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Original Article

Overexpression of the fat mass and obesity associated gene (FTO) in breast cancer and its clinical implications

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Abstract: Background and purpose: Incidence of breast cancer is increasing and seems to be associated with fatty foods, metabolism, and so on. The fat mass and obesity associated gene (FTO) has been intensively investigated in diabetes, obesity and the other diseases. Previous studies have reported that FTO SNPs are associated with breast cancer risk. Here, we investigated the expression of FTO in human breast cancer tissues and its relationship with the clinicopathological features. Methods: In this retrospective study, tissues from 79 patients with breast cancer were collected, as well as 43 cases of adjacent breast tissues. Immunohistochemistry was used to detect the expression of FTO. Statistical analysis was performed to assess the association between FTO expression and the clinicopathological features of breast cancer. Results: FTO was expressed in both mammary epithelial and breast cancer tissues, but with different degree. The expression level of FTO in breast cancer tissues was significantly higher than that in the adjacent breast tissues ($P < 0.001$). The percentage of FTO-positive expression in cases with hormone receptor (HR) negative and HER2 amplification was significantly higher than that in those with HR positive and HER2 negative ($P = 0.001$, $P < 0.001$). The positivity rate of FTO in breast cancer with P53 positive and histological grade 3 seemed to be higher than that with P53 negative and histological grade 1 or 2, respectively ($P = 0.077$, $P = 0.082$). There was no association between FTO expression and age, T stage, LN status, TNM stage, Ki67, and BMI in breast cancer. Besides, FTO expression in HER2-overexpressed subtype was significantly higher than that in Triple-negative and Luminal A/B1 subtypes ($P < 0.001$). Conclusion: Our study suggests that FTO expression may have a vital role in the carcinogenesis of breast cancer, especially in HER2-overexpressed breast cancer.

Keywords: FTO expression, breast cancer, immunohistochemistry

Introduction

Breast cancer is the most commonly diagnosed cancer in women and is the leading cause of mortality among women after lung cancer [1]. Obesity has been reported to increase breast cancer risk [2, 3] and there is evidence that weight loss, as well as decrease in fat consumption may lead to decreased risk for breast cancer [4, 5].

The fat mass and obesity associated protein (FTO) is an AlkB-like 2-oxoglutarate-dependent nucleic acid demethylase with a strong preference for 3-methylthymidine and 3-methyluracil in single-stranded DNA and RNA [6]. The FTO gene is a novel gene and SNPs of FTO have been identified through genome wide association studies (GWAS) to be associated with an increased risk of obesity [7, 8]. Homozygous loss-of-function of FTO was reported to cause severe growth retardation and multiple malformations [9], whereas a duplication of FTO was found to be associated with morbid obesity [10]. Given the strong association of obesity with breast cancer, several studies previously examined the relationship between *FTO* gene polymorphism and the incidence of breast cancer [11, 12]. And the recent study reported the association of rs17817449 (MIR1972-2: FTO) with the mammographic density measures [13].

So far, the expression pattern of FTO protein in breast cancer and its association with clinical

Table 1. Expression of FTO in different types of breast tissue

Breast tissue	N	FTO staining n (%)		*P value*
		Low expression	High expression	
Breast cancer	79	17 (21.5)	62 (78.5)	< 0.001
Para-carcinoma tissue	43	31 (72.1)	12 (27.9)	

pathological parameters remains obscure. Therefore, the aim of this study was to investigate expression of FTO protein in tumor tissues, as well as its potential association with clinical parameters.

Patients and methods

Patients

This retrospective study included 79 consecutive patients (all women) with invasive ductal carcinoma of breast at the First Affiliated Hospital, Guangxi Medical University, China between January 2012 and December 2013, 43 patients in whom juxta-tumor tissues were obtained. The mean age of patients was 50.2±10.1 years (ranged from 30 to 76 years). Among the 79 patients, 60were ER negative and 19 ER positive, 64 were PR negative and 15 PR positive, 44 were Her-2 negative and 35 Her-2 positive. The juxta-tumor tissue was obtained at least 2 cm from the site of breast cancer. All tissues used were from mastectomy without any cancer treatment before. And written informed consent to use the samples for research was obtained from the patients and clinicians. The histopathological diagnoses were made according to the 2003 WHO International Histological Classification of Breast Cancer [14]: for histological grade, 4 were grade I, 48 grade II, and 14 grade III; for pathological stage, 12 were stage I, 36stage II, and 31 stage III.

