DOTAP functionalizing single-walled carbon nanotubes as non-viral vectors for efficient intracellular siRNA delivery

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Abstract

Context: Functionalized single-walled carbon nanotubes (SWNT) with 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) were used as novel and more convenient carriers of small interfering RNA (siRNA).

Objective: To utilize the unique capability of SWNT to be easily modified by functional groups and readily internalized by mammalian cells to bind, condense, stabilize siRNA and enhance its transfection efficiency.

Methods: After SWNT were non-covalently functionalized by cationic DOTAP (SWNT–DOTAP), siRNA interacted with SWNT–DOTAP via static electricity (SWNT–DOTAP/siRNA). Subsequently, the size, zeta potential and morphology of SWNT–DOTAP/siRNA were analyzed. The optimal compression ratio and stability of siRNA were assessed by agarose gel electrophoresis. Furthermore, in prostate carcinoma PC-3 cells, RT-PCR, flow cytometry and sulforhodamine B assays were used to evaluate the silencing activity, transfection efficiency and cell proliferation, respectively.

Results and discussion: The characteristics of SWNT–DOTAP, i.e. an average size of 194.49 nm, a zeta potential of 45.16 mV and lower cytotoxicity than Lipofectamine 2000, indicated that this vector was suitable for siRNA delivery. Moreover, after interaction with SWNT–DOTAP, siRNA of human telomerase reverse transcriptase was bound, condensed and stabilized. In PC-3 cells, SWNT–DOTAP/siRNA exhibited 82.6% silencing activity and 92% transfection efficiency. Furthermore, the complexes inhibited cell proliferation by 42.1%.

Conclusion: SWNT–DOTAP may be a promising siRNA delivery vector for gene-based therapeutic applications in cancer.

Keywords

Cancer therapy, gene silencing, non-viral vector, single-walled carbon nanotubes, siRNA

Introduction

Recent exploration of the molecular biology of cancer raises the possibility of cancer gene therapeutics based on small interfering RNA (siRNA). However, the future of such gene-based therapeutics is dependent on achieving successful delivery (Guo et al., 2011). A delivery system with stability, high transfection efficiency and low cytotoxicity is necessary because siRNA molecules are rapidly degraded by nucleases present under physiological conditions and are not able to enter the cytoplasm to reach intracellular targets (Kurreck, 2009). Viral vectors have been seriously challenged because of safety concerns, such as biological immunogenicity and possible carcinogenicity resulting from insertion mutations (Liang et al., 2012). A growing number of literature reports have encouraged further siRNA delivery studies of non-viral delivery systems.

Single-walled carbon nanotubes (SWNT) are an important class of artificial nanomaterials and exhibit better potency in creating versatile delivery systems due to the following properties: first, they are readily internalized by various mammalian cells along with their cargo (Li et al., 2013) and second, their very large surface can be modified with functional groups and be loaded with nucleic acids, drugs and proteins (Foldvari & Bagonluri, 2008). Based on the specific ability of direct translocation over the plasma membrane and the structural advantage to be loaded with therapeutics, SWNT have become an area of research that is gaining much interest as a non-viral delivery system of siRNA (Lee et al., 2007; Bates & Kostarelos, 2013).

Among the various attempts to deliver siRNA using SWNT, the conjugation of siRNA to SWNT through chemical reactions, such as the chemical conjugation of thiol-modified siRNA to non-covalently functionalized SWNT or the conjugation of siRNA to chemically functionalized SWNT, is complicated and time consuming (Zhang et al., 2006;
Liu et al., 2007; Krajcik et al., 2008; Wang et al., 2008; Liu et al., 2009; Podesta et al., 2009; Al-Jamal et al., 2010; Al-Jamal et al., 2011; Varkouhi et al., 2011; Chen et al., 2012; Battigelli et al., 2013; Wang et al., 2013). However, nanoparticle preparation through electrostatic interactions between the negatively charged nucleic acids and the non-covalently functionalized cationic SWNT may have more potential for siRNA delivery due to the ease and convenience of this technique.

