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Original Paper

Superior Gene Transfer into Solid Tumour Cells than into Human Mobilised Peripheral Blood Progenitor Cells using Helpervirus-free Adeno-associated Viral Vector Stocks

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Autologous peripheral blood progenitor cell (PBPC) grafts can be contaminated with tumour cells that potentially give rise to relapse following myeloablative therapy and PBPC transplantation. Adeno-associated virus (AAV)-based vectors produced by a new adenovirus-free technique are a gene delivery system which may be applicable for tumour cell purging. To test for the host range of these vectors, solid tumours of clinical relevance and normal $CD34^+$ PBPC were selected as target cells for an AAV-vector, encoding the green-fluorescent protein (GFP) as the indicator gene. At a multiplicity of infection (MOI) of 100: 79.94% ± 14.36% (mean ± SEM) of the connective tissue sarcoma cell line (HS-1) and 64.84% ± 6.91% of the cervical carcinoma cell line cells (HeLa-RC) expressed GFP while the other cell lines tested (1 ovarian tumour, 1 germ cell tumour, 1 osteosarcoma, 2 small cell lung cancer) ranged between 2.82% and 11.94%. Optimising the transduction protocol by use of higher MOIs of up to 500 and by pretreatment with the tyrosine kinase inhibitor, genistein, resulted in up to 95.97% and 94.10% green-fluorescent HS-1 and HeLa-RC cells, respectively. In contrast, only 1.39% ± 0.51% of the normal haematopoietic $CD34^+$ progenitor cells expressed GFP at a MOI of 100. The differential infectivity between HS-1 and $CD34^+$ cells was maintained after tumour cell spiking in leucapheresis products. Our observations suggest that AAV-based vectors may prove useful for purging of autologous PBPC grafts from solid tumour cells. © **1999 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

HIGH-DOSE CHEMOTHERAPY followed by haematopoietic stem cell transplantation has improved the survival or response rates of patients with solid tumours, such as sarcomas [1], ovarian cancer [2], germ cell tumours [3] and small cell lung cancer [4]. Stem cell grafts in solid tumour patients can be contaminated with tumorigenic cells [5]. The transfer of suicide genes to tumour cells using retroviral vectors [6], which may also transduce haematopoietic cells in the graft or adenoviral vectors [7] that do not integrate into the genome, has been reported. The primary host of the small parvovirus, adeno-associated virus (AAV), seems to be epithelial cells: wild-type AAV particles have been observed in cervical [8] and in respiratory epithelium [9]. Wild-type AAV is a replication defective virus, which requires helpervirus functions from adenovirus or herpesvirus [10, 11], in order to replicate efficiently and has not yet been associated with any disease, thereby making AAV-based vectors good candidates for gene therapy purposes. One line of investigation using AAV vectors in tumour cells is targeting colon carcinoma, malignant melanoma, ovarian carcinoma as well as lymphoblastoid cells to generate tumour cell vaccines. It has been possible to show transgene expression in melanoma, colon carcinoma and

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ovarian cells, whereas less than 1% of lymphoblastoid cells expressed the reporter gene [12]. Other groups have been able to infect neuroblastoma cells following leptomeningeal spreading [13] or have explored the chemosensitisation activity of wild-type AAV in human tumour cells [14].

