J Membrane Biol (2013) 246:783–791
DOI 10.1007/s00232-013-9548-5

In Vitro Targeted Gene Electrotransfer to Endothelial Cells with Plasmid DNA Containing Human Endothelin-1 Promoter

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Received: 9 January 2013 / Accepted: 17 April 2013 / Published online: 8 May 2013

Abstract Development of recombinant DNA technologies has allowed us to create new delivery systems that target specific cell types and that can be used in gene therapy. One of these targets is vascular endothelium because of its important role in tumor angiogenesis. For tumor endothelium-specific targeting, we prepared plasmid DNA encoding green fluorescent protein under the control of human endothelin-1 promoter (pENDO-EGFP), which is specific for endothelial cells. First we determined gene electrotransfer parameters for improved transfection of endothelial cells evaluating different osmolarity of electroporation buffer, voltages of applied electric pulses, and addition of fetal bovine serum immediately after electroporation to the cells for improved transfection and survival. Transfection efficacy of pENDO-EGFP in different endothelial and nonendothelial cell lines was determined next. Gene electrotransfer efficacy was evaluated using three different methods: fluorescence microscopy, fluorescence microplate reader, and flow cytometry. Our results showed that transfection efficacy was higher when cells were prepared in hypoosmolar compared to isoosmolar electroporation buffer. Furthermore, immediate addition of fetal bovine serum to the cells after pulsing also improved gene electrotransfer into target cells. We proved expression of EGFP under the control of human endothelin-1 promoter in endothelial cells, which was also significantly higher compared to nonendothelial cells. Taken together, we successfully constructed pENDO-EGFP, which was specifically expressed in endothelial cells using improved gene electrotransfer parameters.

Keywords Electroporation · Endothelial cells · Gene electrotransfer · Human endothelin-1 promoter · Osmolarity of electroporation buffer · Transfection efficacy

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Introduction

Gene therapy represents a new and promising approach for treatment of human diseases that have developed as result of gene dysfunction (Cotrim and Baum 2008). In cancer gene therapy, the most commonly used therapeutic genes are those that initiate immune response against the tumor, block the expression of oncogenes, or activate the expression of tumor suppressor genes (Roth and Cristiano 1997). Vectors for gene delivery are divided into two categories: viral and nonviral. Although viral vectors are most frequently used because of their high transfection efficacy, they can cause different side effects (Thomas et al. 2003). Development of nonviral vectors provides safer but less efficient gene delivery compared to viral vectors. Direct injection of naked plasmid DNA into the target tissue, such as muscle, represents the simplest way for nonviral gene delivery (Wolff et al. 1990). However, the expression level of the transgene is inefficient as a result of the low cellular uptake (Wolff and Budker 2005). For improved transfection efficacy of plasmid DNA into the target tissue, several chemical and physical delivery methods have been developed (Niidome and Huang 2002).

Electroporation is a simple physical method for delivery of different exogenous molecules into the target cells in vitro and in vivo. It is based on application of controlled

electric pulses to cells or tissues, which leads to increased cell membrane permeability. Introduction of plasmid DNA into the target tissues using electroporation leads to increased transgene expression compared to transgene expression after direct injection of plasmid DNA (Cemazar et al. 2006; Mir 2009; Faurie et al. 2010). When performing in vitro gene electrotransfer, several parameters, such as composition of plasmid DNA and its concentration, parameters of electric pulses, and composition of electroporation buffer, can influence electrotransfer and should thus be determined for each specific cell line in order to achieve a high transfection yield (Rebersek et al. 2011). One of the goals of gene therapy is also to provide safe, selective, and controlled expression of transgene. Constitutive viral promoters (e.g., CMV, SV40, MoMLV) are most commonly used in mammalian systems because they allow very high levels of transgene expression but cannot be controlled and are not tissue specific (Young et al. 2008; Qin et al. 2010). Furthermore, as a result of their viral origin, in eukaryotic cells, viral promoters are prone to silencing and inactivation in vitro and in vivo compared to eukaryotic promoters (Prösch et al. 1996; Kamensek et al. 2011). Tissue-specific eukaryotic promoters are tightly regulated; they drive transgene expression into appropriate cell types and minimize or exclude inappropriate expression in surrounding, nontarget, cells. Additionally, these promoters also provide long-lasting transgene expression (Papadakis et al. 2004). However, tissue-specific eukaryotic promoters are weaker compared to the stronger viral promoters, resulting in lower transgene expression (Nakamura et al. 2008).

