CLINICAL SIGNIFICANCE OF ADIPOSE TISSUE INVASION IN ER-POSITIVE BREAST CANCER

Qi Liu[1], Zheng Sun[1], Long-long Li[1], Zheng-peng Fu[2], Chun-hong Xu[1]
[1]Department of Breast and Thyroid Surgery, Weifang Traditional Chinese Hospital, Weifang, Shandong, People's Republic of China - [2]Department of Pathology, Weifang Traditional Chinese Hospital, Weifang, Shandong, People's Republic of China

ABSTRACT

Objective*: The aim of this study is to investigate the prognostic significance of adipose tissue invasion in breast cancer patients and its potential value for breast cancer therapy.*

Methods*: A total of 162 patients with early breast cancer were selected from June 2014 to June 2015 in Department of Breast and Thyroid Surgery of Weifang Hospital of Traditional Chinese Medicine. The clinical data of the patients were collected, and the relationship between adipose tissue invasion and clinicopathological factors was analyzed.*

Results*: Among the 162 patients, there were 56 cases of breast cancer patients with adipose tissue invasion and 106 cases without adipose tissue invasion. Univariate analysis showed that tumor histological grade (P=0.041), tumor size (P=0.029), lymph node metastasis (P=0.032), positive ER (P=0.001) and molecular subtype (P=0.01) were related to adipose tissue invasion. In addition, Luminal B was positively correlated with the adipose tissue invasion, while TNBC was negatively correlated with the adipose tissue invasion. Logistic regression analysis showed that tumor histological grade (P=0.014), lymph node metastasis (P=0.003), and positive ER (P=0.044) were significantly correlated with adipose tissue invasion. Patients with adipose tissue invasion (24/56, 42.85%) had a worse prognosis than those without adipose tissue invasion (26/106, 24.58%) at 5-year follow-up (P=0.004).*

Conclusion*: In this study, histological grade, lymph node metastasis and positive ER were found to be associated with adipose tissue invasion of breast cancer, suggesting that adipose tissue invasion is associated with low prognosis of breast cancer and may affect the treatment of ER-positive breast cancer patients.*

Keywords: *Adipose tissue invasion, Breast cancer, Molecular subtype, Estrogen receptor, Prognosis.*

DOI: 10.19193/0393-6384_2022_5_499

Received *January 15, 2022;* ***Accepted*** *June 20, 2022*

Introduction

In recent years, the incidence of breast cancer has been increasing, and its onset age tends to be younger. It is the most common malignant tumor threatening the life and health of women in the world. The treatment methods of breast cancer include surgery, chemotherapy, endocrine therapy, radiotherapy, targeted therapy, etc. However, there are still some patients with recurrence and metastasis, which would affect survival. Obesity is currently recognized as an important factor affecting the prognosis of breast cancer patients, which may be related to the expression of related inflammatory cytokines in adipose tissue[4]. One study has shown that there is a correlation between adipocytes and cancer cells. Cancer cells can activate adipocytes and lead to the up-regulation of expressions of inflammatory factors, tumor necrosis factor, matrix metalloproteinase (MMP) and other adipokines, thus promoting the occurrence and development of tumors. Moreover, adipocytes close to tumors are smaller in size and fewer in number than adipocytes far away from tumor cells[6-8]. For ER (estrogen receptor) and/or PR (progesterone receptor) positive patients, endocrine therapy is the main therapy. Estrogen in postmenopausal women is mainly derived from adipose tissue[3], and obesity

is a high risk factor for recurrence and metastasis of postmenopausal hormone-dependent breast cancer[1-2,5]. Therefore, the purpose of this study is to explore the clinical significance of adipose tissue invasion in the development of breast cancer.

Materials and methods

Case selection

From June 2015 to June 2016, 162 cases of patients with early breast cancer were selected from Department of Breast and Thyroid Surgery of Weifang Hospital of Traditional Chinese Medicine. Their age ranged from 28 to 81 years old, with a median age of 50 years old. All patients were pathologically confirmed to be invasive ductal carcinoma and underwent breast -conserving surgery or modified radical mastectomy or with plastic surgery. After surgery, standard chemotherapy, radiotherapy, endocrine therapy, and targeted therapy were performed according to NCCN guidelines. Patients receiving preoperative neoadjuvant therapy or with stage IV breast cancer, invasive lobular carcinoma, mucinous carcinoma, medullary carcinoma or other pathological types, were excluded.

