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Development of a Nanotechnology Platform for Prostate Cancer Gene Therapy

Arash Hatefi, Ph.D.

The objective of this research is to design and develop a nanocarrier that is able to evade the immune system, circulate in the bloodstream, find its target prostate cancer cells, and transfer therapeutic genes into prostate cancer cells efficiently. The gene carrier is composed of: a) histone H2A peptide (H2A) to condense pDNA into nano-size particles (nanocarriers), b) a PC-3 specific targeting motif (TM) to target prostate cancer cells, c) an endosome disrupting motif (EDM) to disrupt endosome membrane and facilitate escape of the cargo into the cytosol, and d) a nuclear localization signal (NLS) to actively translocate pDNA towards the nucleus of cancer cells. The gene encoding the gene delivery system was synthesized and cloned into a pET21b vector. The vector was genetically engineered and complexed with plasmid DNA (pDNA) to form stable nanoparticles with sizes below 100nm. The nanoparticles were used to deliver reporter genes (pEGFP) to target PC-3 prostate cancer cells. The results demonstrated that the gene delivery system is able to target and efficiently transfact PC-3 cancer cells with minimum cross-reactivity with normal epithelial prostate cells. Furthermore, the gene delivery system by itself did not show any detectable toxicity in the range tested (110ug/ml). An animal protocol has been prepared and approved by IACUC and DOD ACURO.
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**Introduction**

A major obstacle to improving patients’ survival with advanced prostate cancer is progression of the cancer to androgen-independence. Therefore, methods such as gene therapy capable of delay or stop this progression may have a significant impact on improving patients’ health. However, many challenges lie ahead for gene therapy, including improving DNA transfer efficiency to cancer cells, enhancing levels of gene expression, and overcoming immune responses. **The overall objective** of this research is to design and develop a nanocarrier that is able to evade the immune system, circulate in the blood stream, find its target prostate cancer cells, and transfer therapeutic genes into prostate cancer cells efficiently. The gene carrier is composed of: a) histone H2A peptide (H2A) to condense plasmid DNA (pDNA) into nano-size particles (nanocarriers), b) a PC-3 specific targeting motif (TM) to target prostate cancer cells, c) an endosome disrupting motif (EDM) to disrupt endosome membranes and facilitate escape of the cargo into the cytosol, and d) a nuclear localization signal (NLS) to actively translocate pDNA towards the nucleus of cancer cells. An elastin like polymer (ELP) has also been engineered in the vector structure to provide a hydrophilic shield and protect the vector/pDNA complex in the blood stream from the immune system. For simplicity, the vector will be shown as EDM-H2A-NLS- TM or namely GHT2. PC-3 prostate cancer cells are selected as target because they are highly metastatic and characterized to be CAR¯/HER2¯. This means that they are not a good candidate for adenoviral gene therapy or Herceptin anti-HER2 immunotherapy. Therefore, development of a targeted delivery system for this type of prostate cancer cells could be highly beneficial.

CAR: Coxsackie Adenovirus Receptor

HER2: Human Epidermal Growth Factor Receptor 2

**Body**

This is the second year report; therefore, below we have highlighted the studies that have been performed in Year 2 of the DOD grant. We have met all the objectives of Year 1 and our annual report for year 1 has been approved by DOD reviewers. For year 2, months 1-12, Task 1 was proposed. All the deadlines as delineated in the Statement of Work are met in time and we are progressing as planned.

**Task 1- Complex the vector with pDNA (i.e, pEGFP) to form nanocarriers, evaluate and optimize the transfection efficiency in vitro (months 1-12).**

1.1. Vector/pDNA complexes will be formed and particles will be characterized under various physicochemical conditions including pH, temperature, and salt conditions. The nanoparticles will be stabilized and optimum conditions to form nanoparticles will be identified (months 1-5).
- The mean hydrodynamic particle size and charge of vector/pDNA complexes were determined using Dynamic Light Scattering (DLS) and Laser Doppler Velocimetry (LDV) respectively. All vectors were able to complex with pDNA and formed particles with sizes below 100nm. The particle size and charge analysis for GHT2 is shown in Fig. 1. This method has previously been published by our group [1]. The particles demonstrated stability under various pH, temperature (up to 37oC) and salt concentrations (up to 150mM NaCl). Because the nanoparticles demonstrated stability, they were used to transfect various cell lines.

![Fig. 1: Various amounts of vector in 5mM acetate buffer were added to 1 μg of pDNA (pEGFP) to form complexes at different N:P ratios (N-atoms in vector to P-atoms in pDNA) in a total volume of 100μl deionized water. After 30 minutes of incubation, the size and zeta potential of the complexes were measured and reported as mean ± SEM, (n=3). Each mean is the average of 15 measurements and n represents the number of separate batches prepared for the measurements.](image)

1.2. Cell transfection studies will be performed using PC-3 and RWPE-2 cells. Cells will be transfected with vector/pEGFP complexes at various N/P ratios in the presence and absence of chloroquine, bafilomycin, and Nocodazole and the transfection efficiency will be measured. The cell transfection process will be optimized to obtain highest transfection efficiency (months 5-12)

- The transfection efficiency was optimized and the ability of the GHT2 vector to target and transfect PC-3 prostate cancer cells but not RWPE-1 normal epithelial prostate cells is shown in Fig. 2. The details of the method of cell transfection have been previously published by our group [1]. The ability of the vector to escape from endosomes and traffic pDNA towards nucleus has also been shown [1]. Please note that we had originally proposed to use RWPE-2 normal prostate epithelial cells. However, through consultation with Dr. Minko (collaborator) who has extensive experience in cancer therapy we replaced it with RWPE-1. This cell line, i.e., RWPE-1 is a more appropriate cell line and a better representative of normal human prostate cells.
Using a WST-1 cell toxicity assay, the potential toxicity of the GHT2 vector to PC-3 cancer cells was evaluated. The details of this method have previously been published [1]. We did not observe any vector related toxicity in PC-3 cancer cells at any NP ratio tested (Fig. 3). At NP ratio of 18, 23.5µg of vector is used to complex with 1µg of pEGFP.

**Fig. 2:** Demonstration of PC-3 targeted gene delivery. Qualitative and quantitative representation of the PC-3 (CAR⁻/HER2⁻), RWPE-1 (normal cells) and SK-OV-3 cells (CAR⁺/HER2⁻) transfected with vector/pEGFP complexes at N:P ratio of 10 and lipofectamine/pEGFP. The percentage of transfected cells is measured by flowcytometer. This figure shows that the GHT vector can target and transfect prostate cancer cells while preserving normal cells. In contrast, commercially available vectors such as lipofectamine non-selectively transfect all cells which could result in unwanted toxicity to normal cells during the therapy.
1.3. An animal protocol will be prepared and submitted for approval by the IACUC (months 10-12).

