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Dual bio-responsive gene delivery via reducible poly(amido amine) and survivin-inducible plasmid DNA

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Abstract A bioreducible poly(amido amine) (SS-PAA) gene carrier, known as poly (amido-butanol) (pABOL), was used to transfect a variety of cancer and non-cancer cell lines. To obtain cancer-specific transgene expression for therapeutic efficiency in cancer treatment, we constructed survivin-inducible plasmid DNA expressing the soluble VEGF receptor, sFlt-1, downstream of the survivin promoter (pSUR-sFlt-1). Cancer-specific expression of sFlt-1 was observed in the mouse renal carcinoma (RENCA) cell line. pABOL enhanced the efficiency of gene delivery compared to traditional carriers used in the past. Thus, a dual bio-responsive gene delivery system was developed by using bioreducible p(ABOL) for enhanced intracellular gene delivery and survivin-inducible gene expression system (pSUR-sFlt-1 or pSUR-Luc reporter gene) that demonstrates increased gene expression in cancer that has advantages over current gene delivery systems.

Keywords Dual bio-responsive ·
Gene therapy · Reducible polymer ·
Survivin · Tumor cells

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Introduction

Synthetic non-viral vectors are potential carriers for gene therapy due to their ability to condense both large and small nucleic acid molecules, high storage ability, low-host immune response, and easy production in large quantities. However, the major disadvantage of these non-viral vectors is their low transfection efficiency compared to virus-based delivery systems. Commonly used synthetic gene carriers include polycations (De Smedt et al. 2000; Shi and Hoekstra 2004; Benns and Kim 2000) and cationic lipids (Felgner et al. 1995; Miller 1998). Both

synthetic gene carriers condense nucleic acids via electrostatic interactions with the anionic phosphate backbone of nucleic acid chains. The resulting complexes (polymer or lipid/DNA complexes) protect DNA from degradation and facilitate the delivery of therapeutic gene into cells. Unfortunately, however, they are also responsible for carrier-associated toxicity in vitro or in vivo (Eliyahu et al. 2005; Lungwitz et al. 2005).

An integral requirement for the achieving human gene therapy is the use of safe and efficient gene delivery vehicles. In order to achieve efficient gene transfer using non-viral vectors, several barriers that limit nucleic acids delivery must be overcome. These barriers include poor intracellular delivery due to inefficient release of nucleic acids from the non-viral polymeric vector (Schaffer et al. 2000), toxicity resulting from high molecular weight polycations and opsonization in physiologic serum conditions (Chollet et al. 2002; Dash et al. 1999). Therefore, attention has been given to hydrolytically-degradable polymeric gene carriers that break down into lower molecular weight components, thus facilitating intracellular release of therapeutic nucleic acids and limit cell cytotoxicity. Unfortunately, these hydrolytically-degradable systems are only moderately stable in the extracellular environment and hence have limited applications. Consequently, bio-responsive and bio-degradable polymeric vectors are beginning to emerge in the field of non-viral gene therapy. These vectors are hydrolytically stable in the extracellular environment but contain disulfide linkages that are cleaved in the presence of glutathione and/or thioredoxin reductases found intracellularly (Oupicky and Diwadkar 2003; Christensen et al. 2006). Indeed, these polymeric vectors are more stable in the extracellular environment than the aforementioned hydrolyzable gene vectors and still maintain relatively high transfection efficiency and low cytotoxicity.

Exploitation of the relatively high concentrations of glutathione and/or thioredoxin reductases intracellularly has also been successful for intracellular release of drug-conjugates or delivery systems (Saito et al. 2003; Kakizawa et al. 1999). Recently, we developed a synthetic vector based on linear reducible poly(amido amine)s that contain multiple disulfide linkages in their main chain having various side groups such as ABOL, APOL, DMEA, AEEOL, MOPA, MPA (SS-PAAs) (Lin et al. 2006, 2007a, b,

2008). These SS-PAAs were stable in the extracellular environment but prone to fast degradation in a reductive environment analogous to that existing in the cytoplasm of the cell. In the series of polymers, it was revealed that the polymers with more hydrophobic side groups, pABOL and pAPOL, showed higher transfection efficacy and much lower cytotoxicity than 25 kDa branched polyethylenimine (BPEI 25K).

Also noteworthy is that cancer therapy and eradication of tumors in humans is often made difficult due to the upregulation of survival factors which are expressed by tumor cells. These survival factors often execute their function by suppressing apoptosis or regulating cell division. They play an important role in the enhanced viability of tumor cells and the progression of malignancies (Deveraux and Reed 1999; Salvesen and Duckett 2002). Recently, a member of the inhibitors of apoptosis proteins (IAP) family, survivin, has been implicated in both preservation of cell viability and control of mitosis (Ambrosini et al. 1997). The survivin gene is largely undetectable in normal adult tissues and is upregulated in most human cancer cells. It inhibits apoptosis resulting in an unfavorable patient prognosis (LaCasse et al. 1998). Moreover, survivin expression is activated transcriptionally, which suggests that the survivin promoter is a cancer-specific promoter that can be used to drive the expression of therapeutic genes in cancer cells specifically (Li and Altieri 1999).

