Choline kinase knock-down in breast cancer cells using RNA interference is associated with an increase in intracellular lipid droplets and triacylglycerides

K. Glunde[1], V. Raman[1], N. Mori[1], Y. Mironchik[1], Z. M. Bhujwalla[1]

[1]Radiology Department, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Synopsis

Increased choline kinase (ChK) expression and activity are associated with increased malignancy, and may be a major factor for the elevated NMR-detectable levels of phosphocholine (PC) and total choline (tCho) in breast cancer cells. To test this hypothesis, we used small interfering RNA against ChK (siRNA-chk) to specifically knock down ChK expression. ChK activity and its downstream effects were monitored by ^{1}H NMR spectroscopy and fluorescence microscopy. SiRNA-chk treatment significantly reduced PC and tCho levels in breast cancer cells, but not in human mammary epithelial cells. The knock-down of ChK increased the formation of intracellular triacylglyceride-containing lipid droplets indicating differentiation.

Introduction

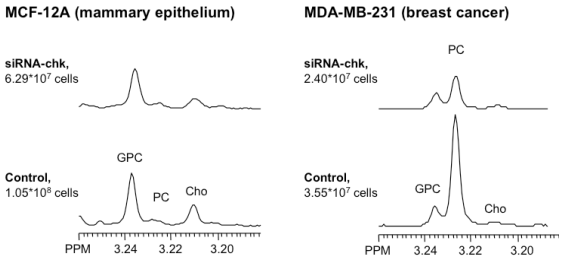
Elevated phosphocholine (PC) and total choline (tCho) levels, typically detected in cancer cells, have been linked to malignant transformation, invasion, and metastasis. Increased choline kinase (ChK) expression and activity is correlated with increased malignancy [1], identifying it as a unique target for cancer cells. We developed a small interfering RNA (siRNA)-based approach to down-regulate ChK. SiRNA treatment is a novel and powerful tool for knocking down gene expression of specific proteins. The effect of siRNA specific for ChK (siRNA-chk) on PC and tCho levels was monitored in MCF-12A human mammary epithelial cells and MDA-MB-231 breast cancer cells using ^{1}H NMR spectroscopy. Lipid fractions were recovered after dual phase extraction [2] and analyzed using ^{1}H NMR spectroscopy. Additionally, siRNA-chk treated cells were stained with Nile Red to visualize intracellular lipid droplets using fluorescence microscopy.

Methods

MDA-MB-231 human breast cancer cells and MCF-12A human mammary epithelial cells (HMECs) were treated with siRNA-chk for 48 hours 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 [2]. Fully relaxed ^{1}H NMR spectroscopy of the water-soluble and lipid fractions was performed on a Bruker Avance 500 spectrometer. Signal integrals were quantified relative to cell number and internal standard concentration. The internal standards used were 3-(trimethylsilyl)propionic-2,2,3,3,-d_4 acid (TSP) for water-soluble and tetramethylsilane (TMS) for lipid samples. 2-D ^{1}H-^{1}H-COSY NMR spectra [3] were acquired from the lipid fractions to detect triacylglyceride signals. Nile Red (9-diethylamino-5H-benzo[α]phenoxazine-5-one) staining of lipid droplets [4, 5] was performed, following siRNA-chk treatment and fixation of cells. Fluorescence microscopy was performed with a Zeiss LSM 410 laser-scanning fluorescence microscope using a 40x/1.2 water immersion lens.

Results

SiRNA-chk treatment of MDA-MB-231 breast cancer cells resulted in a significant ($p<0.05$, $n=5$) decrease of PC and tCho levels by 62 % and 46 % of control values, as shown in Figure 1. No significant changes in ChK mRNA levels and choline phospholipid metabolites were detected after siRNA-chk treatment of MCF-12A HMECs (Fig. 1). The mixed fatty acid signal at 1.3 ppm increased by 67 % of control values (n=2 each), most likely due to an increase in triacylglycerides, in both HMECs and breast cancer cells, as demonstrated in the ^{1}H NMR spectra of the lipid fractions in Figure 2a. The increase in triacylglycerides was verified by 2-D ^{1}H-^{1}H-COSY NMR spectra of the lipid fractions. The number and size of intracellular lipid droplets formed by triacylglycerides, as detected by Nile Red staining and confocal laser-scanning fluorescence microscopy, increased following siRNA-chk treatment in HMECs and breast cancer cells shown in Figure 2b.