Stratification of clinicopathological factors

According to the 2018 NCCN guidelines, the diameter of primary tumor (D) was divided into T1 (D ≤2.0cm), T2 (2.0cm < D ≤5.0cm), and T3 (D > 5.0cm). The lymph node metastasis was defined as negative or positive. Positive ER/PR was defined as ≥1% tumor nuclear staining. Positive HER-2 (human epidermal growth factor receptor 2) was defined as standard immunohistochemistry +++, or positive fluorescence in situ hybridization. Ki-67 was classified as low expression (< 20%) and high expression (≥20%) according to the positive index. According to the expression of breast cancer receptor, breast cancer was divided into four molecular subtypes: Luminal A (ER and/or PR positive, HER-2 negative and Ki-67 < 20%); Luminal B (ER and/or PR positive and HER-2 overexpression or amplification or HER-2 negative and Ki-67≥20%); HER-2 overexpression (ER and PR deletion and HER-2 overexpression or amplification); and, Triple-negative breast cancer (TNBC) (negative expression of ER and PR and HER-2).

Adipose tissue invasion

Adipose tissue invasion (18) refers to the direct invasion of adipose tissue by more than 20 cancer cells or interpenetration of cancer cells in adipose tissue. In this study, adipose tissue was defined as a pure aggregation of more than 20 adipocytes, not including the fibrous tissue in the breast. Adipose tissue included ducts or perilobular and subcutaneous tissue of the breast. Fibroadipose tissue (adipose cells mixed with various types of fibrous tissue) was strictly distinct from adipose tissue(Figure 1).

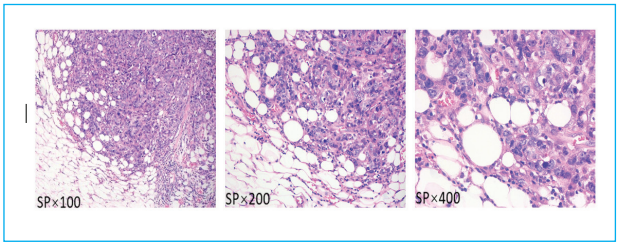

Figure 1:???????

Immunohistochemistry

The samples of breast cancer lesions were fixed with 10% formaldehyde solution, and then cut into 4 μm sections continuously. The sections were dewaxed by xylene and debenzenated by gradient ethanol. After incubating with 3% H2O2, the sections were thoroughly washed with PBS and subjected to antigen retrieval in a microwave oven. ER, PR and HER-2 were repaired with citrate buffer and Ki-67 was repaired with EDTA buffer. After antigen retrieval, the sections were naturally cooled to room temperature, and ER, PR, HER-2 and Ki-67 monoclonal antibodies were added. After PBS rinsing, biotin-labeled secondary antibody was added for incubation at room temperature. Then, the sections were incubated at room temperature with horseradish peroxidase-labeled streptavidin. After thoroughly rinsing with PBS, the sections were developed by DAB, counter-stained with hematoxylin, and mounted with neutral gum. The known positive sections were used as the positive control, and the sections incubated with PBS instead of the primary antibody were used as the negative control. The results were observed and photographed under a microscope.

Statistical method

SPSS 19.0 statistical software was used for analysis. Numeration data were expressed as percentage (%), and analyzed with chi-square test. Binary logistic regression was used to analyze the factors related to adipose tissue invasion. $P < 0.05$ was considered statistically significant.

Results

Relationship between adipose tissue invasion and clinicopathological factors

There were 162 cases of breast cancer in this study, including 56 cases with adipose tissue invasion and 106 cases without adipose tissue invasion. Chi-square test analyzed the relationship between adipose tissue invasion and clinicopathological factors. The results showed that adipose tissue invasion was associated with tumor histological grade (P = 0.041), tumor size (P = 0.029), lymph node metastasis (P = 0.032), positive ER (P = 0.001), and molecular subtype (P = 0.01) (Table 1).