Vascular endothelial cells in tumors represent one of important targets in cancer gene therapy because of their role in tumor angiogenesis. Tumor angiogenesis is the process of formation of new blood vessels from preexisting vasculature and it is necessary for solid tumor progression and metastasis (Tandle et al. 2004; Kamensek and Sersa 2008). Endothelin-1 is a potent vasoconstrictor peptide that is synthesized predominantly in vascular endothelial cells (Harats et al. 1995). In addition to its vasoactive properties, it also stimulates angiogenesis through mitogenic effect on endothelial cells and is also responsive to various stimuli, such as intracellular calcium level, hormones, growth factors, and oxidative stress (Kähler et al. 2001; Grant et al. 2003; Stow et al. 2011). In addition, it has been demonstrated that the expression of endothelin-1 was increased in certain types of tumors, which makes its promoter an appropriate choice for use in gene therapies directed against tumor vasculature (Yamashita et al. 1991; Bagnato et al. 1999; Vlachostergios et al. 2012). Therefore, to specifically target tumor endothelial cells, our aim was to construct recombinant plasmid DNA encoding EGFP under the control of human endothelin-1 promoter (pENDO-EGFP), which is specific for endothelial cells, and to evaluate its transfection efficacy in different cell lines. For this purpose, we first tested several gene electrotransfer parameters for improved transfection efficacy of plasmid DNA in HUVEC cells and murine endothelial cells 2H11. Next, to determine the specificity of recombinant plasmid DNA, its transfection efficacy was determined on different endothelial and nonendothelial cell lines using defined transfection conditions.

Materials and Methods

Chemicals

Sucrose, dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), and magnesium chloride ($MgCI_2$) were purchased from Sigma Aldrich (Taufkirchen, Germany). Trypsin/EDTA, phosphate-buffered saline (PBS), advanced Dulbecco modified Eagle medium (ADMEM), advanced Eagle minimum essential medium (AMEM), L-glutamine, antibiotic gentamicin, and heat-inactivated fetal bovine serum (FBS) were purchased from Gibco (Invitrogen, Paisley, UK).

Cell Lines

Cell lines used in this study were obtained from American Type Culture Collection (ATCC, Manassas, Vancouver, Canada), except the human umbilical vein endothelial cells (HUVEC), which were the gift of Urska Batista (Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia), but which were originally obtained from ATCC. All cells were cultured in a humidified atmosphere with 5 % CO_2 at 37 °C in the following culture media: HUVEC, murine endothelial cells SVEC4-10 and 2H11 in ADMEM supplemented with 5 % FBS, gentamicin, and L-glutamine; and human malignant melanoma cells SK-MEL-28 and murine melanoma cells B16-F1 in AMEM supplemented with 5 % FBS, gentamicin, and L-glutamine. For experiments, cells were maintained in monolayers until they reached 80–90 % confluence.

Construction of Plasmid DNA with Tissue-specific Promoter

Plasmid encoding enhanced green fluorescence protein (EGFP) with no promoter (pEGFP-N1-noCMV promoter; the gift of Claudia Karl, Medical Center, University of Munich, Munich, Germany) was used as a source of EGFP genes. The source of the sequence for human endothelin-1 promoter (Lee et al. 1990) was plasmid pDD297 (the gift of David A. Dean, School of Medicine and Dentistry,

Rochester, NY, USA). Recombinant plasmid expressing EGFP under the control of human endothelin-1 promoter (pENDO-EGFP) was constructed by subcloning the 274 bp promoter fragment from pDD297 into the *Sac*I/*Hin*dIII restriction site of plasmid pEGFP-N1-noCMV promoter using standard molecular biology techniques of restriction and ligation. *Escherichia coli* strain JM107 (Thermo Scientific Molecular Biology, Vilnius, Lithuania) was transformed with prepared pENDO-EGFP using the TransformAid Bacterial Transformation kit (Thermo Scientific Molecular Biology, Vilnius, Lithuania) according to the manufacturer's instructions.

pEGFP-N1 encoding EGFP under the control of CMV promoter (pEGFP-N1, Clontech, Basingstoke, UK) was used as positive control for human endothelial-1 promoter in gene electrotransfer experiments. For experiments, plasmids were purified using the Jetstar Plasmid Maxi Prep isolation kit (Genomed GmbH, Lohne, Germany).

Gene Electrotransfer

To determine gene electrotransfer parameters for improved transfection of endothelial cells, HUVEC and 2H11 cells in exponential growth phase were trypsinized, washed in ADMEM with 5 % FBS, and centrifuged for 5 min at 4 °C at 1,500 rpm (Kendro GmbH Multifuge 1 S-R, Langenselbold, Germany). Final cell suspension, at a concentration of 2.5×10^7 cells/ml, was prepared in electroporation buffer (10 mM K_2HPO_4, 2.5 mM KH_2PO_4, 2 mM $MgCl_2 \times 6H_2O$) containing 125 mM (hypoosmolar, pH 7.2, conductivity 2.1 mS/cm and osmolarity 160 mOsm/kg) or 250 mM (isoosmolar, pH 7.2, conductivity 1.43 mS/cm, and osmolarity 270 mOsm/kg) sucrose content. Cell suspension (40 µl) was mixed with 10 µg/µl of pEGFP-N1, and the prepared mixture (1×10^6 cells) was placed between two parallel plate stainless steel electrodes with a 2 mm gap (van Leeuwen et al. 1999; Cemazar et al. 2002; Peister et al. 2004). Electroporation was performed with eight square-wave-shaped electric pulses at 80, 100, and 120 V for a duration of 5 ms and at a frequency of 1 Hz. To generated electric pulses, a homemade electroporator (Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia) was used. Immediately after pulsing, 50 µl of 100 % FBS (final concentration 50 % v/v) or ADMEM + 5 % FBS (final concentration 2.5 % v/v) was added to the cells, which were incubated at room temperature (25 °C) for 5 min (Delteil et al. 2000). The room temperature was selected on the basis of results of preliminary experiments with different (4, 25, 37 °C) temperatures, which found no difference in transfection efficiency (data not shown). After 5 min of incubation, 2 ml of ADMEM with 5 % FBS was added, and the cells were plated in 10 cm^2 petri dishes (TPP, Trasadingen, Switzerland) for further assays.