We were interested in whether adeno-associated viral vectors, which are known to integrate stably in several cell types without generally infecting human PBPC [15], may be suitable for tumour cell purging from autologous PBPC grafts [16]. To test this hypothesis, several cell lines of tumours which are treated with high-dose chemotherapy and PBPC transplantation in clinical settings were infected with a recombinant AAV-green-fluorescent protein (rAAV-GFP) vector: connective tissue sarcoma (HS-1), cervical carcinoma (HeLa-RC), ovarian tumour (O-AW42), germ cell tumour (BEWO), osteosarcoma (KHOS) and two small cell lung cancer lines (H69 and H146). The green-fluorescent protein gene is a widely used reporter gene. Its gene product can be easily detected by fluorescent activated cell scanning (FACS) analysis [17]. Recently, new AAV-production techniques have been developed which do not require adenovirus infection of AAV-producer cells [18]. These AAV vector stocks are more suitable for possible *in vivo* applications since they do not contain immunogenic adenoviral contaminants. Together with new purification and concentration procedures (modified from [19]), this resulted in highly purified and concentrated helpervirus-free AAV-stocks. In addition to these new rAAV production procedures, new substances and techniques have also recently been identified which should increase overall transduction efficiencies of cells by rAAV. One of these substances, genistein [20], was included in this study. Incubation with the tyrosine kinase inhibitor genistein specifically blocks the phosphorylation of the cellular single-stranded D sequence-binding protein (ssD-BP), which prevents single-stranded AAV vectors from being transcribed to double-stranded DNA before integration into the genome. The tyrosine phosphorylation state of this protein correlates with the efficiency of rAAV-mediated gene transfer inversely and, therefore, genistein might increase transduction efficiencies in cells with a high phosphorylation state of the ssD-BP. Our study showed higher transfection rates as well as GFP-expression for all tested solid tumours compared with $CD34^+$ mobilised PBPC.

MATERIALS AND METHODS

Plasmids

For rAAV production two plasmids were used. The helper-plasmid pDG [18] provides the cell with all genes required for rAAV production in trans; therefore, infection of the transfected 293T cells with wildtype (wt) adenovirus was not required. In addition, this vector does not generate wtAAV particles. The plasmid pTR-UF5 (a generous gift from Dr Muzyczka, University of Florida, Gainsville, U.S.A.) contains the humanised GFP gene under transcriptional control of the cytomegalovirus (CMV) early promoter/enhancer, as well as the neomycin resistance gene being driven by the thymidine kinase promoter, all flanked by the AAV-inverted terminal repeats.

Production, purification and concentration of rAAV particles

For generation of recombinant AAV (rAAV) particles by transient plasmid transfection, 4×10^6 293T cells/dish were grown in 15 cm dishes (Becton Dickinson, Plymouth, U.K.) at a confluency of 40–70% in DMEM (Life Technologies, Paisley, U.K.) supplemented with 10% fetal calf serum (FCS; Sigma) and 0.1 mg/ml penicillin/streptomycin (PS; Life Technologies) at 37°C/5% CO_2. Cells were transfected by the calcium-phosphate co-precipitation method using 2 μg pTR-UF5 and 6 μg pDG. After 18 h, the medium was removed and replaced with fresh medium. Cells were further incubated for another 48 h, after which medium and cells were collected. Recombinant AAV particles were released by four cycles of freeze-thawing (–80°C to 37°C), cell debris was removed by centrifugation and supernatants were pooled. Concentration and purification of the rAAV-containing lysate was performed as previously published by Tamayose and colleagues [19]. In brief: the lysate was passed over a phosphate buffered saline (PBS) pre-equilibrated column containing sulphonated cellulose matrix beads. After thoroughly washing the column with PBS, the rAAV particles were eluted with 10 mM phosphate buffer (pH 7.2) containing 1.0 M NaCl. Eluates were collected in fractions of 4 to 6 ml. Eluted fractions were further purified and concentrated by sucrose-cushion centrifugation. The fractions were loaded on top of 2 layers of sucrose (in PBS), 50% and 30%, respectively, and centrifugated for 5 h in a Beckman Ultracentrifuge (SW40ti swingout-rotor; Beckman, München, Germany) at 250 000 g. The supernatant was discarded and the pellet resuspended in 1 ml Opti-MEM supplemented with 0.1 mg/ml penicillin/streptomycin (both Life Technologies). Experiments were performed with 5 independently produced AAV vector stocks.

Titration of rAAV particles

HeLa-RC (rep/cap transduced HeLa cells; [21]) were plated into 96-well plates at 5×10^3 cells/well in 100 μl DMEM/10% FCS/PS (as above) and incubated for 24 h (37°C/5% CO_2). The next day, the different batches of rAAV were titrated on the HeLa-RC cells in limiting dilutions spaced in log steps ranging from 1:10 to $1:10^{10}$. After 2 h of incubation (37°C/5% CO_2), 100 μl wild-type adeno5 particles at a multiplicity of infection (MOI) of 100 in DMEM were added to the wells. The 96-well plates were further incubated for 48 h at 37°C/5% CO_2. The wells were scored under a fluorescence microscope (Leitz, Stuttgart, Germany) and the titre determined by calculation at which dilution GFP-positive HeLa-RC cells could still be detected. The AAV vector stocks contained 0.5 to 2.0×10^8 infectious particles per ml.

