Selective cell kill of breast cancer cells through choline kinase downregulation as an adjunct therapy

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Introduction: The cellular phosphocholine (PC) and choline-containing compound (tCho) levels are biomarkers for cancers [1-3]. Increased activity of choline kinase (chk), the enzyme converting free choline (Cho) to PC, is associated with a higher histologic tumor grade [4]. Developing novel anticancer therapies that are specific to cancer cells and non-toxic for normal cells is our ultimate goal. We have previously shown that both transient transfection and stable expression of siRNA against choline kinase (siRNA-chk), induced differentiation and reduced proliferation in breast cancer cells [5]. Here we have examined the effects of transient siRNA-chk transfection on PC and tCho levels, and on the viability/proliferation of two breast cancer cell lines, MDA-MB-231 and MCF-7, and a nonmalignant breast epithelial cell line, MCF-12A. We also investigated the effects of combination treatment with transient siRNA-chk transfection and the anti-cancer drug 5-fluorouracil (5-FU) in two breast cancer cell lines, MDA-MB-231 and MCF-7.

Methods: MR study: Cells were transfected with siRNA-chk for 48 hours using oligofectamine. The effect of transfection was determined by the decrease of chk message using reverse transcription-polymerase chain reaction (RT-PCR) analysis. Water-soluble as well as lipid extracts were obtained from control and siRNA-chk-treated cells using a dual-phase extraction method [6]. Fully relaxed ^{1}H MR spectroscopy of the water-soluble phase was performed on a Bruker Avance 500 spectrometer. Signal integrals were quantified relative to cell number and an internal concentration standard. The internal standard used was 3-(trimethylsilyl)propionic-2,2,3,3,-d_4 acid (TSP).

Western blot analysis: Chk protein expression levels were detected with polyclonal choline kinase antibody by detecting horseradish peroxidase on immunoblots. Actin protein levels were detected as a loading control.

MTT assay: 4000 cells were seeded in each well of a 96 well plate and kept overnight at 37 ºC and 5 % CO_2 in a humidified atmosphere. Twenty-four hours later, siRNA-chk was transfected transiently using oligofectamine, as previously described [5]. Cell viability/proliferation was evaluated using the MTT Cell Proliferation Assay (ATCC) 3 days after single or combined treatment of cells with siRNA-chk (48h) and 5µg/ml of 5-FU (24h), and was compared to values obtained with untreated cells.

Results: The MR study (Fig. 1) showed that PC levels were significantly lower in MCF-7 and MDA-MB-231 breast cancer cells, but not in nonmalignant MCF-12A cells following transient transfection of siRNA-chk. The significantly lower chk protein levels (Fig. 2) following transient siRNA-chk transfection in all three cell lines used confirmed successful siRNA-chk transfection and downregulation of chk levels. A significant reduction of cell viability/proliferation in both MDA-MB-231 and MCF-7 cells was detected following transfection using siRNA-chk alone compared to untreated cells, whereas cell viability/proliferation remained almost the same in MCF-12A following siRNA-chk transfection (Fig. 3). Treatment with 5-FU alone resulted in a reduction of cell viability/proliferation of both malignant breast cancer cell lines. Treatment with 5-FU in combination with siRNA-chk transfection increased this reduction of viability/proliferation in MCF-7 and MDA-MB-231 cells.

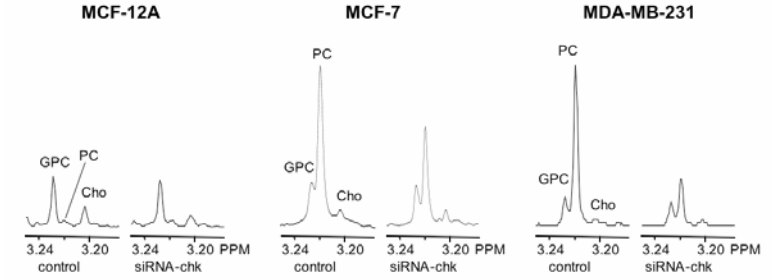

Figure 1: Representative ^{1}H MR spectra (water soluble extract fractions) of the choline phospholipid metabolite region of control and siRNA-chk transfected MCF-12A, MCF-7, and MDA-MB-231 cells.

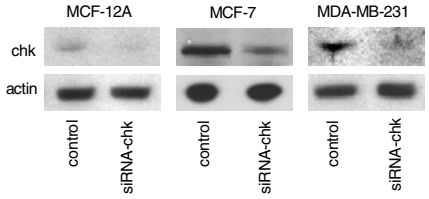

Figure 2: Chk protein levels in control and siRNA-chk transfected MCF-12A, MCF-7, and MDA-MB-231 cells. Actin immunoblotting was performed as a loading control.

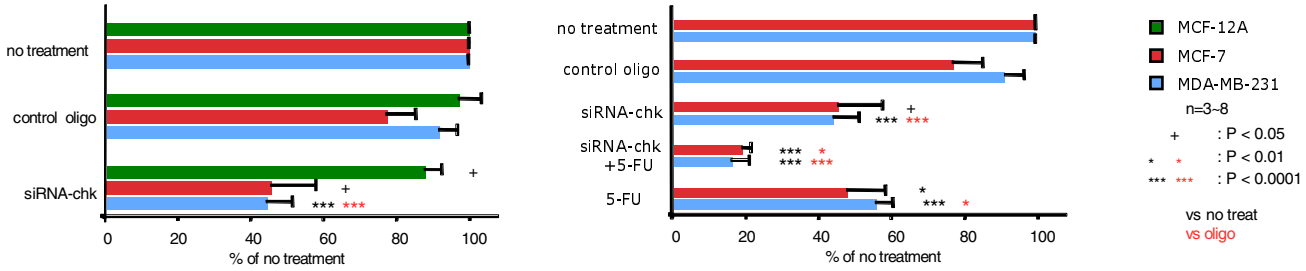

Figure 3: Cell viability/proliferation as determined by MTT assay of cells treated with oligofectamine (Oligo), siRNA-chk, 5-FU, and combination of siRNA-chk + 5-FU in MCF-12A (green bars), MCF-7 (red bars), and MDA-MB-231 (blue bars). Values are mean ± standard error.

Discussion: High-resolution MR spectroscopy of cell extracts demonstrated that chk expression plays an important role in generating the high PC and tCho levels observed in breast cancer cells but not in nonmalignant breast epithelial cells. Chk plays a role in breast cancer cell proliferation, since transient siRNA-chk transfection resulted in a significant inhibition of cell viability/proliferation in MDA-MB-231 and MCF-7 breast cancer cells. However, chk downregulation had an almost undetectable effect on the cell proliferation of nonmalignant MCF-12A breast epithelial cells. The low basal chk and PC levels in these MCF-12A cells may be the reason for this observation. Combined treatment with siRNA-chk transfection and 5-FU reduced the cell viability/proliferation to levels that were significantly lower than either treatment alone in both breast cancer cell lines. These results demonstrate that transient siRNA-chk transfection increased the cell killing effects of 5-FU in breast cancer cells. Chk downregulation with siRNA-chk may provide a novel alternative to enhance the effect of anti-cancer drugs, while normal cells remain unaffected.

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