In this study, we constructed survivin-inducible plasmid DNA (pDNA) encoding the soluble vascular endothelial growth factor receptor 1 (VEGFR1/sFlt-1), a VEGFR antagonist, by inserting the survivin promoter and sFlt-1 into the pCI pDNA (pSUR-sFlt-1). We also constructed pSUR-Luc pDNA as a reporter gene to assess the role of survivin promoter on gene expression. To deliver these genes cancer-specifically we used a bioreducible SS-PAA known as poly (amido-butanol) (pABOL) as shown in Scheme 1.

## Materials and methods

Materials

BPEI (MW: 25 kDa) was purchased from Polysciences, Inc. (Warrington, PA). 4-Amino-1-butanol (ABOL), MTT, terrific broth, ampicillin, and Roswell

Scheme 1 Illustration of experimental concept
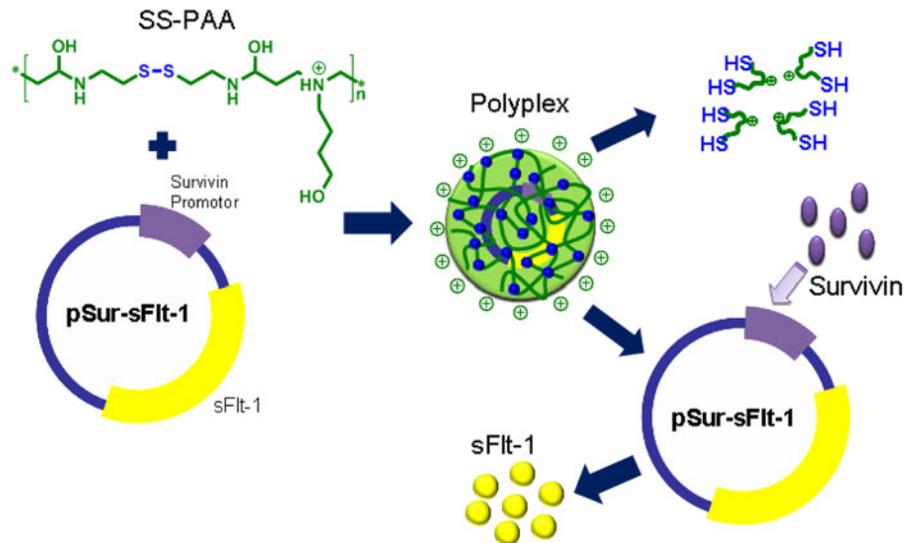
Park Memorial Institute (RPMI-1640) and Dulbecco's Modified Eagle's Medium (DMEM) medium were purchased from Sigma–Aldrich (Milwaukee, WI). Fetal bovine serum (FBS) was purchased from HyClone (Logan, UT, USA). Luciferase assay system with reporter lysis buffer was purchased from Promega (Madison, WI) and the bicinchoninic acid (BCA) protein assay reagent kit was from Pierce Chemical Co. (Rockford, IL). pDNA was propagated in chemically competent DH5α strain (GibcoBRL) and prepared from overnight bacterial cultures by alkaline lysis and column purification with a Qiagen plasmid Maxi kit (Qiagen, Valencia, CA). The concentration of pDNA solution was determined by measuring the absorbance at 260 nm and its optical density at 260–280 nm was in the range of 1.8–1.9. Cell viability was estimated by using MTT.

Synthesis of pABOL

The synthesis of pABOL (Fig. 1) is described in Lin et al. (2006). Briefly, pABOL was synthesized by Michael addition of 4-amino-1-butanol (ABOL, Aldrich) to N,N′-cystamine bisacrylamide (CBA). To do so, CBA and ABOL were dissolved in 1.1 ml methanol/water (4:1, v/v). Polymerization was carried out in the dark at 45°C under N_2. The resulting product was purified by ultrafiltration and collected as solid powder after freeze-drying. The synthesized polymer was characterized by ^{1}H-NMR spectroscopy.
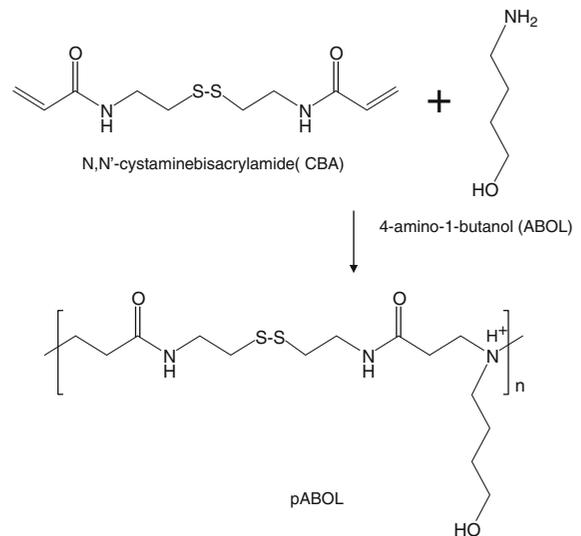
Fig. 1 Synthetic scheme of pABOL reducible gene carrier