Non-covalent functionalized SWNT that are conjugated to synthetic succinylated polyethyleimine (Siu et al., 2014) or to complicated lipids and natural amino acid-based dendrimers (McCarroll et al., 2010) for the purpose of nucleic acid loading have been shown to be efficient in vitro and/or in vivo. However, the treatment of non-covalent functionalized carbon nanotubes (CNT) with cetylpyridinium chloride to introduce pyridinium was disappointing (Varkouhi et al., 2011). Thus far, whether 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), another cationic lipid, can non-covalently functionalize SWNT for siRNA delivery remains unknown. In this study, one novel SWNT–DOTAP/siRNA complex, in which siRNA interacts with vectors of non-covalently SWNT functionalized with DOTAP through simple electrostatics, was investigated (Figure 1). The cellular cytotoxicity and the silencing activity of the vectors were compared to those based on the most widely used transfection agent Lipofectamine. Furthermore, the ability of the complexes to help siRNA inhibit the proliferation of cancer cells was explored.

Materials and methods

Materials and reagents

SWNT (produced by chemical vapor deposition; catalog no. 1109) with metallic catalyst content <5% were purchased from Chengdu Organic Chemicals Co. Ltd (Chengdu, Sichuan Province, China). The human prostate cancer cell line PC-3 (catalog no. TCHu158) was obtained from the Chinese Academy of Sciences Cell Bank, Shanghai, China. DOTAP (methyl sulfate salt) was obtained from Avanti Polar Lipids (Alabaster, AL). Lipofectamine 2000 was purchased from Invitrogen (Breda, The Netherlands). Sulforphodamine B (SRB) and other chemicals were obtained from Sigma Aldrich (Steinheim, Germany). The primers for GAPDH and hTERT were synthesized by Dalian Takara Biotechnology (Dalian, Liaoning Province, China). Negative-control siRNA, anti-human telomerase reverse transcriptase (hTERT) siRNA and carboxytetramethylrhodamine (FAM)-labeled siRNA were synthesized by Gene-Pharma Co., Ltd (Shanghai, China). The sequence of hTRET siRNA was as follows: sense strand, 5’-3’CAGGGUGACCGAGCCACUGTT and antisense strand, 5’-3’ CAGUGCGUGACGUCACCGUUGTT.

Figure 1. Schematic representation of the non-covalently functionalized SWNT with DOTAP.

Purification, cutting and oxidation of SWNT

According to the method reported previously by our group (Li et al., 2013), SWNT were processed with acids (H2SO4 and HNO3) and ultrasound to obtain purified, shortened and oxidized SWNT. Oxidized SWNT were used unless stated otherwise.

Preparation and characterization of SWNT-DOTAP/siRNA complexes

SWNT (5 mg) were mixed with DOTAP solutions (2 mg/ml) using an ultrasonic bath for 3 h after 6 mg of DOTAP was fully dissolved in 3 ml of diethylpyrocarbonate (DEPC) water. The mixture was further ultrasonicated using an ultrasonic probe (250 W, 15 times). The resulting suspension was centrifuged at 4000 rpm for 10 min and repeated to remove aggregates; then, SWNT–DOTAP was obtained as a black supernatant. Absorbance at 808 nm was used to determine the SWNT concentration in the suspension by an UV-Vis spectrophotometer (UV-3600, Shimadzu, Kyoto, Japan). After 200 μl of SWNT–DOTAP was diluted in 1.8 ml of ultra-pure H2O, the size and zeta potential were analyzed in triplicate using a Zetasizer Nano ZS-90 (Malvern, UK) based on quasi-elastic light scattering.

For preparation of SWNT–DOTAP/siRNA complexes, appropriate amounts of SWNT–DOTAP were diluted with DEPC water according to different mass ratios; meanwhile, siRNA stock solution was also diluted to an equal volume. Finally, both the dilutions were mixed and incubated for 20 min at room temperature with gentle vortexing. Transmission electron microscopy (TEM) was conducted to check the morphology of the SWNT–DOTAP/siRNA complexes. Briefly, one drop of the suspension of complexes was dropped onto a copper grid and then dried. Next, the complexes were visualized under a JEM-1400 TEM (JEOL, Tokyo, Japan) operating at 80 kV.

Agarose gel electrophoresis

The siRNA condensed by the vectors was evaluated by a gel retardation assay. Complexes from SWNT–DOTAP and siRNA at different mass ratios were loaded on 1% agarose gels after incubation for 20 min. Electrophoresis was performed at 100 V for 15 min. siRNA was visualized by Goldview staining, and images were obtained from a GelDoc 2000 imager system (Bio-Rad, Munich, Germany).

