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ORIGINAL ARTICLE

Chun-Bao Guo · Ying-Cun Li · Xian-Qing Jin

Chemoprotection effect of retroviral vector encoding multidrug resistance 1 gene to allow intensified chemotherapy in vivo

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Abstract Increasing the expression of human multidrug resistance (MDR) 1 gene in bone marrow cells to prevent or circumvent bone morrow toxicity from chemotherapy agent is a high priority of dose intensification protocols. In this study, we have used a tumor-bearing model to investigate the chemoprotection effect of MDR1 gene by transfecting retroviral vectors containing and expressing the MDR gene in vivo. Hematopoietic progenitor cells were served as target of MDR1 gene transferred by the mediation of retrovirus vector and engrafted into the BALB/c mice with 60Co-γ ray exposure in advance. Doxorubicin (5, 10, and 20 mg/kg) suppressed tumor growth of the xenograft significantly in a dose-dependence mode if supported by suitable peripheral WBC. WBC count revealed that the mice that had received gene-transduced cells showed a significant increase in WBC count compared with their gene-transduced naive counterparts. The function and expression of MDR1 gene were detected by flow cytometry, RT-PCR, and immunohistochemistry (IC) method. MDRl mRNA expression could be detected in BM. Spleens contained measurable amounts of MDRl mRNA. Tail vein blood and tumor tissue detected MDRl DNA but no MDRl mRNA expression. FACS analysis of infected BM cells obtained 6 weeks later showed high levels of P-gp function. Based on these results we conclude that cytostatic drug resistance gene therapy may provide some degree of chemoprotection and so can increase the chemotherapy dose to kill tumor cells.

Keywords Gene therapy · Multidrug resistance · P-glycoprotein · Marrow transplant

C.-B. Guo · Y.-C. Li · X.-Q. Jin (✉)
Laboratory of Surgery, Children's Hospital of Chongqing Medical University, 132 Zhongshan Rd., 400014 Chongqing, People's Republic of China
E-mail: gchunbao@yahoo.com.cn
Tel.: +86-23-63870729

Introduction

Chemotherapy has played an important role in treatment of human cancers besides surgery and radiation. But, some chemotherapeutic agents have toxic side effects to deal with a large tumor burden, so the sensitive normal tissues, such as bone marrow (BM), limit the maximum tolerated doses in some solid tumors such as breast carcinoma. Treatment is at most palliative, remission is of transient duration, and the overall survival for the patient is sometimes minimal [1, 2]. P-glycoprotein (P-gp) is the drug efflux pump to extrude hydrophobic compounds including chemotherapeutic drugs to prevent accumulation and it is widely expressed in normal human tissues. But erythroid and myeloid BM cells do not possess significant amounts of P-gp as a protective factor, so its sensitivity to chemotherapy is a major limitation for high-dose chemotherapy regimens, which result in clinical phenomenon of neutropenia and thrombocytopenia [3–5]. How to prevent myelosuppression and eliminate dose-limit from chemotherapy treatment is of high priority for research.

Hematopoietic stem cells are characterized by their ability to sustain lymphomyeloid hematopoiesis over the lifetime of an animal. Transfer of drug-resistant genes to hematopoietic stem cells has shown success as an experimental strategy for protecting hematopoiesis against drug-induced myelosuppression [6, 7]. During the past few years, significant progress of effective gene therapy has been made. Several murine models of hematopoietic disorders have been successfully corrected using retroviral or lentiviral vectors to target hematopoietic stem cells (HSCs) [8–12]. The success of these preclinical studies has relied on highly efficient gene transfer into murine HSCs and myeloablative conditioning of transplant recipients to ensure high-level engraftment of transduced cells [13, 14]. Retroviral vectors have been used extensively in gene transfer because of their efficient entry into cells and integration into the host genome [15]. Both in vitro and in vivo

studies support that transfer of MDR1 retroviral vectors resulted in a significant increase in P-gp expression in most cases and transplantation of murine BM cells that were infected with MDRl-containing retroviruses into sublethally irradiated recipients resulted in consistently very low levels of MDRl expression in developing hematopoietic cells 6 months after transplantation [16]. Several approaches have been explored for amplifying genetically modified stem cell populations by in vivo selection in order to determine the expression and toxicity associated with retroviral-mediated transfer of the MDRl gene to hematopoietic cells in patients undergoing high-dose chemotherapy and autologous BM transplantation [17, 18]. It has also been reported using purified human CD34+ progenitor cells. When CD34+ cells were cultured in cytokines for 6 days after they were infected with MDRl-containing retroviruses, upto 11% of the resulting progeny expressed MDRl by fluorescence-activated cell sorting (FACS) analysis [19].

With the toxic effects of subsequent high-dose chemotherapy, specific and nonspecific suppression growth of tumor volume and cell replication would be demonstrated [20]. The goal of the studies was to derive preclinical in vivo model to study if the MDR1 gene capable of high-level expression in hematopoietic progenitor cells is resistant to the leukopenia induced by many chemotherapeutic agents from natural products. But for clinical usage, the patients would be with cancer not involving the bone marrow, in which routinely high-dose chemotherapy is combined with autologous BM transplantation. The present study should be useful in humans to prevent marrow toxicity, thereby providing a model for the use of the MDRl cDNA to protect BM during dose intensification protocols during tumor chemotherapy.

