



Monitoring HSV-TK/ganciclovir cancer suicide gene therapy using CdTe/CdS core/shell quantum dots

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ABSTRACT

To be able to label a gene and monitor its migration are key important approaches for the clinical application of cancer suicide gene therapy. Photonic nanomaterials are introduced in this work. One of the most promised suicide genes - herpes simplex virus thymidine kinase (HSV-TK) gene - is successfully linked with CdTe/CdS core/shell quantum dots (QDs) via EDC/NHS coupling method. From confocal microscopy it was demonstrated that plasmid TK intracellular trafficking can be effectively and distinctly traced via monitoring the luminescence of the QDs up to 96 h after transfection of QDs-TK conjugates into HeLa cells. MTT results show that the QDs-TK conjugates have a high efficient cytotoxicity after adding GCV into HeLa cells, whereas the QDs exert no detectable deleterious effects on the cellular processes. The apoptosis induced by QDs-TK conjugates with GCV is distinctly traced partly due to the strong luminescence of the QDs. Our results indicate that photonic nanomaterials, e.g. QDs, provide a tool for monitoring TK gene delivery and anti-cancer activity.

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1. Introduction

Cancer is becoming the leading cause of death worldwide. This fact accentuates the need for a new generation of more effective therapies for cancer. Current treatment of cancer with chemotherapy or radiotherapy is insufficient in selectivity, thus induces toxicity to normal cells [1]. Cancer gene therapy offers a better alternative in this respect since it has higher efficiency and lower side effect characteristics. Cancer gene therapy includes a number of possible approaches, one of which utilizes “suicide” gene systems. Herpes simplex virus thymidine kinase gene (HSV-TK) is one of the most promising “suicide” genes. Cells expressing the HSV-TK gene metabolize ganciclovir (GCV) to ganciclovir monophosphate, which is further converted into ganciclovir triphosphate by cellular kinases. As the resulting compound is an analog of deoxyguanosine triphosphate, inhibition of DNA polymerase and/

or incorporation into DNA will occur, leading to chain termination and tumor cell death [2–4]. Till now HSV-TK/GCV has been the only gene directed enzyme prodrug therapy combination to reach phase III human trials. It was reported that a subsequent phase II trial that utilized a replication defective adenovirus for delivery of HSV-TK into patients with glioma produced a clinically and statistically significant increase in median survival from 38 to 62 weeks over standard chemotherapy (surgery and radiotherapy) [5,6].

However, TK gene can also transfer to normal human cells. Cytotoxic compounds do not discriminate between neoplastic cells and rapidly dividing healthy cells, such as bone-marrow (hematopoietic) precursors and gastrointestinal mucosal epithelial cells, thus leading to a range of toxic side effects such as neutropenia, thrombocytopenia, anemia, and mucositis [7,8]. It might to a certain extent restrict the application of this promising method. Up to now, there is no systematic research of tracing and identifying the intracellular localization of a TK gene. Therefore a stable and good tracing means is very necessary to label TK gene and monitor its migration so that the TK gene is proved to access into tumor tissue before adding GCV to kill tumor cells exclusively without destruction of normal cells.

As a new kind of biological label, quantum dots (QDs) are nanometer-sized semiconducting crystals with unique fluorescent

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properties, which are attracting more and more attention for their potential application in cell imaging and in vivo animal targeting [9,10]. In comparison with organic dyes and fluorescent proteins, QDs are brighter than most conventional fluorescent dyes and have been significantly easier to detect than green fluorescent protein (GFP) considering the background autofluorescence in vivo [11,12]. Further, QDs have wide excitation spectrum, size-tunable and narrow emission spectrum. Another important feature is their long-term photostability which opens the possibility of investigating the dynamics of cellular processes over time, such as continuously tracking cell migration, differentiation, and metastasis [9,13,14].

In this study, we have developed a procedure employing highly luminescent and stable CdTe/CdS core/shell QDs for TK intracellular trafficking and killing effect in cancer therapy during long-term. A schematic representation of the conjugation used in our research is shown in Fig. 1A. After defining the conditions for reproducible conjugation of QDs to plasmid TK, it is found out that the QDs-TK conjugates can transfect with high efficiency and kill tumor cells comparable to the parental TK gene after adding GCV, whereas QDs do not show obvious cytotoxicity. Plasmid TK intracellular trafficking process and cytotoxicity in cancer cells have been successfully traced with luminescent signal, as being illustrated in Fig. 1B.

2. Materials and methods

2.1. Materials

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (99%), sulfo-N-hydroxysulfosuccinimide (sulfo-NHS) (98.5%), Ethidium bromide (EB) and agarose gel were purchased from Sigma–Aldrich. Cell culture media were purchased from Invitrogen. Basel, Switzerland. FuGENE HD transfection reagents were purchased from Roche Applied Science. All the reagents were used without further purification. Deionized water was purified through Milli-Q water purification system and the resistivity was 18.2 MΩ cm. The plasmid pCMV-TK encoding the HSV-tk gene under the control of CMV promoter was kindly presented by Dr. You-Sub Won (Department of Molecular Biology, Institute of Nanosensor and Biotechnology, Dankook University).

2.2. Synthesis of water-soluble CdTe/CdS QDs

Usually, aqueous CdTe QDs are prepared by the reaction between Cd²⁺ and sodium hydrogen telluride (NaHTe) solution following the method described previously [15]. Mercaptopropionic acid (MPA) was used as surfactant stabilizer, and the molar concentration of MPA was varied with fixed Cd²⁺ concentration, pH value and synthetic temperature. It is reported that low thiol ligand concentration is regarded as in favor of a rapid growth of CdTe NCs and an increase of photoluminescence (PL) quantum yield (QY) [16]. In this way, the QY of the bare CdTe core can be highlighted to be 27%.