Construction of pSUR-sFlt-1 plasmid DNA

The survivin promoter sequence was obtained from genomic DNA of 4T1 cell line. The forward primer, 5′-cgc **aga tct** aga tgg gcg tgg ggc ggg ac-3′ included a *Bgl*II cut site (in bold) and *Hin*dIII site within the reverse primer (in bold) 5′-ccc **aag ctt** cct ccg cca aga cga ctc aaa c-3′. The 270 bp product corresponding to −264 to −1 of the murine survivin promoter was digested and inserted into the pCI plasmid (Promega Corp, Carlsbad, CA) to make the pSUR plasmid vector. Human soluble Flt-1 was amplified from

human placenta cDNA (Spring Bioscience) using primers modified to allow ligation into the pSUR plasmid at the XhoI and SalI restriction sites (in bold): forward 5′-cgt **gaa ttc** gct cac cat ggt cta-3′ and reverse 5′-gct **gtc gac** ctg cta tca tct ccg aac tc-3′. The PCR reaction was run at 94°C for 5 min, 10 cycles at 94°C for 15 s, 55°C for 60 s and 72°C for 180 s followed by 25 cycles at 94°C for 15 s, 60°C for 60 s, 72°C for 180 s and an extension for 10 min at 72°C. The length of the expected product was 2,214 bp and was confirmed by running on a 1% agarose gel. The map of pDNA is shown in Fig. 2.

Agarose gel retardation assay

Polymer/pDNA complexes were prepared at various weight (w/w) ratios ranging from 0 to 60 in PBS buffer and incubated for 30 min at room temperature. The polyplexes were then electrophoresed through a 1% (w/v) agarose gel containing ethidium bromide (0.5 µg/ml) in 0.5 X TAE (Tris/acetate/EDTA) buffer at 100 V for 20 min. Naked pDNA was used as a control. The gel was then analyzed on a UV illuminator to observe the position of the complexed pDNA relative to that of naked pDNA.

Fig. 2 Map of pCMV-sFlt-1 (**a**) and survivin-inducible pDNA (pSUR-sFlt-1) (**b**)
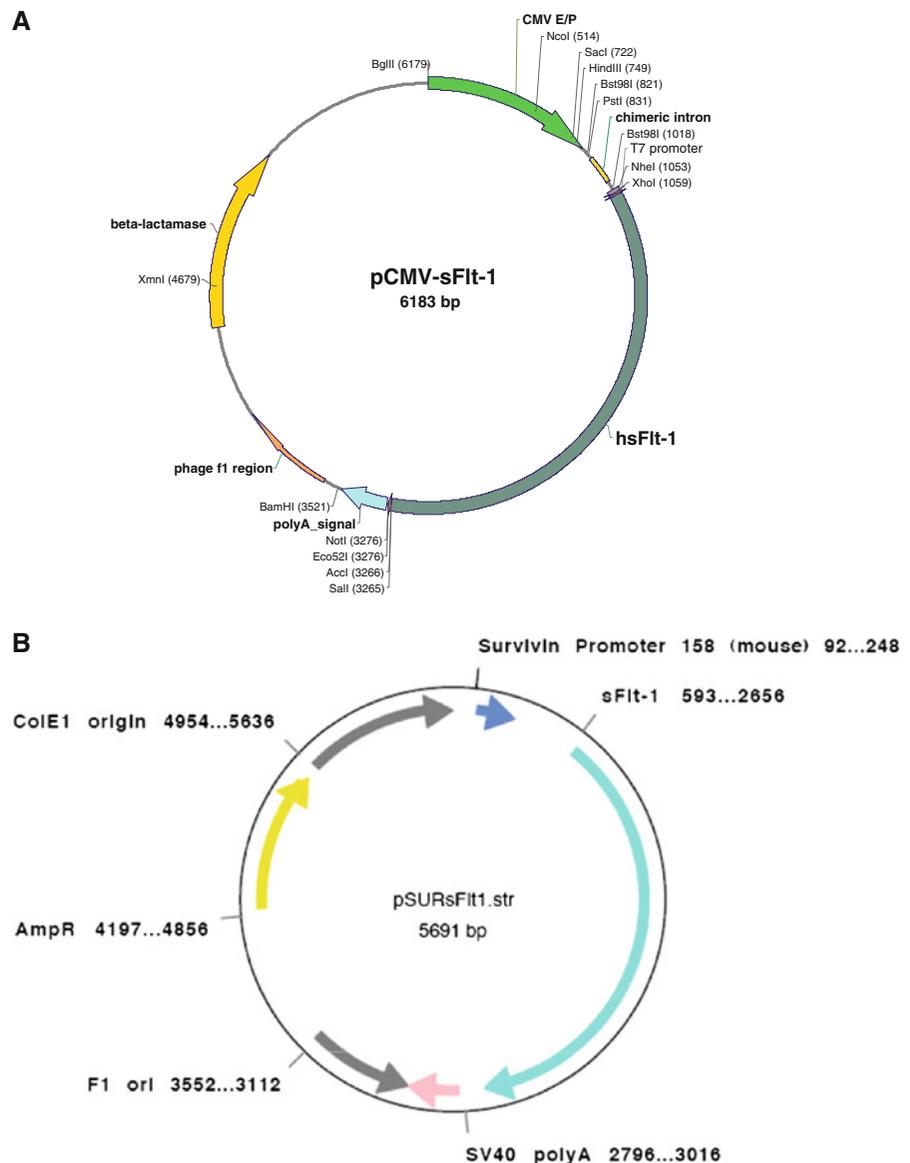
Cell culture

