Image-Guided Combined siRNA and Enzyme/Prodrug Cancer Therapy

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Introduction

Destroying cancer cells while sparing normal tissue is one of the most sought after goals in cancer therapy. In previous work[1,2], we developed an image-guided enzyme/prodrug cancer strategy in which a prodrug-activating enzyme was labeled with multimodal imaging reporters. Prodrug was injected when the enzyme concentration was non-invasively determined to be high in tumor but low in normal tissues. This achieved an efficient therapeutic effect with minimized systemic toxicity. RNA interference (RNAi) is a naturally occurring process that mediates sequence-specific inhibition of gene expression. The discovery of RNAi mediated by small-interfering RNA (siRNA) has provided powerful new tools in cancer therapy. We previously observed that siRNA-mediated downregulation of choline kinase (chk), an enzyme that is upregulated in highly invasive breast tumor, increased the cell kill effects of 5-fluorouracil (5-FU) in breast cancer cells[3]. Here we have synthesized a conjugate, bCD-PEI-PLL, in which the prodrug-activating enzyme bacterial cytosine deaminase (bCD), multimodal imaging reporter labeled poly-$_L$-lysine (PLL), and siRNA delivery vector polyethyleneimine (PEI), were incorporated covalently. The bCD-PEI-PLL/siRNA-chk polyplex mediated combined siRNA and prodrug therapy demonstrated higher therapeutic effect than either prodrug or siRNA treatment given alone *in vitro* and *in vivo*. The data demonstrate that image-guided combined siRNA and prodrug strategy has significant potential in increasing therapeutic efficacy while minimizing damage to normal tissues.

Method and Experiment

bCD was chosen as the therapeutic enzyme due to its high enzymatic stability and the ability to convert the nontoxic prodrug 5-fluorocytosine (5FC) to the active drug 5FU. PLL (M_r = 5.6 kD) was selected as a carrier of the imaging reporters because of its multiple primary amines that are ready for multivalent labeling. T_1-weighted MRI contrast agent Gd^{3+}-DOTA and near-infrared fluorescent probe Cy5.5 were functionalized into PLL because MRI and optical imaging modalities have complementary strengths; the optical reporters allow microscopy of tissue sections at subcellular resolution, while MRI is required for clinical translation. PEI was used as the siRNA delivery vector because of its high transfection efficacy. Rhodamine and fluorescein labeled in PEI and siRNA-chk were used to track the intratumoral delivery of polyplex and disassociation of siRNA from vector in both live cell culture and excised tissue. The conversion of 5FC to 5FU can be detected non-invasively *in vivo* with ^{19}F MR spectroscopy, and the down-regulation of total choline in tumor xenograft can be quantified by ^{1}H MR spectroscopic imaging. Additionally, The bCD-PEI-PLL conjugate (Figure 1A) extravasates into the tumor interstitium due to the enhanced permeability and retention (EPR) effect of the macromolecules.

Results and Discussion

The molar equivalent ratio of bCD hexamer/PLL/PEI in bCD-PEI-PLL was determined as 1/1/1.1. The molecular weight, hydrodynamic radius and zeta potential of bCD-PEI-PLL were determined as 376 kDa, 45.8 nm and 1.9 mV respectively. Kinetic studies with 5FC as substrate revealed that bCD-PEI-PLL had the K_m value of 3.7 mM and k_{cat} value of 69 s^{-1}. bCD-PEI-PLL demonstrated low cytotoxicity with an IC_{50} value of 4.9 μM in human MDA-MB-231 breast cancer cell cultures. Confocal fluorescence microscopic studies showed that bCD-PEI-PLL/siRNA-chk polyplex (N/P = 50) internalized into MDA-MB-231 cells efficiently *in vitro* and FITC labeled siRNA-chk was successfully released from the vector to the cytosol at 4 h after the internalization. Immunoblot studies demonstrated a high chk down-regulation efficiency of the bCD-PEI-PLL/siRNA-chk polyplex in MDA-MB-231 cell culture even with siRNA concentrations as low as 20 nM (Figure 1B). The additive therapeutic effect was evident from the cell viability of 47% with combined siRNA and prodrug therapy, compared to 79% for treatment with polyplex alone and 75% for treatment with the active drug 5FU alone. *In vivo* MRI (Figure 1C) and optical imaging demonstrated the efficient intratumoral delivery of the polyplex within MDA-MB-231 tumors after intravenous injection. Optical images of the tumor sections indicated that siRNA-chk was released from the bCD-PEI-PLL vector between 4 and 24 h after injection. Significantly, *in vivo* MRI and *ex vivo* H&E staining clearly showed the development of large necrotic regions in the tumor at 48 h after polyplex injection (Figure 1D). In contrast there was no detectable necrosis in the liver and kidney, which indicates the absence of acute systemic toxicity.