Materials and methods

Donors and recipient mice

Protocols for animal care and experimental management were approved by the Children's Hospital Scientific Committee, Chongqing Animal Research Institute. Normal BALB/c mice with weight of 20–25 g and between 8 and 14 weeks of age were used as BM donors and tumor burden BALB/c mice as recipients and were all provided by experimental animal center of Chongqing Medical University. The mice were cared for and handled according to the national regulation for experimental animals.

Tumor-derived H22 cell lines from the mouse hepatoma was supplied by the Chongqing Cancer Research Bank (Chongqing, China). The cells were cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37°C in RPMI 1640 medium (LifeTech, Grand Island, NY) supplemented with 10% heat-inactivated FCS (Bio-Whittaker, Walkersville, MD). The medium was changed every 3 days.

Each mouse was inoculated with H22 (supplied by the Chongqing Cancer Research Bank, Chongqing, China) with 5×10^6 cells per animal subcutaneously on the left side of the armpit on day 0. The animals were randomly divided into five groups (8 animals/group) immediately after inoculation and received one of the following treatments: normal group (N), healthy normal animal with doxorubicin 5 mg/kg per week; control group (C), mice engrafted hematopoietic cells without MDR1 gene transfection with doxorubicin of 5, 10, and 20 mg/kg, mice engrafted hematopoietic cells with MDR1 gene transfection and doxorubicin of 5, 10, and 20 mg/kg, respectively. The shortest and longest diameter of the tumor were measured with calipers at 7-day intervals, and tumor volume (mm^3) was calculated using the following standard formula: (the shortest diameter)^2 × (the longest diameter) × 0.5.

MDR1 retroviral packaging cell culture and production of retroviral supernatants

PA317 packaging cell line (kindly provided by Dr. Wang) containing full-length human MDR1 cDNA was cultured at 37°C in 5% CO_2 for 80% plat filled then fed with fresh medium 24 h before harvest of virus-containing supernatants and then concentrated by centrifugation at 40,000*g* for 2 h. Viral supernatants were removed and sterile-filtered (0.45 μm).

Donor BM cells harvest followed by infection with retroviral in vitro

Whole BM was harvested from the BALB/c mice by flushing femurs, tibiae, and humeri with ice-cold Dulbecco's Modified Eagle Medium (DMEM; Gibco, Grand Island, NY). Aliquots of harvested BM (3×10^6 cells) were cocultured with retroviral supernatants. Cocultures were incubated for 24–48 h, Growth factors were included in the suspension culture at the following concentrations: 20 ng/ml murine IL-3 (Amgen, Thousand Oaks, CA), 50 ng/ml human IL-6 (Amgen), and 50 ng/ml murine SCF (Amgen and R&D Systems, Minneapolis, MN) and 2 μg/ml Polybrene (Sigma, St. Louis, MO). The unattached BM cells were aspirated and then counted in a hematocytometer using acetic acid and trypan blue to determine the number of viable nucleated cells (9). Finally, aliquots of $1–2\times10^6$ viable nucleated cells in 0.5 ml or less of PBS were collected in 1-ml tuberculin syringes and kept at room temperature in preparation for injection into irradiated recipient mice.

Transplantation protocol followed by chemotherapy

Recipient tumor burden mice were sublethally irradiated with 1.5 Gy using 60Co-γ source. Donor BM cells was then infused slowly through the central tail vein of the

recipients. Each animal received 10×10^6 BM cells from either the MDR1 transduction BM or BM without MDR1 transduction to show engraftment of non-MDR-expressing BM. Radiation controls were prepared simultaneously to confirm adequacy of the sublethal irradiation dose. Doxorubicin was administered after 3 days of BM transplantation by a single intraperitoneal injection into the lower right quadrant of the peritoneum. Drug doses were 5, 10, 20 mg/kg in accordance with clinical usage and injected every week per experiment. Peripheral blood was collected by tail vein. The refractile viable leukocytes (white blood cells [WBC]) were counted on weeks 0 (before treatment), 1, 2, 3, 4, 5, and 6 on a ultraplane Neubauer's hemocytometer (Hawser Scientific, Pittsburgh, PA).

BM recipients were killed at predetermined time by cervical dislocation. Spleens and tumor tissue were removed en bloc at necropsy and exsanguinated with PBS washing and placed on dry ice immediately. BM cells were flushed from long bones with PBS/1 mmol/l EDTA. Tissue samples for paraffin sections were fixed in 4% paraformaldehyde in PBS, dehydrated, and embedded in paraffin and then subjected to immunohistochemistry. Tissue samples for RNA extraction were frozen in liquid nitrogen immediately upon collection and shipped on dry ice.

P-gp immunohistochemical staining of BM and tumor tissue of recipient mice

Paraffin sections were first submitted for rehydration and rehydrated sections were incubated with the 1:200 dilution of rabbit anti-human primary antibody to P-GP (Santa Cruz Biotechnology, Santa Cruz, CA) or control IgG (1:1000) overnight at 4°C. The tissue sections were washed in PBS, then incubated with a 1:300 dilution of biotinylated secondary sheep anti-rabbit or goat anti-rabbit IgG. After washing with PBS, tissue sections were incubated with an avidin–biotin complex and developed in 0.075% (w:v) 3,3 diaminobenzidine (DAB). After lightly counterstaining with hematoxylin, the sections were dehydrated.

