科技部補助專題研究計畫成果報告
期末報告

陽離子性高分子相關於細胞胞外泌體之應用與開發

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 106-2221-E-041-005-
執 行 期 間 ： 106年08月01日至107年07月31日
執 行 單 位 ： 嘉藥學校財團法人嘉南藥理大學藥學系(含碩士班)

計 畫 主 持 人： 郭榮華
共 同 主 持 人： 詹明修
計畫參與人員： 博士班研究生-兼任助理：王羿忻

報 告 附 件 ： 出席國際學術會議心得報告

中 華 民 國 107 年 08 月 27 日

中 文 摘 要 ： 透過細胞分泌之細胞外囊泡內之信息傳遞者如微RNA，細胞間傳遞被認知為參與各種階段之腫瘤發展和轉移。因此本研究為探索癌細胞被非病毒性基因傳送後分泌之細胞外囊泡內之微RNA表現模式，以更進一步了解癌細胞經基因傳送後細胞間傳遞之分子信息資料。我們使用了二種常用非病毒性載體(lipofetamine 2000及jet polyethylenimine)來傳送綠螢光蛋白質體於HeLa 癌細胞，萃取純化分泌之細胞外囊泡並經次世代定序來分析其RNA組成。由即時聚合酶鏈反應分析印證，我們發現二個共同重複表現於二種載體基因傳送之微RNAs(hsa-miR-143-3p和hsa-miR-193b-3p)，我們並預測其目標作用基因及相關之分子路徑分析，細胞死亡及壓力分子路徑之相關蛋白質表現亦一併分析印證。這二個微RNAs(hsa-miR-143-3p和hsa-miR-193b-3p)之上調節表現可被用於不同非病毒性載體基因傳送之癌細胞基因療法之潛在作用分子目標。

中 文 關 鍵 詞 ： 細胞外囊泡; lipofetamine 2000; jet polyethylenimine; 次世代定序; 基因傳送; 微RNA

英 文 摘 要 ： Intercellular communication is known to be involved in various stages of tumor development and metastasis through the secretion of extracellular vesicles (EVs) containing messengers such as microRNAs (miRNAs). Therefore, this study explored miRNA profiles in cancer cell-derived EVs after non-viral gene delivery in order to better understand the molecular information of intercellular communication in cancer cells after gene delivery. Two commonly used non-viral vectors (Lipofectamine 2000 and jet polyethylenimine) were used for the delivery of gene fluorescent protein plasmid in HeLa cancer cells. EVs were extracted and the contents of their RNA were subjected to the next-generation sequencing. In order to illustrate the common characteristics of non-viral vectors in the cancer cells, two overlapped up-regulated miRNAs (hsa-miR-143-3p and hsa-miR-193b-3p) were confirmed by real-time quantitative reverse transcriptase-polymerase chain reaction in the secreted EVs in response to both lipoplexes and polyplexes. The prediction of target genes and molecular pathways involved in these two miRNAs were determined and the protein expressions related to the pathways of cell death and stress in HeLa cells were identified. Hsa-miR-143-3p and hsa-miR-193b-3p were found to be up-regulated by the use of different non-viral vectors and can thus serve as potential targets of non-viral cancer gene therapy.

英 文 關 鍵 詞 ： Extracellular vesicles ; Lipofectamine 2000; jet polyethylenimine ; next-generation sequencing; gene delivery; microRNAs

Journal of Drug Targeting

ISSN: 1061-186X (Print) 1029-2330 (Online) Journal homepage: http://www.tandfonline.com/loi/idrt20

The microRNA expression profiles in extracellular vesicles from HeLa cancer cells in response to cationic lipid- or polyethylenimine-mediated gene delivery

Chia-Wei Lin, Ming-Shiou Jan & Jung-Hua Steven Kuo

To cite this article: Chia-Wei Lin, Ming-Shiou Jan & Jung-Hua Steven Kuo (2018): The microRNA expression profiles in extracellular vesicles from HeLa cancer cells in response to cationic lipid- or polyethylenimine-mediated gene delivery, Journal of Drug Targeting, DOI: 10.1080/1061186X.2018.1491977

To link to this article: https://doi.org/10.1080/1061186X.2018.1491977

JOURNAL OF DRUG TARGETING
https://doi.org/10.1080/1061186X.2018.1491977

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

The microRNA expression profiles in extracellular vesicles from HeLa cancer cells in response to cationic lipid- or polyethylenimine-mediated gene delivery

Chia-Wei Lin[a], Ming-Shiou Jan[a,b,c] and Jung-Hua Steven Kuo[d]

[a]Institute of Biochemistry, Microbiology and Immunology, Taichung, Taiwan; [b]Immunology Research Center, Medical College of Chung Shan Medical University, Taichung, Taiwan; [c]Division of Allergy, Immunology, and Rheumatology, Chung Shan Medical University Hospital, Taichung, Taiwan; [d]Department of Pharmacy, Chia Nan University of Pharmacy and Science, Tainan, Taiwan

ABSTRACT

Intercellular communication is known to be involved in various stages of tumour development and metastasis through the secretion of extracellular vesicles (EVs) containing messengers such as microRNAs (miRNAs). Therefore, this study explored miRNA profiles in cancer cell-derived EVs after non-viral gene delivery in order to better understand the molecular information of intercellular communication in cancer cells after gene delivery. Two commonly used non-viral vectors (Lipofectamine 2000 and jet polyethylenimine) were used for the delivery of gene fluorescent protein plasmid in HeLa cancer cells. EVs were extracted and the contents of their RNA were subjected to the next-generation sequencing. In order to illustrate the common characteristics of non-viral vectors in the cancer cells, two overlapped up-regulated miRNAs (hsa-miR-143-3p and hsa-miR-193b-3p) were confirmed by real-time quantitative reverse transcriptase-polymerase chain reaction in the secreted EVs in response to both lipoplexes and polyplexes. The prediction of target genes and molecular pathways involved in these two miRNAs were determined, and the protein expressions related to the pathways of cell death and stress in HeLa cells were identified. Hsa-miR-143-3p and hsa-miR-193b-3p were found to be up-regulated by the use of different non-viral vectors and can thus serve as potential targets of non-viral cancer gene therapy.

ARTICLE HISTORY
Received 6 May 2018
Revised 9 June 2018
Accepted 19 June 2018

KEYWORDS
Extracellular vesicles; Lipofectamine 2000; jet polyethylenimine; next-generation sequencing; gene delivery; microRNAs

Introduction

Extracellular vesicles (EVs) are membrane-bound structures that are secreted from most biological cells and are basically composed of lipids, transmembrane proteins and cargo biomolecules [1]. EVs are associated with various cell functions generally including cell waste management, the regulation of immune functions, angiogenesis, tissue regeneration, cancer metastasis and cell-to-cell communication via the transportation of proteins and nucleic acids such as mRNA, short non-coding microRNA (miRNA) and non-coding long RNA [2–4]. Based on their sizes and intracellular origins, EVs can be approximately divided into three types: exosomes (50–200 nm), microvesicles (200–1000 nm) and apoptotic bodies (500–1000 nm) [5]. Among these different types of EVs, exosomes have drawn intensive interest for use in both therapeutic and diagnostic clinic applications due to their capacity to be used as targeted nanomedicine vehicles without the limitations of most synthetic nanoparticles, such as shorter circulating half-lives, poor biocompatibility and a lack of targeting ability [6,7]. Also, growing evidence has revealed that the miRNAs contained in tumour cell-derived exosomes possess unique characteristics in terms of the genetic regulation of cancer progression and metastasis [8,9].

Despite lower transfection efficiency in gene delivery as compared with viral vectors, non-viral vectors own important advantages such as lower immunogenicity, a lower risk of mutagenesis, a higher cargo loading capacity and easy scalability [10–12]. Today, a variety of non-viral vectors have been developed for use in gene delivery and are mainly classified as cationic lipid-based vesicles, polymer-based carriers and inorganic nanoparticles [13–15]. However, the molecular mechanisms of cells underlying the cellular responses to such vector-mediated gene delivery have still not been fully investigated, even as enhanced understanding of these cellular responses is important for achieving efficient and safe gene delivery. So far, various studies primarily based on the toxicogenomics have been conducted in order to mechanistically elucidate the responses in cells after vector-mediated gene delivery [16–19]. Differences in molecular responses within cells after nucleic acid delivery through the use of different vectors have been demonstrated [17,20]. Nonetheless, the molecular information of the distant intercellular communication that occurs in response to vector-mediated gene delivery has yet to be fully explored.

