Genomic signature and toxicogenomics comparison of polycationic gene delivery nanosystems in human alveolar epithelial A549 cells

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Received 18 Nov 2008; Revised 31 July 2009; Accept 12 Aug 2009

ABSTRACT

Background and the purpose of the study: Of the gene delivery systems, non-viral polycationic gene delivery nanosystems have been alternatively exploited as a relatively safe delivery reagents compared to viral vectors. However, little is known about the genomic impacts of these delivery systems in target cells/tissues. In this study, the toxicogenomics and genotoxicity potential of some selected polycationic lipid/polymer based nanostructures (i.e., Oligofectamine® (OF), starburst polyamidoamine Polyfect® (PF) and diaminobutane (DAB) dendrimers) were investigated in human alveolar epithelial A549 cells.

Methods: To study the nature and the ontology of the gene expression changes in A549 cells upon treatment with polycationic nanostructures, MTT assay and microarray gene expression profiling methodology were employed. For microarray analysis, cyanine (Cy3/Cy5) labeled cDNA samples from treated and untreated cells were hybridized on target arrays housing 200 genes.

Results and major conclusions: The polycationic nanosystems induced significant gene expression changes belonging to different genomic ontologies such as cell defence and apoptosis pathways. These data suggest that polycationic nanosystems can elicit multiple gene expression changes in A549 cells upon their chemical structures and interactions with cellular/subcellular components. Such impacts may interfere with the main goals of the desired genemedicine.

Keywords: Gene Delivery Nanosystem, Gene Expression, Genocompatibility, Microarray, Toxicogenomics

INTRODUCTION

Gene therapies such as antisense oligonucleotides (ODNs), ribozymes, DNAzymes and short interfering RNA (siRNA) are emerging as promising futuristic nanoscaled medicines for treatment of genetic diseases in the post-genomic era (1). For any successful gene therapy, basically, genomedicines should be successfully delivered to target sites by appropriate gene delivery carriers, which should possess an appropriate physicochemical properties to cross the biological membranes/barriers with minimal cytotoxicity/genotoxicity on target cells/tissues (2). Due to immunogenicity potentials of the viral vectors (e.g., adenoviral vectors), as in ornithine transcarbamylase gene therapy (3), various nonviral vector systems have been developed (4, 5).

Of these gene delivery systems, the positively charged liposomes are able to condense the negatively charged DNA and form micro/nanostructures of "lipoplexes" which can enter cells via adsorptive endocytosis. These nanostructures release nucleic acids out of the endosomal/lysosomal compartments with the net effect of yielding high uptake and intracellular delivery of genes and oligonucleotides (6, 7), even though the mechanisms have not been fully understood yet. So far, the major challenges for gene therapy have been focused on increasing efficiency of transgene expression, while improving bioavailability and decreasing cellular toxicity appear to be a keystone to achieve maximum transfection efficiency (1).

In fact, the low toxicity and immunogenicity, lack of pathogenicity, and ease of production continue to make nonviral vectors an attractive alternative to viral vectors (8). In numerous studies on vector-cell interactions, however it has been reported that the nonviral vectors can bind and enter cells efficiently although they yield low gene expression. In fact, there exist some critical impediments, which may affect the assembly of the vector particle and its disassembly inside the target cells. These issues are important for achievement of the premeditated

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biological goals and it is believed that the interaction of nonviral vectors with cellular components may provoke undesired signalling paths. Using microarray methodology, previously it was reported that the human epithelial cells (i.e., A431 and A549 cells) treated with cationic lipid/polymer based gene delivery nanosystems can induce multiple intrinsic gene expression changes (9-13). Since the cytogenomic impacts of the cationic lipids and polymers may vary by differences in their structures. The aim of the current investigation, was to compare the pattern of changes in gene expression elicited by Oligofectamine®, Polyfect® and DAB dendrimers within the human alveolar epithelial A549 cells using microarray global gene expression proofing method.

