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 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 1μM V-11-0711 treated cells using the dual-phase extraction method [4]. Fully relaxed 1H NMR spectroscopy of the water-soluble phase was performed on a Bruker Avance 500 spectrometer. Signal integrals of the phosphocholine (PC), glycerophosphocholine (GPC) and 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 polyacrylamide 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 Chk 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].