Therefore, the aim of this study was to better understand the mutually occurring molecular information of intercellular communication in cancer cells after non-viral gene delivery by exploring the miRNA expression profiles within the EVs secreted from cancer cells. We selected HeLa cervical cancer cells as the model tumour cells and cationic liposomes (Lipofectamine 2000 (Lipo)) and linear jet polyethylenimine (PEI), which are two commercial non-viral vectors commonly used for *in vitro* and *in vivo* gene delivery, for the delivery of model gene GFP (green fluorescent protein)

CONTACT Jung-Hua Steven Kuo kuojunghua@yahoo.com.tw Department of Pharmacy, Chia Nan University of Pharmacy and Science, 60 Erh-Jen Road, Sec. 1, Jen-Te, Tainan 717, Taiwan; Ming-Shiou Jan msjan@csmu.edu.tw Division of Allergy, Immunology, and Rheumatology, Chung Shan Medical University Hospital, 110 Jianguo North Road, Sec. 1, Taichung 40201, Taiwan

Supplemental data for this article can be accessed here.

plasmid, which allows for the easy examination of protein expression. EVs were extracted and their RNA contents were subjected to the next-generation sequencing. We focussed on the identification of overlapping differentially expressed miRNAs in the EVs because they are closely related to the regulation of gene expression by non-viral vectors in cancer cells. The target genes and involved molecular pathways of differentially expressed miRNAs were also analysed. The study results provide comprehensive information regarding the intercellular communication of cancer cells in response to non-viral-mediated gene delivery and should be of use in improving the non-viral gene delivery systems used for cancer therapy.

Materials and methods

Materials

Lipo was obtained from Invitrogen Corp. (Carlsbad, CA) and PEI was acquired from Polyplus-transfection Inc. (New York, NY). GFP antibody was purchased from BioVision Inc. (Milpitas, CA). Rabbit anti-caspase-3, anti-cleaved caspase-3, anti-PI3K, anti-phospho-PI3K, anti-Akt, anti-phospho-Akt, anti-mTOR and anti-phospho-mTOR antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA). Rabbit anti-p62, anti-Beclin 1, anti-Igf1, anti-LC3, anti-TGF-β1, anti-phospho-Smad3 and mouse anti-β-actin antibodies were obtained from Novus Biologicals, LLC (Littleton, CO). Mouse anti-IRS-1 and anti-insulin receptor β antibodies were acquired from BD Biosciences (San Jose, CA). Rabbit anti-Smad2, anti-phospho-Smad2 and anti-Smad3 antibodies were purchased from Thermo Fisher Scientific Inc. (Rockford, IL).

Cell culture

HeLa human cervical cells (ATCC CCL-2) were maintained in DMEM medium (Invitrogen) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS; Invitrogen) and 1% (v/v) antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen)) in a humidified atmosphere of 5% CO_2 at 37 °C.

Transfection procedures

Lipoplexes were prepared by vortex mixing at a fixed concentration of 1 μg/mL plasmid DNA encoding enhanced GFP (pEGFP-C1) with various concentrations of Lipo (2.5, 5, 10 and 20 μg/mL) in a total 200 μL serum-reduced medium (Opti-MEM (Invitrogen)) and allowed to stand for 25 min at room temperature before being used for transfection. Similarly, polyplexes were prepared from PEI stock solution at various concentrations of 2.5, 5, 10 and 20 μL/mL. HeLa cells were plated at 40,000 per well in 8-well Lab-Tek chamber slides (Nalge Nunc, Naperville, IL) and incubated overnight (60–70% confluence). After PBS washing, the lipoplexes and polyplexes were added to the cells for 6 h, and then the medium was discarded and replaced by a growth medium. After 18 h of incubation, the cells were fixed in 4% paraformaldehyde-PBS solution, the nuclei were stained with DAPI, and the cells were examined under a fluorescence microscope (Axio Imager 2; Zeiss, Jena, Germany) equipped with a standard GFP/DAPI filter set. The quantification of transfection was analysed as the GFP% fraction of the images of 300 total counted cells. GFP expressions in HeLa cells were also analysed by Western blotting.

Cell viability assay

The cytotoxicity of the lipoplexes and polyplexes was examined using a Cell Counting Kit-8 (Dojindo Laboratories, Tokyo, Japan), which measured the activity of cell dehydrogenases, as previously reported [20]. Briefly, HeLa cells were seeded in 48-well plates at a density of 40,000 cells/well and allowed to adhere overnight (60–70% confluence). After following the same transfection protocols as described above, 10 μL of cell-counting kit solution were added and incubated for 4 h. The absorbance at 450 nm was obtained with 595 nm background corrections. The results are presented as the cell viability percentage (average optical density (OD)/average untreated control OD) ± standard deviation (SD).

EV isolation

The isolation of EVs from untreated HeLa cells and from HeLa cells treated with the optimal condition of lipoplexes or polyplexes was conducted. Cells (2×10^5/well) were seeded in 6-well plates and allowed to reach 60–70% confluence. The cells were then subjected to the 24-h transfection protocols mentioned above, except that the FBS in the growth medium was replaced by exosome-depleted FBS (Invitrogen). After transfection, 5 ml of medium was collected from the cells and centrifuged at 1500 rpm for 5 min to remove dead cells and cell debris. The resulting medium was further filtered with a Millipore 0.22 μm filter to remove particles greater than 220 nm in size. After adding 1 ml of ExoQuick-TC reagent (System Biosciences Inc., Mountain View, CA), the resulting solution was inversion mixed 4 times and incubated overnight at 4 °C. After centrifugation at 1500× *g* for 30 min, EVs were pelleted by removing supernatant and stored at −80 °C for further use.

Characterization of EVs by in situ wet-cell transmission electron microscopy, nanoparticle tracking analysis, and Western blot analysis

The morphology of the PBS-dispersed EVs was observed by JEOL 2010 LaB_6 transmission electron microscopy (TEM) (JEOL Ltd., Tokyo, Japan) with an *in-situ* liquid cell, which can image biological samples in liquid surroundings, at a 200 KV electron acceleration voltage. The particle size and distribution of the PBS-diluted EVs were investigated using a 405-nm laser nanoparticle tracking analysis (NTA) (NanoSight LM10HS, Malvern Instruments Ltd., Worcestershire, UK), and the data were processed using NTA 3.1 software. Western blot analysis was applied to assess the typical biomarker proteins in the EVs (which consisted primarily of exosomes), as previously reported [1]. The total protein concentrations of the EVs were determined using the BCA Protein Assay kit (Pierce Biotechnology, Rockford, IL). Primary rabbit anti-CD63 and anti-CD81 antibodies were obtained from System Biosciences, and primary mouse anti-TSG101 antibody was purchased from Novus Biologicals, LLC (Littleton, CO).

RNA isolation

Total RNA was extracted from purified EVs pooled from three independent samples using an exoRNeasy serum/plasma maxi Kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. The extracted RNA was then concentrated and the concentration and purity of the total RNA was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

Small RNA library construction and sequencing

Small RNA libraries were prepared using the Bioo NEXTflex™ Small RNA-Seq Kit v3 following the protocol provided by the manufacturer (Bioo Scientific, Austin, TX). Briefly, 2 μg of total RNA was used as starting material. After ligation of the RNA 3′- and the RNA 5′ adapter, total RNA samples were reverse transcribed to cDNA libraries and unique indexes were introduced during 18 cycles or fewer of PCR amplification. The products of the PCR amplification were size-selected and purified with Mag-Bind RxnPure Plus magnetic beads (Omega Bio-tek, Norcross, GA). The final cDNA libraries were qualified and quantified using the Agilent 2200 Tapestation instrument (Agilent Technologies, Santa Clara, CA). The successful library preparation was assured by the presence of a strong ~150 bp band. Afterwards, equimolar amounts of small RNA-derived libraries were pooled together and sequenced on a HiSeq 2500 next-generation sequencing platform (Illumina, San Diego, CA).

Analysis of miRNA expression and differentially expressed miRNAs

After sequencing, raw reads were filtered and trimmed using Trimmomatic software to remove low quality bases and reads shorter than 16 bp [21]. The alignment of reads was conducted using the miRDeep2 and Bowtie software packages to map pre-processed read data to the RNA sequence, which was sourced from miRBase v21; the RefSeq from the NCBI Homo sapiens Annotation Release 107; and the piRBase v1.0 databases [22,23]. The differentially expressed miRNA among different samples was determined by DESeq2 package based on a model using the negative binomial distribution [24]. Comparison of untreated HeLa cells with cells treated with lipoplexes or polyplexes were performed. The standard selection criteria used to identify differentially expressed miRNA were as follows: $|\log_2 \text{Fold change}| \geq 0.585$ and p values $<.05$.

