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ORIGINAL ARTICLE

Expression and significance of carcinoembryonic antigen, cancer antigen 153, and cyclooxygenase-2 in breast cancer*

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Abstract

Objective This study aimed to evaluate serum and nipple discharge levels of carcinoembryonic antigen (CEA) and cancer antigen 153 (CA153) and tissue cyclooxygenase-2 (COX-2) expression in breast cancer cases and associations of these proteins with breast cancer metastasis.

Methods The immunohistochemical *Ultra Sensitive*™ *S-P* method was used to detect COX-2 expression in 77 cases of invasive breast carcinoma. Of these cases, 52 exhibited CEA and CA153 in both serum and nipple discharge (electrochemiluminescence method), and associations of these biomarkers with breast cancer prognosis were studied. Sixty cases of benign breast lesion were selected as a control group. Overall survival of breast carcinoma patients was evaluated. COX-2 expression was evaluated relative to clinicopathological features and CEA and CA153 levels, and its role in invasiveness was investigated.

Results Among cases of invasive breast cancer, 72.7% (56/77) were COX-2 immunopositive, compared to 16.7% of benign lesions (χ^2 = 66.745, P = 0.000) percentage of positive cells. COX-2 overexpression in breast cancer correlated positively with histological grade (II vs III; χ^2 = 4.064, P = 0.043), lymph node metastasis (χ^2 = 9.135, P = 0.003), and distant metastasis (χ^2 = 8.021, P = 0.003). However, COX-2 expression did not correlate with age (≤ 50 vs 50 years) or tumor size (≤ 5 vs > 5 cm) (χ^2 = 0.081, P = 0.776 and χ^2 = 3.702, P = 0.054, respectively). Among breast cancer patients, COX-2 overexpression in tumors also correlated with shorter overall survival (P < 0.05). In brief, increased COX-2 expression correlates with worse prognosis and shorter overall survival. Malignant lesions were associated with significantly higher serum and nipple discharge levels of biomarkers, relative to benign lesions (P < 0.05). These biomarkers were present at significantly higher levels in nipple discharge than in serum (P < 0.05). Furthermore, significantly higher nipple discharge levels of CEA and CA153 were observed in COX-2-positive breast carcinoma patients, compared to COX-2-negative patients (P <0.05). Shorter overall survival in cancer patients group related to COX-2 overexpression in tumors (P < 0.05).

Conclusion The study suggests that COX-2 overexpression correlates with poor clinicopathological parameters in breast cancers and might be an important biological marker of invasion and metastasis. The findings of the present study suggest that combined detection of COX-2 tissue expression and CEA and CA153 in serum and nipple discharge could facilitate clinical monitoring and diagnosis of metastasis in patients with breast cancer.

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Key words: breast cancer; carcinoembryonic antigen (CEA); cancer antigen 153 (CA153); cyclooxygenase 2 (COX-2); prognosis

Breast cancer is the most common form of cancer among women, and the annual incidence of related morbidity is increasing worldwide [1–2]. In recent years, China has seen a relatively high incidence of breast cancer, with continued progress toward a peak incidence; currently, a large number of patients are expected to die of breast cancer complications or serious organ metastasis each year [2–3]. Pathogenesis of breast cancer is not yet

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fully clear. Many studies have found that abnormal expression of molecules such as estrogen receptors, progesterone receptors, human epidermal growth factor receptor-2, and Ki-67 can serve as biological indicators, thus guide clinical diagnoses, treatments, and prognostic determinations [4]. Several other molecular biomarkers, such as cyclooxygenase-2 (COX-2), carcinoembryonic antigen (CEA), and cancer antigen 153 (CA153) have also been confirmed to contribute to the evolution of malignancy [2, 4–5]. Cellular conditions such as hypoxia lead to the increased COX-2 expression [5–6]. COX-2 is an inducible enzyme that interferes with tumor development and angiogenesis via the inhibition of apoptosis, which is mediated through suppression of the proapoptotic Bax protein and anti-apoptotic bcl-2 protein overexpression [5–6]. COX-2 overexpression is a frequent feature of malignant disease and is commonly associated with a poor prognosis.

