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Short communication

Co-expression of *MDR1* and HLA-B7 Genes in a Mammalian Cell Using a Retrovirus

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Using a retrovirus, foreign genes can be introduced into mammalian cells. The purpose of this study is to produce a retrovirus that can make the infected cells express two genes; the human multidrug resistance gene (*MDR1*) and the HLA-B7 gene, which is one of the major human histocompatibility complex (MHC) class I genes. For the expression of these genes, the internal ribosome entry site (IRES) was used, which was derived from the encephalomyocarditis (EMC) virus. In order to produce retroviruses, a retroviral vector was transfected into a packaging cell line and the transfected cells were treated with vincristine, which is an anti-cancer drug and a substrate for the *MDR1* gene product. This study revealed that two genes were incorporated into chromosomes of selected cells and expressed in the same cells. The production of the retrovirus was confirmed by the reverse transcription (RT)-PCR of the viral RNA. The retrovirus that was produced infected mouse fibroblast cells as well as the human U937. This study showed that packaging cells produced the retroviruses, which can infect the target cells. Once the conditions for the high infectivity of retrovirus into human cells are optimized, this virus will be used to infect hematopoietic stem cells to co-express *MDR1* and HLA-B7 genes, and develop the lymphocytes that can be used for the immnogene therapy.

Keywords: Hematopoietic stem cell, HLA-B7, *MDR1*, PA317, Retrovirus.

Introduction

Gene therapy is considered as a powerful tool for the treatment of cancer and many inherited diseases (Miller, 1992; Mulligan, 1993). In many cases, foreign genes have been introduced into mammalian cells using a retrovirus-mediated gene transfer, but the efficiency of the transfer, and the stability of the transferred genes, has been limited (Bodine *et al.*, 1990; Olsen *et al.*, 1993). The use of a dominant drug-selectable marker *in vitro* and *in vivo* enables the selection and enrichment of cells expressing the transduced gene.

The chromosome of an amphotropic packaging cell line, PA317, encodes three proteins that are necessary for the production of viral particles. After the transfection, a retroviral vector can be transiently or stably transcribed. The transcripts are packaged into viral particles, which are budded from the packaging cells (Frederick, *et al.*, 1992).

The study of the drug resistance mechanism of cancer cells has led to the identification of some of the genes and molecules that confer drug resistance. Among them, the gene responsible for multidrug resistance in human cells, termed *MDR1*, has been identified (Chen *et al.*, 1986; Ueda, *et al.*, 1987b). *MDR1* encodes a 170- to 180-kDa plasma membrane glycoprotein known as P-glycoprotein(P-gp). P-gps belong to the superfamily of ATP-binding cassette transporters and actively efflux a wide range of structurally diverse amphipathic drugs used to treat cancer (Gottesman and Pastan, 1993). P-gps undergo N-linked glycosylation, but the unglycosylation of P-gps might not affect the function of P-gps in several MDR cell lines (Paek, *et al.*, 1998). For the clinical approach to cancer therapy, target-sensitive immunoliposome can be useful. It contains doxorubicine, one of the substrates of P-gp, and a coupled monoclonal antibody that is specific to cancer cell surface antigen (Nam, *et al.*, 1998). Transfection studies clearly show that the P-gp is responsible for multidrug resistance (MDR) (Ueda, *et al.*,

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1987a). The retrovirus-mediated expression of the *MDR1* cDNA has been shown to confer MDR *in vivo* when the *MDR1*-carrying vector was introduced into the bone marrow cells of mice (Sorrentino *et al.*, 1992). Therefore, the *MDR1* gene could be used as a dominant selectable marker *in vivo* that would allow the selection and enrichment of cells co-expressing the second transduced gene (Sugimoto, *et al.*, 1995a).