Tissue histology and immunohistochemistry

Paraffin-embedded sections on polylysine-coated slides were used for staining. Sections were cut at 4 μm. Immunohistochemical staining was performed with Envision™ two steps. Slides were baked at 65°C for 2 hours, then deparaffinized in xylene and rehydrated in a grade alcohol series. Antigen retrieval was achieved by micromaving in EDTA at PH 9 for 2 min. Endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol for 10 min, and samples were washed with phosphate buffered saline (PBS) (3×5 min). Rabbit monoclonal to FTO (EPR6895, American Abcam Biological Technology Co. LTD.,) was diluted to 1:100 and incubated for 1 hour in a humidity chamber at 37°C. After the period of incubation, samples were washed with PBS (3×5 min) and the horseradish peroxidase (HRP) tagged secondary antibody was then added. The sample was incubated for 30 min at room temperature, and then washed with PBS (3×5 min). Visualization was performed using DAB chromogen for 5-10 min. Control sections were incubated with PBS instead of primary antibody. Sections were counterstained with hematoxylin, dehydrated, and mounted.

The staining results were evaluated according to the immunodetection of stain intensity and number of positive cells [15]. One hundred cells from 5 representative areas from each case were counted. The degree of staining was subdivided as follows: 0, No staining; 1, Focal or fine granular, weak staining; 2, Linear or cluster, strong staining; 3, Diffuse, intense staining. The positive cells in the observed tissue ranged from 0 to 3 in percentage: 0, No staining; 1, < 30%; 2, 30%-70%; 3, > 70%. The samples were categorized as positive and negative based on the sum of the scores as follows: 0-1, (-); 2-3, Positive (+); 4, Positive (++); 5-6, Positive (+++). Positive (++ or +++) was considered as high expression. All cases were reviewed by two pathologists who were blinded.

Statistical analyses

The Fisher exact test was used to compare overexpression of FTO among different groups with SPSS 16.0 for Windows. A value of $P < 0.05$ was considered statistically significant.

Results

Expression of FTO in different breast tissue

FTO was expressed in both mammary epithelial and breast cancer tissues, but with different degree. In noncancerous tissue, FTO expression was detected in part of epithelial cells and mainly in the nuclei. While in the tumorous tis-