Body fluid components are easily detected and therefore serve as ideal diagnostic biomarkers. Serum biomarkers such as CA153 and CEA are considered as prognostic factors and can be evaluated during follow-up [2–3]. Serum protein markers are advantageous because they can be easily used to construct a multiplex tumor-associated autoantibody assay [3, 7]. Nipple discharge evaluation and management can be undertaken with minimal difficulty by performing a careful history and examination and following a logical thought process to link the type of nipple discharge with a suitable mode of treatment [2, 7]. To date, the precise implications of CA153 and CEA screening of both serum and nipple discharge and COX-2 screening of breast cancer tissue, as well as the correlations of these markers with breast carcinoma invasiveness and metastasis, have not yet been investigated. In this study, we evaluated expression of the potential biomarker COX-2 in a panel of mammary tissues and CEA and CA153 levels in both serum and nipple discharge to explore the above markers and associated clinicopathological parameters in breast cancer.

Materials and methods

Patients

The present study was approved by the Rizhao local ethical committee. Written informed consent was obtained from all patients before their participation in the current study. Clinical and pathological information was documented at the time of surgery. Each biopsy slide was subjected to pathological reading, and an overall pathological diagnosis was determined for each subject. Mammary tumor samples were obtained from patients after surgical removal at Rizhao People's Hospital. The samples were fixed in 10% neutral formalin and embedded in paraffin. Hematoxylin and eosin (HE)-stained sections were subjected to pathological diagnosis according to the current World Health Organization (WHO 2012) diagnostic criteria for mammary tumors. All diagnoses were revised by 2 pathologists using the same guidelines to ensure consistency. Tumor grades were determined using modified Bloom–Richardson scores. Grades were obtained by summing the scores for tubule formation, nuclear pleomorphism, and mitotic count (possible scores: 1, 2, or 3). The final scores ranged between 3 and 9 and were divided into 3 grades: I, 3–5 points; II, 6–7 points; and III, 8–9 points. The pathologists were blinded to the clinical histories of cases and the results of immunohistochemical staining assays. A pathological reading was determined for each biopsy slide, and an overall pathological diagnosis was determined for each subject. After revision by 2 pathologists, 77 malignant tumor samples collected at Rizhao People's Hospital from June 2014 to June 2016 were selected for immunohistochemical and survival analyses. The patients ranged in age from 32 to 77 years (mean age: 53.6 years). Sixty cases of benign breast disease (age range: 21–67 years, mean: 43.3 years) were selected as a control group. Patients diagnosed with invasive breast cancer had not received hormone endocrine therapy, anti-neoplastic chemotherapy, or radiotherapy during the last 6 months.

Measurement of CEA and CA153 in serum and nipple discharge samples

All samples were collected before any treatments were initiated. For biomarker analysis, 3 ml of heparinized blood and 0.2 mL of nipple discharge were drawn per individual. The nipple was first cleaned with alcohol swabs to remove cellular debris. Nipple discharge was expressed by manual breast compression, and droplets were collected in an Eppendorf tube. The tube was then stored in a dedicated refrigerator at 4°C. Samples were transported to the laboratory department within 8 h after collection. Viscous samples were diluted up to 20-fold with normal saline before centrifugation and storage at 4°C. Commercial reference control sera were used for quality control and calibration. CEA and CA153 were detected using an electrochemiluminescence method (E-601; Roche, Germany) in the clinical laboratory at Rizhao People's Hospital. In our laboratory, the cut-off values for CEA and CA153 were 3.40 ng/mL and 25.00 U/mL, respectively, in serum and 9.8 ng/mL and 35.00 U/mL, respectively, in nipple discharge. Patients were classified into 2 groups by histological grade: grade II and grade III. Patients were also classified by the levels of CEA and CA153 in peripheral blood or nipple discharge: those with normal levels and those with high levels.

Immunohistochemical evaluation of COX-2 in tumor tissues

Immunohistochemical methods were used to detect COX-2 expression. Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections were then deparaffinized and rehydrated using standard procedures. Serial sections (3–4 μm) were deparaffinized in xylene and hydrated through a graded series of ethanol. The specimens were finally washed in phosphate-buffered saline (PBS) within 5 min and examined under a binocular dissecting microscope. Immunoreactions were processed using the Ultra Sensitive™ S-P Kit (Maixin-Bio, China) according to the manufacturer's instructions, and signals were visualized using a DAB substrate, which stained the target proteins yellow. Negative controls were obtained by substituting PBS for the primary antibody. Tissues known to express COX-2 were used as positive controls. COX-2 positivity was indicated by cytoplasmic staining. The number of COX-2 positive cells was classified semiquantitatively according to the positive rate, and the distribution score was defined by the estimated percentage of positive cells in 5 fields at 400 × magnification and color intensity. In brief, a score was assigned to represent the estimated proportion of positive tumor cells on an entire slide. For each histological section, the percentage of positive cells was scored as: 0, < 5% stained cells; 1, 5–25%; 2, 26–50%; 3, 51–75%; and 4, > 75% stained cells. For staining intensity, values from 0 to 3 were attributed as follows: 0, negative (-); 1, weak staining (light yellow); 2, moderate staining (tan); and 3, strong staining (dark brown). Scores corresponding to the percentage of positive cells and staining intensity were multiplied to obtain a total immunoreactive score (IRS; possible range: 0–12). Samples with an IRS of 0–4 were considered negative; those with an IRS > 4 were considered positive.