Cells and cell culture

Cells of the following lines were obtained from the tumour cell bank of the German Cancer Research Centre and grown in appropriate culture media (all Life Technologies): connective tissue sarcoma (HS-1, RPMI/10% FCS), cervical carcinoma (HeLa-RC, DMEM/10% FCS), ovarian tumour (O-AW42, IMDM/10% FCS), germ cell tumour (BEWO, RPMI/10% FCS), osteosarcoma (KHOS, RPMI/10% FCS) and two small cell lung cancer lines (H69 and H146, RPMI/10% FCS). The $CD34^+$ cells were obtained from mobilised peripheral blood from 6 patients. The investigations on viral gene transfer into mobilised peripheral blood of tumour patients were approved by the Ethical Committee of the Medical Faculty of the University of Heidelberg and informed consent was obtained from each patient. Mononuclear cells were obtained by ficoll-gradient centrifugation (Ficoll, Biochrom KG, Berlin, Germany). Mononuclear cells

were collected from the interphase and washed twice with PBS (Life Technologies). The MACS MultiSort kit (Miltenyi Biotec, Bergisch Gladbach, Germany) was used for immunoselection of $CD34^+$ cells from mobilised peripheral blood apheresis products according to the manufacturers instructions. For assessment of the selection efficiency, $CD34^+$ restaining was performed. $CD34^+$ purity was 97.96% ± 0.64% as determined by FACS. $CD34^+$ cells were cultured in either αMEM (Life Technologies) supplemented with 10% FCS (experiments with serum) or QBSF60 serum-free medium (serum-free experiments; Quality Biological Inc., Gaithersburg, U.S.A.), both with Flt3-ligand (FL; R&D systems, Wiesbaden, Germany), stem cell factor (SCF; R&D systems) at 100 ng/ml and thrombopoietin (TPO; R&D systems) at 20 ng/ml.

Tumour cell spiking of $CD34^+$ selected leucapheresis samples with a rAAV-GFP vector

In some experiments, one day before transduction, 9×10^4 $CD34^+$ cells and 1×10^4 tumour cells per well were admixed and seeded into 24-well plates in QBSF60 medium supplemented with FL and SCF at 100 ng/ml, TPO at 20 ng/ml and 10% FCS.

Transduction

One day before transduction, cells were seeded into 24 well plates at a concentration of 1×10^4 cells/well in 400 µl of their respective media containing FCS. Cells were either not pre-treated with genistein, or incubated for 2 h with 150 µM genistein at 37°C and 5% CO_2 in humidified atmosphere. Thereafter medium was removed, cells were washed and fresh medium was added. In the serum-free experiments, just before transduction, serum-containing medium was removed and cells were washed twice and resuspended in the respective medium without serum. All cell-types were transduced with rAAV particles at MOIs as given in the Results section and incubated in a humidified incubator at 37°C/5% CO_2. 24 h after infection, FCS was added to all experiments to yield a final concentration of 10% (vol./vol.). 60 h after infection, cells were harvested, washed with ice-cold PBS and stored at 4°C (<15 min) until FACS analysis.

FACS analysis

Acquisition and analysis were performed on a FACSCalibur flow cytometer (Becton-Dickinson GmbH, Heidelberg, Germany) mounted with an air-cooled 488 nm Argon laser and equipped with CellQuest software (Becton-Dickinson). 10 000 events were acquired and analysed for GFP expression. GFP was measured on the FL1-channel (short band pass 530 nm filter) and plotted against side scatter. Debris and dead cells were excluded in a forward-sideward scatter plot by gating. Uninfected cells of each group were used as controls, thereby correcting for background fluorescence. Mean fluorescence is given in arbitrary fluorescence units (afu). Tumour cell spiked PBPC samples were stained with anti-human CD45 PerCP (Becton-Dickinson) to discriminate between cells of leucocytic origin and tumour cells. Samples were washed twice with PBS and analysed. For both tumour cells and leucocytes content GFP-expression was determined as described above.

