Choline kinase suppression increases tumor lipid content and vascular volume in a human breast cancer model

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Introduction

Increased choline kinase (Chk) expression is a primary cause of the elevated phosphocholine (PC) and total choline (tCho) levels in breast cancers [1-2], and may play a key role in tumor aggressiveness and altering the tumor microenvironment. Increased ChK activity and high tumor grade have been demonstrated to correlate in clinical tumor samples [2]. Here we have characterized, for the first time, the effect of suppressing Chk on tumor lipid content and vascularization in a human breast tumor model. We studied tumor xenografts from human MDA-MB-231 breast cancer cells stably expressing small interfering RNA specific for Chk under the control of a U6-promoter (siRNA-chk). This molecular biology technique allows for the stable reduction of messenger RNA (mRNA) levels of a gene of choice. Multiparametric co-localized maps of vascular volume, permeability, and total choline and lipid were obtained with ^{1}H MRI/MRSI, and decreased PC levels were confirmed using single-voxel ^{31}P MRS *in vivo* and fully relaxed ^{1}H MRS of tumor extracts.

Methods

Human MDA-MB-231 breast cancer cells stably expressing siRNA-chk under the control of an U6-promoter [1] were orthotopically inoculated into the mammary fat pad of severe combined immune suppressed (SCID) mice. The MDA-MB-231 breast cancer cell clone expressing siRNA-chk, which contained the most significantly reduced Chk mRNA, protein, and PC levels, was chosen for these inoculations [1]. Control mice were inoculated accordingly with empty-vector control cells, or MDA-MB-231 wild-type cells. *In vivo* MR studies were performed on a 4.7T Bruker Biospec spectrometer. Metabolic maps of total choline (TE=272 ms) were obtained from a 4-mm thick central slice, with an in-plane resolution of 1-mm x 1-mm using a 2D CSI sequence with VAPOR water suppression. Quantitative maps of total choline from water suppressed spectra and total lipid obtained from unsuppressed water reference spectra acqired with TE=15 ms were obtained with an in-house IDL program based on the method of Bolan et al [4]. Contrast-enhanced MRI using albumin-GdDTPA was performed to measure vascular volume and permeability [3]. *In vivo* single-voxel ^{31}P MRS and fully relaxed ^{1}H MRS of tumor extracts were performed to confirm decreased tumoral PC levels.

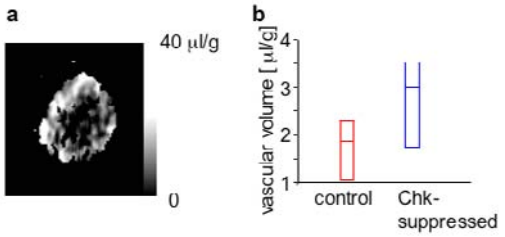

Figure 1: (a) Map of vascular volume, and (b) box-and-whisker plot of vascular volume in Chk-suppressed and control tumors demonstrating increased vascular volume in Chk-suppressed tumors.

Results

MDA-MB-231 breast cancer cells stably expressing siRNA-chk contained significantly decreased Chk mRNA and protein levels reflected by significantly decreased tCho and PC levels [1]. Significantly decreased tumoral PC levels in Chk-suppressed breast tumors confirmed the reduction of Chk levels in these tumors. Tumor growth rates of Chk-suppressed tumors were slower compared to control vector tumors. The vascular volume was significantly higher in Chk-suppressed breast tumors compared to controls (2.8 ± 0.5 versus 1.7 ± 0.5 µl/g, Fig. 1), which is consistent with 2.5-fold higher vascular endothelial growth factor (VEGF) mRNA levels in Chk-suppressed MDA-MB-231 cells than in empty-vector control cells. The lipid signal at 1.3 ppm, obtained using ^{1}H spectroscopic imaging, was significantly higher in Chk-suppressed breast tumors compared to controls (0.88 ± 0.35 M versus 1.45 ± 0.35 M, Fig. 2). The permeability was not altered in Chk-suppressed tumors compared to controls. For the limited numbers of animals studied here (n=3 in each group), the reduction of total choline was not significant. This may be in part due to a significant increase of GPC observed in the Chk-suppressed tumors.

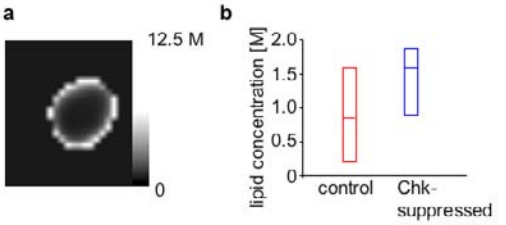

Figure 2: (a) Map of total lipid, and (b) box-and-whisker plot of total lipid in Chk-suppressed and control tumors demonstrating increased total lipid levels in Chk-suppressed tumors.

Discussion

These data indicate that Chk suppression in breast tumors, which is being explored as a therapeutic strategy, has diverse effects on the tumor microenvironment. Chk suppression in breast tumor xenografts resulted in increased levels of vascular volume, which was most likely caused by increased VEGF levels. Treatment of breast tumors with siRNA-chk may thus have an effect on the vascularization of tumors, which in turn may have an impact on drug delivery. The increased lipid levels in breast cancer xenografts containing stably downregulated Chk levels are in good agreement with previous findings that revealed increased levels of triacylglyceride-containing lipid droplets indicating differentiation in Chk-suppressed breast cancer cells [1]. Increased lipid levels in Chk-suppressed breast tumors may be indicative of increased differentiation of these tumors following Chk downregulation. Proton MRSI of the lipid signal at 1.3 ppm may be used to monitor differentiation following targeting of Chk as potential cancer therapy.

References

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