Assignments: Cho, free choline; GPC, glycerophosphocholine; PC, phosphocholine.

Figure 1: ^{1}H NMR spectra of the water-soluble fractions of control (bottom panel) and siRNA-chk-treated (upper panel) in MCF-12A human mammary epithelial cells (left) and MDA-MB-231 breast cancer cells (right). Choline kinase knock-down significantly ($p<0.05$) decreased PC levels in the breast cancer cells.

Discussion

These data demonstrate that choline kinase expression plays an important role in the high PC and tCho levels observed in breast cancer cells. The increase in cellular triacylglycerides following knock-down of choline kinase expression is caused by increased formation of intracellular triacyglyceride-containing lipid droplets. Lipid droplets are a marker of functional differentiation of mammary tissue [6]. Thus, the formation of lipid droplets following ChK knock-down in HMECs and breast cancer cells is consistent with the possibility of inducing differentiation in these cells by inhibiting ChK. ^{1}H MRS proved useful in detecting potential cancer biomarkers, such as increased PC and decreased triacylglyceride levels, and studying the effects of cancer therapies utilizing siRNA technology to target ChK.

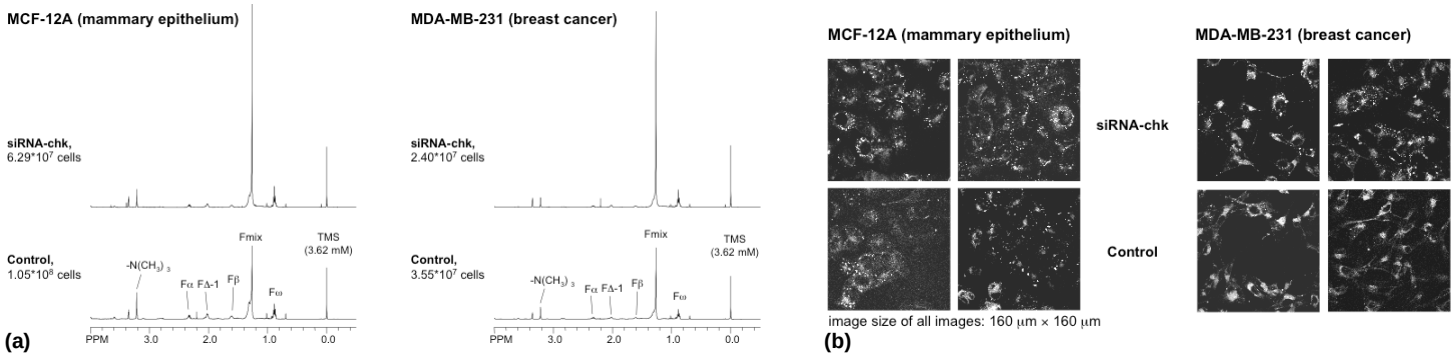


Figure 2: (a) ^{1}H NMR spectra of the lipid fractions and (b) Nile-Red-stained fluorescent lipid droplets in control (bottom panel) and siRNA-chk-treated (upper panel) MCF-12A human mammary epithelial cells (left) and MDA-MB-231 breast cancer cells (right). The mixed fatty acid signal at 1.3 ppm is significantly increased in both cell lines. Assignments: Fα, fatty acid protons neighboring the esterbond; Fβ, fatty acid protons next to Fα; FΔ-1, fatty acid protons neighboring a double-bond; Fmix, mixed fatty acid signal of different methylene protons; Fω, fatty acid CH_3-protons; -$N(CH_3)_3$, methylprotons in phosphatiylcholine; TMS, tetramethylsilane.

References

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