Verifying miRNA expression using real-time quantitative reverse transcriptase-polymerase chain reaction (RT-QPCR)

The differentially overlapped expressed miRNAs from the sequencing results were further verified from three independent samples using RT-QPCR as conducted in our previous experimental protocols [19]. The following primer sequences were investigated: hsa-miR-143–3p, forward GCCTGAGATGAAGCACTGT, reverse GTTG CC TCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACGAGCT; hsa-miR-193b-3p, forward GTTAACTGGCCCTCAAAGTC, reverse GTTGG CTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAGCGG; hsa-miR-451a, forward CGCAAACCGTTACCATTACT, reverse GTTGG CT CTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAACTC; hsa-miR-3960, forward GGCGGCGGCGGAG, reverse GTTGGCTCTGGTGC AGGGTCCGAGGTATTCGCACCAGAGCCAACCCCCCGC. The data were normalised to the internal control 18S.

Prediction of the potential target genes of regulated miRNAs and involved molecular pathway analysis

The potential target genes of the differentially expressed miRNAs were identified and the pathway analysis of the target genes involved was performed using DIANA-TarBase v8, which is a database of experimentally supported miRNA–gene interactions and molecular pathway analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [25].

Analysis of related protein expressions in HeLa cells treated with lipoplexes or polyplexes

In addition to EV isolation, related protein expressions in the untreated HeLa cells and the HeLa cells treated with lipoplexes or polyplexes were examined by Western blot as previously reported [18].

Statistical analysis

The results are shown as mean ± standard deviation ($n = 3$). The differences between the samples from the untreated control HeLa cells and the samples from the treated HeLa cells were analysed using Student's t-test. $p < .05$ was considered statistically significant.

Results

Optimization of transfection

The transfection of HeLa cells was optimised by varying the doses of Lipo or PEI at fixed 1 μg/mL DNA concentration according to low cytotoxicity and high GFP transfection, and the optimal conditions were found to be at 5 μg/mL of Lipo or 10 μL/mL of PEI at 1 μg/mL of DNA (Figure 1). Under optimal conditions, the relative cell viability of the lipoplexes was lower than that of the polyplexes after 24 h of incubation (Figure 1(A)) and both the lipoplexes and polyplexes induced some degree of cell death, as indicated in Figure 1(B). Representative images of optimal GFP transgene expression of lipoplexes or polyplexes in HeLa cells after 24 h of incubation are illustrated in Figure 1(C). The cellular GFP uptake and GFP transgene expression of the optimal lipoplexes were higher than those of the optimal polyplexes (Figure 1(D,E)). In the following sections, the optimal lipoplexes and polyplexes were used, respectively, in the analysis of the EVs secreted from the HeLa cells.

Characterization of isolated EVs

The morphology of EVs was investigated using *in situ* wet-cell TEM (Figure 2(A)). The EVs isolated from the untreated HeLa cells and the HeLa cells treated with lipoplexes or polyplexes, appeared to be typical membrane-like nano-sized vesicles in liquid surroundings. The particle sizes and distributions of the EVs isolated from the three sources were further determined using NTA (Figure 2(B–D)). The EVs isolated from the three sources exhibited similar average particle sizes within the classic size for exosomes (50–200 nm). Distribution patterns with mostly uniform populations were observed for the EVs isolated from the untreated cells and the EVs isolated from the lipoplex-treated cells, while more numerous particle populations were observed for the EVs isolated from the polyplex-treated cells. Finally, the expression of hallmark proteins of exosomes (TSG101, CD63, and CD81) in the isolated EVs confirmed the presence of exosomes (Figure 2(E)).

Analysis of small RNA contents of EVs by next-generation sequencing

The small RNA contents of the EVs isolated from the untreated HeLa cells and from the HeLa cells treated with lipoplexes or with polyplexes were quantified by mapped sequence read counts, and the distributions of those contents as determined by the RNA length of the mapped sequence read counts are illustrated

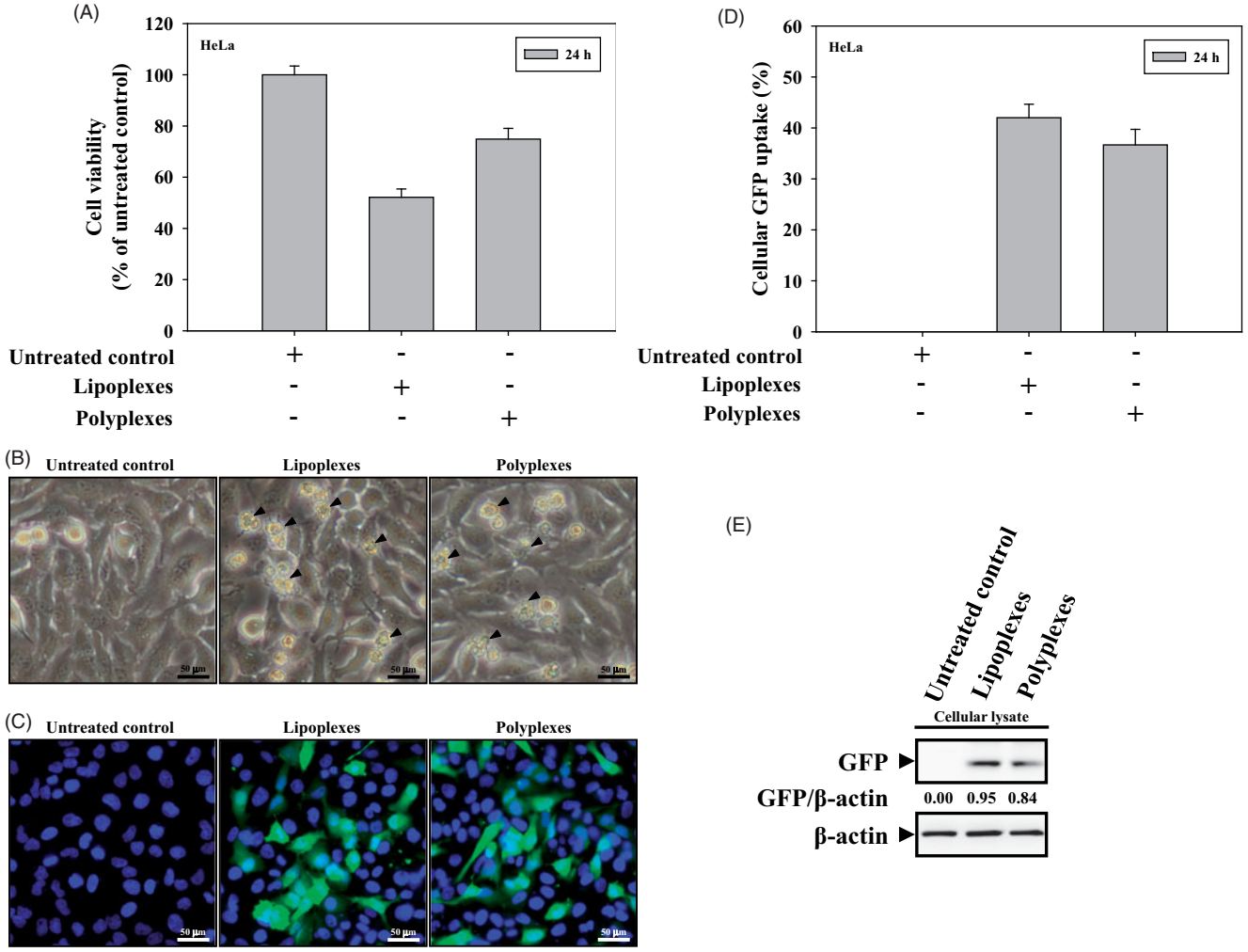

Figure 1. The optimal properties of transfection of HeLa cells by lipoplexes or polyplexes after 24 h of incubation. (A) Cell viability of untreated HeLa cells and HeLa cells treated with lipoplexes or polyplexes. Data shown as percentage of untreated control ((average OD/average untreated control OD) ± SD ($n = 3$)). The data are all $p < .05$ vs. untreated controls. (B) Representative optical images of cell morphology in untreated HeLa cells and HeLa cells treated with lipoplexes or polyplexes (images with ×400 magnification). The arrow heads indicate cell death after treatment of lipoplexes or polyplexes. (C) Representative fluorescence images of transfection (images with ×400 magnification). (D) Quantification GFP gene expression. The data are expressed as the % fraction of cellular GFP uptake from a total of 300 cells ($n = 3$). (E) Western blot analysis of GFP expression, in one representative experiment among three similar experiments. β-actin was the loading control. The ratios of band density were calculated with Alphalmager 2200 software.

in Figure 3. As expected, the isolated EVs contained diverse cargoes of small RNA, including miRNAs, non-coding RNA (ncRNA), messenger RNA (mRNA), ribosomal RNA and miscellaneous RNA (misc RNA). Also, miRNAs represented the majority of the small RNA contents and were investigated in the following study.