Colony-forming cell (CFC) assay

The concentration of haematopoietic progenitor cells was assessed using a semisolid clonogenic culture assay (MethoCult H4433, Stem Cell Technologies Inc. Vancouver, Canada). The culture medium consisted of 30% fetal calf serum, 10% medium conditioned by phytohemagglutinin-stimulated leucocytes, 1 IU recombinant human erythropoietin, 5×10^{-5} M 2-mercaptoethanol, and 0.9% methylcellulose. 5×10^2 $CD34^+$ mobilised PBPC cells were plated in duplicate and incubated at 37°C and 5% CO_2 in humidified atmosphere. After 14 days, colonies were scored using an inverted microscope.

Statistics

Data are given as mean values ± 1 standard error of the mean (SEM). Significance levels were determined by paired Student *t*-test analysis.

RESULTS

Infection of tumour cell lines with rAAV-GFP

To determine the susceptibility of the solid tumour cell lines, these were infected with rAAV-GFP at 100 particles per cell, allowed to proliferate for 60 h and thereafter assayed for GFP expression by FACS. Of all infected HS-1 sarcoma cells, 79.94% ± 14.36% (Figure 1a) expressed GFP over mock-infected control cell levels. The increase in mean fluorescence between mock-infected and rAAV-GFP infected HS-1 cells was 355.15 ± 202.41 afu (Table 1). The percentage of rAAV-GFP infected HeLa-RC cells was 64.84% ± 6.91% (Figure 1b), while the increase in mean fluorescence in infected cells was: 40.74 ± 5.76 afu (Table 1). The other cell lines displayed lower percentages of infected cells, ranging from a mean of 11.94% in the ovarian tumour O-AW42 cells down to 2.82% in small cell lung cancer H69 cells (Figure 1c–g). The corresponding mean fluorescence intensities ranged from a mean of 7.59 afu in germ cell tumour BEWO cells to 0.74 afu in H69 cells (Table 1).

Infection of mobilised peripheral blood $CD34^+$ cells with rAAV-GFP

Fresh $CD34^+$ PBPC cells were infected with rAAV-GFP at 100 particles per cell, allowed to proliferate for 60 h and thereafter assayed for GFP expression by FACS. Of all infected $CD34^+$ cells, 1.39 ± 0.51% (Figure 1h) expressed GFP over mock-infected control cell levels. The difference in mean fluorescence between mock-infected and rAAV-GFP

Table 1. *Expression levels of the green fluorescent protein in tumour cell lines and $CD34^+$ cells following rAAV-GFP transduction*

Cell line	Δ Mean fluorescence intensity (arbitrary fluorescence units)
HS-1	355.15 ± 202.41
HeLa-RC	40.74 ± 5.76
O-AW42	5.85 ± 1.40
BEWO	7.59 ± 0.92
KHOS	7.58 ± 2.57
H69	0.74 ± 0.14
H146	1.24 ± 0.04
$CD34^+$	0.15 ± 0.05

Δ Mean fluorescence intensity, mean GFP-fluorescence of sample corrected for background of uninfected controls in arbitrary fluorescence units (afu). Data given as mean ± SEM ($n = 3$).

