Correlation between choline kinase mRNA and ^{1}H MRS-detectable choline metabolite levels in stable choline kinase knock-down clones of a human breast cancer cell line

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Synopsis

Elevated phosphocholine (PC) and total choline (tCho) levels in breast cancer cells, frequently detected by ^{1}H MRSI, may be caused by an increased expression of choline kinase (ChK). Clones of human breast cancer cells were generated that stably express small interfering RNA to specifically knock down ChK mRNA levels. ChK mRNA, PC, and tCho levels were monitored in several ChK knock-down clones by quantitative real-time reverse transcription-polymerase chain reaction (Q-RT-PCR) analysis and ^{1}H MR spectroscopy. A direct correlation between ChK mRNA level and PC and tCho concentration was observed.

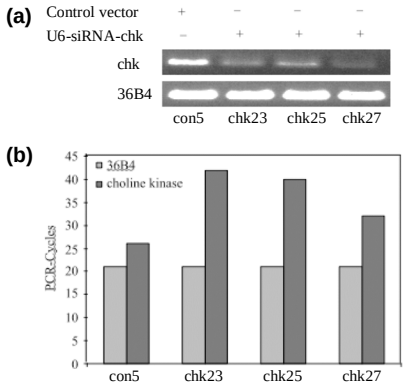

Figure 1: Stable clones of MDA-MB-231 breast cancer cells expressing U6-siRNA-chk. (a) RT-PCR of clones containing either the empty-vector control (con5) or U6-siRNA-chk (chk23, chk25, chk27). ChK message is decreased in clones chk23, chk25, and chk27 as compared to empty-vector control clone con5. (b) Quantitative real-time RT-PCR of con5, chk23, chk25, and chk37 carried out using the iCycler from BIO-RAD. The number of cycles required to amplify ChK message was much higher in chk23, chk25, and chk27 than in con5 validating an effective decrease of ChK mRNA in the stably U6-siRNA-chk transfected clones, while the internal control 36B4 was amplified equally in all samples.

Introduction

Several cancers exhibit elevated phosphocholine (PC) and total choline (tCho) levels, detectable between 3.20 and 3.24 ppm in ^{1}H MR spectra. ^{1}H MRSI detection of total choline levels can be employed clinically to distinguish between malignant *versus* benign breast lesions [1]. Increased choline kinase (ChK) expression and/or activity may cause the increased PC and tCho levels in breast cancers, since a correlation between increased ChK activity and high tumor grade has been demonstrated [2]. To investigate the relation between ChK expression and the corresponding ^{1}H MR-detectable choline metabolite levels, we generated stable clones of highly metastatic breast cancer cells MDA-MB-231 expressing the small interfering RNA (siRNA) specific for ChK under the control of an U6-promoter to knock down ChK messenger RNA (mRNA) levels. The ChK mRNA level of each clone was characterized by quantitative real-time reverse transcription-polymerase chain reaction (Q-RT-PCR) analysis. The PC and tCho levels of clones with substantially reduced ChK mRNA levels were monitored using ^{1}H MR spectroscopy.

Methods

A small interfering hairpin sequence specific for ChK was ligated into the pSHAG vector [3] containing the U6 promoter for RNA Polymerase III catalyzed transcription. The U6 promoter plus hairpin-siRNA for ChK were cut from this vector using restriction endonucleases and ligated into the multiple cloning site of a pCR3.1 vector (Invitrogen) devoid of any promoter. This final construct, referred to as U6-siRNA-chk, was transfected into human MDA-MB-231 breast cancer cells. MDA-MB-231 clones expressing U6-siRNA-chk were selected with G418 (Gibco). 45 clones expressing U6-siRNA-chk were isolated, expanded, and analyzed by Q-RT-PCR using the iQ SYBR Green Supermix and the iCycler (BIO-RAD). Controls were transfected with empty-vectors. Water-soluble cell extract fractions were obtained from U6-siRNA-chk clones with maximally reduced ChK expression as well as from two empty-vector control clones using the dual-phase extraction method [2]. Fully relaxed ^{1}H MR spectroscopy of the water-soluble fractions was performed on Bruker Avance 500 spectrometer. The signal integrals were quantified relative to cell number and concentration of the internal standard 3-(trimethylsilyl)propionic-2,2,3,3,-d_4 acid.