Mouse renal carcinoma (RENCA), mouse mammary carcinoma (4T1), and mouse mammary carcinoma (EMT-6) cell lines were cultured in RPMI medium and human embryonic kidney (293T) cells were cultured in DMEM medium, both containing 10% (v/v) FBS and 1% (v/v) antibiotics. Every other day the medium was replaced until cells became 80% confluent and then subcultured using 0.25% trypsin/ EDTA.

Cell viability assay

The cytotoxicity of polymer/pDNA complexes was investigated using a tetrazolium-based calorimetric assay as previously reported. In this test, viability of cells is assessed by the metabolism of a water-soluble tetrazolium dye, MTT, into insoluble formazan salts (Sigma). Briefly, 293T cells were plated in 12-well plates at 1.5×10^5 cells per well and incubated for 24 h. Polymer/pDNA complexes were added in triplicate to the wells at 1 μg reporter pDNA per well at varying w/w ratios. After 44 h incubation, the cell viability was determined and compared to untreated cells.

Luciferase reporter gene assay

Cells were plated in 12-well plates at 1.5×10^5 cells/ well. Polymer/pDNA complexes were prepared in PBS buffer solution with 1 μg total pDNA/ml. Cells were cultured in 1 ml media containing 10% (v/v) serum until ∼80% confluency was reached, at which time cells were washed with PBS. Media without serum was then added, followed by addition of polymer/pDNA complexes, and the cells were allowed to incubate for 4 h. Media was replaced with fresh media containing serum, and cells were allowed to incubate further for a total of 24 h. Cells were washed with PBS and treated with cell culture lysis buffer (Promega). Luciferase quantification was performed using a luciferase assay system (Promega) on a luminometer from Dynex Technologies, Inc. (Chantilly, VA). The amount of protein in the cell lysate was determined using a standard curve of bovine serum albumin (Sigma) and a BCA protein assay kit (Pierce).

ELISA

In vitro expression level of sFlt-1 by pABOL was carried out on non-cancerous 293T human kidney cells in addition to RENCA cells using pCMV-sFlt-1, and pSUR-sFlt-1 pDNA. Polyplexes (pABOL/pDNA) were prepared using polymer/pDNA weight ratios of 60 in a PBS buffer solution. Other experimental conditions were the same as described above. The level of sFlt-1 after transfection of pCMV-sFlt-1 was determined by using mouse VEGF-R1 ELISA kit (R&D Systems Inc., Minneapolis, MN) according to the recommendation of the manufacturer's protocol. Briefly, the 96-microwell plate, coated with mouse monoclonal anti-sFlt-1 (termed as sVEGF-R1) antibody was washed with wash buffer. Cell supernatant samples were diluted 1:2 by calibrator diluent, and 100 μl of the sample was added into the designated wells. Similarly, sFlt-1 standards ranging from 0.03 to 2 ng/ml, also diluted in calibrator diluent, were added to the microwell plates. The calibrator diluent alone was added in blank well and incubated at room temperature for 2 h. After washing four times with wash buffer, 200 μl of polyclonal antibody against sVEGF R1 conjugated to horseradish peroxidase, were added and incubated at room temperature for 2 h. After washing the wells again four times, tetramethylbenzidine substrate solution was added and incubated at room temperature for 30 min. The enzymatic reaction was stopped by adding 50 μl stop solution to the wells, and absorbance was determined by spectrophotometric reading at 450 nm. The sFlt-1 concentration in the cell supernatant samples was calculated based on the standard curve.

Statistical analysis

Results were reported as the mean ± SEM. The statistical analysis between two groups was determined using a nonpaired t test.
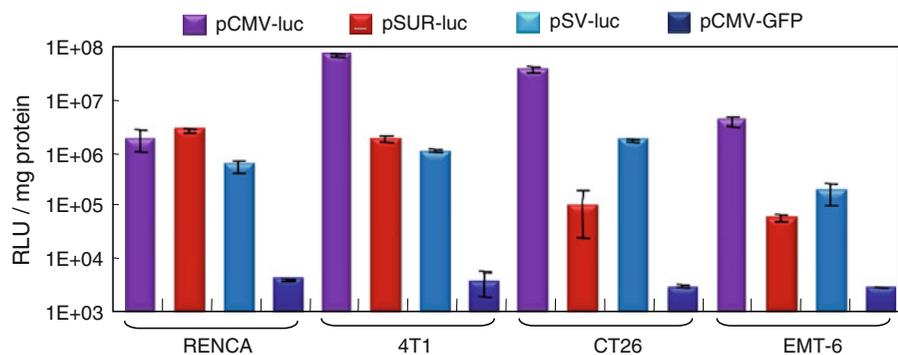
Results and discussion

