Ethanolamine Kinase-1 is the major contributor to Phosphoethanolamine Levels in Breast Cancer Cells

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Introduction: Cells in culture and tumors show increased PC with ^{31}P MRS. An increased signal from phosphoethanolamine (PE) is, however, only observed in tumors but not in culture. This is because while mammalian plasma contains both choline (~10-40 μM) and ethanolamine (~10 μM) (1), most culture media only contain choline (~ 1-20 μM). Therefore, although increased PE has been observed in tumors almost as consistently as increased PC (2), understanding the role of PE in cancer is relatively unexplored. We have previously shown that choline kinase (ChK)-α has a dual choline/ethanolamine kinase activity but ChK-β has no significant role in maintaining PE levels *in vivo* (3). We have also shown that ethanolamine kinase (EtnK)-1siRNA significantly reduces PE levels in triple negative MDA-MB-231 human breast cancer cells. Here we have further investigated the role of ethanolamine kinase-2 (EtnK-2) in contributing to the increased PE observed in cancers, and the effect of various small interfering RNA (siRNA) combinations downregulating ChK and EtnK on cell viability, as potential therapeutic strategies in these triple negative breast cancer cells.

Materials and Methods: Cells were cultured in RPMI-1640 medium supplemented with 21mM choline and 50mM ethanolamine. High-resolution ^{31}P MR spectra of water soluble cell extracts of MDA-MB-231 human breast cancer cells were acquired from cells 48h after siRNA treatment. While 50nM siRNA was used in all individual siRNA treatment, for combination siRNA treatment 50nM each of specific siRNA was used. 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, NM_018638 for EtnK-1, and NM_018208.3 for EtnK-2 were used to design specific siRNA. Silencing of 80-90% message was confirmed using qRT-PCR for the siRNAs used. Approximately 40 million cells were harvested after 24h of treatment and cell extracts were prepared using a dual-phase extraction method based on methanol/chloroform/water (1/1/1; v/v/v) (4). Water soluble fractions were collected, lyophilized and dissolved in 0.6 ml deuterated water containing phenylphosphonic 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 MR spectrometer using a 60^0 pulse, 1s repetition time, 4000 averages and composite pulse proton decoupling. Integrals of metabolites were determined to estimate their absolute concentration relative to PPA. For assaying cell viability, 4000 cells per well were plated in a 96 well plate and transfected with 50nM siRNA and viability assessed 96h post transfection using the Cell Counting Kit-8 according to the manufacturer's protocol.

Results and Discussion: 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 are shown in **Figure 1A**. Quantitative PC and PE levels obtained from the spectra are presented in **Figure 1B**. PC decreased consistently when cells were treated with Chk-α siRNA. In cells treated with EtnK-1 siRNA, a significant reduction in PE levels was observed. ChK-β or EtnK-2 siRNA treatment did not affect PC or PE levels in these cells. These results indicate that ChK-β and EtnK-2 do not have significant choline or ethanolamine kinase activity in these breast cancer cells. Etnk-1 is the major contributor to PE levels in these breast cancer cells with some contribution from Chk-α.