It is well known that histocompatibility is important in the immunologic reaction against tumors in a host. *In vitro* and *in vivo* experiments clinically showed that the difference of MHC, or minor histocompatibility (MiHC) between the donor and the recipient, reduced the tumor recurrence in patients during allogeneic bone marrow transplantation (graft-versus-tumor effect) (Eibl *et al.*, 1996). In this study, we attempted to express HLA-B7 (a human MHC class I gene) along with the *MDR1* gene (drug-selectable marker) in a target cell using a retrovirus.

For the co-expression of the drug-selectable marker, *MDR1* gene, with the non-selectable genes, HLA-B7, we used the internal ribosome entry site (IRES) derived from the encephalomyocarditis (EMC) virus (Kaufman *et al.*, 1991; Morgan *et al.*, 1992). From this construct, a single mRNA is transcribed under the control of an upstream promoter, and the two gene products are translated independently from a single mRNA. The translation of one gene is cap-dependent, and the other is translated under the control of IRES.

In this study, we produced a retrovirus which programs infected target cells to express two foreign genes, *MDR1* gene and HLA-B7 gene, from the stably retroviral vector-integrated packaging cells.

Materials and Methods

Cell culture The amphotropic retrovirus packaging cell line PA317 (American Type Culture Collection Cat.# CRL-9078) and the mouse fibroblast cell line NIH3T3 were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The PA317 cell line was derived from TK$^-$ NIH 3T3 cells by cotransfection with a gene related to phage packaging signals and the Herpes simplex virus thymidine kinase (TK) gene. Introduction of retroviral vectors into these cells resulted in the production of a retrovirus with an amphotropic host range that was capable of infecting cells of many different mammalian species.

U937 is a well-known human immature macrophage (promonocytic) cell line. These cells were cultured in RPMI-1640 supplemented with 10% FBS.

Construction of vectors For the construction of retroviral vectors, a moloney murine leukemia virus (Mo-MLV)-derived retroviral vector, pLXSN, was used. In this plasmid, a 5'-long terminal repeat (LTR) promoter of Mo-MuSV LTR drove the expression of the retroviral mRNA (Miller and Rosman, 1989). For the coexpression of two genes, *MDR1*-IRES-HLA-B7 and HLA-B7-IRES-*MDR1* were inserted into pLXSN using a 5'-Xho I site in the multicloning site (MCS) and a 3'-Nae I site in the Neomycin resistant gene. The schematic structures of retroviral constructs are presented in Fig. 1.

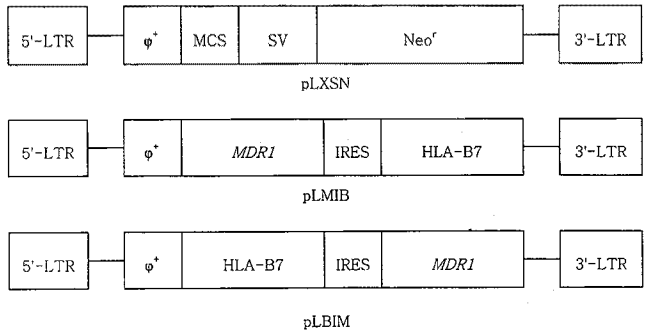

Fig. 1. Schematic structures of pLXSN, pLMIB, and pLBIM. Drawing is not to scale. MCS, multicloning site (EcoRI-HpaI-XhoI-BamHI); SV, early promoter of SV40; LTR, long terminal repeat; φ^+, packaging signal; Neo^r, neomycin resistant gene.

