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ORIGINAL RESEARCH

Transfection of myeloid leukaemia cell lines is distinctively regulated by fibronectin substratum

Gunes Esendagli · Hande Canpinar · A. Lale Dogan · Munir Akkaya · Emin Kansu · Dicle Guc

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Abstract Gene transfer into haematopoietic cells is a challenging approach. The extracellular matrix component fibronectin has been known to modulate the cell cycle dynamics, viability and differentiation of leukaemia cells. Thus, our aim was to investigate the influence of fibronectin substratum on the liposomal transfection of myeloid leukaemia cell lines. Liposomal transfection was performed with K562 and HL-60 as representative lines of transfection-competent and -incompetent myeloid leukaemia cells, respectively. Flow cytometry analyses were performed to determine transfection efficiency monitored by green fluorescent protein (GFP) expression and to assess cell viability and cell cycle status. Quantitation of GFP gene expression and DNA uptake was assayed by real time PCR. The current data showed that the adhesion to fibronectin deteriorated the transfection of K562 cells. In contrary, it enhanced the delivery of plasmid DNA into HL-60 cells. Correspondingly, the adhesion to fibronectin influenced the transfection efficiency mainly by modulating the intracellular presence of plasmid DNA. The cell cycle and viability which is regulated by fibronectin had a minor impact on the success of gene delivery. This phenomenon may be considered as an important factor which may modulate the potential gene transfer approaches for myeloid leukaemia.

Keywords Liposomal transfection · Leukaemia · Fibronectin · Extracellular matrix · Cell cycle

G. Esendagli (✉) · H. Canpinar · A. Lale Dogan · M. Akkaya · E. Kansu · D. Guc
Department of Basic Oncology, Institute of Oncology, Hacettepe University, 06100 Sihhiye, Ankara, Turkey
e-mail: gunese@hacettepe.edu.tr

Introduction

Gene transfer into neoplastic cells offers a promising gateway for studying the roles of specific genes in cancer biology and for developing potential therapeutic approaches. However, genetic modification of certain haematological malignancies including leukaemia, has not been very successful since these cells are generally incompatible for both viral and non-viral methods (Uchida et al. 2002; Marit et al. 2000). Because viral transduction requires a specific cognate molecule expressed on the cell surface, non-viral gene transfer (e.g. cationic lipid-mediated transfection, nucleofection and electroporation) is anticipated as a universal approach (Roddie et al. 2000; Gonzalez et al. 1999; Schakowski et al. 2004). The cationic lipid-mediated transfection to leukaemia cells offers a practical and advantageous method by favouring both ex vivo and in vivo gene transfer (Schakowski et al. 2004; Audouy et al. 2002).

Other than the composition of liposome and DNA complex, the efficiency of transfection is influenced by cell type-dependent variables such as endocytic activity, nuclear translocation, cytotoxicity and cell cycle dynamics (Lechardeur and Lukacs 2002; Brunner et al. 2000). Proliferating cells are better targets for liposomal gene transfer (Mortimer et al. 1999; Pelisek et al. 2002). In contrast, actively dividing leukaemia cells grown in suspension exhibit a low transfection capacity that indicates other possible factors influencing the efficiency of gene transfer (Uchida et al. 2002). Amongst the leukaemia cell lines, K562 is relatively transfection competent. On the other hand, HL-60 cell line has been reported to be reluctant to gene transfer (Uchida et al. 2002; Roddie et al. 2000; Schakowski et al. 2004).

Several cell lines of haematopoietic malignancies, including K562 and HL-60, are capable of binding to fibronectin, a widely distributed extracellular matrix protein. K562 and HL-60 cells express $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrin molecules which serve as receptors for fibronectin and following adhesion to fibronectin, these cells acquire new features such as resistance to apoptosis and cell cycle arrest in G1 phase (Canpinar et al. 2007; Bohnsack and Chang 1994). Furthermore, the interaction between fibronectin and integrins results in cytoskeletal rearrangements and cellular motility (Mitra et al. 2005; Echarri et al. 2007). However, upon detachment from fibronectin, these novel features are forfeited (Canpinar et al. 2007).

The improvement of viral gene transfer into cells of haematopoietic origin in the presence of fibronectin fragments has been previously shown (Moritz et al. 1996; Hanenberg et al. 1997). Fibronectin serves as a mediator for the retrovirus to colocalize at the cell surface. On the other hand, no other mechanism related to the cellular processes affected by fibronectin was identified to modulate the efficiency of gene transfer (Moritz et al. 1996).

In the current study, the influence of adhesion to fibronectin on the liposome-mediated gene transfer into K562 and HL-60, as representative myeloid leukaemia cell lines, was investigated. The current data demonstrate the differential effects of fibronectin adherence on transfection capacity of K562 and HL-60 cells, where HL-60 cell line rendered to be transfection competent.

