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Revisiting Cationic Phosphorus Dendrimers as a Nonviral Vector for Optimized Gene Delivery Towards Cancer Therapy Applications

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KEYWORDS: cationic phosphorus dendrimers; gene delivery; cancer therapy; surface modification

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ABSTRACT

Gene delivery, one important cancer therapy mode, still remains to be challenging due to the shortage of highly efficient and non-toxic non-viral vectors. Here, we revisit the development of cationic phosphorus dendrimers by synthesizing them with different generations (G1-3) and surface ligands (1-(2-aminoethyl) pyrrolidine, 1-(3-aminopropyl) piperidine, or 1-(2-aminoethyl) piperidine) for optimized gene delivery toward cancer gene therapy applications. First, the synthesized dendrimer derivatives were used to compact plasmid DNA (pDNA) encoding enhanced green fluorescent protein (EGFP) to optimize their gene delivery efficiency by varying the dendrimer generations and surface polycationic ligands. We show that all dendrimer/pDNA polyplexes display good cytocompatibility, and the 1-(2-aminoethyl) pyrrolidine-modified protonated G1 dendrimers (1-G1) display the best gene delivery efficiency to HeLa cells under the same conditions through flow cytometry and fluorescence microscopic imaging analyses. Hence, 1-G1 dendrimers were then used as a vector to transfect pDNA encoding both EGFP and p53 protein for cancer gene therapy applications. Our results reveal that under the optimized conditions, the transfection of pDNA induces the significant p53 protein expression as verified through the resulted cell cycle arrest (regulation of p21 and Cdk4/Cyclin-D1 expression) and Western blotting. The cancer gene therapy potential of the polyplexes was finally validated through therapy of a xenografted tumor model after intratumoral injection without systemic toxicity. The developed cationic 1-G1 dendrimers may hold a great promise to be used as a powerful vector for cancer gene therapy applications, as well as for highly efficient gene delivery to other biosystems.

INTRODUCTION

Successful gene delivery relies on the highly efficient and non-toxic vectors. Viral-based gene delivery is currently still one of the most efficient methods to introduce exogenous genes into cells.¹⁻² However, concerns about immunogenicity or long-term oncologic effects of the viral vectors have prompted the development of synthetic non-viral gene delivery systems.³⁻⁴ In general, non-viral delivery systems consist of linear cationic polymers, such as polylysine and their conjugates,⁵⁻⁶ branched polyethylenimine,⁷ poly(amidoamine) (PAMAM) or poly(propyleneimine) dendrimers,⁸⁻¹² cationic lipids,¹³⁻¹⁴ or liposomes.¹⁵⁻¹⁶ These vector systems can electrostatically compact negatively charged DNA to form polyplexes for effective cellular uptake to implement the gene expression.

The major advantages of these non-viral delivery systems over viral vectors can be summarized as simplicity to prepare, stability, easiness to be modified, and biosafety. However, unlike viral vectors that have evolved means to overcome cellular barriers and immune defense mechanisms, non-viral gene carriers consistently exhibit limited transfection efficiency due to numerous extra- and intracellular obstacles.¹⁷ Further, some key properties of non-viral gene carriers such as dendrimers with defined architecture, tunable biocompatibility and a high ratio of multivalent surface moieties to molecular volume also make them highly promising to be developed as synthetic (non-viral) vectors for delivery therapeutic nucleic acids.¹⁸⁻²⁰ Among many types of dendrimers, phosphorus dendrimers possessing biocompatible backbone have been employed as anti-prion agents,²¹⁻²² anti-HIV agents,²³ and stimulator of immune blood cells²⁴⁻²⁵ and human natural killer (NK) cells²⁶ depending on the type of their terminal groups.

Cationic phosphorus dendrimers have also been used as promising gene delivery platforms for numerous biomedical applications.²⁷⁻²⁹ The most widely investigated cationic phosphorus dendrimers bear diethylamine termini with cyclotriphosphazene (CTP) core. They have been used as carriers of plasmids,^{12, 30-31} oligodeoxynucleotides³² and siRNAs.³³⁻³⁵ For instance, Loup et al.¹² disclosed the better transfection efficiency of dendrimers in the presence of serum than in the absence of serum for plasmid transfection. As demonstrated by Padié et al.,³⁶ the surface of CTP-cored phosphorous dendrimers can be decorated with cyclic amines such as pyrrolidine,

morpholine, methyl piperazine and phenyl piperazine. Hence, cyclic amine-modified phosphorous dendrimers have been used for gene delivery applications. For instance, pyrrolidine-terminated dendrimers were found to be low-toxic, efficient to form dendriplexes among other surface cyclic amine modifications for highly efficient gene transfection.³⁶ Dzmitruk et al.³⁵ reported the use of piperidine-terminated phosphorus dendrimers to compact siRNA for possible cancer cell gene silencing. However, the molecular mechanism of antiproliferation and in vivo tumor gene silencing were not accomplished. As a whole, there is a lack of systematic and in-depth exploration of cationic phosphorus dendrimers with different cyclic amine groups for optimized gene delivery and also for cancer gene therapy applications.