- The animal protocol is prepared and approved by the IACUC (Protocol #: 10-070). The approval from DOD Animal Care and Use Review Office (ACURO) has also been obtained.

**Task 2- Inject the nanocarriers in mice bearing xenograft tumor model of prostate cancer and evaluate the transfection efficiency and therapeutic efficacy (months 12-23).**

2.1. To evaluate transfection efficiency, pCMV-luc or pEGFP will be complexed with vectors and used to transfect PC-3 tumors in nude mice. The transfection efficiency in tumors and other tissues will be evaluated using an in vivo imaging system. These studies will be performed using 40 nude mice (months 12-17)

- This task is in progress and has not completed yet.
**Key Research Accomplishments**

a) Developed stable nanoparticles with sizes less than 100nm. This size range makes them suitable for receptor-mediated endocytosis.

b) Formulated nanoparticles with ability to target PC-3 prostate cancer cells with high efficiency but with low binding to normal epithelial prostate cells.

c) The engineered nanoparticles showed no acute cytotoxicity.

**Reportable Outcome**

A) Manuscript: In preparation

B) Presentations:

1- The PI (A. Hatefi) received an invitation from the organizers of the Nanomedicine and Drug Delivery Symposium (NanoDDS’10) to give a talk about this research. This symposium was held on October 3-5, 2010 and the work was presented.

2- The PI (A. Hatefi) also submitted and abstract (Abstract#1847) and presented this work in DoD IMPaCT 2011 conference held in Orlando, Florida.

C) Training: A Research Scientist with experience in molecular biology techniques was hired. She has received training in vector development, vector characterization, mammalian cell culture and transfection, and targeted prostate cancer gene therapy. In addition, a PhD student was trained to perform the studies. The newly recruited PhD student will be conducting the proposed animal studies under Task 2.

D) Grant application: None

E) Patent application: None

**Conclusions**

Using genetic engineering techniques we have created a PC-3 specific gene delivery system that can potentially be used in the treatment of the patients that do not respond to adenoviral gene therapy or Herceptin immunotherapy. The *in vivo* studies in nude mice are designed and will be performed at Rutgers University.

**References**

Appendices


Appendix B: The published abstract for DOD IMPaCT 2011 conference.

Appendix C: Reference 1
Abstract#1847: DEVELOPMENT OF A NANOTECHNOLOGY PLATFORM FOR PROSTATE CANCER GENE THERAPY

Arash Hatefi¹ and Yuhua Wang²

¹²Rutgers, The State University of New Jersey, Piscataway, NJ
²Washington State University, Pullman, WA

Background and Objectives: The objective of this research was to develop a multifunctional vector that can condense therapeutic genes into nano-size carriers, target PC-3 prostate cancer cells specifically and transfect and kill them efficiently. To achieve the objective, we developed a vector composed of four repeating units of histone H2A, a fusogenic peptide, and a PC-3 specific targeting motif. The PC-3 cancer cell model was chosen as a target because it represents a subpopulation of metastatic cancer cells that do not overexpress human epidermal growth factor receptor 2 (HER2) making them non-responsive to treatment with anti-HER2 antibodies such as Herceptin. In addition, PC-3 cells don’t overexpress coxsackie adenovirus receptor which makes it a poor candidate for adenoviral gene therapy. Therefore, there is a need for the development a vector that can fill this gap and used for the treatment of such cancer cells.

Methodologies: The vector was designed, genetically engineered in Escherichia coli, and purified using nickel column chromatography. The vector was complexed with pEGFP (encodes GFP) and pTK (encodes thymidine kinase) to form nanosize particles. The size of the nanoparticles was measured using dynamic light scattering technique by Malvern Nano ZS90 instrument. Vector/pEGFP complexes were used to transfect PC-3 (prostate cancer), RWPE-1 (normal prostate), and SKOV-3 (ovarian cancer) cells. The transfection efficiency was determined using flow cytometry. The vector-related cytotoxicity was also determined by a WST-1 cell toxicity assay. To evaluate the therapeutic efficacy, the vector was complexed with pTK and used to transfect PC-3 cells. Concurrently, PC-3 cells received 25uM of ganciclovir (prodrug) for a period of 7 days. The killing efficiency of vector/pTK complexes was determined by WST-1 cell toxicity assay.

Results: The results demonstrated that the vector is able to condense plasmid DNA into particles with sizes less than 100 nm, target PC-3 cancer cells but not RWPE-1 or SKOV-3 cells, and mediate efficient gene expression. The cell toxicity assay data also showed that the vector by itself does not have any detectable toxicity at its maximum efficiency. The in vitro therapeutic efficacy studies revealed that the vector is able to kill approximately 70% of the PC-3 cells only in the presence of ganciclovir.

Conclusions: Recombinant DNA technology has allowed us to create a targeted multifunctional vector that can specifically kill PC-3 prostate cancer cells with minimal impact on normal prostate cells.

Impact Statement: The proposed nanosystem has a significant impact on prostate cancer gene therapy because it could fill the gap where immunotherapy and adenoviral gene therapy are not effective in treating patients with metastatic prostate cancer.
Keynote Speakers
Joseph DeSimone (University of North Carolina at Chapel Hill)
Teruo Okano (Tokyo Women’s Medical University)

Confirmed Speakers
Christine Allen (University of Toronto)
Mark Davis (California Institute of Technology)
Dennis Discher (University of Pennsylvania)
Iola Duarte (University of Aveiro)
Mohamed El-Sayed (University of Michigan)
Hamid Ghandehari (University of Utah)
Justin Hanes (The Johns Hopkins University)
Arash Hatefi (Washington State University)
W.E. Hennink (Utrecht University)
Leaf Huang (University of North Carolina at Chapel Hill)
Akihiro Kishimura (The University of Tokyo)
Philip S. Low (Purdue University)
Robert Luxenhofer (Dresden University of Technology)
Andrew Mackay (University of Southern California)
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Registration and abstract submission: www.nanodds.org
The field of nanomedicine has seen an exponential growth in the level of activity, investment, and development in recent years as evidenced by the creation of new companies, the launch of new technologies, and the growth of international collaborations. This growth has been mirrored in the number of conferences and academic meetings dedicated to the field, including the NanoDDS symposium.