PCR and RT-PCR analysis

Genomic DNA of different samples was extracted with genomic DNA purification kit (Promega, Madison, WI). Total RNA was extracted with Trizol reagent (Gibico) and was precisely quantified. One microgram of total RNA was reversely transcribed into complementary DNA (cDNA). RT-PCR measurement of MDR1 cDNA was performed. The primer sequences were designed with the Primer Premier 5.0 program (Premier Biosoft International, Palo Alto, CA), Primer Expression 5.0 program (PE Applied Biosystems), and synthesized (Shanghai Sangon Biotech, Shanghai, China). For MDR1 (GenBank accession number http://ajplung.physiology.org/cgi/external_ref?access_num= AJ290968&link_type= GEN M80637), the forward primer is: 5′-CCC ATC ATT GCA ATA GCA GG-3′; reverse primer is, 5′-GTT CAA ACT TCT GCT CCT CA-3′; results in a fragment of 157 bp. A standard PCR was performed with 35 cycles of 60°C for 20 min, 94°C for 4 min, 94°C for 30 s, 57°C for 45 s, 72°C for 60 s, and 72°C for 7 min. For β-actin, the forward primer is; 5′-ACA CTG TGC CCA TCT ACG AGG-3′; the reverse primer is; 5′-AGG GGC CGG ACT CGT CAT ACT-3′; results in a fragment of 621 bp. The optimized PCR was performed with 35cycles of 60°C for 20 min, 94°C for 5 min, 94°C for 30 s, 62°C for 30 s, 72°C for 45 s, and 72°C for 10 min. Part of the PCR product (20 μl) was examined on a 1.2% (w/v) agarose gel, together with 100 DNA size markers, and each lane was scored for the presence or the absence of the expected PCR product. The gel images were digitally captured with a CCD camera.

Flow cytometry analysis and P-gp functionality

P-gp activity was determined by using the daunomycin efflux assay. Cells (10^6) were incubated at 37°C in 5% CO_2 for 30 min in a staining medium (RPMI 1640 with 10% fetal calf serum) containing 7.5 μg of daunomycin (Sigma) per milliliter. After two washes, cells were transferred into daunomycin-free medium and allowed to efflux for 10 min. In parallel experiments, efflux was performed in the presence of 10 mM verapamil, a P-gp inhibitor. Flow cytometric measurements were carried out on a FACScan flow cytometer (Becton Dickinson, San Jose, CA) at an excitation wavelength of 488 nm, using 530/30-nm (green fluorescence) or 585/40-nm (red fluorescence) bandpass filters. Results are presented as histograms of daunomycin fluorescence. Control flow cytometric materials were the murine BM cells.

Statistical analysis

Results were expressed as mean ± SD. For multiple comparisons, continuous parametric data were subjected to analysis of variance (ANOVA) followed by the Student–Newman–Keuls post-hoc test for between-group differences. For intensity of immunostaining score, a Kruskal–Wallis test was used to detect differences across the groups, followed by the Wilcoxon–Mann–Whitney test for differences between the two groups. Results were considered statistically significant at $P < 0.05$.

Results

Production and characterization of BM infected with retroviruses containing MDR cDNA with a period of time in culture

Infected BM showed a 157 bp unique MDR1-specific band (Fig. 1, arrow) representing cellular DNA provirus

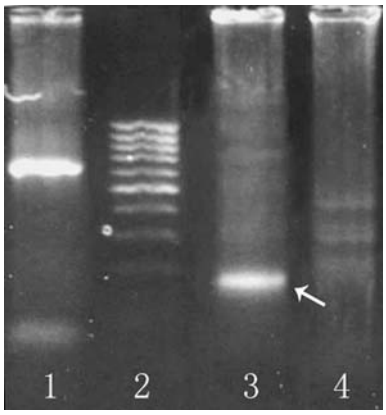

Fig. 1 Exogenous hMDR1 mRNA expression levels assessed by RT-PCR in murine MDR1-BM according to the procedures described in the Materials and Methods section. *Arrow* at right indicates expected size band (157 bp), diagnostic for the human MDRl transgene. Three examples were included per condition and data shown are representative of two independent experiments

junction fragments. BM cells were positive for P-gp expression but showed low levels of expression (Fig. 2b). In comparison, infected BM expressed much higher levels of P-gp (Fig. 2a). The functionality of P-gp was tested in BM by analyzing the daunomycin uptake after 2 h (Fig. 3). Compared to BM, daunomycin accumulation was lower in BM infection with MDR1 gene indicating that these cells overexpressed a functional P-gp. When combined, these data illustrate a significant P-gp expression and functionality in BM cells.

Functionally expression of exogenous hMDR1 in infected BM in vivo

The percentage of BM cells express low levels of MDR1, that is, only 1% positive in N, C groups while the infected BM cells are from 25 to 35% positive for high levels of MDRI expression (Fig. 4). Although the expression of MDRl on infected BM cells was decreased at the examined time, FACS analysis of infected BM cells obtained 6 weeks later showed high levels of MDRl expression over a 6-week period (Fig. 5). Thus, expression of the transduced MDRl gene is stable in BM cells and allows a potential use for these cells in vivo.

Expression of hMDR1 gene in live mice over time

MDRl mRNA expression could be detected in all BM samples from mice that were reconstituted with MDR-BM throughout the experiment. Over 90% of the mice analyzed by MDR1 PCR analysis of their tail vein blood post-transplantation contained the MDR gene (Fig. 6), but not MDRl mRNA. Spleens contained measurable amounts of MDRl mRNA at day 43. Thirty percent of tumor tissue detected MDRl DNA but no MDRl mRNA expression. Neither MDRl DNA nor its transcripts could be detected in any of the control animals that received normal marrow (data not shown). This result indicates that, in this animal, BM stem cells were clearly transduced initially since mature granulocytes containing high levels of MDR protein were present as long as 6 weeks post-transplantation.

