# 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)* $^{31}$P-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. *NIR Imaging:* Female athymic nude mice were inoculated subcutaneously on the left hind flank with DU145 cells (5 x $10^6$) and tumors were grown for 4-6 weeks. A Xenogen IVIS system was used to obtain *in vivo* NIR fluorescent images ($\lambda_{exc}$ 615-665 nm, $\lambda_{em}$ 695-770 nm). Following baseline imaging the mice were injected intravenously with 80 nmol Pyro-PL-BHQ and imaged for up to 28 h. The mean flux originating from the tumor, gut and muscle was quantified. *$^{31}$P-MRS:* DU145 cells (2.5 x $10^7$) were harvested, counted and centrifuged at 1000 rpm for 5 m. The cell pellet was resuspended in 50 mM Tris-HCl buffer (20% $D_2O$) and homogenized on ice for 2 m. Sonicated egg-PtdCho vesicles (25 mg) were incubated for 24 h at 37ºC with whole cell DU145 extracts, or with 2U of PC-PLC and compared to baseline. 31P-MR spectra (256 scans) were acquired on a Varian INOVA 9.4 T spectrometer using a 45° pulse width, TR=2 s; data size 2K; spectral width 5 kHz.

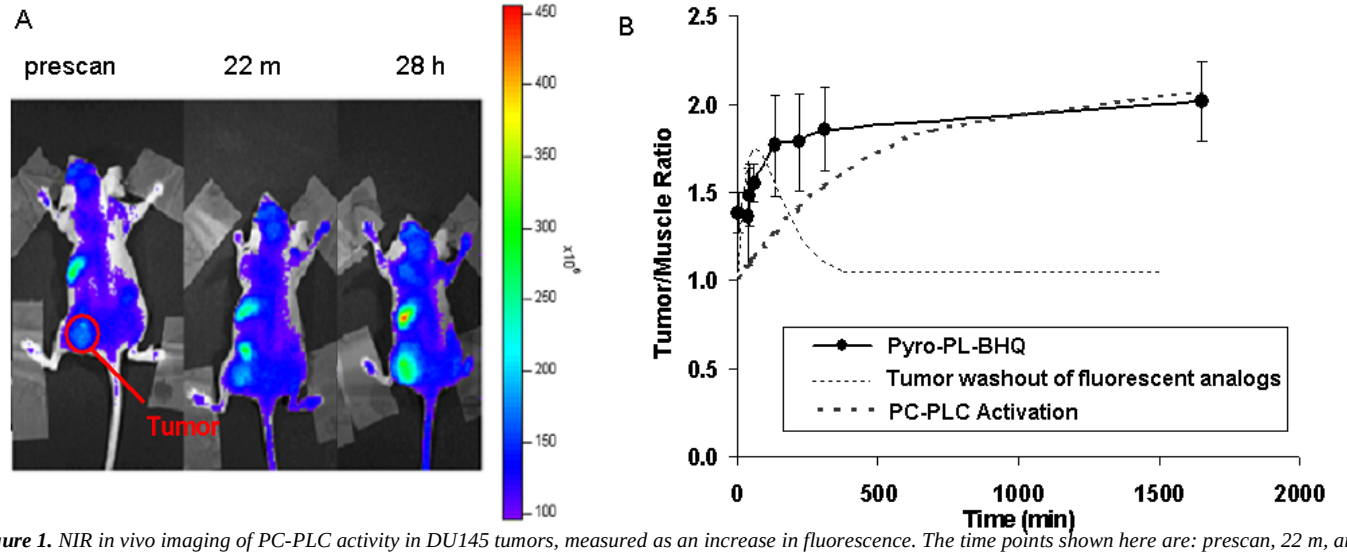


***Figure 1.*** *NIR in vivo imaging of PC-PLC activity in DU145 tumors, measured as an increase in fluorescence. The time points shown here are: prescan, 22 m, and 28 h* ***(A)****. ROIs were drawn around the tumor and normal muscle areas and the ratio of tumor flux:muscle flux was quantified* ***(B)****.*

**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). *$^{31}$P-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.

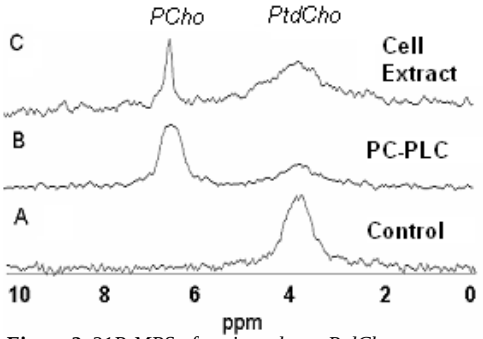


***Figure 2.*** *31P-MRS of sonicated egg-PtdCho vesicles* ***(A)****, after 24 h incubation at 37ºC with 2 U PC-PLC* ***(B)****, or whole cell DU145 extracts* ***(C)****.*

**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**

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