

## Choline kinase alpha has ethanolamine kinase activity and its choline kinase activity is modulated by ethanolamine in breast cancer cells

Tariq Shah<sup>1</sup>, Jannie P Wijnen<sup>1</sup>, Flonne Wildes<sup>1</sup>, Balaji Krishnamachary<sup>1</sup>, Kristine Glunde<sup>1</sup>, and Zaver M Bhujwalla<sup>1</sup>  
<sup>1</sup>Division of Cancer Imaging Research, Johns Hopkins University, Baltimore, MD, United States

**Introduction:** The aberrant choline metabolism of cancers occurs, in large part, due to increased expression of choline kinase (Chk)- $\alpha$ , an enzyme that has been associated with malignant transformation and an aggressive phenotype (1). Since Chk converts choline to phosphocholine the increase of Chk results in increased phosphocholine and total choline (PC, glycerophosphocholine, and free choline) signals in cells and tumors as observed with <sup>1</sup>H MRS. While cells in culture and tumors show increased PC with <sup>31</sup>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  $\mu$ M) and ethanolamine (~10  $\mu$ M) (2), most culture media only contain choline (~1-20  $\mu$ M). 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- $\alpha$  in contributing to the increased PE observed in cancers, and the effect of different concentrations of ethanolamine on the activity of Chk.

**Materials and Methods:** MDA-MB-231 human breast cancer cells were treated with 1 mM or 10 mM ethanolamine (Sigma-Aldrich). In separate groups, similar numbers of cells were transfected overnight with 100 nM Chk- $\alpha$  specific siRNA (Thermo Scientific) and the medium changed to 1 mM or 10 mM ethanolamine. MDA-MB-231 cells without ethanolamine or siRNA treatment were used as controls to determine the endogenous level of metabolites. Approximately 15 million cells were harvested after 24 h treatment and cell extracts were prepared using a dual-phase extraction method based on methanol/chloroform/water (1/1/1; v/v/v) (4). Lyophilized samples were dissolved in deuterated solvent containing pyrophosphonic acid (PPA) that served as concentration standards as well as chemical shift reference. <sup>31</sup>P MR spectra were acquired with a Bruker 11.7 NMR spectrometer using a 60° pulse, a 1 s repetition time 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 <sup>31</sup>P MR spectra of the PC and PE regions from cells treated with 10 mM ethanolamine with or without Chk- $\alpha$  siRNA compared to control MDA-MB-231 cells. Figure 2 shows quantitative data of PC and PE obtained from <sup>31</sup>P MR spectra acquired under various conditions. In untransfected MDA-MB-231 cells, addition of either 1 mM or 10 mM ethanolamine, elevated PE levels as compared to control cells. However, PC signals decreased by 25% and 75% respectively in presence of either 1 mM or 10 mM ethanolamine as compared to control cells. This decreased PC level could be attributed to a competitive inhibition of Chk activity from ethanolamine, which can also act as substrate for Chk. Upon addition of either 1 mM or 10 mM of ethanolamine to MDA-MB-231 cells post transient transfection with 100 nM of Chk- $\alpha$  siRNA, PC levels were significantly reduced at both concentrations as compared to control cells. This decrease was most likely due to the inhibition of Chk activity by Chk- $\alpha$  specific siRNA. PE was also reduced in the 10 mM ethanolamine + Chk- $\alpha$  siRNA treated sample but to a lesser extent compared to PC. Reduction in both PC and PE levels following treatment with Chk- $\alpha$  siRNA confirms in these breast cancer cells the previously observed dual kinase activity of Chk- $\alpha$  (5). These data confirm that Chk- $\alpha$  has ethanolamine kinase activity that can contribute to increased PE levels observed in tumors; studies with ethanolamine concentrations approaching plasma levels are ongoing. We are also investigating the role of Chk- $\beta$  and ethanolamine kinase in the decrease of PC and increase of PE observed with high ethanolamine concentrations in the medium, and the effect of their downregulation on PC and PE. These studies will further unravel mechanisms underlying aberrant choline metabolism, one of the major hallmarks of cancer.

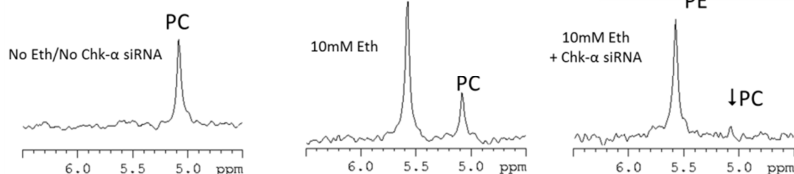


Figure 1: Representative phosphorus spectra from control MDA-MB-231 cells and cells treated with 10 mM ethanolamine (Eth) in the presence or absence of Chk- $\alpha$  siRNA.

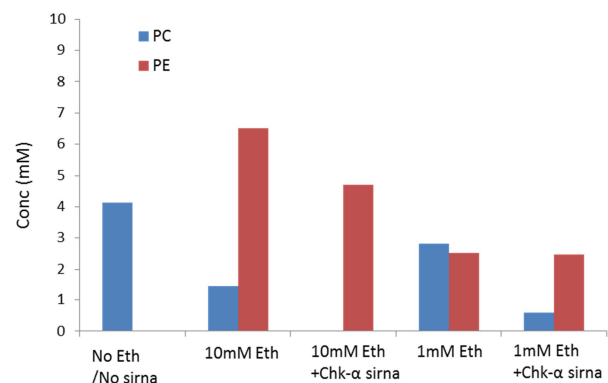


Figure 2: Quantitative PC and PE values obtained from <sup>31</sup>P MR spectra of MDA-MB-231 cells acquired under various conditions.

**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; 5. Gallego-Ortega *et al.*, *PLoS One*. 2009;11:e7819.

**Acknowledgements:** This work was supported by P50 CA103175.