Quantitative 31P magnetic resonance spectroscopy stratifies treatment response to a PI3K/mTOR inhibitor in two distinct breast cancer xenografts

Morteza Esmaeili[1], Tone F. Bathen[1], Olav Engebråthen[2,3], Gunhild Mælandsmo[2], Ingrid S. Gribbestad[1], and Siver A. Moestue[1]

[1]Department of Circulation and Medical Imaging, Norwegian University of Science and Technology (NTNU), Trondheim, Norway, [2]Institute for Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway, [3]Department of Tumor Biology, Institute for Cancer Research, Oslo University Hospital Radiumhospital, Oslo, Norway

PURPOSE: The phosphatidylinositol-3-kinase (PI3K) signaling pathway promotes cell proliferation and survival of cancer cells[1]. Inhibitors of this pathway are under investigation as targeted anticancer treatments[1]. The aim of this study was to develop a phosphorus (^{31}P) high resolution magic angle spinning (HR MAS) magnetic resonance spectroscopy (MRS) protocol for quantifying phosphorylated metabolites of importance in cancer, and to use this method for identifying biomarkers for response to PI3K inhibition.

METHODS: Two genetically distinct breast cancer xenografts[2] representing basal- (n=10) and luminal-like (n=6) subtypes were treated with the dual PI3K/mTOR inhibitor BEZ235 (Selleck Chemicals, Houston, US), receiving 50 mg/kg BEZ235 dissolved in vehicle (30% Captisol/NMP 66:33 v/v) by gavage daily for three days. Control groups (6 basal- and 5 luminal-like) received drug free vehicle only (0.2 ml dose). ^{31}P HR MAS MRS experiments were performed on a 14.1T spectrometer (Bruker Avance III 600 MHz/54 mm US-Plus) equipped with a triplet ^{1}H/^{13}C/^{31}P MAS probe (Bruker BioSpin, Germany). ^{31}P MAS spectra were acquired at 4ºC using an inverse-gated ^{31}P pulse-acquired sequence with ^{1}H decoupling activated during the acquisition using a 30° excitation pulse, 1536 transients, 60 ppm sweep width, a repetition time of 3.62 s, and a total acquisition time of 1 hr 30 min. The chemical shifts were calibrated with respect to the GPC peak at 3.04 ppm. Metabolites were quantified using the PULCON principle[3] and a methylenediphosphonic acid calibration curve obtained under identical conditions. Treatment effect on expression of choline kinase alpha/beta (ChoKA/B), phospholipase A2 group 4 (PLA2GIV), and ethanolamine kinase 2 (ETNK2) was probed using Western blotting. The metabolic degradation during the course of ^{31}P MRS experiments was evaluated.

RESULTS: ^{31}P HR MAS MR spectra acquired from breast xenograft tissues (24.2±4.6 mg) showed high SNR and spectral resolution (Figure 1A). The xenograft models showed distinct metabolic profiles with an inverse PC-GPC relationship (Figure 1A, the control spectra). In basal-like xenografts, BEZ235 treatment induced a significant decrease in PE (-25.6% compared to the control group, P = 0.01) whilst PC (16.5%, P = 0.02) and GPC (37.3%, P < 0.001) were significantly increased (Figure 1B). No significant metabolic changes were observed in luminal-like xenografts. PLA2GIV enzyme levels indicated a 2-fold increase (P = 0.005) in treated basal-like tissue samples compared to vehicle-treated controls, whilst no significant changes were observed in the other enzymes quantified (Figure 1C). Metabolic degradation measurements revealed decreases in PC (up to -20% compared to baseline) and GPC (up to -37%) during the course of ^{31}P MRS.

