Dual Modality Imaging of Phosphatidylcholine-specific Phospholipase C in DU145 Prostate Cancer Cells and Solid Tumors

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Purpose

MRS studies have consistently revealed the presence of elevated levels of phosphocholine (PCho) in various types of cancer cells and solid tumors [1]. It has been shown that PCho levels are correlated with the degree of malignancy in cancer [2, 3], and that PCho levels decrease in response to successful chemotherapeutic treatment [4]. While some studies have associated high levels of PCho with increased choline transport and choline kinase activity [3, 4], others have attributed increases in PCho to the breakdown of phosphatidylcholine (PtdCho) by PtdCho-specific phospholipase C (PC-PLC) activity [5, 6]. However, the relative contributions of the anabolic and catabolic pathways of PCho formation are still uncertain. We have employed a dual modality approach to this problem by combining MRS with a near-infrared (NIR) PC-PLC-activated optical imaging probe in order to directly image the contribution of this catabolic pathway in cancer.

Methods

Two methods were implemented in determining the contribution of PC-PLC activity in the human prostate carcinoma cell line, DU145: 1) In vivo NIR fluorescent imaging using Pyro-PL-BHQ, an NIR enzyme-activated lipid probe that was synthesized in house. Pyro-PL-BHQ is self-quenched until hydrolyzed by PC-PLC separating a fluorescent moiety from a quencher moiety causing up to a 50-fold increase in fluorescence [7], and 2) 31P-MRS of egg-PtdCho vesicles incubated with DU145 whole cell extracts or with PC-PLC. Cell culture: DU145 cells were cultured in a humidified atmosphere of 5% CO2 in air at 37ºC using MEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 2 mM glutamine.

Results

NIR Imaging: At 22 h, Pyro-PL-BHQ accumulation was observed in the gut and the tumor (Figure 1A). At 28 h, the tumor/muscle ratio has increased by 100 % and appears to still be rising. This increase in fluorescence is attributed to the presence of PC-PLC activity within the tumor, since i) this probe is highly specific to the actions of PC-PLC [7] and ii) fluorescent analogs of this probe have previously demonstrated tumor washout by 6 h post-injection (Figure 1B). 31P-MRS: Baseline spectra of egg-PtdCho vesicles show a strong singular peak at 3.8 ppm (Figure 2A). Incubation of PtdCho with PC-PLC resulted in a decrease in PtdCho and an increase in PCho at 6.4 ppm (Figure 2B). Incubation with cell extracts yielded the same decrease in PtdCho and increase at 6.4 ppm (Figure 2C), validating the presence of PC-PLC activity in DU145 cells.

Conclusion

One goal in anticancer drug research is to establish specific molecular markers for the malignant phenotype[8]. In this work, we have employed an NIR PC-PLC activatable probe in combination with MRS to image the level of anabolic PC-PLC activity in cancer. The ability to image both the anabolic and catabolic pathways would lead to an increased understanding of choline phospholipid metabolism as it relates to cancer, and aid in the development of targeted chemotherapy.

References