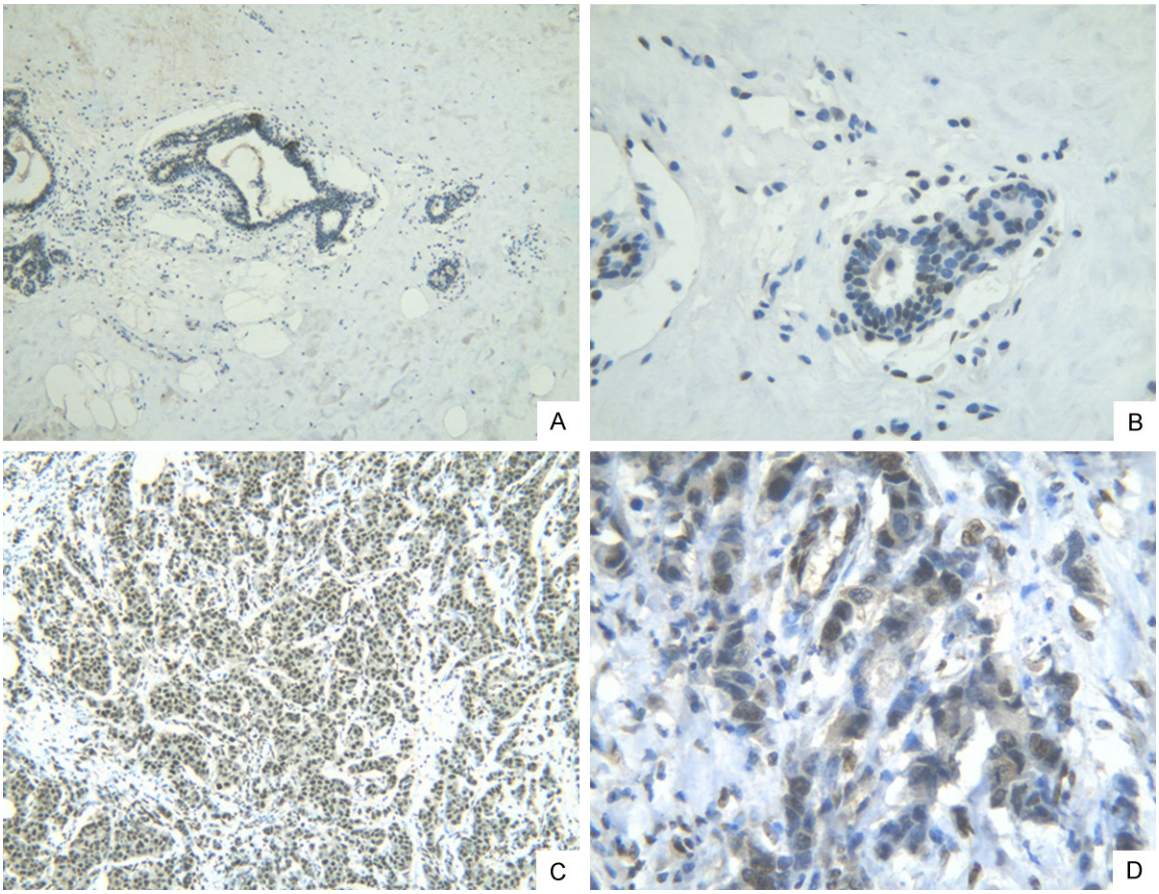

Figure 1. Expression patterns of FTO in mammary epithelial and breast cancer. Mammary epithelial, A. 10×10, B. 10×40; Breast cancer, C. 10×10, D. 10×40. Immunohistochemical staining.

sues, FTO expression was detected in both nuclei and cytoplasm. The intensity of tissue staining for FTO in breast cancer was significantly higher than that in juxta-tumor tissue (78.5% vs. 27.9%, $P < 0.001$) (**Table 1** and **Figure 1**).

Relationship between FTO expression and clinicopathological features in breast cancer

The percentage of FTO-positive expression in cases with hormone receptor (HR) negative (89.3%) and HER2 amplification (97.2%) was significantly higher than that in those with HR positive (52.2%) and HER2 negative (63.7%), respectively ($P = 0.001$, $P < 0.001$). The positivity rate of FTO in breast cancer with P53 positive (90.9%) and histological grade 3 (100%) seemed to be higher than that with P53 negative (73.8%) and histological grade 1 or 2 (75%), respectively, however, the *P* value did not reach the significant level ($P = 0.077$, $P = 0.082$). There was no association between FTO expression and age, T stage, LN status, TNM stage, Ki67, and BMI in breast cancer (**Table 2**).

FTO expression in molecular subtypes of breast cancer

The expression of FTO in HER2-overexpressed subtype (97.1%) was significantly higher than that in Triple-negative (76.2%) and Luminal A/B1 subtypes (52.2%) ($P < 0.001$) (**Table 3**).

Discussion

Obesity is a well-established risk factor for breast cancer [16, 17], and several genes associated with obesity have been intensively investigated in the study of carcinogenesis. Among the genes present in obesity susceptibility loci is the FTO gene, which locates in chromosome 16q12.2. Recently, FTO was reported as a susceptibility marker of breast cancer [18]. The

Table 2. Relationship between FTO expression and clinicopathological features in breast cancer

Variables	N	FTO staining n (%)		*P value*
		Low expression	High expression	
Age (years)				
≤ 50	42	9 (21.4)	33 (78.6)	0.98
> 50	37	8 (21.6)	29 (78.3)	
BMI (kg/m^2)				
< 24.0	48	8 (16.7)	40 (83.3)	0.551
≥ 24.0	28	7 (25.0)	21 (75.0)	
Histological grade				
1	4	1 (25.0)	3 (75.0)	0.082
2	48	12 (25.0)	36 (75.0)	
3	14	0 (0.0)	14 (100.0)	
T stage				
T1	14	1 (7.1)	13 (92.9)	0.389
T2	46	11 (23.9)	35 (76.1)	
T3-4	19	5 (26.3)	14 (73.7)	
LN status				
Negative	34	5 (14.7)	29 (85.3)	0.272
Positive	45	12 (26.6)	33 (73.4)	
ER and/or PR				
Negative	56	6 (10.7)	50 (89.3)	0.001
Positive	23	11 (47.8)	12 (52.2)	
HER-2				
No amplification	44	16 (36.4)	28 (63.7)	< 0.001
Amplification	35	1 (2.9)	34 (97.2)	
TNM stage				
I-II	48	7 (14.6)	41 (85.5)	0.092
III	31	10 (32.2)	21 (67.8)	
Ki-67(%)				
≤ 20	9	3 (33.3)	6 (66.6)	0.396
> 20	70	14 (20.0)	56 (80.0)	
P53				
Negative	42	11 (26.2)	31 (73.8)	0.077
Positive	33	3 (9.1)	30 (90.9)	