		Adipose tissue				
		Yes	No	Total	X^2	P
Age	<35	4	6	10		
	≥35	52	100	152	0.001	0.976
Menstruation	Postmenopausal	33	52	85		
	Premenopausal	23	54	77	1.432	0.213
Grade	I	1	15	16		
	II	50	84	134		
	III	5	7	12	6.386	0.041
T	≤2cm	16	49	65		
	>2cm	40	57	97	4.754	0.029
N	Positive	24	27	51		
	Negative	33	78	111	4.601	0.032
ER	Positive	48	64	112		
	Negative	8	42	50	11.024	0.001
PR	Positive	39	59	98		
	Negative	17	47	64	2.998	0.083
HER-2	Positive	14	26	40		
	Negative	42	80	122	0.004	0.947
Ki-67	Low expression	17	32	49		
	High expression	39	74	113	0	0.982
Molecular subtype	Luminal A	11	22	33		
	Luminal B	34	39	73		
	Her-2 over-expression	10	20	30		
	TNBC	5	31	36	11.445	0.010
Vascular invasion	Yes	17	33	50		
	No	39	73	112	0.01	0.919
perineuronal invasion	Yes	7	12	19		
	No	49	94	143	0.049	0.824
		56	106	162		

Table 1: Correlation between adipose tissue invasion and clinical factors.

However, adipose tissue invasion was not related to age, menstrual status, PR, HER-2, nerve invasion and vascular invasion (Table 1). Further analysis of molecular subtype revealed that Luminal B was positively correlated with the occurrence of adipose tissue invasion (P=0.014, OR=2.275), while TNBC was negatively correlated with the occurrence of adipose tissue invasion (P=0.003, OR=0.237) (Table 2).

	Adipose tissue invasion						95% confidence interval	
	Yes	No	Total	X^2	P	OR	upper limit	lower limit
Luminal A	11	21	33					
Others	45	84	129	0.003	0.957			
Luminal B	29	34	63					
Others	27	72	79	5.990	0.014	2.275	1.171	4.419
Her-2 overexpression	15	25	40					
Others	41	81	122	0.202	0.653			
TNBC	5	31	36					
Others	51	75	126	8.751	0.003	0.237	0.086	0.651

Table 2: Correlation between molecular subtype and adipose tissue invasion.

Binary Logistic regression multivariate analysis

Logistic regression analysis showed that tumor histological grade (P=0.014), lymph node metastasis (P=.003), and positive ER (P=0.044) were significantly correlated with adipose tissue invasion (Table 3).

						EXP(B) of 95% C.I.	
	B	S.E,	Wals	df	Sig.	Exp (B)	lower limit	upper limit
Age	-.008	.026	.099	1	.754	.992	.943	1.043
Grade	-1.181	.478	6.090	1	.014	.307	.120	.784
Menstruation	.402	.642	.393	1	.531	1.495	.425	5.258
T	.225	.151	2.222	1	.136	1.253	.931	1.685
N	1.376	.469	8.606	1	.003	3.960	1.579	9.931
ER	2.132	1.058	4.057	1	.044	8.428	1.059	67.071
PR	-.423	.605	.489	1	.485	.655	.200	2.144
HER2	-.832	.567	2.152	1	.142	.435	.143	1.323
Ki-67	.017	.014	1.428	1	.232	1.017	.989	1.047
Molecular subtype	-.231	.477	.234	1	.628	.794	.312	2.022
Perineuronal invasion	-.573	.567	1.020	1	.312	.564	.185	1.714
Vascular invasion	-.018	.419	.002	1	.966	.982	.432	2.234

Table 3: Survival analysis of adipose tissue invasion after follow-up for 5 years.

All patients were followed up for 5 years. Among 56 patients with adipose tissue invasion, 24 patients (42.85%) had recurrence or metastasis, while in 106 patients without adipose tissue invasion, 26 patients (24.58%) had recurrence or metastasis (P = 0.004).

Discussion

Obesity is a recognized risk factor associated with the occurrence and development of cancer, including breast cancer, endometrial cancer,

esophageal cancer, etc[9]. Breast cancer is also recognized as a malignant tumor closely related to adipose tissue. Adipose tissue mainly includes adipocytes, fibroblasts, and endothelial cells. Adipose tissue is distributed throughout the body. It not only stores excessive energy in the body, but also acts as an endocrine organ, secreting a variety of adipokines and cytokines, and participating in tumor progression and metastasis[10-11]. Studies have shown that there is an interaction between breast cancer tissue and precancerous adipose tissue. Compared with those in the normal microenvironment, adipocytes in the tumor microenvironment have a lower degree of differentiation, and mature adipocytes lose their normal function and can promote the growth of tumor cells[12-13].