Analysis of miRNA expression and differentially expressed miRNAs

As compared with untreated cells, 12 up-regulated and 4 down-regulated differentially expressed miRNAs in the lipoplex-treated cells were identified (Table 1). Also, 2 up-regulated and 7 down-regulated differentially expressed miRNAs were identified in the polyplex-treated cells in comparison with the untreated cells (Table 2). Two overlapping up-regulated miRNAs (hsa-miR-143–3p and hsa-miR-193b-3p) and two overlapping down-regulated miRNAs (hsa-miR-451a and hsa-miR-3960) were detected in both the cells treated with lipoplexes and the cells treated with polyplexes. The numbers of up-regulated and down-regulated differentially expressed miRNAs were higher for the cells treated with lipoplexes than for the cells treated with polyplexes.

Verifying miRNA expression using RT-QPCR

Because the differentially overlapped expressed miRNAs (up-regulated: hsa-miR-143-3p and hsa-miR-193b-3p; down-regulated: hsa-miR-451a and hsa-miR-3960) in the EVs represented the mutual non-viral vector effects of lipid- or PEI- mediated gene delivery, these miRNAs were focussed on in this study and were verified using RT-QPCR (Figure 4). For the cells treated with lipoplexes as well as the cells treated with polyplexes, only the results indicating up-regulated hsa-miR-143-3p and hsa-miR-193b-3p were quantitatively consistent with the sequencing results, whereas hsa-miR-451a and hsa-miR-3960, which were down-regulated according to sequencing data, were quantitatively up-regulated, meaning the results were inconsistent with the sequencing results. Therefore, the verified up-regulated hsa-miR-143-3p and hsa-miR-193b-3p were subjected to further analysis.

Prediction of the target genes and involved molecular pathways of differentially expressed miRNAs

The predicted target genes and KEGG pathway analysis results for the up-regulated hsa-miR-143-3p and hsa-miR-193b-3p in the EVs

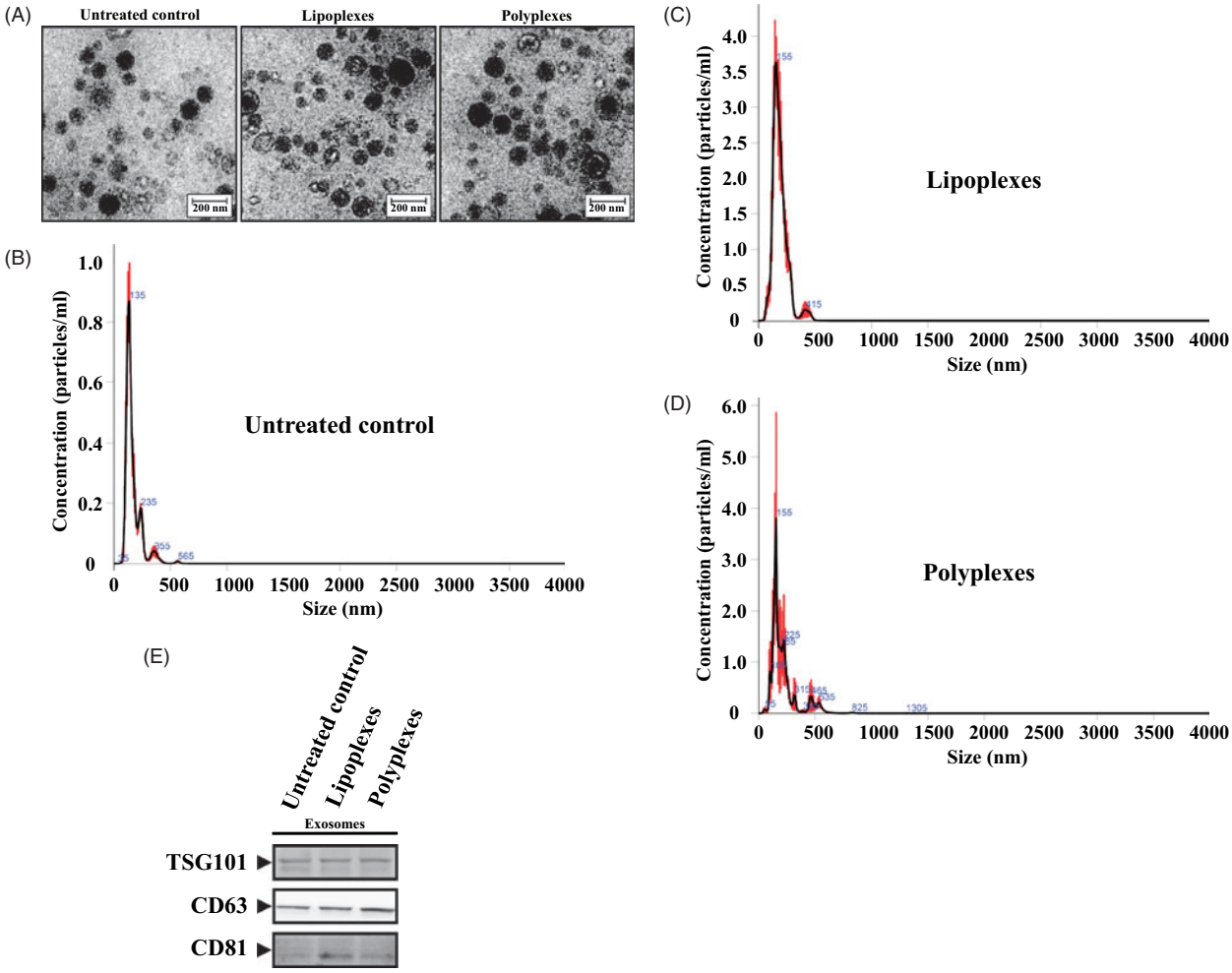

Figure 2. Characterization of isolated EVs. (A) Representative electronic microscopic images of the isolated EVs produced by using *in situ* wet-cell TEM. The size distributions and concentrations of PBS-diluted EVs from (B) untreated, (C) lipoplex-treated and (D) polyplex-treated HeLa cells by NTA. The concentrations shown were multiplied by 10^7. (E) Western blot analysis of hallmark proteins of exosomes (TSG101, CD63 and CD81) from the isolated EVs. The data shown are from one representative experiment among three similar experiments.

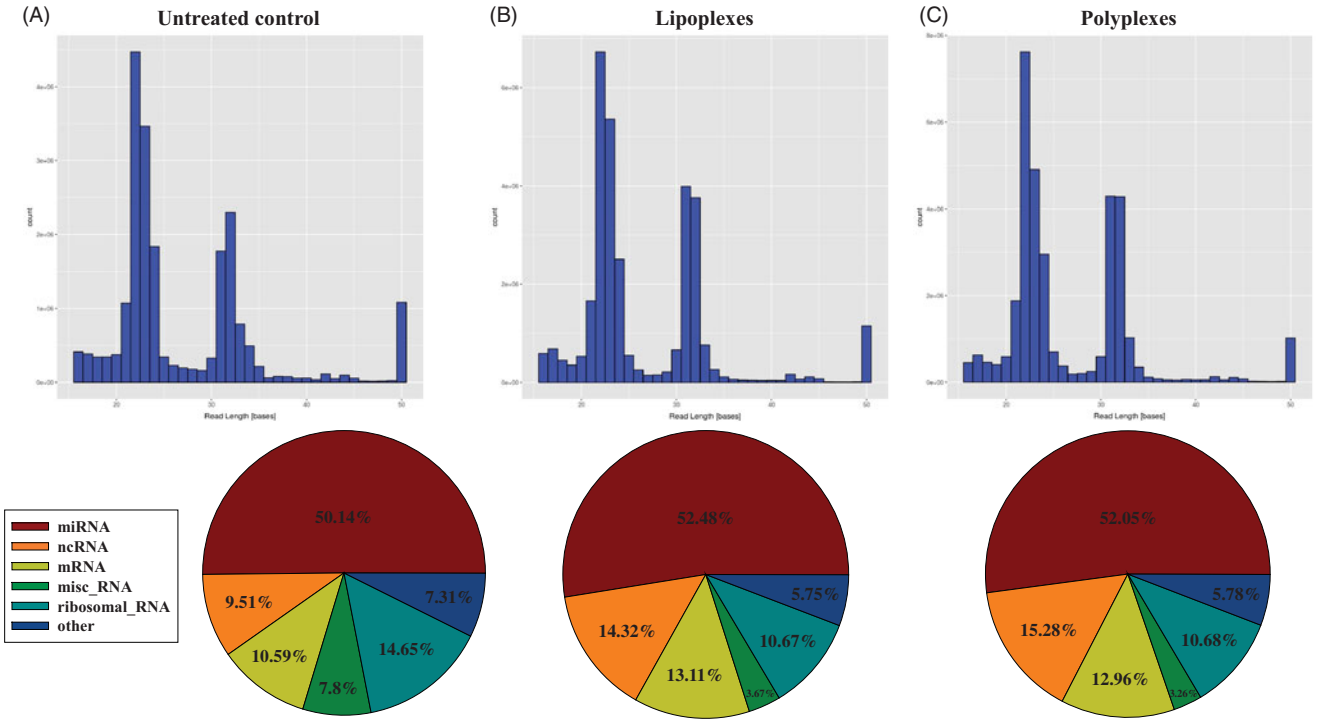

Figure 3. Length distribution of mapped sequenced reads of isolated EVs from (A) untreated, (B) lipoplex-treated and (C) polyplex-treated HeLa cells.

