Int J Clin Exp Med 2020;13(10):7873-7880
www.ijcem.com /ISSN:1940-5901/IJCEM0117475

Original Article

High expression levels of RUNX2 and HER2 indicate a poor prognosis in breast cancer tissues with calcification

Xuemin Liu[1], Yunsuo Zhang[2]

[1]*Department of General Surgery, Zhangjiagang TCM Hospital Affiliated to Nanjing University of Chinese Medicine, Zhangjiagang, Jiangsu Province, China;* [2]*Department of Breast Surgery, Zhenjiang No. 4 People's Hospital, Zhenjiang, Jiangsu Province, China*

Received July 3, 2020; Accepted July 28, 2020; Epub October 15, 2020; Published October 30, 2020

Abstract: Objective: To study the expressions of Runt-related transcription factor 2 (RUNX2) and human epidermal growth factor receptor 2 (HER2) in breast cancer tissues with calcification and their correlation with clinical features and prognosis. Methods: A total of 712 breast cancer patients were selected for this prospective study, including 320 breast cancer patients with calcified foci (Calcification group) and 392 breast cancer patients without (Non-calcification group). The expression levels of RUNX2 and HER2 were detected and quantified by immunohistochemical staining and qRT-PCR after surgery. Furthermore, the correlation between their expression level and the clinical features and prognosis of patients with breast cancer with calcification were analyzed. Results: The positive rates of HER2 and RUNX2 expression in breast cancer patients with calcification were significantly higher than those without ($P<0.05$). High grade; T category, M category, and N category were associated with the high mRNA expression levels of RUNX2 and HER2 ($P<0.05$). The overall survival and disease-free survival of patients with positive expression of RUNX2 and HER2 was notably lower than those of patients with negative expression ($P<0.05$). Conclusion: RUNX2 and HER2 were highly expressed in breast cancer patients with calcification, and as such they may be good biomarkers indicting a poor prognosis for patients with breast cancer.

Keywords: Breast cancer, calcification, Runt-related transcription factor 2, human epidermal growth factor receptor 2, clinical features, prognosis

Introduction

Breast cancer is one of the most common malignant tumors which threatens female health, it accounts for 11.4% of all newly diagnosed tumors and 6.6% of all death cases [1-3]. It has been reported that the incidence of breast cancer rises every year and patients trend to be young adults [4, 5]. Calcification is a common feature in breast disease, and about 42.8% of breast cancer patients are diagnosed with calcification [6]. Studies have shown that breast calcification is an important imaging indicator for the diagnosis of breast cancer and one of the risk factors of breast cancer progression [7-9]. Chemotherapy has shown an effect on calcified foci in breast cancer patients with calcification, and whether the calcified foci shrink or not after chemotherapy affects the prognosis of patients [10].

Runt-related transcription factor 2 (RUNX2) is a tissue-specific transcription factor found in bone, it is important in bone metabolism and participates in the formation of tissue calcification [11]. Recent studies have revealed that RUNX2 overexpression in tumors is closely related to tumor progression [12, 13]. For example, RUNX2 is highly expressed in breast cancer tissue and plays an important role in bone metastasis in breast cancer [14], suggesting that high expression of RUNX2 promotes breast cancer metastasis and affects prognosis. However, the relationship between the expression level of RUNX2 and clinical-pathological characteristics and the prognosis is still unclear, which makes it controversial whether RUNX2 can be used as a molecular marker for the diagnosis, treatment and prognosis in cancer research [15].

Table 1. General and baseline data analysis between the calcification group and non-calcification group

Categories	Calcification group (n=320)	Non-calcification group (n=392)	χ^2	P
Age (Year)				
<50	120	172	2.962	0.085
≥50	200	220		
BMI (kg/m^2)	22.99±2.03	23.27±2.13	1.782	0.075
Pathological type				
Insitu tumor	258	236	34.585	<0.001
Invasive tumor	62	156		
Tumor size (cm)				
>3 cm	104	136	0.379	0.583
≤3 cm	216	256		
TNM staging				
I-II	216	320	18.910	<0.001
III-IV	104	72		
T category				
T1-T2	200	304	19.300	<0.001
T3-T4	120	88		
N category				
N0	204	324	32.849	<0.001
N1	116	68		
M category				
M0	272	362	9.749	0.002
M1	48	30		
Comorbidities				
Hypertension	90	113	0.043	0.837
Type 2 diabetes	78	89	0.274	0.601
Hyperlipidemia	76	90	0.062	0.804

Note: BMI: Body mass index; TNM: tumor node metastasis.

