Choline Kinase over expression increases drug resistance and invasion in MCF7 breast cancer cells

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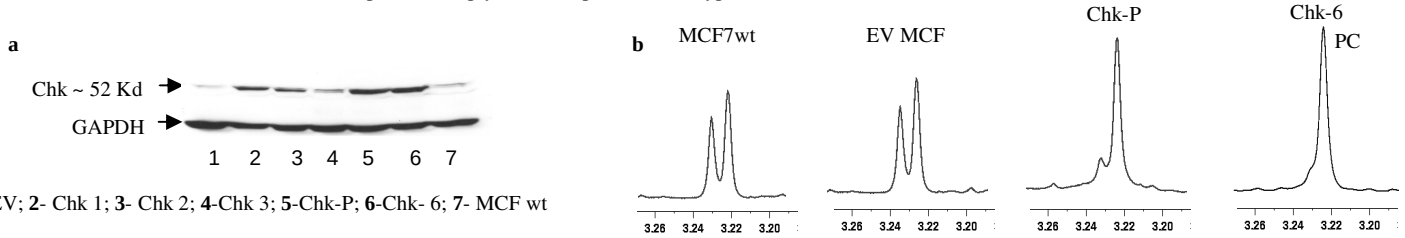
Introduction: Increased choline kinase (Chk) and phosphocholine has been associated with a more aggressive cancer phenotype. We have previously shown that siRNA-mediated downregulation of Chk induces differentiation (1) and increases the effect of 5-Fluorouracil (5-FU) treatment (2) in human breast cancer cells. To further understand the role of Chk in drug resistance and invasion we created Chkα1 over expressing clones of the poorly invasive MCF-7 (Chk MCF7) human breast cancer cell line. The relationship between ChKα1 expression and drug resistance to 5-FU was determined using an MTT assay. Rhodamine-123 efflux in these cells was studied in order to determine the relationship between Chkα1 and drug transport. A magnetic resonance (MR) compatible cell perfusion assay, the Metabolic Boyden Chamber (MBC), was used to dynamically track invasion and metabolism in these cells.

Material and Methods: The Chkα1 gene was cloned into the pEf1α myc/HisA vector. MCF-7 cells were transfected and various clones were obtained and characterized. Chkα1 overexpressing pooled cells (Chk-P) were obtained from cells remaining after the colonies were picked. Overexpression of Chkα1 was confirmed by immunoblot analysis using a custom made Chk antibody (1). The invasion assay was carried out as previously described (3). The MTT assay was carried out using an ATCC assay kit. Briefly, 4000-5000 cells were plated in 96 well plates. The following day cells were treated with 2.5 µg/ml 5-Flourouracil for 24 h. Cells were processed for the MTT assay 48 h later. For flow cytometry studies $1x10^6$ cells/ml were used for each cell line per condition. Rhodamine -123 was added at a final concentration of 0.5 µg/ml in sample tubes while similar controls were left untreated for comparison. Cells were agitated slowly at 37° C. After 45 minutes cells were washed 2X with cold PBS and fixed with 0.5% paraformalehyde.

Results: Fig. 1a shows various clones (lane 2-6) of high Chk expression compared to empty vector MCF-7 (EV) (lane 1) and parental wild type (wt) MCF-7 (lane 7). Fig. 1b shows ^{1}H spectra from the choline region where phosphocholine (PC) is elevated in Chk clones compared to parental MCF7. No significant differences in cell proliferation were detected in these clones compared to empty vector or parental wild type cells.



1- EV; **2**- Chk 1; **3**- Chk 2; **4**-Chk 3; **5**-Chk-P; **6**-Chk- 6; **7**- MCF wt

Figure 1 (a) Immunoblots of Chk levels in total protein from cell lysates of Chk MCF7 clones with increased choline kinase expression. **(b)** Representative expanded ^{1}H spectra of the choline region from empty vector MCF7, parental MCF7 and Chk MCF7 clones obtained from water soluble cell extracts.

Table 1: Significant differences were observed in total choline (tCho) ($p<0.001$) and lactate/tryglycerides (LacTG) ($p<0.007$) from ^{1}H MRS data and PC from ^{31}P MRS data between empty vector and Chk-6 MCF7 cells ($p<0.001$). Values are mean ± SEM (n=3).

