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Original Article

The significance of JARID1B/KDM5B and P16 expression in intraductal proliferative lesions and invasive ductal cancer of the breast

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Abstract: Objective: This study aims to investigate the expression and significance of JARID1B/KDM5B and P16 in intraductal proliferative lesions and invasive ductal cancer of the breast. Methods: The S-P immunohistochemical method was used to observe the expression of JARID1B/KDM5B and P16 in each lesion. Results: The positive expression of JARID1B/KDM5B in normal breast tissue, usual ductal hyperplasia (UDH), flat epithelial atypical hyperplasia (FEA), atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS), and invasive ductal carcinoma (IDC) was observed in 7.39%, 9.68%, 41.18%, 47.83%, 45.45%, and 74.43% cases respectively. The difference in expression of JARID1B/KDM5B was statistically significant between normal breast tissue and FEA, ADH, DCIS, and IDC ($P<0.05$). No statistical difference was seen between normal breast tissue and UDH ($P>0.05$), and between FEA, ADH, and DCIS ($P>0.05$). The positive expression of P16 in normal breast tissue, UDH, FEA, ADH, DCIS, and IDC was observed in 82.95%, 80.65%, 70.59%, 73.91%, 36.36%, and 35.80% cases respectively. The difference in expression of p16 was statistically significant between normal breast tissue, UDH, FEA, ADH, DCIS, and IDC ($P<0.05$). No significant difference was observed between normal breast tissue, UDH, FEA, and ADH ($P>0.05$), respectively, and between DCIS and IDC ($P>0.05$), demonstrating a negative correlated expression of P16 and JARID1B/KDM5B in intraductal proliferative lesions and invasive ductal cancer of the breast. Conclusions: JARID1B/KDM5B and P16 can be used in differential diagnosis of intraductal proliferative lesions and invasive ductal cancer of the breast.

Keywords: Invasive ductal carcinoma of the breast, intraductal proliferative lesions, JARID1B/KDM5B, P16

Introduction

Intraductal proliferative lesions (IDPL) in breast cancer are defined by the WHO as a set of hyperplastic lesions with structural diversity in cytological morphology and histology that mainly occur in the terminal ductal lobular system. In this kind of disease, there are different degrees of correlation with the risk of invasive cancer. This group of lesions is a hot topic of research and is controversial in the field of breast pathology and includes usual ductal hyperplasia (UDH), flat epithelial atypical hyperplasia (FEA), atypical ductal hyperplasia (ADH), and ductal carcinoma in situ (DCIS). IDPL may be non-tumorigenic or tumorigenic, since there are large differences in the proliferation of different lesions.

According to the classification system described in 2012 [1], this definition is not classified among the precursor lesions of breast cancer. However, the biopsies for the diagnosis of UDH patients combined with long-term follow-up results showed that the future risk of invasive cancer increased slightly by about 1.5-2 times in these patients as compared to the general population.

The 2012 edition of the WHO classification describes in detail that clear flat epithelial atypical are neoplastic lesions occurring in terminal lobular units, whereas a pattern of intraductal hyperplasia, that is a histological type of FEA, belongs to the columnar epithelial lesions of the breast. Follow-up data has shown that columnar epithelial hyperplasia lesions progress with a very low risk of breast cancer, and the relative risk is 1.5 times that of the general population [2].

The data demonstrated the presence of direct transition between FEA and low grade DCIS and supported the interpretation of FEA as a stage in the development of low-grade breast cancer [3].

ADH is defined as atypical with low level of DCIS cytology, According to the WHO (2003, 2012 Edition) breast cancer histological classification, ADH is defined as a tumor of intraductal lesions, with a single form of epithelial hyperplasia, and uniform distribution characteristics, that may progress with the moderate risk of breast cancer.

DCIS has a propensity for developing into invasive cancer, but is not necessary for the development of invasive carcinoma. In fact, DCIS is a group of highly heterogenous lesions with similar expression, histological morphology, biological characteristics, and the risk of developing other aspects of invasive breast cancer.

Among several histologic types of breast cancer, invasive ductal carcinoma is generally referred to as adenocarcinoma without other designations and comprises the majority (79%) of breast cancer cases [4].

