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Original Article

The CD200/CD200R expression level and its mechanism of action in hematological malignancy patients

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Abstract: Objective: To explore the CD200/CD200R expression level in the peripheral blood mononuclear cells (PBMC) of hematological malignancy patients and to analyze its mechanism of action. Methods: Thirty hematological malignancy patients who were hospitalized in our hospital from June 2019 to December 2019 were recruited as the study cohort and placed in the disease group, and 30 healthy people were also recruited for the study and placed in the healthy control group. The CD200/CD200R expression level in the two groups' peripheral blood was measured using real-time fluorescent quantitative PCR, and enzyme-linked immunosorbent assays were used to measure the interleukin 17 (IL-17), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) expression levels in the two groups' peripheral blood. We monitored the effects of the effective treatment on the CD200/CD200R level in the hematological malignancy patients. Results: Compared with the healthy group, the CD200 and CD200R mRNA expression level in the PBMC of the disease group was down-regulated, but the IL-17, TNF-α and IFN-γ expression levels in the peripheral blood plasma were up-regulated, and the differences were statistically significant ($P < 0.001$). The CD200 and CD200R levels showed a negative correlation with the IL-17, TNF-α, and IFN-γ expression levels in the hematological malignancy patients ($P < 0.001$). The CD200/CD200R expression level was significantly increased in the PBMC of the effectively treated hematological malignancy patients compared with their pre-treatment expression level, and the difference was statistically significant ($P < 0.001$). Conclusion: CD200/CD200R exhibits a low expression level in hematological malignancy patients, reducing the inhibitory effect on the inflammatory factor expressions, enhancing the inflammatory factors, and mediating the occurrence and development of hematological malignancies.

Keywords: Hematological malignancies, CD200, CD200R

Introduction

Hematological malignancies comprise a group of malignant clonal disorders arising from the hematopoietic tissues [1], including leukemia, multiple myeloma, and lymphoma, and they have a high morbidity and mortality [2, 3]. An imbalance in the autoimmune homeostasis is a key factor leading to the occurrence of hematological malignancies, and numerous immune cells are involved in this process, such as autoreactive T cells, regulatory T cells, B cells, and monocytes. Numerous studies have found that T-cell dysfunction plays a decisive role in the occurrence of hematological malignancies, specifically the excessive activation of Th17 subtype cells, resulting in autoimmune hyperactivity in patients [4].

CD200 is a transmembrane glycoprotein that serves as a component of the immunoglobulin superfamily. It is expressed in a variety of cells, including B cells, T cells, and follicular dendritic cells [5]. The CD200 receptor (CD200R) is also expressed on the surfaces of T cells, macrophages, etc., and CD200 can transmit immunosuppressive signals through its binding to CD200R, and acts as a regulator of autoimmune dysfunction [6]. Consequently, we investigated the CD200/CD200R expression level and its mechanism of action in hematological malignancy patients in order to provide information to help gain an understanding of the hematological malignancy mechanism.

Materials and methods

Objects

Thirty hematological malignancy patients who were hospitalized in our hospital from June 2019 to December 2019 were recruited as the

Table 1. General data - the disease group vs. the healthy group [n (%), $\bar{x} \pm s$]

Group	n	Sex (M/F)	Age (years)	Body mass (kg/m^2)
Disease group	30	19/11	53.56 ± 11.45	23.82 ± 2.09
Healthy group	30	17/13	52.93 ± 12.08	23.57 ± 1.68
χ^2/t		0.278	0.207	0.511
P		0.598	0.836	0.612

study cohort and placed in the disease group, which included 15 patients with non-Hodgkin's lymphoma (NHL), 6 patients with acute myeloid leukemia (AML), and 9 patients with acute lymphoblastic leukemia (ALL). Inclusion criteria (1) Patients over 18 years old. (2) Patients who met the diagnostic criteria for hematological malignancies issued by the *European Organization for Research and Treatment of Cancer and Fungi Research Group* [7]. (3) Patients able to follow medical advice to complete the relevant tests and diagnoses. (4) The patients were informed and signed the informed consent forms. Exclusion criteria: (1) Patients also suffering from active infections, chronic obstructive pulmonary disease, hypertension, or diabetes mellitus. (2) Patients also suffering from liver and kidney dysfunction, or patients with blood loss symptoms within the past month. (3) Patients with mental disorders.