Cell line dependency of the survivin promoter-containing gene

To determine the cell line dependency of the survivin promoter, we transfected survivin-inducible pDNA
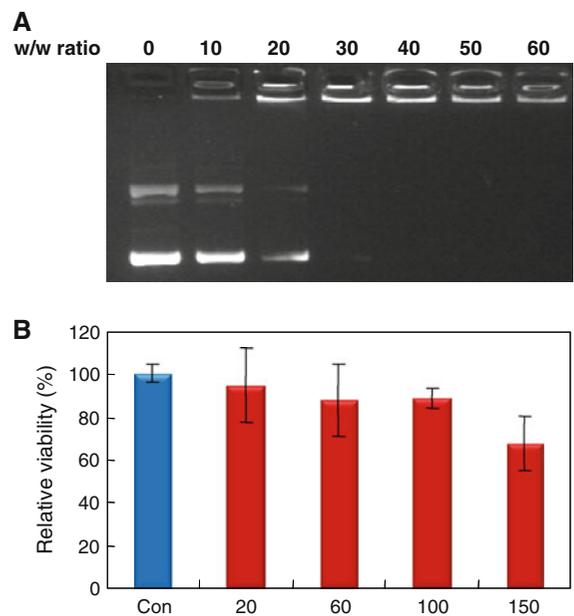
into several cancer cell lines using a prototype polymer, BPEI (25 kDa), as the delivery vehicle. In vitro dependency of the transfection efficiency on cell lines was carried out on RENCA, 4T1, CT26, and EMT-6 cell lines using pCMV-Luc (cytomegalovirus promoter), pSUR-Luc (survivin promoter), pSV-Luc (simian virus 40 promoter), and pCMV-GFP reporter pDNA encoding green fluorescence protein. We previously found that the expression of survivin in cancer cell lines such as RENCA, 4T1, CT-26 and EMT-6, was higher than observed in non-cancerous 293T cell line (data not shown). However, there was no significant difference in quantitative survivin expression has been observed among these cancer cell lines. Additionally, higher amount of survivin expression in cultured cancer cell lines was also confirmed in other reports (Peng et al. 2008; Stauber et al. 2006). Survivin promoter activity was determined from luciferase activity from the cell lysate of transfected cells. pCMV-GFP expressing green fluorescence protein was used as a negative control. In case of all cancer cell lines except RENCA cell line, luciferase activity was found to be higher when transfected with luciferase pDNA containing the CMV promoter than that observed on employing SUR promoter (Fig. 3). Similar luciferase activity was observed in RENCA cells when transfected with pCMV-luc and pSUR-luc pDNA. In CT-26 and EMT-6 cell lines, the luciferase activity transfected with pSV-luc was higher than that exhibited with pSUR-luc, though it was lower than pSUR-luc in RENCA and 4T1 cell lines. These results suggest that the activity of survivin-inducible pDNA varies according to cell lines but can be used for improved expression in RENCA and 4T1 cell lines, which is consistent with other literature previously reported (Bao et al. 2002).

Polyplex formation of pABOL with pDNA and cell toxicity

To identify the formation of pABOL/pDNA polyplexes, we performed agarose gel electrophoresis at different w/w ratios. The positively charged pABOL reducible polymer forms polyelectrolyte complexes with the negatively charged phosphate ions on the backbone of pDNA in the absence of reducing agent such as 1,4-dithiothreitol. As the value of the w/w ratio of pABOL/pDNA increased to 30, no free pDNA was detected on the agarose gel indicating polyplex formation at neutral to positive charge (Fig. 4a). When polyplexes were incubated with 2.5 mM DTT,

Fig. 4 Polyplex formation profile (**a**) and cellular viability (**b**) of pABOL with pDNA

Fig. 3 Luciferase activity driven by CMV, survivin, and SV40 promoters in cell lines as indicated

pABOL was unable to condense pDNA and the results were in conformity with observation found in case of the free pDNA control as reported previously (data not shown). Dynamic light scattering and ζ-potential measurements showed that pABOL condenses pDNA efficiently, forming polyplexes with small particle sizes (<200 nm) and possessing a positive surface charge (>+20) (data not shown).

In order to optimize the w/w ratio of pABOL to pDNA for improved transfection and still maintain low carrier-associated toxicity, we have evaluated pABOL/pDNA polyplex effects on cellular viability in 293T cells over a range of w/w ratio using a MTT assay against cells not receiving any formulated pDNA treatment on 293T cells. Results show that the cell viability was decreased slightly to 90% at w/w ratio 60 when compared to untreated control (Fig. 4b). It was also observed that even at w/w ratio 100 only 10% cytotoxicity was imposed. Therefore we have performed all the experiments in this study at w/w ratio 60, as there was no significant toxicity.