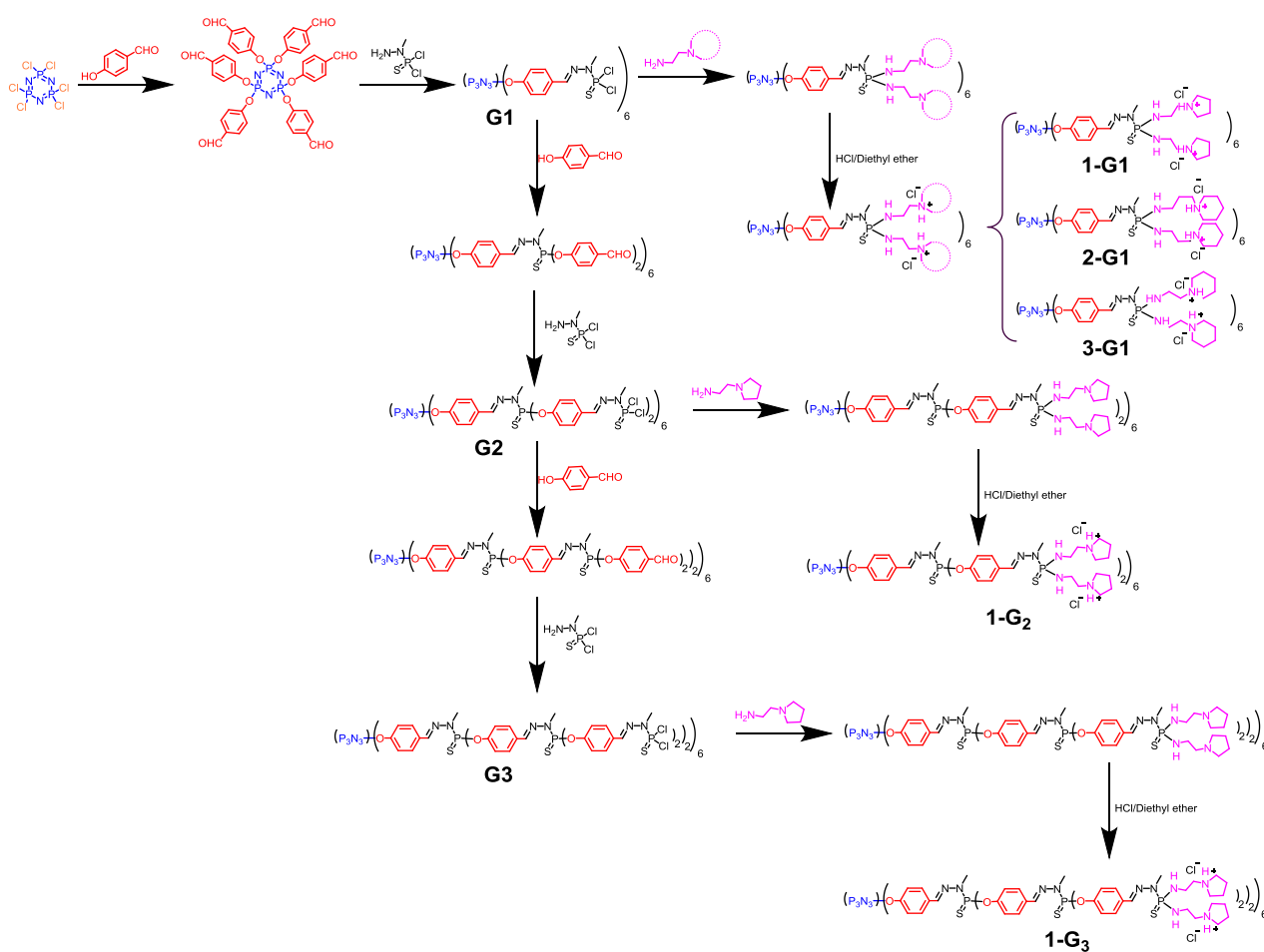


Figure 1. Synthesis of cationic phosphorous dendrimers with varying generations and cyclic amine group.

Here, in this work, we revisited cationic phosphorus dendrimers with different generations and

cyclic amine groups as a non-viral vector for optimized gene delivery towards cancer therapy applications. We first synthesized and characterized the first, second and third generations of phosphorus dendrimers ended with protonated cyclic amine groups (Figure S1 and Figure 1). Then, plasmid DNA (pDNA) encoding enhanced green fluorescent protein (EGFP) was used as a model to test the gene compaction ability of the dendrimers, the cytotoxicity of the dendrimer/pDNA polyplexes, and gene delivery efficiency. After screening, the optimized dendrimer derivative was used as a vector to condense pDNA encoding both EGFP and p53 protein for cancer gene therapy applications through investigation of p53 protein expression validated from cell cycle arrest (regulation of p21 and Cdk4/Cyclin-D1 expression) and Western blotting. Finally, we verified the potential to the polyplexes for gene therapy of a xenografted tumor model after intratumoral injection. According to our thorough literature investigation, this work is a systematic and in-depth exploration of cationic phosphorus dendrimers in gene delivery towards cancer therapy applications.

Table 1. The prepared cationic phosphorus dendrimers.

Generation	Numbers of terminal groups per dendrimers			Series
1	12	1-G`1	1-G1	1
2	24	1-G`2	1-G2	
3	48	1-G`3	1-G3	
				2
1	12	3-G`1	3-G1	3
1	12	2-G`1	2-G1	

n-G`n represents non-protonated dendrimer derivative; n-Gn represents the protonated cationic

dendrimer derivatives.

RESULTS AND DISCUSSION

Synthesis and Characterization of Cationic Dendrimer Derivatives. As shown in Table 1 and Figure 1, we prepared five different cationic phosphorus dendrimers with varying generations and cyclic amine groups. First, generation 1-3 (**G1-3**) dendrimers were prepared and characterized according to the literature.³⁷ Then, the dendrimers were reacted with 1-(2-aminoethyl) pyrrolidine (1-a), 1-(3-aminopropyl) piperidine (2-a), or 1-(2-aminoethyl) piperidine (3-a) to form 1-G`1, 1-G`2, 1-G`3, 3-G`1, or 2-G`1, and protonated to create the final cationic 1-G1, 1-G2, and 1-G3 dendrimers. These dendrimers were fully characterized through different NMR techniques (Figure S2-S6, see details in Supporting Information).