Materials and methods

Cell culture

HL-60 and K562 cell lines were cultured in RPMI 1640 medium supplemented with 10% foetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified 5% CO_2 incubator. The cells (2×10^5) were cultured in suspension or seeded into 24-well culture plates coated with fibronectin (40 μg/mL; Invitrogen, Carlsbad, CA, USA). To obtain fibronectin-adherent cells after an overnight incubation, non-adherent cells were gently washed out with PBS. A PBS-EDTA solution (0.5%, v/v) was used for detachment from fibronectin. Otherwise specified, all reagents were obtained from Sigma (St. Louis, MO, USA).

MTT assay

The methylthiazolyltetrazolium (MTT) assay was used to evaluate cell viability after the exposure of cell-cycle regulatory drugs. Briefly, 50 μL cell suspension containing 2×10^4 K562 cells or 1×10^4 HL-60 cells was seeded into 96-well plates and 50 μL of aphidicolin or hydroxyurea (in various concentrations) was added to each well. After 48 or 72 h of incubation, 25 μL of MTT solution (Sigma, 1 mg/mL final concentration) was added. The formazan crystals produced were solubilised by adding 80 μL lysing buffer (pH 4.7) composed of 23% SDS dissolved in a solution of 45% dimethylformamide. Optical densities (OD) were read at 570 nm using a microplate reader (Spectramax Plus; Molecular Devices, Sunnyvale, CA, USA). The cells incubated in culture medium alone served as a control for cell viability (untreated wells). All assays were performed in quadruplicate and mean OD values were used to estimate the percentage of cell death. Thus, the subtoxic doses of hydroxyurea (0.25 mM for both cell lines) and aphidicolin (0.5 μM for HL-60; 0.1 μM for K562) were preliminarily determined.

Liposomal transfection

Transfection of pIRES2-EGFP plasmid (Clontech, Palo Alto, CA, USA) carrying enhanced green fluorescent protein (EGFP) gene into K562 or HL-60 cells

was performed with a lipid-based transfection reagent, Lipofectamine2000™ (Invitrogen). This vector carries a modified internal ribosomal entry site (IRES) sequence enabling improved translation of downstream gene compared to conventional IRES carrying vectors. Transfection conditions were preliminarily optimized with suspension cultures. The cells (3×10^4) were transfected with a DNA-liposome ratio 1:2, in 500 μL serum-free RPMI 1640 medium (4 ng plasmid/μL medium). Basic transfection procedures were performed as instructed by the manufacturer. After 5 h of incubation, FBS was added into the wells to a 10% final concentration. In the experiments with cell cycle modulating agents (aphidicolin or hydroxyurea), the cells were washed prior to transfection.

Flow cytometric analysis of transfection efficiency

EGFP expression was determined as reporter gene of transfection at 488 nm using an EPICS XL-MCL flow cytometer (Beckman Coulter, Fullerton, CA, USA) at 48 h post-transfection. Untransfected cells gone through the same treatments, were used as autofluorescence controls. EGFP expression levels were calculated with the mean fluorescence intensity (MFI) ratio of the $EGFP^{+}/EGFP^{-}$cell populations.

Quantification of intracellular presence of plasmid and EGFP gene expression by real-time PCR

Total RNA and DNA were isolated with QIAamp® RNA and DNA Blood Mini Kit (Qiagen, Valencia, CA, USA), respectively, according to manufacturers' instructions. Isolated DNA was used for the quantification of plasmid uptake whereas, for the gene expression analyses, plasmid DNA was completely removed from RNA samples by DNase treatment (DNA-free™ kit, Ambion, Austin, TX, USA). cDNA was synthesized from 0.2 μg of RNA, using oligo(dT) primers and RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania).

PCRs were carried out in a reaction mixture containing 1× DNA Master SYBR Green I (Roche Diagnostics, Mannheim, Germany), 0.125 μM primer oligonucleotides and 3.5 mM $MgCl_2$ using conditions 30″ at 95 °C; 30″ at 60 °C; 30″ at 72 °C) on a Rotor-Gene 6000™ cycler (Corbett Research, Sydney, Australia). Beta-actin was amplified as a reference house keeping gene with the primers, sense 5′-CTG GAA CGG TGA AGG TGA CA; antisense 5′-AAG GGA CTT CCT GTA ACA ATG CA, (product size: 139 bp). Amplification of EGFP gene was performed with the primers, sense 5′-TGG TGC CCA TCC TGG TCG A; antisense 5′-TTG CCG GTG GTG CAG ATG AAC, (product size: 123 bp). Each reaction was carried out in duplicates. Comparative Ct (ΔΔCt) method was used for the relative quantification of target gene expression (Pfaffl 2001). Data obtained from other transfection groups were normalized against transfections in suspension cultures.