DNA transfection and the generation of a stable vector-producing cell line Packaging cells were plated at 1×10^5 cells per 60 mm-dish on day 1 and transfected with 10 μg of the retroviral vector on day 2. For the transfection, the vector was placed into 250 μl of a HEPES-buffered saline (HBS) and 16 μl of 2M $CaCl_2$ was added. After tapping the tube for 30 seconds, the tube was incubated at room temperature until a fine, hazy blue precipitate developed (approximately 45 m). After the media were removed from the dish containing packaging cells, the Ca^{2+}-DNA complex was added on the cell monolayer and the dish was gently rocked. The dish was incubated for 20 m in a clean bench and 10 ml of a fresh medium without serum was added. The treated cells were grown at 37°C in a humidified incubator with 5% CO_2. After 4 h, the media was removed and the new medium with 10% FBS was added. On day 3, cells were transferred from the 60 mm-dish to a 100 mm-dish using the trypsin-EDTA (GibcoBRL Cat.# 15400-054). On day 4, the cells were incubated with 40 ng/ml vincristine until colonies formed.

Infection and titration NIH 3T3 cells were plated at 8×10^4 cells per well in a 6-well plate and incubated overnight at 37°C in a humidified incubator with 5% CO_2. The 10-fold serial dilutions of the viral stock to be titrated were added into wells with 6 μg/ml of polybrene and the plates were placed in the incubator overnight. The next day, cells were incubated with 30 ng/ml vincristine for 10-14 days. When the colonies were countable, they were stained with crystal violet.

Preparation of genomic DNA and the polymerase chain reaction (PCR) Cells were trypsinized from the plate and counted using a hemacytometer and tryphan blue. After washing with a phosphate buffered saline (PBS), the 1×10^6 cells were resuspended in 900 μl of the extraction buffer (pH 8.0) (100 mM NaCl, 10 mM Tris-Cl (pH 8.0), 25 mM EDTA (pH 8.0), and 0.5% SDS), 100 μl of 1 mg/ml proteinase K added and incubated at 50°C for 12-16 h. After incubation, the cells were extracted with 1 volume of phenol : chloroform (1 : 1) and the nucleic acids were

precipitated by 3M sodium acetate and 100% ethanol. The precipitate was washed with 70% ethanol, 100 μl of TE (pH 8.0) added and incubated at 65°C for 1 h.

For the PCR, the sequences of forward and reverse primers for the *MDR1* gene were 5'-cccatcattgcaatagcag-3' (2596 to 2614 in *MDR1* gene) and 5'-gttcaaacttctgctcctga-3' (2733 to 2753 in *MDR1* gene), respectively. Those of the reverse primer for the HLA-B7 gene was 5'-ttcccgttctccaggtatct-3' (580 to 599 in HLA-B7 gene). The components of the PCR mixture were 200 ng of genomic DNA, 25 pmol of each primer, 5 μl of 10 × reaction buffer, 6.5 μl of 2.5 mM dNTP and 0.5 μl of 5 units/μl Taq polymerase. The final volume was adjusted to 50 μl with D.W.

Preparation of the viral RNA and RT-PCR The viral soup was harvested from plates in which transfected packaging cells were grown, and treated with a TRI-zol LS reagent (GibcoBRL Cat.# 10296-010). Chloroform was added in this mixture and the mixture was centrifuged. The upper phase was collected and the nucleic acids were precipitated by isopropanol. The prepared RNA was amplified to DNA by RT-PCR. The components of the RT reaction mixture were 4 μl of 5 × reaction buffer, 25 pmol of reverse primer, 14 units of RNasin, 1 μl of 25 mM dNTP, and 1 unit of the AMV-reverse transcriptase. After incubation at 37°C for 2 h, the same components of the PCR mixture of genomic DNA were added.

FACS analysis The cells to be analyzed were counted using the same method as that of genomic DNA. The 1×10^6 cells were washed with a washing solution, which was PBS supplemented with 2% FBS.

For the detection of P-gp, we used a human P-gp-specific antibody (Ab), MRK16 (KAMIYA BIOMEDICAL COMPANY Cat.# MC-012) as a primary Ab, and a goat anti-mouse IgG conjugated with Fluorescein (FITC) (Southern Biotechnology Associates, Inc. Cat.# 1080-02) as a secondary Ab. After washing, the cells were mixed with the primary Ab, and incubated on ice for 1 h. Ab-stained cells were washed and incubated with secondary Ab on ice for 30 m. The fluorescence-stained cells were washed twice and resuspended with PBS.