Formation of Dendrimer/pDNA Polyplexes for Optimized Gene Transfection. pDNA encoding EGFP (pDNA-EGFP) was employed to evaluate the gene transfection efficiency of the designed vectors *in vitro*. After formation of dendrimer/pDNA polyplexes under different N/P ratios (the molar ratio of positive charge of the dendrimers to phosphates in the pDNA backbone) through electrostatic interaction (Figure 2A), agarose gel retardation assay was utilized to check the DNA compaction ability of the positively charged phosphorous dendrimers (Figure 2B-F). It is clear that the pDNA-EGFP is able to be completely compressed by the cationic phosphorous dendrimers under different N/P ratios. For the n-G1 (n=1, 2 and 3) dendrimers that possess the same number of positive charges, the DNA condensation ability follows the order of 1G1 (N/P = 1 or above) > 3G1 (N/P = 2 or above) > 2G1 (N/P = 4 or above), imply that the DNA condensation ability is dependent on the type of cyclic amine groups (Figure 2B-D). This could be due to the distinct cyclic amine termini that have different dendrimer spatial structures to have different electrostatic compactions of DNA. Namely, the dendrimers with terminal pyrrolidine rings may render them with higher charge densities (compared to mass) than those with the terminal piperidine rings due to the smaller Mw and less crowded terminal packing of the pyrrolidine rings. Further, compared to 3-a, 2-a have one

more methylene group, hence 2G1 dendrimers have slightly decreased molecular rigidity and charge/mass ratio as compared to 3G1 dendrimers, thus having less DNA compaction ability than 3G1. Therefore, for optimized gene compaction, 1a-modified dendrimers should be better than 2a- and 3a-modified dendrimers with the same generation.

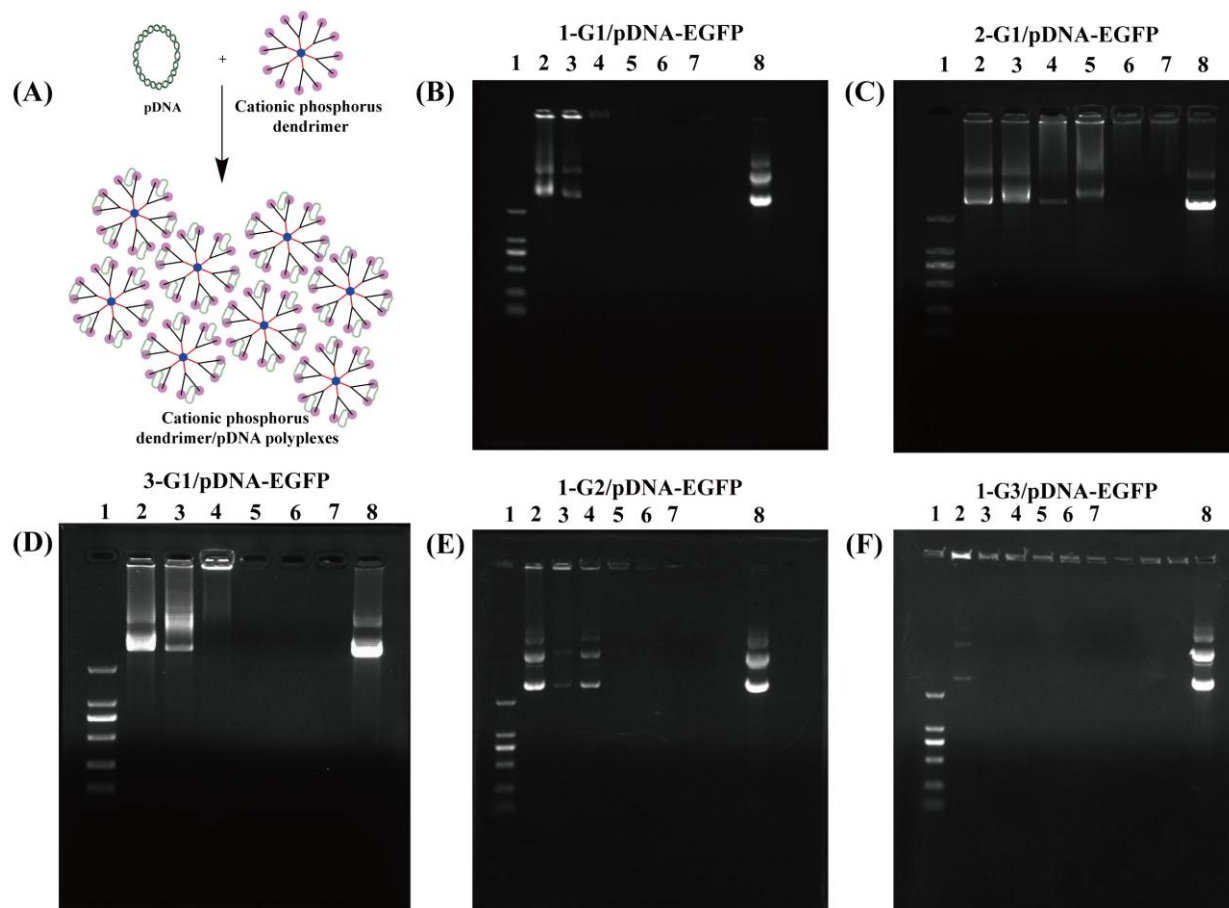


Figure 2. (A) Preparation of the dendrimer/pDNA polyplexes for gene delivery; (B-F) Gel retardation assay of pDNA-EGFP complexed with 1-G1, 2-G1, 3-G1, 1-G2 and 1-G3 at various N/P ratios. Lane 1: DNA marker, lane 2: N/P = 0.25:1, lane 3: N/P = 0.5:1, lane 4: N/P = 1:1, lane 5: N/P = 2:1, lane 6: N/P = 4:1, lane 7: N/P = 6:1, and lane 8: free pDNA-EGFP.