Previous studied have demonstrated that the expression level of human epidermal growth factor receptor 2 (HER2), a tumor marker, can predict the recurrence and prognosis of breast cancer [16]. Therefore, we conducted this long-term follow-up study to investigate the relationship between the expression of RUNX2 and HER2 and the clinical-pathological characteristics as well as the prognosis of breast cancer patients with calcification.

Materials and methods

General data

Seven hundred and twelve patients with breast cancer in the department of Gynecology of Zhenjiang No. 4 People's Hospital from March 2015 to July 2019 were recruited in this prospective study, including 320 breast cancer patients with calcified foci (Calcification group) and 392 breast cancer patients without calcified foci (Non-calcification group). These patients were aged from 26-70 years with an average age of 48.7±10.0 years old. Informed consent was signed by all patients. This study was approved by the Ethics Committee of Zhenjiang No. 4 People's Hospital.

Inclusion and exclusion criteria

Inclusion criteria: Patients met the criteria of breast cancer diagnosis and TNM staging referring to the Diagnosis and Treatment of Breast Cancer of the China Anti-Cancer Association (2019 edition) [17]; patients aged over 18 years old; patients who had no radiotherapy or chemotherapy history before surgery. Exclusive criteria: Patients without complete clinical data; patients with severe heart, liver, kidney and other diseases; patients with mental disorders or cerebrovascular diseases who could not cooperate with this study; patients who could not be followed-up; and patients with other cancers.

Methods

The qualitative analysis of HER2 and RUNX2 in breast cancer tissues: Breast cancer tissues were obtained from Zhenjiang No. 4 People's Hospital after the breast cancer surgery, which were stored at -80°C. Then, the tissues were embedded in wax and sectioned in a routine method. The protein expression level of HER2 and RUNX2 was detected by a streptavidin-peroxidase method according to the procedures of the kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., China). The percentage of positive cells in 5 randomly selected fields * the staining degree was counted under a microscope. The results were scored: ≤1% = 0 points; 2-10% = 1 point; 11-50% = 2 points; 51-80% = 3 points; 81-100% = 4 points. The staining degree was classified by negative (0 points), weak positive (1-4 points), positive (5-7 points), and

Table 2. Comparison of positive rate of RUNX2 and HER2 expression the calcification group and non-calcification group

Categories	Calcification group (n=320)	Non-calcification group (n=392)	χ^2	P
RUNX2			4.363	<0.03
Positive	220	240		
Negative	100	152		
HER2			30.570	<0.00
Positive	172	130		
Negative	148	262		

Note: RUNX2: Runt-related transcription factor 2; HER2: human epidermal growth factor receptor 2.

Table 3. Comparison of positive rate of HER2 expression between RUNX2 positive and negative patients

Categories	RUNX2 positive (n=460)	RUNX2 negative (n=252)	χ^2	P
HER2			9.302	0.002
Positive	215	88		
Negative	245	164		

Note: RUNX2: Runt-related transcription factor 2; HER2: human epidermal growth factor receptor 2.

strong positive (8-12 points). The positive rate (%) = (weak positive case + positive cases + strong positive case) number/total case number * 100.

The relative quantification of HER2 and RUNX2 mRNA in breast cancer tissues: Total RNA of breast cancer tissues was extracted using a Trizol kit (Molecular Research Center, Cincinnati, OH, USA), which was reverse transcribed into cDNA via a reverse transcription kit (Fernentas, Waltham, MA, USA). The reaction system (25 μL): SYBR premix (2x), 12.5 μL. Forward and reverse primers were synthesized by Ruibiotech Company (Guangzhou, China). RUNX2 mRNA primers: 5'-ACCCACGAATGCACTATCCA-3' and 5'-GCTTCCATCAGCGTCAACAC-3'; HER2 mRNA primers: 5'-GATCAACTGCACCCACTCCTGT-3' and 5'-ACCAGCAGAATGCCAACCACC-3'; internal reference GAPDH: 5'-GTCGTAGCAAACCACCAAGC-3' and 5'-TGTGGGTGAGGAGCACATAG-3'. The PCR reaction system (50 μL): 1× Taq man buffer, 3.5 mmol/L $MgCl_2$, 200 μmol/L dATP, dCTP, and dGTP, 400 μmol/L dUTP, 1.25 U AmpliTaq Gold, 0.5 U AmpErase UNG, fluorescent probe 20 nmol/L, 100 ng cDNA and corresponding templates. The reaction conditions: pre-denaturation at 94°C, 4 min, 95°C, 40 s, 60°C, 30 s, 72°C, 30 s, 35 cycles, and 72°C extension, 1 min. The expression of mRNA was quantified by $2^{-\Delta\Delta CT}$ method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the internal reference.