Most of the breast diseases can allow for a clear diagnosis in conventional HE sections and most differential diagnoses can be determined by microscopy. However, difficulties are encountered with central necrosis UDH and intermediate DCIS, UDH and ADH of florid hyperplasia, ADH and low level of DCIS, DCIS and identification of expansive growth infiltration cancer. The application of the concept of stem cells and immunohistochemical characteristics of different cell types can contribute to the diagnosis and differential diagnosis of these lesions. KDM5B-mediated histone H3K4 demethylation contributes to the silencing of retinoblastoma target genes in senescent cells, presumably by compacting chromatin and by silencing certain genes. Previous studies have found that KDM5B depletion stimulated p16 transcription and suppressed tumor cell growth in vitro and in vivo, suggesting that it plays a role in cell growth regulation in human cancer [5]. In this study the expression of JARID1B/KDM5B and P16 were detected by using immunohistochemistry to investigate the intraductal proliferative lesions of the breast (IDPL) and their application to the diagnosis and differential diagnosis of invasive ductal carcinoma.

Methods

Case selection

This study included tumor tissues surgically resected from patients who visited Beijing Tongren Hospital and Beijing Civil Aviation Hospital between 2008 and 2014. The patients ranged from 16 to 77 years of age (mean age of 47 years). Study qualifiers included: diagnosis of invasive ductal carcinoma (IDC) in 176 cases, UDH in 31 cases, FEA in 17 cases, ADH 23 in cases, and low/intermediate DCIS grade in 22 cases. Additionally, other qualifier characteristics included no preoperative chemotherapy, radiation therapy, or non-steroidal anti-inflammatory drug therapy. The diagnoses were confirmed by pathology and the integrity of clinical data, demographic, clinical, and pathologic data were computed from patient files, surgical reports, and pathology reports. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Capital Medical University. Written informed consent was obtained from all participants.

Immunohistochemistry

Immunohistochemical staining was performed on 4 μm sections of formalin-fixed, paraffin-embedded surgical tumor samples. The sections were mounted, deparaffinized in xylene thrice, and rehydrated through 100%, 90%, 80%, and 70% ethanol followed by Tris-buffered saline (pH 7.4). Antigen retrieval was performed using 10 mM citrate buffer, pH 6.0, heated in a pressure cooker for 5 min. The blocking of endogenous peroxidases was accomplished by incubating the sections in 3% hydrogen peroxide (H_2O_2; Maixin Biotechnology; Fuzhou, China) for 5 min. The sections were incubated with rabbit anti-JARID1B/KDM5B antibody (1:100; DAKO; Hamburg, Germany) and rabbit anti-P16 antibody (Maixin Biotechnology) overnight at 4°C. Immunostaining for JARID1B/KDM5B was performed using the S-P immunohistochemistry kit according to the manufacturer's instructions (Maixin Biotechnology). The sections were counterstained with hematoxylin for nuclear staining and were then examined for the extent and intensity of nuclear and non-nuclear staining in tumor cells and for background staining by two independent observers in a blinded manner. Discordant scores were resolved by review and consensus agreement or by a third observer.

For p16 immunohistochemical (IHC) scoring, nuclear or/and cytoplasmic staining was identified as positive staining. For JARID1B/KDM5B IHC scoring, nuclear staining was identified as

Table 1. The expression of JARID1B /KDM5B and P16 in breast lesions

Groups	Numbers	KDM5B		χ^2 value	*P* value	P16		χ^2 value	*P* value	r_s	*P value*
		Negative	Positive (%)			Negative	Positive (%)				
Normal tissues	176	163	13 (7.39)			30	146 (82.95)				
UDH	31	28	3 (9.68)	0.006	0.940	6	25 (80.65)	0.098	0.754	-0.668	0.000
FEA	17	10	7 (41.18)	4.833	0.028	5	12 (70.59)	0.188	0.664	-0.509	0.037
ADH	23	12	11 (47.83)	0.175	0.676	6	17 (73.91)	0.054	0.816	-0.422	0.045
DCIS	22	12	10 (45.45)	0.025	0.873	14	8 (36.36)	6.421	0.011	-0.500	0.018
IDC	176	45	131 (74.43)	8.010	0.005	113	63 (35.80)	0.003	0.958	-0.303	0.000

Note: χ^2 value: A comparison with a previous group of tables; r_s: The correlation of positive expression rate and staining intensity between KDM5B and p16 in UDH, FEA, ADH, DCIS, IDC.

positive staining. The extent of positively stained cells was estimated and classified on a five-point scale as follows: grade 0, <10%; grade 1, 10%-25%; grade 2, 25%-50%; grade 3, 50%-75%; grade 4, >75%. The intensity of the positive staining was categorized into three groups: weak 1), moderate 2), and strong 3). A final IHS score was obtained by multiplying the score for the extent and the score for intensity as follows: 0, negative (-); 1-4, weakly positive (+); 5-8, moderately positive (++); and 9-12, strongly positive (+++).

