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Multifunctional QD-based co-delivery of siRNA and doxorubicin to HeLa cells for reversal of multidrug resistance and real-time tracking

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ABSTRACT

Co-delivery of siRNA and chemotherapeutic agents has been developed to combat multidrug resistance in cancer therapy. Recently, we developed a series of quantum dots (QDs) functionalized by β-cyclodextrin (β-CD) coupled to amino acids, some of which can be used to facilitate the delivery of siRNA. In this study, two CdSe/ZnSe QDs modified with β-CD coupled to L-Arg or L-His were used to simultaneously deliver doxorubicin (Dox) and siRNA targeting the MDR1 gene to reverse the multidrug resistance of HeLa cells. In this co-delivery system, Dox was firstly encapsulated into the hydrophobic cavities of β-CD, resulting in bypass of P-glycoprotein (P-gp)-mediated drug efflux. After complex formation of the mdr1 siRNA with Dox-loaded QDs via electrostatic interaction, significant down-regulation of mdr1 mRNA levels and P-gp expression was achieved as shown by RT-PCR and Western blotting experiments, respectively. The number of apoptotic HeLa cells after treatment with the complexes substantially exceeded the number of apoptotic cells induced by free Dox only. The intrinsic fluorescence of the QDs provided an approach to track the system by laser confocal microscopy. These multifunctional QDs are promising vehicles for the co-delivery of nucleic acids and chemotherapeutics and for real-time tracking of treatment.

1. Introduction

Poly-therapy is the combination of various treatment methods with different mechanisms to merge their individual advantages and surmount obstacles in the treatment of disease [1–4]. Co-delivery carriers are able to achieve the simultaneous transport of different therapeutic agents in the target tissue and enhance the treatment effects. For example, co-delivery of paclitaxel with interleukin-12-encoded plasmid DNA in cationic core–shell nanocomplexes to the same cells inhibited cancer growth in a mouse model of breast cancer more effectively than the individual administration of either paclitaxel or the plasmid [5]. The therapy agents delivered by the same vehicle were in close proximity and exerted synergistic effects on the target tissue.

To date, various co-delivery platforms for siRNA and chemotherapy agents have been developed to reverse multidrug resistance in cancer cells. For instance, a cationic liposome that co-delivered doxorubicin with siRNA targeted to MDR1 and BCL2 mRNA in MDR lung cancer cells suppressed cellular resistance and enhanced cell death induction [6]. Liposomes possess a relatively large payload, but the application of this delivery system has been limited by their complicated construction and their poor performance in vivo due to uptake in non-specific tissues [7]. Todd et al. developed dendrimer-based nanoglobular carrier to contemporaneously deliver siRNA and Dox to achieve combination effect of chemotherapy and gene therapy [8]. Mesoporous silica nanoparticles (MSNP) was innovatively used to co-deliver mdr1 siRNA to down-regulate P-gp expression and Dox to induce apoptosis at lower dosages [9]. More recently, hierarchical nano-assemblies were formulated as a facile nano-vector for siRNA and drug delivery [10]. Although the existing carriers have performed well as co-delivery systems, we believe that introducing intrinsic fluorescence to the vector will allow for observation of the co-delivery in vivo and in vitro after administration.

Recently, we synthesized a series of QDs with small size (4–5 nm), a positive charge, low toxicity ($IC_{50} > 100$ μg/mL), high biocompatibility, high colloidal stability in biological buffer and strong optical emission properties that allow the QDs to have the

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potential to be carriers for in vitro and in vivo drug delivery [11–13]. Among these QDs, the core/shell CdSe/ZnSe QDs modified with β-CD coupled to L-Arg or L-His have been demonstrated a lower cytotoxicity and a higher transfection efficiency in siRNA delivery. The long fluorescence half-life allows for QDs to be easily and stably monitored in gene silencing studies. The addition of β-CD was applied to improve the solubility and biocompatibility of the QDs.