CdTe/CdS core/shell QDs were prepared according to our previous publication [15]. The tailor-designed CdTe QDs (QY = 27%) were capped with 3 monolayers (ML) of CdS shell to obtain the green emission CdTe/CdS core/shell QDs with QY up to 69%. The average size of the aqueous core–shell QDs was measured to be only about 3.9 nm (TEM result was shown in Fig. 2B), which successfully discards the drawback of the large size (12–30 nm) water-soluble QDs after the multilayer coating, e.g., phospholipids, silica, or polymer, etc. This is because that the small dimension would dramatically improve the biological labeling and detection efficiency.

2.3. Conjugation of plasmid TK with QDs

As shown in Fig. 1A, the plasmid TK was conjugated to CdTe/CdS QDs classically by using EDC and sulfo-NHS (S-NHS) as cross-linking reagent [17,18]. Firstly, 0.1 ml of QDs (60 μM) was mixed with 10 μl of EDC and sulfo-NHS successively in phosphate buffer saline (PBS, pH 7.4) with the molar ratio of 1:100:200. After 30 min of magnetic stirring, 0.3 ml of equivalent molar plasmid TK (20 μM) was added into the reacted bottle and stirred at room temperature for another 3 h. In this way, the amide linkage can be formed through the amino of the purine or pyrimidine bases of the plasmid TK gene and the active carboxyl of QDs. In order to remove the excess small molecules, e.g., EDC and sulfo-NHS, the resulting samples were centrifuged in Microcon Centrifugal Filter Devices (50,000 Nominal Molecule Weight Limit). The conjugation of plasmid TK and QDs was confirmed by the agarose gel (0.8% w/v) electrophoresis technique. Agarose gel electrophoresis was run at 150 V/cm, for 10 min.

2.4. Cell culture and cell transfection experiment

The transfection procedure was performed using FuGENE HD (Roche) according to the manufacturer's instructions. Briefly, HeLa cells (ATCC Number: CCL-2) were maintained at 37 °C under 5% CO₂ in Dulbecco's modified Eagle's medium-high glucose (DMEM-HG) (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Invitrogen), penicillin (100 U/ml), and streptomycin (100 μg/ml) were plated 24 h before transfection at a density of 1 × 10⁶ cells per 35 mm well in antibiotic- and serum-free medium. FuGENE HD transfection reagent (5 μl) and the QDs-TK conjugates (10 μl, C_{QDs} = 15 μM) were diluted in opti-MEM (300 μl) and incubated at room temperature for 15 min. Then complexes were added to cell cultures in fresh antibiotic- and serum-free medium to the final volume of 1000 μl for transfection. Replace the medium with serum-containing medium 4–8 h after transfection. Approximately 24 h post-transfection, cells were prepared for confocal microscopy.

2.5. Cytotoxicity of QD, QDs-TK conjugates and QDs-TK conjugates with GCV

HeLa cells were seeded into 96-well plates at a density of 2 × 10⁴ cells/well, and then treated with different concentrations of QDs (Fig. 3A) at different time (Fig. 3B) or QDs-TK conjugates with the final concentration of 0.15 μM at different time points (Fig. 4C) after transfection under the humidified atmosphere (5% CO₂) and in the dark. Viability of HeLa cells was assessed by conventional MTT assay.

To measure the cytotoxicity of conjugates with GCV, cells were plated at a density of 1 × 10⁶ cells/well in 35 mm well 24 h before transfection. The procedure was expressed as above described. After 24 h the viable cells were seeded in 96-well plates at a density of 2 × 10⁴ cells/well, the cells were treated in the absence or presence of varying concentrations of GCV (0, 1, 10, 50, 100 μg/ml). After incubation for 72 h, viability of cells was assessed by conventional MTT assay.

2.6. Cell viability assay (MTT)

Following treatment cell viability was assessed by colorimetric 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assays. 20 μl stock MTT (5 mg/ml) was added to each well in 96-well plates, and cells were then incubated for 4 h at 37 °C. The optical density (OD) value of each well was read by a microplate reader (Sanyo Company) at 570 nm. The survival rate of cells was expressed as A/B 100%, where A was the absorbance value from the experimental cells and B was that from the control cells.

2.7. Confocal laser scanning microscopy

After transfection for 96 h, the cells were washed twice again with chilled PBS and the nuclei were stained with Hoechst 33258 (5 μg/ml) for 5 min. Then the cells were washed twice with PBS. The plasmid TK intracellular location and cytotoxicity was observed by confocal laser scanning microscopy. The confocal laser scanning microscopy was carried out using a Olympus FV1000 microscope equipped with multi-line argon LASER, 405, 488 nm, and 30 mW Laserclass 3D laser.

2.8. Statistical analysis

Data were expressed as mean ± SD. The statistical significance of the data was compared by Student's *t*-test. Analysis of variance (ANOVA) was used to analyze the differences among the different groups. Results were considered significantly at *P* < 0.05.

3. Results

3.1. Characteristics of water-soluble CdTe/CdS QDs

Compared with Rodanmin-6G, the QY of the 3 ML CdTe/CdS core/shell QDs was calculated to be 69%. As shown in Fig. 2C, the CdTe/CdS core/shell QDs are found to be stable and their optical properties in a sealed vial remain invariable over one year's storage under ambient condition. Even after the EDC mediated condensation reaction with TK gene, the optical properties are still stable without any fluorescent quenching since being stored at about 4 °C for several months (shown in Fig. 2A). Hereby, they are capable to act as the fluorescent marker in the cell imaging.

