Detection of altered adipose tissue composition in breast cancer patients using MR spectroscopy

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Target Audience: Clinical oncologists specializing in breast cancer, cancer biologists investigating the role of adipose in promoting cancers, scientists studying metabolism in obesity, and MR spectroscopists.

Purpose: Obesity has reached epidemic levels and is associated with diseases such as diabetes, dyslipidemia, metabolic syndrome. Obesity has also been identified as a risk factor for the development of breast cancer (BrCa). A 30-year follow up study of Danish BrCa patients found that a BMI ≥ 30kg/m^2 at the time of diagnosis was a prognostic factor for developing distant metastases and for death as a result of BrCa [1]. The relationship between adipose tissue and BrCa, however, is complex and the biological mechanism by which increased adiposity stimulates BrCa growth is poorly understood. We hypothesize that independent of increased adiposity (or fat mass), lipid metabolism is altered in the vicinity of the tumor, and the altered metabolic milieu is favorable to BrCa progression. In this pilot study, we examined the fatty acid (FA) composition of breast adipose tissues from BrCa patients using *ex vivo* MR spectroscopy (MRS) and found differences between the adipose located proximal to the tumor versus that at a distal location within the same breast. Our results indicate that localized alterations occur in FA metabolism in the tumor microenvironment in BrCa.

Experimental Methods: Peritumoral adipose tissue (PTA, located within the same quadrant as the tumor) and distal adipose tissue (DSA, located within the other 3 quadrants with no tumor) were collected from mastectomy specimens from BrCa patients (n=7). Patient/tumor characteristics were as follows: age: 42-51; BMI: 24.0-34.2; lesion size 0.5cm-4.8cm; all but one were ER positive; and four were node positive; three were node negative. PRESS localized proton MR spectra of PTA and DSA were acquired from 1μL voxels in the specimens on a 14.1T Bruker Avance imager without water suppression using recycle time, TR 40s, echo delay time, TE 7.5ms, spectral width 8000Hz and 16k complex points. Peak integrals of FA functional groups, corrected for T_2 relaxation time, were measured from the spectra from which the fractions of mono-unsaturated (f_M), poly-unsaturated (f_P), and saturated (f_S) FA were calculated using previously reported methods [2]. The following FA ratios were compared between PTA and DSA depots: mono-unsaturated to saturated FA (f_M/f_S), poly-unsaturated to saturated FA (f_P/f_S), and total unsaturated to saturated FA (f_U/f_S). The corresponding receiver operating characteristic (ROC) curves were compared to pathologically determined node status and tumor size by using the area under the curve (AROC) and computing the 95% confidence intervals (CI) based on percentile boot straps.

Results: Fatty acid composition was dependent on the location of the breast adipose tissue in BrCa patients as shown in Table 1.

Table 1. Fatty acid composition in peritumoral and distal adipose depots in breast cancer patients

Adipose site	f_M	f_P	f_S	f_M/f_S	f_P/f_S
Distal (DSA)	0.35±0.06	0.29±0.02	0.36±0.06	1.00±0.29	0.82±0.14
Peritumoral (PTA)	0.44±0.04	0.28±0.03	0.29±0.03	1.56±0.30	0.98±0.15

PTA from BrCa patients had elevated f_M and decreased f_S compared to DSA, although both specimens were located within the same breast. Changes in the fractions of monounsaturated and saturated fatty acids resulted in significant elevations in f_M/f_S (p=0.01) and f_P/f_S (p=0.04) in the PTA. Adipose tissue from breasts with no tumor burden (from patients undergoing prophylactic mastectomy) had f_M/f_S =1.12±0.18 and f_P/f_S =0.57±0.03 (n=2). ROC analysis revealed that several MRS measures of FA composition in PTA and DSA have potential for predicting pathologically determined node status and tumor size (>2cm), two prognostic markers for breast cancer aggressiveness, as shown by their AROC and 95% CI given in parentheses (Table 2). Values of AROC closer to 1 or 0 indicate high predictive potential. PTA f_M and f_M/f_S had positive predictive value for node status and f_P had negative predictive value. Interestingly, DSA f_M and f_M/f_S had positive predictive value for tumor size (>2cm), while f_P had negative predictive value for the same.

Table 2. Prognastic potential of MRS measures for breast cancer pathology

Adipose site	*MRS measure*	*AROC*	*95%CI*	*Predicted pathology*
PTA	f_M	0.94	0.89 – 0.99	Node status
PTA	f_M/f_S	0.78	0.65 – 0.94	Node status
PTA	f_P	0.04	1.19×10^{-4} – 0.08	Node status
DSA	f_M	0.81	0.68 – 0.99	Tumor size>2cm
DSA	f_S	0.27	0.17 – 0.46	Tumor size>2cm
DSA	f_M/f_S	0.76	0.61 – 1.00	Tumor size>2cm

Discussion: Our results suggest that in BrCa, localized alterations in lipid metabolism occur in the vicinity of the tumor. The FA profile in the peritumoral region that we detected using MRS might represent a unique metabolic milieu that is permissive to cancer progression. Elevated f_M/f_S is consistent with increased activity of fatty acid desaturases, such as stearoyl-CoA desaturase, which is known to be upregulated in other cancers as well as obesity [3]. Bioenergetic demands and increased membrane synthesis in the context of cancer progression might be responsible for the altered FA profile in the tumor microenvironment.

Conclusions: In BrCa, lipid metabolism is altered in the adipose tissue adjacent to the tumor. The unique FA profile in the PTA might be a metabolic signature for the presence of BrCa. Our study suggests that *in vivo* MRS may have potential for developing noninvasive biomarkers for invasive BrCa.

References: 1. Ewertz M, Jensen MB, Gunnarsdottir KA, et al. Effect of obesity on prognosis after early-stage breast cancer. *J. Clin. Oncol.* 29: 25-31 (2011). 2. Ren J, Dimitrov I, Sherry AD, et al. Composition of adipose tissue and marrow fat in humans by 1H NMR at 7T. *J. Lipid Res.* 49: 2055-62 (2008). 3. Ntambi JM. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. *J. Lipid Res.* 40:1549-58 (1999).
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