The NanoDDS symposium, now entering its eighth year, began in Omaha, Nebraska in 2003 as a US-Japan mini-symposium organized by Dr. A. Kabanov and Dr. K. Kataoka. According to Dr. Ruth Duncan, it was the first scientific meeting of its kind. NanoDDS has since established itself as an internationally-recognized, medium-sized and focused symposium highlighting new discoveries and developments in the field of nanomedicine while also serving as a forum to discuss the issues of pre-clinical and clinical development of nanomedicines. The intimate format of NanoDDS sets it apart from all other meetings of its kind, fostering new, fruitful collaborations, and encouraging networking, discussion, creativity and innovation between trainees (graduate students and post-doctoral fellows) and some of the world’s leading academic scientists and industry specialists. These interdisciplinary interactions should aid in fostering new, fruitful collaborations, leap-step advances, and ground-breaking discoveries.

**SCIENTIFIC PROGRAM**

**Sunday October 3rd, 2010**

**First Keynote Presentation**
Molecular Design of Intelligent Surfaces for Drug and Cell Delivery - Teruo Okano, Tokyo Women’s Medical University, Japan

**Session 1: Nanomedicines in Cancer (Part One)**
- Image Guided Design of Liposome-Based Cancer Therapy - Christine Allen, Univ. of Toronto, Canada
- Ligand-Targeted Molecules for Imaging and Therapy of Cancer and Inflammatory Diseases - Philip S. Low, Purdue University

**Session Two: Nanomedicines in Cancer (Part Two)**
- Nanoparticle Delivery of siRNA for Cancer Therapy - Leaf Huang, University of North Carolina-Chapel Hill
- Architectural Influence of Nanocarriers on Tumor Distribution and Toxicity - Hamid Gandehari, University of Utah

**Session Three: Clinical Translation of Nanomedicines**
- Industrial Session and Roundtable - From Bench to Bedside: Tom Redelmeier of Northern Lipids Inc., David Owen of Starpharma, Derek O’Hagan of Cerulean Pharma Inc., Muthiah Manoharan of Alnylam Pharmaceuticals

**Monday October 4th, 2010**

**Second Keynote Presentation**
Engineering Better Medicines and Vaccines - Joseph DeSimone, University of North Carolina-Chapel Hill

**Session Four: Novel Nanoformulation Technologies**
- Facile Production of Nanoparticles for Difficult to Deliver Therapeutics: hydrophobic drugs, peptides, and siRNA - Robert Prud’homme, Princeton Univ.

**Wednesday October 5th, 2010**

**Session Six: Nanomedicine Research Reports**
- Following Dynamic Biological Processes through NMR Spectroscopy and Metabonomic Profiling - Iola Duarte, University of Aveiro, Portugal
- Novel polymeric hollow capsules “Picosomes” - Akihiro Kishimura, University of Tokyo, Japan
- Doubly-Amphiphilic Poly(2-oxazoline) with Unusual Microenvironment as High-Capacity Drug Delivery Systems - Robert Luxenhofer, Dresden University of Technology, Germany
- SPECT Imaging of in vivo siRNA Delivery - Olivia Merkel, Philips Univ.-Marburg, Germany
- Molecular and Quantitative Pharmacology of siRNA Oligonucleotides Delivered via Receptor-Mediated Endocytosis - Xin Ming, University of North Carolina at Chapel Hill

**Poster Sessions and Oral Presentations**
- Polymersomes to Filomicelles - thickness, shape, flexibility, charge - Dennis Discher, University of Pennsylvania
- Thermosensitive Polymeric Micelles for Tareted Delivery – H. Hennek, Utrecht Univ., Netherlands
- Strategies to Enhance Nanoparticle Transcytosis - Randall Mrskny, University of Bath, United Kingdom
- The Mucus Barrier to Viral and Non-Viral Gene Therapy - Justin Hanes, Johns Hopkins University

**GENERAL INFORMATION**

**Symposium Location**
The symposium will be held at the Hilton Omaha, Nebraska’s only 4 diamond hotel, located at 1001 Cass Street and within easy walking distance of the Old Market, Nebraska’s premiere arts and entertainment district. The Hilton Omaha is only a short drive from Omaha’s airport, as well as many other attractions including the Holland Performing Arts Center, Joslyn Museum, Durham Western Heritage Museum, and the Henry Doorly Zoo.

**Accommodations**
Accommodations are available at the Hilton Omaha at reduced conference rates. Alternate accommodations may also be found at several nearby hotels. Please visit the symposium website www.nanodds.org for additional information.

**Symposium Registration**
To register for the symposium, or for additional information, please visit the symposium website www.nanodds.org. Fees are $125 for graduate students and post-doctoral fellows, $400 for academics, and $1,200 for industry participants. These fees include admission to all three days of the symposium, an abstract book, name badge and conference packet, continental breakfasts, lunches, and refreshment breaks.

**Call for Posters**
The poster session will be the highlight of the symposium with time dedicated for poster viewing and discussion with the authors. A number of posters will be selected for oral presentations based on their scientific merit, innovation and clarity. Those interested in presenting their work during the poster session of the symposium should please visit www.nanodds.org for abstract submission guidelines and formats. Please email your abstract to nanodds@unmc.edu by Friday, August 27, 2010.

**CONTACT**
questions about NanoDDS’10 may be directed to: nanodds@unmc.edu

www.nanodds.org
A designer biomimetic vector with a chimeric architecture for targeted gene transfer

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1. Introduction

In recent years, scientific interest in nature, particularly in the field of life sciences, has grown tremendously. The science of biomimetics has the potential to advance many areas of technology including vector development for gene therapy research. Biomimetic vectors are designed to mimic the viral characteristics in order to overcome the cellular barriers. For administered therapeutic genes to successfully reach the nuclear target cells, a carrier (vector) should be designed to overcome cellular barriers. Accordingly, the vector should be able to: a) condense DNA from a large micro-meter scale to a smaller nano-meter scale suitable for endocytic uptake and protection from nuclelease degradation, b) be recognized by specific receptors on the target cells and internalize, c) promote the escape of the gene from the endosomal compartment into the cytosol, and d) assist the translocation of DNA from the cytosol to the nucleus [1]. While lipoplexes provide high transfection efficiency, their reproducibility and cytotoxicity remain a major concern [2]. On the other hand, cationic polyplexes are robust and relatively biocompatible but they suffer from poor gene-transfer efficiency [1]. Polymeric carriers for targeted gene transfer are generally synthesized using chemical synthetic methods. This results in the production of polymers with random sequences, distribution of molecular weights, and a limited ability to attach functional motifs at precise locations [3]. Consequently, the major limiting factor in polymeric gene delivery remains the establishment of a systematic correlation between polymer structure and gene transfection. In contrast to synthetic methods, genetic engineering techniques allow biosynthesis of limitless combinations of biomimetic motifs where there is full control over the vector architecture, the sequence and length at a molecular level. As a result, the relationship between vector structure and gene transfer efficiency can be recognized both at the molecular and nano-scale. A novel technique has previously been reported by Ghandehari's group to genetically engineer and express highly cationic biopolymers with tandem repeating units in E. coli expression system [4].