Conclusion

In summary, we synthesized and characterized a novel bCD-PEI-PLL/siRNA-chk polyplex that demonstrated high enzymatic stability, efficient intracellular uptake and excellent transfection efficacy. *In vitro* studies demonstrated the additive therapeutic efficacy achieved by the combined siRNA and prodrug cancer strategy. *In vivo* MRI and optical imaging showed efficient intratumoral delivery of the polyplex and significant tumor cell kill after the image-guided combined treatments. *Ex vivo* H&E staining verified the efficient therapeutic effect without acute toxicity. Further characterizations of *in vivo* total choline down-regulation and tumor growth delay after the combined treatment are currently underway.

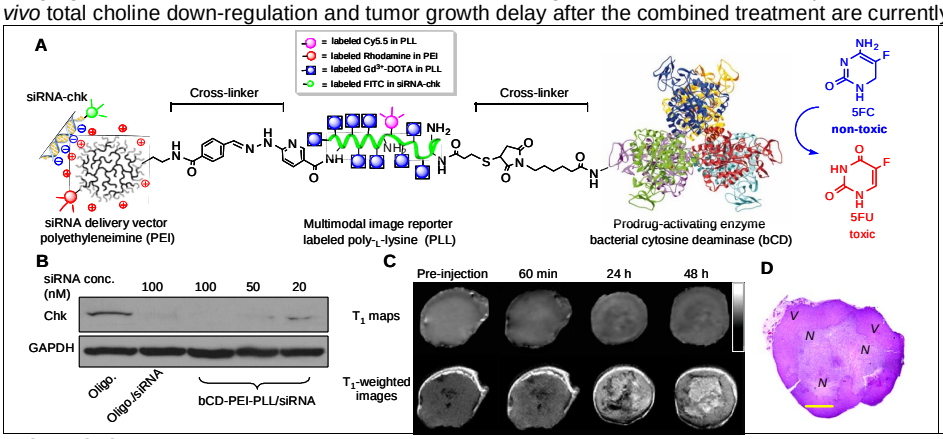

Figure 1 (A) Schematic of bCD-PEI-PLL/siRNA-chk polyplex. (B) Immunoblot of choline kinase in MDA-MB-231 cells after the treatment with the polyplex (N/P =50, siRNA conc.: 100 nM) for 48 h. Oligfectamine alone and oligofectamine/siRNA-chk lipoplex were used as the control. (C) *In vivo* dynamic T_1 map and T_1-weighted images of a wild type human breast MDA-MB-231 tumor xenograft after intravenous injection of the polyplex (380 mg/kg, N/P = 50). 5FC (160 mg/kg) was injected *i.v.* 24 h after the polyplex administration. (D) H&E stained histological sections of the tumor treated with the polyplex for 48 h (the same tumor in panel C). Purple hematoxylinphilic regions (***V***) indicate viable tumor tissues, and the eosinophilic area indicates tumor necrosis (***N***). *Scale bar*: 1.0 mm.

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