	EV	Chk-MCF7	EV	Chk-MCF7	EV	Chk-MCF7	EV	Chk-MCF7
[rel.u]	0.17day		0.5day		1.0day		1.5 day	
tCho	2.17 ± 0.5	6.6 ± 0.39	2.85 ± 0.5	7.3 ± 0.37	4.67 ± 0.41	9.11± 0.11	5.6 ± 0.78	9.7 ± 0.4
PC	17.9 ± 4.9	56.2 ± 3.0	24.9 ± 1.6	70.3 ± 7.2	28.1 ± 3.2	75.2 ± 10.5	31.8 ± 6.0	80.5 ± 9.0
LacTG	3.2±0.195	5.0 ± 1.6	2.9 ± 0.2	4.55 ± 0.7	3.1 ± 0.3	3.9 ± 0.3	3.56 ± 0.65	4.0 ± 1.0

Chk MCF7 cells were significantly more resistant to 5-FU compared with EV and parental MCF7 cells (Fig. 2a), and effluxed more rhodamine-123 compared to wild type MCF7 cells (Fig. 2b).

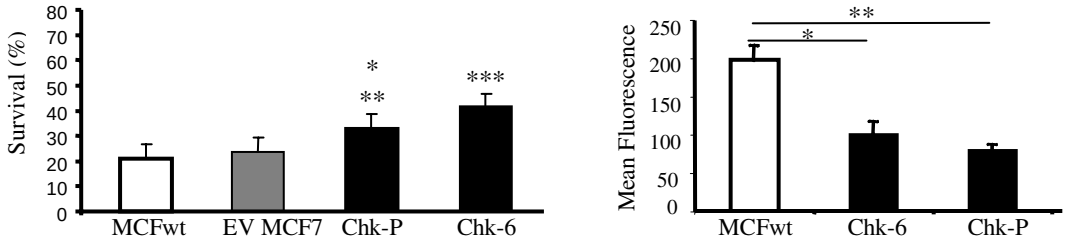


Figure 2 (a) MTT assay of Chk MCF7 cells treated with 2.5 µg/ml 5-Flurouracil for 24 h. Values are mean ± SD (n=3 * $p<0.05$ vs EV; ** $p<0.02$ vs MCF-7wt; *** $p<0.001$ vs MCF-7wt and EV MCF-7). **(b)** Intracellular accumulation of rhodamine-123. Values are mean ± SE. (n = 3, * $p< 0.05$ and ** $p<0.01$).

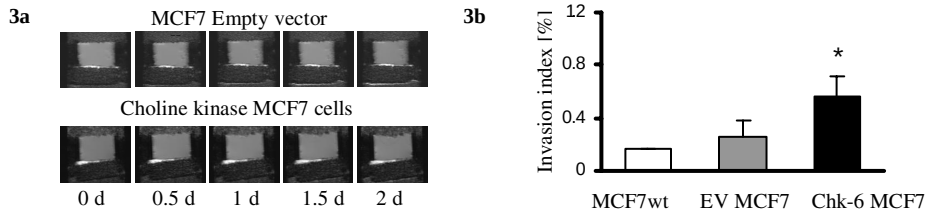


Figure 3 (a) T1-weighted ^{1}H MR images of Matrigel over 2 days showing invasion of Matrigel by cells. **(b)** Quantitative time-dependent invasion indices I(t) obtained from intracellular profile at 1.5 days from three cell types. Values are mean ± SD (Empty vector and Chk-6; n=3, MCF7wt; n=1). * $p < 0.04$ EV *vs* Chk-6 MCF7.

Quantitative time-dependent invasion indices I(t) demonstrated that over expression of Chkα1 resulted in a small but significant increase of invasion compared to empty vector (EV) and wt MCF-7 cells (Figure 3).

Discussion: Overexpression of Chk in poorly invasive MCF-7 breast cancer cells resulted in increased resistance to 5-FU, increased efflux of rhodamine-123 and a small but significant increase in invasion. These data further support targeting Chk in the treatment of breast cancer.

References: [1] Glunde et al. Cancer Res 2005, 65:11034-43; [2] Mori et al. Cancer Research (in press), 2007; [3] Ackerstaff E et al. Neoplasia 2007, 3:222-35.