In vitro transfection activity of polymers

In vitro characterization of pABOL, linear polyethylenimine (LPEI), and BPEI was carried out on non-cancerous 293T human kidney cells in addition to RENCA cells using pCMV-Luc reporter pDNA. Polyplexes (pABOL/pCMV-Luc) were prepared using polymer/pDNA ratios (w/w) ranging from 20:1 to 100:1 in a PBS buffer solution. LPEI/pDNA and BPEI/pDNA polyplexes were prepared at N/P ratios of 5:1 and 10:1, which are optimal ratios for LPEI and BPEI. Other experimental conditions are the same as described above. Total protein and luciferase activity in cell lysates at 24 h post-transfection were determined. As shown in Fig. 4, the transfection efficiency of pABOL was much greater than that of LPEI, though comparable to that of BPEI in both normal cells (Fig. 5a) and cancer cells (Fig. 5b). This result suggests that pABOL itself does not have tumor cell specificity. Moreover, pABOL/pDNA polyplexes showed very little cytotoxicity as shown in a previous report (Lin et al. 2006).

Cancer cell-inducible expression
of survivin-containing therapeutic gene

We then asserted that the survivin promoter exhibits cancer specificity gene expression in the RENCA cell
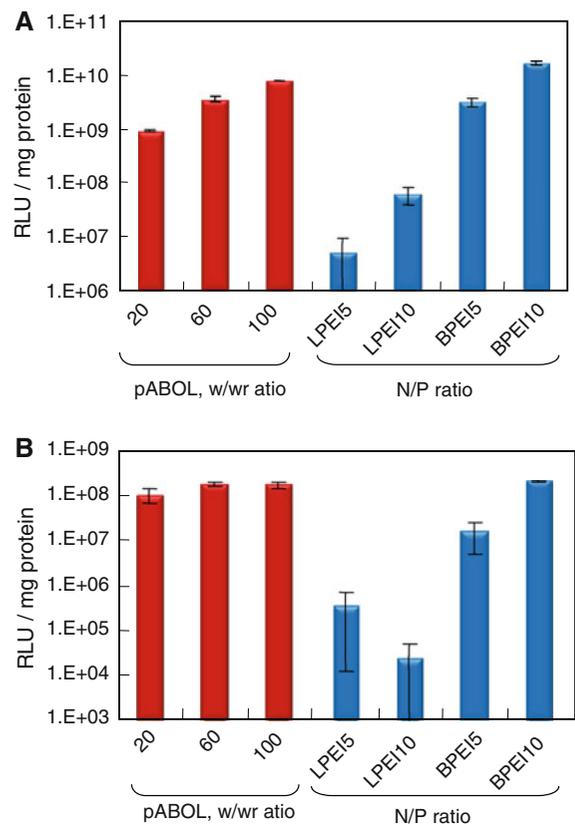
Fig. 5 Transfection activity of pABOL, LPEI, and BPEI in 293 T cells (a) and RENCA cells (b). Cells (1.5 × 10⁵) were seeded in a 12-well plate 24 h before the addition of polymer/pDNA polyplexes (1 μg pCMV-Luc and various amount of polymers)

line. Polyplexes were prepared using pABOL and pCMV-Luc, pSUR-Luc, pCMV-sFlt-1 and pSUR-sFlt-1 pDNAs at a w/w ratio of 60:1, which is optimal for transfection. Other experimental conditions were the same as described above. Promoter activity was determined from the luciferase activity in cell lysates from transfected cells. Relative luciferase activity in RENCA cancer cells was standardized by 293T cells. Survivin-inducible pSUR-Luc pDNA showed 11-fold higher transfection efficiency than pCMV-Luc pDNA did (Fig. 6a). Expression of sFlt-1, a potent and selective antagonist of VEGF was reported to inhibit VEGF-mediated angiogenesis by binding to VEGF (Kim et al. 2005, 2006). In order to analyze the expression of sFlt-1, ELISA was performed as reported previously (Kim et al. 2005). The production of sFlt-1 in media for survivin promoter was much higher than that of CMV promoter ($P < 0.01$),
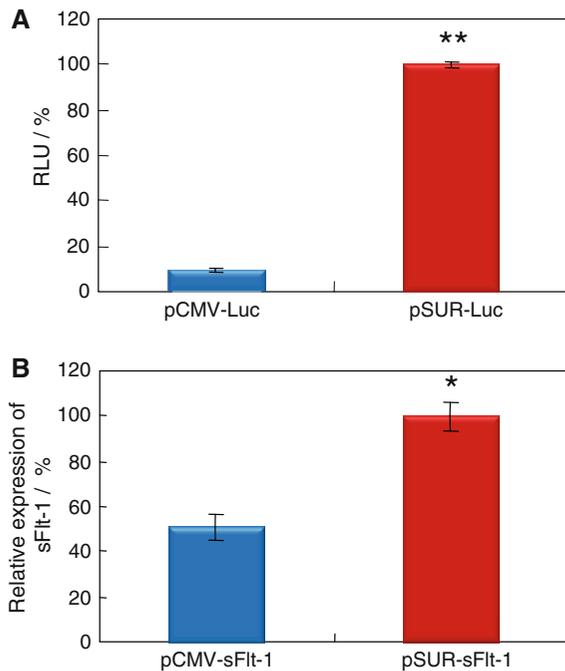
Fig. 6 Specificity of survivin-inducible pDNA in cancer cells. **a** Luciferase activity of pABOL/pDNA polyplexes. **b** Secreted sFlt-1 in media after transfection of pABOL/pDNA polyplexes. Activities (%) in RENCA cells were standardized by those of 293T cells. * $P < 0.01$ and ** $P < 0.001$ compared to CMV promoter

suggesting that survivin expression in cancer cells boosts the expression of the therapeutic gene, sFlt-1 (Fig. 6b).