Sustained hematologic improvement in animals that received transplants

It is evident that the transplantation of MDRl-BM cells afforded chemoresistance against doxorubicin, because there was relatively no apparent decrease (compared with N and C groups) in the peripheral WBC (Fig. 7). This effect can be attributed to the

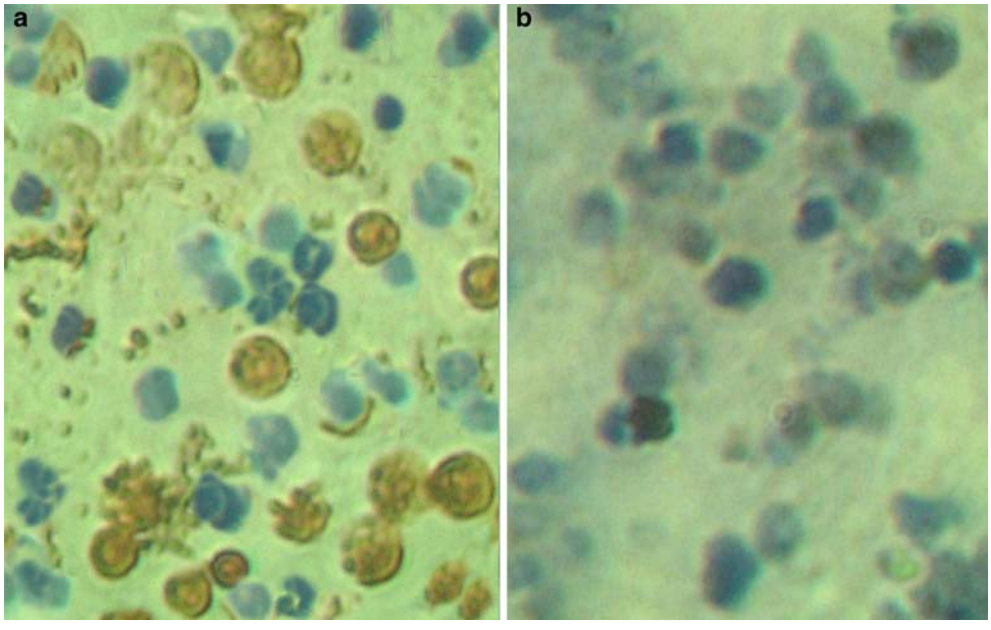

Fig. 2 Immunolocalization of hMDR1 in MDR1-infected BM. Positively stained cells appear in *brown* color due to binding of the antibody-activated 3,3-diaminobenzidine (DAB) chromogen. In MDR1-infected BM strong positive immunoreactivity to P-gp is present (**a**), but the control BM is negative (**b**). Original magnification, ×200