Table 1. The differentially expressed miRNAs with statistically significant changes (p values $< .05$) in EV content from HeLa cells in response to lipoplexes.

Precursor ID	miRNA	$\log_2$ Fold change	p Value
Up-regulated			
hsa-let-7b	hsa-let-7b-3p	1.269	.007
hsa-mir-100	hsa-miR-100-3p	1.429	.008
hsa-mir-143[a]	**hsa-miR-143-3p**[a]	1.219	.009
hsa-mir-193b[a]	**hsa-miR-193b-3p**[a]	1.204	.011
hsa-mir-196a-1	hsa-miR-196a-5p	1.200	.020
hsa-mir-23a	hsa-miR-23a-5p	1.128	.028
hsa-mir-23b	hsa-miR-23b-5p	1.048	.037
hsa-mir-26a-2	hsa-miR-26a-2-3p	0.919	.040
hsa-mir-33b	hsa-miR-33b-3p	1.106	.041
hsa-mir-432	hsa-miR-432-5p	1.007	.043
hsa-mir-6126	hsa-miR-6126	1.071	.048
hsa-mir-92a-1	hsa-miR-92a-1-5p	0.855	.048
Down-regulated			
hsa-mir-1275	hsa-miR-1275	−1.265	.017
hsa-mir-451a[a]	**hsa-miR-451a**[a]	−1.235	.022
hsa-mir-4461	hsa-miR-4461	−1.041	.045
hsa-mir-3960[a]	**hsa-miR-3960**[a]	−1.075	.046

[a]Overlapped miRNAs between lipoplexes and polyplexes.

Table 2. The differentially expressed miRNAs with statistically significant changes (p values $< .05$) in EV content from HeLa cells in response to polyplexes.

Precursor ID	miRNA	$\log_2$ Fold change	p Value
Up-regulated			
hsa-mir-193b[a]	**hsa-miR-193b-3p**[a]	1.324	.008
hsa-mir-143[a]	**hsa-miR-143-3p**[a]	1.144	.015
Down-regulated			
hsa-mir-423	hsa-miR-423-5p	−1.298	.007
hsa-mir-3960[a]	**hsa-miR-3960**[a]	−1.411	.009
hsa-mir-451a[a]	**hsa-miR-451a**[a]	−1.257	.020
hsa-mir-122	hsa-miR-122-5p	−1.018	.022
hsa-mir-193b	hsa-miR-193b-5p	−1.214	.025
hsa-mir-92b	hsa-miR-92b-5p	−1.171	.030
hsa-mir-4488	hsa-miR-4488	−1.127	.036

[a]Overlapped miRNAs between lipoplexes and polyplexes.

isolated from HeLa cells in response to either lipoplexes or polyplexes are summarised in Table 3 (detailed analysis is included in Supplementary Table 1). The pathways involved were very diverse and various pathways related to cell death and stress, such as the cell cycle, p53 signalling pathway and mTOR signalling pathway, were also identified. The non-viral vectors such as Lipo and PEI are well-known for their considerable cytotoxicity and have also been found to be involved in various pathways related to cell death such as the p53 signalling pathway and mTOR signalling pathway [19]. Therefore, we focussed on these pathways related to cell death and stress for further investigation.

Analysis of related protein expressions of cell death and stress in HeLa cells treated with lipoplexes or polyplexes

Since pathways related to cell death and stress were identified in the EVs in response to lipoplexes or polyplexes, the related protein expressions in the cells were simultaneously examined (Figure 5). The protein levels of cleaved caspase-3, which is related to apoptosis, were detected in HeLa cells treated with lipoplexes or polyplexes (Figure 5(A)). The protein expression levels of cleaved caspase-3 in cells treated with lipoplexes were greater than those in cells treated with polyplexes. This indicated that apoptosis was more up-regulated in cells treated with lipoplexes than in those treated with polyplexes. For cells treated with lipoplexes or polyplexes, autophagy was also induced from the observation of the increases of LC3-II/LC3-I ratios and the decreases of p62 and

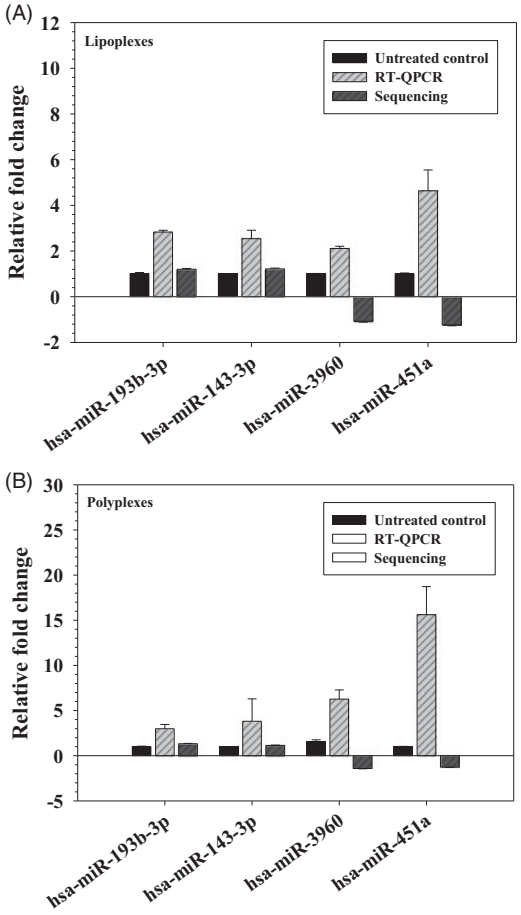

Figure 4. Confirmation of sequencing results of the differentially overlapped expressed miRNAs by RT-QPCR in isolated EVs from (A) lipoplex-treated and (B) polyplex-treated HeLa cells. The data from microarray are represented with mean values and the data from RT-QPCR are shown as means ± standard deviation ($n = 3$).

beclin-1 (Figure 5(B)). Both the treatment with lipoplexes and treatment with polyplexes resulted in the down-regulation of PI3K, AKT, and mTOR phosphorylation as compared with the levels of such phosphorylation in the untreated cells (Figure 5(C)). This implied that the inhibition of the mTOR signalling pathway resulted in the induction of autophagic cell death after treatment with lipoplexes or treatment with polyplexes. In the upstream of the mTOR signalling pathway, key proteins (Igf1, insulin receptor β, and IRS-1) were also down-regulated in the cells treated with lipoplexes or polyplexes (Figure 5(D)). Finally, key proteins (TGF-β1, Smad2 and Smad3) in the TGF-beta signalling pathway, which is closely related to autophagy and plays an important role in tumour micro-environments, were up-regulated after treatment with lipoplexes or treatment with polyplexes (Figure 5(E)) [26]. However, greater up-regulation of the above proteins in cells treated with lipoplexes was shown as compared with their up-regulation in cells treated with polyplexes.