3.2. Cytotoxicity assay of water-soluble CdTe/CdS QDs

It was known that certain QD types may be cytotoxic to various cells, however, this cytotoxicity is concentration dependent and time dependent. At present studies HeLa cells were grown for 24 h

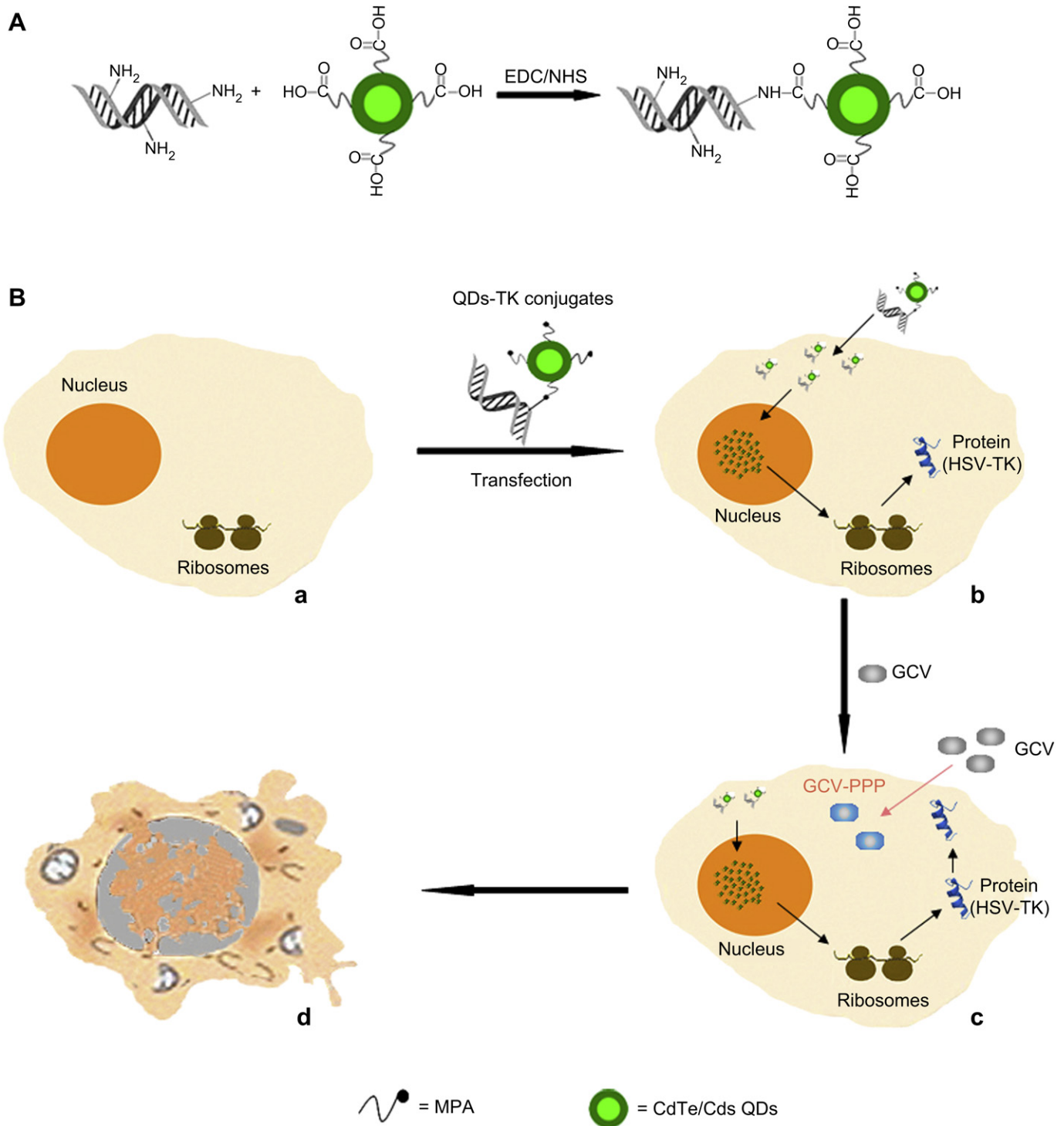


Fig. 1. Schematic illustration of the process for our investigation. (A) Covalent coupling between carboxylic acid ($-\text{COOH}$) coated QDs and primary amines ($-\text{NH}_2$) on plasmid using EDC/NHS as coupling reagents. (B) Intracellular tracking and cytotoxicity in tumor cells of the QDs-TK conjugates by effect of GCV. a) Tumor cell before transfection. b) Transfecting with the QDs-TK conjugates in tumor cell, then transcription in nucleus and translation in ribosome to express HSV-TK gene. c) Cell expressing the HSV-TK gene converted GCV to ganciclovir triphosphate by cellular kinases. d) As the resulting compound is an analog of deoxyguanosine triphosphate, inhibition of DNA polymerase and/or incorporation into DNA will occur, leading to chain termination and tumor cell death.

with the medium containing free QDs with the final concentrations ranging from 0.5 to 3 μM (Fig. 3A). The cell viability was measured using MTT assay. Results showed that the viability of cell growth with the QDs was decreased with increasing the QDs concentration. When the concentration was exceeded to 1.5 μM , the cell viability was lowered to 80%. Cytotoxicity of 1 μM CdTe/Cds QDs at different

time with Hela cells was shown in Fig. 3B, the viability of cell growth with the QDs was decreased with increasing time. After incubating for more than 72 h, the cell viability was lower to 80%. The cytotoxicity of QDs was dependent on the dose and time. When the dose was 1 μM or less and the cells were incubated for less than 72 h, there was no obvious influence on cell growth and development.