Evaluation of patients' survival: The follow-up was conducted by outpatient re-examination, hospitalization information inquiries, and telephone calls from March 2015 to July 2019, whereas lost cases were eliminated from the study. Overall survival (OS) was defined as the time from the start of study enrollment or chemotherapy to the death of the patients. Disease-free survival (DFS) was defined as the date of pathological diagnosis to the last point of follow-up or disease progression. The last follow-up date was July 18th, 2019.

Statistical analysis

SPSS 17.0 (IBM SPSS Statistics, Chicago, IL, USA) was used for the statistical analysis. Quantitative data was presented as mean ± standard deviation ($\overline{x} \pm sd$). If the data was in a normal distribution with homogeneity of variance, it was analyzed by unpaired t-test (t), otherwise the data was processed by rank sum test (Z). The comparison among multiple groups was processed by one-way analysis of variance (ANOVA) and Tukey post hoc test. The counting data was presented as cases/percentage (n/%), and the comparison between groups was performed with a Pearson's Chi-square test or Fisher's exact test (χ^2). The survival rate was analyzed by Kaplan-Meier method and Log-rank test. $P<0.05$ indicated a statistically significant difference.

Results

Comparison of general and baseline data between breast cancer patients with or without calcification

There was no statistical difference between the two groups in terms of age, body mass index (BMI), tumor size, and comorbidities ($P>0.05$).

Table 4. Comparison of relative expression levels of RUNX2 and HER2 between the calcification group and non-calcification group

Categories	Calcification group (n=320)	Non-calcification group (n=392)	χ^2	P
RUNX2 mRNA expression level	2.16±0.31	1.68±0.24	23.280	<0.001
HER2 mRNA expression level	1.25±0.41	1.03±0.38	7.416	<0.001

Note: RUNX2: Runt-related transcription factor 2; HER2: human epidermal growth factor receptor 2.

Table 5. Comparison of the mRNA expression of RUNX2 and HER2 among breast cancer with calcification and different pathological features

Categories	n	RUNX2 mRNA expression level	HER2 mRNA expression level
Age (Year)			
<50	120	2.14±0.34	1.26±0.43
≥50	200	2.17±0.30	1.24±0.39
t		0.823	0.427
P		0.411	0.670
Tumor size (cm)			
>3 cm	104	2.16±0.44	1.27±0.45
≤3 cm	216	2.15±0.42	1.23±0.38
t		0.196	0.830
P		0.844	0.407
TNM staging			
I-II	216	1.86±0.32	1.00±0.30
III-IV	104	2.42±0.54	1.46±0.59
t		11.602	9.250
P		<0.001	<0.001
T category			
T1-T2	200	2.01±0.37	1.08±0.31
T3-T4	120	2.24±0.49	1.32±0.40
t		4.754	6.002
P		<0.001	<0.001
N category			
N0	204	1.81±0.41	1.07±0.32
N1	116	2.54±0.59	1.34±0.41
t		13.620	6.812
P		<0.001	<0.001
M category			
M0	272	1.87±0.36	1.04±0.37
M1	48	2.56±0.57	1.36±0.43
t		11.072	5.387
P		<0.001	<0.001

Note: RUNX2: Runt-related transcription factor 2; HER2: human epidermal growth factor receptor 2; TNM: tumor node metastasis.

TNM category, T category, N category, M category, and tumor types between the two groups showed statistically significant difference (P< 0.05) (**Table 1**).

Comparison of positive expression of RUNX2 and HER2 between breast cancer patients with or without calcification

The positive rates of HER2 and RUNX2 in the calcification group was significantly higher than those in the non-calcification group (P<0.05). Further analysis demonstrated that the positive rate of HER2 expression in RUNX2 positive patients was notably higher compared with RUNX2 negative patients (P<0.05) (**Tables 2** and **3**).