The common practice in gene therapy using viral and non-viral vector utilizes nonspecific promoters such as CMV and SV40 which can be expressed in tumor as well as in non-tumor cell with high efficiency. However these promoters also induce high toxicity. To circumvent this drawback and to achieve higher specificity towards the tumor cells against the normal cells, pDNA having a tumor-specific promoter has been utilized for transcriptional tumor targeting. There are several reports that demonstrated the employment of tumor-inducible promoters, specific to cancer cell lines. For example prostate-specific antigen (PSA) promoter for prostate cancer, a-fetoprotein (AFP) promoter for hepatocarcinoma and several other promoters such as hTERT promoter, E2F1 promoter, and VEGF promoter (Zhu et al. 2006; Huang et al. 2006; Jakubczak et al. 2003; Kang et al. 2008). Unfortunately, most of these promoters cannot be used universally to treat tumors

of various origins and they display relatively weak transcriptional activity.

Recently, a novel tumor-inducible promoter, the survivin promoter, that displays a tumor-specific activity in a wide range of tumors, has been reported (Li and Altieri 1999; Altieri 2003; Johnson and Howerth 2004). Several research groups showed that the survivin promoter were capable of preventing the toxic effects of the therapeutic gene both in vitro and in vivo preserving the antitumor activity of the gene (Wu et al. 2005; Chen et al. 2004). Therefore, survivin promoter could be a potential candidate for targeted cancer gene therapy. However, to attain high activity of tumor-specific promoter gene function, an efficient gene delivery vector is needed. We previously reported bioreducible poly(amido amine)s (SS-PAAs) that combines efficient gene delivery with low cytotoxicity. The presence of the disulfide linkages and their intracellular reduction is thought to be responsible for triggering efficient gene release resulting improved transfection capabilities and reduced cell toxicity compared to non-degradable vectors. In this study, we combined the tumor-specific gene expression system and a reducible polymeric gene vector exhibiting a strong transfection profile for enhanced cancer-specific therapeutic gene expression in RENCA cells. The gene having CMV promoter lacks cancer cell specificity as it shows high gene expression both in normal cells as well as in cancer cells. However, survivin promoter-containing gene has higher cancer cell specificity because it promotes relatively higher gene expression than CMV promoter in cancer cells as shown in Fig. 5. This combination of tumor cell specific gene expression and improved polymeric gene carrier has potential use due to its higher efficacy and much desired cancer cell specific gene expression.

Conclusions

In conclusion, we constructed pDNA containing a survivin-inducible promoter capable of driving transgene expression in a cancer-specific manner for cancer gene therapy. Moreover, to deliver pDNA into cells with high efficacy, we synthesized and used a bioreducible gene carrier. This polymer formed stable nanoparticles with pDNA and mediated significant expression of therapeutic gene in cell culture with

low cytotoxicity. By using a survivin-inducible pDNA/pABOL polyplex system, greater expression of sFlt-1 was obtained in cancer cells when compared to its expression in a normal cell line. Finally, we believe that this combination of cancer specificity and reducible gene delivery system make it an excellent candidate for tumor therapy in vitro and in vivo.

References

Altieri DC (2003) Survivin and apoptosis control. Adv Cancer Res 88:31–52

Ambrosini G, Adida C, Altieri DC (1997) A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 3:917–921

Bao R, Connolly DC, Murphy M et al (2002) Activation of cancer-specific gene expression by the survivin promoter. J Natl Cancer Inst 94:522–528

Benns JM, Kim SW (2000) Tailoring new gene delivery designs for specific targets. J Drug Target 8:1–12

Chen JS, Liu JC, Shen L et al (2004) Cancer-specific activation of the survivin promoter and its potential use in gene therapy. Cancer Gene Ther 11:740–747

Chollet P, Favrot MC, Hurbin A et al (2002) Side-effects of a systemic injection of linear polyethylenimine-DNA complexes. J Gene Med 4:84–91

Christensen LV, Chang CW, Kim WJ et al (2006) Reducible poly(amido ethylenimine)s designed for triggered intracellular gene delivery. Bioconjug Chem 17:1233–1240

Dash PR, Read ML, Barrett LB et al (1999) Factors affecting blood clearance and in vivo distribution of polyelectrolyte complexes for gene delivery. Gene Ther 6:643–650

De Smedt SC, Demeester J, Hennink WE (2000) Cationic polymer based gene delivery systems. Pharm Res 17:113–126