Statistical analysis

SPSS version 17.0 statistical software (SPSS Inc., USA) was used for data analysis. Numerical data were assessed using the χ^2 test or Fisher's exact test, as appropriate. CEA and CA153 levels are expressed as means and standard deviations (mean ± SD). As the data related to these markers did not exhibit Gaussian distributions, the nonparametric Mann–Whitney U-test was used to determine differences between the benign and malignant groups. Correlations of COX-2 staining with CEA and CA153 expression were assessed using a Spearman's rank correlation coefficient test. Relationships of this dichotomous variable with other clinicopathological correlates were established using the χ^2 test or Fisher's exact test, as appropriate. Analyses were performed using GraphPad Prism V5.0 software (GraphPad Software Inc., USA). Values were considered statistically significant at a P value < 0.05.

Results

Comparison of CEA and CA153 levels in malignant and benign groups

Fig. 1 showed the serum and nipple discharge levels of CEA and CA153 in the malignant and benign groups. The biomarker levels in serum (Fig. 1a) and nipple discharge (Fig. 1b) were significantly higher in the malignant group, than the benign group ($P < 0.05$). Furthermore, in the malignant group, the levels of both biomarkers were significantly higher in nipple discharge than in serum ($P < 0.05$; Fig. 1c).

COX-2 expression and relationships with biological parameters

Cytoplasmic COX-2 expression was detected in both breast cancer and benign breast lesion tissues. The COX-2 expression findings in the benign and malignant groups

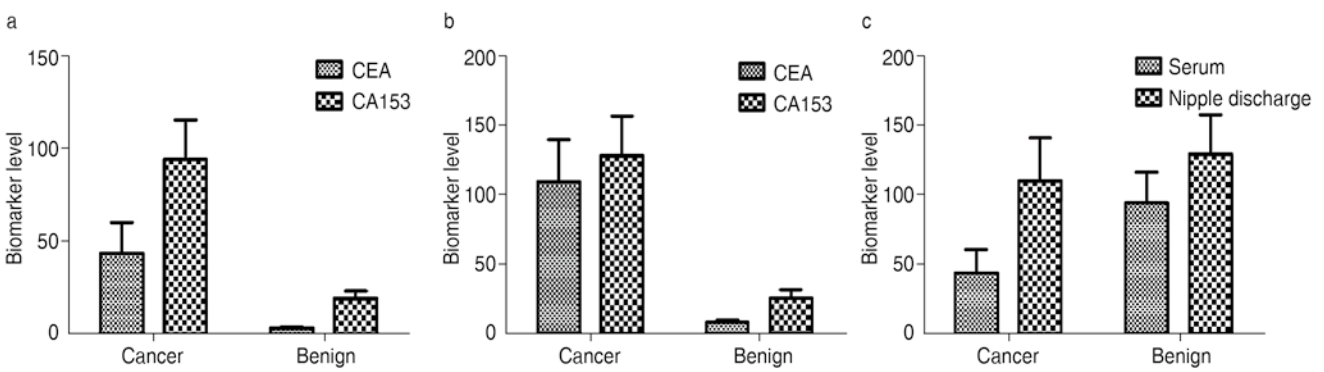

Fig. 1 Comparison of biomarker levels among groups. (a) Comparisons of serum biomarker levels and (b) nipple discharge biomarker levels between malignant and benign groups; (c) comparison of serum and nipple discharge biomarker levels in the malignant group.