Assessment of cell cycle and viability

Cell viability was analysed by flow cytometry using propidium iodide (PI) exclusion method. The percentage of PI positive dead cells was calculated after subtraction of background present in the control cell cultures.

For the cell cycle studies, briefly, the cells were fixed in 90% ethanol. After washing twice in PBS, cells were incubated with 10 μg/mL ribonuclease (Sigma) and 50 μg/mL PI (Sigma) for 20 min. The amount DNA stained with PI was determined by flow cytometry and the resulting histograms were analysed using the Multicycle software (Phoenix Flow System, San Diego, CA, USA) to calculate the percentage of cell cycle phases.

Statistical analysis

All values are expressed by arithmetic mean ± standard deviation (SD). Statistical difference between experimental groups was determined using Student's paired or unpaired *t*-test where appropriate. Differences were regarded as statistically significant when $P \leq 0.05$.

Results

The effect of fibronectin adherence on transfection efficiency in K562 and HL-60 cells

There are numerous lipid-based transfection systems available. In this study, Lipofectamine reagent and a plasmid with a universal CMV promoter were

preferred owing to a relatively high efficiency reported with leukaemia cells in previous studies (Uchida et al. 2002; Brunner et al. 2000).

Following 48 h post-transfection, only a small percentage of HL-60 cells cultured in suspension were positive for EGFP. HL-60 cells bound to fibronectin had an increased $EGFP^+$ percentage, on the other hand, when the cells were readily detached from fibronectin and transfected, a decline in the transfection efficiency was observed. Interestingly, an opposite situation was achieved with the K562 cell line (Figs. 1a, 3c). For both of the cell lines, transfection of fibronectin-adherent cells yielded an increased expression of EGFP, i.e. fluorescence intensity. The level of EGFP expression in the cells detached from fibronectin was comparable with suspension cells (Fig. 1b).

The analysis of EGFP mRNA expression normalised with the cultures transfected in suspension supported the increased versus decreased transfection