MATERIAL AND METHODS

Materials

Human alveolar epithelial A549 cell line was from ECACC, (Salisbury, UK). Trireagent™, Ethidum Bromide, isopropanol, chloroform, formaldehyde and diaminobutane (DAB16) denrimer were from Sigma (Poole, UK). Cationic lipid Oligofectamine, Dulbecco's modified Eagle's medium (DMEM) containing 25 mM HEPES, Opti-MEM®I, fetal bovine serum (FBS), penicillin G, streptomycin, L-glutamine, dithiotheritol (DTT), SuperScript II reverse transcriptase, salmon sperm DNA and RNase/DNase free ddH_2O were purchased from Invitrogen, (Paisley, UK). The deoxynucleotide triphosphate monomers (dNTPs) and random hexamer primers (pdN6) and cyanine fluorescent dyes (Cy3 and Cy5) were from Amersham Life Science (Little Chalfont, UK). Slide arrays (housing 161 human genes, 32 replicas and 7 *Arabidopsis* controls) and hybridization buffer were from MWG Biotech Ltd (Milton Keynes, UK). Tissue culture treated multi-well plates and flasks were from Corning Costar (High Wycombe, UK). RNasin™ was from Promega (Southampton, UK). The QIAquick PCR purification kit was from QIAGEN (Crawley, West Sussex, UK).

Methods

Cell culture and transfection

A549 cells were cultured at a seeding density of 5.0×10^4 cells per cm^2 onto the 6-well plates using normal culture medium (DMEM supplemented with 10% FBS, 100 units/ml penicillin G and 100 µg/ml streptomycin), which were then subjected to transfection with the polycationic reagents.

To prepare Oligofectamine nanoliposomes, briefly, 2 µl of Oligofectamine were gently diluted in 15 µl serum-free medium (SFM), Opti-MEM®I, and then incubated at room temperature for 15 min. For OF-DNA lipoplexes, 2 µl OF nanoliposomes were gently mixed with salmon sperm DNA (2 µg DNA in 100 µl SFM) and incubated at room temperature for 15 min. After preparation of the OF/OF-DNA nanoliposomes, they were diluted in 800 µl of SFM and introduced to the A549 cells at 40-50% confluency for 4 hrs incubation at 37°C. Similarly, DAB or PF polymers alone (20 µg/ml) or as complexed with a salmon sperm DNA (4 µg/ml) were introduced to the A549 cells at 40-50% confluency for 4 hrs incubation at 37°C. Cells were then washed with SFM and replenished with normal culture medium (DMEM supplemented with 10% FBS) and incubated at 37°C for 24 hrs, after which they were subjected to the microarray genotoxicity analyses.

Viability assessment, Zeta potential and particle sizing of polyplexes

Cell counting and methylthiazoletetrazolium (MTT) assay used for cytotoxicity analysis. For MTT assay, briefly, cultured cells in 96-well plates were exposed to transfection reagents in the presence or absence of DNA for 4 hrs. They then washed once with phosphate buffered saline (PBS), replenished with normal culture medium and incubated at 37°C for 24 hrs. The normal culture medium replaced with 200 µl fresh media and then 50 µl MTT reagent (2.5 mg/ml in PBS) added to each well. Following incubation at 37°C for 4 hrs, medium was removed and cells were exposed to 200 µl of DMSO and 50 ml of Sorenson buffer (pH 7.4). Cultures incubated for 30 min at 37°C and then UV absorbance measured at 570 nm using a spectrophotometric plate reader, (ELx808™, BioTek, USA).

The zeta potentials of the polycationic nanostructures (by itself or as complexed with DNA) were determined using Malvern ZetaSizer 3, Malvern Instruments Ltd., (Malvern, Worcestershire, UK) as explained previously (13).

Total RNA isolation and in-direct labeled cDNA micoarray

Total RNA was isolated, from treated and untreated cells, using Trireagent and examined for quantity and quality as described previously (11). For indirect labelling method, the amino-allyl (aa) conjugated dUTP (aa-dUTP) was exploited to label the cDNA (aa-cDNA), and the aa-cDNA was labelled with fluorescent cyanine dye (Cy3 or Cy5) which was subjected to several purification prior to hybridization, as described previously (13).

