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Can proton MRS provide useful information for characterizing estrogen receptor status in breast cancer?

Estrogen receptor (ER) status has been used in the clinical management of breast cancer both as a predictive factor for treatment and as a prognostic factor for survival [1]. Compared with ER-positive cancer, ER-negative cancer has a poorer clinical outcome and shorter median survival [2–3]. ER-negative cancer was more aggressive, with bigger tumor size, more prominent tumor infiltration showing non-mass type enhancements on magnetic resonance imaging (MRI) features [4]. ER-negative tumors showed higher intratumoral microvessel density than did ER-positive tumors [5].

ER-negative breast carcinoma was also associated with an increased choline kinase (ChoK) activity [6]. The ChoK and its product, phosphocholine (PCho), have been implicated in human carcinogenesis. Elevated level of total choline-containing compounds (tCho) is a tissue proliferative marker for malignant tumor [7]. In this study, we reported a quantitative proton magnetic resonance spectroscopy (^{1}H-MRS) study to further investigate if the tCho level shows difference between ER-positive and ER-negative breast cancers. The aim of our study was to determine whether *in vivo* ^{1}H-MRS can provide useful information for characterizing ER status in breast cancer.

Forty-seven breast cancer patients, who were scanned with the MRI/proton magnetic resonance spectroscopy (MRS) protocol from June 2004 to December 2006, were included in this study. The inclusion criteria were patients with biopsy confirmed diagnosis of malignant lesions that measured ≥1.8 cm on magnetic resonance (MR) images. All 47 patients had histopathologically an invasive ductal carcinoma. The ER status was examined by pathologists at hospital and was considered negative if immunoperoxidase staining of tumor cell nuclei in the biopsy specimen was <5%. This study protocol was approved by the Institutional Review Board and was HIPAA compliant. All patients gave written informed consent.

The MRI study was carried out using a 1.5 T MR scanner with a standard bilateral breast coil (Philips Medical Systems, Cleveland, OH). The imaging protocol consisted of high-resolution precontrast imaging from the concerned breast, bilateral dynamic contrast-enhanced imaging, and proton MRS. After the MRI study was completed, single-voxel ^{1}H-MRS was carried out using a point-resolved spin-echo sequence. The spectroscopic voxel was carefully positioned to maximize the coverage of the contrast-enhanced lesions while minimizing the inclusion of adipose tissue. The voxel size was from 2.4 to 8.0 ml. The jMRUI software package (http://sermn02.uab.es/mrui/) was used for time–domain analysis. AMARES [8], a widely used quantitation tool for MRS data, was employed to fit spectra. In this study, a Gaussian lineshape model was chosen to quantify the tCho peak. The Cramer-Rao lower bound (CRB) was used as a measure of fitting accuracy [9]. Absolute quantification of tCho concentration was obtained using the water peak from the unsuppressed spectrum, fit at 4.7 p.p.m., as an internal reference. The tCho

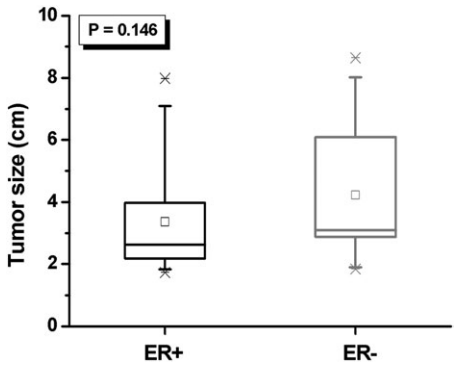

Figure 1. Box plot showing comparison of tumor size in estrogen receptor-positive (ER+) and estrogen receptor-negative (ER−) breast cancer groups. The tumor size was larger in ER− group than in ER+ but not reaching significant level ($P = 0.146$, Student's t-test).

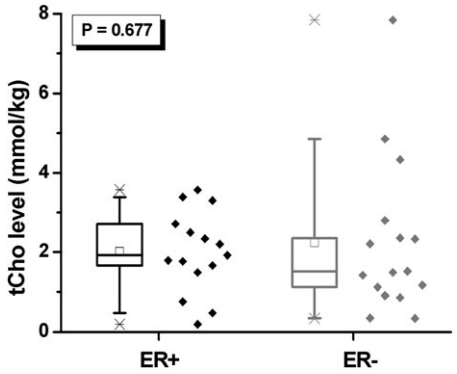

Figure 2. Box plus data plot showing comparison of total choline-containing compounds (tCho) concentration in estrogen receptor-positive (ER+) and estrogen receptor-negative (ER−) breast cancer groups. There was no significant difference in the tCho level between these two groups ($P = 0.677$, Student's t-test).