Discussion

EVs have been found to be involved in intercellular communication between cancer cells and the surrounding microenvironments through their shuttling of cargoes such as miRNAs during various stages of tumour development and metastasis [27]. Recently, the applications of non-viral anti-cancer gene delivery systems for cancer therapy have been successfully investigated both *in vitro* and

Table 3. The prediction of target genes and KEGG pathway analysis for the differentially overlapping expressed miRNAs.

miRNAs	KEGG pathway	*p* Value	No. of target genes	No. of miRNAs involved
Up-regulated				
hsa-miR-143-3p	Prion diseases	3.70E-17	5	2
hsa-miR-193b-3p	Fatty acid biosynthesis	5.49E-14	1	2
	Cell cycle	8.21E-08	39	2
	Fatty acid metabolism	1.29E-07	6	2
	Lysine degradation	1.00E-06	15	2
	Proteoglycans in cancer	7.28E-06	36	2
	Protein processing in endoplasmic reticulum	1.90E-05	40	2
	Glioma	8.81E-05	19	2
	Spliceosome	.000110598	32	2
	Selenocompound metabolism	.000164303	9	2
	DNA replication	.000221063	15	2
	Bladder cancer	.000310883	15	2
	Hippo signalling pathway	.000375311	28	2
	Hepatitis B	.000732559	33	2
	Adherens junction	.00144921	17	2
	Base excision repair	.001740291	10	2
	Non-small cell lung cancer	.001740291	16	2
	Melanoma	.00252441	20	2
	Viral carcinogenesis	.002899506	41	2
	Oocyte meiosis	.005644064	26	2
	Small cell lung cancer	.005644064	22	2
	Estrogen signalling pathway	.006828861	23	2
	Biosynthesis of unsaturated fatty acids	.008240064	3	2
	p53 signalling pathway	.008240064	19	2
	Chronic myeloid leukaemia	.010474011	18	2
	Endocytosis	.012703556	38	2
	mTOR signalling pathway	.013908739	17	2
	Focal adhesion	.013908739	42	2
	Central carbon metabolism in cancer	.013908739	17	2
	Pathways in cancer	.016538475	65	2
	Fc gamma R-mediated phagocytosis	.020119298	21	2
	Thyroid cancer	.021039712	9	2
	Regulation of actin cytoskeleton	.023668553	37	2
	RNA transport	.024817205	32	2
	Pancreatic cancer	.025021395	17	2
	One carbon pool by folate	.03714743	7	1
	Prostate cancer	.03750987	21	2

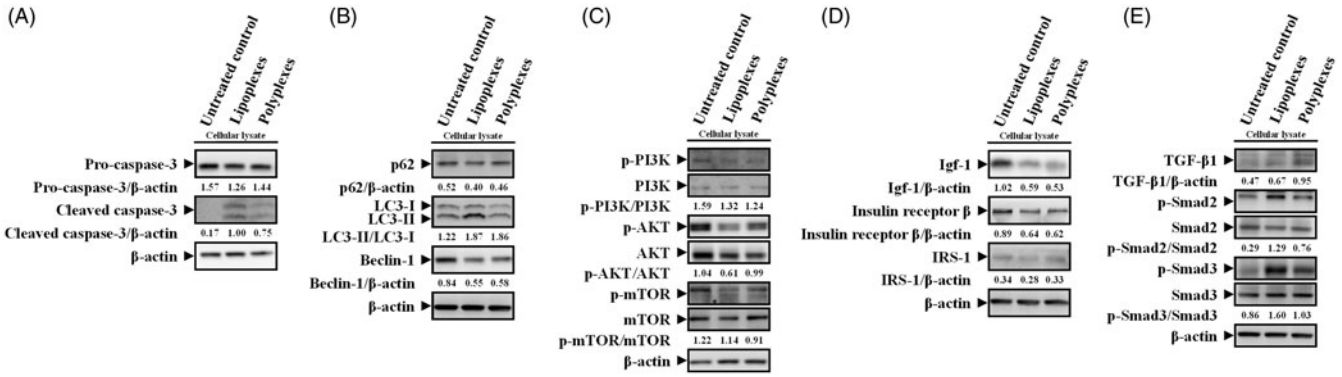

Figure 5. Western blot analysis of key proteins of (A) apoptosis (pro-caspase-3, and cleaved caspase-3), (B) autophagy (p62, LC3-I, LC3-II, and Beclin-1), (C) mTOR signalling pathway (phosphorylated PI3K (p-PI3K), PI3K, phosphorylated Akt (p-AKT), AKT, phosphorylated mTOR (p-mTOR) and mTOR), (D) upstream of mTOR signalling pathway (Igf1, insulin receptor β, and IRS-1), and (E) TGF-beta signalling pathway (TGF-β1, phosphorylated Smad2 (p-Smad2), Smad2, phosphorylated Smad3 (p-Smad3), and Smad3). The data shown are from one representative experiment among three similar experiments. β-actin was used as the loading internal control. The ratios of band density were calculated with AlphaImager 2200 software.

in vivo [28]. Also, the intrinsic anti-tumour property of non-viral vectors has been demonstrated to influence the regulation of gene expressions in cancer cells [17,29]. Still, the molecular information involved in intercellular communications after the non-viral delivery of genetic material into cancer cells has yet to be completely explored. Therefore, the aim of this study was to focus on the intercellular molecular information in cancer cells after non-viral gene delivery by exploring the miRNA profiles within cancer cell-derived EVs. In a previous study, the common effects on the regulation of miRNA expressions in cells treated with Lipo as well as cells treated with PEI were demonstrated [19]. Furthermore, vector-related differences in miRNA delivery have been shown to occur in different cellular responses, including those involving molecular mechanisms related to cell death and survival [20]. The differences in the vector-related effects of Lipo and PEI are also reflected in the differences in cytotoxicity and transfection efficiency (Figure 1), and these differences resulted, in turn, in the differentially expressed miRNAs in the secreted EVs (Tables 1 and 2). In this study, we focussed on the identification of differentially overlapped expressed miRNAs in EVs in order to illustrate the

common characteristics of non-viral vectors in cancer cells. The differences between Lipo and PEI in terms of differentially expressed miRNAs will be further investigated.

In our study, polymer (polyethylene glycol (PEG)) precipitation was used for the isolation of EVs from HeLa cells. By applying pre-isolation 0.22 μm filtration, mostly exosomes (50–200 nm) were confirmed in our samples according to the characteristics of exosomes presented in Figure 2. However, the presence of other types of EVs in the isolated EVs cannot be completely excluded.

Based on both the sequencing and RT-QPCR studies, overlapped up-regulated hsa-miR-143–3p and hsa-miR-193b-3p were identified in the EVs secreted in response to both lipoplexes and polyplexes (Figure 4). In previous reports, hsa-miR-143–3p has been observed in the secreted EVs of prostate and lung cancers and served as tumour suppressor targeted TGF-beta signalling pathway in colorectal cancer [30–32]. In our study, various pieces of evidence regarding the activations of key protein expressions of the TGF-beta signalling pathway in EVs (Figure 5), were consistent with these observations. Also, hsa-miR-193b-3p has been found to be up-regulated in chemotherapy resistant oesophageal cancer cell lines and has been found to act as a tumour suppressor in T-cell acute lymphoblast leukaemia [33,34]. However, the function of hsa-miR-193b-3p in cancer-secreted EVs has not been reported. The direct links of key proteins of apoptosis and autophagy in Figure 5 to hsa-miR-143–3p and hsa-miR-193b-3p in EVs need to be verified in the future. Also, the putative target cells of cancer-secreted EVs after gene delivery need to be further identified. In our study, the selection criteria used to identify differentially expressed miRNA were set as $|\log_2 \text{Fold change}| \geq 0.585$ and p values $<.05$. The selection criteria used were based on the data handling capacity and statistical significance. Using different selection criteria could lead to different panels of miRNAs for the analysis. Also, a variety of powerful biological network analytical tools, such as protein–protein interaction (PPI) network analysis, Cytoscape, and the Database for Annotation, Visualization and Integrated Discovery (DAVID), will be helpful for the studying complex cellular processes and functions of the predicted genes in our study in order to overcome the current study's limitation in terms of lacking functional and big data on the miRNA profiles. In this study, we mainly focussed on hsa-miR-143–3p and hsa-miR-193b-3p, which represent the regulation of gene expression by common non-viral vectors in cancer cells. The other differentially expressed miRNAs, which relate to individual characteristics of non-viral vectors, will be a focus of our future investigations.

Finally, the overexpression of GFP resulting in oxidative stress in cells has been reported, especially in HeLa cells, where GFP overexpression has been reported to result in the up-regulation of various genes associated with inflammation [35]. Since the induction of oxidative stress can activate redox signalling pathways that result in cell death, the relationships among oxidative stress, GFP overexpression and delivery systems in our study need to be further investigated.

Conclusion

Our exploration of the miRNA profiles in cancer cell-derived EVs provides insightful information regarding intercellular communication after non-viral gene delivery. In particular, the overlapping up-regulation of two miRNAs (hsa-miR-143-3p and hsa-miR-193b-3p) was found to be a shared outcome of using different non-viral vectors, and these miRNAs could potentially serve as targets of non-viral cancer gene therapy.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by grant MOST 106–2221-E-041–005 from the Ministry of Science and Technology, Taiwan.