Comparison of relative expression levels of RUNX2 and HER2 between breast cancer patients with or without calcification

The mRNA expression level of RUNX2 and HER2 in the calcification group was remarkably higher than those in the non-calcification group (P<0.05) (**Table 4**).

Comparison of the mRNA expression levels of RUNX2 and HER2 among breast cancer patients with calcification and different pathological features

There was no significant difference in the mRNA expression levels of RUNX2 and HER2 in breast cancer tissues of patients with different ages and tumor sizes (P>0.05). Whereas patients with high grades in TNM staging, T category, M category, and N category had high mRNA expression levels of RUNX2 and HER2 (P<0.05) (**Table 5**).

Multivariate Cox regression analysis of prognosis of breast cancer patients with calcification

The factors with statistically significant difference in univariate Cox regression analysis were screened to proceed with multivariate Cox regression analysis using the adverse prognosis event as the dependent variable. The data revealed that RUNX2 and HER2 expression levels as well as TNM staging, T category, M category, and N category were independent

Table 6. Multivariate Cox regression analysis of prognosis of breast cancer patients with calcification

Variables	b	S_b	χ^2	P	95% CI	
					Upper bound	Lower bound
Age	0.702	1.622	1.624	0.156	0.075	47.265
Tumor size	0.526	0.684	2.698	0.524	0.169	2.068
TNM staging	0.826	0.264	4.862	0.038	1.521	3.886
T category	0.765	0.342	9.532	0.000	1.113	4.136
N category	0.658	0.312	8.213	0.002	1.045	2.878
M category	0.578	0.264	7.135	0.007	1.074	2.965
RUNX2	0.495	0.127	8.135	0.003	1.269	2.109
HER2	0.266	0.078	5.108	0.017	1.126	1.532

Note: RUNX2: Runt-related transcription factor 2; HER2: human epidermal growth factor receptor 2; TNM: tumor node metastasis; CI: confidence interval.

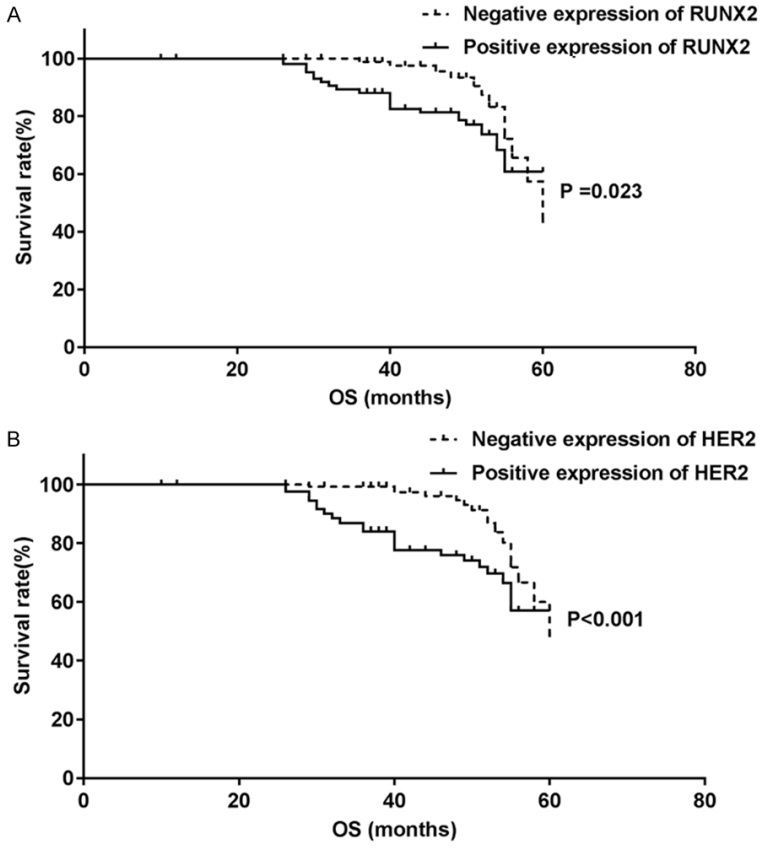

Figure 1. Comparison of OS between positive and negative expression of RUNX2 and HER2A. A. Comparison of OS between positive and negative expression of RUNX2 in breast cancer patients with calcification; B. Comparison of OS between positive and negative expression of HER2 in breast cancer patients with calcification. RUNX2: Runt-related transcription factor 2; HER2: human epidermal growth factor receptor 2; OS: overall survival.

risk factors that influenced prognosis among breast cancer patients with calcification (P< 0.05) (**Table 6**).