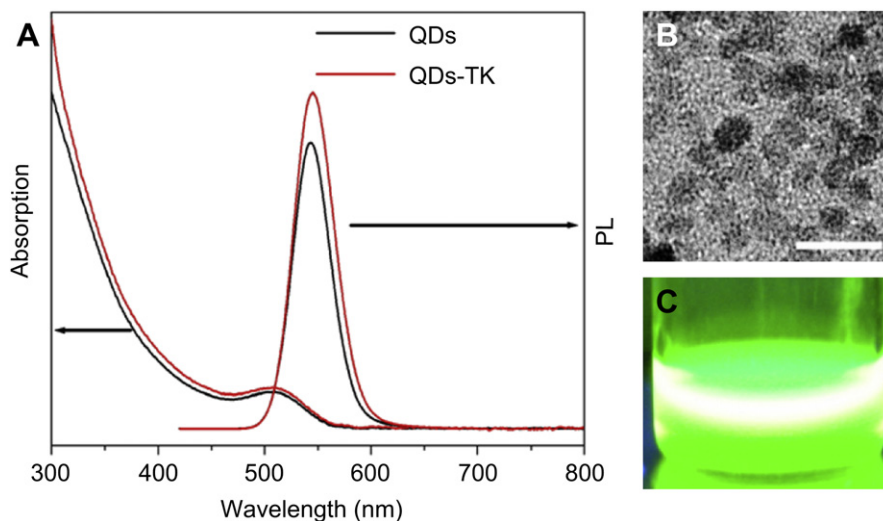


Fig. 2. (A) Absorption and PL spectrum of QDs and QDs-TK conjugates. (B) TEM image of the CdTe/CdS core/shell QDs, the scale bar indicates 10 nm. (C) The photo of the CdTe/CdS core/shell QDs under an ultraviolet lamp excitation.

3.3. Confirmation of QDs-TK conjugation and cytotoxicity

Gel electrophoresis was employed to confirm QDs-TK conjugation, based on QDs' green emission or Ethidium bromide's red fluorescence. Because the thiol carboxylic acid stabilized QDs carry a highly negative charge density under the electrophoretic buffer solution, they migrate quickly through the gel matrix under electric field. The mobility of the bio-molecules in electrophoresis is determined by mass/charge ratio of bio-molecules. As shown in Fig. 4A, the green fluorescence bands of QDs and QDs labeled TK gene are observed under the excitation of a 365 nm lamp. Because the sizes (equal to the mass/charge ratio) of the QDs-TK conjugates are larger than the QDs, they migrated more slowly than pure QDs and separated well in 0.8% AGE, which strongly suggests the presence of the corresponding conjugates. In addition, the AGE electrophoresis of bare TK gene and QDs-TK conjugates dyed with EB was also performed (Fig. 4B). Because the sizes (equal to the mass/charge ratio) of the QDs-TK conjugates are larger than TK gene, they

migrated more slowly than pure TK gene. Therefore we can confirm the successful coupling between the QDs and TK gene from the gel electrophoresis. The cytotoxicity of QDs-TK conjugates was determined by MTT assay at different time points (after transfecting for 6, 12, 24, 48, 72 and 96 h) with the medium containing the complex with the final concentration of QDs (0.15 μ M) (Fig. 4C). The results indicate that concentration of QDs-TK conjugates have no obvious effect on cell viability until transfecting for 96 h.

3.4. Antitumoral activity by the QDs-TK conjugates plus GCV on *Hela* cells

The main goal of this study was to evaluate the therapeutic potential due to the combination of QDs and plasmid TK. Fig. 5 showed the cytotoxic effect of ganciclovir in *Hela* cells transfected with the QDs-TK conjugates by MTT assay. The results were expressed as the percentage of cell viability with respect to control cells without GCV. The results showed that GCV treatment