Development of this technology provided the basic tools for the design and development of more complex non-viral gene delivery systems, namely Designer Biomimetic Vectors (DBVs). Herein, we report a vector which is an ensemble of molecules of biological and synthetic origins for targeted gene transfer.

We hypothesize that a DBV with chimeric architecture can be developed to target model cancer cells, mimic viral characteristics to break through intracellular barriers, and mediate efficient gene transfer. The major challenge to this approach is to preserve the functionality of each motif in the vector structure because one domain could interfere with the other and render the vector ineffective. To test the hypothesis, a vector was genetically engineered to contain at precise locations: i) four tandem repeating units of N-terminal domain of histone H2A peptide (4HP), ii) a synthetic single chain HER2 targeting motif (TM), iii) a fusogenic peptide (FP) named GALA [5], and iv) a cathepsin D substrate (CS) (Fig. 1). The positioning of a cathepsin D cleavage site in between 4HP and TM allows for dissociation of the targeting motif from the vector inside the endosomes where cathepsin D is abundant [6].

2. Materials and methods

2.1. Cloning and expression of the targeted vector

The gene encoding 4HP-CS-TM was designed; expression optimized, and synthesized by Integrated DNA Technologies (San Diego, CA) with N-terminal Ndel and C-terminal HindIII restriction sites. The
synthesized gene was double digested with NdeI and HindIII (New England Biolabs, Ipswich, MA) restriction enzymes and cloned into a pET28b expression vector to make pET28b:4HP-CS-TM. The parent pET28b vector was purchased from EMD Biosciences (Gibbstown, NJ). The successful cloning of the 4HP-CS-TM gene in pET28b vector was verified by DNA sequencing. Subsequently, the fusogenic peptide gene (GALA) was synthesized by Integrated DNA Technologies with N-terminal Ncol and C-terminal NdeI restriction sites and cloned into pET28b:4HP-CS-TM expression vector to make pET28b:FP-4HP-CS-TM. The expression vector was transformed into E. coli BL21(DE3) pLysS (Novagen, San Diego, CA), grown using Barnstead-Labline MAX Q 4000 shaking incubator, and expressed by the addition of IPTG to a final concentration of 0.4 mM at 30 °C. Cells were collected, lysed, and centrifuged for 40 min at 30,000 g (4 °C) to pellet the insoluble fraction. The soluble fraction was recovered and loaded onto a Ni-NTA column (Amersham Biosciences, Piscataway, NJ) for purification. The column was washed with 40 volumes of wash buffer containing 100 mM NaH2PO4 (pH = 8), 10 mM Tris, 6 M GdnHCl, and 20 mM imidazole and eluted with buffer containing 100 mM NaH2PO4, 10 mM Tris, 4 M GdnHCl, and 250 mM imidazole. The purity of the vector was confirmed by loading 10 µg of DBV onto SDS-PAGE. The 66 kDa bovine serum albumin (BSA) with ca. 96% purity (Sigma) was used as control. The expression of the DBV was confirmed by western blot analysis (1 µg loading) using mouse monoclonal anti-6XHis. The amino acid content of DBV was analyzed by Commonwealth Biotechnologies Inc. (Richmond, VA). The purified vector was stored at −20 °C after addition of 50% glycerol. The exact molecular weight of the purified FP-4HP-CS-TM (DBV) was determined by mass spectrometry at Washington University mass spectroscopy core facility. Using the same protocol, 4HP-CS-TM which lacks fusogenic peptide (DBV/GALA) was expressed and purified.

2.2. Preparation of DBV stock solution

Before use, DBV was precipitated out of the storage buffer (50 mM NaH2PO4, 10 mM Tris, 4 M GdnHCl, 250 mM imidazole, and 50% glycerol) by adding saturated ammonium sulfate solution (4.1 M) in small increments while incubating on ice. The salted out vector was collected in a microfuge tube by centrifugation at 5000 g and removal of supernatant. Subsequently, DBV was re-dissolved in 5 mM acetic acid buffer pH 3.5 to make ca. 2.0 mg/ml stock solution. This stock solution was used in ensuing studies.

2.3. Hemolysis assay

The pH activated membrane lysis of GALA was determined by hemolysis assay. Thoroughly washed sheep red blood cells were purchased from Innovative Research (Novi, MI) and constituted to 108 cells/1 ml and supplemented with different amounts of vectors. The assays were performed in phosphate buffered saline (PBS) pH 7.4 and 5.5. After mild shaking and incubation for 1 h at 37 °C, the absorbance of lysate was measured at 541 nm. One percent Triton X-100 was used as a positive control whereas PBS (pH 7.4 and 5.5) was used as a negative control. DBV without GALA (DBV/GALA) at pH 5.5 was used as vector control. The data is reported as mean ± s.d., (n = 3). The statistical significance was evaluated using t-tests (p < 0.05).

2.4. Recognition of cathepsin D substrate by cathepsin D enzyme

Cathepsin D was purchased from Calbiochem (Gibbstown, NJ) and diluted to 6 nM with 50 mM Glycine-HCl buffer and 0.01% Triton X-100 as per manufacturer’s protocol. Fifteen µg of vector was incubated with 2 units of Cathepsin D at 37 °C for 2, 4, and 18 h. The enzymatic reaction was stopped by adding SDS-PAGE sample loading buffer and boiling for 5 min at 95 °C. Samples were loaded on 15% Tris-glycine SDS-PAGE gels and visualized by coomassie staining.