Hybridization of Cy-dye coupled aa-cDNA

Prior to hybridization, the glass arrays (duplicate for each experiment) were prepared with the Gene-Frames to improve the reliability of process. The array supplier's protocol was performed for hybridization with slight modifications as described previously (13). The slide arrays were then scanned for Cy3 (green laser 532 nm; filter FM570-10) and Cy5 (red laser 635 nm; filter FM665-12) at gain 35, resolution normal (10 micron) and line average 1

using Affymetrix 428 Array Scanner (CA, USA) or TECAN (Switzerland).

Analysis of cDNA microarrays

The microarray data analyses were performed according to our previous reports (12, 13). Briefly, data were normalized using LOWESS method typically reported as an 'expression ratio' or the base 2 logarithm of the expression ratio of the treatment (T) to the untreated control (UT). Genes were assumed to be up- or down-regulated if they revealed an expression ratio of >2 and <0.5 (>1 and <-1 for $\log_2$ transformed data), respectively. The hierarchical clustering was applied to altered genes based upon Euclidean distance metric with 99% confidence interval.

To determine the functional expression of altered genes, the expression analysis systematic explorer (EASE) was also exploited as described previously (14).

For statistical assessments, one way analysis of variance (ANOVA) followed by multiple comparison test (post-hoc) and/or unpaired two-tailed t-test were used with an assumption of p value less than 0.05 for significant differences using GraphPad prism software (http://www.graphpad.com/).

RESULTS AND DISCUSSION

The use of viral vector technology in gene therapy trials, despite high transfection efficiency, has been complicated by safety issues such as unexplained cytotoxicity and immunogenicity in target cells/tissue (5). Thus, non-viral vectors (e.g., cationic lipids and polymers) have been exploited as potentially safer alternatives (15, 16). Of these, cationic lipid/polymer based nanostructures have been the most studied delivery systems (17, 18), however, surprisingly no substantial information is available about their genomic impacts and their interaction with subcellular components despite their cytotoxic potentials (19, 20). Thus, this study designed to examine the genocompatibility and toxicogenomics of cationic nanostructures in human alveolar epithelial A549 cells. Previously by using microarray-based gene expression profiling, it was shown that cationic lipids (e.g., Oligofectaime™) and polymers (e.g., DAB8 and DAB16) can elicit undesired changes in gene expression in the human epithelial cells (9-13). To compare the genomic impacts of the cationic lipids and dendrimers, in the current investigation the effect of OF and PF and DAB nanostructures on the pattern of gene expression of the human alveolar epithelial A549 cells by using a low-density cDNA microarrays housing 200 gene spots was investigated.

Particle size, zeta potential and cytotoxicity assessments

Particle size analysis yielded 134 ± 23, 87 ±18 and 115 ± 27 nm for OF, PF and DAB16 nanostructures, respectively. Evaluation of Zeta potential gave 25.4 ± 5.8, 44.7 ± 3.0 and 31.8 ± 8.0 (mV) for OF, PF and DAB16 nanostructures respectively. Upon complexation with DNA, their surface charges were diminished significantly (data not shown). The cell survival MTT assay revealed a significant cytotoxicity in A549 cells upon exposure to the designated amount of these nanostructures (Figure 1). The relationship between physicochemical properties and biological impacts of nonviral gene delivery systems have already been well documented (21-23). The zeta potential of cationic OF liposomes was reduced from about +30 mV to approximately +5 mV when it was complexed with DNA. However, complexation with DNA increased the size of OF:DNA nanostructures (~250 nm). Similar results were also observed for PF and DAB16. In agreement with previous studies (24,25), MTT survival assay revealed a marked cytotoxicity in A549 cells upon treatment with these nanostructures in a concentration dependent manner even though their complexation with DNA resulted in reduced cellular toxicity. It is speculated that the reduced zeta potential of the lipoplexes/polyplexes might reduce the prevalence of their interactions with the negatively charged cell membrane, and accordingly cytotoxicity is deemed to be diminished. However, this is not always the case as reported for increased cytotoxicity induced by cationic dendrimers upon complexation with DNA (26).