Thirty healthy patients who underwent physical examinations at our hospital during the same period and had qualified results were also recruited for the study and placed in the healthy control group. The differences between the two groups in terms of sex, age, and body mass were not statistically significant (P > 0.05) (**Table 1**), so the two groups were comparable. This study was approved by our hospital's ethics committee (approval no. 2019-252-01).

Therapeutic methods

ALL the patients were treated with chemotherapy using VDLP (vincristine + prednisone + daunorubicin + asparaginase). The NHL patients were treated with CHOP (mabthera + cyclophosphamide + adriamycin + vincristine + prednisone) chemotherapy, and the AML patients received chemotherapy with HA (homoharringtonine + cytarabine). Each patient with hematological malignancies received two cycles of chemotherapy in a 21-day cycle.

Instruments and reagents

Human lymphocyte separation medium (Sigma, P7794, USA), TRIzol (ThermoFisher Scientific, 155960-18), reverse transcription PrimeScript RT Master Kit (Takara, Japan, RR036A), Sysmex XT-2100L automatic hematology analyzer and real-time fluorescence quantitative PCR instrument (ABI, USA, model: 7500). ELISA kits for interleukin 17 (IL-17), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) (R&D, USA, DTA00D, DIF50, D1700).

Testing methods

(1) We collected 5 mL of fasting venous blood from the disease group before admission, and 5 mL of fasting venous blood from the healthy group on the day of the physical examination into vacuum anticoagulant tubes, which were shaken gently, and sent to the laboratory department. (2) Real-time fluorescence quantitative PCR was used to measure the CD200/CD200R expression level in PBMC. The PBMC of each specimen was first isolated using Ficoll density gradient centrifugation, and the total RNA of the samples was extracted using the TRIzol method. cDNA was prepared for reverse transcription for future use according to the kit's instructions. PCR amplification reactions were performed using the SYBR Green embedded dye method. The relative CD200 and CD200R expression levels in the observation group and the control group were calculated using the 2-ΔΔCt method and by taking β-actin as an internal reference gene. The PCR procedure and primers are shown in **Table 2**. (3) The plasma IL-17, TNF-α, and IFN-γ protein concentrations were measured using enzyme-linked immunosorbent assays (ELISA), and the operator strictly followed the kit's instructions.

Statistical methods

SPSS 21.0 software was used for the data processing, and GraphPad prism 8.0 was used to draw the figures. The enumeration data were presented as rate (%), and χ^2 tests were performed. The measurement data were expressed as the mean ± standard deviation ($\bar{x} \pm s$), and t-tests were used. A correlation analysis was carried out using Spearman's rank correlation

Table 2. The PCR procedure and primers

	CD200	CD200R
Upstream	CCGTCAACAAAGGCTATTGG	GACCAGAGAGGGTCTCACCA-
Downstream	ATITAGGGCTCTCGGTCCTG	CCGCTI'CGGCCACTAAGAAG
PCR procedure	Step 1: 94°C 3 min, Step 2: 94°C 1 min, 60°C 1 min, 72°C 1 min, 30-40 cycles; Step 3: 72°C 5 min, Step 4: 4°C forever.	

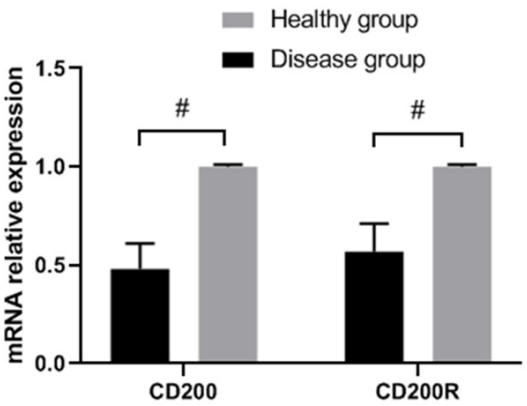

Figure 1. The CD200/CD200R expression level in PBMC - the disease group vs. the healthy group. # indicates P < 0.001.

test. *P* < 0.05 indicated a statistically significant difference.