Comparison of OS among RUNX2 and HER2 positive and negative breast cancer patients with calcification

The OS of RUNX2 positive patients was 53.746 months (95% CI: 52.051-55.440), which was significantly lower than 57.142 months of RUNX2 negative patients (95% CI: 55.643-58.640; χ^2=5.102, P= 0.023). The OS of HER2 positive patients was 52.593 months (95% CI: 55.824-58.418), which was considerably lower than 57.121 months of HER2 negative patients (95% CI: 50.326-53.674) (χ^2=11.420, P<0.001) (**Figure 1**).

Comparison of DFS among RUNX2 and HER2 positive and negative breast cancer patients with calcification

The DFS of patients expressing RUNX2 was 41.534 months (95% CI: 31.129-42.871), which was notably lower than 45.230 months of RUNX2 negative patients (95% CI: 40.340-42.728; χ^2=10.120, P=0.002). The DFS of patients expressing HER2 was 41.345 months (95% CI: 39.722-43.278), which was remarkably lower than 44.232 months of HER2 negative patients (95% CI: 41.037-45.963; χ^2=8.858, P=0.003) (**Figure 2**).

Discussion

Previous studies have shown that breast cancer patients with calcification displayed special pathological characteristics, including high malignancy and poor tumor differentiation [18]. Our study also uncovered that TNM staging, T category, N category, and M category of breast cancer patients with calcification are significantly different compared

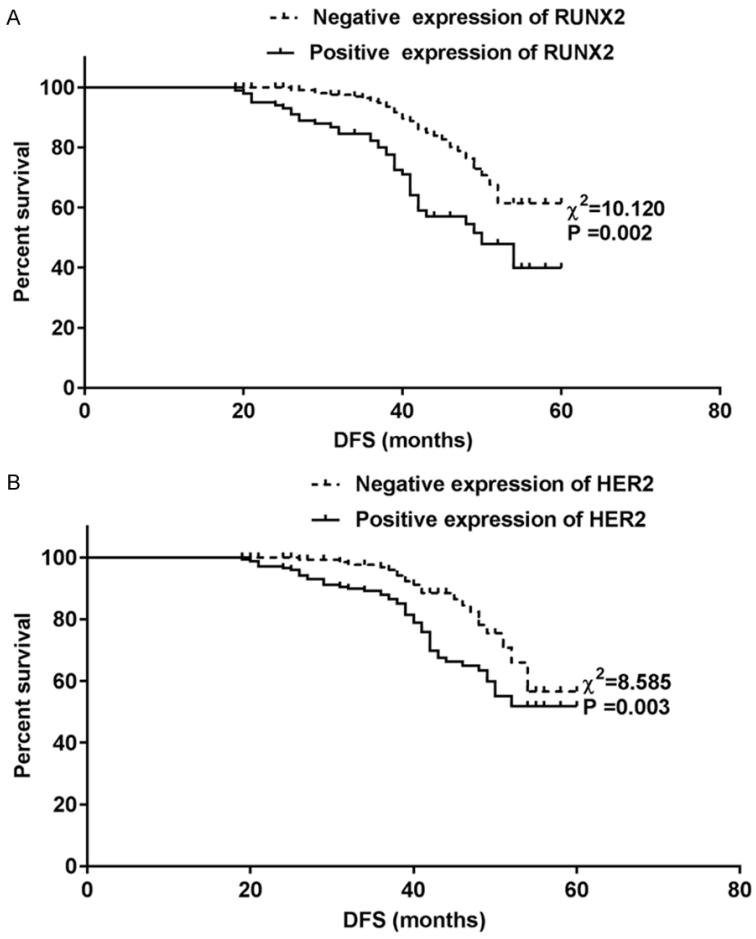

Figure 2. Comparison of DFS between positive and negative expression of RUNX2 and HER2A. A. Comparison of DFS between positive and negative expression of RUNX2 in breast cancer patients with calcification; B. Comparison of DFS between positive and negative expression of HER2 in breast cancer patients with calcification. RUNX2: Runt-related transcription factor 2; HER2: human epidermal growth factor receptor 2; DFS: disease-free survival.

with those of non-calcified breast cancer patients, indicating that breast cancer with calcification is more malignant along with having lower differentiation.