In this study, the hydrophobic cavities of β-CD were used to encapsulate doxorubicin (Dox) and were combined with the corresponding siRNA to create a chemo-gene therapy [14,15]. We used multifunctional β-CD-L-Arg-QDs and β-CD-L-His-QDs as multifunctional nanocarrier-based delivery systems (NDS) to co-deliver Dox and siRNA targeting the MDR1 gene, which is responsible for multidrug resistance in cancer cells. The schematic representation of formation of Dox-loaded and siRNA-bound L-amino acid-β-CD-modified QDs and the rational process of reversing multidrug resistance by these complexes is shown in Scheme 1. The QDs show potential in the simultaneous transport of the siRNA and doxorubicin across the cell membrane to down-regulate the expression of P-gp and induce apoptosis of the cancer cell with a lower dose of Dox. We expect that these QDs can (i) bind with and penetrate negatively charged cell membranes through endocytosis, (ii) provide endosomal release of the siRNA and Dox, and (iii) down-regulate the expression of P-gp, increase Dox accumulation in nucleus and induce apoptosis in multidrug resistant cancer cells. The effectiveness of the two QDs as NDS for mdr1 siRNA and Dox co-delivery was evaluated using a doxorubicin-resistant cervical carcinoma cell line.

2. Materials and methods

2.1. Materials and reagents

All reagents were purchased from Sigma–Aldrich unless indicated otherwise and were used without additional treatment. β-CD-L-Arg-QDs and β-CD-L-His-QDs were synthesized based on our previous work [12,13]. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were obtained from HyClone Inc. The anti-P-gp monoclonal antibody and β-actin monoclonal antibody were obtained from Santa Cruz (USA). The mdr1 siRNA and FAM-labeled negative siRNA were purchased from Dharmacon (USA). Doxorubicin (Dox) was purchased from Keygene Biotech Co. (Nanjing, China).

2.2. Preparation and characterization of Dox-loaded QDs and Dox-loaded QDs-siRNA complexes

Dox-loaded QDs were prepared as follows: Dox and β-CD-L-Arg-modified QDs were dissolved in PBS at various mass ratios (m_{QDs}:m_{Dox} = 1:0.5, 1:1, 1:1.5, 1:2, 1:4), followed by ultrasonic agitation in a Type 60 Sonic Dismembrator (Fisher Scientific) at a power level of 30 for 4 h. The β-CD-L-His-QD-encapsulated Dox was prepared in the same manner.