References

[1] Vader P, Mol EA, Pasterkamp G, et al. Extracellular vesicles for drug delivery. Adv Drug Deliv Rev. 2016;106:148–156.
[2] Gopal SK, Greening DW, Rai A, et al. Extracellular vesicles: their role in cancer biology and epithelial-mesenchymal transition. Biochem J. 2017;474:21–45.
[3] Maas SL, Breakefield XO, Weaver AM. Extracellular vesicles: unique intercellular delivery vehicles. Trends Cell Biol. 2017;27:172–188.
[4] Pitt JM, Kroemer G, Zitvogel L. Extracellular vesicles: masters of intercellular communication and potential clinical interventions. J Clin Investig. 2016;126:1139–1143.
[5] Shahabipour F, Barati N, Johnston TP, et al. Exosomes: nanoparticulate tools for RNA interference and drug delivery. J Cell Physiol. 2017;232:1660–1668.
[6] Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharm Sin B. 2016;6:287–296.
[7] Wu CY, Du SL, Zhang J, et al. Exosomes and breast cancer: a comprehensive review of novel therapeutic strategies from diagnosis to treatment. Cancer Gene Ther. 2017;24:6–12.
[8] Tomasetti M, Lee W, Santarelli L, et al. Exosome-derived microRNAs in cancer metabolism: possible implications in cancer diagnostics and therapy. Exp Mol Med. 2017;49:e285.
[9] Zhou Y, Zhou G, Tian C, et al. Exosome-mediated small RNA delivery for gene therapy. Wiley Interdiscip Rev RNA. 2016;7:758–771.
[10] Guan S, Rosenecker J. Nanotechnologies in delivery of mRNA therapeutics using nonviral vector-based delivery systems. Gene Ther. 2017;24:133–143.
[11] Hill AB, Chen M, Chen CK, et al. Overcoming gene-delivery hurdles: physiological considerations for nonviral vectors. Trends Biotechnol. 2016;34:91–105.
[12] Pahle J, Walther W. Vectors and strategies for nonviral cancer gene therapy. Expert Opin Biol Ther. 2016;16:443–461.
[13] Del Pozo-Rodríguez A, Solinís MÁ, Rodríguez-Gascón A. Applications of lipid nanoparticles in gene therapy. Eur J Pharm Biopharm. 2016;109:184–193.
[14] Falagan-Lotsch P, Grzincic EM, Murphy CJ. New advances in nanotechnology-based diagnosis and therapeutics for breast cancer: An assessment of active-targeting inorganic nanoplatforms. Bioconjug Chem. 2017;28:135–152.
[15] Wagner E. Polymers for nucleic acid transfer-an overview. Adv Genet. 2014;88:231–261.
[16] Akhtar S. Cationic nanosystems for the delivery of small interfering ribonucleic acid therapeutics: a focus on toxicogenomics. Expert Opin Drug Metab Toxicol. 2010;6:1347–1362.
[17] Beyerle A, Irmler M, Beckers J, et al. Toxicity pathway focused gene expression profiling of PEI-based polymers for pulmonary applications. Mol Pharm. 2010;7:727–737.

[18] Lin CW, Jan MS, Kuo JH. Autophagy-related gene expression analysis of wild-type and *Atg 5* gene knockout mouse embryonic fibroblast cells treated with polyethylenimine. Mol Pharmaceutics. 2014;11:3002–3008.

[19] Lin CW, Jan MS, Kuo JH. Exploring microRNA expression profiles related to the mTOR signaling pathway in mouse embryonic fibroblast cells treated with polyethylenimine. Mol Pharm. 2015;12:2858–2868.

[20] Lin CW, Jan MS, Kuo JH. The vector-related influences of autophagic microRNA delivery by Lipofectamine 2000 and polyethylenimine 25K on mouse embryonic fibroblast cells. Eur J Pharm. Sci 2017;101:11–21.

[21] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–2120.

[22] Friedländer MR, MacKowiak SD, Li N, et al. MiRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. Nucleic Acids Res. 2012;40:37–52.

[23] Langmead B, Trapnell C, Pop M, et al. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 2009;10:R25.

[24] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.

[25] Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, et al. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions. Nucleic Acids Res. 2018;46:D239–D245.

[26] Liu FL, Mo EP, Yang L, et al. Autophagy is involved in TGF-β1-induced protective mechanisms and formation of cancer-associated fibroblasts phenotype in tumor microenvironment. Oncotarget. 2016;7:4122–4141.

[27] Kosaka N, Yoshioka Y, Fujita Y, et al. Versatile roles of extracellular vesicles in cancer. J Clin Investig. 2016;126:1163–1172.

[28] Kozielski KL, Rui Y, Green JJ. Non-viral nucleic acid containing nanoparticles as cancer therapeutics. Expert Opin Drug Deliv. 2016;13:1475–1487.

[29] Omidi Y, Barar J, Akhtar S. Toxicogenomics of cationic lipid-based vectors for gene therapy: impact of microarray technology. Curr Drug Deliv. 2005;2:429–441.

[30] Lawson J, Dickman C, MacLellan S, et al. Selective secretion of microRNAs from lung cancer cells via extracellular vesicles promotes CAMK1D-mediated tube formation in endothelial cells. Oncotarget. 2017;8:83913–83924.

[31] Rodriguez M, Bajo-Santos C, Hessvik NP, et al. Identification of non-invasive miRNAs biomarkers for prostate cancer by deep sequencing analysis of urinary exosomes. Mol Cancer. 2017;16:156.

[32] Su J, Liang H, Yao W, et al. MiR-143 and MiR-145 regulate IGF1R to suppress cell proliferation in colorectal cancer. PloS One. 2014;9:e114420.

[33] Hummel R, Sie C, Watson D, et al. MicroRNA signatures in chemotherapy resistant esophageal cancer cell lines. World J Gastroenterol. 2014;20:14904–14912.

[34] Mets E, Van der Meulen J, Van Peer G, et al. MicroRNA-193b-3p acts as a tumor suppressor by targeting the MYB oncogene in T-cell acute lymphoblastic leukemia. Leukemia. 2015;29:798–806.

[35] Ganini D, Leinisch F, Kumar A, et al. Fluorescent proteins such as eGFP lead to catalytic oxidative stress in cells. Redox Biol. 2017;12:462–468.

行政院科技部補助出席國際學術會議報告

107 年 8 月 8 日

報告人姓名	郭榮華 嘉南藥理大學藥學系		
時間 會議地點	07/22/2018-07/24/2018 New York Hilton Midtown Manhattan Hotel, New York City, New York , USA		
會議名稱	（中文）2018 控制釋放協會年會暨展覽會 （英文）2018 Annual Meeting & Exposition of the Controlled Release Society		
發表論文題目	（中文）**脂質及聚乙烯亞胺基因傳送系統對於人類子宮頸癌海拉氏細胞衍生之細胞胞外泌體內 MicroRNA 資料之探討** (英文) **Exploring The Microrna Profiles In Hela Cell-derived Extracellular Vesicles In Responses To Cationic Lipid- Or Polyethylenimine-mediated Gene Delivery**		

報告內容應包括下列各項：

一、參加會議經過

本屆 2018 年的控制釋放協會年會暨展覽會 (2018 Annual Meeting & Exposition of the Controlled Release Society)為期三天(07/22/2018-07/24/2018)，在纽约市 (New York, USA)會議中心舉行。此次會議為藥學及控制釋放領域中最為重要的會議之一，今年有超過 300 篇壁報論文、超過 50 位邀請演講、超過 30 廠商設攤展示的相關研究領域學業界參與這場盛會。來自全世界各大製藥公司的研發人員都聚集於此，一方面發表最新的研究成果，可以了解未來控制釋放與創新應用方向，另一方面提供控制釋放從事相關領域工作者一個交流平台。本次 plenary session 所邀請的是來自 Purdue University (USA) 之 Prof. Kinam Park 演講與討論 A Long Walk to PLGA 、Harvard Medical School and Massachusetts General Hospital (USA) 之 Prof. Rakesh Jain 演講與討論 Re-engineering the Tumor Microenvironment to Improve Cancer Treatment、 Central PA Chapter of the National Pancreas Foundation (NPF) (USA) 之 Dr. Lora Kelly 演講與討論 Pancreatic Cancer: A Survivor's Journey、Imperial College London (UK) 之 Prof. Molly Stevens 演講與討論 Exploring and Engineering the Cell-material Interface for Drug Delivery，因此對於學界在研究成果如何轉換成兼具實用與價值的產品，都可以在這個會議中獲益無窮。而本次科學會議涵蓋的主題非常廣泛及實用，如 Micro- and Nanoparticle Delivery、Delivery Technologies in Cosmetics, Personal Care, and Household Products 、3D Printing in Drug Delivery 、 Nucleic Acid Delivery、Regional Delivery、Advanced Immunotherapy For Neuro- Oncology、Polymer Therapeutics Clinical Translation:、Antibody-Drug Conjugates、Toward an Improved Understanding of Complex Drug Substances and Complex Formulations Through GDUFA Regulatory Research Program、Parenteral Controlled Release、 Cells as Delivery Vehicles、Manufacture, Characterization, Measurement, and Stability、Implant Formulation Optimization and In Vivo Performance、Digitally Controlled Drug Delivery、Delivery of Proteins, Peptides and Vaccines、Encapsulation for Industrial Applications、Tropical and Transdermal Delivery、Research Highlight Talk 等、及各種各樣的 FOCUS GROUP，另外 Pearls of Wisdom Session 討論 Targeted Nano-Drugs、Technology Forums 展示產品科學技術平台、Industry Roundtables 討論如何轉換構想成為成功的產品，令人目不暇給，實屬挖寶之旅。