2.5. Particle size, charge, and stability analysis

The mean hydrodynamic particle size and charge measurements for vector/pDNA complexes were performed using Dynamic Light Scattering and Laser Doppler Velocimetry respectively using Malvern Nano ZS90 instrument and DTS software (Malvern Instruments, UK). Various amounts of vector in 5 mM acetic acid buffer (pH 3.5) were added to 1 µg of pDNA (pEGFP) to form complexes at different N:P ratios (2 to 10) in a total volume of 100 µl deionized water. For example, to prepare N:P ratio of 10, 14 µg of vector was used to complex with 1 µg of pEGFP. After 15 min of incubation, the size and zeta potential of the complexes were measured and reported as mean ± SEM, (n = 3). Each mean is the average of 15 measurements and n represents the number of separate batches prepared for the measurements.

The stability of vector/pDNA nanoparticles (N:P 10) was studied at various pH conditions using a Malvern Nano ZS90 equipped with an automated titrator and the provided DTS software. For pH titrations, NaOH (0.1 and 0.5 M) and HCl (0.1 M) were used and the measurements were performed in triplicate.

2.6. Serum stability study

The neutralization of pDNA negative charges by DBV was examined using gel retardation assay in the absence of serum. One microgram pDNA (pEGFP, Clontech, CA, USA) was complexed with the DBV at N:P ratio of 10, incubated at room temperature for 15 min, and the mobility of pDNA was visualized on an agarose gel by ethidium bromide staining. To examine the effect of serum on the stability of particles, fetal bovine serum (Invitrogen, CA, USA) was added to the complexes at a final concentration of 10% (v/v) and incubated for 30 min at 37 °C. The complexes were then electrophoresed on a 1% agarose gel and pDNA mobility was visualized by ethidium bromide (Sigma, Milwaukee, WI) staining.

To evaluate the ability of the vector in protecting pDNA from endonucleases, complexes were formed in a microfuge tube and incubated with serum for 30 min. Subsequently, sodium lauryl sulfate was added (10%) to the tubes to decomplex pDNA from the vector. The released pDNA was electrophoresed on agarose gel and visualized by ethidium bromide staining.

2.7. Cell culture and transgene expression

SK-OV-3, MDA-MB-231, and NIH3T3 cells were seeded in 96-well plates at 4 × 103 cells/well and incubated overnight at 37 °C. Cells were transfected with vector/pEGFP complexes at various N:P ratios (equivalent of 1 µg pEGFP) in media supplemented with insulin, transferrin, selenium, ovalbumin, dexamethasone, and fibronectin. After 4 h, the media was removed and replaced with fresh media supplemented with 10% serum. When used, 100 µM chloroquine or 100 nM bafilomycin A1 (Sigma, Milwaukee, WI) was added to the media at the time of transfection. When applicable, 10 µM nucodazole (Sigma, Milwaukee, WI) was added 1 h prior to transfection to
overnight incubation at 37 °C humidity fresh McCoy media supplemented with 10% serum followed by pEGFP complexes for 2 h. The media was removed and replaced with McCoy media supplemented with 10% serum and incubated over-night test groups is expressed as percent of the control where the control is absorbance was measured at 440 nm. The measured absorbance for Science, Indianapolis, IN) was added, incubated for 4 h, and well received PBS only. The next day WST-1 reagent (Roche Applied serum. The expression of green fluorescent protein was measured by flow cytometry. The data are reported as mean ± s.d., n = 3. The statistical significance was determined using t-test (p < 0.05). 2.8. Inhibition assay SK-OV-3 cells were seeded in 96 well tissue culture plates at 4 x 10^4 cells/well in 200 µl McCoy media supplemented with 10% serum. After overnight incubation, cells were washed and incubated in serum free media for 4 h. A serial dilution of targeting peptide was prepared (0, 0.035, and 1.2 nM) and added to the cells followed by the addition of vector/pEGFP complexes (N:P 10). Cells were then incubated at 37 °C for 2 h followed by replacing the media supplemented with 10% serum. The expression of green fluorescent protein was measured by flow cytometry. The data are reported as mean ± s.d., n = 3. The statistical significance was determined using t-test (p < 0.05). 2.9. Cell viability assay SK-OV-3 cells were seeded in 96-well plates at 4 x 10^4 cells/well in McCoy media supplemented with 10% serum and incubated over-night. Cells were treated with serial dilutions of vector or vector/pEGFP complexes for 2 h. The media was removed and replaced with fresh McCoy media supplemented with 10% serum followed by overnight incubation at 37 °C humidified CO2 atmosphere. The control well received PBS only. The next day WST-1 reagent (Roche Applied Science, Indianapolis, IN) was added, incubated for 4 h, and absorbance was measured at 440 nm. The measured absorbance for test groups is expressed as percent of the control where the control is defined as %100 viable. The data are reported as mean ± s.d., n = 3. The statistical significance was evaluated using a t-test (p < 0.05). The morphology of the SK-OV-3, MDA-MB-231 and NIH3T3 cells before and after transfection was examined by an inverted light microscope.

3. Results 3.1. Cloning, expression and identification of DBV Using the cloning strategy shown in Fig. 2a, the DBV gene was cloned into pET28b and the fidelity of the sequence to the original design was confirmed by DNA sequencing. The DBV was expressed in E. coli and purified under stringent conditions with a 5 mg/l yield using affinity chromatography (Fig. 2b). The purity of the DBV in comparison to BSA (Fig. 2b, lane 2) is estimated to be above 96%. We have previously demonstrated that highly cationic vectors can be expressed in E. coli under similar conditions [4,7]. The exact molecular weight of the DBV was also determined by mass spectrometry (MALDI-TOF) to be 28,522 Da (Fig. 2c). The result of amino acid content analysis demonstrated that in general the theoretical and experimental values are in close agreement (supplementary Table 1).

Fig. 2. Cloning, expression, and characterization of the purified targeted vector. a) An overview of the cloning strategy used to clone GALA-4HP-CS-TM gene into pET28b expression vector. b) SDS-PAGE (left panel) of the purified DBV (GALA-4HP-CS-TM). PM stands for protein marker; Lane 1 is purified DBV and lane 2 is BSA with ca. 96% purity. Lane 3 is the expressed DBV identified by western blot analysis (right panel). c) The MALDI-TOF picture of the purified DBV. The molecular weight was determined to be 28,522 Da.

3.2. Hemolytic assay The ability of the DBV to lyse the membranes at low pH was examined by incubating the red blood cells with the vector at two different concentrations and pH conditions. In comparison to negative control group (buffer only), the results revealed that the vector was lytic only in acidic environment (pH 5.5). No significant cell lysis was observed at pH 7.4 or with the DBV/GALA at pH 5.5 (Fig. 3a). In addition, no significant difference in hemolytic activity of DBV between 1 and 10 µg concentrations was observed.

