Effects of anti-$CXCR_4$ monoclonal antibody 12G5 on proliferation and apoptosis of human acute myelocytic leukemia cell line HL-60*

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【Abstract】 **Objective**: To investigate the expression of $CXCR_4$ on HL-60 cell line and the proliferation, apoptosis of HL-60 cell line cocultured with bone marrow stromal cells, so as to assess the possibility of 12G5, an anti-$CXCR_4$ monoclonal antibody, in eradicating the minimal residual disease. **Methods**: The activity of SDF-1 was inhibited by 10 μg/ml 12G5. After treatment with 12G5, the status of adhesion was observed, and the adhesion rates, apoptosis and cell cycles were detected after 24 h of treatment. Cell growth rates were measured by trypan blue exclusion. Cell growth curve was plotted, and the expression of PCNA and apoptosis related protein including PCNA, Bcl-2 and Fas were detected with immunohistochemical technique. **Results**: (1) There was middling degree expression of $CXCR_4$ on HL-60 membrane. From 0 h to 6 h, as the time of 12G5 incubation along, the expression of $CXCR_4$ decreased gradually. (2) After treatment for 24 h, the adhesion rates in the experiment group and the control were (39.4±7.9)% and (51.4±5.9)%, respectively. (3)After treatment for 24 h, the percentage of HL-60 cells in G_0/G_1 phase were (55.21±4.9)%, and that in S phase and G_2/M phase were (30.40±4.1)% and (14.39±5.2)%, respectively, with the corresponding proportions being (44.67±2.2)%, (45.30±3.7)%, and (10.03±2.6)% in the control. (4) The percentage of apoptotic HL-60 cells was (8.95±1.7)% in the experiment group, compared to (3.97±2.4)% in the control. (5)The survival rates of HL-60 cells decreased markedly at 48 h to 96 h, and the proliferation slowed down at this time duration. (6)The expression of PCNA and Bcl-2 down-regulated significantly, but the Fas protein expression was up-regulated. **Conclusion**: 12G5 could inhibit the capability of adhesion and proliferation of HL-60 cells and it can induce more cells to enter G_0/G_1 phase and promote apoptosis. It may be helpful by inhibiting the bioactivity of SDF-1 with 12G5 in the therapy of marrow residual disease.

【Key words】 SDF-1/$CXCR_4$; monoclonal antibody; acute leukemia; proliferation; apoptosis; drug resistance; marrow residual disease

In acute leukemia, a few leukemia cells could prevent from being killed by induction remission chemotherapy. And became of minimal residual disease (MRD) and the support of bone marrow stromal cells, these residual cells keep proliferating and finally result in the relapse of leukemia[1]. Furthermore, bone marrow stromal cells can affect migration, election, proliferation, differentiation, apoptosis of leukemia clone and can also make for drug resistance by complicated mechanisms, so modifying the leukemic hemotopoietic microenvironment may become a new strategy in the treatment of acute leukemias.

Stromal cell derived factor-1, SDF-1, excreted by marrow stromal cells, along with its receptor, $CXCR_4$, expressed on hematopoietic and leukemic cells, plays an important role in hematopoiesis, angiogenesis, and carcinogenesis. Although it was reported that local SDF-1 had the anti-tumor effect in melanoma[2], more results showed that SDF-1/$CXCR_4$ axis played an active pole in the infiltration of lung cancer and pancreatic cancer[3,4]. Moreover, SDF-1/$CXCR_4$ axis was closely related to mobilization, infiltration and adhesion of leukemia cells. This ligand/receptor sys-

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tem may play a key role in MRD formation[5]. But the above-mentioned conclusions only suggested the interaction between leukemia cells and marrow stroma, which lead us to study the influence of SDF-1/$CXCR_4$ on leukemia cells in marrow, namely effects on biological property, such as proliferation and apoptosis, *etc.* Meanwhile, it has been reported that beta1 integrin such as VLA-4 or VLA-5 and their ligands VCAM-1 play a vital role in the bio-effects network of SDF-1/$CXCR_4$. Furthermore, interaction between VLA-4 on leukemia cells and fibronectin on stromal cells was a decisive factor for the formation of MRD in acute myelogenous leukemia[6-8]. Therefore changes in bio-activities of SDF-1/$CXCR_4$ system may affect MRD formation through multi-pathways including changes of VLA-4 or VLA-5.

According to these evidences, in the present study, we designed to block the bioactivity of SDF-1 with 12G5, an anti-$CXCR_4$ monoclonal antibody, at a dose of 10 μg/ml[9], then observed the adhesive status of HL-60 cells with invert microscope, and detected the cell survival rates by means of trypan blue exclusion. Meanwhile, the expression of $CXCR_4$ on HL60 cell line, the cell cycles, apoptosis rates and the molecules related with proliferation and apoptosis including PCNA, Bcl-2 and Fas were detected with flow cytometry analysis and immunohistochemical technique. The aims of this experiment was to clarify the effect of SDF-1 on survival and proliferation of leukemia cells in bone marrow, in a bid to develop a new strategy in MRD treatment through modifying the hematopoietic microenvironment.

MATERIALS AND METHODS

Samples Fourteen marrow samples were all obtained from patients with de-novo or relapsed acute leukemias. Among these patients, according to FAB classification, 1 case was ANLL-M2, 1 case was M6, 3 cases were M3, 4 cases were ALL-L1 and 5 cases were L2. HL-60 cell line was purchased from China Academy of Science.

Reagents and equipments The antibodies 12G5(MAB170, IgG2a) were purchased from R&D Systems(USA). Flow cytometry (FACS-calibur) used in this work was from BD Corporation, USA. CO_2 incubator (SHELD-M2300) was manufactured in SIM International (USA), and invert microscope (DMIRD) was manufactured in Leica Corporation(Germany).

Cell culture Mononuclear cells from bone marrow samples were maintained in PRMI-1640 medium supplemented with 10 ng/ml bFGF, 10% fetal calf serum, 10% horse serum, 100 μ/ml penicillin, 100 μg/ml streptaquaine and 10^{-6} mol/L hydrocortisone at 37 C in humidified air with 5% CO_2. Stromal cells were grown from these mononuclear cells and PRMI-1640 medium were replaced once a week. When confluent monolayer was formed, stromal cells were digested by pancreatin and seeded at 1×10^4/ml together with HL-60 cells seeded at 1×10^6/ml in PRMI-1640 medium supplemented with only 10% fetal calf serum in 12-well or 24 well culture board.

Samples were randomized into 2 groups, with adding of 10 μg/ml 12G5 for the experiment group and without adding of 12G5 for the control. Being used to detect $CXCR_4$ expression, HL-60 cells were cultured in RPMI supplemented with 10% fetal calf serum.

Adhesiveness assay After treatment by 12G5 for 6 h, the adhesive states of HL-60 cell was detected by invert microscope. After treatment for 24 h, supernatant was collected and centrifuged at 1 000 r/min for 10 min and deposit was diluted with 0.5 ml of 0.01 mol/L PBS. After mixed on a shaker, 50 μl mixtures were used for the cell count. The adhesion rate was calculated as follows: (total cell number − cell number in supernatant after incubation)/ (total cell quantity) × 100%.

Typan blue exclusion At 0 h, 24 h, 48 h, 72 h and 96 h after treatment with 12G5, HL-60 cells were collected and equal volume of 0.2% typan blue was added. After stained for 8 min, viable cells were determined under light microscope. The cells without staining were classified as viable cells. On the contrast, strongly staining cells were dead cells. Cell survival rate was, in fact, the per-