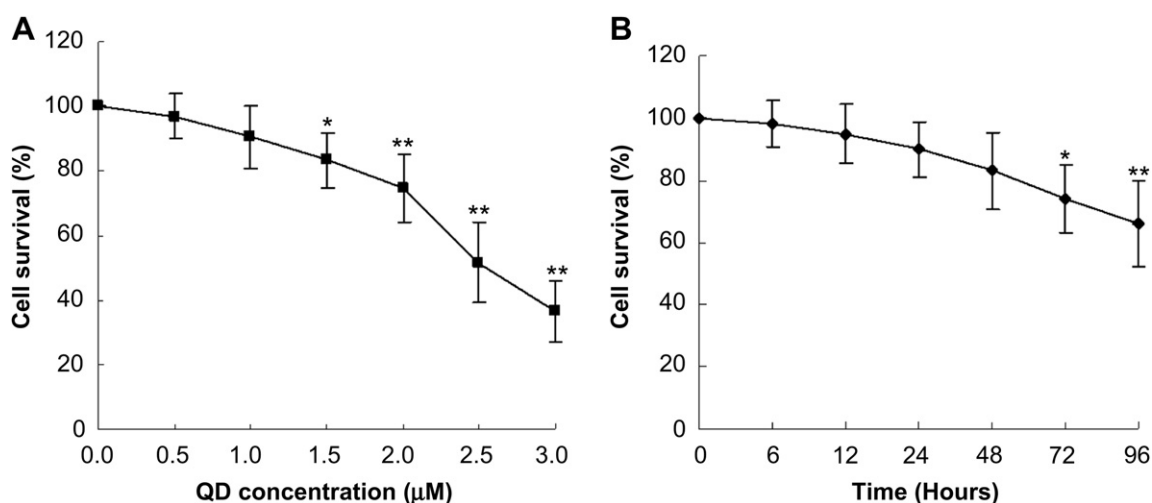


Fig. 3. (A) Cytotoxicity of CdTe/CdS QDs in different concentrations with *Hela* cells in 24 h. (B) Cytotoxicity of 1 μ M CdTe/CdS QDs in different time with *Hela* cells. Viability of *Hela* cells after treated with CdTe/CdS QDs in DMEM medium at 37 $^{\circ}$ C in the humidified atmosphere with 5% CO₂. The cell viability was calculated as a percentage from the viability of the control (untreated) cells. The viability of the control cells was considered as 100%. The results are means \pm SE from three independent experiments. * P < 0.05 versus control group, ** P < 0.01 versus control group.

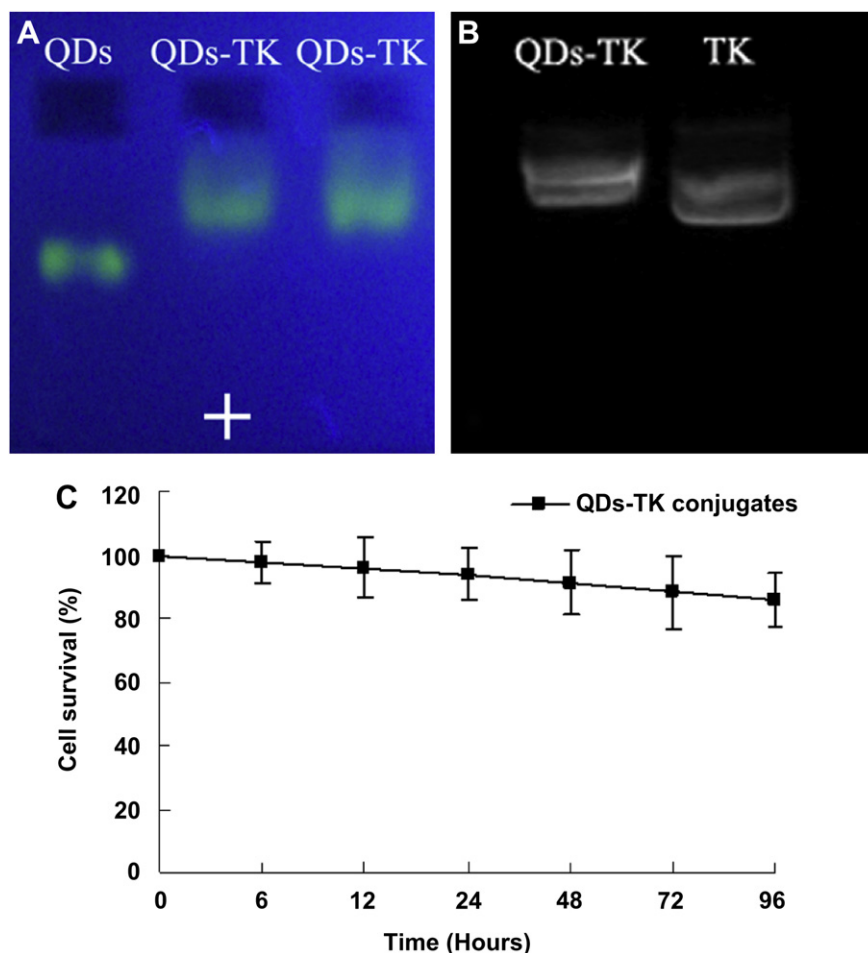


Fig. 4. (A) Digital photo of the QDs-based AGE gel electrophoresis for probing the TK gene under the excitation of a 365 nm ultraviolet lamp. The positive pole of the electrophoresis was signed inset. Left panel showed pure QDs while the other two panels showed QDs-TK conjugates. (B) The AGE electrophoresis of bare TK gene and QDs-TK conjugates dyed with EB. Left panel showed QDs-TK conjugates while right panel showed pure TK gene. (C) Cytotoxicity of QDs-TK conjugates in different incubation time without GCV after transfected with HeLa cells. The cell viability was calculated as a percentage from the viability of the control (untransfection) cells. The viability of the control cells was considered as 100%. The results are means \pm SE from three independent experiments.

promoted does-dependently death of HeLa cells transfected with the QDs-TK conjugates. The minimum effective concentration of GCV was 10 $\mu\text{g/ml}$, there was no significant cytotoxicity at 1 $\mu\text{g/ml}$ GCV. For all tested strategies, the highest reduction in cell viability was obtained at 96 h after transfected with QDs-TK conjugates plus GCV. Fig. 6 showed the morphology of HeLa cells incubated with 10 $\mu\text{g/ml}$ GCV at 96 h after transfected with the 10 μl of QDs-TK conjugates (15 μM), 10 μl of TK plasmid (15 μM) alone, and 10 μl of QDs (15 μM) alone, respectively. Microscopic studies partly confirmed the biochemical assays of cellular viability. Compared with the non-transfected cells, the morphology of cells transfected with TK and the QDs-TK conjugates all showed granular cytoplasm, undefined nuclei, and evidence of bleb during incubation of GCV. However, the morphology of cells transfected with QDs was similar with the normal cells. The results showed the concentration of QDs used in this experiment was no toxicity to HeLa cells, and the death of cells had resulted from HSV-TK/GCV.

