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PRELIMINARY STUDY OF RETROVIRAL MEDIATED TRANSFER OF THE HUMAN mdr-1 GENE INTO MURINE AND HUMAN HEMATOPOIETIC STEM/PROGENITOR CELLS

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To investigate the characteristics of multidrug-resistance and transplantation of modified stem/progenitor cells by multidrug-resistant gene (mdr-1 gene), we established PA317/MDR-1 cell line which producing retroviruses by transfecting the retroviral vector PHaMDR1/A into packging cell line PA317 by Lipofectin. The virus titer of the supernatants was 1.2 $\times 10^5$ cfu/ml. We transfected the murine hematopietic cells collected from 5-FU pretreated mice and they showed the ability to reconstitute the long-term hematopoiesis of pre-irradiated mice. After 4 months, both of bone marrow cells and peripheral blood cells of transplanted mice still contained mdr-1 gene. We also transfered mdr-1 gene into human bone marrow CD34+ cells selected by using magnetic cell sorting system. PCR analysis showed that transduced CD34+ cells maintained the mdr-1 cDNA. A fraction of CFU-GM originated from transfected CD34+ cells had the charactor of resistance to Taxol. It is indicated that mdr-1 gene can be transduced into murine and human stem/proginitor cells through retroviral mediated gene transfer and it protects the transfected cells from cytotoxic drugs.

Key Words: Stem/progenitor cells, mdr-1 gene , Gene transfer , Retroviral mediated

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INTRODUCTION

Chemotherapy is still one of the major treatment for malignancies. The successful use of chemotherapy for cancer has been limited by the toxicity of those drugs on hematopoietic tissues such as bone marrow which is the most sensitive tissue to this type of therapy. This results in increased infections and the administration of the optimal doses and schedules of chemotherapeutic drugs is not possible due to the sensitivity to chemotherapy-induced marrow suppression. If we transduce mdr-1 gene into hematopoietic stem/progenitor cells, the cells will be protected and resistant to cytotoxic drugs therefore the doses of the drugs could be promoted from toxic effects of chemotherapy. It is suggests that mdr-1 gene therapy may help prevent myelosuppression following high doses of chemotherapy.

MATERIALS AND METHODS

Vector Production and Assay

The retroviral packaging cell line PA317 was grown in Dulbecco's modified Eagle medium (DMEM, Gibco) with 10% fetal calf serum(FCS). Producer cell line PA317/MDR-1 was created by transfering it the vector PHaMDR-1/A which containing the human mdr-1 cDNA using lipofectin (according to Promega Technical Bulletin) and it was selected by cultured in

DMEM with 200ng/ml colchicine. Viral supernatant was harvested after 6 hours culture of PA317/MDR-1 cells in the Iscove's modified Dulbecco medium (IMDM, Gibco) containing 10% horse serum (HS), 10% FCS and 5×10^{-7}mol/L hydrocortisone sodium hemisuccinate at 32 ℃ in 5%CO_2. The titration of retroviruses in the cell-free supernatants produced was preferred by a standard method using NIH3T3 cells.[1]

Selection of Murine and Human Hematopietic Cells

Murine hematopoietic cells were collected from the bone marrow of babl/c mice 48 hours after the injection of 5-fluorouracil (5-FU) at the concentration of 150mg/kg and mononuclear cells were obtained using Ficoll-Hypaque centrifugation. Human bone marrow obtained from the ribs of patients from Thoracic Surgery, who did not have evidence of bone marrow involvement. After isolation of mononuclear cells by using Ficoll-Hypaque centrifugation, CD34+ cell selection was performed by using the method as previously described.[2]

Transduction Procedure

Murine hematopoietic cells (5×10^5/ml) were transduced over 24 hours in a system containing 10%HS, 10%FCS, 8μg/ml polybrene (Sigma) and 50% retroviral supernatant. Human CD34-selected cells (1×10^5/ml) were prestimulated at 37 ℃ with 5%CO_2 in a saturated humidified atmosphere in IMDM medium supplemented with 10% HS, 10% FCS, 5×10^{-7}mol/L hydrocortisone, 50ng/ml stem cells factor (SCF), 100ng/ml GM-CSF, 100U/ml IL-3 for 24 hours. Then added the equal volume retroviral supernatant with the same cytokines and 8μg/ml polybrene. The cells then incubated at 37 ℃ with 5% CO_2 in a saturated humidified atmosphere for another 12 hours. After transduction, the cells were used for colony-forming cell assay and PCR analysis.