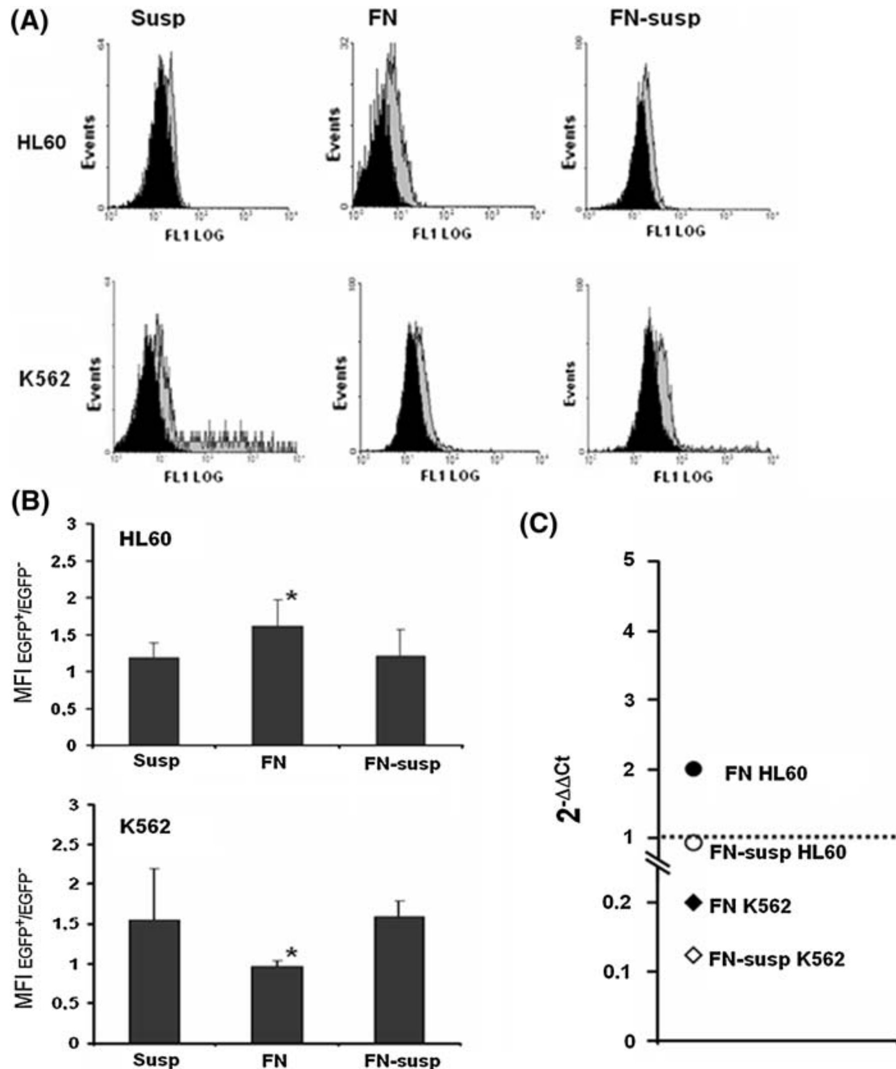

Fig. 1 Efficiency of liposomal gene transfer into fibronectin-adherent HL-60 and K562 cells. **a** $EGFP^+$ cells were analysed with flow cytometry and **b** the levels of expression were calculated using the mean fluorescence intensity (MFI) values ($n = 3$, $*P < 0.05$). **c** EGFP gene expression was determined by real-time RT-PCR. The data obtained from the fibronectin-adherent cells or from the cells detached from fibronectin were normalized against their suspension counterparts. *Dashed line* crossing at the value $2^{-\Delta\Delta Ct}=1$ indicates an equal expression level between suspension and fibronectin-adherent or fibronectin-suspension groups (*Susp* suspension cells, *FN* fibronectin-adherent cells, *FN-susp* cells detached from fibronectin)

efficiency observed in fibronectin-adherent HL-60 and K562 cells, respectively (Fig. 1c).

Adhesion to fibronectin increases the intracellular presence of plasmid DNA in HL-60 but not in K562 cells

The indispensable factors influencing the transfection efficiency are the cellular uptake of cationic lipid DNA complexes and intracellular stability of plasmid DNA (Lechardeur and Lukacs 2002). Therefore, the intracellular presence of plasmid DNA was determined. HL-60 cells adhered to fibronectin throughout or prior to the transfection deposited higher amounts of plasmid DNA compared to suspension counterparts. On the other hand, interaction with fibronectin deprived the intracellular presence of plasmid in K562 cells (Fig. 2).

Cell cycle dependence of transfection in fibronectin-adherent leukaemia cells

Cell cycle status and mitotic activity modulate the competence of target cells for liposomal transfection (Brunner et al. 2000; Mortimer et al.1999). We investigated the impact of cell cycle regulation by fibronectin adherence on the transfection of leukaemia cells. Both fibronectin-adherent HL-60 and K562 cell lines were accumulated in G1 phase and upon detachment, synchronously proceeded into S phase (Fig. 3a). To determine the impact of cell cycle arrest on transfection, suspension cells were also treated with hydroxyurea and aphidicolin arresting the cell cycle in G1 and pre-S phases, respectively (Fig. 3a). The ratio of the cells in G1 phase was also calculated in comparison with asynchronous cells grown in suspension. The G1 accumulation in the hyrdroxyurea- and aphidicolin-treated cells was comparable with the cells adhered to fibronectin and the cells detached from fibronectin, respectively (Table 1; Fig. 3b).

Unlike fibronectin-adherent cells, the cells synchronised in fibronectin, hydroxyurea, and aphidicolin treatment groups were allowed to proceed through the cell cycle at the time of incubation with cationic lipid DNA complexes. Therefore, during 5 h of transfection, the cells detached from fibronectin and hydroxyurea-treated cells advanced from G1 towards S phase, and aphidicolin-treated cells were released from pre-S phase block. The percentage of $EGFP^{+}$ HL-60 cells was significantly higher in fibronectin-adherent cells than in the other groups. In comparison to HL-60, transfection efficiency in K562 cells appeared to be more dependent on the cell cycle status. For both of the cell lines, pre-S accumulation with aphidicolin resulted in inefficient transfection.