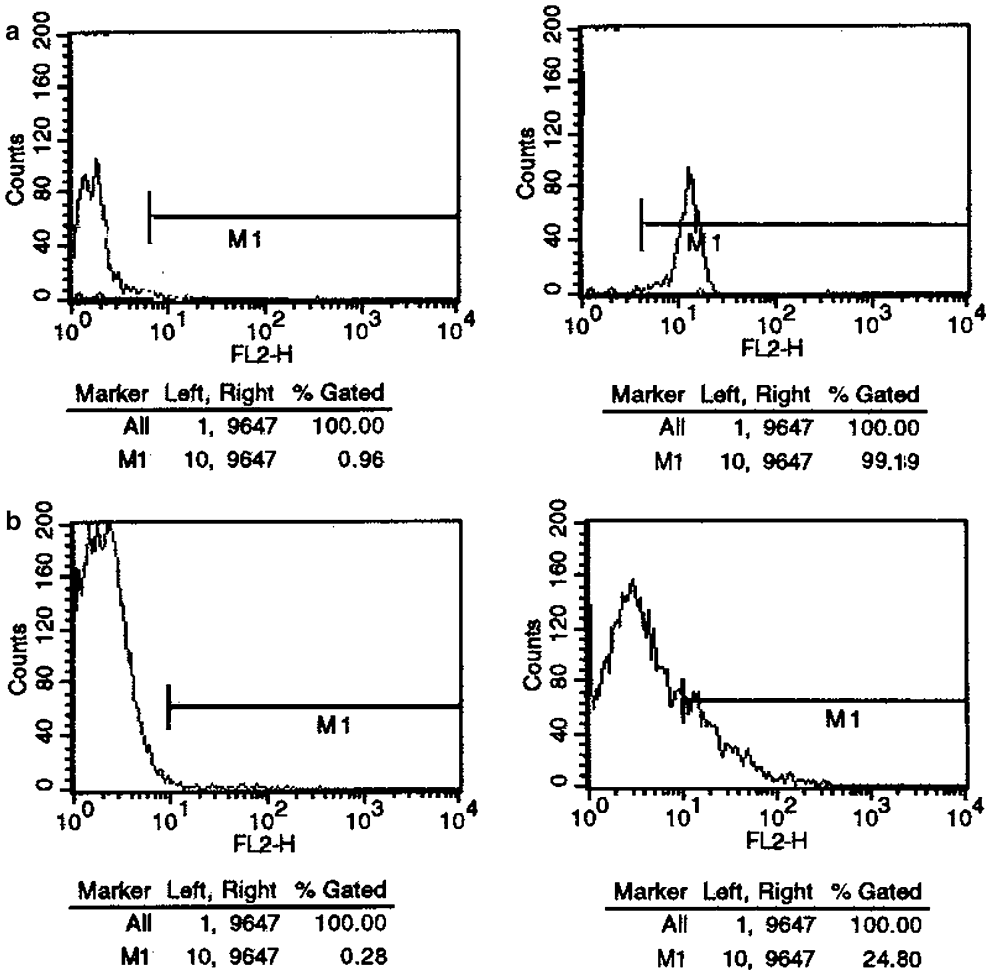

Fig. 3 The daunomycin efflux assay of P-gp activity on BM after the MDR1 gene vector transfection in vitro. The distinct population to the right of the gate (*vertical line* on graphs) shows the daunomycin fluorescence of daunomycin-loaded cells and the left of the gate reflects daunomycin-depleted cells. *y* axis, cell count; *x* axis, daunomycin fluorescence. The percentages of daunomycin-depleted cells (corrected for untransduced cells) were 76.48% for MDR1-infected BM (**b**) whereas, it is 1.87% for the control BM (**a**)

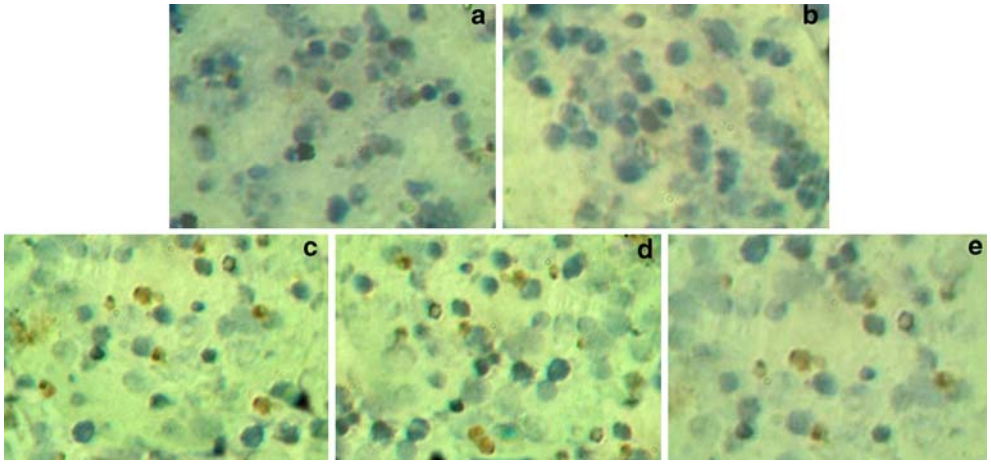

Fig. 4 Stable expression of hMDR1 product of different doses of doxorubicin chemotherapy group in 6 weeks after MDR1-BM was transplanted. Many positive cells for P-gp can be observed among the groups (**c** 5 mg/kg group; **d**, 10 mg/kg group; **e** 20 mg/kg group) with MDR1-BM transplanted mice and undergoing different doses of doxorubicin chemotherapy. But there were no significant differences. The positive P-gp immunoreactivity was not observed in the normal group (**a**) and control group (**b**) mice engrafted hematopoietic cells without MDR1 gene transfection. Original magnification, ×200

MDRl gene, because these animals express MDRl mRNA in their BM (Figs. 4, 5). It appears that BM cells expressing the human MDRl gene maintain this function after transplantation to irradiated host animals for a minimum of 6 weeks. In animals that were reconstituted with normal BM, the same amount of the chemotherapeutic agent led to a significant WBC decrease (Fig. 7). So, engraftment of normal BM cells did not protect against the myelosuppressive activity of chemotherapy (Fig. 7).