To check the dependence of the dendrimer generation, we investigated the DNA compaction ability of the 1a-modified G1-3 dendrimers (Figure 2B, E-F). It can be seen that the DNA compaction ability is in the order of 1G3 (N/P = 0.5 or above) > 1G1 (N/P = 1 or above) > 1G2 (N/P = 2 or above). It seems that unlike the generation dependence of gene delivery efficiency of PAMAM dendrimer,³⁸ the DNA compaction ability of phosphorous dendrimers with the same

terminal structure has no clear dendrimer generation dependence due to their intrinsic rigid molecular backbones. In this context, one has to comprehensively consider all facts to optimize their gene delivery efficiency.

The hydrodynamic size and surface potential of the polyplexes have been known to be important factors in influencing the cytotoxicity, intracellular uptake, and release of the gene cargos.³⁹⁻⁴¹ The selection of N/P ratios at 10-30 was based on the fact that a large N/P ratio would lead to a compacted structure and a relatively high positive surface potential of the polyplexes, which facilitates enhanced cellular uptake of the polyplexes. As shown in Figure 3A, 1-Gn/pDNA-EGFP (n = 1, 2 and 3) polyplexes are all in the size range of ~250 nm. Nevertheless, the size of the 2-G1 and 3-G1 are around ~ 400 nm. It appears that the 1-a-modified dendrimers have better gene compaction ability than 2-a- and 3-a-modified 2-G1 and 3G1 dendrimers, in agreement with the gel retardation assay data. In addition, under the same 1-a modification, there is no distinct generation-dependent difference in the hydrodynamic size of the dendrimer/pDNA polyplexes for 1G1-3 dendrimers. On the other hand, with the increase of N/P ratio, more dendrimers participate in the compaction of pDNA, thereby leading to decreased size of the polyplexes until reach saturation to have a slightly increased size.

The surface potentials of the dendrimer/pDNA-EGFP polyplexes were then measured (Figure 3B). Clearly, 1-Gn/pDNA-EGFP (n = 1, 2 and 3) polyplexes under various N/P ratios show a surface potential of approximately 20-30 mV. However, the surface potentials of 2-G1 and 3-G1 dendrimers complexed with pDNA-EGFP under different N/P ratios are all around ~10 mV. This suggests that polyplexes of dendrimers with the pyrrole ammonium group possess much more positive surface potential than those with the piperidine ammonium group, presumably due to the adaptability of the pyrrole tertiary ammonium group to pH.³⁶ It should be noted that with the continuous increase of N/P ratio, the interaction between dendrimers and pDNA could reach saturation and the surface potentials of polyplexes do not have a significant change since only the dendrimers covered onto the surface of the polyplexes contribute to the surface potential of the polyplexes. According to the literature,⁴² vector/DNA polyplexes with a size of around 200 nm and a positive surface potential of

20-30 mM are suitable for gene delivery. Hence, the N/P ratio of 20 was selected for the subsequent experiments.