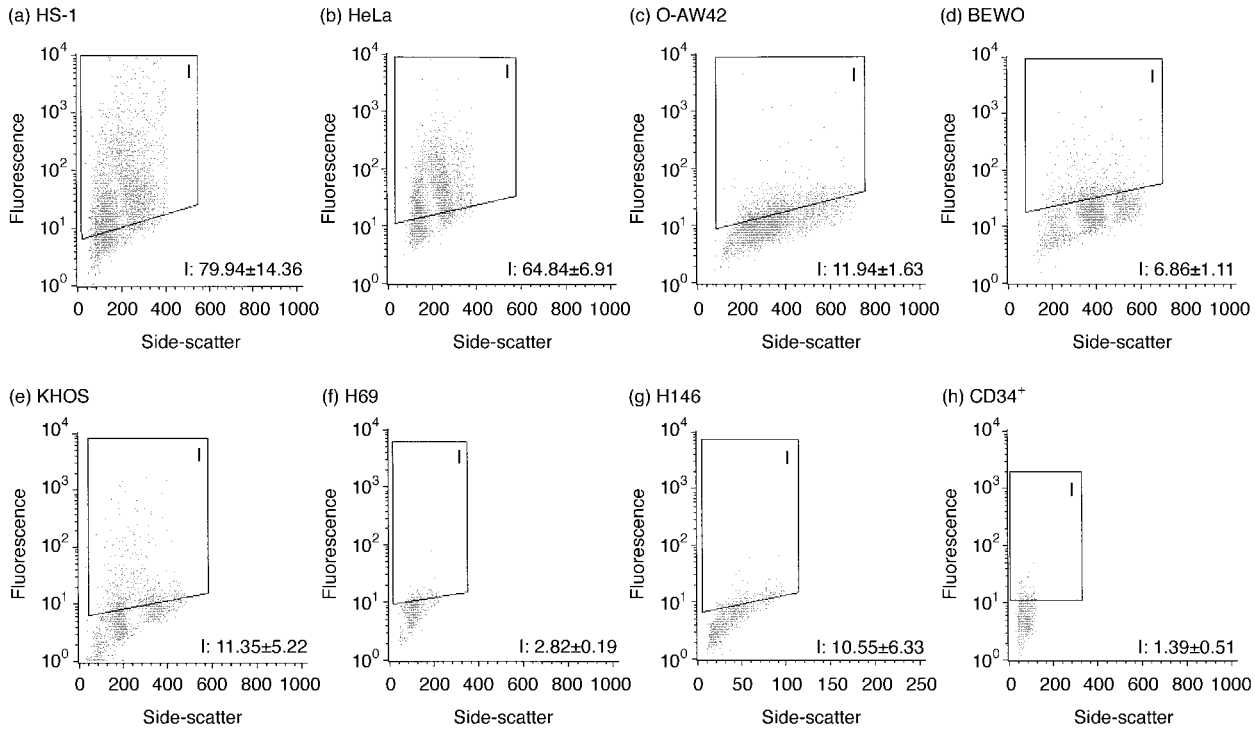

Figure 1. Representative plots of GFP expression of connective tissue sarcoma (HS-1; a), cervical carcinoma (HeLa-RC; b), ovarian tumour (O-AW42; c), germ cell tumour (BEWO; d), osteosarcoma (KHOS; e), two small cell lung tumours (H69; f, H146; g) and CD34$^+$ haematopoietic progenitor (h) after infection with rAAV-GFP particles at a MOI of 100 in serum-containing medium. Each gate was set against respective mock infected control cells. The percentage of infected (I), GFP-expressing cells are given for each cell line. Data are depicted as mean values ± 1 standard error of the mean (SEM) ($n = 3$).

infected samples was 0.15 ± 0.05 afu (Table 1). The infection rate and GFP expression level of CD34$^+$ PBPC was thus lower than that of all tumour cell lines investigated.

The cloning efficiency of both rAAV-GFP infected and mock-infected mobilised PBPC derived CD34$^+$ cells was assessed in a semi-solid colony-forming cell assay. Infection of the human mobilised PBPC CD34$^+$ cells with the rAAV-GFP vector at a MOI of 100 did not have a negative effect on their cloning-efficiency, rather an increase in CFC was noted as shown in Table 2.

Optimising AAV-GFP transduction efficiencies for tumour cell purging

In order to make rAAV vector-based tumour cell purging clinically applicable, transduction efficiencies have to be as high as possible in tumour cells, whilst infection of CD34$^+$ haematopoietic progenitor cells in the graft should be minimal. For these experiments the connective tissue sarcoma cell line HS-1 and the cervix carcinoma line HeLa-RC were selected. All media used during transduction were serum-free to avoid possible binding of rAAV to serum-components. The effect of genistein on transduction efficiencies was tested by either pre-incubating cells with or without genistein (150 μM for 2 h).