Next, to determine the transfection efficacy of tissue-specific plasmid DNA pENDO-EGFP, endothelial HUVEC, 2H11, and SVEC4-10 cells as well as nonendothelial SK-MEL-28 and B16-F1 cells were prepared in hypoosmolaric electroporation buffer and transfected with pENDO-EGFP or pEGFP-N1 using eight electric pulses at 100 V with a duration of 5 ms and a frequency of 1 Hz. Cell were then incubated for 5 min with 100 % FBS and plated for further assays.

Determination of Cell Survival

Cell survival of 2H11 and HUVEC cells was determined by counting viable cells with a hemocytometer. Because of the different growth rate of the cells (with 2H11 cells having a doubling time of 19.9 h and HUVEC cells having a doubling time of 25.4 h), cell survival was determined 24 h after transfection for 2H11 cells and 48 h for HUVEC cells. Cells were trypsinized, washed in ADMEM with 5 % FBS, centrifuged for 5 min at 25 °C and 1,500 rpm, and resuspended in ADMEM. Round-shaped cells with halos around them were defined as viable and were counted. Small, pycnotic cells with a wrinkled cell membrane were considered to be dead cells. Percentage of cell survival was obtained as a ratio of the number of viable cells from treated samples and the number of viable cells from control samples.

Determination of EGFP Expression

Expression of EGFP was determined by several different methods. The number of cells expressing EGFP and its intensity was determined by fluorescence microscopy (Olympus TH2-200, Hamburg, Germany). Images were taken under visible and fluorescence light conditions 5, 24, and 48 h after transfection at ×40 objective magnification with an Olympus U-MWIB filter (excitation 460–495 nm and emission 510–550 nm) with a digital color camera (Olympus ColorViewIII) connected to the fluorescence microscope. Images were then processed with Cell^A Imaging software (Soft Imaging System GmbH, Munster, Germany). For each treated and nontreated sample, five different observation fields were captured. These fields were marked so that the same observation fields were captured at different time points. Obtained images were analyzed by ImageJ software 1.410 (NIH, Bethesda, MD, USA). First, images taken at different times after transfection were stacked together; then the threshold was adjusted and fluorescence intensity was determined for each stack. Gene electrotransfer efficacy was determined as a ratio between the number of cells expressing EGFP and

the total number of cells, which were counted under phase contrast. EGFP fluorescence intensity was also measured by a fluorescence microplate reader Tecan Infinite 200 (Tecan, Mannedorf, Switzerland). Suspension of cells from control and treated samples were prepared 5 and 24 h after transfection. Aliquots of cells, containing 100,000 cells in 100 µl of medium, were distributed to three wells of a 96-well black microtiter plate with a clear bottom (Grainer, Bio-One, Nurenberg, Germany). The fluorescence intensity of EGFP was measured at 485 nm excitation and 535 nm emission wavelengths. In addition, in the experiments evaluating the transfection efficacy of pENDO-EGFP and pEGFP-N1 in different cell lines, the percentage of transfected cells expressing EGFP and the median fluorescence intensity of EGFP were quantified by flow cytometry (BD FACSCanto II; Becton–Dickinson, San Jose, CA, USA). For these measurements, cells were trypsinized 48 h after transfection and centrifuged for 5 min at 25 °C at 1,500 rpm. Cells were then resuspended in 200 µl of PBS, transferred to polystyrene round-bottom tubes (Becton–Dickinson), and analyzed by flow cytometry, identifying the percentage of EGFP-positive (fluorescent) cells and measuring the median fluorescence intensity of the EGFP. Laser excitation was 488 nm and the number of events was 20,000. Experiments were performed in duplicate and repeated three times independently.

Statistical Analysis

Statistical analysis was performed by SigmaPlot 11.0 (Systac Software Inc., San Jose, CA, USA). Significance tests were carried out by the Holm-Sidak method after one-way analysis of variance (ANOVA); $p < 0.05$ values were considered as significant. The values were expressed as arithmetic mean (AM) ± standard error of the mean (SE).