The ability of the complexes to protect siRNA against degradation was also characterized using agarose gel electrophoresis according to the method described by Gao et al. (2011). Samples of siRNA, either in aqueous solution or complexes, were mixed in a 1:1 volume ratio with antibiotics-free Roswell Park Memorial Institute-1640 (RPMI-1640) medium supplemented with 20% fetal bovine serum (FBS) and incubated at 37°C. At 6 h, after the complexes were
untreated or destroyed by the detergent, Triton-X100. Aliquots containing siRNA of each sample were loaded on an agarose gel, and intact siRNA was evaluated by electrophoresis.

**Flow cytometry and fluorescence microscopy to assess transfection efficiency**

Human prostate cancer PC-3 cells were cultured in RPMI-1640 medium containing 10% of FBS, 1 × 10^5 U L⁻¹ penicillin and 1 × 10^6 µg L⁻¹ streptomycin at 37°C and 5% CO₂ in a humidified incubator. FAM-labeled siRNA were added to the cells and incubated at 37°C for 6 h after the cells (1 × 10^5 cells/well) were seeded into six-well plates and cultured overnight. The cells were washed twice with phosphate-buffered saline (PBS) before collection. Then, the transfection efficiency of the cells was determined by flow cytometry (BD FACS) using CELLQuest software (Franklin Lakes, NJ) with excitation at 488 nm and emission at 525 nm. Alternatively, after the cells were washed twice with PBS, they were immobilized by acetone and imaged by a fluorescence microscope.

**Cytotoxicity and cell inhibition assay**

The PC-3 cells (8 × 10^3 cells/well) were seeded into 96-well plates and cultured overnight. After 2.5–10.0 µg/ml of SWNT–DOTAP or Lipofectamine 2000, which contain the concentrations in siRNA complexes, were separately added and incubated with cells for 48 h, SRB assays were performed to measure the cytotoxicity of the SWNT–DOTAP and Lipofectamine 2000 (Vichai & Kirtikara, 2006). In addition, cell inhibition was assessed by a SRB assay. Briefly, after PC-3 cells were seeded and cultured as above, cells were transfected with the anti-hTRRT siRNA complexes for 6 h at final concentrations of 4 mg/L functionalized SWNT–DOTAP (calculated with SWNT concentration) and 100 nM siRNA. Subsequently, the medium was removed, and fresh medium was added. The SRB assay determined cell inhibition 48 h after incubation at 37°C. Values are the mean ± SD of three independent experiments (n = 5 wells in each individual experiment).

**Gene silencing**

PC-3 cells were seeded in six-well plates at a density of 1.2 × 10^5 cells/well and incubated continuously with growth medium for 48 h after siRNA transfection for 6 h. Then, the cells were collected for the extraction of total RNA using a TRizol single-step RNA extracting kit. cDNA synthesis and PCR amplification were accomplished according to the kit manual. The hTERT primer (200 bp) sequences are as follows: sense, 5'-3'-CAGG AAGAGTGTCTGAGCAGAA and antisense, 5'-3'-CAGGCAGTAAGC CATGTTCA; for GAPDH primers (450 bp): sense, 5'-3'-CTCAGACACCCATGGGAAAGTTGA and antisense, 5'-3'-ATGATCTTG AGGCTGTTGTCATA. Reaction parameters were the following: 94°C pre-degeneration for 5 min; 94°C degeneration for 30 s, 60°C annealing for 30 s and 72°C extension for 60 s, with the above cycled for 45 times; and then re-extension at 72°C for 7 min. A total of 5 µL of PCR products was used for agarose gel electrophoresis and then imaged with the gel analysis system.

**Statistical analysis**

Quantitative data are expressed as the mean ± SD. Differences between groups were analyzed using an analysis of variance followed by Dunnett’s post-test using SPSS 17.0 statistical software (Chicago, IL), and p values < 0.05 were considered statistically significant.