Statistical analysis

All statistical analyses were performed using the SPSS statistical software package, version 19 (SPSS Inc. Chicago, IL, USA) for Windows. The χ^2 test and Spearman's correlation coefficient were used to test for any associations between the levels of JARID1B/KDM5B and p16 proteins. A $P<0.05$ was considered to be statistically significant for all tests.

Results (Table 1)

The positive expression rate of JARID1B/KDM5B in the adjacent normal tissues, UDH, FEA, ADH, DCIS, and IDC was in 7.39% (13x), 9.68% (3x), 41.18% (7x), 47.83% (11x), 45.45% (10x), and 74.43% (131x) respectively. The difference was statistically significant in comparisons between normal breast tissues and FEA, ADH, DCIS, IDC ($P<0.05$); between UDH and ADH, DCIS, IDC; FEA and IDC; and between ADH and IDC ($P<0.05$). No statistically significant differences were observed between normal breast tissues and UDH, and between FEA and DCIS, ADH ($P>0.05$) (**Figure 1**).

The positive expression rate of p16 in the adjacent normal tissues, UDH, FEA, ADH, DCIS, and IDC was 82.95% (146x), 80.65% (25x), 70.59% (12x), 73.91% (17x), 36.36% (8x), and 35.80% (63x) respectively. The difference in expression was statistically significant in comparisons between normal breast tissues and DCIS, IDC; between UDH and DCIS, IDC; between FEA and DCIS, IDC; and between ADH and DCIS ($P<0.05$). No statistically significant differences were observed between UDH and ADH, and between normal breast tissues and FEA, ADH ($P>0.05$) (**Figure 2**).

Statistical analyses demonstrated that JARID1B/KDM5B and p16 protein expression in breast intraductal proliferative lesions (UDH, FEA, ADH, DCIS) and invasive ductal cancer were negatively correlated ($r_s=-0.668$, $r_s=-0.509$, $r_s=-0.422$, $r_s=-0.500$, $r=-0.303$, $P<0.05$).

Discussion

JARID1B (KDM5B/PLU-1/RBP2-H1) is a member of the highly conserved family of jumonji/ARID1 (JARID1) histone 3 K4 (H3K4) demethylases, which show nuclear localization of the gene product, an AT-rich DNA interaction domain characteristic of the JmjC family of demethylases, that specifically targets H3K4 [1]. These are involved in tissue development, cancer, and normal stem cell biology [6]. The highest levels of expression of PLU-1 mRNA are seen in testis, with low levels of expression being observed in the placenta, lymph node, and thymus. In the normal mammary gland, mPlu-1 mRNA is expressed in the embryo in the developing mammary bud, and during pregnancy in the adult, suggesting a role in the proliferation of the developing and differentiating tissue [7]. JARID1B was originally isolated as a gene that was overexpressed in breast carcinomas. Subsequently, JARID1B dysregulation has been reported in several types of solid tumors, including prostate cancer, melanoma, and

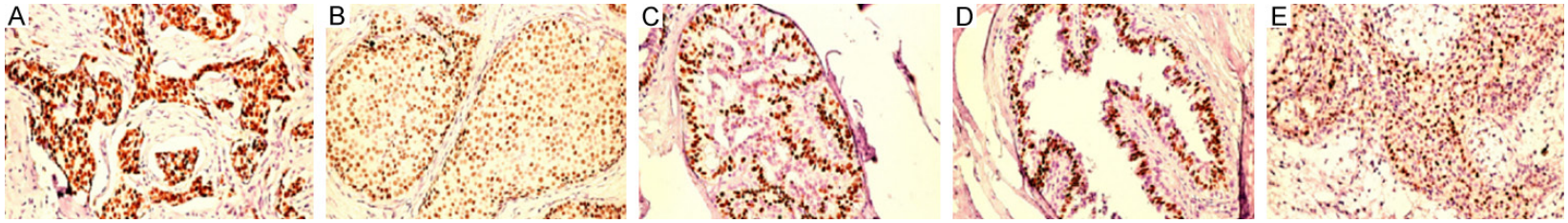

Figure 1. The positive expression of JARID1B/KDM5B in lesions. A: IDC, B: DCIS, C: ADH, D: FEA, E: UDH.