Deveraux QL, Reed JC (1999) IAP family proteins-suppressors of apoptosis. Genes Dev 13:239–252

Eliyahu H, Barenholz Y, Domb AJ (2005) Polymers for DNA delivery. Molecule 10:34–64

Felgner PL, Tsai YJ, Sukhu L et al (1995) Improved cationic lipid formulations for in vivo gene therapy. Ann NY Acad Sci 772:126–139

Huang SL, Wu Y, Yu H et al (2006) Inhibition of Bcl-2 expression by a novel tumor-specific RNA interference system increases chemosensitivity to 5-fluorouracil in Hela cells. Acta Pharmacol Sin 27:242–248

Jakubczak JL, Ryan P, Gorziglia M et al (2003) An oncolytic adenovirus selective for retinoblastoma tumor suppressor protein pathway-defective tumors: dependence on E1A, the E2F-1 promoter, and viral replication for selectivity and efficacy. Cancer Res 63:1490–1499

Johnson ME, Howerth EW (2004) Survivin: a bifunctional inhibitor of apoptosis protein. Vet Pathol 41:599–607

Kakizawa Y, Harada A, Kataoka K (1999) Environment-sensitive stabilization of core-shell structured polyion complex micelle by reversible cross-linking of the core through disulfide bond. J Am Chem Soc 121:11247–11248

Kang YA, Shin HC, Yoo JY et al (2008) Novel cancer anti-angiotherapy using the VEGF promoter-targeted artificial zinc-finger protein and oncolytic adenovirus. Mol Ther 16:1033–1040

Kim WJ, Yockman JW, Lee M et al (2005) Soluble Flt-1 gene delivery using PEI-g-PEG-RGD conjugate for anti-angiogenesis. J Control Release 106:224–234

Kim WJ, Yockman JW, Jeong JH et al (2006) Anti-angiogenic inhibition of tumor growth by systemic delivery of PEI-g-PEG-RGD/pCMV-sFlt-1 complexes in tumor-bearing mice. J Control Release 114:381–388

LaCasse EC, Baird S, Korneluk RG et al (1998) The inhibitors of apoptosis (IAPs) and their emerging role in cancer. Oncogene 17:3247–3259

Li F, Altieri DC (1999) Transcriptional analysis of human survivin gene expression. Biochem J 344:305–311

Lin C, Zhong Z, Lok MC et al (2006) Linear poly(amido amine)s with secondary and tertiary amino groups and variable amounts of disulfide linkages: synthesis and in vitro gene transfer properties. J Control Release 116:130–137

Lin C, Zhong Z, Lok MC et al (2007a) Novel bioreducible poly(amido amine)s for highly efficient gene delivery. Bioconjug Chem 18:138–145

Lin C, Zhong Z, Lok MC et al (2007b) Random and block copolymers of bioreducible poly(amido amine)s with high- and low-basicity amino groups: study of DNA condensation and buffer capacity on gene transfection. J Control Release 123:67–75

Lin C, Blaauboer CJ, Timoneda MM et al (2008) Bioreducible poly(amido amine)s with oligoamine side chains: synthesis, characterization, and structural effects on gene delivery. J Control Release 126:166–174

Lungwitz U, Breunig M, Blunk T et al (2005) Polyethylenimine-based non-viral gene delivery systems. Eur J Pharm Biopharm 60:247–266

Miller AD (1998) Cationic liposomes for gene therapy. Angew Chem Int Ed Engl 37:1769–1785

Oupicky D, Diwadkar V (2003) Stimuli-responsive gene delivery vectors. Curr Opin Mol Ther 5:345–350

Peng XC, Yang L, Yang LP et al (2008) Efficient inhibition of murine breast cancer growth and metastasis by gene transferred mouse survivin Thr34 → Ala mutant. J Exp Clin Cancer Res 27:46

Saito G, Swanson JA, Lee KD (2003) Drug delivery strategy utilizing conjugation via reversible disulfide linkages: role and site of cellular reducing activities. Adv Drug Deliv Rev 55:199–215

Salvesen GS, Duckett CS (2002) IAP proteins: blocking the road to death's door. Nat Rev Mol Cell Biol 3:401–410

Schaffer DV, Fidelman NA, Dan N et al (2000) Vector unpacking as a potential barrier for receptor-mediated polyplex gene delivery. Biotechnol Bioeng 67:598–606

Shi F, Hoekstra D (2004) Effective intracellular delivery of oligonucleotides in order to make sense of antisense. J Control Release 97:189–209

Stauber RH, Rabenhorst U, Rekik A et al (2006) Nucleocytoplasmic shuttling and the biological activity of mouse survivin are regulated by an active nuclear export signal. Traffic 7:1461–1472

Wu B, Wang Y, Ren JH et al (2005) Molecular cloning of survivin gene promoter and detecting its specific activity in Hela cell. Chin J Cancer Biother 12:116–119

Zhu ZB, Makhija SK, Lu Be et al (2006) Targeting mesothelioma using an infectivity enhanced survivin-conditionally replicative adenoviruses. J Thorac Oncol 1:701–711