Results and Discussion

Effect of Voltage Amplitude, Pulsing Buffer Osmolarity, and Postpulsation Addition of Serum on Transfection Efficacy on Endothelial Cells

We tested several gene electrotransfer parameters that were based on data in the literature for improved transfection efficacy of pEGFP-N1 in HUVEC and 2H11 cells: electroporation buffer osmolarity, voltage of electric pulses, and immediate addition of 100 % FBS or ADMEM + 5 % FBS to the cells after pulsing. The results showed a significant increase of percentage of transfected HUVEC cells with increasing voltage of electric pulses regardless of electroporation buffer osmolarity (Fig. 1). Nevertheless, the percentage of transfected HUVEC cells was ~2.0

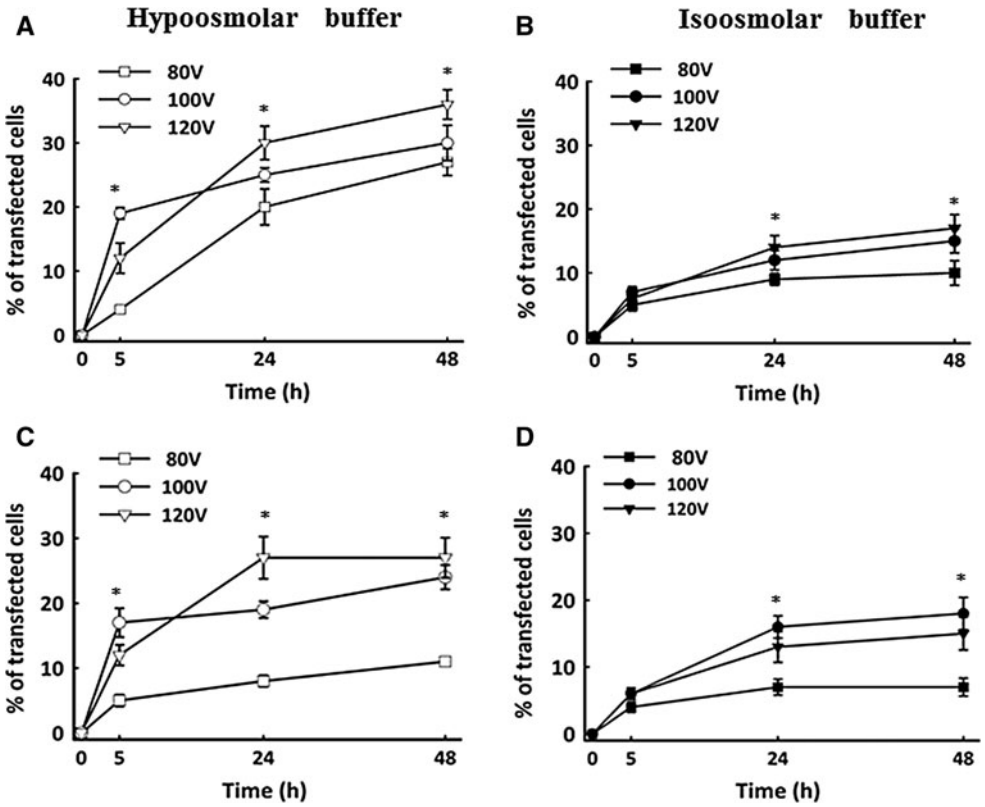

Fig. 1 Effect of electroporation buffer osmolarity on percentage of transfected HUVEC cells using different electric pulses. FBS (**a**, **b**) or ADMEM + 5 % FBS (**c**, **d**) was immediately added to the cells after pulsing. Experiments were performed in duplicate and repeated 3 times; results are expressed as mean ± SE (*$p < 0.05$ compared to all other groups)