Results

The CD200/CD200R expression levels in the PBMC-disease group vs. the healthy group

The mRNA expression levels of CD200 and CD200R in PBMC were down-regulated in the disease group compared with the healthy group, and the difference was statistically significant (t=21.840, 16.780, P < 0.001), as shown in **Figure 1**.

The plasma IL-17, TNF-α, and IFN-γ expression levels - the disease group vs. the healthy group

The plasma IL-17, TNF-α, and IFN-γ expression levels were up-regulated in the disease group compared to the healthy group, and the differences were statistically significant (t=12.260, 24.770, and 15.460; all P < 0.001) (**Table 3**).

Analysis of the correlation of CD200 and CD200R with the IL-17, TNF-α, and IFN-γ expression levels in the hematological malignancy patients

CD200 and CD200R showed a negative correlation with the IL-17 (t=-0.685, -0.621), TNF-α (t=-0.713, -0.682), and IFN-γ (t=-0.6393, -0.581) expression levels in the hematological malignancy patients (all P < 0.001) (**Table 4**).

Monitoring the CD200/CD200R expression level in the peripheral blood PBMC of the hematological malignancy patients through effective treatment

A total of 13 patients achieved complete clinical remission, including 7 with NHL, 2 with AML, and 4 with ALL. The peripheral blood specimens were further collected from 13 patients, and it was found that the CD200/CD200R expression level was up-regulated compared with the pre-treatment levels, and a statistically significant difference was found (t=14.527 and 13.674, P < 0.001) (**Figure 2**).

Discussion

The clinical treatment of hematological malignancies primarily depends on chemoradiotherapy. High doses of chemotherapeutic agents and radiation are effective at killing tumor cells while exerting a certain destructive effect on vascular endothelial cells and lymphocytes, whose destruction can inhibit bone marrow hematopoiesis and immune function during radiotherapeutic intervention [8, 9], leading to a poor chemo-radiotherapeutic effect and affecting the prognosis [10, 11]. This is mainly associated with the complex regulation of immune signaling networks involved in hematological malignancies [12]. Hence an in-depth understanding of the specific mechanisms of immune homeostasis imbalance in hematological malignancy patients is of great significance for the diagnosis and treatment of hematological malignancies.

CD200 is a leukocyte differentiation antigen that is widely expressed in a variety of cells and tissues [13, 14]. However, due to its short intracellular segment, CD200 lacks modulators or binding sites that can transmit intracellular signals. Unlike CD200, the expression of its receptor CD200R is more restricted to cells in the

Table 3. The plasma IL-17, TNF-α, and IFN-γ expression levels - the disease group vs. the healthy group ($\bar{x} \pm s$)

Group	n	IL-17 (pg/mL)	TNF-α (pg/mL)	IFN-γ (pg/mL)
Disease group	30	63.95 ± 8.13	88.34 ± 9.56	60.42 ± 7.45
Healthy group	30	41.37 ± 5.97	37.96 ± 5.72	33.52 ± 5.94
t		12.260	24.770	15.460
P		< 0.001	< 0.001	< 0.001