Microarray analysis

The fluorescent superimposed images represented a uniform hybridization of the Cy3- and Cy5-labeled probes on array housing 200 gene spots for OF induced gene expression in A549 cells (Figure 2). Similar fluorescent intensity for both Cy3-labeled untreated and Cy5-labeled treated samples (Figures 2B and C, respectively) indicates high reproducibility of the technique as it has been found for the replicated control genes (data not shown).

As shown in figure 3, the scatter plots of gene expression revealed significant changes in gene expression in A549 cells treated with OF, PF and DAB16. The gene spots highlighted in solid circles represent the genes with 2-fold or more expression changes in the treated versus untreated controls. A greater number of up/down regulated genes observed for OF and DAB16 compared to PF. The exact identities of the altered genes are presented in table 1.

Gebhart and Kabanov (26) reported that the cationic polymers such as starburst PAMAM dendrimers (Superfect) had higher transfection potential than lipid transfection reagents (e.g., Lipofectin, LipofectAMINE, CeLLFECTIN and DMRIE-C) with lower toxic impacts. Similar to our results, it has been reported (26) that polymer based cationic

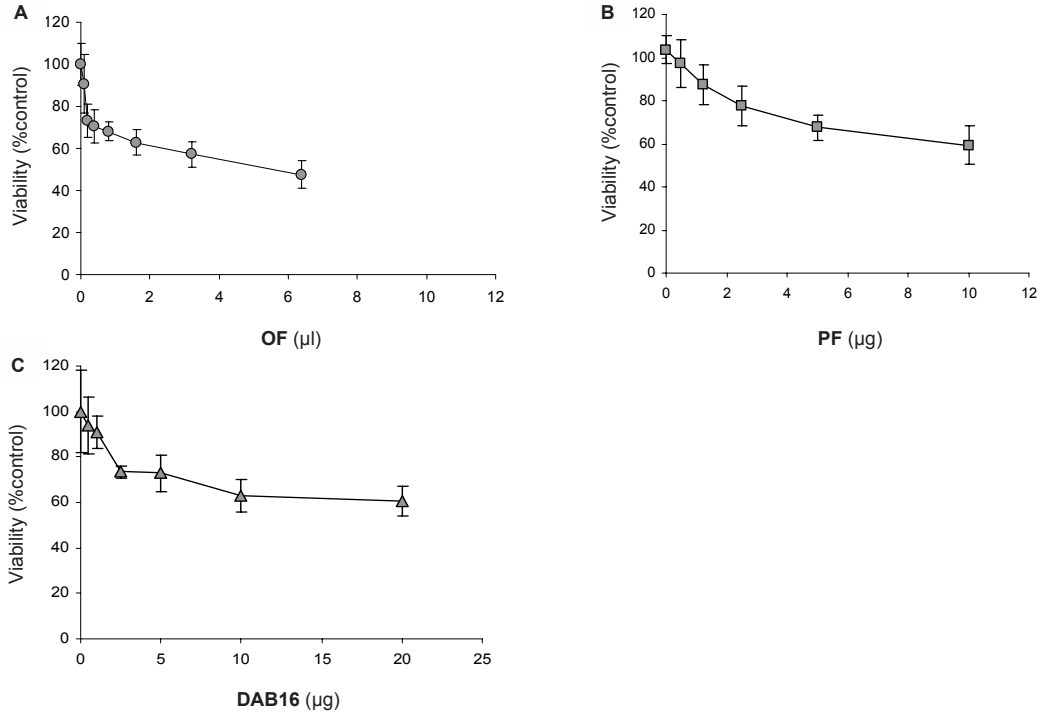

Figure 1. 1 MTT cytotoxicity analysis of A549 cells treated with Polyctionic lipid/polymer based nanostructures. A, B and C represent MTT assays OF, PF and DAB16, respectively. Data represent mean values ± S.D. for 6 replicates.

nanostructure confers somewhat higher transfection activity with relatively lower cytotoxicity. The accuracy and reproducibility of methodology of gene expression of cDNA microarray re-validated by means of the coefficient of variance (CV) and percentages of the all genes studied from the untreated cells of different experiments (Figure 4) which studied. Insignificant gene expression changes as well as a CV less than 15% among untreated replications from various experiments clearly indicate high reproducibility of the microarray methodology.