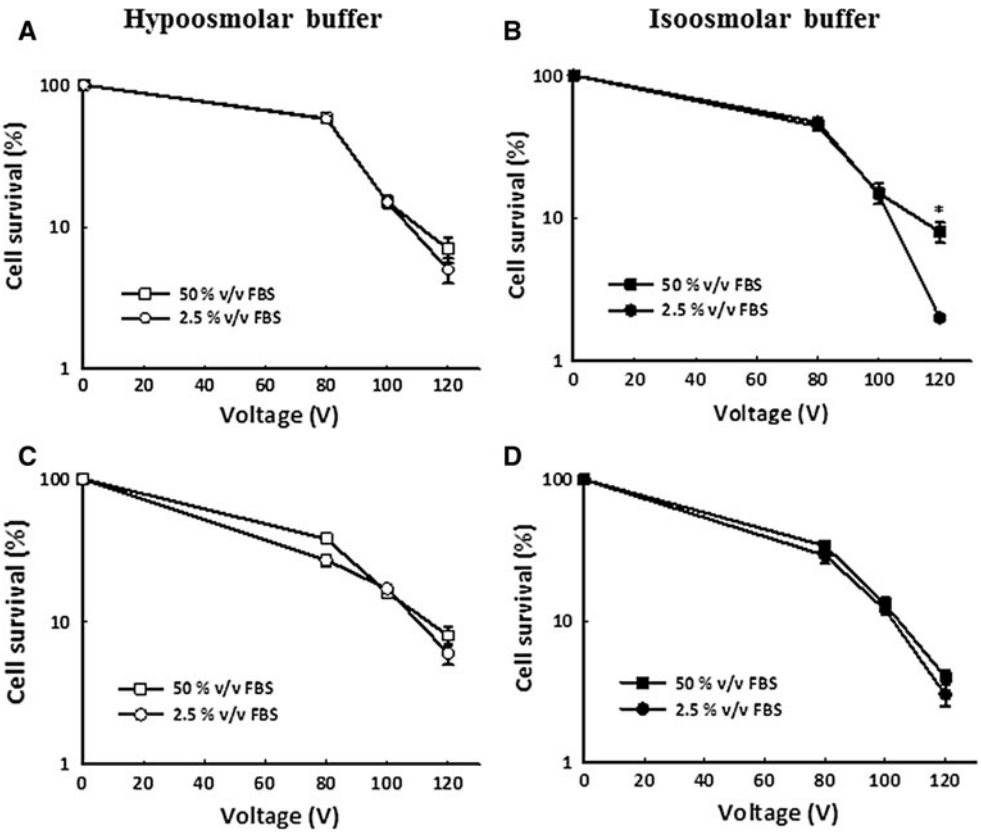

Fig. 2 Effect of electroporation buffer osmolarity on HUVEC (**a**, **b**) and 2H11 (**c**, **d**) cell survival using different electric pulses. Experiments were performed in duplicate and repeated 3 times independently. Results are expressed as mean ± SE (*$p < 0.05$ compared to all other groups)

times higher when cells were prepared in hypoosmolar electroporation buffer compared to isoosmolar electroporation buffer. Furthermore, the immediate addition of 100 % FBS to the cells after pulsing (Fig. 1a, b) improved gene electrotransfer efficacy in both electroporation buffers by ~1.3 times compared to immediate addition of ADMEM + 5 % FBS (Fig. 1c, d). Interestingly, at 5 h after transfection, the number of transfected cells exposed to 100 V was higher compared to the number of transfected cells exposed to 120 V.

The reason for the observed results, which were significant, is currently not known, but it might be related to the more toxic effects of higher voltages on cells that took a longer time to recover (more than 5 h), and consequently the transcription of plasmid DNA was delayed. Similar to HUVEC cells, fluorescence intensity of EGFP in 2H11 cells was significantly higher at 80 and 100 V using hypoosmolar electroporation buffer with an immediate addition of 100 % FBS compared to isoosmolar buffer and addition of ADMEM + 5 % FBS, for 2.0 and 3.6 times, respectively. Most of the studies on gene electrotransfer in vitro utilizes isoosmolar electroporation buffer (Kanduser et al. 2009; Pavlin et al. 2011); however, the results of our study demonstrate that electroporation of cells in hypoosmolar buffer lead to better transfection efficacy. Our results are also in agreement with the results of the study by Golzio et al. (1998). They demonstrated 2.0-fold higher number of cells expressing reporter gene β-galactosidase when Chinese hamster ovary cells (CHO) were pulsed in hypoosmolar electroporation buffer compared to isoosmolar buffer. In that study, addition of FBS to the cells after pulsing was not tested, but its use has been reported in more recent studies (Delteil et al. 2000; Haberl et al. 2010). Delteil et al. (2000), using CHO growing in suspension, demonstrated that addition of 50 % v/v FBS significantly increased transfection efficiency. Our results confirmed these results and extended them to morphologically different cell lines, i.e., endothelial cell lines. In addition, it was shown that the uptake of small molecules is also increased by the use of hypoosmolar buffer in B16F1 and CHO cells when the cells are exposed to electric pulses of varying amplitude-to-distance ratios, ranging from 600 to 1,000 V/cm (Usaj and Kanduser 2012).

Effect of Gene Electrotransfer Parameters on Cell Survival

Besides transfection efficacy, we also determined the effect of gene electrotransfer parameters on HUVEC and 2H11 cells survival at 48 or 24 h after transfection, respectively. As shown in Fig. 2, cell survival decreased with increasing voltage of electric pulses in both cell lines, regardless of