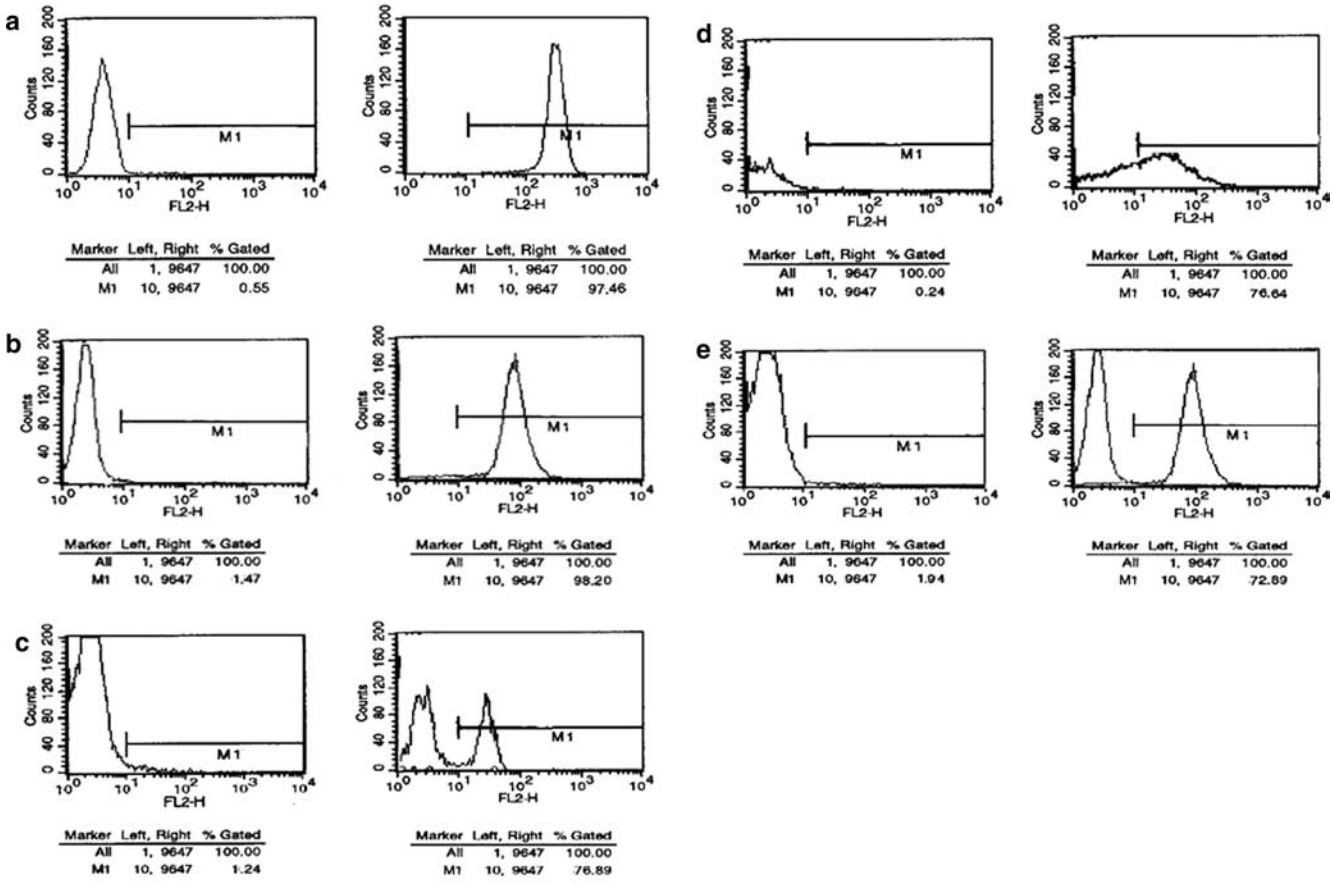

Fig. 5 The daunomycin efflux assay of P-gp activity of recipient mice BM 6 weeks after MDR1-BM was transplanted. The percentages of daunomycin-depleted cells (corrected for untransduced cells) were 24.35 (**c,** 5 mg/kg group), 23.6 (**d**, 10 mg/kg group), and 28.15% (**e**, 20 mg/kg group), whereas 3.09% and 3.27% for the control group (**a**) and normal group (**b**), respectively, indicating functional P-gp overexpression in MDR1-BM transplanted mice

Effect of doxorubicin on tumor growth

Peritoneum injection of doxorubicin (5, 10, and 20 mg/kg) suppressed tumor growth of the xenograft significantly. On day 14, the volume of the xenograft was significantly smaller in the groups treated with 20 mg doxorubicin than in the group treated with 10 mg doxorubicin. The chemotherapeutic agent, doxorubicin, led to a significant tumor growth suppression over time, and reached a peak at 6 weeks. This effect can be attributed to the transplanted MDR-BM, which render protection against doxorubicin in BM (Fig. 8).

Discussion

It is evident that undergoing high-dose therapy would, if successful, significantly ameliorate some of the malignancies that prevent large-scale investigation and widespread use of such treatments. These high-dose chemotherapy trials clearly support the concept that many human tumors have a steep dose-response curve, particularly in response to alkylating agents [3, 16, 21–24]. There are several major myeloid hematopoietic growth factors, such as interleukin-3, 7, erythropoietin, and GS-CSF, [25, 26] which can reduce the period of dangerous neutropenia by accelerating hematopoietic recovery, but it is evidenced that these growth factors have failed to completely eliminate chemotherapy-associated neutropenia and were much less effective in patients with reduced BM reserves. The autografting of hematopoietic stem cells harvested from BM or peripheral blood to rescue patients from high-dose therapies that initiate severe myelosuppression has been intensively explored in many solid tumors [27–29]. Response rates in these trials tend to be high, but both the duration of these remissions and overall survival rates as well as the associated morbidity and mortality vary widely with tumor types, amount of prior therapy, and the general performance status of these patients [30].

In the present study, we described the establishment of a preclinical in vivo gene transfer, hematopoietic stem cells autograft, and high-dose chemotherapy system. Because our objective here was to study the effect of hMDR1 gene undergoing high-dose therapy in neutropenia and thrombocytopenia, it was important to develop this system to investigate the basis for clinical protocols. The versatility of this system is