Table 3. FTO expression in molecular subtypes

IHC-based molecule subtype	N	FTO staining n (%)		*P value*
		Low expression	High expression	
Luminal A or B1	23	11 (47.8)	12 (52.2)	< 0.001
HER-2 overexpression	35	1 (2.9)	34 (97.1)	
Triple-negative	21	5 (23.8)	16 (76.2)	

FTO protein is expressed in various tissues, including breast tissue, which encodes a 2-oxoglutarate-dependent nucleic acid demethylase [19]. A previous study demonstrated that loss of the FTO gene protects individuals from obesity due to increased energy expenditure and reduced adipose tissue [20]. And another study found that overexpression of FTO led to increased food intake and causes obesity in mice [21]. The issue of whether FTO is involved in carcinogenesis has also been explored. Several studies reported that FTO SNPs were associated with breast cancer risk [12, 18, 22]. Kaklamani et al [18] also reported the expression of FTO in normal and breast carcinoma tissues. However, the study sample was small and limited to the American people. The FTO protein expression in breast cancer tissue and relationship with pathological parameters in Chinese women is still unclear. In the present study, we examined 79 breast cancer specimens from Chinese women and found FTO to be overexpressed in breastcarcinoma compared with paracarcinoma normal breast tissue. The fact that FTO is expressed in human breast cancer tissue as well as its role in nucleic acid demethylation may point toward a direct effect of FTO in breast cancer.

Another interesting observation was that the expression of FTO was related to the clinical and pathological features. In our study, the percentage of FTO-positive expression in cases with HR negative and Her-2 amplification was significantly higher than in those with HR positive and Her-2 negative. These results were inconsistent with the study reported by Kaklamani et al [18], which showed no significant difference in tumor FTO expression in ER+ vs. ER-, PR+ vs. PR- or Her2+ vs. Her2- cancers. The discrepancy may be due to the difference in race and patient size. Furthermore, the positivity rate of FTO in breast cancer with P53 positive and histological grade 3 seemed to be higher than that with P53 negative and histological grade 1 or 2. The P53 is a tumor suppressor gene encoding for a nuclear phosphoprotein thought to regulate proliferation of normal cells. *P53* mutations result in a nonfunctional protein that accumulates in tumor cell nuclei and appears to be involved in the development and/or progression of several neoplastic dis-

eases including human breast cancer [23]. The relationship of FTO with P53 implied that FTO might be involved in the development and/or progression of breast cancer. In addition, the expression of FTO was significantly higher in HER2-overexpression subtype than that in triple-negative and luminal A/B1 subtypes. This study is the first to show that FTO expression correlates with breast cancer specific subtypes.

Besides, we also explored the relationship between FTO expression and BMI in breast cancer patients because of the strong association of FTO with obesity. Our study found no significant difference in FTO expression between cases with BMI < 24.0 kg/m^2 and BMI ≥ 24.0 kg/m^2. However, it should be noted that our study sample is small and cancer is one class of consumptive disease. This result may be affected by these factors.

In conclusion, our study suggests that FTO expression may have an essential role in the carcinogenesis of breast cancer, and a relationship with the development and aggressiveness of breast cancer, especially in HER2-overexpression breast cancer. Further large studies are needed to figure out the relationship of FTO expression with breast cancer and to explore the mechanisms underlying the relationship.

Disclosure of conflict of interest

None.

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References

[1] Siegel R, ND, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013; 63: 11-30.

[2] Wolk A, Gridley G, Svensson M, Nyrén O, McLaughlin JK, Fraumeni JF, Adam HO. A prospective study of obesity and cancer risk (Sweden). Cancer Causes Control 2001; 12: 13-21.

[3] Huang Z, Hankinson SE, Colditz GA, Stampfer MJ, Hunter DJ, Manson JE, Hennekens CH, Rosner B, Speizer FE, Willett WC. Dual effects of weight and weight gain on breast cancer risk. JAMA 1997; 278: 1407-11.

[4] Harvie M, Howell A, Vierkant RA, Kumar N, Cerhan JR, Kelemen LE, Folsom AR, Sellers TA. Association of gain and loss of weight before and after menopause with risk of postmenopausal breast cancer in the Iowa women's health study. Cancer Epidemiol Biomarkers Prev 2005; 14: 656-61.