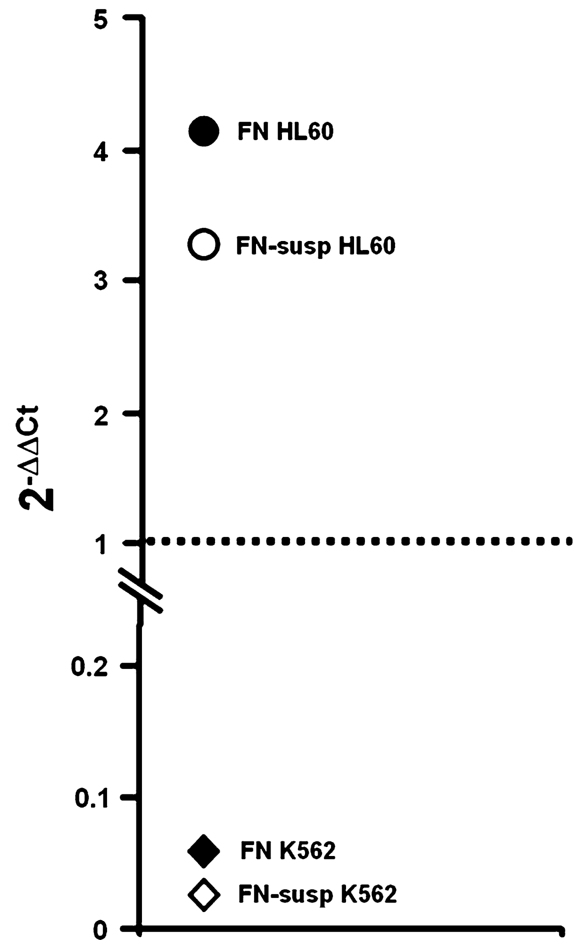

Fig. 2 Intracellular presence of plasmid DNA was quantified by real-time PCR. The data obtained from the fibronectin-adherent cells or from the cells detached from fibronectin were normalized against their suspension counterparts. *Dashed line* crossing at the value $2^{-\Delta\Delta C_t}=1$ indicates an equal amount of plasmid DNA in suspension and fibronectin or fibronectin-suspension groups (*FN* fibronectin-adherent cells, *FN-susp* cells detached from fibronectin)

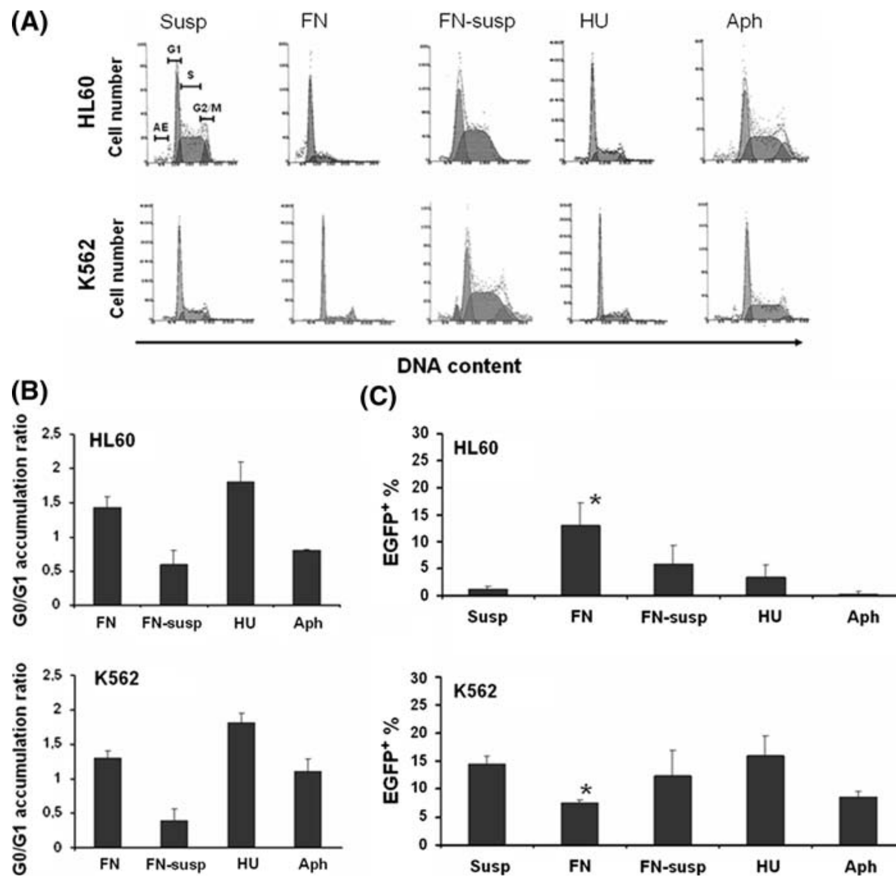

Fig. 3 Effect of cell cycle on the fibronectin-adherent K562 and HL-60 transfection. Cell cycle was analysed as described in the Materials and Methods. In panel **a**, representative cell cycle histograms of HL-60 and K562 cells maintained under several conditions are shown. **b** The change in the ratio of G1 phase was determined in comparison with suspension counterparts (percentages of G1 accumulation are given in Table 1). **c** The percentage of $EGFP^+$ cells were determined by flow cytometry ($n = 3$, $*P < 0.05$) (*Susp* suspension cells, *FN* fibronectin-adherent cells, *FN-susp* cells detached from fibronectin, *HU* hydroxyurea, *Aph* aphidicolin, *AE* uneuploid cells)

Table 1 HL-60 and K562 cells accumulated in G1 phase (%)

	Susp.	FN-adh.	FN-susp.	HU	Aph.
HL-60	41.3 ± 5.6	68.6 ± 1.5*	27.3 ± 2.5*	73.6 ± 7.3*	35.5 ± 2.1
K562	32 ± 11.5	60.6 ± 1.5*	18.6 ± 2.5*	58 ± 4.5*	35 ± 2.6