Table 1 COX-2 expression in different groups

Groups	*n*	COX-2		χ^2 value	*P* value
		-	+		
Breast carcinoma	77	21	56	82.020	0.000
Benign leision	60	50	10		

Table 2 Relationships between COX-2 expression and biological parameters of breast cancer

Biological parameters	*n*	COX-2		χ^2 value	*P* value
		Negative	Positive		
Age at diagnosis (years)					
≤ 50	36	12	34	0.081	0.776
> 50	41	9	22		
Tumor size (cm)					
≤ 5	65	15	50	3.702	0.054
> 5	12	6	6		
Histoligical grade					
II	57	19	38	4.064	0.043
III	20	2	18		
Lymph node metastasis					
Present	50	8	42	9.135	0.003
Absent	27	13	14		
Distant metastasis					
Present	22	1	21	8.021	0.005
Absent	55	20	35		

were shown in Table 1. In benign tissues, COX-2 was weakly expressed in only 15.4% (10/60) of samples. In contrast, immunohistochemical analysis revealed some degree of positivity in 72.7% (56/77) of the examined malignant tumors, and the COX-2 positive rate was higher among breast cancers than among benign lesions (χ^2 = 82.020, P < 0.01).

Table 2 described the relationships between COX-2 expression and clinicopathological parameters of breast cancer. COX-2 overexpression correlated significantly with histological grade (II vs III; χ^2 = 4.064, P = 0.043), lymph node metastasis (χ^2 = 9.135, P = 0.003), and distant metastasis (χ^2 = 8.021, P = 0.003). However, COX-2 expression did not correlate with age at diagnosis (≤ 50 vs > 50 years) or tumor size (≤ 5 vs > 5 cm) (χ^2 = 0.081, P = 0.776 and χ^2 = 3.702, P = 0.054, respectively).

Comparison of nipple discharge biomarkers levels by COX-2 expression

Fig 2 illustrates the comparison of CEA and CA153 levels in nipple discharge from COX-2-positive and -negative cases of breast carcinoma. The respective CEA and CA153 levels were 126.42 ± 34.18 and 134.45 ± 32.57 in the COX-2-positive group, and 72.89 ± 33.41 and 98.76 ± 35.19 in the COX-2-negative group. The levels of CEA and CA153 in nipple discharge were significantly higher in COX-2-positive breast carcinoma patients, compared to COX-2-negative patients (P < 0.05 for both). COX-2 overexpression in tumors also correlated with significantly shorter overall survival among cancer patients (P < 0.05). These results demonstrate that increased COX-2 expression is related to worse prognosis and shorter overall survival (data not shown).

Follow-up

The follow-up durations ranged from 3 months to 2 years, and the levels of above-mentioned indicators were detected through dynamic blood draws. Patient survival was monitored from January 2014 to August 2016 via telephone communication and periodic visits to Rizhao People's Hospital. Overall survival was defined as the period (months) between surgical tumor resection and death related to the malignant process. Patients who died of any other cause were not included in this analysis. Patients were censored if the follow-up period was < 6 months. Efficacy assessments were performed at 6-week intervals. Progressive disease (PD) and stable disease (SD) were assessed after the start of adjuvant treatment, and treatment responses and disease progression were investigated according to the modified Response Evaluation Criteria in Solid Tumors (RECIST version 1.0). A complete response was recorded when the tumor had disappeared completely, and a partial response was recorded when the largest diameter of the tumor shrank by > 30%; "any response" was recorded for any degree of response or a decrease in size without mention of the tumor dimensions. SD was recorded for cases of no sign of recurrent disease within 6 months or changes in tumor size, and PD was recorded if a tumor size increase of any degree was observed. Progression-free survival (PFS) was calculated from the first date of study drug administration until PD or death from any cause. We evaluated heterogeneity in PFS among patients according to treatment before study entry (i.e., no local treatment vs surgical or local radiotherapy vs whole-breast

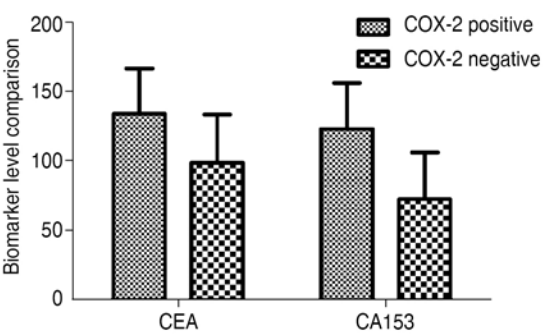

Fig. 2 Biomarker levels in nipple discharge from COX-2 positive and negative breast carcinoma patients.