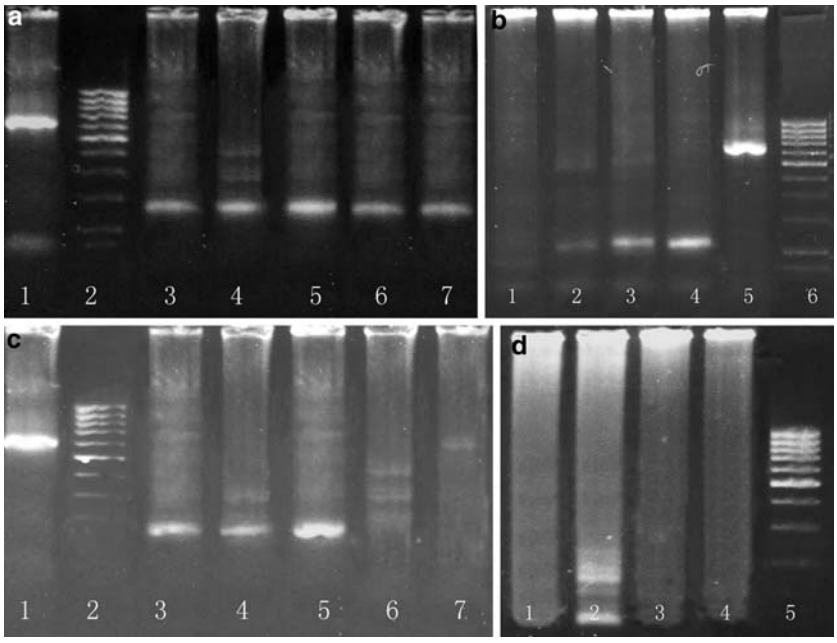

Fig. 6 DNA and RNA analysis of BM, peripheral blood, spleen, tumor of transplant recipients 6 weeks after transplantation with hMDR1-BM using MDR-specific primer (expected size band, (157 bp), diagnostic for the human MDRl transgene). Conditions were as described in Materials and Methods. **a** Human MDRl specific signal obtained from mouse BM DNA and RNA, *lane 1* β-actin (internal control); *lane 2* marker; *lanes 3–4* bone marrow DNA; *lane 5–7* bone marrow RNA. **b** Human MDRl signal from mouse peripheral blood DNA and RNA, lanes 1–2, peripheral blood RNA; lanes 3–4, peripheral blood DNA; lane 5, β-actin (internal control); lane 6, marker. **c** signal obtained from mouse spleen DNA and RNA, Lane 1, β-actin (internal control); lane 2, marker; lanes 3–5, spleen DNA; lane 6–7, spleen RNA. **d** signal obtained from mouse tumor DNA and RNA, Lanes 1–2, tumor RNA; lanes 3–4, tumor DNA; lane 5, marker

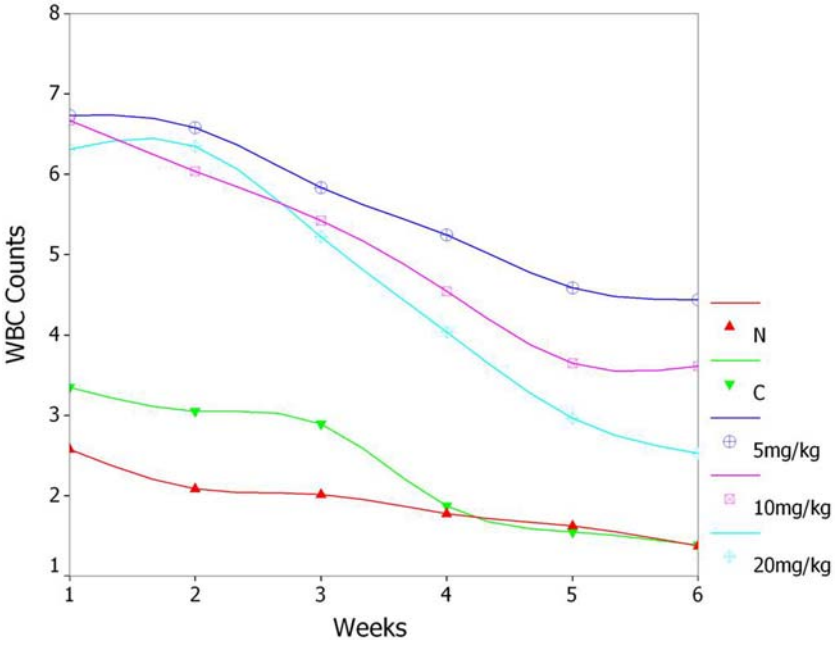

Fig. 7 Time course of WBC after chemotherapy in recipients 5 weeks after BM transplantation. In normal BALB/c mice, the same amount of the same chemotherapeutic agent led to a significant decrease, with the WBC decreasing over several days, reaching nadirs at 6 weeks. There was just little decrease in the peripheral WBC when compared with normal BALB/c mice (significant difference)

evident in our ability to detect the expression of hMDR1, WBC, in BM, spleen and tumor tissue, and functional assay of P-gp activity by different methods during MDR1 gene transfer and the high-dose chemotherapy period.

Several studies have reported that the presence of growth factors, primarily stem cell factor and interleukin-3 and -6, causes an increase in the number of proliferating stem cells available for retroviral gene transfer [31, 32]. Others have demonstrated the efficacy of this