3.5. *In vitro* cellular imaging and death labeling of QDs-TK conjugates

In order to determine the localization of QDs or QDs-TK conjugates in cells and assess the cells' morphological changes, a fluorescence imaging study of HeLa cells transfected with QDs or QDs-

TK conjugates with or without GCV are shown in Fig. 7. The confocal fluorescent image showed QDs or QDs-TK conjugates were observed in the whole cell after transfected for 96 h, and HeLa cells transfected with QDs-TK conjugates (Fig. 7L) exhibited no significant difference in fluorescence intensity of intracellular QDs, as well as cell morphology compared with QDs alone (Fig. 7D), suggesting TK intracellular localization can be traced by QDs. In addition, HeLa cells transfected with QDs-TK conjugates during the 96 h incubation of GCV showed cell apoptosis such as shrinkage and deformation with chromatin aggregation by Hoechst nuclear stain (Fig. 7P), while cells transfected with QDs-TK presented normal morphology in the absence of GCV (Fig. 7L), QDs with GCV also did not induce morphological changes (Fig. 7H). Interestingly, apoptotic cells have stronger fluorescent intensity than normal cells (Fig. 7P), indicating QDs can be a good fluorescence maker for imaging cell apoptosis.

4. Discussion

In present study, we demonstrate one mean of monitoring TK gene delivery and anti-cancer activity in vitro studies using water-soluble CdTe/Cds QDs with emission wavelength of 543 nm linked to plasmid pCMV-TK encoding HSV-TK gene.

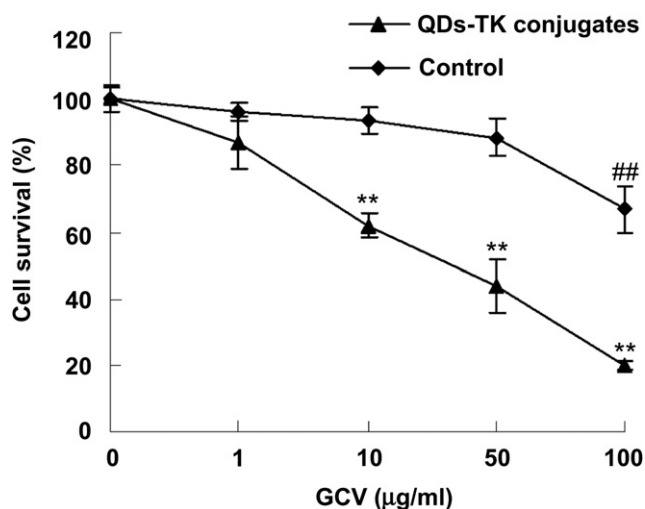


Fig. 5. Effect of ganciclovir concentration on the viability of HeLa cells after transfecting with or without the QDs-TK conjugates for 96 h. Results showed as the concentration of GCV reached 10 µg/ml, 39% HeLa cells transfected with the QDs-TK conjugates dead while only 5% HeLa cells without transfection. The cytotoxicity of GCV was dose dependent. 100 µg/ml GCV caused 34% HeLa cells without transfection dead. ** $P < 0.01$ versus the HeLa cells transfected with the QDs-TK conjugates, which were not treated with GCV; ## $P < 0.01$ versus the HeLa cells without transfection, which were not treated with GCV.

QDs have proved to be ideal optical probes for biological imaging. Traditional QDs synthesized in organic phase must be transferred into aqueous solution for the biocompatibility through surface modification. Whereas, this process often leads to a decrease of the PL QY, weak water-solubility and stability, and increase of the hydrophilic radius, which will strongly limit the applications in biology and biomedicine, especially mentioning the nucleus imaging, because the localization of QDs to the nucleus is

size-dependent (<5 nm) [19]. On the contrary, the water-solubility and biocompatibility of the CdTe QDs synthesized directly in aqueous solution using thiol ligands as stabilizer are better and easier to control just by changing the surface active groups. However, the traditional direct synthesis of QDs in aqueous phase often brought about low QY and stability, which needs powerful developments of water-soluble QDs' preparation and surface modification technique to improve the quality. For example, after a CdS shell passivation, both the QY and photostability or chemical stability of the CdTe QDs is increased greatly [20,21]. In addition, the cytotoxicity of the CdTe QDs could be greatly dropped down via passivating with CdS shell [22]. As a result, the aqueous phase synthesized core/shell QDs-based probes should be widely used in biological and biomedical fields. QDs are highly resistant to metabolic degradation and keep their fluorescent properties after chemical modification, thus we prepared highly luminescent and stable CdTe/CdS core/shell quantum dots in this study.

Next, covalent conjugation of plasmid TK to our tailor-designed QDs modified with –COOH group was obtained using EDC/NHS as coupling reagents. Existing key points, however, lie in maintaining activity of TK and luminescence of the QDs, as well as avoiding the cytotoxicity of QDs for labeling and tracking TK in cancer therapy. In fact, the cytotoxicity of QD can be effectively avoided in some cases [23–25]. In our research, HeLa cells were incubated with QDs for 24 h following a measurement of cell mitochondrial activity through MTT viability assay. The results showed that the viability of cell growth with the QDs decreased with increasing the QDs concentration. When the dose was 1 µM or less, there was no influence on cell growth and development. Thus low dosage of QDs did not have discernable adverse effects on the targeted cells in vitro compared with the same cells not exposed to such QDs.