In this study, we found that the expression levels of RUNX2 and HER2 were significantly higher in breast cancer patients with calcification than those of patients without calcification. RUNX2 is a transcription factor that regulates bone differentiation and participates in the formation of tissue calcification. It can also enhance the activity of osteopontin (OPN) promoter and promote malignant lesions of the breast [19-21]. RUNX2 upregulation in breast cancer patients with calcification suggests that RUNX2 may play an important regulatory role in the calcification of breast cancer [22]. HER2 is known as an indicator for the treatment and prognosis of breast cancer patients, whose expression is positively correlated with TNM staging, tumor size, and lymph node metastasis [23]. It has been reported that HER2 overexpression promotes tumor cell proliferation and malignant transformation [16]. HER2, similar with the estrogen receptor and progesterone receptor, is overexpressed in breast tissue, which activates related signaling pathways and leads to tumor proliferation, increased invasion and metastasis [24-26]. Our research further demonstrated that with the increase of TNM staging grade or the development of lymphatic or distant metastases, the expression levels of RUNX2 and HER2 were increased in breast cancer tissues of patients with calcification, suggesting that the upregulation of RUNX2 and HER2 was correlated with the malignancy of breast cancer. The study of the relationship between RUNX2 and HER2 expression illustrated that the positive rate of HER2 expression in RUNX2 positive patients was significantly higher than that of RUNX2 negative patients; and the positive rate HER2 is positively correlated with the positive rate of RUNX2. Therefore, we suspected that RUNX2 positive expression, the same as HER2 positive expression, may be related to the prognosis of the patient.

In addition, we also found that patients with high expression of RUNX2 or HER2 have shorter OS and DFS compared with patients with low expression, thus high expression of RUNX2 or HER2 may indicate poor prognosis. Previous studies have found that the expression of RUNX2 in breast cancer tissues is negatively correlated with ER, indicating that RUNX2 may be related to the occurrence and progression of

ER-negative breast cancer patients [27]. RUNX2 expression is positively correlated with low histological grade, high tumor stage, and high HER2 expression level, suggesting that high expression of RUNX2 in breast cancer tissues indicates a poor prognosis [28]. Another study has reported that high expression of RUNX2 is negatively correlated with the OS and DFS of patients, and high expression of RUNX2 is an independent risk factor of the prognosis of breast cancer patients [29]. In addition, RUNX2 promotes the development and metastasis of tumors through epithelial-mesenchymal transition (EMT), which easily occurs in non-Luminal type A breast cancer [30]. Approximately 65%-75% of advanced breast cancers have bone metastases. Breast cancers with high expression of Runx2 have high bone metastasis potential, leading to a poor prognosis [31]. Studies have revealed that high expression of HER2 plays an important role in lymph node metastasis, metastasis relapse and poor prognosis of breast cancer [32]. Breast cancer patients with HER2 upregulation demonstrate a shorter survival rate due to high potential of tumor invasion and recurrence after surgery [33, 34].

However, a multi-center study with larger sample size and longer follow-up period is needed in the future to verify our results and to study the effects of RUNX2 and HER2 expression on the 5-year survival of patients.

In summary, RUNX2 and HER2 play crucial roles in the progression of breast cancer with calcification. High expression levels of RUNX2 and HER2 indicate a poor prognosis. RUNX2 and HER2 may be potential biomarkers to indicate the malignancy and prognosis of breast cancer with calcification.

Acknowledgements

This study was supported by Research project on maternal and child health care fund, Jiangsu Provencial Commission of Health and Family Planning for *Based on the application of bI-RADS type 4 breast untouchable lesion imaging combined with serum IL-6. CA153 and other tests in breast cancer screening* (F201681).

Disclosure of conflict of interest

None.

Address correspondence to: Yunsuo Zhang, Department of Breast Surgery, Zhenjiang No. 4 People's Hospital, No. 20 Zhengdong Road, Zhenjiang 212001, Jiangsu Province, China. Tel: +86-13775364532; E-mail: zhangyunsuo4yzj@163.com

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

[2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.

