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Expression and function of CXCR2, CXCR7 of acute leukemic cells in rat

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ABSTRACT

Objective: To investigate the expression and function of chemokine receptor CXCR2 and CXCR7 in the rat with acute leukemia. **Methods:** Flow cytometry and RT-PCR were used to detect the CXCR2, CXCR7 expression on the bone marrow cell surface of the acute leukemia group and the control group. **Results:** The bone marrow cell surface CXCR2, CXCR7 relative fluorescence intensity of the observation group was significantly higher than the control group ($P<0.05$). The CXCR7 expression of the extramedullary infiltration group was significantly higher than non-extramedullary infiltration group ($P<0.05$).The CXCR2, CXCR7mRNA median expression level of the observation group was higher than the control group. The CXCR2 expression and CXCR7 expression of the observation group was positively correlated, and the correlation coefficient was 0.782 ($P<0.01$). **Conclusions:** The chemokine receptor CXCR2 and CXCR7 are highly expressed in acute leukemia, which may be associated with the occurrence of leukemia.

1. Introduction

Acute leukemia (AL) is a hematological disease originated from the malignant proliferation, differentiation and apoptosis of hematopoietic stem cell. Epidemiological survey showed that the AL occurrence is closely related the environment, occupation and genetic factors, and among them the genetic factor is considered to be the most important[1,2]. Studies have shown that chemokine receptor (CXCR) is related to the occurrence, development and metastasis of cancer. Cell growth and proliferation were regulated by many regulatory factors. The high affinity of CXCR2 can be combined with many chemokines and mediated inflammation. As one of the important CXCR family members, it plays an important role in leukemia cell survival, invasion and metastasis[3]. Recently, CXCR7 was discovered as one of the members of the CXCR family. There is not much research on the effects of CXCR2 and CXCR7 on acute leukemia at home and abroad. Based on the above background, this paper detected the bone marrow CXCR2 and CXCR7 expressions of the mice bone marrow in the observation group, and the control group by flow cytometry and RT-PCR, then compared the differences, and made a preliminary study on the relationship of CXCR2 and CXCR7 with acute leukemia.

2. Materials and methods

2.1. Construction of materials and animal models

Acute myeloid leukemia cell line HL60 cells were provided by the hematology department laboratory of medical college of southern medical university. All BALB/ N female nude mice 55 were purchased from the Experimental Animal Center of Southern Medical University, which were divided into the observation group (n=40) and the control group (n=15). The observation group was first irradiated by 3Gy60CO, after observing for 24 h, gave 4×10^6 HL60 cells in logarithmic growth phase by intraperitoneal injection, the

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control group were injected with the same volume of 0.2 mL normal saline without irradiation.

2.2. Reagents and instruments

Anti-mouse CXCR2-PE monoclonal antibody, anti-mouse CXCR7-PE monoclonal antibody were purchased from BD Biosciences, Trizol, chloroform, RT-PCR kit, DEPC, TaKaRa *Taq*™, DNA Marker (DL-2000) were purchased from TAKARA corporation, DaLian. Flow cytometry was purchased from Beckman Coulter Corporation, 37 degrees incubator was purchased from Beijing Changfeng Instrument Factory, PCR amplification was purchased from British Techne Corporation, gel imaging analyzer was purchased from Sigma (USA), ultra-low temperature freezer was purchased from China Haier.

2.3. Methods

2.3.1. CXCR 2, CXCR7 expressions of bone marrow cells by flow cytometry

Fresh bone marrow 2 mL was collected, and placed in ordinary centrifuge at 1 700 rpm. It was centrifuged for 5 min at room temperature, and the supernatants were removed. The middle layer of white blood cells was removed into another clean test tube. 3 mL PBS buffer solution was added, and it was centrifuged for 5 minutes at 1 500 rpm, room temperature, then supernatant were abandoned. 100 μL PBS buffer solution was added, and 25 μL of the mixture liquid was injected into three clean EP tubes. 10 μL PE-CXCR2 antibody, PE-CXCR7 antibody and isotype control antibody was added respectively, then was mixed after repetitive beat by pipette and incubated at 4℃ refrigerator for 45 minutes. 4 mL PBS buffer solution was added and centrifuged for 5 min at 500 rpm, supernatant was abandoned. 200 μL PBS buffer solution was added, and it was placed in the flow cytometry. 488 nm wavelength was selected for flow detection.

2.3.2. CXCR2, CXCR7 expressions by RT-PCR

Fresh bone marrow were collected at asepsis into EDTA tubes, lymphocyte separation medium were obtained and mononuclear cells were isolated by density gradient centrifugation. It was washed with PBS buffer twice, centrifuged at 1 500 rpm for 5 minutes at room temperature, the supernatants were removed. After blotting up, it was frozen and preserved at -80℃. Total RNA was extracted by one-step Trizol, the samples OD260/OD280 ratio was controlled in a range from 1.8-2.0. cDNA synthesis was carried out. The primer sequence and primers were designed by Shanghai Sangon Biological Engineering Services Ltd. CXCR2 upstream primer: CTT TTC TAC TAG ATG CCG C, downstream primer AGA TGC TGA GAC ATA TGA ATT T, amplified product was 417 bp; CXCR7 upstream primer: TGG GTG GTC AGT CTC GT, downstream primer CCG GCA GTA GGT CTC AT, amplified product was 294 bp; GAPDH was used as an internal control, upstream primer: ACC ACA GTC CAT GCC ATC AC, downstream primer TCC ACC ACC CTG TTG CTG TA, amplified product was 452 bp. The total reaction volume was 20 μL, amplification condition was 94 ℃, pre-denatured for 2 min; it was denatured at 94℃ for 30 s, primer annealing at 55℃/53℃ for 30 s, extension at 72 ℃ for 1 min, 30 cycles in total, then it was stopped at 4℃. After that, 5 μL product was obtained for 2% agarose gel electrophoresis. Quantity-One software was used for image gray scale analysis, internal reference GAPDH optical density values were used to standardized CXCR2 and CXCR7 mRNA absorbance values, CXCR2/GAPDH and CXCR7/GAPDH ratio was used as the relative content of CXCR2 and CXCR7 expression levels.