**Results and discussion**

**Characteristics of SWNT–DOTAP**

After pristine SWNT were treated by acids and ultrasound, their solubility in aqueous solution was slightly increased (Figure 2A), and their size and zeta potential were 126.41 ± 9.13 nm and −22.35 ± 3.68 mV, respectively (Figure 2B). Furthermore, along with non-covalent functionalization by DOTAP, SWNT were dispersed into a stable aqueous suspension, and their solubility in PBS (pH 7.4) gradually improved (Figure 2A). The concentrations of SWNT in the SWNT–DOTAP mixture were measured by a spectrophotometer to be 126.15 µg/mL. The size and zeta-potential of SWNT–DOTAP were 194.49 ± 8.51 nm and 45.16 ± 4.89 mV, respectively (Figure 2C). Compared with the oxidized SWNT, the size was bigger and the zeta potential reversed from negative to positive. Figure 2(D) represents the in vitro cytotoxicity of SWNT–DOTAP. The results showed that treatment of PC-3 cells with low concentrations (2.5 µg/ml) of SWNT–DOTAP and Lipofectamine 2000 produced no significant cytotoxicity. In addition, the higher concentrations of SWNT–DOTAP (5 and 10 µg/ml) did not cause any significant change in cell viability. However, exposure of PC-3 cells to the higher concentrations of Lipofectamine 2000 (5 and 10 µg/ml) led to significant inhibition of proliferation compared with the control group (p < 0.05). In addition, there were significant differences in cytotoxicity between the 5 and 10 µg/ml SWNT–DOTAP groups and the corresponding Lipofectamine 2000 groups.

Recent studies have shown that the length, metal contaminants in impure SWNT, degree of aggregation, bound functional group(s) and type of functionalization might be key parameters in obtaining an efficient and non-cytotoxic SWNT-based delivery system (Pulskamp et al., 2007; Wick et al., 2007; Firme Iii & Bandaru, 2010). After carefully optimizing these physicochemical parameters, CNT can be used in biomedical applications as biocompatible materials (Lanone et al., 2013). In this study, according to a method reported previously (Li et al., 2013; Wang et al., 2013), pristine SWNT were first treated with acids (H₂SO₄ and HNO₃) and sonication to remove metal contaminants, shorten the length and oxidize the ends to reduce the aggregation. Furthermore, carboxyl groups in oxidated SWNT allow for the possibility of combining with a cation to form a salt, thereby enhancing the solubility of SWNT. The results of the improved solubility of SWNT in PBS (Figure 2A) and the demonstrated lower toxicity compared to Lipo2000 (Figure 2D) suggest that the hydrophobic lipid chains of DOTAP are anchored onto the SWNT. The hydrophilic ends of DOTAP facilitate SWNT solubilization and enhances its biocompatibility, which is similar to phospholipid–polyethylene glycol playing a role in the non-covalent functionalization of SWNT (Liu et al., 2009).
Figure 2. Characterization of SWCNT-DOTAP nanoparticles. (A) Solubility in PBS. Left to right: pristine SWNT, oxidized SWNT and SWNT–DOTAP. (B) Particle size and zeta potential of oxidized SWNT. (C) Particle size and zeta potential of SWNT–DOTAP. (D) Cytotoxicity of SWNT–DOTAP and Lipo2000 in PC-3 cells. *p<0.01 versus control group, ^p<0.01 versus respective groups of lipo2000.
The size analysis for fine particles is divided into direct and indirect analysis. Although the direct analysis of size using electron microscopy is intuitive, its field of view is very limited, and only a partial region is observed. In contrast, the indirect analysis of laser particle using Zetasizer Nano ZS90 instruments is based on the spherical assumption and DSL principle. Although clubbed and flaky particles, such as CNT with a high aspect ratio, can lead to inaccurate results, the laser particle analysis method can measure relatively large amounts of sample, and the results can provide information on the dispersibility and uniformity of the sample. If the fine particles in a sample liquid are unstable, the results of multiple measurements will be inconsistent. If the particle size distribution is not uniform, there will be more than one peak in measurements of the sample. To fully disclose the characteristics of the sample, a combination of direct and indirect analyses, i.e. TEM and laser particle analysis, was used in our study. The sizes of three measurements are highly repetitive for SWNT–DOTAP, suggesting that our carrier was stable. Only one peak was presented in our carrier, suggesting that the sizes of our carrier were uniform. Furthermore, TEM images showed that nanoscale particles can be obtained by non-covalent SWNT functionalized with DOTAP. These results and the strong positive charges of SWNT–DOTAP suggest that it is suitable for siRNA delivery.

**Bound, condensed and stabilized siRNA**

SWNT–DOTAP showed a positive zeta potential of 45.16 mV, pointing to its ability to form complexes with negatively charged siRNA. Therefore, complexes of siRNA with SWNT–DOTAP were further studied using agarose gel electrophoresis by increasing the mass ratio between the two components (Figure 3A). Compared with naked siRNA, an increase of SWNT–DOTAP in the complexes weakened the band of siRNA, suggesting that the migration of duplex siRNA was retarded gradually. The complete interaction of all siRNA with SWNT–DOTAP was obtained at mass ratios above 1:2, as determined by the disappearance of the siRNA band in the agarose gel.

