A Synergistic Theranostic Approach Using a Targeted siRNA Drug Delivery Platform and a Phthalocyanine-Loaded Dendrimer for Ovarian Cancer Treatment

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ABSTRACT SUMMARY

We have developed a novel combinatorial therapeutic approach actively targeting ovarian cancer using a luteinizing hormone releasing hormone (LHRH) conjugated dendrimer siRNA delivery system capable of silencing DJ-1, a scavenger of reactive oxygen species (ROS), and a phthalocyanine (Pc) loaded dendrimer platform with its mechanism of action conferring cytotoxicity through the production of ROS after light activation. By suppressing ROS-defensive intracellular system with targeted siRNA nanoparticles we were able to successfully enhance the therapeutic efficacy of photodynamic therapy, as well as use the Pc’s theranostic capabilities to provide image guided drug delivery to ovarian cancer cells.

INTRODUCTION

One of the distinctive features of cancer cells, compared to non-transformed cells, is an excessive intracellular level of toxic reactive oxygen species (ROS) as well as overexpression of certain receptors specific for that cancers activation and growth. In addition, cancer cells have developed a ROS defense system, which provides resistance to ROS-induced therapies. The current work is the first attempt to selectively target and treat ovarian cancer by using a novel combinatorial approach based on the synergetic effect of a noninvasive, specifically-targeted photodynamic therapy (PDT) as an exogenous ROS-generating source associated with the siRNA-mediated suppression of the DJ-1 gene, a main player in the intracellular ROS-defense mechanism. Thus, we have developed the required dendrimer-based nanomedicine platforms for tumor targeted delivery of a PDT agent, phthalocyanine (Pc), and DJ-1 specific siRNA, respectively. Owing to the outstanding near-infrared (NIR) optical properties of Pc, our platform has significant potential as a theranostic agent for simultaneous fluorescence image-guided drug delivery and noninvasive treatment of deep tumors by PDT.

EXPERIMENTAL METHODS

The development of the Pc-based theranostic agent (Pc–LHRH) was achieved by the initial encapsulation of monosubstituted Pc into a generation 4 polypropyleneimine (PPI G4) dendrimer. After encapsulation, maleimide-PEG-NHS was added to the solution with ~10-fold molar excess. The NHS on PEG reacted with the amine groups on the periphery of PPI G4 for 1h. Covalent conjugation of LHRH peptides onto the distal end of the PEG layer was achieved by the reaction of the maleimide group on PEG with the cysteine of LHRH peptide. The preparation procedure for siRNA delivery system involved the complexation of siRNA with PPI G4 dendrimers into nanoparticles via electrostatic interactions. To improve biocompatibility and targeted delivery, the surface of the resulting complexes was additionally modified with PEG and LHRH peptide as described above. The ability of nanoparticles to internalize in cancer cells and suppress the expression of targeting mRNA genes was studied by flow cytometry and qPCR, respectively. In vivo body distribution experiments were carried out on nude mice bearing subcutaneous xenografts of human ovarian cancer cells. The fluorescent images were recorded using a Li-Cor Pearl Animal Imaging System at 10 h after iv injection with Pc-LHRH. During the combinatorial treatment ovarian cancer cells (A2780 and ES2) were concurrently incubated with both siRNA-PPI G4 ([siRNA] = 1 μM) and Pc-LHRH ([Pc] = 0.063 μg/mL) for 48 hrs in the dark. Afterwards, cancer cells were exposed to 120 mW/cm² laser diode light of 670 nm for 5 min. The cells viability and ROS level were evaluated with modified Calcein AM and DCFH-DA assays, respectively.

RESULTS AND DISCUSSION

Clinical application of phthalocyanines as theranostic agents is substantially limited by poor water solubility, aggregation and insufficient selectivity for cancer cells. To address these issues, we have developed a novel dendrimer-based theranostic platform for tumor-targeted delivery of hydrophobic Pc. The loading of Pc into the dendrimer interior prevented aggregation of encapsulated photosensitizers in aqueous solutions and thereby preserved their photophysical properties required for efficient fluorescence imaging and PDT. The use of PEG and LHRH peptide significantly diminished cytotoxicity of the developed platform and substantially enhanced its internalization by the cancer cells. We demonstrated that body distribution of the developed nanocarrier can be determined based on the intrinsic fluorescence properties of encapsulated Pc, validating its role as a theranostic agent (Fig. 1A). The in vivo imaging experiments also revealed that the LHRH targeted nanocarrier is capable of an efficient accumulation into cancer tumor. Moreover, it was demonstrated that without the need to release the drug from the carrier the prepared formulation can efficiently generate intracellular ROS upon laser activation to injure cancer cells (Fig. 1B).
Moreover the DJ-1 specific siRNA delivered to the LHRH-positive cancer cells by the prepared delivery system was able to achieve a 40% decrease in the mRNA expression of the targeted gene (Fig. 2B). This provides the basis that developed siRNA and Pc nanomedicine platforms can successfully reach and internalized by their intended target with high efficiency and specificity.

To demonstrate an efficiency of the developed combinatorial approach, the ovarian cancer cells were treated with cocktail of LHRH-targeted complexes loaded with either DJ-1 siRNA (1 μM) or Pc (0.063 μg/mL) for 48 hrs. According to our data, the selected incubation time (48 hrs) was essential to allow the degradation of the DJ1 mRNA and intramolecular digestion of the corresponding protein before application of PDT. After exposure of the cancer cells to laser diode light of 670 nm for 5 min, we detected 40% decrease in viability of the cells concurrently treated with both DJ-1 siRNA and Pc in comparison to the cells exposed to PDT alone (Fig. 3B). Moreover, the enhancement in cell apoptosis was accompanied by a 20% increase in intracellular ROS level compared to cells treated with PDT alone (Fig. 3B).

CONCLUSION
We have successfully synthesized a non-toxic, and targeted delivery system capable of therapeutically delivering PDT drugs and siRNA. Thus, the cytotoxic effect of the combinatorial treatment achieved with the developed delivery systems is superior to each of the two treatments applied separately.

REFERENCES

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