Vascular endothelial growth factor overexpression increases phosphocholine levels in a human breast cancer model

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Synopsis

Vascular endothelial growth factor (VEGF) plays an important role in tumor angiogenesis and the response of tumors to hypoxia. To investigate the role of VEGF in cancer cells, we generated stable clones of VEGF-A overexpressing human MDA-MB-231 breast cancer cells. ^{1}H and ^{31}P NMR spectroscopy was employed to determine the effects of VEGF-A overexpression on choline phospholipid metabolism. MDA-MB-231 tumors derived from VEGF-A overexpressing cells exhibited a significant increase in phosphocholine and a decrease in glycerophosphocholine levels, compared to tumors derived from control empty-vector transfected cells. The cellular total choline concentration was not affected by VEGF-A overexpression.

Introduction

An increased cellular phosphocholine (PC) level, leading to an increased total choline (tCho) level, is one of the signatures of cancer. This elevation is closely related to malignant transformation, invasion, and metastasis. The tCho levels can be utilized in the clinic to distinguish between malignant *versus* benign breast lesions using ^{1}H MRSI. Angiogenesis is an important factor in tumor development. Tumor associated angiogenesis is mediated by the migration and proliferation of host endothelial cells [1]. VEGF is an essential angiogenic factor in tumor vascularization, and increased expression of VEGF is related to cancer aggressiveness and metastasis. In this study we have investigated the effect of VEGF overexpression on choline phospholipid metabolism using ^{1}H and ^{31}P NMR spectroscopy.

Methods

Full-length cDNA for VEGF-A (pHUVEGF2.1) from Genentech was cloned into the eucaryotic expression vector pCR3.1 under control of a constitutive HCMV promoter. MDA-MB-231 human breast cancer cells were stably transfected with this VEGF-A construct or an empty-vector as control, and selected with 700µg/ml G418. MDA-MB-231 clones overexpressing VEGF-A (n=4) or empty-vector controls (n=4) were inoculated into the mammary fat pad of severe combined immune deficient (SCID) mice. Perchloric acid (PCA) extracts were obtained from four VEGF-A overexpressing tumors and from four vector-control tumors as described previously [2]. Tumor volumes were approximately 900-1000 mm^3. Fully relaxed ^{1}H NMR spectroscopy of the tumor extracts was performed on a Bruker Avance 500 NMR spectrometer, using 3-(trimethylsilyl)propionic-2,2,3,3,-d_4 acid (TSP) as an internal concentration standard. ^{31}P NMR spectra were acquired on a GE Omega 400 NMR spectrometer. Enzyme-linked immunosorbent assays (ELISA) were performed to determine the expression level of human VEGF in the solid tumors, using DuoSet kit (R & D systems, Inc.).

Results

ELISA of the solid tumors revealed significantly increased VEGF levels in the VEGF-A overexpressing tumors (4.6 ± 1.6 [pg]/[µg protein], n=3) as compared to empty-vector control tumors (1.72 ± 0.88 [pg]/[µg protein], n=3). Representative ^{1}H NMR spectra are shown in Figure 1, and representataive ^{31}P NMR spectra are shown Figure 2. Data from the ^{1}H NMR spectra are summarized in Figure 3. Solid MDA-MB-231 tumors overexpressing VEGF exhibited significantly ($p<0.05$) increased PC and significantly ($p<0.01$) decreased GPC levels compared to empty-vector control tumors. The levels of tCho (free choline (Cho) + PC + GPC) remained unchanged by VEGF overexpression in solid MDA-MB-231 tumors. The empty-vector MDA-MB-231 tumors contained relatively high GPC levels as compared to levels typically observed in previous studies. This may have been caused by the relatively large size of the tumors utilized in this study, and tumor pH [3] or other factors may have increased GPC levels to atypically high values.

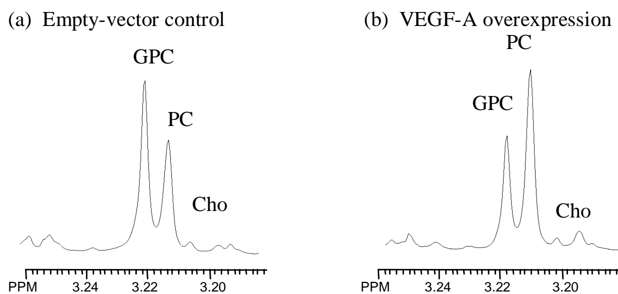

Figure 1: Representative ^{1}H NMR spectra displaying the choline phospholipid metabolite region of PCA extracts from MDA-MB-231 tumors. (a) MDA-MB-231 tumors with empty-vector (control) and (b) MDA-MB-231 tumors overexpressing VEGF-A.

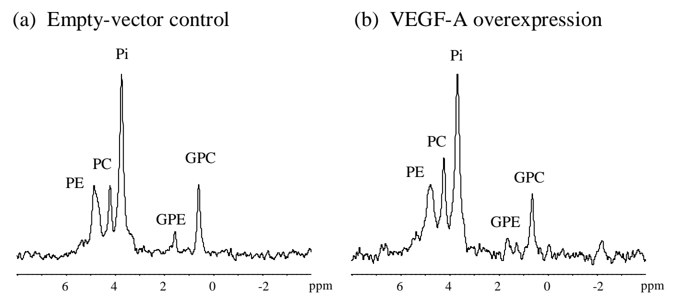

Figure 2: Representative expanded ^{31}P NMR spectra (–3 ppm to 8 ppm) of PCA extracts from MDA-MB-231 tumors. (a) MDA-MB-231 tumors with empty-vector (control) and (b) MDA-MB-231 tumors overexpressing VEGF-A.

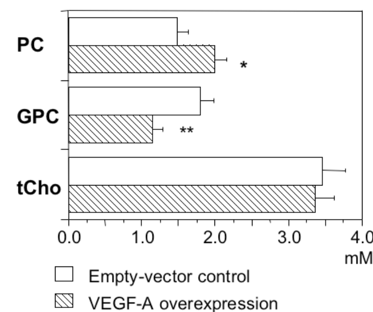

Figure 3: Quantitation of choline phospholipid metabolite levels from the ^{1}H NMR spectra of MDA-MB-231 tumor PCA extracts. Values are mean ± standard error. *represents $p<0.05$, **represents $p<0.01$.

Discussion

Our data demonstrate that an increase in VEGF-A levels in solid breast tumors may, in part, cause the elevated PC and decreased GPC levels typically detected in breast tumors. Increased PC levels are associated with malignant transformation [4]. We have previously shown that treatment with a non-specific inhibitor of cyclooxygenase (COX), indomethacin, resulted in decreased PC and increased GPC levels in human mammary epithelial cells (HMECs) and human vascular endothelial cells (HUVECs) [5, 6]. Thus, VEGF overexpression and COX-1/-2 inhibition have opposing effects on the cellular PC and GPC concentrations in HMECs. These observations are also consistent with studies, which demonstrate that COX-1 and/or COX-2 inhibition can down-regulate VEGF expression in cancer cells, thereby decreasing tumor-associated angiogenesis [1, 7, 8].

References & Acknowledgements

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Assignments in Figures:

Cho, free choline; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; PC, phosphocholine; PE, phosphoethanolamine; Pi, inorganic phosphate; tCho, total choline (Cho+GPC+PC).