The effect of choline kinase-α inhibition on lipid metabolism of breast cancer cells

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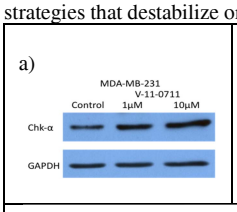
Introduction: Phosphatidylcholine (PtdCho) is the major phospholipid in eukaryotic membranes and plays a critical role in membrane structure and cell signaling. Choline kinase (Chk) is the initial enzyme that catalyzes the transfer of a phosphate group from ATP to choline with magnesium as cofactor to form phosphocholine (PC) in PtdCho biosynthesis. 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-alpha (siRNA-Chk) and the resultant decrease of Chk-alpha protein level significantly reduced proliferation in breast cancer cells [4-5] and tumors [6]. We have shown that the Chk-alpha inhibitor, V-11-0711, which reduces the function of Chk-alpha (reduced PC level) by binding to the active site and inhibiting its catalytic activity but not by reducing Chk-alpha protein level, did not reduce the proliferation of triple negative MDA-MB-231 (up to 10μM) and SUM149 (up to 1μM) breast cancer cells. However, cell proliferation was reduced when Chk-alpha protein level was downregulated by siRNA-Chk with or without V-11-0711. To further investigate the effect of the inhibitor on lipid metabolism in these breast cancer cells, we have used ^{1}H NMR of the lipid phase of cell extracts and compared the levels of fatty acid, phosphatidylethanolamine (PtdE), and PtdCho after treatment with V-11-0711.

Methods. Cell culture and treatment: MDA-MB-231 cells were grown in RPMI-1640 medium with 10% fetal bovine serum (FBS). SUM149 cells were grown in DMEM/F-12 (1:1) medium with 5% FBS, 5 μg/ml insulin, and 0.5 μg/ml hydrocortisone. Cells were treated with 0.1% DMSO (vehicle control), 0.1-10 μM V-11-0711 (Vertex Pharmaceuticals (Europe) Ltd) in culture media for 48 h. **NMR study**: Cells were collected and water-soluble as well as lipid extracts were obtained from vehicle control, and 0.1μM - 10μM V-11-0711 treated cells using the dual-phase extraction method [4]. Fully relaxed ^{1}H NMR spectroscopy of the lipid-soluble phase was performed on a Bruker Avance 500 spectrometer. Signal integrals of the methyl group (-CH_3) in fatty acid (Fmix) at ~0.9 ppm, methylene group (-(CH_2)n-) in Fmix at ~1.3 ppm, PtdE at ~3.1ppm and choline group (-N(CH_3)$_3$) in mainly PtdCho at ~3.2 ppm were determined and normalized to cell number, and compared to the standard (TMS). **Immunoblot analysis**: 50μg of protein from cell lysates at 48 h post-treatment was resolved on 7.5% acrylamide gel using a polyclonal Chk-alpha antibody (custom made) and a monoclonal GAPDH antibody (Sigma). **Cell viability/proliferation**: After 48h of treatment with either DMSO (control), or V11-0711, cells were collected for NMR. Cells were counted in each flask, and control and treated-cell numbers were compared. The cell numbers were expressed as %, with DMSO-treated cell number equal to 100% (% Cell number).