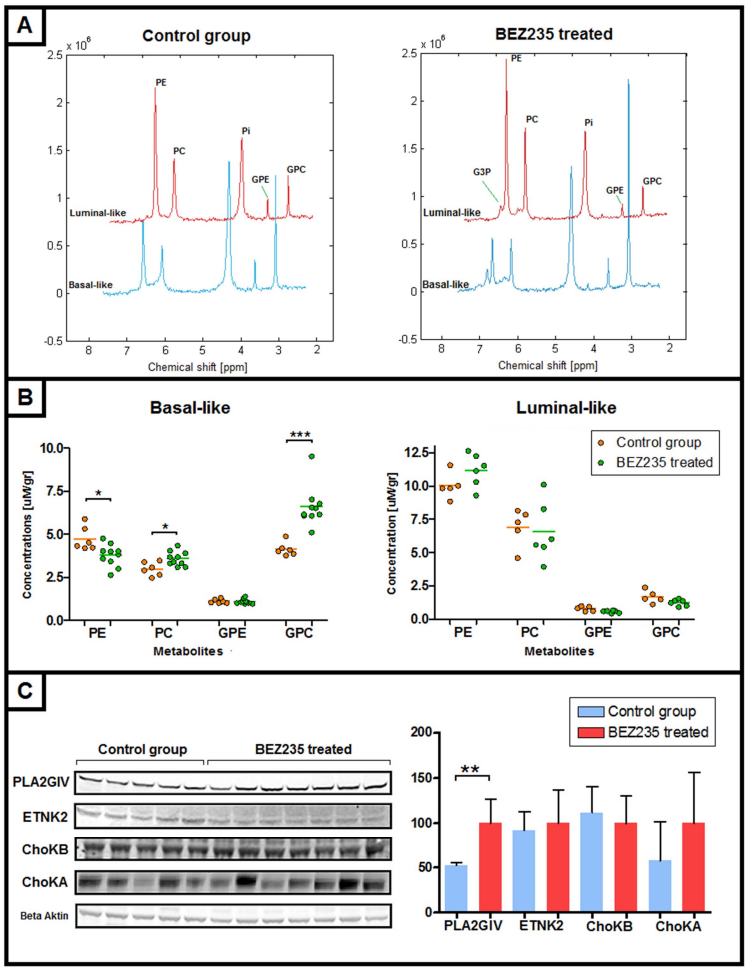

Figure 1: (A) Representative ^{31}P MAS spectra of control group (left column) and BEZ235-treated (right column) obtained from luminal-like (red) and basal-like (blue) breast xenograft tissues. Assigned peaks include; phosphoethanolamine (PE), phosphocholine (PC), inorganic phosphate (Pi), glycerophosphoethanolamine (GPE), glycerophosphocholine (GPC), glycerol-3-phosphate (G3P). (B) BEZ235 induced significant changes in PE, PC, and GPC levels in basal-like, but not in the luminal-like xenografts. (C) Among the proteins quantified in basal-like xenografts tissues (a post-HR MAS analysis), only PLA2GIV showed a significant change after treatment compared to the control group.

DISCUSSION AND CONCLUSION: ^{31}P HR MAS MRS was successfully used to demonstrate treatment-associated metabolic alterations in basal-like, but not luminal-like xenografts. This is consistent with the higher PI3K signaling activity previously described in the basal-like xenografts[2]. Treatment-induced PE reduction is reported previously, but only few studies have found increased PC levels to be associated with targeted therapies. Choline kinases are involved in the intracellular PE and PC regulations, but did not change by BEZ235 treatment. However, the increased GPC could be explained by increased activity of PLA2GIV. The results suggest that the individual choline- and ethanolamine-containing metabolites are promising biomarkers for monitoring the response to targeted anti-cancer drugs. Compared to ^{1}H HR MAS MRS, ^{31}P HR MAS MRS provides additional information in cancer therapy monitoring; (i) PE and GPE can be detected by ^{31}P MRS due to the higher spectral resolution (ii) the quantitative analysis is more sensitive due to the improved spectral resolution and the low background signal. The degradation of metabolites was not depending on xenograft type or treatment, thereby allowing the use of the protocol for comparing concentrations of ^{31}P-containing metabolites across animal models and treatments. If this ^{31}P HR MAS MRS protocol can be translated to *in vivo* ^{31}P MRS, our findings suggest that ^{31}P MRS may be a powerful tool in clinical therapy monitoring.

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