Murine Bone Marrow Transplantation

2×10^6 transduced murine hematopoietic cells were injected into irradiated balb/c mice (irradiated at 88.9 ren/min total of 8.0 Gy with ^{60}Co). After 4 months of hematopoietic reconstitution, the transplanted mice were killed and the bone marrow cells were used for colony-forming cell assay and PCR analysis.

Colony-Forming Cell (CFC) Assay

The murine hematopoietic cells were cultured at 2×10^5 / ml in RPMI-1640 medium (Gibco) supplemented with 25%HS, 20% WEHI-3 cell culture medium, 3% argar. Cells were plated in 1ml each 35mm plate. The plates were incubated at 37 ℃ in 5% CO_2 and a saturated humidified atmosphere. Hematopoetic colonies were scored after 12-14 days. Human colony-forming cell assay was performed as previously described.[3] For drug-resistance colony-forming cell assay, Colchicine and Taxol were added into the murine and human CFC culture system respectively. The concentrations of Colchicine and Taxol were 0, 10, 30, 50, 60 ng/ml and 0, 5, 10, 15, 20 ng/ml, respectively.

Polymerase Chain Reaction

cDNA from transducted cells[4] was used for PCR analysis. The sequence of upstream primer is 5'-CCCATCATTGCAATAGCAGC-3' and downstream primer is 5'-CTTCAAACTTCTGCTCCTGA-3'. Amplifications conditions were as follows: 95 ℃ for 5 min, then 30 cycles of 94 ℃ for 30 sec, 55 ℃ for 30 sec, 72 ℃ for 1 min, followed by extension at 72 ℃ for 10 min.10μl of each reaction were separated on 2% agarose gel (promega) and visualized in ultraviolet light by ethidium bromide staining.

RESULTS

Construction of PA317/MDR-1 Cell Line and the Titration of Retrovirus

PCR analysis shows that the mdr-1 gene has successfully transduced into the packaging cell line, PA317 (Figure)and it is named as PA317/MDR-1. The MDR-1 retrovirus-containing supernatant harvested from the PA317/MDR-1 culture medium had a titer of 1.2×10^5cfu/ml.

Frequency Analysis of mdr-1 Gene Transduction

After transferred the mdr-1 gene into the murine hematopoietic cells, the CFC of transduced cells were 10, 9, 12/2×10^5 cells with colchicine at the concen-

tration of 50ng/ml. In contrast, the non-transduced cells can not forming colonies under the same condition. The CFC in colchicine-free condition were 142,140,146/2×10^5 cells, so the transduction frequency of murine hematopoietic cells was 7.2% (10+9+12/142+140+146). By the same method the transduction frequency of human CD34-selected cells was 2.7%(5+2+3+1/115+97+89+108).

MDR-1 Gene Expression in Transduced Cells

After gene transfer, mdr-1 gene could be detected in the human CD34+ cells, both bone marrow and peripheral blood cells from the transplanted mice after 4 months of recovery by using PCR analysis (Figure 1).

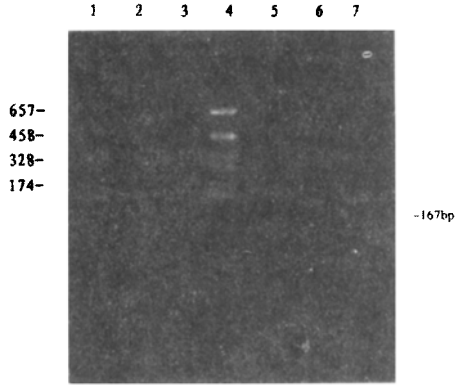

Fig 1. PCR analysis of mdr-1 gene transfected cells
Lane 1: PA 317 mdr-1 cells
Lane 2: Transduced human CD34+ cells
Lane 3: PA 317 cells
Lane 4: DNA marker
Lane 5: Transduced murine peripheral blood cells 4 months after transplantation
Lane 6: Tranceduced murine bone marrow cells 4 months after transplantation
Lane 7: Murine cells control

Function of mdr-1 Gene Transduced Cells

Drug-resistant colony forming cell assay shows that the mdr-1 gene transducted cells have the ability of drug-resistance than the non-transduced cells (Figure 2, 3). The mdr-1 gene trasduced murine hematoopoietic cells also have the ability of hematopoietic reconstitution of pre-irradiated mice, the target gene still remained after 4 months of transplantation.