Susp suspension, *FN-adh* fibronectin-adherent, *FN-susp* cells detached from fibronectin, *HU* hydroxyurea, *Aph* aphidicolin ($n = 3$, $*P < 0.05$)

Adhesion to fibronectin protects the cells from transfection-induced cell death

Cytotoxicity mediated by the cationic lipid DNA complexes reduces the number of successfully transfected cells, consequently deteriorates the efficiency of transfection. Toxicity was pronounced in the suspension K562 cells which were more efficiently transfected. Although the transfection efficiency was increased in the cells detached from fibronectin, ratio of dead cells was comparable to fibronectin-adherent K562 cells. On the other hand, fibronectin-adherent HL-60 cells were less susceptible to transfection-induced cell death. A slight increase in the cell death was also observed in the HL-60 cells detached from fibronectin (Table 2; Fig. 4).

Table 2 Cell death (%) in transfected and untransfected HL-60 and K562 cells

	Susp.	FN-adh.	FN-susp.
HL-60			
Untransfected	1.8 ± 0.2	2 ± 0.17	3.9 ± 0.14
Transfected	4.5 ± 1.7	2.9 ± 1.1*	4.9 ± 2.1
K562			
Untransfected	2.8 ± 0.17	2.9 ± 0.9	4.6 ± 0.8
Transfected	35.1 ± 7.8	12.9 ± 3.1*	11.1 ± 2.5*

Susp suspension, *FN-adh* fibronectin-adherent, *FN-susp* cells detached from fibronectin ($n = 3$, $*P < 0.05$)

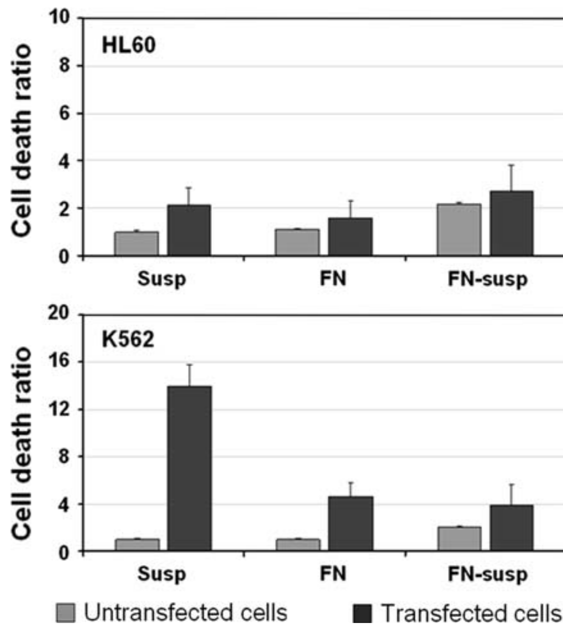

Fig. 4 The effect of liposomal transfection on cell viability was determined by flow cytometric analysis of propidium iodide (PI) incorporation. The ratio of cell death was calculated in comparison with control suspension cells ($n = 3$, $*P < 0.05$) (*Susp* suspension cells, *FN* fibronectin-adherent cells, *FN-susp* cells detached from fibronectin). Percentages of cell death are given in Table 2

Discussion

Gene transfer into the cells of haematopoietic origin is preferentially performed by retroviral transduction. The carboxy-terminal fragment of fibronectin can bind viral particles, therefore increases the transduction efficiency independent of the cellular processes (Moritz et al. 1996). The increase in viral titer on the surface of cells may directly enhance the receptor mediated entry (Moritz et al. 1996; Hanenberg et al. 1997), and the latter events such as intracellular transport and integration of DNA to nucleus are simply mediated by viral vectors. On the other hand, liposomal transfection of DNA is mainly regulated by host cells. Thus, cellular dynamics (e.g. cell cycle, viability, DNA degradation during intracellular trafficking) affected by adhesion to fibronectin can indirectly influence the efficiency of transfection (Lechardeur and Lukacs 2002; Brunner et al. 2000).

In this study, the influence of cellular dynamics regulated by fibronectin substratum on the liposomal transfection of K562 and HL-60 was evaluated as representative lines of transfection-competent and -incompetent myeloid leukaemia cells, respectively. The current data showed that the adhesion to fibronectin could differentially influence the transfection efficiency mainly by modulating the intracellular presence of plasmid DNA. The cell cycle and viability which is regulated by fibronectin had a minor impact on the success of gene delivery.