二、與會心得

這次我報告的壁報論文，被大會安排在壁報論文場次中張貼並與其他有興趣的研究學者一同討論，在壁報張貼期間許多相關領域的專家都前來參觀壁報，也提出許多有用的意見及討論，使我能知道自己研究的盲點何在及本實驗在生技製藥界的價值及重要性，因此受益匪淺。此外在會議進行期間也有許多出版社、廠商設攤展示，進行一連串示範及演講活動，對於往後在研究中所使用相關材料及儀器亦十分有所幫助。此次獲益良多，更對未來的研究主題有正面的幫助。由於此會議所涵蓋之研究領域很廣且選擇性也多，有更多國際的交流與世界各國的精英們學習，也是重要的收穫。

三、攜回資料名稱及內容

大會相關論文手冊、廠商 DM

Exploring the microRNA profiles in HeLa cell-derived extracellular vesicles in responses to cationic lipid- or polyethylenimine-mediated gene delivery

Presenting Author: Jung-Hua Kuo, Chia Nan University of Pharmacy and Science, Taiwan

Co-Authors: Chia-Wei Lin, Chung Shan Medical University, Taiwan

Introduction: After the non-viral delivery of genetic material into cancer cells, the molecular mechanisms of intercellular communication are still not precisely understood. Among the various biological functions of cell-derived extracellular vesicles (EVs), the most well-known is that of intercellular communication, which they accomplish by shuttling molecular messengers such as microRNA (miRNA) to target cells. Therefore, the aim of this study is to probe the miRNA profiles in cancer cell-derived EVs in responses to cationic non-viral gene delivery systems for better understanding the molecular mechanisms of intercellular communication in cancer cells after non-viral gene delivery.

Methods: Lipofectamine 2000 and linear jet polyethylenimine are used for the delivery of gene fluorescent protein plasmid in HeLa cervical cancer cells. EVs are extracted and the contents of RNA are subject to next generation sequencing on a HiSeq 2500 platform (Illumina, USA). The differentially expressed miRNAs were identified and confirmed by real-time quantitative reverse transcriptase-polymerase chain reaction.

Results: Lipoplexes resulted in more up- and down-regulated expressed miRNAs than those of polyplexes. Two overlapped up-regulated miRNAs (hsa-miR-143-3p and hsa-miR-193b-3p) were quantitatively consistent with the results from sequencing data.

Conclusion: Our findings might provide further molecular information of intercellular communication in cancer cells mediated by non-viral gene delivery systems.

Acknowledgments: This study was supported by grant MOST 106-2221-E-041-005 from the Ministry of Science and Technology, Taiwan.

References: Pitt, J.M.; Kroemer, G.; Zitvogel, L. *J Clin Invest.* 2016, 126, 1139-1143;

Pahle, J.; Walther, W. *Expert Opin Biol Ther.* 2016, 16, 443-461.

Learning Objectives

Evaluate the miRNA profiles in HeLa cell-derived extracellular vesicles in responses to non-viral gene delivery.

Analyze the differentially expressed miRNAs by next generation sequencing.

Identify the molecular mechanisms of intercellular communication in cancer cells after non-viral gene delivery.

Print this Page for Your Records.

Dear Junghua Kuo,

Thank you for submitting your abstract Exploring The Microrna Profiles In Hela Cell-derived Extracellular Vesicles In Responses To Cationic Lipid- Or Polyethylenimine-mediated Gene Delivery to the 2018 Controlled Release Society Annual Meeting being held July 22-24, 2018, at the New York Hilton Midtown Manhattan Hotel in New York City, New York, U.S.A. On behalf of the CRS Programming Committee, we are pleased to inform you that your abstract has been accepted for a poster presentation.

IMPORTANT INFORMATION

- *NEW! Poster size is A0 (Width: 84.1 cm Height: 118.9 cm) or (Width: 33.11" Height 46.81")*
- *Poster Author Sessions: authors will either present on Monday, July 23 or Tuesday, July 24.*
- *Poster number assignments and presenter schedule will be sent after May 24.*

As part of the abstract submission process, it is understood that acceptance of your abstract by the program committee would constitute your participation as a presenter. Your poster number will be sent to you in the next few weeks. At that time, we will let you know the specific time you will be presenting your poster during each session.

You may display your information in figures, tables, text, photographs, etc. Please prepare all illustrations neatly and legibly beforehand, in a size sufficient to read at a distance of three (3) feet. A series of typewritten sheets attached to the poster board is not acceptable. Materials will be available to fasten your material to the poster board.

For your convenience, please visit the 2018 CRS Annual Meeting website at 2018.controlledreleasesociety.org for registration and hotel information. On-line registration will be opening on or around March 30th, 2018. Please be sure to register prior to May 24, 2018 to receive the early-bird rate.

Please note that all presenters are expected to cover their own travel and lodging and pay the registration fees.

ALL presenters are required to register for the meeting.

If you have any questions, please feel free to contact the CRS Meetings Department at jsalabritas@controlledreleasesociety.org.

We look forward to meeting you at what promises to be an exciting and informative program. For more information regarding the meeting and the Controlled Release Society, please go to our website at 2018.controlledreleasesociety.org.

See you in New York City!

Kind Regards,

The 2018 Annual Meeting Programming Committee

Hamid Ghandehari, Chair

Annual Meeting Programming Chair

Twan Lammers

Annual Meeting Programming Committee Member

http://abstractsonline.com/notify/NotifySummary.asp?Action=Confirm&MKey={A5A4647C-C6BB-4667-A811-03281BB43B74}&CKey={59E47811-0D9C-4834-8AE

Yu-Kyoung Oh
Annual Meeting Programming Committee Member

Your Response: Confirm

Controlled Release Society
1120 Route 73, Suite 200
Mt. Laurel, NJ 08054, U.S.A.
Tel: +1/856-439-1385
Fax: +1/856-439-0525
Email: info@controlledreleasesociety.org

106年度專題研究計畫成果彙整表

計畫主持人：郭榮華					**計畫編號：**106-2221-E-041-005-	
計畫名稱：陽離子性高分子相關於細胞胞外泌體之應用與開發						
成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			1	篇	The microRNA expression profiles in extracellular vesicles from HeLa cancer cells in response to cationic lipid- or polyethylenimine-mediated gene delivery. J Drug Target. 2018 Jul 24:1-9. doi: 10.1080/1061186X.2018.1491977.
		研討會論文			1		Exploring The Microrna Profiles In Hela Cell-derived Extracellular Vesicles In Responses To Cationic Lipid- Or Polyethylenimine-mediated Gene Delivery. 2018 Annual Meeting & Exposition of the Controlled Release Society
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	

		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
參與計畫人力	本國籍	大專生			0	人次	
		碩士生			0		
		博士生			1		王羿忻
		博士後研究員			0		
		專任助理			0		
	非本國籍	大專生			0		
		碩士生			0		
		博士生			0		
		博士後研究員			0		
		專任助理			0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）							

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：■已發表　□未發表之文稿　□撰寫中　□無
   專利：□已獲得　■申請中　□無
   技轉：□已技轉　■洽談中　□無
   其他：（以200字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   本計畫完成利用生物晶片來探討陽離子性高分子複合体在最佳轉染條件下，對於人類子宮頸癌HeLa細胞所分泌之EVs內所含RNA信息資料，可以幫助我們了解其與癌症細胞間之溝通作用機轉以避免臨床應用上之副作用。本計劃所設計的高分子載體核酸製劑在一般實驗室中就可以大量置備，不僅使成本下降而且減少因缺乏再現性造成分析結果的困難之可能，是一種具有發展潛力達到治療的方式。又根據衛生署資料顯示，癌症是近年來台灣區十大死亡原因的首位。近來利用調控細胞自我吞噬之運作功能為基礎所發展之miRNA-base 抗癌症治療策略，是以了解細胞死亡機轉之分子運作模式而啟動細胞死亡分子開關，是一個期望能夠達到使用毒性低又能有效提昇的方式，本計畫了解陽離子性高分子miRNA輸送系統對細胞自我吞噬作用之影響，在治療癌症的應用潛力無窮。

4. 主要發現
   本研究具有政策應用參考價值：□否　■是，建議提供機關科技部,
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：■否　□是
   說明：（以150字為限）