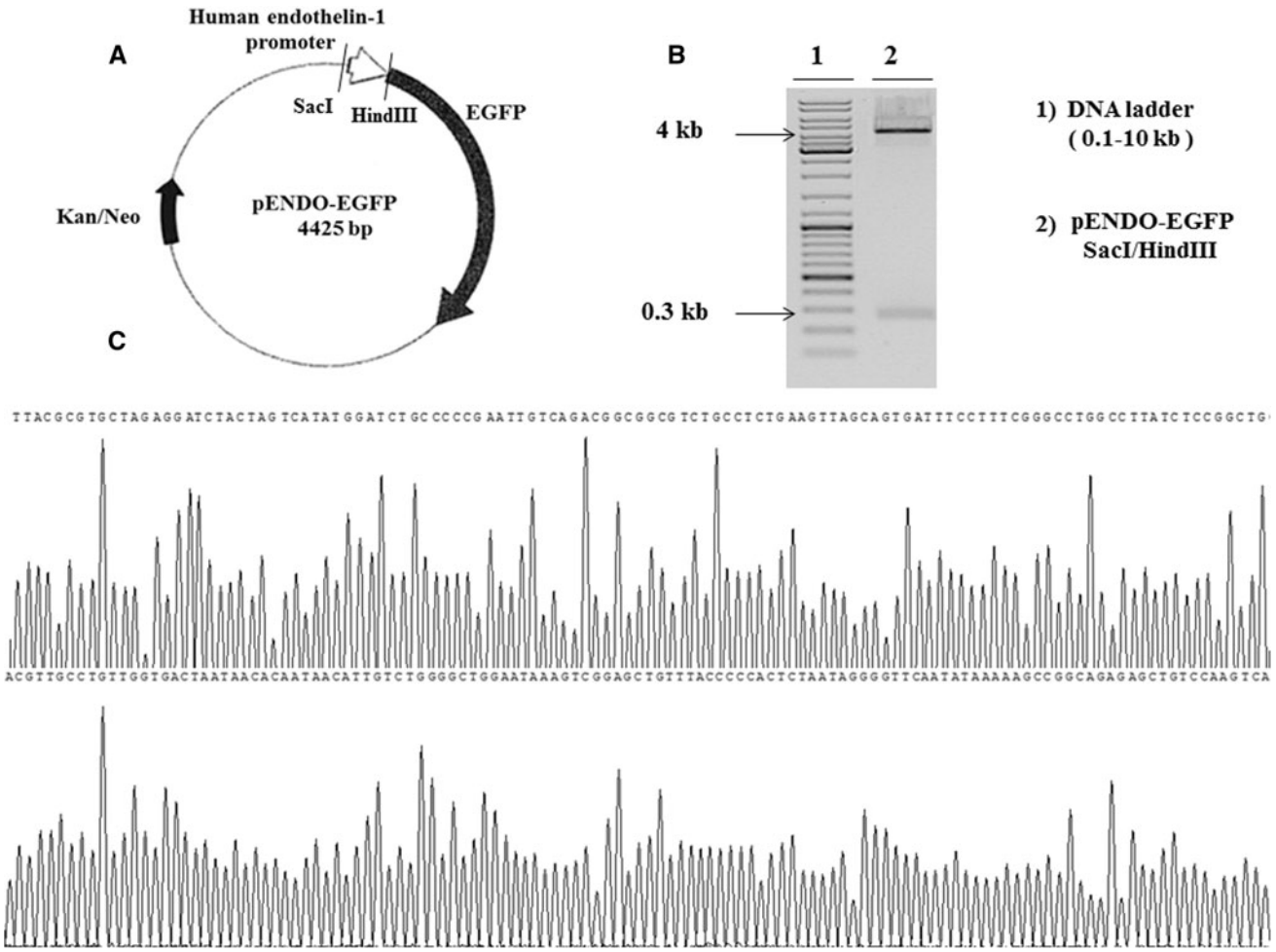

Fig. 3 Map of recombinant plasmid DNA pENDO-EGFP. **a** Confirmation of successful plasmid construction by restriction digest (**b**) and by DNA sequencing (**c**). **b** Sample 2 is linearized recombinant plasmid DNA resulting from ligation of a 247 bp promoter fragment from pDD297 and pEGFP-N1-noCMV vector restricted with *Sac*I and *Hin*dIII

electroporation buffer osmolarity and addition of FBS. A significant difference in cell survival for both cell lines was observed only when electric pulses at 120 V were applied. The survival of cells exposed to electric pulses in isoosmolar buffer and with the addition of ADMEM + 5 % FBS was significantly lower compared to the survival of cells in other groups at this voltage (120 V). Exposure of cells to this high-voltage electric pulse may lead to membrane breakdown, resulting in cell death. Addition of serum can protect cells against the free exchange of macromolecules across the permeabilized membrane, which could in turn lead to increased cell survival (Delteil et al. 2000).

On the basis of the results on transfection efficacy and cell survival, we selected the following electroporation parameters for the further in vitro transfection of cells: hypoosmolar electroporation buffer, electric pulses at 100 V, and immediate addition of 100 % FBS to the cells after pulsing. Compared to other cell lines, tumor and transformed endothelial cell lines appeared to be very sensitive to transfection with electroporation. For example, for gene electrotransfer to murine fibrosarcoma LPB cells, adenocarcinoma TS/A cells and CHO cells, electric pulses of voltage ranging from 120 to 160 V were applied without greatly reduced survival, and an even higher number of pulses was used in some studies (Rols and Teissie 1989; Golzio et al. 1998; Mesojednik et al. 2008; Kamensek et al. 2011).

Construction of Plasmid DNA with Tissue-specific Promoter and Transfection of Endothelial and Nonendothelial Cells

Recombinant plasmid DNA encoding EGFP under the control of human endothelin-1 promoter for specific targeting of tumor endothelial cells (Fig. 3a) was successfully constructed. Construction of pENDO-EGFP was verified with *Sac*I and *Hin*dIII restriction digestion (Fig. 3b) and with the Macrogene (Amsterdam, The Netherland)

standard DNA sequencing service (Fig. 3c). Both DNA sequencing and digestion with restriction enzymes revealed that the construction of recombinant plasmid DNA as described in Materials and Methods was successful.