[5] Prentice RL, Caan B, Chlebowski RT, Patterson R, Kuller LH, Ockene JK, Margolis KL, Limacher MC, Manson JE, Parker LM, Paskett E, Phillips L, Robbins J, Rossouw JE, Sarto GE, Shikany JM, Stefanick ML, Thomson CA, Van Horn L, Vitolins MZ, Wactawski-Wende J, Wallace RB, Wassertheil-Smoller S, Whitlock E,Yano K, Adams-Campbell L, Anderson GL, Assaf AR, Beresford SA, Black HR, Brunner RL, Brzyski RG, Ford L, Gass M, Hays J, Heber D, Heiss G, Hendrix SL, Hsia J, Hubbell FA, Jackson RD, Johnson KC, Kotchen JM, LaCroix AZ, Lane DS, Langer RD, Lasser NL, Henderson MM. Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA 2006; 295: 629-642.

[6] Gerken T, Girard CA, Tung YC, Webby CJ, Saudek V, Hewitson KS, Yeo GS, McDonough MA, Cunliffe S, McNeill LA, Galvanovskis J, Rorsman P, Robins P, Prieur X, Coll AP, Ma M, Jovanovic Z, Farooqi IS, Sedgwick B, Barroso I, Lindahl T, Ponting CP, Ashcroft FM, O'Rahilly S, Schofield CJ. The obesity-associated FTO gene encodes a 2-oxoglutaratedependent nucleic acid demethylase. Science 2007; 318: 1469-1472.

[7] Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007; 316: 889-894.

[8] Qi Q, Kilpeläinen TO, Downer MK, Tanaka T, Smith CE, Sluijs I, Sonestedt E, Chu AY, Renström F, Lin X, Ängquist LH, Huang J, Liu Z, Li Y, Asif Ali M, Xu M, Ahluwalia TS, Boer JM, Chen P, Daimon M, Eriksson J, Perola M, Friedlander Y, Gao YT, Heppe DH, Holloway JW, Houston DK, Kanoni S, Kim YM, Laaksonen MA, Jääskeläinen T, Lee NR, Lehtimäki T, Lemaitre RN, Lu W, Luben RN, Manichaikul A, Männistö S, Marques-Vidal P, Monda KL, Ngwa JS, Perusse L, van Rooij FJ, Xiang YB, Wen W, Wojczynski MK, Zhu J, Borecki IB, Bouchard C,

Cai Q, Cooper C, Dedoussis GV, Deloukas P, Ferrucci L, Forouhi NG, Hansen T, Christiansen L, Hofman A, Johansson I, Jørgensen T, Karasawa S, Khaw KT, Kim MK, Kristiansson K, Li H, Lin X, Liu Y, Lohman KK, Long J, Mikkilä V,Mozaffarian D, North K, Pedersen O, Raitakari O, Rissanen H, Tuomilehto J, van der Schouw YT, Uitterlinden AG, Zillikens MC, Franco OH, Shyong Tai E, Ou Shu X, Siscovick DS, Toft U, Verschuren WM, Vollenweider P, Wareham NJ, Witteman JC, Zheng W, Ridker PM, Kang JH, Liang L, Jensen MK, Curhan GC,Pasquale LR, Hunter DJ, Mohlke KL, Uusitupa M, Cupples LA, Rankinen T, Orho-Melander M, Wang T, Chasman DI, Franks PW, Sørensen TI, Hu FB, Loos RJ, Nettleton JA, Qi L. FTO genetic variants, dietary intake and body mass index: insights from 177,330 individuals. Hum Mol Genet 2014; 23: 6961-6972.

[9] Boissel S, Reish O, Proulx K, Kawagoe-Takaki H, Sedgwick B, Yeo GS, Meyre D, Golzio C, Molinari F, Kadhom N, Etchevers HC, Saudek V, Farooqi IS, Froguel P, Lindahl T, O'Rahilly S, Munnich A, Colleaux L. Loss-of-function mutation in the dioxygenaseencoding FTO gene causes severe growth retardation and multiple malformations. Am J Hum Genet 2009; 85: 106-111.

[10] van den Berg L, de Waal HD, Han JC, Ylstra B, Eijk P, Nesterova M, Heutink P, Stratakis CA. Investigation of a patient with a partial trisomy 16q including the fat mass and obesity associated gene (FTO): fine mapping and FTO gene expression study. Am J Med Genet A 2010; 152A: 630-637.