As shown in Table 1, it was found that the changes in gene expression can fall into a number of various functional genomic ontologies. The up-regulated genes by OF nanoliposomes included the genes involved in apoptosis, oxidative stress and external/biotic stimulus (e.g., IL9R, DUSP1, CSK, CSE1L); while the down-regulated genes were related to the cell growth and/or maintenance, cell proliferation and apoptosis (e.g., CDK4, TNFRSF6, SEP2, PSMA4). The over-expressed genes by PF dendrimers included genes involved in apoptosis, inflammatory response, vesicle-mediated transport, oxidative stress and external and biotic stimulus (e.g., IL9R, MMP8, NOL1); while the down-regulated genes were mainly related to the apoptosis, external and biotic stimulus (e.g., CSA2, MET, RPL6, CDK7, TNFRSF6). The over-expressed genes by DAB16 dendrimers included genes involved in apoptosis, inflammatory response, vesicle-mediated transport, oxidative stress and external and biotic stimulus (e.g., CD14, IL9R, RXRA, ITL2, TGFA, TRA1, TPR, CCNA1); while the down-regulated genes were mainly related to the apoptosis, external and biotic stimulus (e.g., CSE1L, CCNH, CDC2, ERCC1, PSMB4, MET, NOL1, PCNA, BAG1, MSH6).

Among the genes upregulated by OF, PF and DAB16 (Table 1), IL9R gene appeared to be upregulated by all of these nanostructures. It encodes IL9 receptor protein, which is a cytokine receptor that specifically mediates the biological effects of IL9. The ligand binding of this receptor leads to the activation of various JAK kinases and STAT proteins, which connect to different biologic responses, in particular some genetic studies suggest an association of this gene with development of asthma (27). It should be noted that lipid and polymer based formulations which were used for pulmonary drug/gene delivery may inadvertently activate IL9R and accordingly its downstream signalling paths.

The OF upregulated CSE1L is believed to carry nuclear localization signal which may play a role both in apoptosis and in cell proliferation (28). It was downregulated by PF and DAB16 dendrimers (Table 1). This may speculate that the structural differences cause such opposite impacts. The c-src tyrosine kinase (CSK) was upregulated in A549 cells similar to the what was previously observed in A431 cells (9), which may be involved in cell growth and/or maintenance. The DUSP1 may play an important role in the human cellular response

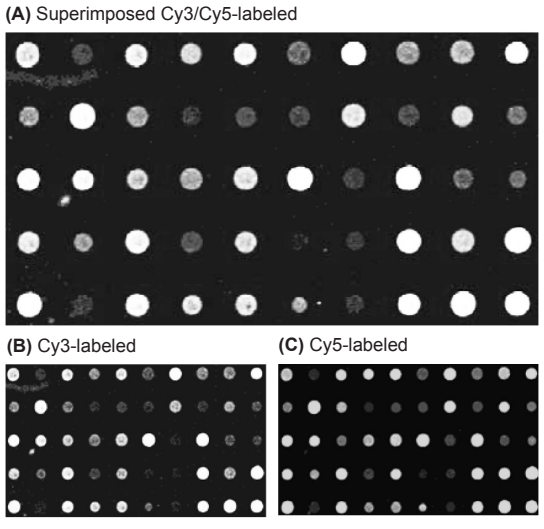

Figure 2. Typical fluorescent images of hybridized cDNA microarray. A) Superimposed image of Cy3- and Cy5-labeled cDNA. B) Cy3-labeled hybridized cDNA of untreated control cells. C) Cy5-labeled hybridized cDNA of treated cells.

to environmental stress as well as in the negative regulation of cellular proliferation (29).These researchers also reported that the DUSP1 is an essential endogenous regulator of the inflammatory response to lipopolysaccharide that can increase interleukins (i.e., IL6, IL10) and chemokines (i.e., CCL3, CCL4, CXCL2). Contradictorily, PF and DAB16 induced modest downregulation, indicating distinct impact of the cationic lipid. Among the genes that are downregulated by OF in A549 cells, ASAH1 and PTEN revealed only modest downregulation. The ASAH1 mutations have been shown to be associated with a lysosomal storage disorder known as Farber disease and codes for the N-acylsphingosine amidohydrolase (30). The PTEN functions as a tumor suppressor by negatively regulating AKT/PKB signaling pathway and plays crucial role in lung cancer (31).

