Image-guided molecular targeting of COX-2 using cationic liposomes containing siRNA and multi-modality imaging reporters

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Introduction: The cyclooxygenase (COX) pathway is the focus of several clinical trials targeting diseases with inflammatory components such as cancer. COX-2, an inducible cytoplasmic enzyme previously shown to be upregulated in different cancers such as that of the colon (1) and the breast (2), catalyzes the conversion of arachidonic acid to active lipid mediators known as prostaglandins (PGs) and thromboxanes. We previously demonstrated that silencing COX-2 using a small interfering RNA (siRNA) molecule stably expressed in the poorly differentiated and metastatic breast cancer cell line MDA-MB-231 delayed tumor formation and inhibited extrapulmonary experimental metastasis (3). Magnetic resonance (MR) image-guided delivery of COX-2 siRNA within tumors would therefore be an attractive treatment option. Several vehicles for systemic drug delivery, including cationic liposomes, are currently being evaluated for siRNA delivery and imaging (4-6). In this study we investigated the possibility of directly loading COX-2-specific siRNA into DOTAP:DOPE (DD) (3:1 molar ratio) and DOTAP:DOPE:DOPE_PEG2000 (DDP) (3:0.95:0.05 molar ratio) cationic liposomes. We used fluorescently labeled COX-2 siRNA and three different types of contrast agents to detect the delivery of this complex (lipoplex) using fluorescent and magnetic resonance imaging (MRI) in MDA-MB-231 breast cancer cells and tumors *in vivo* and determined the specific downregulation of COX-2 in cells.

Materials and Methods: Cationic liposomes with the base formulation DD and DDP were prepared by lipid hydration and extrusion through polycarbonate membrane with 100 nm pore size. siRNA with the following sequence for COX-2 – sense 5′-CAUUCCCUUCCUUCGAAAUdTdT-3′ and antisense 5′-AUUUCGAAGGAAGGGAAUGdTdT-3′ and a control siRNA against GAPDH with the sequence sense 5′- UGGUUUACAUGUUCCAAUAUU-3′ and antisense 5′-PUAUUGGAACAUGUAAACCAUU-3′ were directly loaded during the hydration step into cationic liposomes. COX-2 siRNA was additionally labeled with fluorescein 5(6)-isothiocyanate (FITC) for cell studies and with DY647 for tumor studies. To monitor the delivery of siRNA using MRI, Gd-DTPA-bis(oleylamide) (Gd-BOA) was added to the base formulation in place of varying fractions of the DOTAP, and Magnevist® or Feridex® were loaded directly during the hydration step. For the cell studies we used DD and DDP liposomes loaded with contrast agents. For the *in vivo* studies DDP liposomes and DY647 labeled COX-2 siRNA were selected based on results from the cell studies. The prepared lipoplex was injected intravenously (IV) and mice were imaged with a Xenogen IVIS 200 optical imaging device and MRI before and following the IV injection. The distribution and localization of lipoplex was examined at different time points using optical imaging. For MRI experiment we incorporated a commercially available T_2 contrast agent Feridex® along with with DY647-labeled COX-2 siRNA into DDP liposomes to monitor delivery of siRNA using MRI. Tumors were imaged for 3 h on a 9.4 T horizontal Bruker magnet using T_2-3D RARE (rapid acquisition with rapid enhancement) and T_2*-3D FLASH sequences.

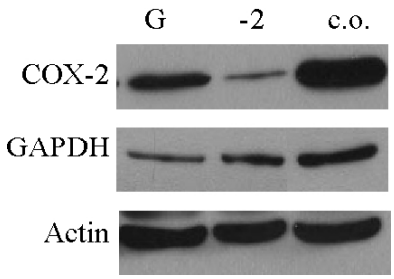

Figure 1. Western blot analysis of COX-2 and GAPDH in MDA-MB-231 breast cancer cells transfected with DD-COX-2 and DD-GAPDH lipoplexes loaded with Feridex®. (G: siRNA for GAPDH, -2: siRNA for COX-2, c.o.: MDA-MB-231 cells only).

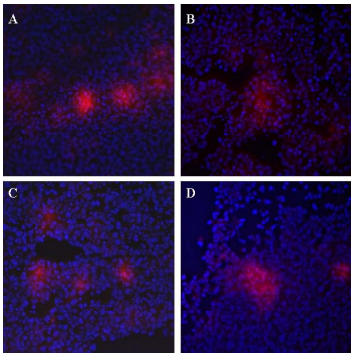

Figure 2. Fluorescent microscopy images of 10 μm tumor sections. Red: DY647-COX-2-siRNA, blue: DAPI-nucleus. (A) 0.5h, (B) 3h, (C) 8h, (D) 24h.

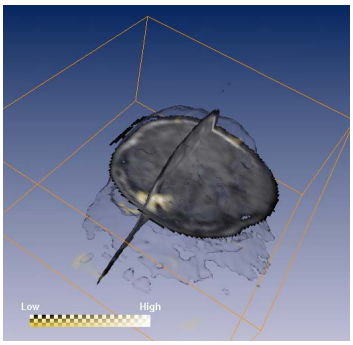

Figure 3. 3D reconstructed image (using Amira™) of difference in intensity in an MDA-MB-231 tumor at baseline and 120 min after administration of the complex. The bright areas show difference in intensity indicating delivery.

Results: MDA-MB-231 cells were transiently transfected using DD and DDP liposomes loaded with COX-2 and GAPDH siRNA and the above-mentioned contrast agents. Immunoblotting demonstrates specific downregulation of COX-2 using DD and DDP liposomes. When Magnevist® or Gd-BOA was incorporated within liposomes non-specific knockdown of COX-2 protein was observed 24 h following transfection (data not shown). On the other hand, incorporation of Feridex® did not affect transfection efficiency: DD liposomes loaded with Feridex® exhibited specific silencing of COX-2 protein *in vitro* (Fig.1). Following IV injections of DDP-COX-2 siRNA lipoplex at least 3 mice were studied at 0.5, 3, 8 and 24 h post injection. Lipoplex was observed in the tumor sections 0.5 h post injection (Fig. 2A). At 3 and 8 h the lipoplex was still detectable in the tumor but with lower concentration (Fig. 2B, C). At 24 h the lipoplex was more diffused (Fig. 2D). Distribution in the organs showed higher accumulation in the lungs, liver and kidney 24 h post injection. The analyses of MR images demonstrated the presence of some fraction of Feridex labeled lipoplex in the periphery of the tumor (Fig. 3).

Discussion: Our findings demonstrate that metastatic MDA-MB-231 breast cancer cells can be efficiently transfected using COX-2 siRNA complexed with DD or DDP liposomes. These results also suggest that COX-2 siRNA can be directly loaded into cationic DD and DDP liposomes without changing its functionality. The incorporation of MR contrast agents within liposomes creates the possibility of noninvasively imaging delivery of siRNA and determining the vascular and metabolic response of these tumors to the siRNA therapy. Preliminary data demonstrate intratumoral delivery of COX-2 siRNA *in vivo* using optical and MR imaging.

References

(1) Wang and DuBois, 2007, (2) Diaz-Cruz et al., 2006, (3) Stasinopoulos et al., 2007, (4) Hogrefe R.I., et al., 2006, (5) Pirollo K.F. et al., 2006, (6) Sioud M et al., 2003.

Acknowledgements

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