For the detection of HLA-B7, the cells were incubated with mouse anti-human HLA-B7 Ab that was conjugated with R-phycoerythrin (PE) (Serotec Ltd. Cat.# MCA986PE) on ice for 1 h. The stained cells were washed twice and resuspended with PBS.

The fluorescence staining level of the cells was analyzed using a FACSCalibur (BECKTON DICKINSON). The median of the cells was calculated by Cell-Quest.

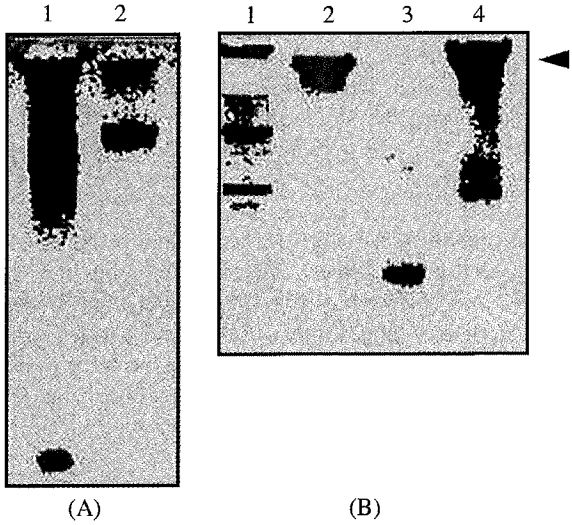

Fig. 2. PCR analysis of genomic DNA of transfected cells. (A) The genomic DNA of pLXSN- (lane 1) and pLMIB-transfected PA317 cells (lane 2). (B) The 10 μl of 50 μl PCR products used the pLMIB (positive control; lane 2), and genomic DNA of pLXSN- (lane 3) and pLMIB-transfected PA317 cells (lane 4) as a template. Lane 1 is 1kb ladder. The forward primer of the *MDR1* gene and the reverse primer of the HLA-B7 gene were used for PCR. PCR band is indicated by an arrowhead.

Co-culture of PA317 with U937 and the selection of retrovirus-infected U937 For the co-culture of transfected PA317 cells with U937 cells, we used RPMI-1640 which contains 10% FBS as a culture media. Because PA317 cells (normal fibroblast cells) grow on the surface of the plate and U937 cells (transformed cells) grow in suspension, it is possible that U937 cells are separated from PA317 cells. After the co-culture for 2 days and separation of the U937 cells, isolated U937 cells were treated with 10 ng/ml of vincristine for 2 weeks. Selected U937 cells were analyzed by FACS as described above.

Results

Retroviral vectors are stably integrated into the chromosome of packaging cells To select the transfected PA317 with a retroviral vector, the cells were selected with vincristine, and genomic DNAs were prepared. After the transfection of retroviral vectors, pLXSN and pLMIB, the packaging cells were selected with 40 ng/ml of vincristine. After 10 days, vincristine-resistant colonies were used for the preparation of genomic DNA. The result of PCR, in which the prepared DNA was used as a template, was that genomic DNA prepared from pLMIB-transfected PA317 was only amplified (Fig. 2). This result means that pLMIB-transfected PA317 is resistant to vincristine, and the resistance is due to the integration of the retroviral vectors. The same result was shown in the pLBIM-transfected PA317 cells (data not shown).

The packaging cells, in which retroviral vectors are stably integrated, express P-gps To test whether or not P-gps are expressed on the cell membrane of pLMIB-transfectant, these cells were stained as described in Materials and Methods. As shown in Fig. 3, the fluorescence peaks and the medians of the fluorescent intensity of the transfectants suggest that *MDR1* gene-transfected cells express human P-gps, and that the expression level of *MDR1* through the cap-dependent manner is higher than that through the IRES-dependent manner.

