Role of Choline Kinase and Ethanolamine Kinase Isoforms in Modulating Phosphoethanolamine Levels in Breast Cancer Cells

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Introduction: The aberrant choline metabolism of cancers occurs, in large part, due to increased expression of choline kinase (Chk)-α, an enzyme that has been associated with malignant transformation and an aggressive phenotype (1). Since Chk converts choline to phosphocholine the increase of Chk-α results in increased phosphocholine (PC) and total choline (sum of PC, glycerophosphocholine, and free choline) signals in cells and tumors as observed with ^{1}H MRS. While cells in culture and tumors show increased PC with ^{31}P MRS, an increased signal from phosphoethanolamine (PE) is observed in tumors but not in culture. This is because while mammalian plasma contains both choline (~10-40 μM) and ethanolamine (~10 μM) (2), most culture media only contain choline (~ 1-20 μM). Therefore, although increased PE has been observed in tumors almost as consistently as increased PC (3), understanding the role of PE in cancer is relatively unexplored. As an initial step to understanding the increase of PE in tumors, here we have investigated the role of Chk-α and -β isoforms and ethanolamine kinase isoform 1 (EthnK1) in contributing to the increased PE observed in cancers, and the effect of different concentrations of ethanolamine on PC and PE.

Materials and Methods: For high-resolution ^{31}P MRS studies of water soluble cell extracts of MDA-MB-231 human breast cancer cells, cells were treated with 50 nM siRNA (Thermo Scientific) and the medium was changed to various ethanolamine concentration. All siRNAs were custom designed using Thermo scientific siRNA design center. Accession numbers NM_001277.2 for Chk-α, NM_005198.4 for Chk-β and NM_018638 for EthnK1 were used to design specific siRNA. Knockdown of 80-90% message was confirmed using qRT-PCR for the siRNAs used here. Approximately 40 million cells were harvested after 24 h of treatment and cell extracts were prepared using dual-phase extraction method based on methanol/chloroform/water (1/1/1; v/v/v) (4). Lyophilized samples were dissolved in deuterated solvent containing phenolphosphonic acid (PPA) that served as a concentration standard as well as a chemical shift reference. ^{31}P MR spectra were acquired on a Bruker 11.7T NMR spectrometer using a 60^{0} pulse, 1 s repetition time, 4000 averages and composite pulse proton decoupling. Integrals of metabolites were determined to estimate their absolute concentration relative to PPA.

Results and Discussion: Figure 1 shows representative ^{31}P MR spectra of the PC and PE regions from cells treated with various siRNAs with or without ethanolamine in the culture medium. Figures 2a-d show quantitative PC and PE levels obtained from ^{31}P MR spectra acquired under various conditions. PC decreased consistently when cells were treated with Chk-α siRNA, but in the absence of Chk-α siRNA only with an ethanolamine concentration of 1 mM and 10 mM in the culture media (Figure 1c and Figure 2a). Reduction in both PC and PE levels following treatment with ChK-α siRNA confirms the previously observed dual kinase activity of ChK-α (Figure 1b, 1c, 2a). We additionally investigated the role of Chk-β ethanolamine kinase activity in these cells and did not find PC or PE reduction when cells were treated with Chk-β siRNA in the presence of ethanolamine (Figures 1d-e and Figure 2c). These results indicate that Chk-β does not have a significant role in conferring choline or ethanolamine kinase activity in these breast cancer cells.

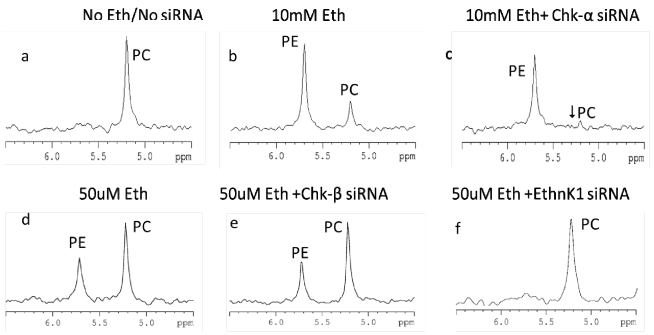

Figure 1: Representative ^{31}P MR spectra showing expanded regions of PC and PE metabolites from untreated MDA-MB-231 cells and cells treated with various ethanolamine (Eth) concentrations and Chk-α siRNA, Chk-β siRNA, EthnK1 siRNA alone or in combination.

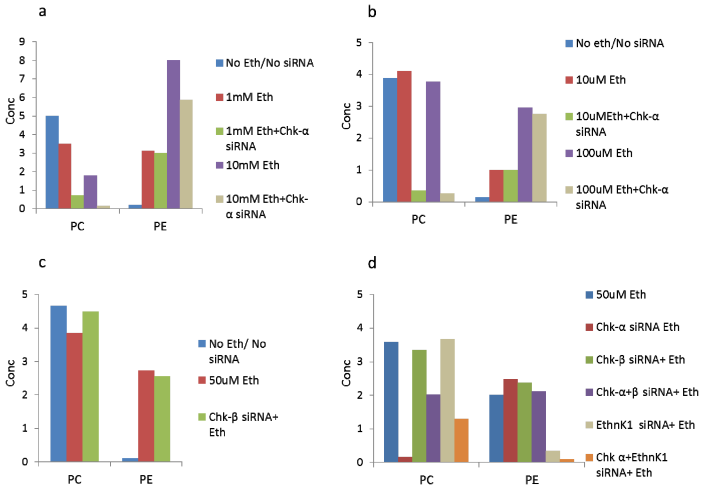

Figure 2: Quantitative PC and PE values obtained from ^{31}P MR spectra of MDA-MB-231 cells acquired under various treatment conditions.

Figures 1 and 2d show that when cells were treated with EthnK1 siRNA, a significant reduction in PE levels was observed. It was intriguing to see that PC was not significantly reduced when cells were treated with Chk-α+Chk-β or ChK-α+Ethnk1 siRNA compared to Chk-α siRNA alone. These results indicate that both Chk-α and EthnK1 contribute to PE levels *in vivo,* with the latter having a primary role in PE biosynthesis. We are currently evaluating the effects of these siRNA treatment combinations on cell viability.

References: 1.Ramírez de Molina A *et al*., Biochem Biophys Res Commun 2002; 296:580-3; 2. Ahiboh *et al.,* Tropical Journal of Pharmaceutical Research, 7: 953-959, 2008; 3. Podo F. NMR Biomed 1999; 12:413–439; 4. Glunde *et al*., Can Res 2008;68:172-80.

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