Table 2. Cloning efficiency of rAAV infected (MOI 100) and uninfected CD34$^+$ cells

	CFU-GM	BFU-E	Total
rAAV (MOI 100)	110, 118	55, 41	165, 159
Control	68, 75	24, 32	92, 107

All assays were performed in duplicate and the results of both assays are shown in the table. 5×10^2 CD34$^+$ cells were seeded per assay 60 h after transduction. The number of colony-forming units granulocyte-macrophage (CFU-GM) and burst-forming units erythrocyte (BFU-E) were counted after a 14-day culture period in methyl-cellulose containing assays. rAAV, recombinant AAV; MOI, multiplicity of infection.

In HS-1 cells as well as in HeLa-RC cells there was a trend towards higher mean percentages of infected cells in serum-free (Table 3) compared with serum containing medium at a MOI of 100 (Table 1). There was a linear dose response between MOI and the mean infection rate (per cent GFP expressing cells; Figure 2) and the ratio of infectious particles per cell (Table 3) between a MOI of 100 and 300 in HS-1 cells and between a MOI of 100 and 500 in HeLa-RC cells. Furthermore, in HeLa-RC cells a steep rise in the infection rate could be detected between a MOI of 100 and 200 from 67.59–90.64%, suggesting that AAV-receptors were not saturated at the first dose level and that optimal MOIs need to be determined for each cell line. A correlation between the MOI and mean fluorescence intensity was observed over the whole range of MOI for HS-1 (no genistein, $r = 0.987$, $P = 0.02$; genistein, $r = 0.986$, $P = 0.023$) and HeLa-RC (no genistein, $r = 0.985$, $P = 0.024$; genistein, $r = 0.984$, $P = 0.025$). Inhibition of ssD-BP protein phosphorylation by genistein slightly but consistently increased the proportion of infected tumour cells if the infection rate was below 90% and in 4 of 6 assays with an infection rate of over 90%. At these high proportions of infected cells a further increase in afu could be obtained by addition of genistein.

Table 3. Susceptibility of tumour cell lines and CD34$^+$ PBPC to transduction with a rAAV-GFPvector

Transduction conditions	HS-1 (afu)	HeLa-RC (afu)	CD34$^+$ (afu)
MOI 100	665.73 ± 175.69	67.77 ± 18.20	0.14 ± 0.18
MOI 100 + Gen.	1499.10 ± 444.84	104.59 ± 38.61	0.46
MOI 200	2475.96 ± 1095.33	214.54 ± 61.52	0.70 ± 0.33
MOI 200 + Gen.	2696.03 ± 1619.16	263.31 ± 79.62	0.49
MOI 300	3383.36	269.55	0.34
MOI 300 + Gen.	5331.59	337.71	0.05
MOI 500	5710.55	427.16	0.61
MOI 500 + Gen.	6454.33	550.54	0.89

MOI, multiplicity of infection; Gen., genistein; Data given as mean ± SEM when multiple experiments were performed ($n = 3$)

In CD34$^+$ cells a maximal infection rate of 4.29% was reached at a MOI of 300 which could not be increased by a higher concentration of infectious particles or by enabling a better intracellular processing of the AAV-vector using genistein (Figure 2). This suggests that the AAV-vector uptake by CD34$^+$ cells is a rate-limiting step.

Tumour cell spiking of CD34$^+$ selected leucapheresis samples was performed with 10% HS-1 cells. MOI calculations were based on the tumour content, so that a 10-fold lower number of AAV particles could be employed for the mixture of CD34$^+$ cells and tumour cells. This still proved to be effective in maintaining the differential infectivity of tumour cells over CD34$^+$ cells and the positive effect of genistein on the infection rate (Table 4).

DISCUSSION

In this study we showed higher infection rates as well as GFP-expression for all tested solid tumour cell lines compared with CD34$^+$ mobilised PBPC. The rAAV-GFP vector production procedure used did not require wild-type adenovirus co-infection, since all the adenoviral genes required for AAV-helper function are contained on a helper plasmid [18]. Also, this vector is known not to produce wtAAV [18]. This is an advantage for possible *in vivo* applications of this vector-system, since rAAV-batches produced with this procedure do not contain immunogenic adenoviral contaminants nor contaminating and replicating wtAAV. Furthermore, a purification and concentration step during rAAV production was introduced using a modified purification and concentration procedure by Tamayose and colleagues [19]. One recent publication by Alexander and colleagues [22] pointed to the importance of purification steps in rAAV production procedures. They reported that contaminations of reporter proteins in rAAV virus stock could result in artificially-introduced reporter protein over-estimation by the process of pseudo-transduction.