3.3. Recognition of cathepsin D substrate by cathepsin D enzyme The availability of the cathepsin D substrate to the protease was examined by incubating the DBV with cathepsin D enzyme. The molecular weight of the undigested DBV (FP-4HP-CS-TM) is 28.5 kDa (Fig. 3b, lane 2) whereas the molecular weights of the digested byproducts (i.e., FP-4HP and TM) are estimated to be approximately...
The results show that after 2 h of incubation the vector is partially digested and as the incubation time increased the concentration of byproducts increased. After 18 h of incubation, cathepsin D enzyme non-specifically digested the band at 21.5 kDa resulting in the byproducts with molecular weights less than 7 kDa.

### 3.4. Particle size, charge, and stability analysis

The size and charge of the nanoparticles was examined at different N:P (positively charged nitrogen to negatively charged phosphate) ratios. It was observed that nanoparticles with sizes below 100 nm can be obtained. For example, at N:P ratio of 10, the pDNA was condensed into nanoparticles with 89 ± 4.5 nm size and +22 ± 5 mV surface charge (Fig. 4a). The serum stability of the nanoparticles was visualized by agarose gel mobility assay. It was shown that the condensed pEGFP in these nanoparticles was fully neutralized and stable in the presence of 10% serum (Fig. 4b, lanes 2 and 3). Furthermore, while DBV was able to effectively protect pEGFP from degradation by the serum nucleases (Fig. 4b, lane 4), the uncondensed pEGFP was susceptible to degradation by the endonucleases (Fig. 4b, lane 5). The stability of the nanoparticles formed at N:P 10 was also studied in a broad pH range. These particles demonstrated stability in a pH range of 4 to 9, but started to lose integrity at pH 10 or higher (Fig. 4c).

### 3.5. Identification of optimum N:P ratio for cell transfection

SK-OV-3 cells were transfected with the vector/pEGFP complexes at various N:P ratios to identify the optimum ratio for transfection. While gene transfer at all N:P ratios were observed, the highest level of transfection efficiency was observed among N:P ratios of 8 to 12.
For example, at the N:P ratio of 10, 30±4% of cells were transfected. The total fluorescent intensity at the N:P ratio 10 is 607,000±68,200 light units which is a measure of total GFP expression. As a positive control we used lipofectamine to validate the transfection protocol. The results showed that 36±5% of cells were transfected when lipofectamine/pEGFP was used.

### 3.6. HER2 targeted gene transfer

An inhibition assay was performed to demonstrate internalization of nanoparticles via HER2. The results of this assay revealed that as the concentration of the targeting peptide (competitive inhibitor) increased, the levels of gene expression decreased (Fig. 6a). When 1.2 nM of the competitive inhibitor was used, the total green expression...
fluorescent protein expression reduced from 507,000 ± 73,000 to 118,000 ± 30,000 light units. This is approximately 80% inhibition in gene expression.

HER2 targeted and cell selective gene transfer was examined by using vector/pE GFP complexes at N:P 10 to transfect HER2 positive SK-OV-3 (ovarian cancer) and HER2 negative MDA-MB-231 (breast cancer) and NIH3T3 (fibroblasts) cells. The results showed that 30 ± 4% of the SK-OV-3 cells were transfected, whereas the percent transfected cells was extremely low for both MDA-MB-231 and NIH3T3 cell lines (<0.1%) (Fig. 6b). Lipofectamine was able to non-selectively transfect all cell lines with relatively high efficiency.

3.7. Overcoming the endosomal barrier and microtubule mediated gene transfer

To examine the effect of fusogenic peptide (GALA) on enhancing escape of cargo into cytosol, SK-OV-3 cells were transfected with DBV in the presence and absence of bafilomycin A1 (Baf) and chloroquine (Ch). In the absence and presence of bafilomycin A1 the percentage of transfected cells was significantly reduced from 30 ± 4 to 3.6 ± 3, respectively (Fig. 7a). However, no significant difference between the transfection efficiency was observed in the absence or presence of chloroquine (Fig. 7a). In addition, when cells were transfected with DBV without GALA (DBV/GALA), a significant reduction in percent transfected cells from 30 ± 4 to 16 ± 3 was observed (Fig. 7b). No significant difference in terms of percent transfected cells was observed when SK-OV-3 cells were transfected with DBV or DBV/GALA plus chloroquine (Fig. 7b). To examine microtubule mediated transport of the nanoparticles towards the nucleus, SK-OV-3 cells were transfected in the absence and presence of nocodazole (Noc). The results illustrated significant reduction in percent transfected cells from 30 ± 4 to 6 ± 5 and total green fluorescent expression from 607,015 ± 68,186 to 38,550 ± 5565 (Fig. 7a).

3.8. Cell viability

The results of the WST-1 cell viability assay in SK-OV-3 cells exhibited no significant cell toxicity at a concentration of up to 80 µg/ml (Fig. 7c). The effect of vector/pE GFP complexes on cell viability was also evaluated qualitatively by monitoring the cells morphology. No signs of stress were observed in SK-OV-3, NIH3T3 or MDA-MB-231 cells before and after transfection with nanoparticles.

4. Discussion

The main objective of this research was to demonstrate the ability to package multiple motifs from different origins and diverse functions into one vector while preserving the functionality of each. Several experiments were conducted to evaluate the functionality of each motif in the vector structure in terms of efficient pDNA condensation, cell targeting, pH dependent endosome membrane disruption, translocation towards nucleus via microtubules and mediating gene transfer.

The gene encoding DBV was cloned (Fig. 2a) and expressed in E. coli and the results demonstrate successful expression and purification of the DBV to high purity (Fig. 2b). The mass spectroscopy result determined that the exact molecular weight of the DBV is in close agreement with the expected theoretical value of 28,561 Da (Fig. 2c). This confirms the expression of the DBV with exact length and sequence in a biological system. The vector was further characterized physicochemically and biologically to assess the functionality of each motif in the vector structure.

At the N-terminus of the vector structure, a synthetic pH-responsive pore-forming peptide (GALA) is designed to assist in escape of the cargo into cytosol [8]. Therefore, the pH responsiveness of the targeted vector in terms of lysing red blood cells under acidic pH 5.5 and physiologic pH 7.4 was evaluated. This feature of GALA is extremely important as it minimizes the possibility of causing cell damage while circulating in the blood stream. The results exhibits that the vector did not have hemolytic activity at pH 7.4, but was able to significantly lyse the red blood cells at pH 5.5 (Fig. 3a). This demonstrates that positioning of GALA at the vector’s N-terminus preserves its amphiphatic fusogenic functionality.