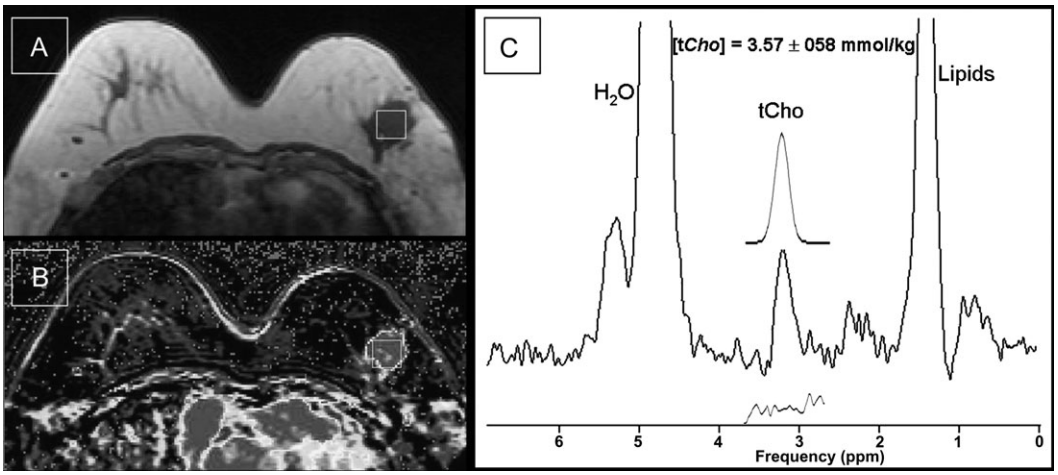

Figure 3. Magnetic resonance imaging/magnetic resonance spectroscopy of a 52-year-old patient with an estrogen receptor-positive invasive ductal carcinoma. The lesion was 2.6 cm and the spectroscopic voxel (size $2 \times 2 \times 2$ cm^3) was superimposed on the hypointense lesion in the precontrast axial image (A) and on the contrast-enhanced lesion in the color-coded subtraction image (B). An elevated total choline-containing compounds (tCho) peak is clearly visible at 3.20 p.p.m. in the spectrum (C). The measured tCho was 3.57 ± 0.58 mmol/kg.

concentration was calculated using measured T1 and T2 values for intensity correction [10].

Of 47 patients, 27 (57%) had ER-positive cancers and 20 (43%) had ER-negative cancers. The progesterone receptor (PR) status was available for 42 patients, including 22 ER-positive patients (20 PR positive and two PR negative) and 20 ER-negative patients (19 PR negative and one PR positive). The mean age was 51 years (range 31–68 years) for ER-positive patients and 47 years (range 31–68 years) for ER-negative patients ($P = 0.264$). The mean tumor size was 3.4 cm (range 1.8–7.1 cm) for the ER-positive group and 4.2 cm (range 1.8–8.6 cm) for the ER-negative group ($P = 0.146$) (Figure 1). On the basis of the criterion (i.e. CRB <100%), tCho detection rate was higher in ER-negative group (16 of 20, 80%) than in ER-positive group (15 of 27, 56%), but not reaching significant level ($P = 0.083$). For these 31 lesions with tCho detection, the measured tCho levels ranged from 0.19 to 7.84 mmol/kg (mean ± standard deviation 2.13 ± 1.96 mmol/kg), which are well within the previously published *in vivo* tCho concentration [11]. The ER-positive group had a lower mean tCho concentration than the ER-negative group, as shown in Figure 2, but no significant difference was observed (2.01 versus 2.24 mmol/kg, $P = 0.677$). Figure 3 shows a representative MRI and MRS measurement of a patient with ER-positive breast cancer. The precontrast axial image of one lesion showed low signal intensity (A). The spectroscopic voxel (size $2 \times 2 \times 2$ cm^3) is placed in the hypointense lesion on the precontrast axial image (A) and on the contrast-enhanced lesion in the subtraction image (B). A tCho peak is visible on the water–fat-suppressed spectrum (C). The Gaussian model fitting on the tCho peak produces a measurement of $[tCho] = 3.57 \pm 0.58$ mmol/kg.

Carcinogenesis relates to ER negativity in ductal carcinoma of breast [5]. Choline transport rates and ChoK activity were found to increase remarkably in the breast cancer cells with elevated expression of PCho [12]. A significant association between ChoK overexpression with high histological tumor grade and ER-negative status was observed [6]. The association was possibly mediated through higher cell proliferation [13]. In our study, the tCho detection rate of *in vivo* ^{1}H-MRS in ER-negative group, although higher, wan not significantly different from that of ER-positive group, and also the absolute tCho levels did not appear to be related to ER status. The reason why our finding was not significant might be due to the heterogeneity of the breast cancer tissue. As shown in Figure 2, the large range in tCho concentration may reflect the heterogeneous nature of breast lesions. Gribbestad et al. [14] reported that phosphatidylcholine, a precursor of tCho-derived phospholipids, also showed a large variation even among the same tumor types. In addition to the intrinsic heterogeneous nature of breast tumors, the limitation of *in vivo* ^{1}H-MRS detection may also contribute to a complicated tCho distribution pattern. tCho detection may be difficult in diffuse-enhancement type cancers because of the intermingling of tumor cells with adipose tissues. Diffusive-enhancement type cancer showed a much lower overall tCho level than mass type cancer [10]. From our present study, it was therefore indicated that *in vivo* quantitative ^{1}H-MRS cannot provide useful information for characterizing ER status in carcinoma of the breast.

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