Packaging cells produce the retrovirus To determine

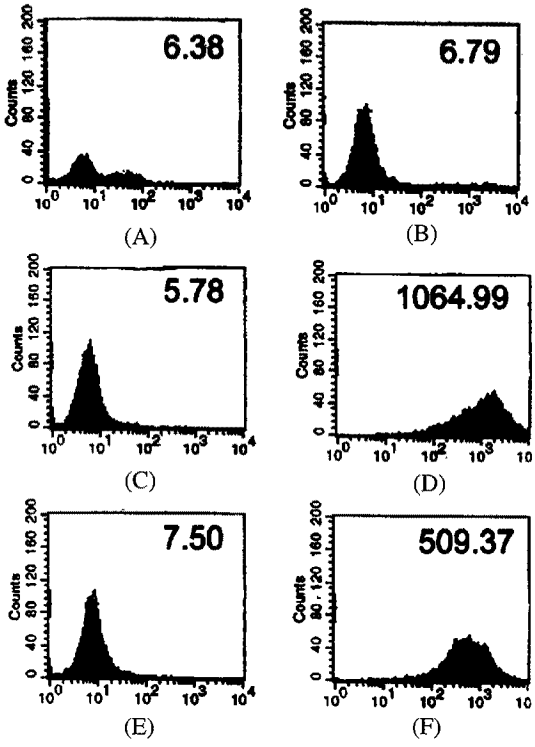

Fig. 3. FACS analysis of pLMIB- and pLBIM-transfected PA317 cells. Normal PA317 cells (A, B), pLMIB-transfectants (C, D), and pLBIM-transfectants (E, F) were stained with only secondary Abs (A, C, E) and with primary Abs and secondary Abs (B, D, F). X- and Y-axis represent the fluorescent intensity and number of cells, respectively. The numbers of each panel represent medians. Each cell was counted to 1×10^4 by FACSCalibur.

whether the retrovirus is produced from drug resistant cells or not, the viral RNA was prepared from the viral soup and RT-PCR was done. After the selection, the surviving cells were re-plated in 24-well plates. When the cells were confluently grown, the viral soup was harvested. From the viral soup, the viral RNA was prepared as described in Materials and Methods. The results of RT-PCR (Fig. 4) showed that pLMIB-transfected PA317 cells produce the retrovirus, which had the *MDR1* gene as well as the HLA-B7 gene.

The retrovirus infects target cells To determine the retrovirus titer of transfected cells, the conventional titration method was used, as described in Materials and Methods. The titers of pLBIM- and pLMIB-transfected cells were 2×10^3 CFU/ml and 4×10^3 CFU/ml, respectively.

To test whether or not the retrovirus infects U937 cells and if infected cells express P-gps and HLA-B7s, the pLMIB-transfected PA317 cells were co-cultured with U937 cells for 2 days. After the isolation of the U937 cells, cells were selected with vincristine and analyzed by FACS using MRK16 (anti-P-gp Ab) and anti-HLA-B7 Abs. The results (Fig. 5) showed that transduced U937 cells by retrovirus expressed HLA-B7s and P-gps.

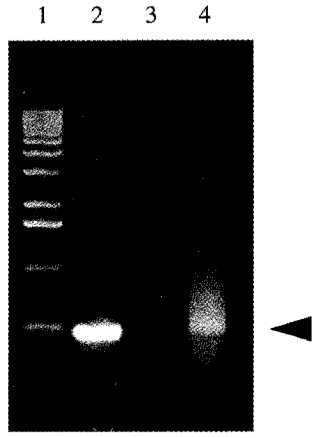

Fig. 4. RT-PCR analysis of viral RNA. The PCR products used the pLMIB (positive control; lane 2), and viral RNA prepared from the viral soup of pLXSN- (lane 3) and pLMIB-transfected PA317 (lane 4) as a template. Lane 1 is 1kb ladder. The forward and reverse primers of the *MDR1* gene were used for PCR. The PCR band is indicated by an arrowhead.

