Multifunctional Drug Delivery System for Combinatorial Treatment of Prostate Cancer

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ABSTRACT SUMMARY

To enhance efficacy of advanced prostate cancer treatment, we developed novel multifunctional platform for simultaneous co-delivery of chemotherapeutic drugs, intracellular nanoheaters, and suppressors of cancer cellular resistance specifically to prostate cancer cells.

INTRODUCTION

The efficacy of conventional chemotherapy for advanced prostate cancer treatment is significantly limited by (i) the insufficient specificity of the drugs for the tumors and (ii) the rapid development of cancer cell resistance during treatment. Thus, a substantial enhancement in efficacy of prostate cancer treatment is possible by the development of combinatorial therapeutic approach, which includes (i) the targeted delivery of high drug concentration directly to the cancer cells and (ii) simultaneous suppression of major mechanisms of cancer cell resistance. Furthermore, results from a number of clinical trials have provided evidence for the significant improvement in the effectiveness of cancer treatment by conventional chemotherapy in conjunction with hyperthermia [1]. However, heat delivery via external applicators has not resulted in a highly-positive therapeutic ratio [1]. Therefore, the development of new methods for the co-delivery of heat, chemotherapeutic agents and suppressors of cell resistance specifically to cancer cells is highly desirable. Consequently, we designed a novel highly efficient drug delivery system (DDS) that contains four main components: (1) iron oxide nanoparticles as intratumoral “nanoheaters,” and carriers of active ingredients; (2) an anticancer drug; (3) siRNA as suppressors of cancer cell resistance, and (4) LHRH peptide as a targeting moiety to the prostate cancer cells.

EXPERIMENTAL METHODS

Oleic acid stabilized iron oxide nanocrystals were synthesized and their surface was modified with two different polymer layers in tandem such as poly (maleic anhydride-alt-1-octadecene) (PMAO) and polyethyleneimine (PEI). Transmission electron microscopy (TEM) and Dynamic Light Scattering (DLS) were used to characterize the size and shape of iron oxide nanoparticles. Heat production was measured by exposing a solution of nanoparticles to an alternated magnetic field created by a custom-made induction heating system. To encapsulate doxorubicin (DOX) into iron oxide nanoparticles, the nanoparticles were suspended in the drug loading solutions under continuous stirring. The drug loading efficiency into iron oxide nanoparticles was evaluated by using HPLC. DOX loaded iron oxide nanoparticles in the presence of amine terminated PPI G5 dendrimers were employed to condense BCL2 siRNA into complexes via electrostatic interaction. An ability of drug loaded nanoparticles and PPI G5 dendrimers to provoke siRNAs complexation was evaluated by gel retardation assay. PEGylation was carried out by coupling linear MAL-PEG-NHS to the PPI G5 amino groups on the surface of siRNA complexes. Furthermore, LHRH peptide was conjugated to the distal end of PEG. Atomic Force Microscope (AFM) and DLS were additionally employed to characterize the properties of developed DDS. The ability of nanoparticles to penetrate cancer cells, internalize siRNAs, and suppress the expression of targeted mRNA was studied by confocal microscopy and quantitative RT-PCR (qPCR), respectively. The Pearl imaging system was additionally employed to evaluate the organ distribution of DDS.

RESULTS AND DISCUSSION

The iron oxide nanoparticles based DDS was designed to fulfill the following tasks: 1) to concurrently transfers anticancer drugs, intracellular nanoheaters, and suppressors of cancer cellular resistance to the prostate cancer cells, 2) to shields itself from the immune system, and 3) to specifically targets prostate cancer cells to diminish adverse side effects. Thus, the developed iron oxide nanoparticles capable of generating heat upon exposure to alternating magnetic field (AMF) created by an induction heating system (Fig. 1) and can be employed as nanoheaters for targeted hyperthermia [2].

In addition to heat delivery, the proposed approach for nanoparticle surface modification allowed us to employ the developed iron oxide nanoparticles as a cargo for concurrent delivery of chemotherapeutic drugs and siRNA to prostate cancer cells. Thus, the hydrocarbon chains of the PMAO intercalate into the inner hydrophobic oleic acid layer on the iron oxide surface and form a hydrophobic reservoir for anticancer drugs loading (Fig. 2A). It was demonstrated that DOX loading
efficiency of nanoparticles was 70% w/w. Under basic conditions, PMAO can electrostatically adsorb the second layer of a positively charged PEI. The positive charge on the nanoparticles surface (+50mV) facilitates electrostatic binding of siRNA and enhanced their cellular internalization [3]. Moreover, we demonstrated the possibility to control the amount of SPION in the inner structure siRNA-iron oxide complexes by introducing additional condensation agent, PPI G5, to the reaction mixture (Fig. 2B). According to an AFM study, 200 nm uniformly distributed complexes were formed and the decrease in the amount of iron oxide nanoparticles compared to PPI G5 in the condensation mixture resulted in the sufficient reduction of nanoparticles in the inner structure of the complexes (Fig. 3A-C). The ability to change iron oxide nanoparticles content in the formulated complexes allows us to control the amount of drug and heat output delivered by DDS. To enhance steric stability of DDS and reduce their uptake by cells of the reticuloendothelial system, PEG was employed to modify the DDS surface (Fig. 2C) [4]. Furthermore, LHRH peptide as a ligand to corresponding LHRH receptors that are overexpressed in the plasma membrane of many types of cancer cells was conjugated to the distal end of PEG polymer to direct the DDS specifically to the prostate cancer tumor and limit the cytotoxic effect of chemotherapy on healthy organs (Fig. 2D) [4]. Our developed DDS was aimed to overcome resistance in the cancer cells via suppression of targeted proteins with siRNA. It was found that BCL2 protein overexpression protects cancer cells from apoptosis induced by chemotherapy and hyperthermia. In vitro and in vivo study demonstrated the feasibility of our DDS to sufficiently enhance the delivery of DOX and siRNA into PC3 prostate cancer cells (Fig. 4).

Analyzing the effect of the active components of DDS on the cellular resistance, it was found that BCL2 targeted siRNA significantly suppressed the expression of the corresponding gene (Fig. 3D). Furthermore, concurrent delivery of BCL2 siRNA and local nanoheaters to the prostate cancer cells significantly enhanced the apoptosis induction activity of DOX (Fig. 5).

CONCLUSION

Our results demonstrate the high potential of the developed DDS for targeted co-delivery of anticancer drugs, intracellular nanoheaters, and siRNA specifically to the prostate cancer cells.

REFERENCES


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