myeloid and lymphoid lineages [15]. Accordingly, CD200 can function by binding to a receptor expressed on other cells (e.g. CD200R). At the time of binding to CD200R as its receptor, CD200 transmits response signals that affect diversified physiological systems. CD200/CD200R has been found to play an essential role in maintaining pulmonary macrophage homeostasis and protecting lung from excessive injury caused by inflammatory reactions [16]. Immune homeostasis imbalance plays a key role in the occurrence of hematological malignancies. Patients usually present with a hyper-expression of the Th1/17 cytokines, breaking their autoimmune tolerance and producing autoantibodies, thereby causing disease. Studies in animal models have shown that the pathological progression of CD200/CD200R myelodysplastic syndrome in model mice is markedly accelerated, and the progression of myelodysplastic syndrome is fully terminated or delayed following the up-regulation of CD200 expression [17]. Additionally, CD200R can promote the conversion of macrophages from M1 to M2 type [18] inhibiting the production of its inflammatory factors such as IL-17, TNF-α and IFN-γ [19]. Consequently, CD200/CD200R binding can affect the production of cytokines and lower the expression levels of the proinflammatory cytokines (IL-17, TNF-α, and IFN-γ). IL-17, a representative of the Th1/17 cytokines, not only accelerates the recruitment and activation of neutrophils, it also induces the granulocyte-giant cell stimulating factor and IFN-γ, synergizes with TNF-α, and enhances the proinflammatory effect, and then further aggravates the condition. TNF-α, an initial initiator of the immune inflammatory reaction, can induce a release of various proinflammatory factors such as IL-1 and IL-6, which in turn initiate a waterfall cascade of inflammation exacerbating the patient's condition. Our study found that the CD200/CD200R signaling molecules showed low expression levels in hematological malignancy patients and were negatively correlated with the IL-17, TNF-α, and IFN-γ expression levels. These results indicated that the down-regulation of the CD200 expression will enhance the expression of the proinflammatory cytokines. Although CD200/CD200R molecules can suppress the immune system response and maintain the immune tolerance of the body, the ability to produce an inhibition of immunity after CD200/CD200R binding will be correspondingly weakened when the expression of CD200 is down-regulated, which is conducive to the expression of the pro-inflammatory cytokines and which ultimately causes an imbalance in the immune homeostasis. It has also been shown that the severe chronic enteritis symptoms will be attenuated in mice with high expressions of CD200 molecules compared with wild-type mice [20]. Therefore, we monitored the CD200/CD200R expression level in hematological malignancy patients using the effective treatment described in this study and found that the CD200/CD200R expression level in patients who achieved clinical complete remission was significantly up-regulated compared with the pre-treatment levels. These results indicated that CD200/CD200R1 signaling molecules are associated with the severity of hematological malignancies and have the potential to become indicators for monitoring hematological malignancy patients' conditions in the future.

In conclusion, CD200/CD200R has a low expression level in hematological malignancy patients, reducing the inhibitory effect on the expression of the inflammatory factors, enhancing the inflammatory factors, and mediating the occurrence and development of hematological malignancies. However, because the study population was solely from our hospital and the cohort was small, it might not have the representability of general patients. Therefore, these results should be interpreted and generalized with caution.

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Disclosure of conflict of interest

None.

Table 4. Analysis of the correlation of CD200 and CD200R with the IL-17, TNF-α, and IFN-γ expression levels in hematological malignancy patients

	Correlation with IL-17 in the plasma of hematological malignancy patients		Correlation with TNF-α in the plasma of hematological malignancy patients		Correlation with IFN-γ in the plasma of hematological malignancy patients	
	r	P	r	P	r	P
C200	-0.685	< 0.001	-0.713	< 0.001	-0.639	< 0.001
CD200R	-0.621	< 0.001	-0.682	< 0.001	-0.581	< 0.001

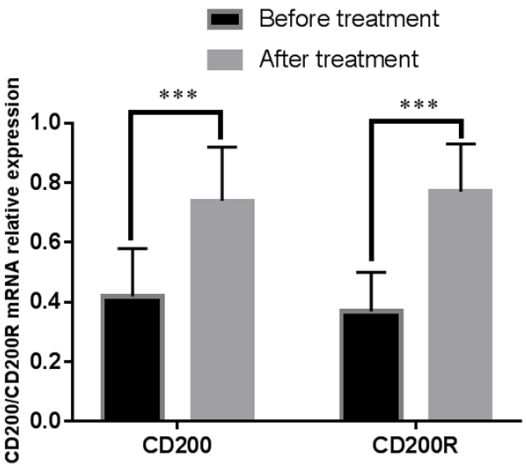

Figure 2. The CD200/CD200R expression level in the peripheral blood PBMC of hematological malignancy patients through effective treatment. *** indicates P < 0.001.

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