Previous study evaluated the relationship between CT attenuation of adjacent breast adipose tissue and breast cancer prognosis, and the results showed that patients with enhanced CT attenuation of adjacent breast adipose tissue had a poor survival rate[14]. TAT HU and HU difference were independent predictors for RFS in patients with breast cancer[14]. Some study also quantified the fat around breast cancer tumor tissue by breast MRI, and found that in patients with early breast cancer, the fat around the tumor was positively correlated with the proportion of pathologically involved axillary lymph nodes[15].

The correlation between tumor and adipose tissue was also evaluated by mammography, and tumors with burr sign in mammography were correlated with adipose invasion[16]. Consistently, the results of this study showed that adipose tissue invasion was positively correlated with lymph node metastasis and histological grade, and the prognosis of breast cancer patients with adipose tissue invasion was worse. Other study[17] has shown that adipose tissue invasion was not associated with lymph node metastasis, tumor grade, tumor size, and menopause, but was related to the metastatic outcome of breast cancer patients. However, these studies suggest that adipocyte phenotypes around breast cancer tumors are altered, and adipocyte invasion, like vascular invasion, is also an important predictor of prognosis in breast cancer patients[18].

Another important finding in this study was that adipose tissue invasion was positively correlated with ER; Luminal B was also positively correlated with the occurrence of adipose tissue invasion; while, TNBC was negatively correlated with the occurrence of adipose tissue invasion. Estrogen is involved in a variety of physiological processes of human growth and development, and the positive rate of ER is about 50-80% in breast cancer[19]. Estrogen is mainly from ovarian in premenopausal women. In postmenopausal women, ovarian estrogen can be neglected and estrogen is mainly derived from adipose tissue. The Cytochrome P450 aromatase (P450arom) is a kind of microsomal enzyme, which is involved in the process from the 19 carbon steroid (androgens, such as androstenedione and testosterone) to 18 carbon steroid (estrogen, such as estradiol and estradiol), one of the last step of estrogen biosynthesis[20]. In patients with ER and/or PR positive breast cancer, the growth pattern was hormone-dependent. Endocrine therapy is the main treatment after the resection of hormone receptor positive breast cancer. Tamoxifen is the treatment for premenopausal women. Ovarian function inhibition combined with aromatase inhibitors in the treatment for premenopausal women with high risk factors, and aromatase inhibitors are also used for the treatment of breast cancer in postmenopausal women. Aromatase inhibitors are the drugs that block the action of aromatase to reduce estrogen levels in the body.

There are two types of aromatase inhibitors: irreversible steroidal inhibitors such as exemestane, and non-steroidal inhibitors such as anastrozole and letrozole, all of which have been approved by the FDA. Aromatase is hypothesized to be a major driver of postmenopausal estrogen-dependent breast cancer in adipose tissue. In postmenopausal women, obesity and weight gain are associated with a significantly increased risk of ER+/PR+ breast cancer, partly due to increased local estrogen production by breast adipose tissue[21]. This result was consistent with the results of Moriuchi H et al., which found that compared with other types of cancer (such as basal-like tumors), Luminal breast cancer seemed to show more interactions with stromal tissue[16]. Therefore, we also suppose that adipose tissue invasion may also affect endocrine therapy for ER positive breast cancer, but further studies are needed to verify this assumption.

In conclusion, the results of our study demonstrate that adipose tissue invasion is an important factor affecting the prognosis of breast cancer, and it is significantly correlated with ER-positive and Luminal type, which suggests that endocrine therapy of hormone receptor-positive breast cancer may be affected.