There have been many reports using QDs for labeling cells, live embryos, tumor cells, antibodies, proteins, and single-stranded oligonucleotides [9,26–28]. Until now QDs labeling of plasmid DNA has few attempts due to its large size and the perception that

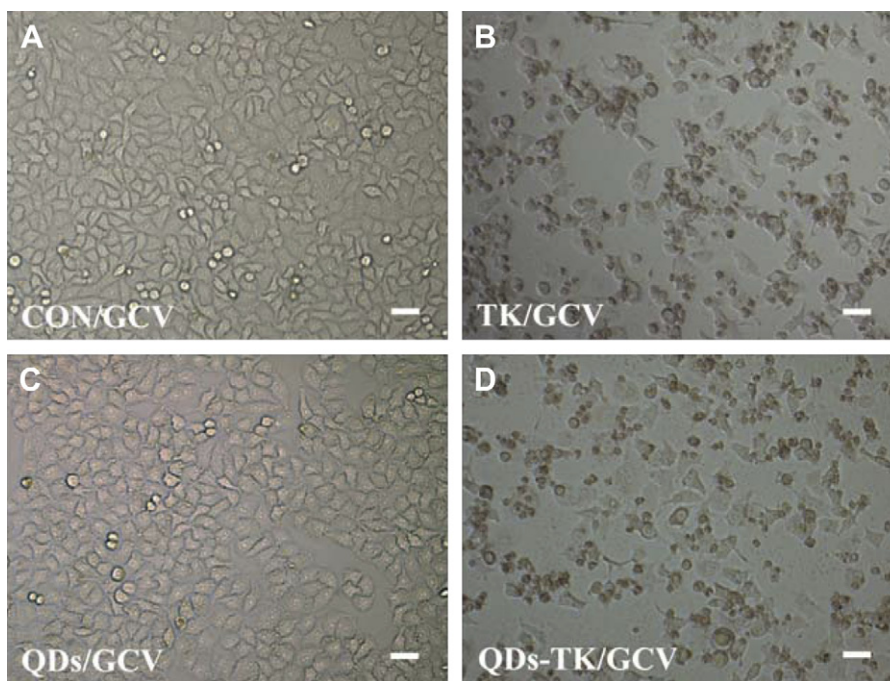


Fig. 6. Morphology of HeLa cells incubated with 10 µg/ml GCV for 96 h after transfected with TK, QDs, and the QDs-TK conjugates respectively. (A) The normal HeLa cells were treated with GCV as a control; (B) The cells transfected with 10 µl of TK plasmid (15 µM) alone were treated with GCV; (C) The cells transfected with 10 µl of QDs (15 µM) alone were treated with GCV; (D) The cells transfected with 10 µl of QDs-TK conjugates (15 µM) were treated with GCV. The scale bar indicates 100 µm.

