Choline kinase downregulation by siRNA reduces phosphocholine and total choline, and enhances treatment of breast cancer cells with 5-Fluorouracil

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Introduction

The increase of cellular phosphocholine (PC) and choline-containing compounds (tCho) is one of the most widely established characteristics of cancer [1-3]. This elevation is closely related to malignant transformation, invasion, and metastasis. We have previously shown that both transient transfection and stable expression of siRNA against choline kinase (siRNA-chk), the enzyme that converts choline (Cho) to phosphocholine, induced differentiation and reduced proliferation in breast cancer cells [4]. Here we have examined the effect of transient siRNA-chk transfection on PC and tCho levels and in combination with the anti-cancer drug 5-fluorouracil (5-FU) on the viability and proliferation of two breast cancer cell lines, MDA-MB-231 and MCF-7, with different choline kinase expression levels.

Methods

NMR study: MDA-MB-231 cells were transfected with siRNA-chk for 48 hours using oligofectamine as 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 the dual-phase extraction method [5]. Fully relaxed ^{1}H NMR spectroscopy of the water-soluble phase was performed on a Bruker Avance 500 spectrometer. Signal integrals were quantified relative to cell number and internal standard concentration. The internal standards used was 3-(trimethylsilyl)propionic-2,2,3,3,-d_4 acid (TSP).

MTT assay: MDA-MB-231 cells were grown in RPMI-1640 medium without phenol red supplemented with 10% FBS. MCF-7 cells were cultured in EMEM without phenol red supplemented with 10% FBS. Both cell lines were maintained in a humidified atmosphere with 5% CO_2 in air, at 37 ºC. 4000 cells were seeded in each well of a 96 well plate and cultured overnight at 37 ºC. Twenty-four hours later, 98 nM of siRNA-chk was transfected transiently using oligofectamine, as previously described [4]. Oligofectamine without siRNA was used as a negative control. Cell viability/proliferation was evaluated using the MTT Cell Proliferation Assay (ATCC) 4 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 NMR study (Fig. 1a and 1b) showed that PC and tCho were significantly lower in the malignant MDA-MB-231 breast cancer cells, following transient transfection of siRNA-chk. Consistent with our previous observations, both MDA-MB-231 and MCF-7 cells showed a significant reduction of viability/proliferation following transient transfection using siRNA-chk alone (Fig. 2a and 2b) compared to untreated cells. Treatment with oligofectamine alone resulted in a small reduction of viability/proliferation for both cell lines (Fig. 2a and 2b) compared to untreated cells. Treatment with 5-FU alone resulted in a reduction of viability/proliferation of both cell lines (Fig. 2a and 2b). Treatment with 5-FU in combination with siRNA-chk transfection enhanced this reduction of viability/proliferation in both cell lines (Fig. 2a and 2b).

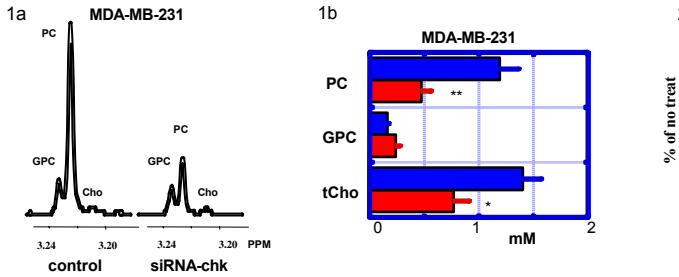

Figure 1: (a) Representative ^{1}H NMR spectra of the choline phospholipid metabolite region of MDA-MB-231 cells from [4]. (b) Choline phospholipid metabolite levels quantitated from ^{1}H MR spectra of control (■) and siRNA-chk treatment (■). Values are mean ± standard error. ∗ : P<0.05, ∗∗: P<0.01, compared with control.

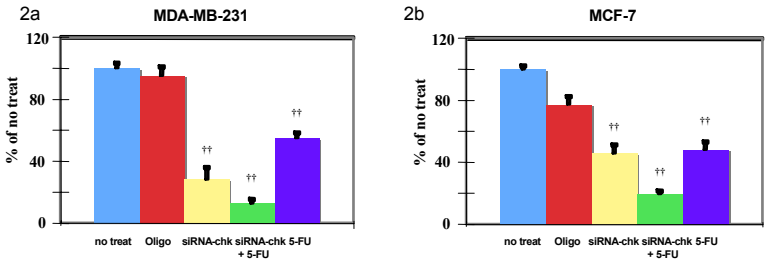

Figure 2: Cell viability/proliferation as determined by MTT assay of cells treated with oligofectamine (Oligo), siRNA-chk, 5-FU, and combination of siRNA-chk + 5-FU in (a) MDA-MB-231 and (b) MCF-7 cells. Values are mean ± standard error. ++ ; P<0.001, compared with no treatment. Values are mean ± standard error.

Discussion

The NMR spectra demonstrated that choline kinase expression plays an important role in the high PC and tCho levels observed in breast cancer cells. MDA-MB-231 cells, which typically exhibit higher choline kinase expression compared to MCF-7 cells [4], were more sensitive to siRNA-chk transfection, although the response to 5-FU was comparable for both cell lines. MDA-MB-231 cells are also more invasive and metastatic compared to MCF-7 cells.

Combined treatment with siRNA-chk transfection and 5-FU reduced proliferation to levels that were comparable but significantly lower than either treatment alone in both cell lines. These results demonstrate that transient transfection of siRNA-chk sensitized breast cancer cells to treatment with 5-FU. ChK inhibition may provide a novel alternative to enhance the effect of anti-cancer drugs.

References

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