To verify the specific targeted expression of EGFP under the control of human endothelin-1 promoter, different endothelial and nonendothelial cell lines were transfected with pENDO-EGFP, as well as with pEGFP-N1, using gene electrotransfer parameters determined in the first part of the study (Fig. 4a, b). The expression of EGFP under the control of human endothelin-1 promoter was demonstrated in all three endothelial cell lines at 24 h after transfection, but not in nonendothelial cell lines (Fig. 4b). As expected, the fluorescence intensity of cells was higher when cells where transfected with pEGFP-N1, where EGPF is under the control of a constitutive promoter (Kamensek et al. 2011), compared to the cells transfected with pENDO-EGFP, where EGFP is under the control of a tissue-specific promoter.

Differences in transfection efficacy of pEGFP-N1 and pENDO-EGFP in endothelial and nonendothelial cell lines, which were obtained by fluorescence microscopy, were also confirmed by flow cytometry analysis (Fig. 5a, b). Flow cytometry analysis showed that 17.3 ± 0.1 % of SVEC4-10, 13.8 ± 2.0 % of HUVEC, 8.0 ± 0.7 % of B16-F1, and only 1.8 ± 0.1 % of SK-MEL-28 cells expressed EGFP when the cells were transfected with pENDO-EGFP (Fig. 5b). Surprisingly, only 3.0 ± 0.4 % of endothelial cells 2H11 expressed EGFP. Furthermore, there was a significant increase in fluorescence intensity of expressed EGFP in SVEC4-10 and HUVEC cells compared to the BF16-F1, SK-MEL-28, and 2H11 cells

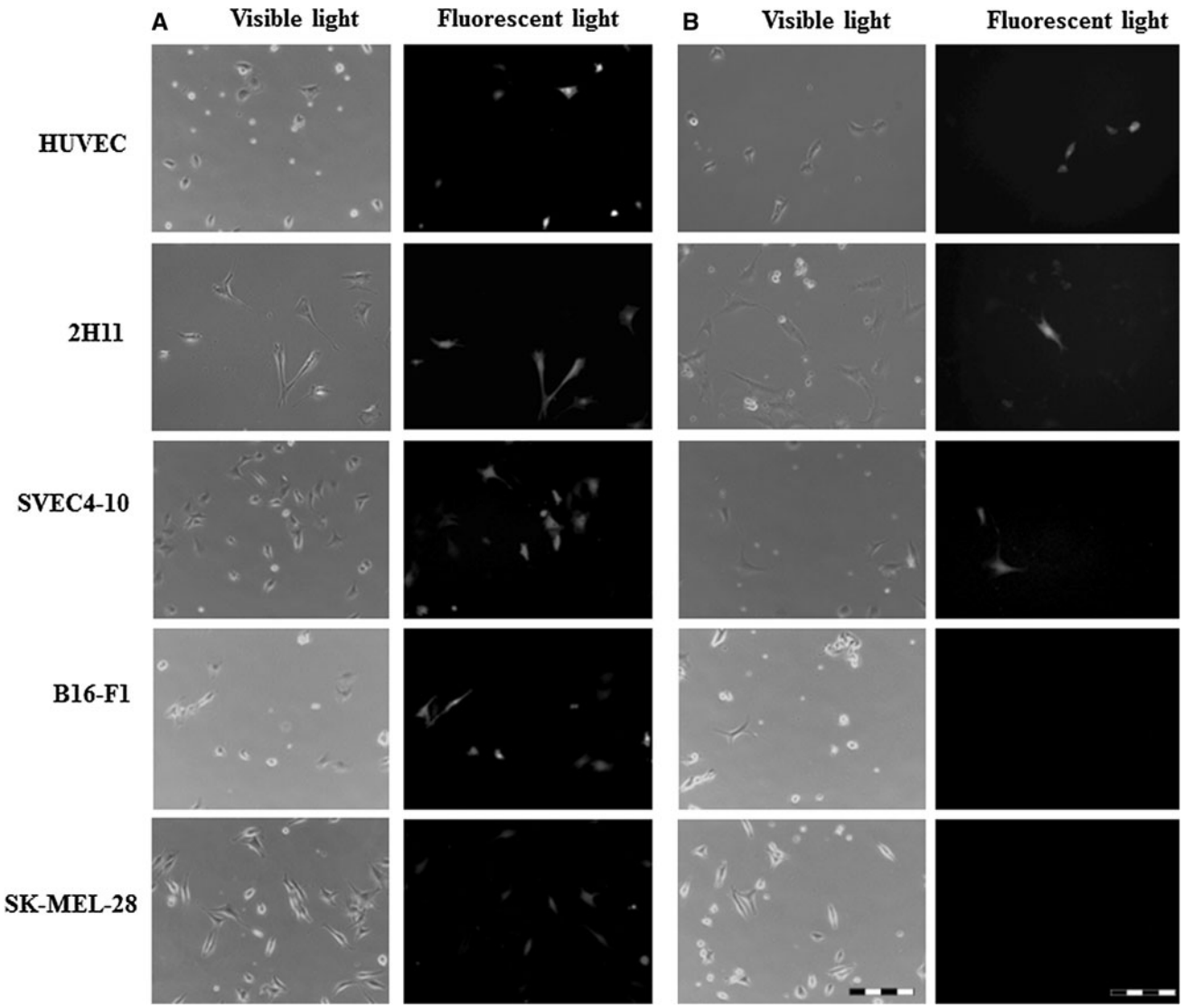

Fig. 4 Different endothelial and nonendothelial cell lines transfected with pEGFP-N1 (**a**) and pEDNO-EGFP (**b**). Images were taken under visible and fluorescence light conditions 24 h after transfection at ×10 objective magnification *scale bar* 200 μm