The TNFRSF6, which was downregulated by all of the nanosystems, is a member of TNF-receptor superfamily and contains a death domain. It plays a central role in the physiological regulation of programmed cell death, and in the pathogenesis of various malignancies and diseases of the immune system. The interaction of this receptor with its ligand allows formation of a death-inducing signalling complex that includes Fas-associated death domain protein (FADD), caspase 8, and caspase 10 (32). Suppression of this gene by OF, PF and DAB may indicate influence of common properties of these nanostructures (perhaps through their positive surface charges) on target cells.

PF appeared to alter the expression of a lower number of genes compared to OF and DAB, indicating to be possibly a safer gene delivery nanosystem. Apart from the upregulated IL9R induced by PF, proteins

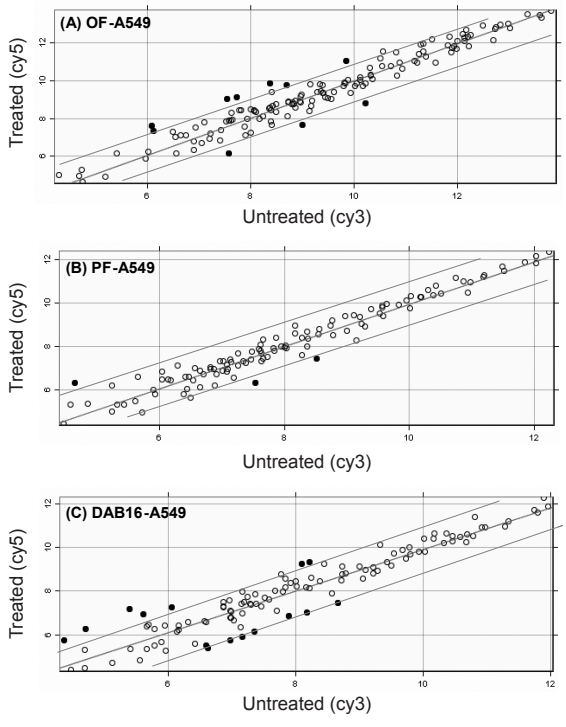

Figure 3. Scatter plots of gene expression changes induced by cationic lipid/polymer based nanostructures in A549 cells. A, B and C represent scatter plots for Oligofectamine (OF), Polyfect (PF) and diaminobutane (DAB) treated cells, respectively. Data show Log_2 transformed gene expression values. The reference lines shown indicate the intensity ratios of genes exhibiting no changes (0), under-expression by 2-fold (-1) or over-expression by 2-fold (1).

of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Most MMP's are secreted as inactive proproteins, which activated when cleaved by extracellular proteinases. However, the enzyme encoded by this gene is stored in secondary granules within neutrophils and activated by autolytic cleavage. Its function is degradation of type I, II and III collagens. The gene is part of a cluster of MMP genes, which are, localized in chromosome 11q22.3. Among downregulated genes, for example, the proto-oncogene MET product is the hepatocyte growth factor receptor and encodes tyrosine-kinase activity. The primary single chain precursor protein is post-translationally cleaved to produce the alpha and beta subunits, which are disulfide linked to form the mature receptor (33). The protein encoded by CDK7 is a member of the cyclin-dependent protein kinase (CDK) family. CDK family members are highly similar to the gene products of Saccharomyces cerevisiae CDC28, and Schizosaccharomyces pombe CDC2, and known as important regulators of cell cycle progression. This protein forms a trimeric complex with cyclin

Table 1. Polyctionic lipids/polymers induced gene expression changes in A549 epithelial cells.