Results and Discussion: A slight increase of Chk-α protein was observed after 48 h treatment with V-11-0711 in MDA-MB-231 cells (Figure 1). In SUM149 cells, Chk-α protein level was almost stable after V-11-0711 treatment (Figure 1). There was no significant reduction of % cell number in MDA-MB-231 cells treated with up to 10μM V-11-0711 (Figure 2). 0.1μM V-11-0711 treatment did not affect % cell number, but 1μM V-11-0711 treatment significantly reduced % cell number in SUM149 (Figure 2). Fatty acid and PtdE levels did not change significantly after V-11-0711 treatment in both cell lines (Figure 4). PtdCho levels decreased slightly dose dependently in MDA-MB-231, which was not significant. In SUM149 PtdCho levels decreased dose dependently and significantly following treatment with 1μM V-11-0711 (Figure 4). We previously showed that significant reduction of PC under these conditions did not markedly affect proliferation of breast cancer cells if Chk-alpha protein levels were not reduced. Our results here show that cells can survive if PtdCho levels are not reduced significantly even if PC levels are low. The two cell lines we used showed different effects against V-11-0711. MDA-MB-231 cells were more resistant to V-11-0711 and showed no significant reduction of PtdCho levels. This may be partly due to different homeostatic regulation of related enzymes such as Chk-alpha in these cells that increased Chk-alpha protein level after treatment. Taken together, these data demonstrate that reduction of PC has little affect on the proliferation of breast cancer cells as long as Chk-alpha protein levels and PtdCho levels are not reduced. Chk-alpha protein and PtdCho, but not PC, may be essential in cancer cell proliferation. These data support the development of strategies that destabilize or downregulate Chk-alpha protein and PtdCho.



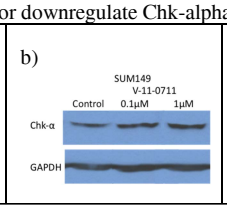
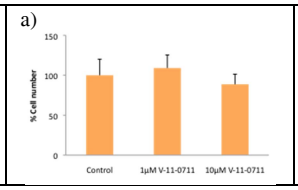


Figure 1: Chk-α protein expression levels in a) MDA-MB-231 and b) SUM149 cells treated with 0.1% DMSO (Control) or V-11-0711. 50 μg of protein was loaded. GAPDH: loading control



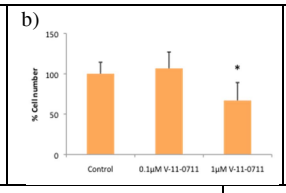


Figure 2: % Cell number of a) MDA-MB-231 and b) SUM149 cells. Values are mean ± SD. * : P < 0.05 *vs* Control

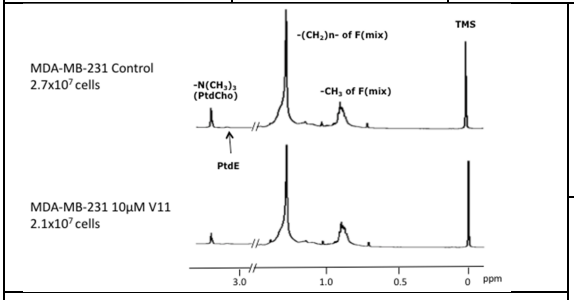
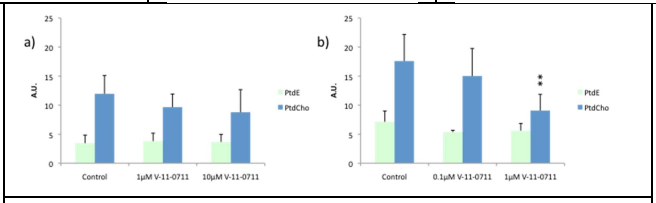


Figure 3: Representative ^{1}H NMR spectra of the lipid phase of MDA-MB-231 cells. TMS: tetramethylsilane (internal standard), F(mix): Fatty acid, PtdE: phosphatidylethanolamine, PtdCho: phosphatidylcholine.



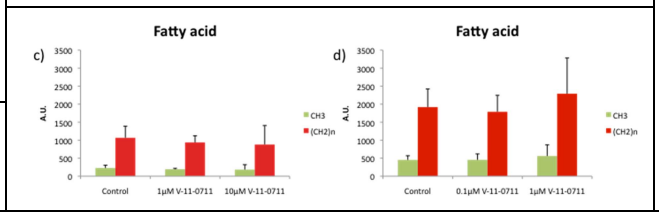


Figure 4: Lipid amounts (A.U.: arbitrary unit / cell) in a) & **c)** MDA-MB-231 and b) & d) SUM149 cells treated with 0.1% DMSO (Control) or V-11-0711 for 48 h. Values are mean ± SD.

** : 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).
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