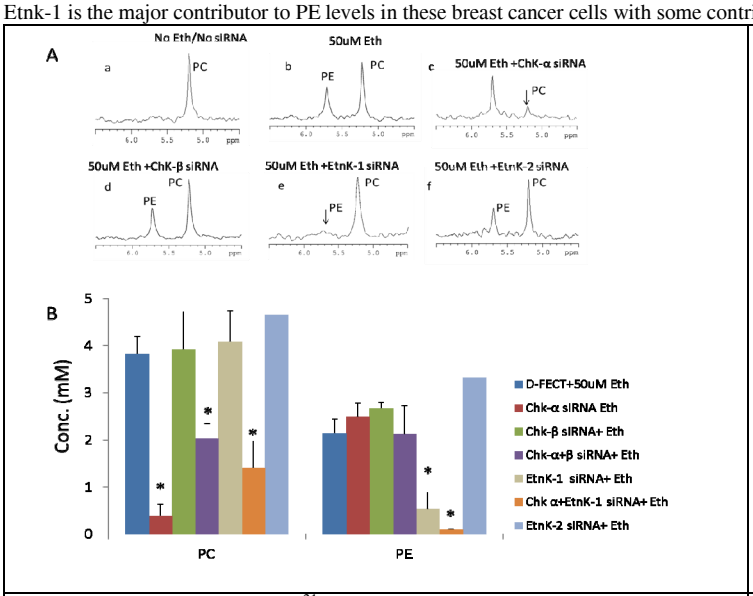

Figure 1: **(A)** Representative ^{31}P MR spectra showing expanded regions of PC and PE metabolites from untreated MDA-MB-231 cells and cells treated with various siRNA alone or in combination. **(B)** Quantitative PC and PE values obtained from ^{31}P MR spectra of MDA-MB-231 cells acquired under various siRNA treatment conditions (* p<0.05 *vs* D-FECT, ChK-βsiRNA, EtnK-1 siRNA, EtnK-2 siRNA); †p<0.05 *vs*. D-FECT, ChK-αsiRNA, ChK-β siRNA, ChK-α+β siRNA, EtnK-2 siRNA).

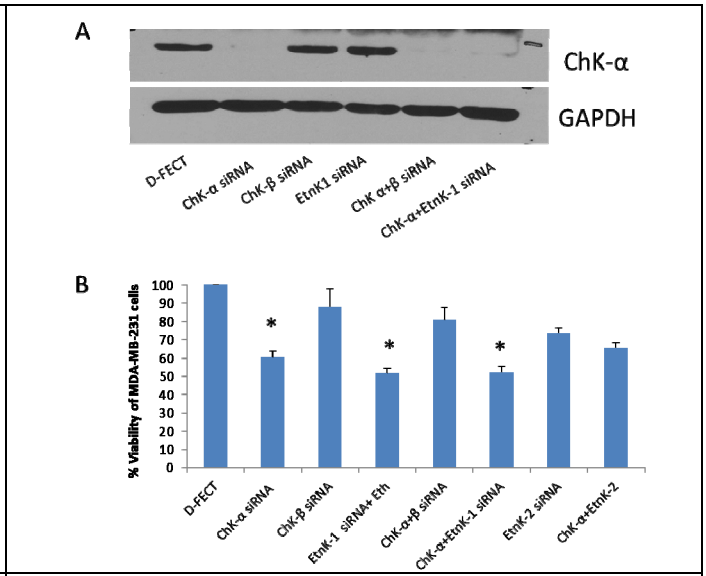

Figure 2: **(A)** Immunoblot showing ChK-α expression level following treatment with various siRNA treatment conditions. **(B)** Cell viability obtained 96h post transfection with various siRNA treatment conditions in MDA-MB-231 breast cancer cells (* p <0.05 *vs* D-FECT, ChK-β, ChK-α+β and EtnK-2).

Interestingly, the decrease of PC was significantly attenuated when cells were treated with ChK-α+ChK-β or ChK-α+EtnK1 siRNA compared to ChK-α siRNA alone. This attenuation was not due to a compensatory increase of Chk-α protein as shown in the immunoblots in **Figure 2A** that would buffer the PC levels. **Figure 2B** shows cell viability data following treatment with ChK and EtnK siRNA. ChK-β siRNA did not have a significant effect on cell viability whereas ChK-α and EtnK1 siRNA resulted in ~ 60% and 50% reduction of cell viability respectively. Combined treatment with ChK-α+ EtnK-1 siRNA was not more effective than treatment with the individual siRNA alone, suggesting that both siRNA act through the same path in decreasing cell viability. The reduction of cell viability caused by treatment with EtnK-1 siRNA indicates its therapeutic potential that warrants further investigation.

References: 1. Ahiboh *et al.,* Tropical Journal of Pharmaceutical Research, 7: 953-959, 2008; 2. Podo F. NMR Biomed 1999; 12:413–439; 3. Shah *et al.,* ISMRM, 1086, 2014; 4. Glunde *et al*., Can Res 2008;68:172-80. **Acknowledgements:** This work was supported by P50 CA103175.