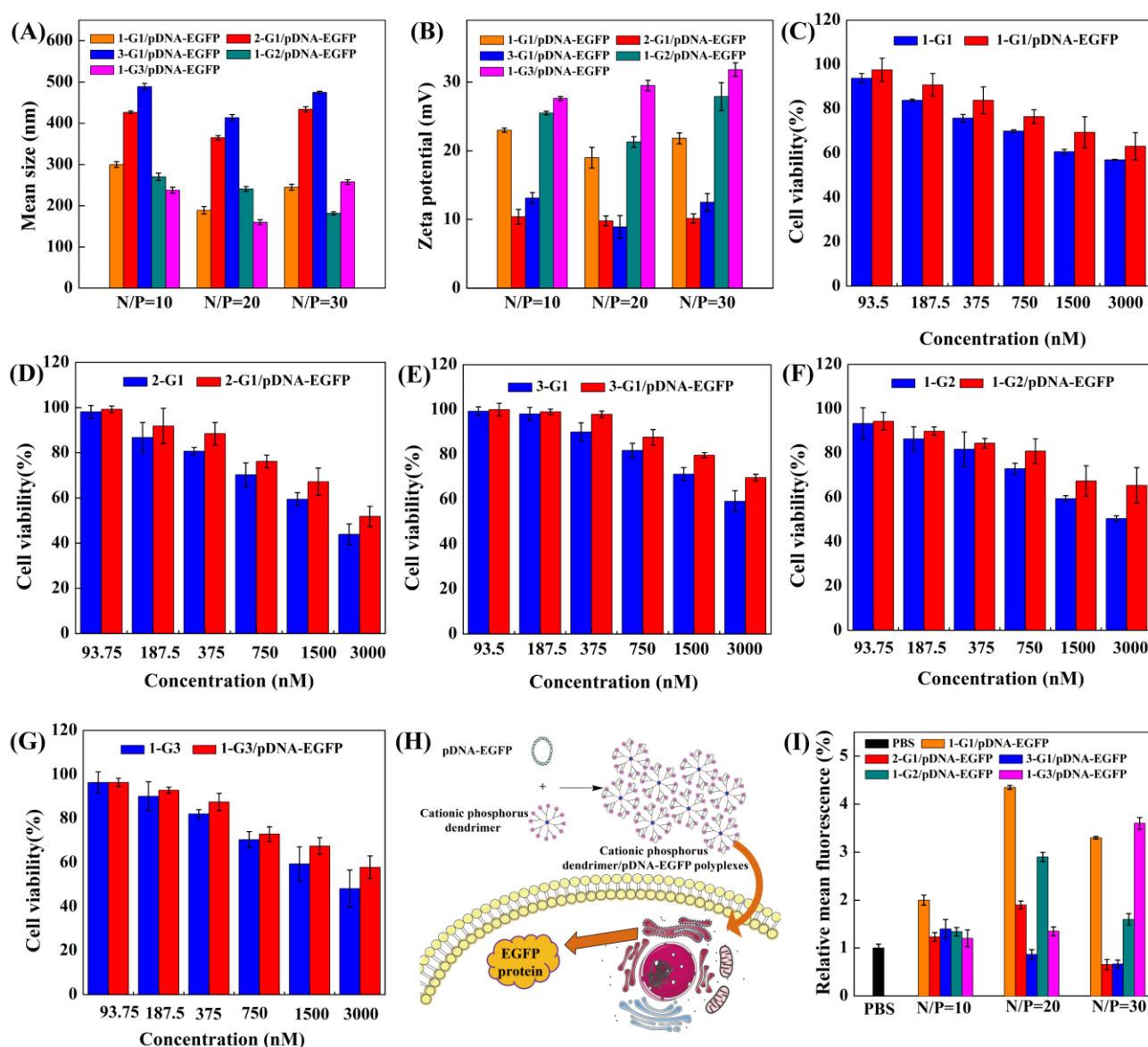


Figure 3. (A) Mean hydrodynamic size and (B) zeta-potential of pDNA-EGFP complexed with 1-G1, 2-G1, 3-G1, 1-G2 and 1-G3 at different N/P ratios; (C-F) CCK8 assay of HeLa cells treated with dendrimers and dendrimer/pDNA-EGFP polyplexes at different dendrimer concentrations for 24 h. Data are presented as mean \pm SD (n = 6); (H) Schematic diagram of the gene transfection of dendrimer/pDNA-EGFP polyplexes to express EGFP protein; (I) Relative EGFP protein expression in the HeLa cells after transfected with the dendrimer/pDNA-EGFP polyplexes at different N/P ratios as measured by flow cytometry.

Further, the cytotoxicity of the dendrimer/pDNA-EGFP was tested via CCK8 viability assay of HeLa cells (a human cervical carcinoma cell line, Figure 3C-F). The cell viability displays a concentration-dependent decreasing trend. At the highest concentration of dendrimers and dendrimer/pDNA (3000 nM, at the N/P ratio of 20), cells display a viability around > 50%. In any case, at the same dendrimer concentration, the dendrimer/pDNA polyplexes show a lower cytotoxicity than the dendrimers, possibly due to the complexation of pDNA that results in reduced positive charges of the dendrimers, in agreement with the literature.⁴³

Next, we evaluated the transfection efficiency of the polyplexes to express EGFP in HeLa cells (Figure 3H-I) through fluorescence microscopic imaging and flow cytometry assay. Since the change of N/P ratio has been reported to have an effect on the gene transfection efficiency in cancer cells,⁴⁴ polyplexes with the N/P ratios at 10-30 were tested. Flow cytometry data reveal that cells transfected with polyplexes formed using dendrimers with pyrrolidine amine termini display much more EGFP expression than those with piperidine amine termini at the same N/P ratios, with the best gene transfection efficiency achieved at an N/P ratio of 20 (Figure 3I). Fluorescence microscopic images qualitatively show the similar results (Figure S7). Therefore, the 1-G1 dendrimers were selected as a vector to transfect the tumor suppressor p53 gene for the subsequent study.

Formation of Dendrimer/pDNA Polyplexes for Gene Therapy of Cancer Cells. The primary objective was to investigate the gene therapy effect in cancer cells. The tumor suppressor p53 pDNA (p53, encoding both EGFP and p53 protein) was complexed with 1-G1 dendrimers to form polyplexes for therapeutics of cancer cells (Figure 4A). Firstly, the ability of 1-G1 to compact pDNA-p53 was evaluated using gel retardation assay (Figure 4B). Apparently, the pDNA-p53 is able to be completely compressed by 1-G1 dendrimers at an N/P ratio of 1 or greater. Secondly, the hydrodynamic size and surface potential of the polyplexes were tested. The surface potential and hydrodynamic size of the polyplexes are ~30 mV and ~200 nm under various N/P ratios (Figure 4C-D), which are suitable for further gene delivery applications. Since the p53 plasmid contains gene segment decoding EGFP, the EGFP gene was used as a reporter gene to first evaluate the transfection and expression efficiency of the 1-G1/pDNA polyplexes. Flow cytometry assay reveals

that the best gene transfection efficiency can be achieved at an N/P ratio of 20 (Figure 4E), which can be further confirmed through qualitative fluorescence microscopic imaging of HeLa cells expressing the EGFP (Figure S8). Therefore, the N/P ratio of 20 was selected for the subsequent experiments. Cytotoxicity assay results reveal that more than the cells possess a viability of 60% after treated with the polyplexes at the highest dendrimer concentration (3000 nM) at the N/P ratio of 20 (Figure 4F), implying the good cytocompatibility of the polyplexes.