Gene ID	Gene description	OF	PF	DAB16
NM_000591	cd14 antigen precursor; cd14	1.21	1.31	3.36+
NM_002186	interleukin 9 receptor; il9r	2.82+	3.12+	2.95+
NM_002957	retinoid x receptor, alpha; rxra	1.60	0.91	2.58+
AF040965	unknown protein it12	1.25	1.06	2.47+
NM_003236	transforming growth factor, alpha; tgfa	1.28	1.24	2.27+
NM_003299	tumor rejection antigen (gp96) 1; tra1	1.03	1.22	2.18+
NM_003292	translocated promoter region (to activated met oncogene); tpr	1.48	1.55	2.12+
NM_003914	cyclin a1; ccna1	1.59	1.41	1.96+
NM_002789	proteasome (prosome, macropain) subunit, alpha type, 4; psma4	0.37	0.84	1.72
NM_002424	matrix metalloproteinase 8 preproprotein; mmp8	1.46	1.90	1.64
NM_004383	c-src tyrosine kinase; csk	2.61+	1.33	1.52
NM_032959	dna directed rna polymerase ii polypeptide j, isoform b; polr2j	2.26+	1.31	1.26
NM_002953	ribosomal protein s6 kinase, 90kd, polypeptide 1; rps6ka1	0.65	1.00	1.08
NM_000314	phosphatase and tensin homolog (mutated in multiple advanced cancers 1); pten	0.59	1.20	1.07
NM_000660	transforming growth factor, beta 1; tgfb1	1.92+	0.73	1.03
NM_001799	cyclin-dependent kinase 7; cdk7	1.21	0.54-	1.02
XM_091463	hypothetical protein xp_091463; loc162281	2.78+	0.89	1.00
AF182645	chondrosarcoma-associated protein 2; csa2	0.68	0.42-	1.00
NM_001237	cyclin a; ccna2	0.65	1.00	1.00
NM_004315	n-acylsphingosine amidohydrolase (acid ceramidase); asah	0.59-	1.00	1.00
NM_002945	replication protein a1 (70kd); rpa1	0.56-	1.04	1.00
NM_015129	septin 2; sep2	0.36-	1.00	1.00
NM_002947	replication protein a3 (14kd); rpa3	0.65	1.19	0.95
NM_000075	cyclin-dependent kinase 4, isoform 1; cdk4	0.38-	0.88	0.86
NM_001961	eukaryotic translation elongation factor 2; eef2	0.63	1.68	0.84
NM_004417	dual specificity phosphatase 1; dusp1	1.97+	0.75	0.76
NM_000970	ribosomal protein l6; rpl6	0.95	0.54-	0.70
NM_005319	h1 histone family, member 2; h1f2	1.00	0.79	0.64
NM_002690	polymerase (dna directed), beta; polb	0.79	0.61	0.62
NM_000043	apoptosis (apo-1) antigen 1; tnfrsf6	0.58-	0.57-	0.61
NM_021067	kiaa0186 gene product; kiaa0186	2.26+	1.00	0.59-
NM_000179	muts homolog 6 (e. coli); msh6	0.89	0.89	0.56-
NM_004323	bcl2-associated athanogene; bag1	1.17	1.00	0.55-
NM_002592	proliferating cell nuclear antigen; pcna	0.74	1.00	0.48-
NM_006170	nucleolar protein 1 (120kd); nol1	1.54	1.89	0.46-
NM_000245	met proto-oncogene precursor; met	0.79	0.46-	0.44-
NM_002796	proteasome (prosome, macropain) subunit, beta type, 4; psmb4	0.76	1.14	0.43-
NM_001983	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence); ercc1	1.36	1.00	0.43-
NM_001786	cell division cycle 2 protein, isoform 1; cdc2	1.54	0.84	0.42-
NM_001239	cyclin h; ccnh	0.68	0.61	0.42-
NM_001316	cse1 chromosome segregation 1-like (yeast); cse1l	2.09+	0.64	0.41-

Ratios of the treated over untreated cells are given and the over-expressed (+) and under-expressed (–) genes are indicated.