It was also mentioned that the engineered cathespin D substrate in the vector structure is to facilitate dissociation of the targeting motif inside endosomes by cathespin D enzyme. The vector was incubated with cathespin D enzyme to evaluate the accessibility of the substrate to the enzyme. The results showed that the substrate was readily available to the protease (Fig. 3b, lane 2 and 3). This means that the dissociation of the targeting peptide from the vector can occur under in vitro conditions, but direct in vivo measurement of the kinetics of dissociation is not feasible at this point. Nevertheless, this observation suggests that the probability for the vector to shed the targeting motif inside endosomes exists. Because the targeting motif is designed to facilitate internalization of nanoparticles, its removal after receptor mediated endocytosis could result in better exposure of GALA on the nanoparticle surface facilitating interaction with the endosome membrane. In the next steps, we examined the ability of the DBV to condense pDNA into stable nanosize carriers, vector mediated gene transfer, and the effectiveness of TM in targeting cells over-expressing HER2.

While full length histone H1, H2, H3, and H4 have been used as gene transfer agents with limited success [9], we have utilized the N-terminal domain of histone H2A (residues 1–37) which is sufficient to condense plasmid DNA (pDNA) into particles suitable for cellular uptake [10]. However, one major problem associated with the use of short peptides is their limited capability to form stable nanosize particles with pDNA. It has been shown that peptides as short as 20 amino acids are able to condense pDNA into nanosize particles, but they fail to form stable nanoparticles under the complex physiological environment [11]. To overcome this hurdle, cationic peptides can be polymerized to enhance their affinity towards pDNA resulting in stable particles [7,12]. As a starting point, we designed four tandem repeating units of histone H2A in the vector structure and examined the ability of the vector to form stable nanocomplexes with pDNA. It was observed that at N:P ratios of 2 to 10, the pDNA was condensed into nanoparticles with less than 100 nm size with slight positive charge (Fig. 4a). Nanoparticles in this size range are shown to be suitable for receptor mediated endocytosis as they can fit into clathrin-coated vesicles [13].

The stability of the nanoparticles in the presence of serum and protection of pDNA from endonucleases were also investigated. It was observed that the pE GFP in these nanoparticles was fully neutralized, remained stable in the presence of 10% serum (Fig. 4b, lanes 2 and 3) and effectively protected pDNA from degradation by the serum nucleases (Fig. 4b, lane 4). These results suggest that the DBV effectively condensed pE GFP shielding it from serum endonucleases.

To observe the behavior and stability of nanoparticles under various pH conditions, the size of the nanoparticles was measured over titration of a broad pH range. This is an important measurement because these nanoparticles are expected to be exposed to not only pH 7.4 of cell cytoplasm but the low pH of endosomes. While these particles demonstrated stability in the pH range of 4 to 9, they started to lose integrity at pH 10 or higher (Fig. 4c). This was expected as lysine residues in the vector structure neutralize at pH values close to their pKa (i.e., 10.8) resulting in unstable nanoparticles. Stability of nanoparticles at pH 4.0 is an indication that they may survive the low pH environment of late endosomes. The stability of nanoparticles at this low pH could provide the opportunity for the fusogenic peptide (GALA) to more effectively interact with the endosome membranes and disrupt their integrity.

So far, we have demonstrated that the vector containing four repeating units of histone H2A is able to condense pDNA into stable
nanoparticles in the presence of serum and broad pH range. The next logical step is to evaluate the ability of the vector to mediate gene transfer. SK-OV-3 cells were transfected with the vector/pEGFP complexes at various N:P ratios to identify the optimum ratio for transfection studies. While the highest levels of transfection efficiency was observed at N:P ratios of 8 to 12 (Fig. 5), we chose to use N:P 10 in subsequent studies. As a positive control we used lipofectamine to validate the transfection protocol. It is of paramount importance to appreciate that non-targeted commercially available transfection reagents such as lipofectamine are usually formulated to flocculate into large size particles, in this case 645 ± 37 nm, so that they can precipitate readily onto the cells surface for maximum transfection efficiency. Such gene transfer reagents may not be suitable for systemic gene delivery not only due to their large particle size but non-specific binding to normal cells. For targeted nanoparticles, size below 200 nm is crucial in order to mediate efficient gene transfer because such nanoparticles need to fit into clathrin-coated vesicles for efficient receptor mediated endocytosis [13]. Consequently, higher rate of gene transfer for lipofectamine makes it a better gene transfer reagent for in vitro cell transfection studies but less effective vector for systemic gene therapy.

It has been demonstrated that presence of a targeting motif on the surface of nanoparticles significantly enhances their internalization into target cells [14]. In this study, as a model targeting motif, a single chain 57 amino acid affibody with high affinity towards HER2 (picomolar) was designed in the vector structure [15]. An inhibition assay was performed to demonstrate the functionality of the affibody on the surface of the nanoparticles and their internalization via HER2-mediated endocytosis. This was evaluated by pre-treatment of SK-OV-3 cells with the targeting peptide to saturate the receptors followed by transfection of the cells with vector/pEGFP complexes. The results of this assay revealed that as the concentration of the targeting peptide (competitive inhibitor) increased, the levels of gene expression decreased (Fig. 6a). This signifies that the nanoparticles were utilizing HER2 to enter the cells.

In addition, to examine the ability of the vector to target HER2 positive cells with high specificity, three well characterized cell lines (SK-OV-3, MDA-MB-231, and NIH3T3) were selected as a model to test this hypothesis. Orlova et al. [15], have previously demonstrated that the HER2 targeted affibody is able to selectively target SK-OV-3 ovarian cancer cells which over-express HER2. As negative controls we chose the breast cancer cell line MDA-MB-231 which has very low levels of HER2 expression [16] and NIH3T3 fibroblasts which over-express FGF2 receptors but not HER2. A similar approach by others has been employed to test the targetability of immunoliposomes [17,18]. To test the hypothesis, we used the vector/pEGFP complexes at N:P 10 and transfected MDA-MB-231 breast cancer and NIH3T3 fibroblast cells. The high level of transfection efficiency in SK-OV-3 cells versus extremely low levels in MDA-MB-231 and NIH3T3 fibroblasts cells demonstrates that HER2 targeted motif in the vector structure facilitated internalization of particles in HER2 positive cells resulting in significant gene expression.