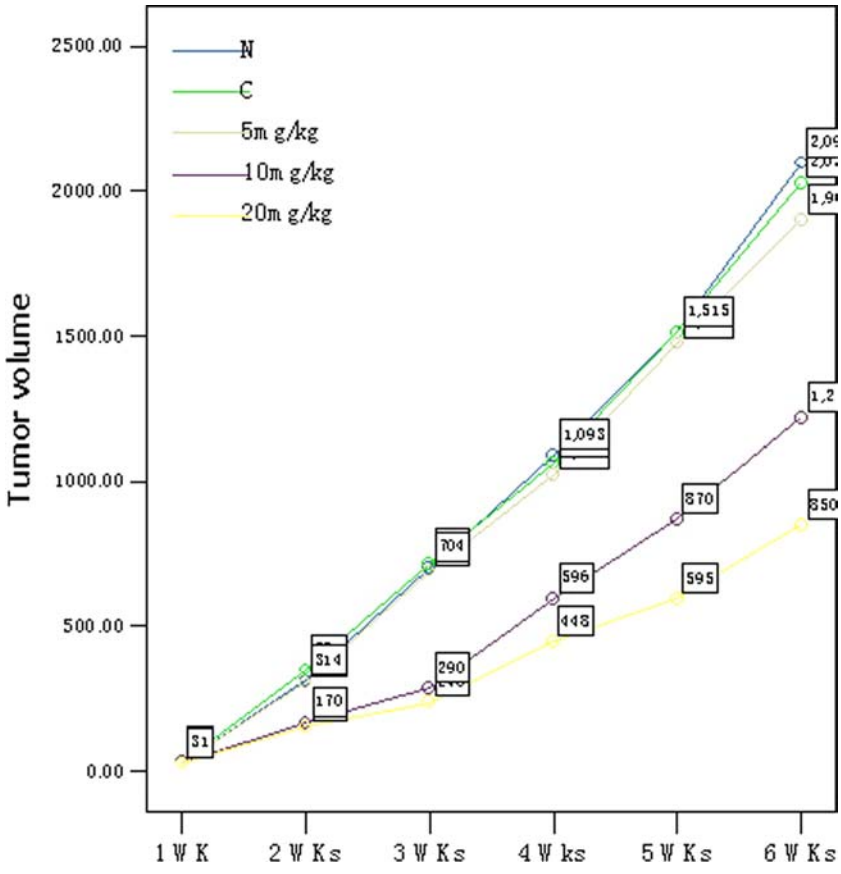

Fig. 8 Effect of doxorubicin on the growth of hematopoietic cancer xenografts in BALB/c mice. The volume of the xenograft (mm^3) was significantly smaller in the groups treated with 20 mg/kg doxorubicin mice than in the control group and normal group that were treated with 5 mg/kg doxorubicin 5 weeks after the cancer xenograft was established in BALB/c mice. * $P < 0.05$ versus control by one-way ANOVA followed by Scheffe's F procedure

procedure in enriching the BM of live animals for cells expressing high levels of MDR. Some others have raised questions about the safety of using MDR1 vectors for clinical myeloprotection strategies because the expanded grafts caused a myeloproliferative syndrome in transplanted mice [33, 5, 34]. But our data show discrepancy with these findings that by decreasing the proliferative rate with alternate cytokine mixtures, a modification of this system could be used for BM transplant and gene therapy applications. A possible explanation may be that shorter periods of culture with MDR1 vectors in the presence of early acting cytokines could result in a low incidence of this complication and the myeloproliferative syndrome is a consequence of the ex vivo expansion rather than any direct result of the MDR1 vector per se.

Previous studies [2, 35, 36] have demonstrated that retroviral gene transfer of the MDRl gene to normal BM progenitors and transplantation into BALB/c mice results in functional gene expression and increased resistance to paclitaxel in vivo. The data presented in this paper extend these observations and provide strong evidence to the fact that hematopoietic progenitor cells infection is possible with appropriate MDR1-containing retroviral vectors and producer lines containing human genes. All animals contain MDR1 DNA in spleen and BM specimens and maintain a stable MDR expression by retroviral-mediated gene transfer to the hematopoeitic in vivo. The expression of the MDRl gene product was stable over a 6-week period. It is possible that transduced BM cells can express high levels of MDR in vivo on exposure of the cells to MDR-responsive chemotherapeutic agents. MDR-infected BALB/c mice were resistant to the myelosuppressive effect of doxorubicin chemotherapy, since the WBC count can maintain a significant level to avoid neutropenia and thrombocytopenia.

To our knowledge, previous study has reported a therapeutic or a chemoprotective effect of MDR1 gene for phase I clinical trial [37]. By definition, a phase I clinical trial has a toxicity endpoint. So the question of a therapeutic or a chemoprotective effect is addressed in the study design. We utilize the experiment of a well-characterized MDR-infected BALB/c mice system, and exploited the relatively easy access to BM cells that constitutively and functionally expresses the human MDRl gene to explore the therapeutic use of MDR1 gene. The present studies confirm that this level of expression is stable for several weeks in vivo and sufficient to confer resistance against a panel of chemotherapeutic agents currently in clinical use. In addition, the present study demonstrated that doxorubicin suppressed the growth of hematopoietic cancer xenografts, in contrast to previous reports regarding the toxicity and feasibility of retroviral-mediated MDRl gene transfer, [38, 39] Our study offers evidence for the first time to the fact that doxorubicin at high doses suppresses the growth of tumors transplanted in BALB/B mice. Our further research reveals that tumor volume

was strongly associated with the degree of apoptosis of tumor cells, indicating that doxorubicin inhibited tumor growth mainly by inducing apoptosis and suppressing cell replication in vivo (data not shown), but the precise mechanisms for these phenomena need further clarification. For further support a rationale for conducting clinical trials where dose intensification of MDRl-dependent drugs could result in improved response and survival in cancer patients remains to be investigated in clinical studies. Taken together, these results provide us a framework that will allow us to extend this model to examine, in vivo, the optimal conditions for transfer of the MDRl gene into normal hematopoietic progenitor cells and its response to high-dose chemotherapy.

In conclusion, our present studies addressing the feasibility of transplanting MDR-BM cells and dose intensification of MDRl-dependent drugs have been informative and suggest the possibility of new approaches to gene therapy for cancer in improved response and survival with cancer patients. We succeeded in establishing stable and long-lasting MDR-BM grafts, and showed a high degree of chemoresistance and suppressed growth of tumor volume in animals receiving these transplants. If this strategy is successful, then this methodology could be applied to other genetic diseases. It appears that this approach remains to be investigated in clinical studies.

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