References

1) NaNa, Keum, Darren, et al. Adult Weight Gain and Adiposity-Related Cancers: A Dose-Response Meta-Analysis of Prospective Observational Studies(J). Jnci Journal of the National Cancer Institute, 2015.
2) Chen G C, Chen S J, Zhang R, et al. Central obesity and risks of pre-and postmenopausal breast cancer: a dose-response meta-analysis of prospective studies(J). Obesity Reviews, 2016.
3) Brown KA . Obesity and Breast Cancer: Mechanisms and therapeutic implications(J). Front Bio, 2012, 4(7): 2515-2524.
4) CoradiniD, Gambazza S, Oriana S, et al.Gene expression profile of normal breast tissue and body mass index.(J).Breast Cancer. 2020 Nov 13. DOI: 10.1007/s12282-020-01183-z. Online ahead of print
5) Park J W, Han K, Shin D W, et al. Obesity and breast cancer risk for pre-and postmenopausal women among over 6 million Korean women(J). Breast Cancer Research and Treatment, 2020: 1-12.
6) Nieman KM, Romero IL, Van Houten B, Lengyel E (2013) Adipose tissue and adipocytes support tumorigenesis and metastasis. Biochim Biophys Acta Mol Cell Biol Lipids 1831: 1533-1541
7) Divella R, De Luca R, Abbate I et al (2016) Obesity and cancer: the role of adipose tissue and adipocytokines-induced chronic inflammation. J Cancer 7: 2346-2359.
8) Nieman KM, Kenny HA, Penicka CV et al (2011) Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. Nat Med 17: 1498-1503.
9) Nana K, Greenwood DC, Hoon L D, et al. Adult Weight Gain and Adiposity-Related Cancers: A Dose-Response Meta-Analysis of Prospective Observational Studies(J). J Natl Cancer Inst, 2015(3): 3.
10) Duong M N , Geneste A , Fallone F, et al. The fat and the bad: Mature adipocytes, key actors in tumor progression and resistance.(J). Oncotarget, 2017, 8(34).
11) Guaita-EsteruelasS, Gumà, J, Masana L, et al. The peritumoral adipose tissue microenvironment and cancer. The roles of fatty acid-binding protein 4 and fatty acid-binding protein 5(J). Molecular & Cellular Endocrinology, 2017: S0303720717300783.
12) Engin A B,Engin A, Gonul I I. The effect of adipocyte-macrophage cross-talk in obesity-related breast cancer(J). Journal of Molecular Endocrinology, 2019, 62(3).
13) Fletcher S J, Sacca P A, Pistone-Creydt M, et al. Human breast adipose tissue: characterization of factors that change during tumor progression in human breast cancer(J). Journal of Experimental & Clinical Cancer Research, 2017, 36(1): 26.
14) Lee J W, Kim S Y, Lee H J, et al. Prognostic Significance of CT-Attenuation of Tumor-Adjacent Breast Adipose Tissue in Breast Cancer Patients with Surgical Resection(J). Cancers, 2019, 11(8).
15) J.-P. Obeid, R. Stoyanova, D. Kwon, et al. Multiparametric evaluation of preoperative MRI in early-stage breast cancer: prognostic impact of peri-tumoral fat(J). Clinical & Translational Oncology, 2017.
16) MoriuchiH, Yamaguchi J, Hayashi H, et al. Cancer Cell Interaction with Adipose Tissue: Correlation with the Finding of Speculation at Mammography(J). Radiology, 2016: 56.
17) Khalil A A,Ilina O, Gritsenko PG, et al. Collective invasion in ductal and lobular breast cancer associates with distant metastasis(J). Clinical & Experimental Metastasis, 2017.
18) JunzoY, Hiroshi O, Kazukuni N, et al. Prognostic impact of marginal adipose tissue invasion in ductal carcinoma of the breast.(J). American Journal of Clinical Pathology, 2008(3): 382-388.
19) Wittliff J L . Steroid-hormone receptors in breast cancer.(J). Cancer, 2015, 53(S3): 630-643.
20) SibyS, Bulun S E. A highly complex organization of the regulatory region of the human CYP19 (aromatase) gene revealed by the Human Genome Project.(J). Journal of Clinical Endocrinology &Metabolism(10): 4600-2.
21) Munsell M F, Sprague B L, Berry D A, et al. Body Mass Index and Breast Cancer Risk According to Postmenopausal Estrogen-Progestin Use and Hormone Receptor Status(J). Epidemiologic Reviews(1): 114.

Corresponding Author:
Chun-hong Xu
Department of Breast and Thyroid Surgery, Weifang Traditional Chinese Hospital, 1055 Weizhou Road, Weifang 261000, Shandong, People's Republic of China
Email: xuch0624@163.com
(China)