[11] Kaklamani V, Yi N, Sadim M, Siziopikou K, Zhang K, Xu Y, Tofilon S, Agarwal S, Pasche B, Mantzoros C. The role of the fat mass and obesity associated gene (FTO) in breast cancer risk. BMC Medical Genetics 2011; 12: 52.

[12] Zhang B, Li Y, Li L, Chen M, Zhang C, Zuo XB, Zhou FS, Liang B, Zhu J, Li P, Huang ZL, Xuan H, Li W, Chen ZD. Association study of susceptibility loci with specific breast cancer subtypes in Chinese women. Breast Cancer Res Treat 2014; 146: 503-514.

[13] Stone J, Thompson DJ, Dos Santos Silva I, Scott C, Tamimi RM, Lindstrom S, Kraft P, Hazra A, Li J, Eriksson L, Czene K, Hall P, Jensen M, Cunningham J, Olson JE, Purrington K, Couch FJ, Brown J, Leyland J, Warren RM, Luben RN, Khaw KT, Smith P, Wareham NJ, Jud SM, Heusinger K, Beckmann MW, Douglas JA, Shah KP, Chan HP, Helvie MA, Le Marchand L, Kolonel LN, Woolcott C, Maskarinec G, Haiman C, Giles GG, Baglietto L, Krishnan K, Southey MC, Apicella C, Andrulis IL, Knight JA, Ursin G, Alnaes GI, Kristensen VN, Borresen-Dale AL, Gram IT, Bolla MK, Wang Q, Michailidou K, Dennis J, Simard J, Pharoah P, Dunning AM, Easton DF, Fasching PA, Pankratz VS, Hopper JL, Vachon CM. Novel associations between common breast cancer susceptibility variants and risk-predicting mammographic density measures. Cancer Res 2015; 75: 2457-67.

[14] Böcker W. WHO classification of breast tumors and tumors of the female genital organs: pathology and genetics Verh Dtsch Ges Pathol 2002; 86: 116-119.

[15] Li H, Zhang L, Lou H, Ding I, Kim S, Wang L, Huang J, Di Sant'Agnese PA, Lei JY. Overexpression of decoy receptor 3 in precancerous lesions and adenocarcinoma of the esophagus. Am J Clin Pathol 2005; 124: 282-287.

[16] Huang Z, Hankinson SE, Colditz GA, Stampfer MJ, Hunter DJ, Manson JE, Hennekens CH, Rosner B, peizer FE, Willett WC. Dual effects of weight andweight gain on breast cancer risk. JAMA 1997; 278: 1407-1411.

[17] Trentham-Dietz A, Newcomb PA, Storer BE, Longnecker MP, Baron J, Greenberg ER, Willett WC. Body size and risk of breast cancer. Am J Epidemiol 1997; 145: 1011-1019.

[18] Kaklamani V, Sadim M, Siziopikou K, Zhang K, Xu Y, Tofilon S, Agarwal S, Pasche B, Mantzoros C. The role of the fat mass and obesity associated gene (FTO) in breast cancer risk. BMC Med Genet 2011; 12: 52.

[19] Gerken T, Girard CA, Tung YC, Webby CJ, Saudek V, Hewitson KS, Yeo GS, McDonough MA, Cunliffe S, McNeill LA, Galvanovskis J, Rorsman P, Robins P, Prieur X, Coll AP, Ma M, Jovanovic Z, Farooqi IS, Sedgwick B, Barroso I, Lindahl T, Ponting CP, Ashcroft FM, O'Rahilly S, Schofield CJ. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 2007; 318: 1469-1472.

[20] Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Brüning JC, Rüther U. Inactivation of the Fto gene protects from obesity. Nature 2009; 458: 894-898.

[21] Church C, McMurray F, Girard C, Banks GT, Teboul L, Wells S, Brüning JC, Nolan PM, Ashcroft FM, Cox RD. Overexpression of Fto leads to increased food intake and results in obesity. Nat Genet 2010; 42: 1086-1092.

[22] Hernández-Caballero ME, Sierra-Ramírez JA. Single nucleotide polymorphisms of the FTO gene and cancer risk: an overview. Mol Biol Rep 2015; 42: 699-704.

[23] Allred DC, Elledge R, Fuqua SA, Brown RW, Chamness GC, Osborne CK, McGuire WL. Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. J Natl Cancer Inst 1993; 85: 200-206.