2.4. Statistical analysis

All data was analyzed with SPSS 16.0 software. Semi-quantitative expression levels of CXCR7 and CXCR2 were non-normal distribution, the measurement data was expressed as the median. The two samples rates or several sample rates of the enumeration data were evaluated by pearson *Chi*-Square. Two independent samples were compared by wilcoxon rank sum test. Correlation analysis was analyzed with the Speraman correlation analysis. α =0.05 is the level of significance, P<0.05 was regarded as statistically significant difference.

3. Results

3.1. Flow cytometry detection of bone marrow mononuclear cells CXCR2 and CXCR7 expression

CXCR2 expression in the observation group [(13.5±4.0)%] was significantly higher than that in the control group [(2.4±1.3)%] (P=0.01),and the CXCR7 expression of the observation group [(9.3±2.6)%] was significantly higher than that in the control group [(2.7±1.7)%] (P=0.003).

3.2. CXCR2 and CXCR7 gene expression

CXCR2 mRNA median expression levels of the observation group and the control group were 0.389 (0.173-0.658) and 0.074 (0.032-0.111) respectively, the CXCR2 expression in the observation group was significantly higher than that in the control group (P=0.008). CXCR7 mRNA median expression levels of the observation group and the control group were

0.422 (0.065-0.795) and 0.069 (0.037-0.122) respectively, the CXCR7 expression in the observation group was significantly higher than that in the control group (P=0.004).

3.3. Correlation analysis

CXCR2 and CXCR7 relative fluorescence intensity of the nude mice in the observation group were determined by the spearman rank correlation coefficient test, the result showed positive relationship between CXCR2 and CXCR7 (r=0.782, P<0.01).

4. Discussion

Tumorigenesis is a multi-step, multi-stage and multi-gene complex process, which is the final manifestation of the loss of control of cell growth and proliferation. After analysis on all the factors of cell growth and proliferation, it is considered that the chemokine receptor is closely related to the tumor occurrence[5-12]. Chemokine receptor belongs to the seven transmembrane G protein-coupled receptors. In the current seven kinds, CXCR and CXCR2 can combine with most of the chemokine receptors and participate in the body's inflammation and defense responses[13]. Oladipo *et al* considered that it can be expressed moderately in human microvascular endothelial cells and promote the vascular endothelial cell survival, proliferation and inhibit apoptosis, which then play an important role in the producing process of the tumor blood vessels[14-19]. CXCR7 is a new family member of the chemokine receptor with less expression in normal cells, while it can be mainly seen in tumor cell lines, activated endothelial cells, as well as fetal liver cells. Maciej considered that CXCR7 is highly expressed in leukemia cells, which can produce chemotaxis and induce neutrophil cell migration, then mediated large number of neutrophils locally concentrated which lead to peripheral tissue damage and increase the probability of cancer[20-25]. A number of studies by domestic and foreign scholars indicate that SDF-1/CXCR ligand system can activate many intracellular signaling pathways which lead to the activation of some transcription factors, such as interleukin-8 (IL-8), NF-kB, *etc*. The activation of the transcription factor can further promote endogenous chemokine and receptor expressions, while many of the transcription factor itself (IL-8, NF-kB) can form tumor molecular biomarker, thus promote tumor growth[26,27]. The study found that the bone marrow CXCR2 and CXCR7 expressions in nude mice with acute leukemia were significantly higher than non-maligance hematology disease nude mice, the bone marrow CXCR2 mRNA expression in acute lymphoblastic leukemia nude mice was higher than the non-hematologic malignancies nude mice, the CXCR7 mRNA expression in acute lymphoblastic leukemia nude mice was higher than the non-hematologic malignancies nude mice, and the CXCR2 and CXCR7 relative fluorescence intensity were positively correlated in the AL nude mice. We considered the high expression of CXCR2 and CXCR7 may be related to the occurrence of acute leukemia, and some literature suggests that the CXCR7 expression was significantly higher than CXCR2 of patients with acute leukemia extramedullary infiltration, which showed that CXCR7 high expression is closely related to its development and occurrence of this disease. Although some studies show that the CXCR7 expression levels is low *in vitro*, Burton *et al* analyzed blood samples of tumor cell lines by RT-PCR and flow cytometry, the results show that the acute leukemia has high expression of CXCR7, which is consistent with our results.

There is less research on CXCR2 in hematological tumor, but studies at home and abroad all considered CXCR2 can be activated by a variety of upstream factors. CXCR2 itself can promote high expression by autocrine or paracrine activation pathways, and then activated partial transcription factor by the activation of many cells intracellular signal transduction pathways, and can promote angiogenesis and tumor growth by the partial migration of chemotaxis vascular endothelial cells[28,29]. SINGH S can inhibit the growth of various tumors by blocking the function of CXCR2, further proved that CXCR2 possesses an angiogenesis effect and produce consistent effects in different tumor development processes[30].

In summary, the roles and relationship of CXCR2 and CXCR7 in hematological malignances have become the focus recently. With the increasing understanding of the relationship between CXCR2 and CXCR7 with acute leukemia and the invasive mechanism, its antagonists can provide new ideas and methods in the clinical treatment process.

Conflict of interest statement

We declare that we have no conflict of interest.

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