While initial reports suggested high infection rates and expression of rAAV vectors in human CD34$^+$ cells [23, 24], recent results point to a less efficient ability of rAAV vectors to infect these cells [15] and haematopoietic cell lines [12]. Ponnazhagan and colleagues [25] showed that susceptibility of CD34$^+$ cells to rAAV varied from donor to donor, which seemed to be the result of heterogenous AAV-receptor expression. In all our donors, we found a consistently low

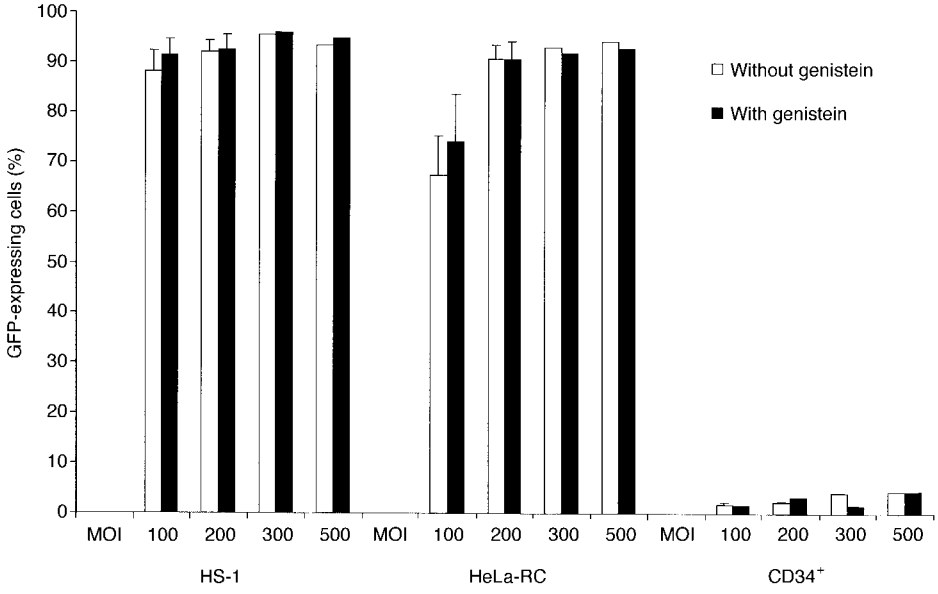

Figure 2. Percentage GFP-expressing HS-1, HeLa-RC and CD34$^+$ cells after infection with a rAAV-vector at MOIs of 100 to 500 without or with pretreatment of cells with genistein. Uninfected cells of each cell type served as controls. Data given as mean values ± 1 standard error of the mean (SEM).

Table 4. Tumour cell (HS-1) spiking of CD34+ selected leucapheresis samples with a rAAV-GFP vector

Transduction conditions	% GFP expressing cells		Δ Mean fluorescence intensity (afu)	
	HS-1	CD34+	HS-1	CD34+
MOI 100	80.24	1.70	390.91	5.45
MOI 100 + gen.	89.76	6.57	822.01	11.60
MOI 200	84.74	1.82	1343.45	2.11
MOI 200 + gen.	87.39	1.97	1281.25	2.7

Tumour (HS-1) and CD34+ cells were mixed 1:10 and then transduced with rAAV-GFP. Transduction was performed in serum-free media without or with genistein (gen.). For each cell population the percentage of GFP-positive cells and the GFP-fluorescence intensity was determined. MOI, multiplicity of infection.