We then asked the question whether the presence of GALA in the vector backbone had any effect on endosomal escape and transgene expression. GALA is expected to effectively increase the delivery of pDNA into the cytosol via membrane destabilization of acidic endocytic vesicles containing vector/pDNA complexes. This was assessed by transfecting SK-OV-3 cells in the absence and presence of bafilomycin A1 and chloroquine. Chloroquine is a buffering agent known to disrupt the endosomal membrane by increasing the pH of the endosome environment and facilitating escape of the cargo into cytosol [19]. In contrast, bafilomycin A1 hampers the acidification of the endosome environment by inhibiting the vacuolar ATPase endosomal proton pump which significantly reduces the escape of the cargo into cytosol [20]. When SK-OV-3 cells were transfected in the presence of bafilomycin, the transfection efficiency was significantly reduced highlighting the fact that the acidic pH of the endosomes is necessary for the escape of the nanoparticles into cytosol (Fig. 7a). This could be another reason to believe that the GALA motif in the vector structure became functional at low pH of endosomes. In addition, observing no significant increase in transfection efficiency in the presence of chloroquine implies that most of the internalized nanoparticles could escape into cytoplasm without remaining trapped inside the endosomes (Fig. 7a).

Furthermore, when SK-OV-3 cells were transfected with DBV without GALA (DBV/GALA), a significant reduction (ca. 50%) in transfection efficiency was observed in comparison to DBV (Fig. 7b). We expected to observe ca. 90% reduction in transfection efficiency close to the level of DBV in the presence of bafilomycin (i.e. 3.6 ± 3). This interesting observation could be attributed to the presence of histag (6 consecutive histidines) in the vector structure which may have disrupted endosome membranes due to the proton sponge effect [7,21]. Assuming that one pEGFP molecule is packaged in one nanoparticle, at N:P ratio of 10 approximately 9400 histidine residues exist. This could be an indication that to efficiently disrupt endosomes both fusogenic activity and proton sponge effect could be utilized simultaneously.

We also transfected cells with DBV/GALA in the presence of chloroquine to evaluate whether the observed 50% reduction in transfection efficiency was due to entrapment of nanoparticles inside the endosomes. The results elucidated that a significant subpopulation of the nanoparticles did remain trapped inside endosomes and could not escape into cytoplasm to mediate gene transfer (Fig. 7b). These results in combination with the results obtained from the hemolysis assay suggest that GALA played a significant role in enhancing gene expression due to its pH-dependent fusogenic activity.

To date, many effective non-viral gene delivery systems have been developed with the ability to overcome the barriers of gene delivery to the cytoplasm. However, studies have shown that the cellular uptake of plasmid DNA does not correlate with efficient cell transfection [22]. Although there is limited understanding of the cellular and molecular mechanisms involved with synthetic vector mediated gene transfer, transfection efficiency appears to be essentially limited by inefficient trafficking of DNA to the site of gene transcription in the nucleus [23]. Thus, an active translocation of pDNA from cytosol to the nucleus could reduce cytosolic residency time and minimize the possibility of pDNA degradation by the cytoplasm endonucleases. To examine whether the nanoparticles in this study utilized microtubules to reach nucleus, SK-OV-3 cells were transfected in the presence and absence of nocodazole, a reagent known to depolymerize microtubule structure [24,25]. The results revealed significant reduction in transfection efficiency when microtubule network was disrupted (Fig. 7a). Therefore, it can be deduced that the nanoparticles may have exploited microtubules to reach the nucleus. Histone H2A is shown to bear an inherent nuclear import signal in its structure which utilizes microtubules to be actively transported to the nucleus [10]. Therefore, presence of this signal sequence could have contributed to microtubule mediated transfer of the nanoparticles to the perinuclear membrane. Although it would be unlikely for the nanoparticles in this study with an average 89 nm particle size to go through the 30 nm diameter nuclear pore complex, it is highly that they enter the nucleoplasm during the mitosis phase when the nuclear membrane dissolves.

So far, we have examined each motif in the vector structure and showed that by correct positioning in the vector backbone, the functionality of each motif can be preserved. This can be explained by the fact that during the pDNA condensation process, hundreds of vector molecules participate to condense one molecule of pDNA. For example, at N:P ratio of 10, approximately 1570 vector molecules each containing 60 positively charged residues are employed to condense one pEGFP moleculc (9462 negative charges). Therefore, it is probable to have a fraction of fully functional targeting motifs and FPs in the
vector/pDNA complex architecture. Thus, the nanocomplex is rendered ready to bind to the receptors, fuse with the endosome membranes, and utilize the microtubules for active translocation of genetic material to the cell nucleus.

To examine whether the vector/pEGFP complexes had any effect on the viability of the cells and in turn negatively affecting cell transfection efficiency, SK-OV-3 cells were incubated with the vector or vector in complex with pEGFP. The results exhibited no significant cell toxicity at a concentration of up to 80 µg/ml (Fig. 7c). This shows that the vector was not toxic and could not negatively impact transfection efficiency. In our transfection studies with MDA-MB-231 and NIH3T3 we did not observe any change in morphology or signs of stress which could be another indication that the vector is relatively non-toxic.

One last question that still remains unanswered is why all cells were not transfected since the vector seems to have overcome the major cellular barriers. Besides the dose of administered pEGFP, the answer could be due to the abundance of entry gates (i.e., HER2) on the surface of cancer cells which determines the number of particles that can be internalized. The relationship between the number of receptors on the cells surface and transfection efficiency has been demonstrated in adenoviruses which rely on coxsackie adenovirus receptor (CAR) to enter cells [26]. In addition, it is likely that not all SK-OV-3 cancer cells will over-express HER2 as cancer cell populations are usually heterogeneous. Nonetheless, other more complex explanations could be involved with this process which opens the door for more mechanistic studies to unravel the mysteries of efficient gene transfer. The results obtained from this study justify further evaluation of nanoparticles in terms of therapeutic efficacy, immunogenicity and toxicity in animal models.

5. Conclusion

We have successfully demonstrated that a multi-domain designer vector with complex chimeric architecture can retain individual functionality of components. This allows for the creation of efficient and targeted systems that can be fine-tuned for various gene delivery needs. Such systems can be modified, equipped with a variety of targeting motifs, and programmed to transfer genes to various cell types with applications in gene therapy for cancer, cardiovascular disease, cystic fibrosis and wound healing among others.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jintimp.2009.03.007.

References