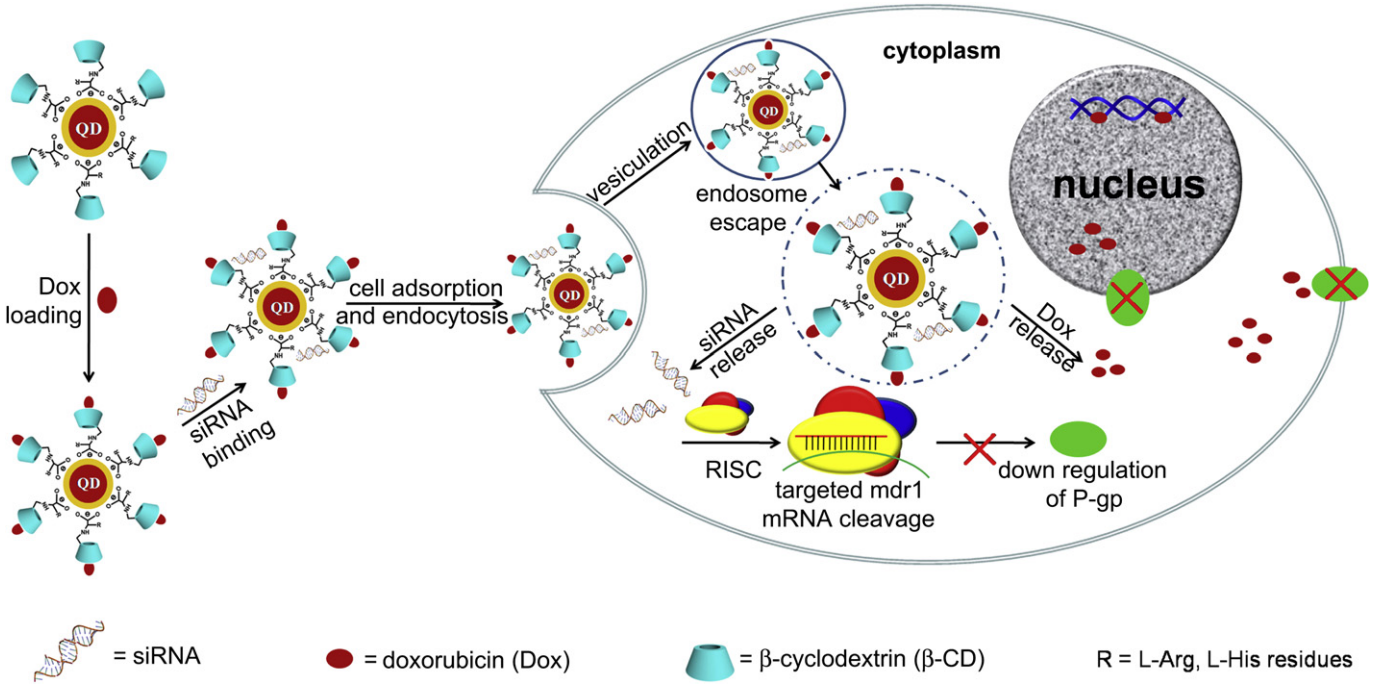
The UV–visible absorption spectra of free Dox and Dox-loaded QDs were scanned from 300 nm to 600 nm with a Cary 300 spectrophotometer (Varian, USA). The fluorescence emission spectra from 500 nm to 700 nm were obtained using an RF-5301PC spectrofluorophotometer (Shimadzu, Japan). The formation of Dox-loaded QDs was confirmed using an infrared spectrometer (Bruker, Germany) and comparing the infrared spectrum to that of free Dox. To investigate the Dox release mechanism, two Dox-loaded QDs were incubated in PBS (pH 7.4) or acetate buffer (pH 5) at 37 °C for 72 h, and their fluorescence emission spectra were measured at different time points.

Dox-loaded and siRNA-bound QD-complexes (Dox/β-CD-L-Arg-QDs-siRNA, Dox/β-CD-L-Arg-QDs-siRNA) were prepared as previously described [10]. In short, two Dox-loaded QDs were incubated with siRNA at room temperature for 1 h to form the complexes.

The particle sizes and morphology of the two Dox-loaded QDs and the two Dox-loaded QD-siRNA complexes were determined by TEM (JEOL. Ltd. Inc., Japan), and their zeta potentials were measured under room temperature using a zeta potential analyzer (Beckman Coulter Inc., USA).

2.3. Cell lines, culture conditions and the establishment of a doxorubicin-resistant cell line

The HeLa human cervical carcinoma cell line was obtained from the Experimental Animal Center at Sun Yat-Sen University. A doxorubicin-resistant cell line (HeLa/Dox) was established by stepwise and continuous exposure of the parental HeLa cells to an increasing concentration of Dox as described previously [16,17]. After a 1-year period of culturing, the mdr1 mRNA expression levels (Fig. S1A) and P-gp levels (Fig. S1B) were remarkably elevated compared to the HeLa parental cell line. Fig. S1C showed that Dox sensitivity of the HeLa/Dox cell line (IC_{50} = 8.0 μg/mL after 24 h) decreased approximately 23 fold compared to the original HeLa cells (IC_{50} = 0.35 μg/mL after 24 h). The HeLa cells and HeLa/Dox cells were routinely



Scheme 1. Schematic representation of siRNA adsorption and Dox loading onto L-amino acid-β-CD-modified QDs and illustration of multifunctional QDs as co-delivery platforms for mdr1 siRNA and Dox to reduce multidrug resistance in cancer cells.