Results

Three U6-siRNA-chk expressing MDA-MB-231 clones with substantially reduced ChK mRNA levels were obtained as demonstrated by conventional and quantitative real-time RT-PCR shown in Figure 1. ^{1}H MR spectra of these clones revealed that the clone chk23, which exhibited the most drastically reduced ChK mRNA levels, also displayed the most pronounced decrease in cellular PC (50 % of control) and tCho (70% of control) levels as shown in Figure 2. The second largest decrease of ChK mRNA levels, detected in clone chk25, also resulted in reduced PC (70% of control) and tCho levels (75% of control) (Fig. 2). PC and tCho levels in clone chk27, exhibiting slightly decreased ChK mRNA levels, remained unchanged (Fig. 2).

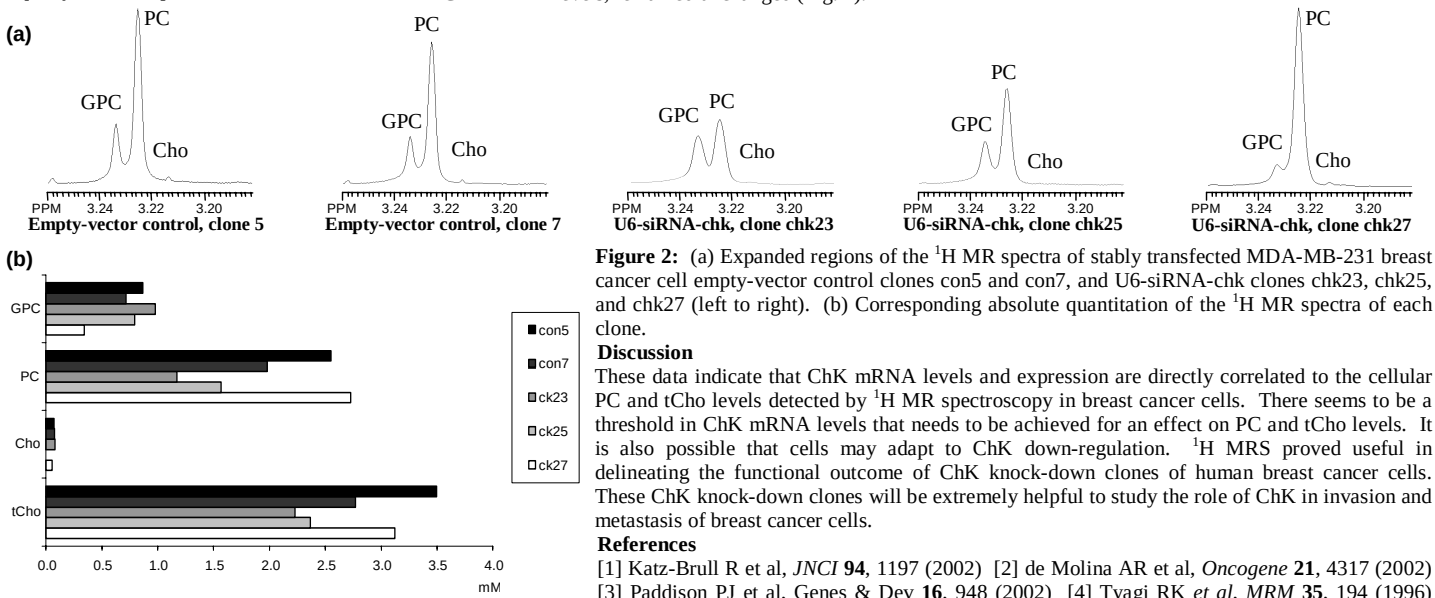

Figure 2: (a) Expanded regions of the ^{1}H MR spectra of stably transfected MDA-MB-231 breast cancer cell empty-vector control clones con5 and con7, and U6-siRNA-chk clones chk23, chk25, and chk27 (left to right). (b) Corresponding absolute quantitation of the ^{1}H MR spectra of each clone.

Assignments: Cho, free choline; GPC, glycerophosphocholine; PC, phosphocholine; tCho, total choline (Cho+GPC+PC).

Discussion

These data indicate that ChK mRNA levels and expression are directly correlated to the cellular PC and tCho levels detected by ^{1}H MR spectroscopy in breast cancer cells. There seems to be a threshold in ChK mRNA levels that needs to be achieved for an effect on PC and tCho levels. It is also possible that cells may adapt to ChK down-regulation. ^{1}H MRS proved useful in delineating the functional outcome of ChK knock-down clones of human breast cancer cells. These ChK knock-down clones will be extremely helpful to study the role of ChK in invasion and metastasis of breast cancer cells.

References

[1] Katz-Brull R et al, *JNCI* **94**, 1197 (2002) [2] de Molina AR et al, *Oncogene* **21**, 4317 (2002) [3] Paddison PJ et al, Genes & Dev **16**, 948 (2002) [4] Tyagi RK *et al*, *MRM* **35**, 194 (1996)

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