The extracellular matrix regulates cytoskeletal rearrangements implicated in cellular motility and endocytosis. Especially, the interactions between fibronectin and integrin molecules direct the endocytic activity and intracellular trafficking of raft/caveolae domains (Mitra et al. 2005; Echarri et al. 2007). Recently, a possible role for fibronectin in gene transfer was suggested in a non-viral vector—protein adsorption method. There, the polyethlenimine (PEI)/DNA complexes were adsorbed to fibronectin substratum and an increase in DNA internalization was achieved with NIH/3T3 fibroblasts (Bengali et al. 2007). A different strategy was applied in our study for myeloid leukaemia cells, such as culturing the HL-60 and K562 cells on the fibronectin matrix prior to transfection. The hard-to-transfect cell line HL-60 harboured increased amounts of plasmid DNA compared to its suspension counterparts. The increased ability for the uptake of cationic lipid DNA complexes may be influenced by the potential of HL-60 cells to differentiate into mature leukocytes with an increased ability for endocytosis (Collins 1987; Esendagli et al. 2009).

An increase in the liposome-mediated gene transfer can be achieved with the haematopoietic cells grown on stromal or fibroblast cell line monolayers (Marit et al. 2000). Our findings on leukaemia cells support the importance of stromal components for an efficient

gene delivery. However, in the study of Marit et al. (2000), the K562 cells grown on monolayers were transfected with 18.3% efficiency. We and other groups have reported approximate efficiencies for K562 cultured in suspension under optimised transfection conditions and using different reporter systems (Schakowski et al. 2004; Brunner et al. 2000). We monitored both mRNA and protein levels of the reporter, EGFP, to evaluate the success of transfection. Intriguingly, in contrary to protein levels, gene expression was found to be slightly increased in fibronectin adherent K562 cells when compared with the cells detached from fibronectin. This may indicate an altered transcription-translation process for the plasmid in fibronectin adherent K562 cells. Moreover, the plasmid used in our study carries an EGFP gene to be translated through an internal ribosomal entry site (IRES) sequence which may also be responsible of dissimilar levels of mRNA and protein expression.

Upon adhesion to fibronectin, the haematopoietic cells display lowered mitotic activity (Bengali et al. 2007). Fibronectin-adherent HL-60 and K562 cells reversibly accumulate in the G1 phase of cell cycle (Canpinar et al. 2007). Intriguingly, mitotic activity has been reported as an important parameter for lipid-mediated transfections, in vitro (Mortimer et al. 1999). Moreover, certain phases of the cell cycle can influence the endocytosis, intracellular transport and the nuclear uptake of plasmid DNA. The cells arrested in G1 phase exhibit lower reporter gene activity whereas the transgene expression can be restored when the G1-synchronised cells are allowed to proceed through the cell cycle (Brunner et al. 2000; Mortimer et al. 1999). Correspondingly, this phenomenon was observed with the K562 cells. On the other hand, the number of $EGFP^+$ HL-60 cells was higher in the fibronectin-adherent group with an increased ratio of the cells synchronized in G1-phase. For HL-60 cells, the cell cycle status and mitotic activity may be secondary factors for an efficient gene delivery. However, as expected, the increased presence of intracellular plasmid DNA was critical for HL-60.

In contact with fibronectin, several haematopoietic cells acquire a novel type of drug resistance mechanism, the cell adhesion mediated drug resistance (CAM-DR), that has been proposed as an important determinant of drug response and contributes to the survival of tumour cells (Canpinar et al. 2007; Hazlehurst and Dalton 2001; Damino et al. 2001). The liposome-mediated transfection interferes with the cell viability. The toxicity arisen from the cationic lipid-DNA complex may vary according to chemical composition of the liposome, the amount of DNA, and the type of the host cell (Uchida et al. 2002; Pelisek et al. 2002; Nguyen et al. 2007). Therefore, the efficiency of gene delivery is generally inversely proportional to the cell viability. In our study, the viability of fibronectin-adherent HL-60 cells was not impaired and even a higher efficiency of transfection was achieved. These cells may be protected from the transfection-induced cell death by a cell-adhesion mediated resistance mechanism. CAM-DR to chemotherapeutic drugs can be reversed in tumour cells by detachment from fibronectin (Canpinar et al. 2007; Damino et al. 2001). According to our results, the toxicity of cationic lipid-DNA complexes may not be potent enough to surpass the resistance soon after the K562 cells were released from fibronectin.

In summary, adhesion to fibronectin rendered the HL-60 cells to have (1) increased capacity of endocytosis (presence of intracellular plasmid DNA), (2) resistance to transfection-induced cell death, and (3) lowered proliferation rate (G1 accumulation) leading to the enhancement of liposomal transfection. Although upon adhesion to fibronectin the latter two properties were also gained by K562 cells, decrease in the intracellular presence of plasmid critically reduced the efficiency of transfection. The distinct regulation of DNA transfection in myeloid leukaemia cell lines upon adhesion to extracellular matrix components appears to be an important factor for the non-viral gene therapy approaches for haematological malignancies. Our descriptive observations may stimulate further investigations on the applications of liposomal gene transfer for myeloid leukaemia.