infectability of CD34+ cells with the rAAV-GFP vector. Recent investigations have provided possible explanations for the low expression level of recombinant AAV-introduced transgenes in human haematopoietic progenitor cell populations. Summerford and Samulski [26] showed that the receptor mediating AAV infection is a membrane-associated heparan sulphate proteoglycan. Possibly this receptor is expressed at low levels on human haematopoietic progenitors. Qing and colleagues [20] reported that the tyrosine phosphorylation state of the cellular single-stranded D sequence-binding protein correlated with the efficiency of rAAV-mediated gene transfer. They observed that in both human erythroid leukaemia cell lines as well as human CD34+ progenitors, this protein was in a phosphorylated form, resulting in a significantly lower efficiency of rAAV-mediated gene transfer. rAAV vectors might be able to infect human CD34+ progenitor cells, though phosphorylation of the single-stranded D sequence-binding protein could inhibit efficient expression. Both can contribute to low infection rates and/or expression of rAAV vectors in human CD34+ cells. Our results only showed a marginally higher infection rate of genistein-treated CD34+ cells suggesting that AAV-uptake rather than AAV single- to double-strand synthesis is the rate limiting step.

Since human haematopoietic CD34+ progenitors could only be infected with the rAAV-GFP vector with very low efficiency (1.39% ± 0.51%) and with the knowledge that cells of epithelial origin seem to be the natural host of wild-type AAV [8, 9], AAV-based vectors might prove to be useful for application in progenitor cell purging setups. To this end, connective tissue sarcoma (HS-1), cervical carcinoma (HeLa-RC), ovarian carcinoma (O-AW42), osteosarcoma (KHOS), chorion carcinoma (BEWO) and small cell lung cancer (H69 and H146) cell lines were infected with a rAAV-GFP vector at a MOI of 100. Of the HS-1 sarcoma cells, 79.94% ± 14.36% were infected with the vector and expressed GFP (Δ mean fluorescence: 355.15 afu ± 202.41 afu), whilst lower levels were seen with the cervical carcinoma HeLa-RC cells (64.84 ± 6.91%; Δ mean fluorescence: 40.74 afu ± 5.76 afu). The other cell lines could be less readily infected (range: 2.82–11.94%; Figure 1 and Table 1), though all with higher efficiency and expression levels than the mobilised peripheral blood CD34+ cells. Further investigation with the HS-1 and HeLa-RC cell lines revealed that an increase in the MOI, the use of serum-free media during transduction as well as the use of the ssD-BP inhibitor genistein could further increase the transduction efficiencies and GFP expression levels in these cells (Table 3).

The linear increase in GFP fluorescence intensity with higher MOIs in HS-1 and HeLa-RC cells at percentages of infected cells over 90% may reflect multiple integrations of the rAAV vector. The 10-fold higher GFP expression level in HS-1 cells than in HeLa-RC cells may be due to a higher number of AAV receptors on HS-1 cells.

A possible toxic effect of rAAV infection on human CD34+ progenitor cells was assayed using semi-solid methyl-cellulose cultures. The results of the clonogenic assays did not indicate any inhibitory effects on the cloning efficiency nor on the differentiation ability of the CD34+ cells by the rAAV vector.

As shown, connective tissue sarcoma as well as cervical carcinoma cells were particularly susceptible to recombinant AAV-mediated gene transfer. This is of clinical importance for connective tissue sarcomas, since these are a growing indication for high-dose chemotherapy followed by PBPC support [1] and residual sarcoma cells have been detected in patients' blood or bone marrow [1, 27]. Though no indication for high-dose chemotherapy followed by PBPC support, cervical tumours have a high incidence in women and new treatment strategies, especially for advanced stage might include rAAV-mediated gene therapeutic approaches.

Our finding of a differential infection rate and expression of rAAV-based vectors in sarcoma cells and haematopoietic cells, may allow the use of rAAV vectors for purging of PBPC grafts contaminated with these solid tumour cells. Our data showed that in spiking experiments, where the MOI was calculated on the proportion of tumour cells in the graft, the differential infectability and expression between HS-1 tumour cells and CD34+ cells was maintained, suggesting that the AAV-particles are able to 'find' their targets even if they are diluted in a graft, and absorption by CD34+ cells was minimal. Others recently showed that rAAV-mediated gene transfer was surprisingly more efficient in primary tumours than in human tumour cell lines [12]. Therefore, we assume that the differential infectivity between mobilised PBPC and primary tumour cells in autografts could be more pronounced than shown in our experimental setup with tumour cell lines. Possible tumour cell suicide genes that may be investigated along these lines are the thymidine kinase gene [28] and the cytosine deaminase gene [29].

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