[3] Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I and Bray F. Global Cancer observatory: cancer today. Inter Agen Res Cancer 2018.

[4] Dora L, Agrawal S, Panda R and Abraham A. Optimal global cancer observatory: cancer today breast cancer classification using Gauss-Newton representation based algorithm. Expert Syst Appl 2017; 85: 134-145.

[5] Duffy MJ, Harbeck N, Nap M, Molina R, Nicolini A, Senkus E and Cardoso F. Clinical use of biomarkers in breast cancer: up-dated guidelines from the European Group on Tumor Markers (EGTM). Eur J Cancer 2017; 75: 284-298.

[6] Nalawade YV. Evaluation of breast calcifications. Indian J Radiol Imaging 2009; 19: 282-286.

[7] Sener SF, Sargent RE, Lee C, Manchandia T, Le-Tran V, Olimpiadi Y, Zaremba N, Alabd A, Nelson M and Lang JE. MRI does not predict pathologic complete response after neoadjuvant chemotherapy for breast cancer. J Surg Oncol 2019; 120: 903-910.

[8] Qi XM, Chen AX, Zhang P, Zhang W, Cao XC and Xiao CH. Mammographic calcification can predict outcome in women with breast cancer treated with breast-conserving surgery. Oncol Lett 2017; 14: 79-88.

[9] Maxwell AJ, Clements K, Hilton B, Dodwell DJ, Evans A, Kearins O, Pinder SE, Thomas J, Wallis MG and Thompson AM. Risk factors for the development of invasive cancer in unresected ductal carcinoma in situ. Eur J Surg Oncol 2018; 44: 429-435.

[10] An YY, Kim SH and Kang BJ. Residual microcalcifications after neoadjuvant chemotherapy for locally advanced breast cancer: comparison of the accuracies of mammography and MRI in predicting pathological residual tumor. World J Surg Oncol 2017; 15: 198.

[11] Komori T. RUNX2, an inducer of osteoblast and chondrocyte differentiation. Histochem Cell Biol 2018; 149: 313-323.