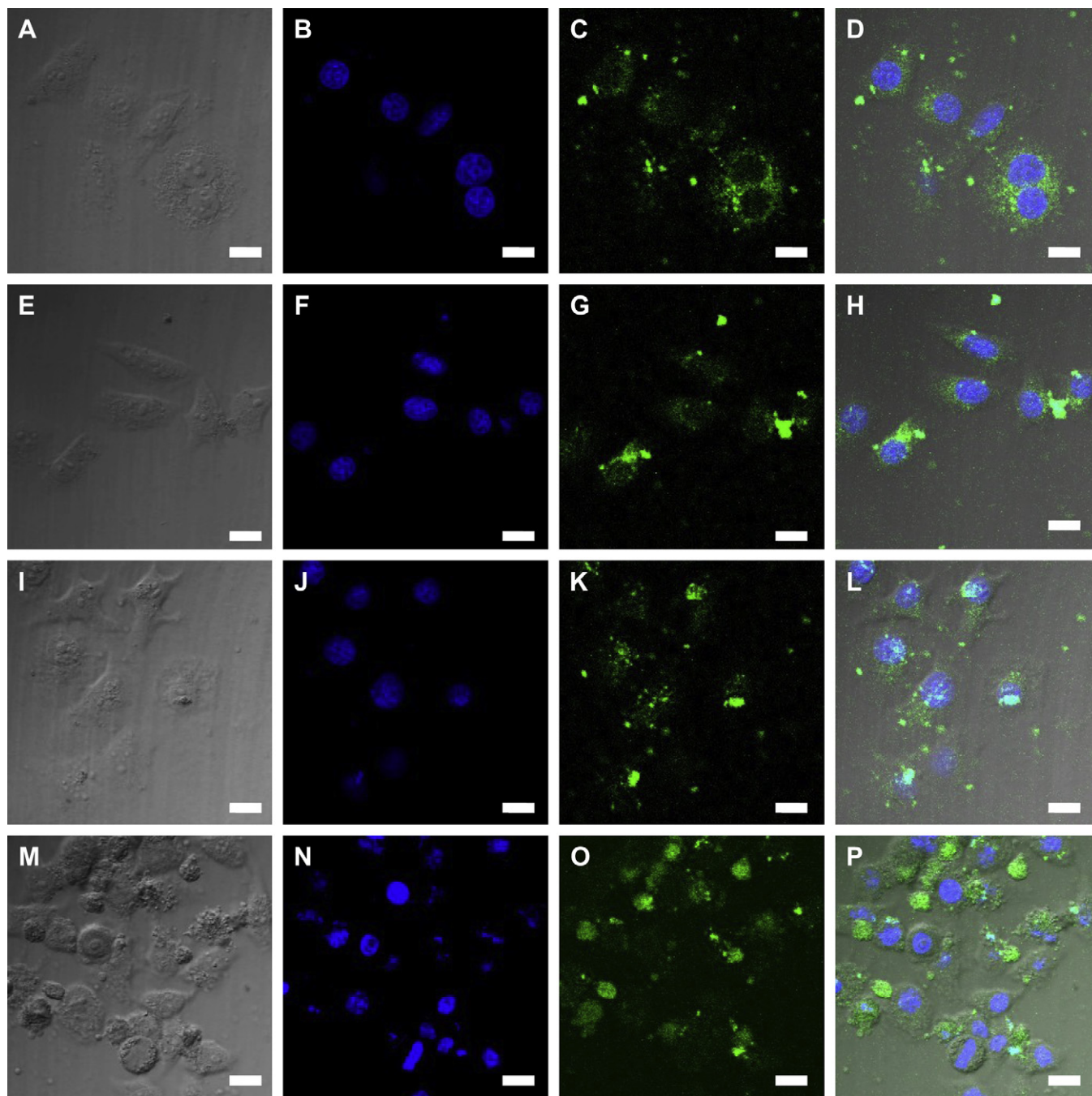


Fig. 7. The imaging of HeLa cells after transfection 96 h with the QDs or QDs-TK conjugates with or without GCV by staining with the Hoechst 33258, and observing under confocal microscopy. Confocal micrographs of HeLa cells transfected QDs without (A–D) or with GCV (E–H), as well as transfected QDs-TK conjugates without (I–L) or with GCV (M–P): (A, E, I, M) visualization of cells in bright-field; (B, F, J, N) visualization of nuclei stained with Hoechst 33258; (C, G, K, O) visualization of QDs or QDs-TK conjugates in the intracellular compartment; (D, H, L, P) overlay of the three micrographs. The scale bar indicates 10 μm .

any covalent modification of the DNA template might alter the integrity of the plasmid [29]. An ideal probe for imaging the intracellular trafficking of DNA should ensure that the “tagged” plasmid DNA remains functional and thereby serves as a template for gene transcription upon arrival in the nucleus. Srinivasan et al. reported that conjugation of plasmid DNA with phospholipid-coated QDs was accomplished using a peptide nucleic acid–N-succinimidyl-3-(2-pyridylthio) propionate (PNA–SPDP) linker. These QD–DNA conjugates were capable of expressing the reporter protein, enhanced green fluorescent protein [30]. However, the huge cost of the SPDP–PNA linker would limit its application in vivo. In this study,

we have developed a simple procedure for labeling plasmid TK using our highly luminescent and stable CdTe/Cds core/shell quantum dots modified with –COOH group using EDC/NHS as coupling reagents. To confirm QDs–TK conjugation, we used gel electrophoresis detection method based on QDs’ green emission or Ethidium bromide’s red fluorescence respectively. Our MTT results in HeLa cell line showed the QDs–TK conjugates has been fully functional in its antitumor activity. In order to eliminate the influence of QDs cytotoxicity for living cell imaging, the QDs–TK conjugates were also incubated with HeLa cells even up to 96 h. The results showed the concentration of the QDs–TK conjugates we used in this experiment

was no obvious toxicity to Hela cells. Accordingly the death of cells had resulted from HSV-TK/GCV but QDs.

Suicide gene therapy is a unique form of drug delivery system that allows for negative selection of malignant cells using a prodrug approach. It is an exciting strategy currently in clinical trial in the treatment of a number of tumors, such as prostate cancer [31], glioblastoma [6] and nasopharyngeal carcinoma [32]. However, normal human cells also expressing TK enzymes due to low selection, the potential of GCV may cause long-term organ damage and increased risk of side effects. As a result, it might restrict the application of this promising method. Therefore it is important for development of new strategies for labeling and monitoring TK in order to kill cancer selectively without damage of normal cells. There have been a few reports about tracing HSV-TK suicide gene therapy in living cells and animals by fluorescence agents [33,34]. Jacobs and coworkers constructed a model fusion gene comprising green fluorescent protein (GFP) and HSV-TK genes. Although they demonstrated that fusion gene approach provides a paradigm of monitoring therapeutic suicide gene expression *in vitro*, it could not trace intracellular location of TK gene at long and real time. Moreover lower photostability and higher immunogenicity of fluorescent protein GFP limited its application [33]. To solve the problem we employed an inorganic fluorophore–quantum dots as tools for labeling and monitoring TK, because QDs have high fluorescence intensity and photostability for long time. In present study, we transfected the QDs-TK conjugates into Hela cells for TK localization for 96 h. Once QDs-TK conjugates entered into the cells, GCV was added to the transfected cells. Confocal microscopy showed after transfected with QDs-TK conjugates plus GCV for 96 h, the nucleus of Hela cell exhibited the classical morphological characteristics of apoptosis, including reduction in nuclear size, chromatin condensation, and DNA fragmentation by staining with the Hoechst nuclear staining. Moreover apoptotic cells have stronger fluorescence intensity by QDs imaging and labeling. These results indicate that QDs provide new approach for TK tracing and antitumoral effect in cancer therapy during long-term.

5. Conclusions

In this study, the long-term imaging of the intracellular localization and therapy effect of TK gene was first demonstrated by linking the water-soluble CdTe/CdS QDs to plasmid pCMV-TK gene using EDC/NHS as coupling reagents. Taking the advantage of photostability and nanometer-size, the QDs could label and image TK gene up to 96 h after being transfected to Hela cells. The cell viability assay demonstrated that the death of tumor cells was due to HSV-TK/GCV. Moreover the apoptosis induced by HSV-TK/GCV is distinctly traced partly via strong fluorescence of the QDs. These results provide useful insight towards using the QDs-TK conjugates in future studies to gain a better understanding of the efficiencies of the various processes involved early cancer theranostic even cancer therapy *in vivo*.

Acknowledgments

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