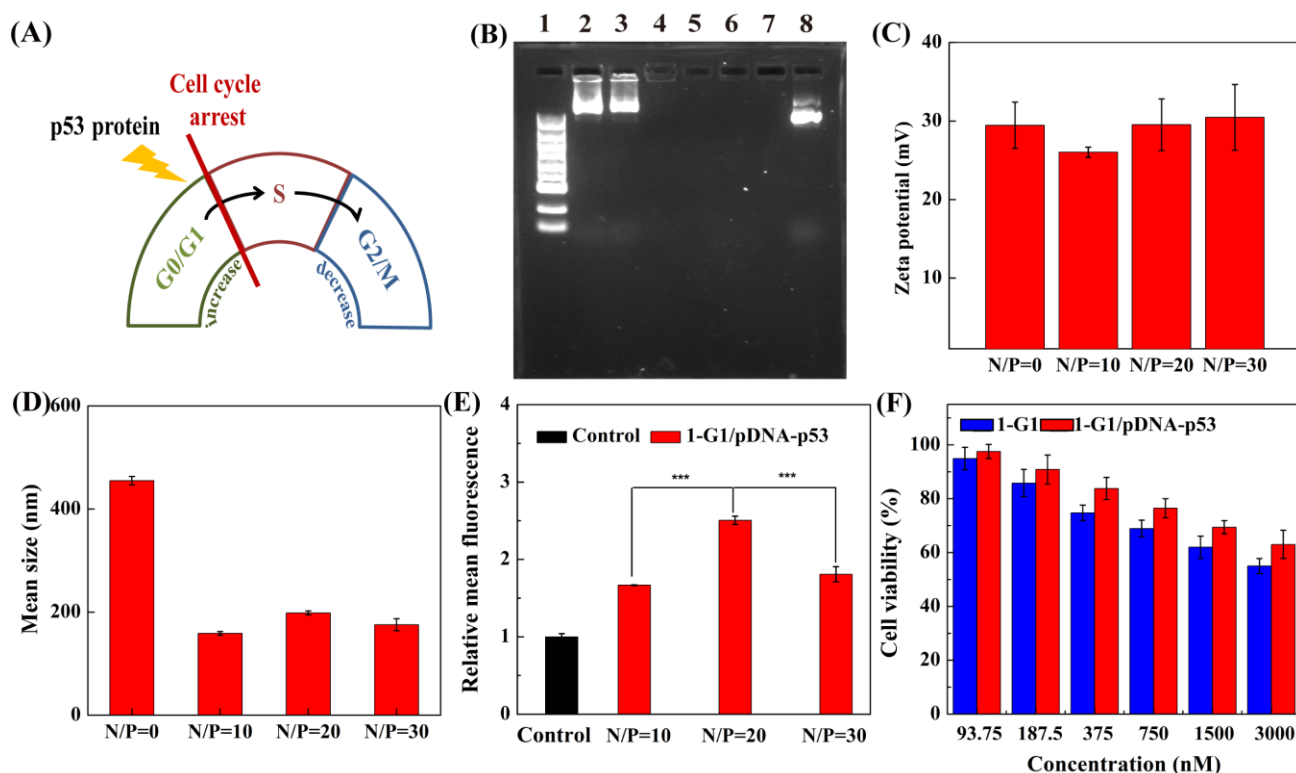


Figure 4. (A) Schematic diagram of p53 protein expression-mediated G0/G1 phase arrest; (B) Gel retardation assay of p53 plasmid DNA complexed with 1-G1 at various N/P ratios (Lane 1: DNA marker, lane 2: N/P = 0.25:1, lane 3: N/P = 0.5:1, lane 4: N/P = 1:1, lane 5: N/P = 2:1, lane 6: N/P = 4:1, lane 7: N/P = 6:1, and lane 8: P53 plasmid DNA); (C) Zeta potential and (D) mean hydrodynamic size of p53 plasmid DNA complexed with 1-G1 at various N/P ratios; (E) Relative EGFP protein expression in HeLa cells transfected with the 1-G1/pDNA polyplexes at different N/P ratios as measured by flow cytometry analysis; (F) CCK8 assay of HeLa cells treated with the polyplexes at different dendrimer concentrations for 24 h.

To check the effective p53 protein expression after the transfection of the polyplexes to cancer

cells, we investigated the block of cell cycle progression in G1 stage in response to DNA damage or other stresses (Figure 4A).⁴⁵⁻⁴⁷ Flow cytometric analysis of the cell cycle distribution was performed (Figure 5A-B). In the PBS group, cells contain 2n chromosomes (phase G0/G1, ~40%) and few cells are in an active DNA synthesis stage (phase S, ~25%) or already engage in the mitosis process (phase G2/M, ~ 35%). Meanwhile, for the 1-G1 and free pDNA-p53, cells are in the phase S (~25%) without a substantial increase of the G0/G1 phase (50%) after 48 h transfection. However, in the 1-G1/pDNA-p53 polyplex group, there are more potent promoters of G1/S cell cycle arrest than other groups (phase G0/G1, ~ 65%; phase G2/M, ~ 5%; and phase S, ~ 30%). The cells in the mitosis process (phase G2/M, ~ 35%) have a substantial decrease after 48 h gene transfection.

To elucidate the molecular mechanism of the cell cycle arrest, the cell cycle-related mRNAs were inspected using quantitative real time-polymerase chain reaction (rt-qPCR) assay. HeLa cells treated with PBS were used as negative control. It is well known that p21 gene was among the first p53 protein target genes to be identified and is now recognized as an encoder for a major cell cycle checkpoint control protein.⁴⁸⁻⁵⁵ The p21 protein functions primarily by binding to and inhibiting Cyclin-D1/Cdk-4 complexes.⁵⁶⁻⁵⁷ Progression of the mammalian cell cycle is regulated by Cdks and regulatory subunits cyclins, cell cycle progression is triggered by partial phosphorylation of Rb by Cyclin-D1/Cdk-4 complexes, and p21 protein disrupts this interactions and inhibits cell cycle progression.⁵⁸ So the relative mRNA level of p53, p21, Cyclin-D1 and Cdk-4 were examined. As shown in Figure 5C, around 6.5-fold and 3.8-time increase in mRNA of p53 and p21, respectively can be observed in cells treated with polyplexes, which is much higher than other groups. Meanwhile, the mRNA levels of Cdk-4 and Cyclin-D1 decrease to 50-60% in the polyplex group when compared to other groups. Rt-qPCR evaluation implies that pDNA-p53 can be transfected by 1-G1 dendrimers to express p53 gene, consistent with the results of confocal microscopy and flow cytometry.