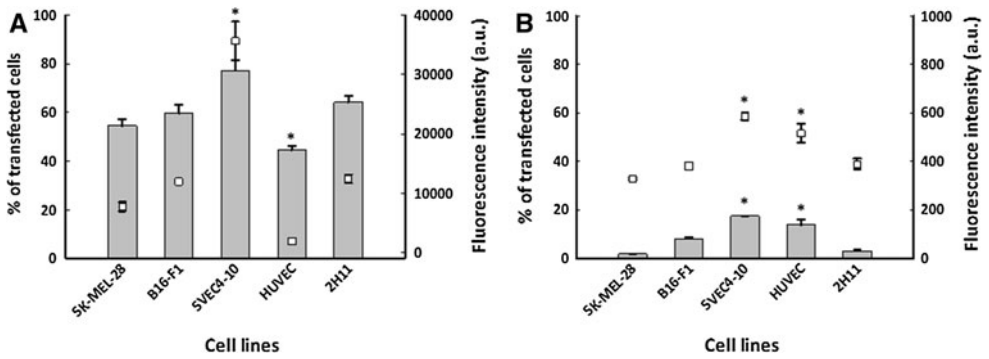

Fig. 5 Transfection efficacy of pEGFP-N1 (**a**) and pENDO-EGFP (**b**) in endothelial (SVEC4-10, HUVEC and 2H11) and nonendothelial (B16-F1 and SK-MEL-28) cell lines. Percentage of cells expressing EGFP (*bar plot*) and fluorescence intensity of EGFP expression (*scatter plot*) were measured 48 h after transfection by flow cytometry. Experiments were performed in duplicate and repeated 3 times. Results are expressed as mean ± SE (*$p < 0.05$ compared to all other groups)

after transfection of cells with pEDNO-EGFP. Jager et al. (1999) have provided evidence that expression of reported gene β-galactosidase under the control of hybrid human prepro-endothelin-1 promoter was almost 10-fold higher in human microvascular endothelial cells HMEC and porcine aorta endothelial cells PAE compared to murine fibroblasts NIH 3T3 and radbomyosarcoma TE671. For construction of hybrid promoter of retroviral vector, human prepro-endothelin-1 promoter was introduced within the 3′ viral long terminal repeat. Lee et al. (1990) also demonstrated that the expression of prokaryotic chloramphenicol acetyltransferase gene under the control of human endothelin-1 promoter sequence cloned upstream of the SV40 promoter was 8-fold higher in bovine aortic endothelial cells BAEC compared to NIH 3T3 and human cervix carcinoma cells HeLa. The number of cells expressing EGFP and the fluorescence intensity of cells were much higher when the cells, regardless of their origin, were transfected with pEGFP-N1 compared to cells transfected with pENDO-EGFP. Higher fluorescence intensity in cells transfected with pEGFP-N1 was an expected result, as constitutive promoters are known to drive high expression of transgene soon after transfection (Kamensek et al. 2011). However, a lower number of transfected cells can be attributed to the fact that the endothelin-1 promoter is active in activated tumor endothelial cells, and therefore, in our experimental setup, which used transformed endothelial cell lines, the number of transfected cells with pENDO-EGFP was most probably underestimated (Yamashita et al. 1991; Bagnato et al. 1999; Vlachostergios et al. 2012). Because electroporation in principle should enable an equal amount of both plasmids to enter the cells, in cells transfected with pENDO-EGFP, the reporter gene was not transcribed as a result of the low level of endothelin-1 promoter activity and therefore was not detectable by either fluorescence microscopy or flow cytometry. Further studies measuring the amount of plasmid DNA present in the cells are needed to clarify this discrepancy.

In conclusion, we successfully constructed recombinant DNA plasmid encoding EGFP under the control of endothelial cell-specific endothelin-1 promoter, and we showed that its expression is more pronounced in endothelial cells compared to cells of other origins. The constructed plasmid with specificity for endothelial cells provides a tool for further studies of vascular targeted approaches in tumor treatment.

Acknowledgments This work was financially supported by Slovenian Research Agency (program P3-0003, projects J3-4259 and J3-4211) and conducted within the scope of the EBAM European Associated Laboratory (LEA) and COST Action TD1104. The authors thank Dr. Julija Hmeljak, Masa Bosnjak, Miroslava Lavric, Lara Prosen, Dr. Jaka Cemazar, Dr. Marusa Lokar, and Dr. Jaka Lavrencak for their help with preparing cell lines for experiments and flow cytometry measurements.

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