Discussion

In this study, we identified the packaging cell, PA317, integrated with the *MDR1* gene and the HLA-B7 gene that expresses P-gp. Furthermore, these PA317 can produce the retrovirus, which has RNAs derived from the retroviral vector, pLMIB, and enables the infection of the target cells. Infected cells appear to have *MDR1* and HLA-B7 genes derived from pLMIB. It means that these retroviruses modify target cells and make these cells express P-gps and HLA-B7s.

We showed by a FACS analysis that vincristine-resistant PA317 cells express the P-gps. However, it was difficult to show that HLA-B7 is expressed on the cytoplasmic membrane of the same cells, since HLA-B7 is the human MHC class I molecule that consists of two polypeptide chains. Those are α, or heavy chain encoded in the MHC, and a smaller non-covalently associated chain, β_2-microglobulin (β_2m), which is not encoded by the MHC gene (Charles and Paul, 1996). The PA317 cell line is derived from mouse fibroblasts and express β_2m of mouse. This β_2m cannot make the HLA-B7 express on the cell surface. For this reason, we did not show the expression of HLA-B7 on the cell membrane of the PA317 cells. Therefore, it was necessary to show that HLA-B7 is expressed on the surface of human target cells, which are infected by the retrovirus we produced. In this context, Fig. 5 shows that infected U937 cells express HLA-B7s after the U937 cells were co-cultured with transfected PA317 cells.

It was reported that titers of the retrovirus, which contain a non-selectable gene and the *MDR1* gene as a selectable marker, were variable. Titers of the retrovirus, which contains glutathione S-transferase pi (Doroshow *et al.*, 1995) or the HSV TK gene (Sugimoto *et al.*, 1995b) as a non-selectable gene, were 2×10^6 CFU/ml and 7.8×10^4 CFU/ml, respectively. In our experiments, the retrovirus titers produced by a mixed

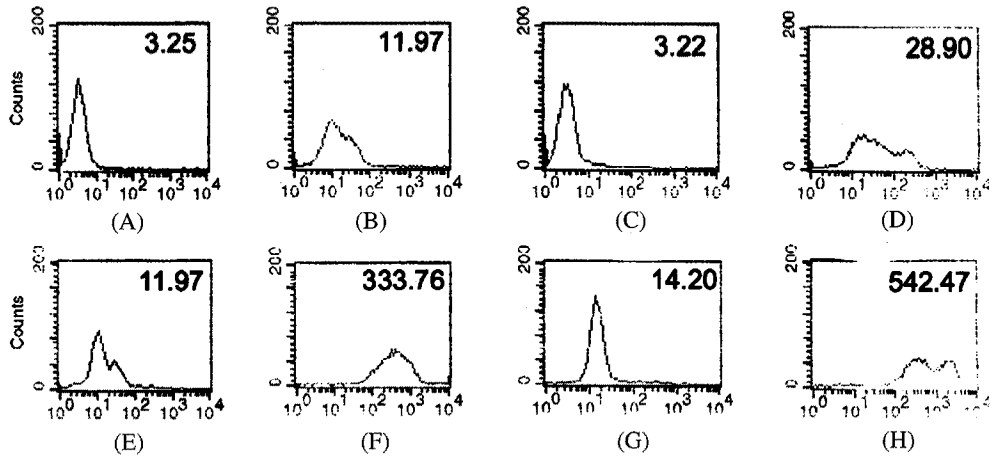

Fig. 5. FACS analysis of retrovirus-infected U937 cells. Normal U937 cells (A, B, C, D) and retrovirus-infected U937 cells (E, F, G, H) were stained with mouse anti-human IgG conjugated with PE (negative control;A, E), mouse anti-human HLA-B7 conjugated with PE (B, F), goat anti-mouse IgG conjugated with FITC (secondary Ab;C, G), and MRK16 (primary Ab) and secondary Ab (D, H). X- and Y-axis represent the fluorescent intensity and number of cells, respectively. The numbers of each panel represent the medians. Each cell was counted to 1×10^4 by FACSCalibur.