radiotherapy), because some patients with no evidence of disease might have artificially influenced PFS. Follow-up information was available for 63 of the patients. Patients were classified into COX-2-negative and -positive groups. Of the evaluable patients, 42 achieved SD, and 21 achieved PD. The overall response rate was 37.2%, with a median PFS of 26 months [95% confidence interval (CI) = 11.1–23.6] and a median overall survival of 65 months (95% CI = 40.7–70.2). In the univariate analysis, patients with detectable CEA and CA153 in nipple discharge had significantly increased risks of disease progression (hazard ratio [HR] = 1.61) and death (HR = 1.53; $P < 0.05$). In the univariate and multivariate analyses, COX-2-positive patients who expressed both CEA and CA153 in nipple discharge and serum had significantly increased risks of disease progression (HR = 1.62) and death (HR = 1.66; $P < 0.05$). Kaplan–Meier survival curves further demonstrated survival differences among patients by COX-2 expression status and combined (nipple discharge + serum) biomarker status ($P < 0.05$).

Discussion

Breast cancer is a major public health issue, which accounts for 23% of all cancers in women worldwide, and has an incidence more than twofold higher than cancer at any other site [1–3]. In recent years, the incidence of breast cancer has increased significantly worldwide. Accordingly, numerous studies have sought to determine the most effective ways to evaluate and treat breast cancer, assess therapeutic effects, correctly evaluate prognosis, and identify postoperative recurrences. Mammography is an important diagnostic method mainly used for breast cancer screening. The resolution and calcification detection rates associated with mammography can be further improved by computer assistance [8]. However, the ability of this technique to diagnose early breast cancer is limited [3, 8] and it is mainly used to diagnose advanced stages of the disease. A previous report demonstrated the clinical diagnostic significance of serum CEA, CA153, and CA125 levels and provided details regarding the management of breast cancer recurrence and metastasis [2–3, 7]. The associations of high serum CEA and CA153 levels with poor prognoses have been validated [2–3, 7]. However, the use of serum tumor markers for breast cancer diagnosis is somewhat limited by factors such as relatively limited sensitivity and specificity in stand-alone assays, as levels of these markers reflect tumor burdens. Nipple discharge is a common complaint among women [2–3, 7, 9]. In patients with early or localized breast cancer, serum CA153 levels do not clinically facilitate diagnosis [7].

Nipple discharge evaluation and management are relatively simple in the context of a careful history and examination and a logical thought process that links the type of discharge with a suitable mode of treatment. However, nipple discharge may be a sign of serious abnormality within the breast. Discharge is classified as normal or abnormal, depending on features such as laterality, cycle variation, quantity, color, or presentation [3, 7, 9–10]. Nipple aspiration has been described as a quick, painless, and noninvasive method for collecting breast epithelial cells and extracellular fluid from the breast ductal and lobular epithelium [11]. However, the ability to obtain adequate fluid has consistently been associated with the following 4 factors: age between 35 and 50 years, earlier age at menarche, non-Asian ethnicity, and history of lactation [6–7]. The nipple aspiration fluid collection rate among native Chinese women is relatively lower than that among women of non-Asian ethnicity. In the present attempt to validate whether biomarkers in nipple discharge might serve as novel breast cancer biomarkers, we examined the levels of CEA and CA153, which are known breast cancer tumor markers, in both serum and nipple discharge samples from patients with benign and malignant breast lesions, as well as COX-2 expression in tissues, to explore the significance and combined predictive value of these markers in breast cancer cases and for determining the prognosis of breast papillary cancer cases. Our study revealed that CEA and CA153 levels were higher in nipple discharge than in serum, and the combined detection of CEA and CA153 in both nipple discharge and serum was significantly higher than that in serum or nipple discharge alone. The clinical results from our study groups revealed that CEA and CA153 in nipple discharge could serve as novel biomarkers of breast cancer prognosis [3]. The human mammary gland comprises discrete ductal alveolar systems in which the breast epithelium exfoliates cells and secretes fluids into the luminal compartment of the gland. Nipple discharge is located in or originates from mammary ducts, where benign and malignant breast tumors are generally found. Nipple discharge in the ducts of non-lactating women contains concentrated proteins secreted from the breast ductal epithelium. These unique cellular and biochemical components, which reflect the true alveolar-ductal system microenvironment, has led to nipple discharge being recognized as a potential gold mine of biomarkers for early breast cancer diagnosis. In addition, biochemical compounds of physiopathological interest are found at higher concentrations in breast ductal secretions than in matched serum samples.