For further elucidation of the molecular mechanism of the cell cycle arrest, the cell cycle-related proteins and p53 were inspected using Western blotting *in vitro* (Figure 5D-E). Around 5.1-fold and 4.2-fold increases of p53 and p21 protein expression, respectively can be seen in cells

treated with the polyplexes, which is higher than all other groups. The G1-phase arresting effect of HeLa cells treated with the polyplexes is related to the down-stream expression level of Cdk-4/Cyclin-D1 that is regulated by kinase inhibitor p21 protein. In order to prove the progression of cell cycle is regulated by Cdks and regulatory subunits cyclins, the expression level of Cdk-4 and Cyclin-D1 proteins was also tested. Clearly, the expression of both Cdk-4 and Cyclin-D1 distinctly decreases in HeLa cells after treated with the polyplexes. In contrast, 1-G and free p53 do not cause significant changes in Cdk-4 and Cyclin-D1 level in HeLa cells. These results demonstrate the excellent gene therapy of cancer cells through the transfection of dendrimer/pDNA polyplexes.

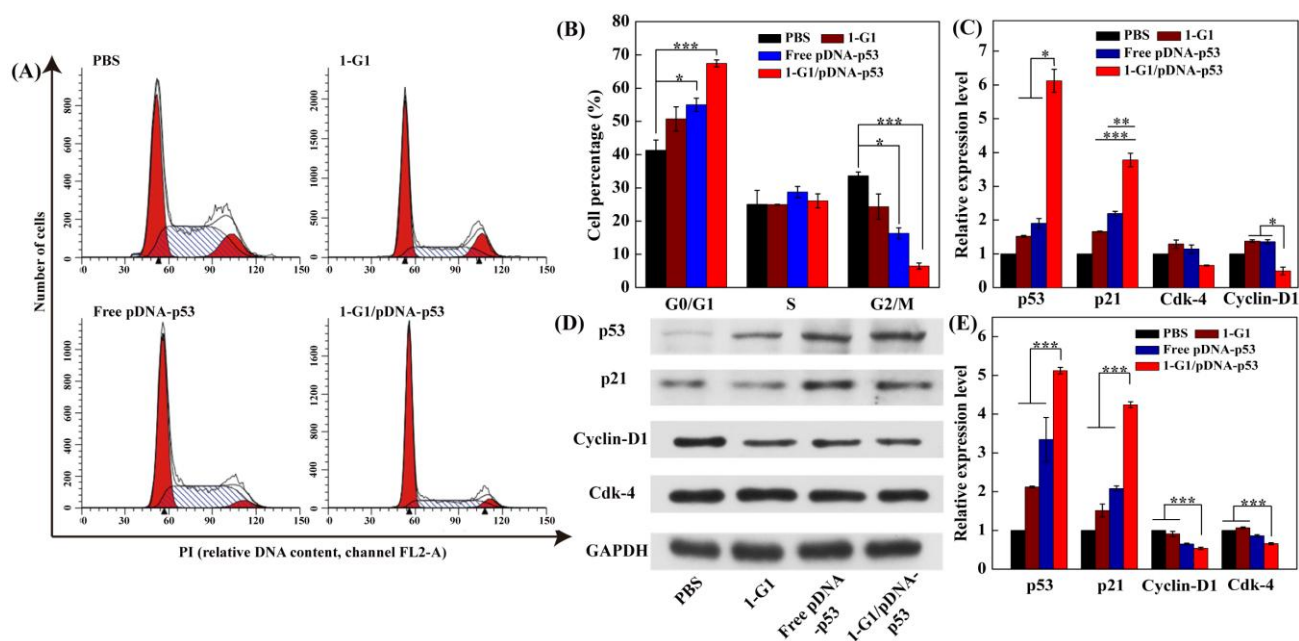


Figure 5. (A) Cell cycle analysis of HeLa cells treated with PBS, 1-G1, free pDNA-p53 and 1-G1/pDNA-p53 polyplexes at an N/P ratio of 20, respectively for 48 h. (B) The percentages of the cellular distribution in different cell cycle phases of the HeLa cells. (C) Validation of relative expression level of mRNA related to G0/G1 phase in HeLa cells after incubation with PBS, 1-G1, free pDNA-p53 and 1-G1/pDNA-p53 polyplexes, respectively for 48 h. (D) Western blot assay of the expression of proteins related to G0/G1 phase in HeLa cells transfected with 1-G1/pDNA-p53 polyplexes (at an N/P ratio of 20). PBS and free pDNA-p53 were used as controls. The GAPDH protein was used as an internal control. (E) Quantitative analysis of proteins related to G0/G1 phase from the Western blot assay data *in vitro* (D).

Dendrimer/pDNA Polyplexes for Gene Therapy of a Tumor Model in Vivo. Next, the polyplex transfection-mediated cancer gene therapy was investigated using a xenografted tumor model (Figure 6A). Normal saline (NS) was used as control, besides the free pDNA-p53 and 1G1 controls. After transfection for 3 days, the tumor tissue was processed to isolate the tumor cells for Western blot assay of the protein expression (Figure 6B-C). Obviously, the tumor cells treated with the 1-G1/pDNA-p53 polyplexes have higher p53 and p21 protein expression level than all other groups ($p < 0.001$), and lower Cdk-4 and Cyclin-D1 expression level than other groups, in agreement with the *in vitro* transfection data (Figure 5D-E). These results suggest that the developed phosphorus dendrimers with the optimized surface cyclic amine termini and generation are able to condense pDNA from degradation *in vivo* to implement effective tumor gene transfection *in vivo*.