We further confirmed that SWNT–DOTAP/siRNA complexes at a mass ratio of 1:3 can be successfully condensed into a nanostructure by TEM. It can be observed that the shape of SWNT appears to be nanofibrous (Figure 3B, void arrows), and the most of surface and end of SWNT can be coated with DOTAP (Figure 3B, dotted arrows). Due to the condensation of siRNA, increased electron-rich areas (dark) were seen on the nanotube surface (Figure 3B, solid arrows). This is consistent with previous data, which showed that the degree of complexation between two oppositely charged components, ammonium-functionalized multiwalled carbon nanotubes prepared by a 1,3 dipolar cycloaddition reaction
and siRNA, can be determined by the formation of electron-rich areas on the nanotube surface (Podesta et al., 2009). Furthermore, the TEM image of the complexes showed that the very large surface of the SWNT-DOTAP/siRNA complex was not yet occupied except for the electron-rich areas (Figure 3B). This characteristic may help to further load antitumor drugs onto the surface of SWNT-DOTAP/siRNA, thus enabling synergistic antitumor therapy by combining antitumor drugs with siRNA to enhance their efficiency.

Based on our results, a mass ratio of 1:3 (siRNA:SWNT–DOTAP) was selected to perform all further experiments unless otherwise indicated.

To detect whether the siRNA in the complexes was degraded, incubated complexes in the experimental environment, i.e. RPMI-1640 medium containing FBS, were tested by gel electrophoresis. As shown in Figure 3(C), an intact siRNA (lane 1), as the positive control, showed bright bands. There were few bands in the naked siRNA 6 h after incubation. In contrast, siRNA was recovered from SWNT–DOTAP/siRNA complexes after treatment of the complexes by Triton-X100 (lane 4), suggesting that siRNA in SWNT–DOTAP was protected and kept relatively stability.

In brief, SWNT–DOTAP successfully condensed siRNA into compact nanoparticles that were ready for transfection (Figure 3A and B). Moreover, SWNT–DOTAP/siRNA complexes could maintain the stability of siRNA in media (Figure 3C).

**Delivery ability, RNAi efficiency and tumor cell inhibition**

To quantify the internalization of siRNA into cells via flow cytometry, FAM-labeled siRNA was used. Under the conditions of siRNA:SWNT–DOTAP mass ratios = 1:3 and 100 nM siRNA, approximately 92% of the cells were positive for FAM when the vector was SWNT–DOTAP (Figure 4A). This can be compared to the 83% of positive cells obtained from a standard lipofection protocol, suggesting that there is no essential difference in the transfection efficiency between lipofection and SWNT–DOTAP transfection. Furthermore, Figure 4(B) shows that normal cells had little fluorescence background, and the fluorescence of cells transfected by SWNT–DOTAP/FAM-labeled siRNA was mainly located inside the cell rather than in the media or at the cell surface. The results suggested that the high transfection efficiency indicated by flow cytometry was not a false positive.

Figure 4(C) shows the hTERT mRNA levels detected by RT-PCR. The silencing efficiency of hTERT was corrected for delivery ability; thus, the percentage silencing reported is only related to transfected cells. The negative siRNA had little effect on hTERT mRNA expression, indicating that the siRNA used was specific for hTERT mRNA inhibition. The GAPDH siRNA delivered by lipofection was used as a positive control; its silencing indicated that the operation, reagents and experimental conditions, such as the concentration of siRNA and the amount of carrier, were appropriate. Incubation of cells with siRNA complexes based on SWNT–DOTAP produced observable RNA interference. The expression level of the hTERT gene was suppressed by 82.6%. This result implies that the specific siRNA was successfully released from SWNT–DOTAP and was able to fully realize its gene silencing potential in the cell.