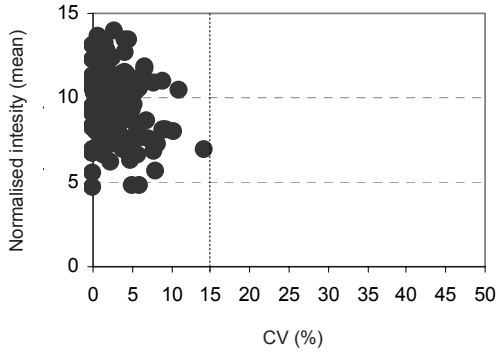

Figure 4. Microarray data validation analysis using precision assessment. Data show the coefficient of variance (CV) of the untreated controls from different experiments.

H and MAT1, which functions as a Cdk-activating kinase (CAK). It is an essential component of the transcription factor TFIIH, which is involved in transcription initiation and DNA repair. This protein is thought to serve as a direct link between the regulation of transcription and the cell cycle (34).

Marked gene expression changes also observed in A549 cells treated with DAB16 dendrimer (Table 1). Among the genes upregulated by DAB16, CD14 is a surface protein preferentially expressed on monocytes/macrophages. It binds lipopolysaccharide binding protein and recently has been shown to bind apoptotic cells (35). Transforming growth factors (TGFs) are biologically active polypeptides that reversibly confer the transformed phenotype on cultured cells. Alpha-TGF shows about 40% sequence homology with epidermal growth factor (EGF; MIM 131530) and competes with EGF for binding to the EGF receptor (MIM 131550), stimulating its phosphorylation and producing a mitogenic response. The protein encoded by CCNA1 belongs to the highly conserved cyclin family, whose members characterized by a dramatic periodicity in protein abundance through the cell cycle. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns, which contribute to the temporal coordination of each mitotic event. This cyclin was found to bind to important cell cycle regulators, such as Rb family proteins, transcription factor E2F-1, and the p21 family proteins (36). The oncogene BCL2 is a membrane protein that blocks a step in a pathway leading to apoptosis or programmed cell death. The protein encoded by this gene binds to BCL2 and referred to as BCL2-associated athanogene. It enhances the anti-apoptotic effects of BCL2 and represents a link between growth factor receptors and anti-apoptotic mechanisms. At least three protein isoforms are encoded by this mRNA through the use of alternative translation initiation sites, including a non-AUG site (37).

Upon these findings, it can proposed that the cationic gene delivery nanosystems can induce gene changes via various pathways, even though there exist some overlaps between OF, PF and DAB16 in terms of changes in gene expression. It appears that these nanosystems may induce specific but different gene signatures as the majority of altered genes different for each entity (see Table 1). It could be evoked that such results may simply be reflective of the low number of genes present on the used arrays. Alternatively, it may be a function of the responsiveness of A549 cells to these gene delivery nanosystems since it has been well documented that the transfection efficiency depends on characteristics of the lipids as well as cell types (12). In fact, the nature and structural architecture of the gene delivery nanosystems could be the key determinants of the extent and type of the altered genes in target cells/tissue. It has been previously shown that PAMAM and DAB dendrimers can exert different gene changes in A431 and A549 cells and in particular had opposing effects on epidermal growth factor receptor expression (10, 38).

CONCLUSION

Based on the results of the present and previous reports, it may speculate that the target cells are able to distinguish the xenobiotics and act distinctly and specifically to compensate their impacts upon their biological directionalities and activities. Thus, the gene expression patterns/profiles seems to be a prerequisite for gene therapy. The genocompatibility and toxicogenomics platform can confer a better understanding upon biosafety of any desired nano-scaled gene delivery systems at a genomic level, where only a single desired genetic change sought in gene therapy in most cases. In fact, any inadvertent gene changes induced by the delivery system itself should take into account since it may exacerbate, attenuate or even mask the desired effects of the genomedicine resulting in critical implications in vivo or ex vivo.

ACKNOWLEDGMENT

The authors are grateful to the technical supports kindly provided by Prof. Saghir Akhtar, SA Pharma, Sutton Coldfield, United Kingdom.

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