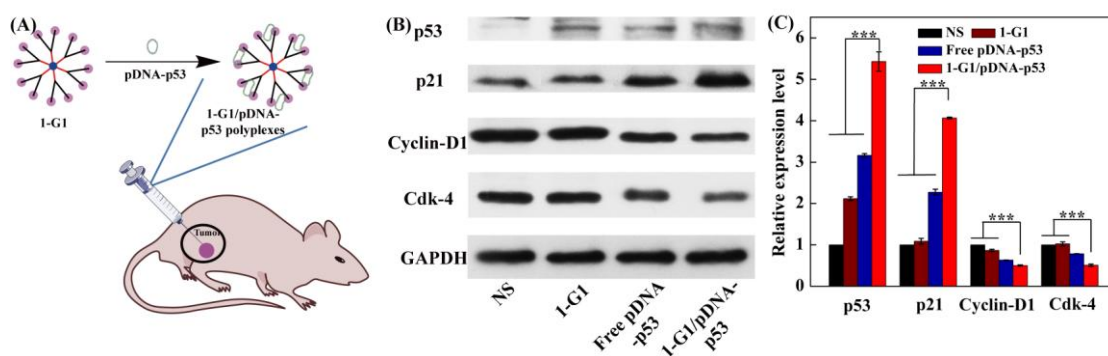


Figure 6. (A) Preparation of the dendrimer/pDNA polyplexes for gene delivery *in vivo*; (B) Western blot assay of the expression of the proteins related to G0/G1 phase in xenografted tumor cells at 4 days post treatment of NS, 1-G1, free pDNA-p53 and 1-G1/pDNA-p53 polyplexes (20 μ g pDNA/mouse for pDNA groups). The GAPDH protein was used as an internal control. (C) Quantitative analysis of the G0/G1 phase-related protein expression level from the Western blot data *in vivo*.

We further used H&E staining to evaluate the *in vivo* biocompatibility of the 1-G1/pDNA-p53 polyplexes (Figure S9). Similar to the control group injected with NS, no significant organ abnormality or damage is observed in the major organs of the mice such as the heart, liver, spleen,

lung, and kidney, declaring that the treatment of the 1-G1/pDNA-p53 polyplexes is quite safe and does not induce any toxic effects. Our results suggest that the designed 1-G1 can be used for safe gene delivery applications.

Conclusions

In conclusion, we revisited the development of cationic phosphorous dendrimers for optimized gene delivery toward cancer gene therapy applications. Cationic phosphorous dendrimers with different generations and surface cyclic amine groups were synthesized, characterized, and used to first compact pDNA-EGFP to form polyplexes and then deliver the polyplexes to cancer cells in vitro in order to screen those with the optimized gene delivery efficiency. 1-G1 dendrimers with pyrrolidine ammonium termini were proven to be the most promising vector that was further used to transfect pDNA-p53 to induce the cell cycle arrest through the regulation of p21 and Cdk4/Cyclin-D1 expression and p53 protein expression in vitro and in vivo. The developed cationic pyrrolidine ammonium-terminated 1-G1 dendrimers may be used for highly efficient gene delivery to different biosystems for gene therapy of various diseases.

Experimental Section

Synthesis of 1-(2-aminoethyl) pyrrolidine-modified G1 (1-G`1). All reactions were carried out in organic solvents using standard high vacuum and dry-argon techniques. 1-(2-Aminoethyl) pyrrolidine (1-a, 27.12 mmol, 3.096 g) and N, N-diisopropylethylamine (DIPEA, 27.12 mmol, 3.505 g) were added to solution of **G1** (2.26 mmol, 0.786 g, in 40 mL tetrahydrofuran (THF)) at 0 °C. The reaction mixture was stirred overnight at room temperature and then concentrated under reduced pressure. Then, 100 mL of pentane was added to the residue and the resulting precipitate was filtered off and dried under reduced pressure to afford **1-G`1** as a powder in 95.7% yield.

Similarly, 1-(3-aminopropyl) piperidine (2-a)-modified G1 (**2-G`1**) and 1-(2-aminoethyl) piperidine (3-a)-modified G1 (**3-G`1**) were synthesized by reacting 2-a and 3-a, respectively under

the above conditions with a starting G1 amount of 0.786 g. Likewise, 1-(2-aminoethyl) pyrrolidine (1-a)-modified G2 (**1-G`2**) and G3 (**1-G`3**) were also synthesized under the same conditions with starting G2 and G3 amount of 2.162 g and 2.419 g, respectively. The reaction conditions for all dendrimer derivatives are listed in Table S1.

Synthesis of cationic phosphorus dendrimers. Hydrogen chloride solution (1 M in diethyl ether, 1.2 mmol, 1 mL) was added to a solution of dendrimers of different generations (**1-G`1**, 0.1 mmol; **2-G`1**, 0.1 mmol; **3-G`1**, 0.1 mmol; **1-G`2**, 0.05 mmol; and **1-G`3**, 0.025 mmol) in 15 mL of THF at 0 °C. The solution was stirred for 4 h and then dried under reduced pressure to afford cationic phosphorus dendrimers as a white powder in around 95% yield for all products. All animal experiments were carried out after approval by the ethical committee for animal care of Donghua University and according to the policy of the National Ministry of Health. See more experimental details in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Part of experimental details and additional experimental data.

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Notes

The authors declare no competing financial interest.

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