B7 family of costimulatory molecules. B7-H2 was reported in 2000, and B7-H2 protein was expressed on the immature DC surface of monocytes. B7-H2 binds to the corresponding receptor ICOS (inducible co-stimulator) on T cells and plays an immunomodulatory role. ICOS is the third member of the CD28/CTLA-4 family. The structure is homologous to CD28 and CTLA-4, which plays an important role in the regulation of T cell activation and Th2 cell development [7]. B7-H1 can be expressed on a variety of cell surface, through the PD-1/B7-H1 signal to inhibit the T cell immune response, promote tumor immune escape and tumor progression [8]. The corresponding receptor molecule PD-1 can be expressed on the surface of activated $CD8^+$ T cells, mediating T cell function depletion [9]. In general, B7 family ligand molecules expressed on antigen-presenting cells such as dendritic cells, mononuclear macrophages and CD28 family receptor molecules are more expressed on T cells; T cell activation of the dual signal theory is based on this understanding. However, recent studies have shown that B7 family molecules also had inducible expression on T lymphocytes and had important biological functions. It has been found that the high expression of B7-H2 in glioblastoma, gastric cancer and hematological tumor cells had the effect of promoting tumor growth [10–13]. B7-1, B7-H1, B7-H2 and ICOS mRNA could be found in colorectal cancer cells and tumor-infiltrating lymphocytes (TILs), it is speculated that B7-H1, B7-H2 costimulatory molecules may be beneficial to the formation of Th2 cytokine-like immune responses in the microenvironment of colorectal cancer patients [14]. For the first time, B7-H2-ICOS was expressed in the microenvironment of human colorectal cancer, but the expression of B7-H2 protein in TILs cell type and clinical significance has not been elucidated.

Since $CD8^+$ T cells are the “central rule” of anti-tumor immune response, in our study, the $CD8^+$ T lymphocytes were selected as the target cell group, and at some time, compared with $CD4^+$ T cells. The fresh tissue samples of patients with colorectal cancer were examined to find the expression of B7-H2 on $CD8^+$ T cells and $CD4^+$ T cells. Clinical significances of B7-H2 expression on $CD8^+$ T cells and $CD4^+$ T cells were analyzed. In addition, by constructing tumor-bearing BALB/c mice, we further explored the expression of B7-H2 on $CD8^+$ T cells and $CD4^+$ T cells in mouse tumors and spleens on day 5, day 10, day 15, day 20, day 25. In conclusion, the study through patients' fresh tissue samples and tumor-bearing mice models explored the expression and clinical significance of B7-H2 on $CD8^+$ T cells and $CD4^+$ T cells in colorectal cancer microenvironment.

2. Materials and methods

2.1. Patients and samples

From July 2015 to July 2016, Fresh tumor tissue and the corresponding non-tumor tissue samples were obtained from 25 untreated patients with pathologically confirmed colorectal cancer at The Department of Gastrointestinal Surgery, The First Affiliated Hospital of Soochow University. The corresponding non-tumor tissue samples were taken at least 5 cm away from the visible tumor margin. The evaluation of tumor stages was based on TNM stages formulated by American Joint Committee on cancer (AJCC). The study was approved by the ethics committee of the First Affiliated Hospital of Soochow University Written and informed consent was obtained from patients and healthy donors.

2.2. Fresh colorectal tissues

Single cell suspensions of tumor and non-tumor samples were obtained after digestion. Briefly, tissues were cut in pieces and placed in petri dishes containing 0.1% Collagenase IV (Sigma, USA) and incubated at 37 °C for 90 min. Samples were then grinded and filtered through membranes with 30-μm pore. Tissue-derived mononuclear cells were obtained after centrifugation and suspended in PBS subsequently. We analyzed the expression levels of B7-H2 on $CD4^+$ T cells and $CD8^+$ T cells in Fresh tissues by flow cytometric analysis.

2.3. Cell lines and animals

The CT26 cell line was purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Twenty four BALB/c mice (aged 8 weeks) were purchased from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China). All experimental animals were raised under specific-pathogen-free (SPF) conditions at the Experimental Animal Center of Soochow University. Animal work was performed using an institutional protocol that was approved by the Animal Care and Use Committee of Soochow University.

2.4. Tumor-bearing mouse model

The mouse experiments were repeated three times independently. Every time, there were twenty four BALB/c mice (aged 8 weeks). Twenty mice were chosen at random to be injected with 0.5 mL CT26 cells (2×10^6 cells/ml) at the right abdomen. The remaining four mice injected with 0.5 mL normal saline were used as a control group. Four tumor-bearing mice were chosen at random to be killed every 5 days. Tumor and spleen samples were removed for analysis on day 5, day 10, day 15, day 20, day 25 respectively. Single cell suspensions of tumor and spleen samples were extracted. Then the expression levels of B7-H2 on $CD4^+$ T cells and $CD8^+$ T cells in tumor and spleen samples were detected by flow cytometric analysis.

2.5. Antibodies and flow cytometric analysis

Tissue-infiltrating leukocytes were stained with fluorochrome-conjugated monoclonal antibodies and then detected by multicolor flow cytometry (Beckman Coulter, Brea, CA). The antibodies used are listed as follows: anti-human-CD3-PE/Cy7, anti-human-CD4-PE/Cy5, anti-human-B7-H2-PE, anti-mouse-CD3-FITC, anti-mouse-CD4-PE/Cy5, anti-mouse-B7-H2-PE were ordered from BioLegend (San Diego, CA, USA).

2.6. Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0 software (LaJolla, CA). Statistical analysis for normally distributed values was performed using Student's *t*-test or ANOVA. Fisher's exact test was performed for clinical significance analysis. The values of $p < .05$ were considered to be statistically significant.

3. Result

3.1. Expression of B7-H2 on infiltrating $CD8^+$ T cells and $CD4^+$ T cells in colorectal cancer microenvironment

By detecting 25 pairs of colorectal tissues through flow cytometry, the expression of B7-H2 on $CD8^+$ T cells in tumor tissues was higher than in non-tumor tissues (Fig. 1A). Further analysis showed that the expression of B7-H2 on $CD8^+$ T cells in tumor tissue (10.88 ± 9.943%) was significantly higher than in non-tumor tissue (5.440 ± 4.814%), $p = .0042$ (Fig. 1B). At the same time, B7-H2 expression on $CD4^+$ T cells in tumor tissues was higher than in non-tumor tissues (Fig. 1A), but further analysis showed that the expression of B7-H2 on $CD4^+$ T cells in tumor tissues (9.560 ± 8.362%) was not significantly different from in non-tumor tissues (6.840 ± 4.327%), $p = .0843$ (Fig. 1B). Also, in non-tumor tissues, the expression of B7-H2 on $CD8^+$ T cells (5.440 ± 4.814%) was not significantly different from on $CD4^+$ T cells