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References

Audouy SA, de Leij LF, Hoekstra D, Molema G (2002) In vivo characteristics of cationic liposomes as delivery vectors for gene therapy. Pharm Res 19:1599–1605

Bengali Z, Rea JC, Shea LD (2007) Gene expression and internalization following vector adsorption to immobilized proteins: dependence on protein identity and density. J Gene Med 9:668–678

Bohnsack JF, Chang J (1994) Activation of beta 1 integrin fibronectin receptors on HL-60 cells after granulocytic differentiation. Blood 83:543–552

Brunner S, Sauer T, Carotta S, Cotten M, Saltik M, Wagner E (2000) Cell cycle dependence of gene transfer by lipoplex, polyplex and recombinant adenovirus. Gene Ther 7: 401–407

Canpinar H, Esendagli G, Kansu E, Gunel-Ozcan A, Guc D (2007) Adhesion of beta1 integrin to fibronectin regulates CAM-DR phenotype via $p21^{WAF1/cip1}$ in HL-60 acute myeloid leukemia (AML) cells. Turk J Med Sci 38: 97–104

Collins SJ (1987) The HL-60 promyelocytic leukemia cell line: proliferation, differentiation, and cellular oncogene expression. Blood 70:1233–1244

Damino JS, Hazlehurst LA, Dalton WS (2001) Cell adhesion mediated drug resistance (CAM-DR) protects the K562 chronic mylogenous lekemia cell line from apoptosis induced by BCR/ABL inhibition, cytotoxic drugs and gamma-irradiation. Leukemia 15:1232–1239

Echarri A, Muriel O, Del Pozo MA (2007) Intracellular trafficking of raft/caveolae domains: insights from integrin signaling. Semin Cell Dev Biol 18:627–637

Esendagli G, Canpinar H, Yilmaz G, Kaymaz FF, Kansu E, Guc D (2009) Fibronectin promotes the phorbol 12-myristate 13-acetate (PMA)-induced macrophage differentiation in myeloid leukemia cells. Int J Hematol 89:167–172

Gonzalez R, Vereecque R, Wickham TJ et al (1999) Increased gene transfer in acute myeloid leukemic cells by an adenovirus vector containing a modified fiber protein. Gene Ther 6:314–320

Hanenberg H, Hashino K, Konishi H, Hock RA, Kato I, Williams DA (1997) Optimization of fibronectin-assisted retroviral gene transfer into human CD34+ hematopoietic cells. Hum Gene Ther 8:2193–2206

Hazlehurst LA, Dalton WS (2001) Mechanisms associated with cell adhesion mediated drug resistance (CAM-DR) in hematopoietic malignancies. Cancer Metastasis Rev 20: 43–50

Lechardeur D, Lukacs GL (2002) Intracellular barriers to non-viral gene transfer. Curr Gene Ther 2:183–194

Marit G, Cao Y, Froussard P et al (2000) Increased liposome-mediated gene transfer into haematopoietic cells grown in adhesion to stromal or fibroblast cell line monolayers. Eur J Haematol 64:22–31

Mitra SK, Hanson DA, Schlaepfer DD (2005) Focal adhesion kinase: in command and control of cell motility. Nat Rev Mol Cell Biol 6:56–68

Moritz T, Dutt P, Xiao X, Carstanjen D, Vik T, Hanenberg H, Williams DA (1996) Fibronectin improves transduction of reconstituting hematopoietic stem cells by retroviral vectors: evidence of direct viral binding to chymotryptic carboxy-terminal fragments. Blood 88:855–862

Mortimer I, Tam P, MacLachlan I, Graham RW, Saravolac EG, Joshi PB (1999) Cationic lipid-mediated transfection of cells in culture requires mitotic activity. Gene Ther 6:403–411

Nguyen LT, Atobe K, Barichello JM, Ishida T, Kiwada H (2007) Complex formation with plasmid DNA increases the cytotoxicity of cationic liposomes. Biol Pharm Bull 30:751–757

Pelisek J, Engelmann MG, Golda A et al (2002) Optimization of nonviral transfection: variables influencing liposome-mediated gene transfer in proliferating vs quiescent cells in culture and in vivo using a porcine restenosis model. J Mol Med 80:724–736

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45

Roddie PH, Paterson T, Turner ML (2000) Gene transfer to primary acute myeloid leukaemia blasts and myeloid leukaemia cell lines. Cytokines Cell Mol Ther 6:127–134

Schakowski F, Buttgereit P, Mazur M et al (2004) Novel non-viral method for transfection of primary leukemia cells and cell lines. Genet Vaccines Ther 2:1

Uchida E, Mizuguchi H, Ishii-Watabe A, Hayakawa T (2002) Comparison of the efficiency and safety of non-viral vector-mediated gene transfer into a wide range of human cells. Biol Pharm Bull 25:891–897