[12] Colden M, Dar AA, Saini S, Dahiya PV, Shahryari V, Yamamura S, Tanaka Y, Stein G, Dahiya R and Majid S. MicroRNA-466 inhibits tumor growth and bone metastasis in prostate cancer by direct regulation of osteogenic transcription factor RUNX2. Cell Death Dis 2017; 8: e2572.
[13] Ogata T, Nakamura M, Sang MJ, Yoda H, Hiraoka K, Yin DJ, Sang MX, Shimozato O and Ozaki T. Depletion of runt-related transcription factor 2 (RUNX2) enhances SAHA sensitivity of p53-mutated pancreatic cancer cells through the regulation of mutant p53 and TAp63. PLoS One 2017; 12: e0179884.
[14] Wang W, Chen B, Zou RL, Tu XY, Tan SL, Lu H, Liu ZJ and Fu JJ. Codonolactone, a sesquiterpene lactone isolated from Chloranthus henryi Hemsl, inhibits breast cancer cell invasion, migration and metastasis by downregulating the transcriptional activity of RUNX2. Int J Oncol 2014; 45: 1891-1900.
[15] Hu B, Fan CL, Xu XJ, Zhao M, Liu YK and Li FF. Analysis of the expression level and clinical significance of RUNX2 gene in breast cancer. Acta Univ Med Anhui 2016; 51: 1356-1359.
[16] Nicolini A, Ferrari P and Duffy MJ. Prognostic and predictive biomarkers in breast cancer: past, present and future. Semin Cancer Biol 2018; 52: 56-73.
[17] Chinese Anti-Cancer Association and Committee of Breast Cancer Society. Guidelines and norms for breast cancer diagnosis and treatment by the China Anti-Cancer Association (2019 Edition). Chin Oncol 2019; 29: 609-680.
[18] Celebi F, Pilanci KN, Ordu C, Ağacayak F, Alço G, İlgün S, Sarsenov D, Erdoğan Z and Özmen V. The role of ultrasonographic histologic grade, findings to predict molecular subtype and hormone receptor status of breast cancer. Diagn Interv Radiol 2015; 21: 448-453.
[19] Lin ME, Chen TM, Wallingford MC, Nguyen NB, Yamada S, Sawangmake C, Zhang J, Speer MY and Giachelli CM. Runx2 deletion in smooth muscle cells inhibits vascular osteochondrogenesis and calcification but not atherosclerotic lesionformation. Cardiovasc Res 2016; 112: 606-616.
[20] Scimeca M, Giannini E, Antonacci C, Pistolese CA, Spagnoli LG and Bonanno E. Microcalcifications in breast cancer: an active phenomenon mediated by epithelial cells with mesenchymal characteristics. BMC Cancer 2014; 14: 286.
[21] Huan JL, Li X, Qin XJ, Gao ZG, Pan XF and Zhao ZD. Expression and clinical significance of osteopontin in calcified breast tissue. Asian Pac J Cancer Prev 2012; 13: 5219-5223.
[22] Dalle-Cafbonare L, Innamorati G and Valenti MT. Transcription factor RUNX2 and its application to bone tissue engineering. Stem Cell Rev 2012; 8: 891-897.
[23] Song B, Wang L, Zhang Y, Li N, Dai H, Xu HF, Cai HF and Yan JY. Combined detection of HER2, Ki67, and GSTP1 genes on the diagnosis and prognosis of breast cancer. Cancer Biother Radiopharm 2019; 34: 85-90.
[24] Leehy KA, Truong TH, Mauro LJ and Lange CA. Progesterone receptors (PR) mediate STAT actions: PR and prolactin receptor signaling crosstalk in breast cancer models. J Steroid Biochem Mol Biol 2018; 176: 88-93.
[25] Rimawi MF, Schiff R and Osborne CK. Targeting HER2 for the treatment of breast cancer. Annu Rev Med 2015; 66: 111-128.
[26] Schrijver W, Suijkerbuijk K, van Gils CH, van der Wall E, Moelans CB and van Diest PJ. Receptor conversion in distant breast cancer metastases: a systematic review and meta-analysis. J Natl Cancer Inst 2018; 110: 568-580.
[27] Zhang P, He XG, Xu XD, Zhang L, Liu X, Liu YK, Jiang Y, Wang SY and Li FF. Effect of transcription factor RUNX2 on the ability of epithelial-mesenchymal transition in human breast cancer MDA-MB-231 cells. Chin J Pathophysiol 2019; 35: 577-583
[28] Onodera Y, Miki Y, Suzuki T, Takagi K, Akahira J, Sakyu T, Watanabe M, Inoue S, Ishida, Ohuchi TN and Sasano H. RUNX2 in human breast carcinoma: its potential roles in cancer progression. Cancer Sci 2010; 101: 2670-2675.
[29] Chen C, Zhang LN and Gu L. Expression and clinical significance of Runt-related transcription factor 2 and osteopontin in breast cancer. Tumor 2019; 2: 107-115.
[30] Ferrari N, Mckonald L, Morris JS, Cameron ER and Blyth K. RUNX2 in mammary gland development and breast cancer. J Cell Physiol 2013; 228: 1137-1142.
[31] Leong DT, Lim J, Goh X, Pratap J, Pereira BP, Kwok HS, Nathan SS, Dobson JR, Lian JB, Ito Y, Voorhoeve PM, Stein GS, Salto-Tellez M, Cool SM and van Wijnen AJ. Cancer-related ectopic expression of the bone-related transcription factor RUNX2 in nonosseous metastatic tumor cells is linked to cell proliferation and motility. Breast Cancer Res 2010; 12: R89.
[32] Sandra J, Vesa H, Suvi KL, Due EU, Tadele DS, Edgren H, Kallioniemi O, Perälä M, Mælandsmo GM and Sahlberg KK. Drug-screening and genomic analyses of HER2-positive breast cancer cell lines reveal predictors for treatment response. Breast Cancer Targ Ther 2017; 9: 185-198.
[33] Hayes DF. HER2 and breast cancer-a phenomenal success story. N Engl J Med 2019; 381: 1284-1286.
[34] Tang M, Schaffer A, Kiely BE, Daniels B, Simes RJ, Lee CK and Pearson SA. Treatment patterns and survival in HER2-positive early breast cancer: a whole-of-population Australian cohort study (2007-2016). Br J Cancer 2019; 121: 904-911.