population of pLBIM- and pLMIB-transfected PA317 cells were 2×10^3 CFU/ml and 4×10^3 CFU/ml, respectively. These titers are lower than those reported by other groups described previously. These results can be explained in three ways: (1) The packaging cells may have lost their packaging ability. The PA317 cells are transfected with the packaging construct DNA and HSV TK gene. Therefore, after HAT (hypoxanthine, aminopterin, and thymidine) selection, the survived cells are supposed to produce the retrovirus. (2) The reason for the low titer appears to be that the condition of infection was not optimized. It was reported that the determination of optimum conditions for the density of cell seeding, length of incubation, and temporary storage of a virus-containing medium is needed for high titers (Parente and Wolfe, 1996). We used the conventional method for titration, as described in Materials and Methods. However, our results (data not shown) showed that the titer was increased about 10-fold when the concentration of serum was 2%. Therefore, for the higher titer, the condition of infection needs to be optimized. (3) The sizes of the genes could be too big to be packaged for high titer. By overcoming these three problems, it will be possible to produce the high-titer packaging cells. Additionally, it was reported that packaging cells have high titers after multiple superinfection (Parente and Wolfe, 1996). This report makes it possible to produce the higher-titer packaging cell lines.

We showed that the cDNA, whose translation was cap-dependent, was expressed at higher levels than the same cDNA whose translation was IRES-dependent (Fig. 3). This result is similar to the result of the other group (Sugimoto, *et al.*, 1995a). They reported that the cap-dependent translation is more effective than the IRES-dependent translation in retrovirus-transduced cells. These results show that the two types of retroviruses can be produced and that effects of the modified cells by one type of retrovirus may be different from those of modified cells by the other type in immunogene therapy. Therefore, it is necessary to determine which type of retrovirus will be infected in the target cells.

After hematopoietic stem cells were infected with the retrovirus that was produced in these experiments, these cells can express P-gp and MHC class I and the modified stem cells are able to produce lymphocytes *in vitro*. For the immunogene therapy, the stem cells or lymphocytes will be useful for autologous bone marrow transplantation.

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References

Bodine, D. M., McDonahg, K. T., Brandt, S. J., Ney, P. A., Agricola, B., Byrne, E. and Nienhuis, A. W. (1990) Development of a high-titer retrovirus producer cell line capable of gene transfer into rhesus monkey hematopoietic stem cells. *Proc. Natl. Acad. Sci. USA* **87**, 3738-3742.

Charles, A. D. and Paul, T. (1996) ImmunoBiology. 2nd ed. 4:3-4 : 4. Current Biology Ltd./Garland Publishing Inc.

Chen, C. J., Chin, J. E., Ueda, K., Clark, D. P., Pastan, I., Gottesman, M. M. and Roninson, I. B. (1986) Internal duplication and homology with bacterial transport proteins in the mdr 1 (P-glycoprotein) gene from multidrug-resistant human cells. *Cell* **47**, 381-389.

Doroshow, J. H., Metz, M. Z., Matsumoto, L., Winters, K. A., Sakai, M., Muramatsu, M. and Kane, S. E. (1995) Transduction of NIH3T3 cells with a retrovirus carrying both human MDR1 and glutathione *S*-transferase pi produces broad-range multidrug resistance. *Cancer Res.* **55**, 4073-4078.

Eibl, B., Schwaighofer, H., Nachbaur, D., Marth, C., Gachter, A.,

Knapp, R., Bock, G., Gassner, C., Schiller, L., Petersen, F. and Niederwieser, D. (1996) Evidence for a graft-versus-tumor effect in a patient treated with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. *Blood* **88**, 1501-1508.