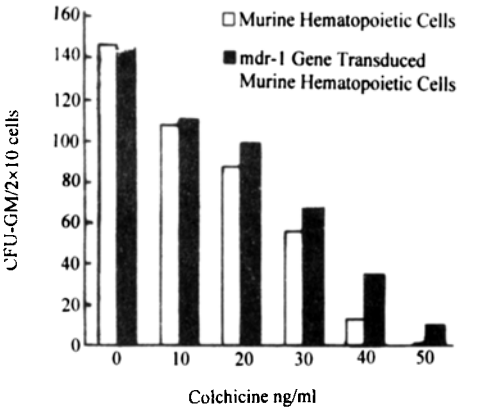

Fig 2. Capability of CFU-GM forming of mdr-1 gene transduced murine hematopoietic cells in medium containing colchicine

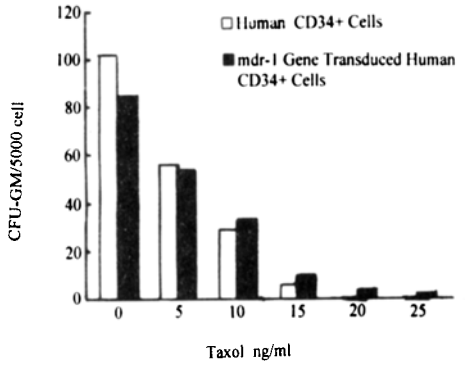

Fig 3. Capability of CFU-GM forming of mdr-1 gene transduced human CD34+cell in medium containing taxol

DISCUSSION

The stem cell and its self-renewing capacity form an ideal candidate for gene therapy. The mdr-1 gene codes for an ATP-dependent efflux pump protein, the p-glycoprotein, with MW 170-180 KD, which is located in the plasma membrane of cells. This pump extrudes chemotherapy agents from the inside to the outside of the cells, and thus maintains the intracellular levels of these drugs at low levels and the cells are protected. The expression of mdr-1 gene in stem cells is benefit to the body while it is harmful if it is overexpressed in tumor cells. These suggest that mdr-1 gene could be introduced into stem cells to protect these cells from the cytotoxic effects of chemotherapy.

For this study, we have established a cell line which producing retrovirus containing mdr-1 gene. We have transfected it into the murine hematopoietic cells and human CD34+ cells. Data show that mdr-1 gene can be succesfully transfered into both murine and human hematopoietic cells by using retrovirus, and the transfected murine bone marrow cells still have the ability of hematopoietic reconstitution. The mdr-1 gene expressed at least over 4 months in murine hematopoietic cells after transduction. A fraction of CFU-GM originated from transfected murine hematopoietic cells and human CD34+ cells has the charactor of resistance to cytotoxicdrugs.It is indicated that mdr-1 gene can protect the transduced cells from cytotoxic drugs.

In our study the transduction frequency of human CD34-selected cells was lower than that of the murine hematopoietic cells.It is reported that the infection of retrovirus is associated with the expression of the retroviral receptors on the cells.[5] Human stem cells prestimulated by cytokines such as SCF, IL-3, G-CSF, GM-CSF can increase the viral transduction frequency by promoting the expression of the receptors.[6]

mdr-1 gene can not only be used as a functional gene, it is also can be used as a marker gene. We can detect the mdr-1 gene by using molecularbiologic techniques and detect the P-glycoprotein by using McAbs.[7,8] Our study suggests that stem/progenitor cells transduced with chemoprotection gene should provide greater flexibility in the administration of higher doses of chemotherapeutic agents in the patients with cancer. It is indicated that retroviral mediated gene transfer of the human mdr-1 gene is a very effective mean for protective gene therapy of hematopoietic stem cells.

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