Prognosis is directly related to factors such as tumor size, lymph node involvement, and the presence of distant metastasis. Large tumors with lymph node involvement or distant metastases indicate a poor prognosis and worse overall and disease-free survival [1–2, 4].

COX-2 has been investigated in the context of several human cancers and was found to correlate with disease evolution. In our study, only 15.4% of benign

lesions weakly expressed COX-2; in contrast, breast cancer tissues were more likely to exhibit some degree of COX-2 positivity. Among breast cancer cases, we observed positive correlations of COX-2 expression with a histological high grade (III), lymph node metastasis, and distant metastasis. Our results also revealed that patients with elevated COX-2 expression had shorter survival times. The strong correlations of COX-2 expression with breast cancer prognostic factors suggested that increased COX-2 expression was associated with worse prognosis, as observed in our survival analysis. We suggest that differences in COX-2 expression in breast cancer patients are related to variations in tumor behavior, thus confirming the association between COX-2 expression and disease aggressiveness. In addition to shorter overall survival, the positive correlation of COX-2 expression with breast carcinoma was verified, thus suggesting a worse prognosis. In our study, we observed shorter survival among patients with higher levels of COX-2 expression in tumors. Therefore, COX-2 inhibitor anti-inflammatory drugs could potentially be used to treat mammary tumors. Our results demonstrate correlations of increased COX-2 expression with worse prognosis and shorter overall survival. These findings suggest that COX-2 overexpression in breast carcinoma might be an important biological marker of invasion and metastasis, and the combined detection of COX-2 could yield better early markers for clinical metastasis monitoring of patients with breast cancer.

Conclusions

Our results demonstrate that increased COX-2 expression correlated with a worse prognosis and shorter overall survival. Our study suggests that COX-2 overexpression correlates with poor clinicopathological parameters in breast cancer patients and might be serve as an important biological marker of invasion and metastasis. Further, our findings suggest that COX-2 overexpression may be considered a negative prognostic marker of breast cancer. The combined detection of COX-2 with CEA and CA153 in both serum and nipple discharge provides a better early marker for the clinical monitoring of metastasis in patients with breast cancer.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Lakhani SR, EllisIO, SchnittSJ, *et al*. WHO classification of tumours of the breast. Lyon: IARCPress, 2012. 1–117.
2. Liang Y, Li Z, Wang G. Expression of HIF-1α in breas tcancer and precancerous lesions and the relationship to clinicopathological features. Chinese-German J Clin Oncol, 2014, 13: 23–28.
3. Wang G, Qin Y, Zhang J, *et al*. Nipple discharge of CA15-3, CA125, CEA and TSGF as a new biomarker panel for breast cancer. Int J Mol Sci, 2014, 15: 9546–9565.
4. Witteveen A, Kwast AB, Sonke GS, *et al*. Survival after locoregional recurrence or second primary breast cancer: impact of the disease-free interval. PLoS One, 2015,10: e0120832.
5. Kim MJ, Kim HS, Lee SH, *et al*. NDRG2 controls COX-2/PGE2-mediated breast cancer cell migration and invasion. Mol Cells 2014, 37: 759–765.
6. Wrensch MR, Petrakis NL, Gruenke LD, *et al*. Factors associated with obtaining nipple aspirate fluid: analysis of 1428 women and literature review. Breast Cancer Res Treat, 1990, 15:39–51.
7. Zhao S, Mei Y, Wang J, *et al*. Different levels of CEA, CA153 and CA125 in milk and benign and malignant nipple discharge. PloS One, 2016, 21: e0157639.
8. He YK, Xu GH, Ren J, *et al*. Mammography combined with breast dynamic contrast-enhanced-magnetic resonance imaging for the diagnosis of early breast cancer. Oncol Transl Med, 2016, 2: 165-168.
9. Piura E, Piura B. Autoantibodies to tumor–associated antigens in breast carcinoma. J Oncol, 2010, 2010: 264926.
10. Wang GP, Zhang H, Zhang ZF, *et al*. Clinical and pathological characteristics of intraductal proliferative lesions and coexist with invasive ductal cancer. Chinses-German J Clin Oncol 2013, 12: 574–580.
11. Sauter ER. Analysis of nipple aspirate fluid for diagnosis of breast cancer: an alternative to invasive biopsy. Expert Rev Mol Diagn, 2005, 5: 873–881.

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