The effect of hTERT siRNA on cell inhibition is shown in Figure 4(D). Treatment with naked siRNA had no significant cytotoxicity to PC-3 cells, indicating that successful delivery was not achieved by naked siRNA, and negative siRNA delivered by SWNT–DOTAP did not cause any significant change in cell viability, indicating that there is no off-target effect due to non-specific siRNA. However, exposure of PC-3 cells to SWNT–DOTAP/siRNA complexes led to significant cell inhibition compared with the vector group (p < 0.05): the inhibition rate of the specific siRNA reached 42.1%. The results suggested that with the help of SWNT–DOTAP, hTERT siRNA was translocated into the cell cytoplasm and exerted its effects.

hTERT is a catalytic subunit of the telomerase complex. Telomerase maintains telomere ends by the addition of the telomere repeat, endowing immortality to cancer cells; even
if knockdown of hTERT is completely accomplished by the introduction of siRNA, cancer cells can still proliferate until their telomere ends are sufficiently shortened. Therefore, in theory, cancer cells will not stop proliferating within 48 h after knockdown of hTERT. However, studies have shown that the down-regulation of hTERT expression via siRNA quickly impairs cell growth both in vitro and in vivo (Xia et al., 2012), and the anti-proliferative effects of the same sequence hTERT siRNA used by us can be sustained by telomere shortening, as a result of prolonged inhibition of the telomere lengthening activity of telomerase, or by loss of telomere lengthening-independent functions of hTERT (Gandellini et al., 2007). Thus, with the help of cationic SWNT–DOTAP, siRNAs targeting hTERT can be delivered to tumor cells and quickly inhibit their growth.

Our SWNT–DOTAP/siRNA complexes exhibited a 92% transfection rate (Figure 4A) and an 82.6% silencing activity (Figure 4C). The complexes could also mediate the successful inhibition of specific siRNA (Figure 4D). In contrast, Varkouhi et al. reported that a disappointing silencing activity and higher cytotoxicity were obtained with two other functionalized CNT, CNT–polyethylenimine (PEI) and CNT–pyridinium, when used to deliver siRNA (Varkouhi et al., 2011). However, they referred to other literature reports to encourage further nucleic acid delivery studies with other types of functionalized CNT and thought that the type of functionalization of CNT might play an important role in obtaining an ideal CNT-based delivery system. In this study, the type of functionalization, in that CNT were non-covalently functionalized by DOTAP, and the processing procedure differed from their study, which most likely contributed to the different results. In accordance with our results, Siu et al. reported that after SWNT were functionalized non-covalently with succinated PEI (PEI–SA), the silencing rate of SWNT–PEI/siRNA was 79.4% (Siu et al., 2014). Wang et al. reported that after SWNT were chemically functionalized with PEI, the transfection rate of SWNT–PEI/siRNA was approximately 87% (Wang et al., 2013). Based on the non-covalent functionalization of SWNT with DSPE–PEG2000–

Figure 4. The delivery efficiency, RNAi efficiency and tumor cell inhibition of the SWNT–DOTAP/siRNA complexes in PC-3 cells. (A and B) Transfection efficiency of the complexes detected by flow cytometry and fluorescence microscopy, respectively. FAM-labeled siRNA were used. (C) Gene silencing assessed by RT-PCR. (D) Cell inhibition measured by SRB assays. **p < 0.01 versus the vector group.
amino, when the chemical conjugation of thiol-modified siRNA to DSPE–PEG–amine (PEI–SA/CNT/siRNA) was used, the inhibition ratio of tumor cell reached 44.53% (Chen et al., 2012). On the premise of similar transfection and silencing efficiency, as well as cell growth inhibition, trivial steps, time-consuming processes and the risk and toxicity of chemicals are avoided in the protocol we use to prepare our vector. Thus, the protocol developed by us is more safe, simple and convenient.

The delivery of RNA to cells represents the limiting step in the development of RNA interference protocols (Chen et al., 2012). Our results suggested that non-covalently functionalized SWNT with DOTAP (SWNT–DOTAP) show favorable physical and biological characteristics, which can be effectively assembled with siRNA into nanostructures. These results also give rise to the possibility of combination therapy with siRNA and other drugs because the remaining large surface areas of SWNT allows for a high-loading capacity of other drugs. Furthermore, SWNT can bind, condense and stabilize siRNA via electrostatic interactions. Notably, SWNT–DOTAP/siRNA complexes are able to achieve siRNA delivery, RNAi efficiency and tumor cell growth inhibition (Figure 5).

Conclusion

In conclusion, the nanoscale particles conveniently formed by SWNT–DOTAP and siRNA via electrostatic interaction were able to disperse SWNT, condense siRNA and silence the expression of hTERT and exhibit the properties of low toxicity and more efficient anti-tumor effects. Thus, SWNT–DOTAP/siRNA complexes might be an attractive alternative as a novel and simple gene delivery system in therapeutic applications.

Declaration of interest

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