Frederick, M. A., Roger, B., Robert, E. K., David, D. M., Seidman, J. G. John, A. S. and Kevin, S. (1992) *Short Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New York.

Gottesman, M. M. and Pastan, I. (1993) Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* **62**, 385-427.

Kaufman, R. J., Davies, M. V., Wasley, L. C. and Michnick, D. (1991) Improved vectors for stable expression of foreign genes in mammalian cells by use of the untranslated leader sequence form EMC virus. *Nucleic Acids Res.* **19**, 4485-4490.

Miller, A. D. (1992) Human gene therapy comes of age. *Nature* **357**, 455-460.

Miller, A. D. and Rosman, G. J. (1989) Improved retroviral vectors for gene transfer and expression. *Biotechniques* **7**, 980-990.

Morgan, R. A., Couture, L., Elroy-Stein, O., Ragheb, J., Moss, B. and Anderson, W. F. (1992) Retroviral vectors containing putative internal ribosome entry sites: development of a polycistronic gene transfer system and applications to human gene therapy. *Nucleic Acids Res.* **20**, 1293-1299.

Mulligan, R. C. (1993) The basic science of gene therapy. *Science* **260**, 926-932.

Nam, S. M., Cho, J. E., Son, B. S. and Park, Y. S. (1998) Convenient preparation of tumor-specific immunoliposomes containing doxofubicin. *J. Biochem. Mol. Biochem.* **31**, 95-100.

Olsen, J. C., Johnson, L. G., Wong-sun, M. L., Moore, K. L., Swanstrom, R. and Boucher, R. C. (1993) Retrovirus-mediated gene transfer to cystic fibrosis airway epithelial cells: effect of selectable marker sequences on long-term expression. *Nucleic Acids Res.* **21**, 663-669.

Paek, N. S., Namgung, J., Lee, J. J., Choi, Y. J., Kim, T. H. and Kim, K. W. (1998) Effect of α-glycosidase inhibitor in multidrug resistant cell lines. *J. Biochem. Mol. Biochem.* **31**, 269-273.

Parente, M. K. and Wolfe, J. H. (1996) Production of increased titer retrovirus vectors from stable producer cell lines by superinfection and concentration. *Gene Therapy* **3**, 756-760.

Sorrentino, B. P., Brandt, S. J., Bodine, D., Gottesman, M. M., Pastan, I., Cline, A. and Nienhuis, A. W. (1992) Selection of drug-resistant bone marrow cells *in vivo* after retroviral transfer of human MDR1. *Science* **257**, 99-103.

Sugimoto, Y., Aksentuevich, I., Murray, G. J., Brady, R. O., Pastan, I. and Gottesman, M. M. (1995a) Retroviral coexpression of a multidrug resistance gene (*MDR1*) and human α-galactosidase A for gene therapy of fabry disease. *Human Gene Ther.* **6**, 905-915.

Sugimoto, Y., Hrycyna, C. A., Aksentijevich, I., Pastan, I. and Gottesman, M. M. (1995b) Coexpression of a multidrug-resistance gene (MDR1) and herpes simlex virus thymidine kinase gene as part of a bicistronic messenger RNA in a retrovirus vector allows selective killing of MDR1-transduced cells. *Clin. Cancer Res.* **1**, 447-457.

Ueda, K., Cardarelli, C., Gottesman, M. M. and Pastan, I. (1987a) Expression of a full length cDNA for the human "MDR1" gene confers resistance to colchicine, doxorubicin, and vinblastine. *Proc. Natl. Acad. Sci. USA* **84**, 3004-3008.

Ueda, K., Clark, D. P., Chen, C. J., Roninson, I. B., Gottesman, M. M. and Pastans, I. (1987b) The human multidrug-resistance (mdr1) gene. cDNA cloning and transcription initiation. *J. Biol. Chem.* **262**, 505-508.