Unraveling the role of choline kinase in cancer

Noriko Mori[1], Flonne Wildes[1], Kristine Glunde[1,2], Catherine Hudson[3], and Zaver M Bhujwalla[1,2]

[1]*JHU ICMIC Program, The Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, Maryland, United States,* [2]*The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine,* [3]*Vertex Pharmaceuticals (Europe) Ltd, Abingdon, Abingdon, Oxfordshire, United Kingdom*

Introduction: Choline kinase (Chk) is the enzyme that catalyzes the first step in phosphatidylcholine biosynthesis. The reaction involves the transfer of a phosphate group from ATP to choline with magnesium ions as cofactor to form phosphocholine (PC). The increase of cellular PC and total choline-containing compound (tCho: PC + glycerophosphocholine (GPC) + free choline) is one of the most widely established characteristics of cancer, and is primarily due to increased levels of Chk. The elevation of Chk and PC is closely related to malignant transformation, invasion, and metastasis [1-3]. We have previously shown that downregulation of mRNA and protein levels of Chk-α significantly reduced proliferation in breast cancer cells [4, 5] and tumors [6]. Here we investigated the effect of the Chk inhibitor, V-11-0711, which reduces the function of Chk by binding to the active site and inhibiting the catalytic activity. We have examined Chk-α protein expression levels, cell viability/proliferation, and PC, GPC, and tCho levels of MDA-MB-231 human breast cancer cells following treatment with this inhibitor.

Methods. Cell culture and treatment of Chk inhibitor (V-11-0711): MDA-MB-231 (ATCC) metastatic human breast cancer cells were grown in RPMI-1640 medium supplemented with 10% FBS. Cells were treated with 1-10 μM V-11-0711 (Vertex Pharmaceuticals (Europe) Ltd) or 0.1% DMSO (vehicle control) in culture media for 48 h. **NMR study**: MDA-MB-231 cells were collected and water-soluble as well as lipid extracts were obtained from control, 1μM and 10 μM V-11-0711 treated cells using the dual-phase extraction method [4]. Fully relaxed ^{1}H NMR spectroscopy of the water-soluble phase was performed on a Bruker Avance 500 spectrometer. Signal integrals of the phosphocholine (PC), glycerophosphocholine (GPC), and free choline peaks were quantified as mM relative to cell number, cell volume (2050 μm^3) and an internal concentration standard. Approximately $3x10^7$ cells were harvested for cell extraction. **Immunoblot analysis**: 50μg of protein from cell lysates at 48 h post-treatment were resolved on 7.5% acrylamide gel using a polyclonal Chk-α antibody (custom made) and a monoclonal GAPDH antibody (Sigma). **Proliferation assay**: CCK-8 assay (Dojindo Molecular Technologies, Inc. MD) was performed using manufacturer's instructions after cells were treated for 48 hours, and 3 days after medium was changed to culture medium at 48 h post-treatment. Values were compared to untreated cells.

Results and Discussion: After 48 h treatment with V-11-0711, Chk-α protein levels were stable or increased slightly (Figure 1). The cell proliferation assay using MDA-MB-231 cells showed that at 48h post-treatment with 10μM V-11-0711 a reduction of cell viability of 15% was detected compared to untreated cells (Figure 2). The level of PC and tCho significantly decreased after treatment with V-11-0711 dose dependently, while GPC levels did not change significantly (Figures 3 and 4). Treatment with 10μM V-11-0711 reduced PC to non-detectable level. In contrast we have previously shown that the inhibition of Chk expression significantly reduced the proliferation of cancer cells [4, 5]. The small increase of Chk-α levels after treatment with the inhibitor may be a compensatory effect in response to reduced PC. Our results here indicate that reduction of PC under these conditions does not markedly affect the proliferation of breast cancer cells if Chk-α protein levels are not reduced. These data are consistent with results obtained by Miyake *et al.,* [7] on the potential role of Chk as a chaperone protein, and suggest that the Chk protein but not PC may be essential in cancer cell proliferation. The data support the development of strategies that destabilize or downregulate Chk.

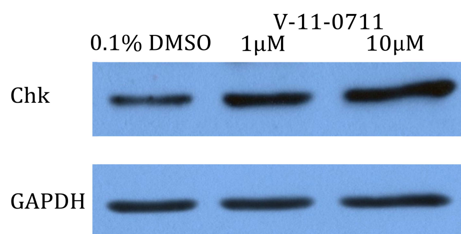

Figure 1: Chk-α protein expression levels in MDA-MB-231 cells treated with DMSO or V-11-0711. 50 μg of protein was loaded on 7.5% reducing SDS-PAGE gel. GAPDH: loading control.

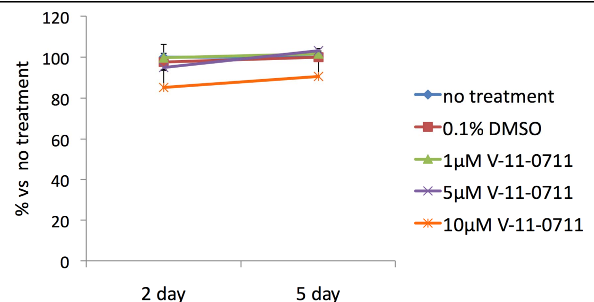

Figure 2: Cell viability/proliferation as determined by the CCK-8 assay in MDA-MB-231 cells treated with1, 5 or 10 μM of V-11-0711 for 48 hours. Cells were treated for 2 days and changed to fresh medium, and assays were done at day 2 and day 5 after starting treatment. Values are mean ± standard error. n=3

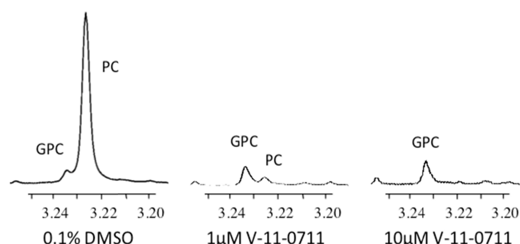

Figure 3: Representative ^{1}H NMR spectra expanded to show the 3.20-3.25 ppm region of MDA-MB-231 cells. PC: phosphocholine; GPC: glycerophosphocholine.

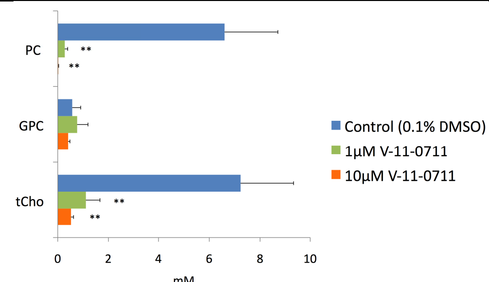

Figure 4: Levels of PC, GPC and tCho in mM quantified from ^{1}H MR spectra of MDA-MB-231 treated with DMSO or V-11-0711. Values are mean ± standard deviation. ** represents P < 0.01 *vs* control.

References and Acknowledgements: [1] Aboagye E *et al*, *Cancer Res*, 59, 80 (1999); [2] Ackerstaff E et al, *Cancer Res,* 61, 3599 (2001); [3] Ramirez de M *et al*, *Oncogene,* 21,4317 (2002); [4] Glunde K *et al*, *Cancer Res*, 65, (2005); [5] Mori N *et al*, *Cancer Res*, 67, (2007); [6] Krishnamachary B *et al*, *Cancer Res*, 69, (2009); [7] Miyake T *et al.,* Oncogene (2011). This work was supported by NIH R01 CA73850 and P50 CA103175.