Choline kinase-α protein but not its activity is necessary in breast cancer cell proliferation

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Introduction: Choline kinase (Chk) is the enzyme that catalyzes 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-α. High levels of Chk and PC are closely related to malignant transformation, invasion, and metastasis [1-3]. We have previously shown that downregulation of mRNA using siRNA against Chk (siRNA-chk) and the resultant decrease of Chk-α protein levels significantly reduced proliferation in breast cancer cells [4-5] and tumors [6]. We observed that the Chk-α inhibitor, V-11-0711, which reduces the function of Chk-α by binding to the active site and inhibiting its catalytic activity, did not reduce the proliferation of triple negative MDA-MB-231 metastatic human breast cancer cells. However, MDA-MB-231 cell proliferation was reduced when Chk-α protein level was downregulated by siRNA-chk. To further investigate this effect in the triple negative inflammatory breast cancer cell line, SUM149, we have examined Chk-α protein expression levels, cell viability/proliferation, and PC, GPC, and tCho levels following treatment with this inhibitor and siRNA-chk.

Methods. Cell culture and treatment: SUM149 cells were grown in DMEM/F-12 (1:1) medium supplemented with 5% FBS, 5 μg/ml insulin, and 0.5 μg/ml hydrocortisone. Cells were treated with DMSO (vehicle control), DharmaFECT (D-FECT, transfection reagent, Thermo Fisher scientific Inc.), 1-10 μM V-11-0711 (Vertex Pharmaceuticals (Europe) Ltd) and/or siRNA-chk in culture media for 48 h. **NMR study**: SUM149 cells were collected and water-soluble as well as lipid extracts were obtained from vehicle control, 0.1μM and 1 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), choline peaks were quantified as mM relative to cell number, cell volume and an internal concentration standard. **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 instruction 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: The cell proliferation assay using SUM149 cells showed that the treatment with 10μM V-11-0711 resulted in 33% reduction of cell viability compared to untreated cells. However there was no significant reduction of viability in cells treated with up to 5μM V-11-0711 (Figure 1). After 48 h treatment with 0.1μM and 1μM V-11-0711, Chk-α protein levels in SUM149 were stable (Figure 2). The level of PC and tCho significantly decreased after treatment with V-11-0711 dose dependently. GPC levels decreased significantly only with the treatment of 1μM V-11-0711 (Figures 3 and 4). Treatment with 1μM V-11-0711 reduced PC to almost non-detectable level (Figures 4). In contrast we have previously shown that the inhibition of Chk expression significantly reduced the proliferation of cancer cells [4-5]. 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. To investigate if the reduced protein level of Chk-α affects the proliferation, we used siRNA-chk. siRNA-chk reduced Chk-α protein to an undetectable level (Figure 5), and proliferation was significantly reduced whether V-11-0711 was present or not (Figure 6). Our results demonstrate that reduction of PC has little affect on the proliferation of breast cancer cells as long as 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-α as a chaperone protein, and suggest that the Chk-α protein may be essential in cancer cell proliferation. The data support the development of strategies that destabilize or downregulate Chk-α protein [7].

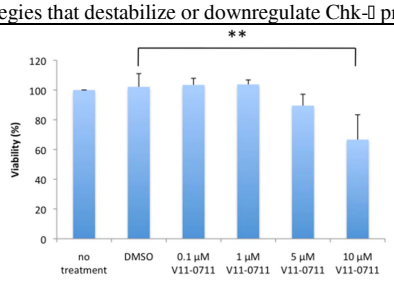

Figure 1: Cell viability/proliferation as determined by the CCK-8 assay in SUM149 cells treated 0.1 - 10 μM of V-11-0711 for 48 h. Values are mean ± standard deviation. ** : P < 0.01.

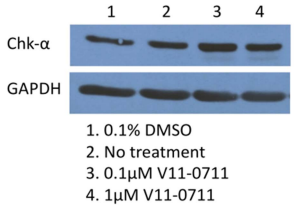

Figure 2: Chk-α protein expression levels in SUM149 cells treated with DMSO or V-11-0711. 50 μg of protein was loaded on 7.5% reducing SDS-PAGE gel. GAPDH: loading control.

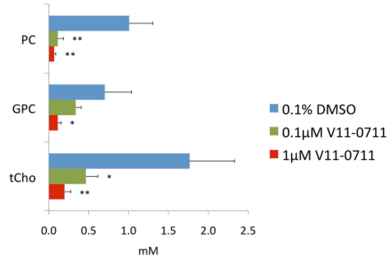

Figure 3: Levels of PC, GPC and tCho in mM quantified from ^{1}H MR spectra of SUM149 treated with DMSO or V-11-0711. Values are mean ± standard deviation.
* : P < 0.05 and ** : P < 0.01 *vs* DMSO

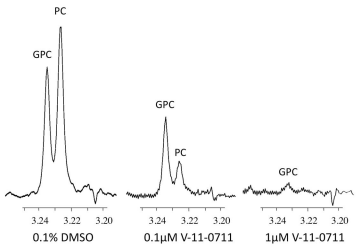

Figure 4: Representative ^{1}H NMR spectra expanded to show the 3.20-3.25 ppm region of SUM149 cells.
PC: phosphocholine; GPC: glycerophosphocholine

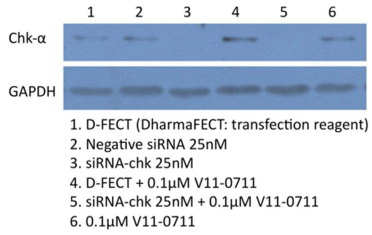

Figure 5: Chk-α protein expression levels in SUM149 cells treated with siRNA and/or V-11-0711. 50 μg of protein was loaded on 7.5% reducing SDS-PAGE gel. GAPDH: loading control.

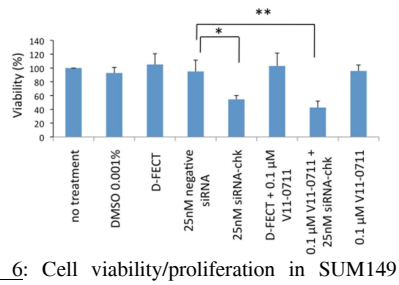

Figure 6: Cell viability/proliferation in SUM149 cells treated with siRNA and/or 0.1 μM of V-11-0711 for 48 h. Cells were treated for 2 days and changed to fresh medium, and assays were done at day 5 after starting treatment. * : P < 0.05 & ** : P < 0.01

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.