Elevated choline kinase protein expression and activity correlate with increased MRS-detected phosphocholine levels in ovarian carcinomas

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Introduction

Choline phospholipid metabolism is altered in several cancers, such as breast, ovarian, prostate, and brain tumors, providing a diagnostic marker for noninvasive cancer detection and monitoring of therapy [1]. Ovarian tumor progression is characterized by an increased of the ^{1}H magnetic resonance spectroscopy (MRS)-detected total choline (tCho) resonance at 3.20-3.24 ppm [2]. Phosphocholine (PCho) was demonstrated to be the predominant choline metabolite contributing to this increase in tCho with ovarian tumor progression [2]. The consistently elevated PCho levels in ovarian carcinoma cells were due to an increase in the overall activity of phosphatidylcholine-specific phospholipase (PC-PL) C and D, combined with increased rates of choline kinase activity [2]. We have here, for the first time, correlated choline kinase (Chk) protein expression levels with the PCho concentrations detected in nontumoral human ovarian surface epithelial cells immortalized by stable transfection with SV40 large-T antigen and with the human telomerase reverse transcriptase gene (hTERT), and the human serous ovarian carcinoma cell lines OVCAR3, IGROV1, and SKOV3.

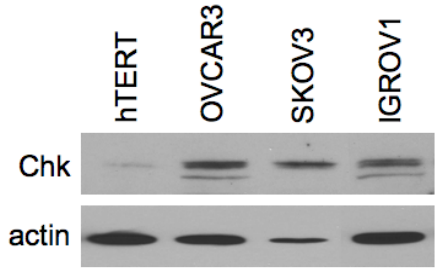

Figure 1: Immunoblotting with choline kinase (Chk) antibody and actin loading controls of the human serous ovarian carcinoma cell lines OVCAR3, SKOV3, and IGROV1 compared to nontumoral ovarian surface epithelial cells immortalized by stable transfection with the human telomerase reverse transcriptase gene (hTERT).

Methods

Nontumoral immortalized hTERT cells, and the human serous ovarian carcinoma cell lines OVCAR3, IGROV1, and SKOV3 were maintained in cell culture as previously described [2]. For Chk detection by immunoblotting, protein lysates were prepared from hTERT, OVCAR3, IGROV1, and SKOV3 cells by homogenizing approximately 3×10^6 cells with lysis buffer as previously described [3]. Fifty µg of total protein was loaded in each lane, and two lanes were loaded with molecular weight standard (BenchMark, Life Technologies, Rockville, MD). Proteins were resolved by 10% sodium dodecyl sulfate-poyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotting were performed as previously described [3]. The polyclonal rabbit anti-human Chk antibody (IgG) used for Chk detection was raised against a hydrophilic synthetic peptide representing the amino acids 73-90 of human Chk α (Proteintech Group, Inc., Chicago, IL) [3]. Using this antibody, human Chk was detected at an apparent molecular weight of approximately 48 kDa. Basal Chk activity was assayed at 25 °C by ^{1}H and ^{31}P MRS methods as previously described [2, 4]. MRS analyses were performed at 9.4 and 16.4 T on Bruker Avance spectrometers.

Results

The human ovarian carcinoma cell lines OVCAR3, SKOV3, and IGROV1 consistently expressed higher levels of Chk protein as compared to nontumoral immortalized hTERT cells (Fig. 1). This elevation in Chk protein expression in three ovarian carcinoma cell lines correlated well with an increase in their Chk activity (Fig. 2a) and their intracellular steady-state PCho and tCho concentrations (Fig. 2b) as detected by ^{1}H MRS.

Discussion

We have previously shown that human ovarian carcinoma cell lines exhibit increased tCho and PCho concentrations as compared to normal and immortalized ovarian surface epithelial cells [2]. Here we have demonstrated for the first time that this increase in tCho and PCho was caused, at least in part, by an elevation of the cellular Chk protein expression levels in human ovarian carcinoma lines. These elevated Chk protein levels most likely resulted in increased Chk activities, significantly contributing to the elevation in PCho and tCho. Increased Chk protein expression was previously also detected in human breast [3], colorectal [5], prostate [5], and lung [6] cancers. We have previously shown that Chk silencing with small interfering RNA specific to Chk (siRNA-chk) may be a valuable therapeutic approach in breast cancer [3]. The finding that Chk expression is consistently increased in human ovarian carcinoma cell lines opens up future possibilities for siRNA-chk-mediated Chk silencing for treatment of ovarian cancers. MRS detection of PCho and tCho can be a valuable tool for characterizing the cancerous choline metabolite profile as a diagnostic marker, and to evaluate and follow future Chk-targeted anticancer therapies in ovarian cancer.

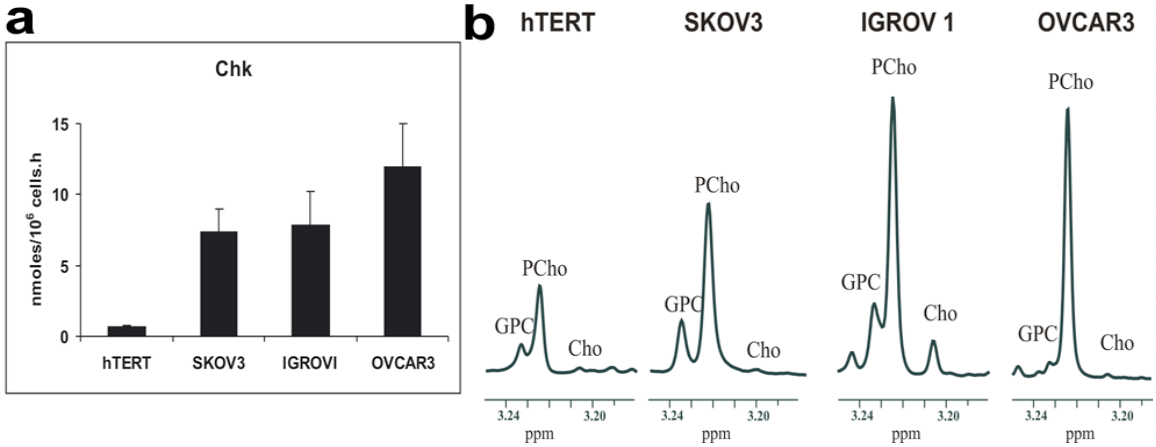

Figure 2: (a) Basal activity rates (mean ± SD) of Chk in nontumoral hTERT and human carcinoma cells. (b) Representative expanded ^{1}H MR spectra (16.4T) of water-soluble extracts of epithelial ovarian immortalized hTERT and human ovarian carcinoma cells. Cho, free choline; GPC, glycerophosphocholine; PCho, phosphocholine.

References: [1] Glunde K et al, *Expert Rev Mol Diagn* 6, 821-9 (2006) [2] Iorio E et al, *Cancer Res* 65, 9369-76 (2005) [3] Glunde et al, *Cancer Res* 65, 11034-43 (2005) [4] Podo F et al, Anticancer Res 16, 1399-412 (1996) [5] Ramirez de Molina A et al, *Biochem Biophys Res Commun* 296, 580-3 (2002) [6] Ramirez de Molina A et al, *Lancet Oncol* 8, 889-97 (2007). This work was supported by Accordo di Collaborazione Italia-USA N. 530F/0F29 and NIH P50 CA103175 (JHU ICMIC Program).