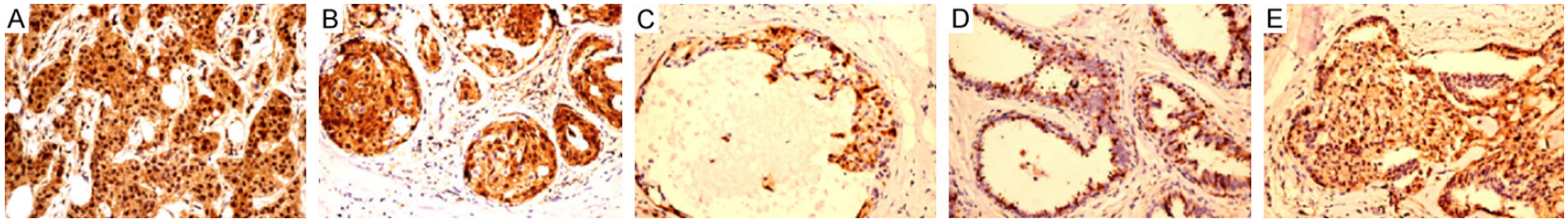

Figure 2. The positive expression of P16 in lesions. A: IDC, B: DCIS, C: ADH, D: FEA, E: UDH.

bladder cancer [8]. In cancer, JARID1B functions as a transcriptional regulator of oncogenes, e.g. BRCA1 in breast cancer, via direct interaction with promoter sites [9].

The expression of JARID1B is associated with invasive and in situ components in primary breast cancers and is expressed only weakly or not at all in benign tumors. Thus, JARID1B expression is closely associated with the malignant phenotype in breast cancer. Catchpole et al have used a mouse model and human cell lines to show that expression of the protein is required for embryonic survival and that this nuclear protein can affect cell growth and ERα signaling in the mammary gland, and in breast cancer [10].

Roesch showed that JARID1B is able to suppress angiogenesis and metastasis, supporting the notion that JARID1B itself is a potential tumor suppressor. In melanocytic tumors, JARID1B is highly expressed in benign nevi, which typically are characterized by oncogene-induced senescence. However, in aggressive primary melanomas and melanoma metastases, there are only single cells with high JARID1B expression (5%-10% of the total population) [11].

The p16/INK4a gene, also known as MTS1, is located on chromosome 9p21. p16 plays a key role in regulating senescence induction, which is the reverse effect on cell cycle regulation than that is seen in cancers; namely, inhibition of cell division and proliferation. p16 acts through the retinoblastoma pathway to inhibit cyclin-dependent kinases, leading to G1 cell cycle arrest and senescence [12]. Loss of this inhibition provided by the p16 protein in a cell leads to dysplasia, cell division, and eventually complete loss of control over cell proliferation, leading to cancer [13].

p16 is one of the important tumor suppressor genes and its expression has been confirmed in many human tumors to be associated with gene mutation and promoter methylation of p16 protein as a specific inhibitor of the cyclin-CDK complex and the conversion regulation of the G-S phase of the cell cycle. Thus, its expression plays a key role in the induction of cell differentiation and cell senescence [14]. At the same time, many observations revealed that the expression pattern of the p16 gene is significantly altered in cervical cancer, endometrial cancer, colon cancer, and especially in patients with human papilloma virus (human papilloma, virus, HPV) infection in cervical cancer, where the expression level was increased along with changes in cell nucleus to cell cytoplasm and nucleus coloring. The abnormal expression of P16 has become a high-grade cervical intraepithelial neoplasia (CIN) marker and is an important sign of cervical cancer. Some researchers have studied p16 gene expression in primary breast cancer and in endometrial cancer [15]. High levels of p16 expression are observed in primary cancer and in various cell lines with the pRb protein, which may be due to the feedback loop mechanism, where ectopic p16 expression inhibits RB transcription. In other human cancers, the expression of p16 seems to be a poor prognostic factor, possibly due to the growth of p16 positive cells with a rapid increase in gene mutation rate [16]. Geradts and Ingram [17] found p16 to be the most common target of cell cycle deregulation in invasive breast carcinomas. In recent years, an independent study has shown high protein expression of p16 in breast cancer, and that this high level of expression is an indicator of poor prognosis.

The study, in which immunohistochemical detection of 72 cases of p16 positive patients with infiltrating ductal carcinoma was performed, the expression of p16 was increased, with the loss of normal nuclear expression, but increased cytoplasmic or whole cell expression. The abnormal expression of p16 in the poorly differentiated carcinoma was also high [18]. This study found positive expression of p16 in the nucleus and/or cytoplasm, with diffuse cytoplasmic expression.

Intraductal proliferative lesions (IDPL) are a group of lesions that mainly occur in the terminal ductal lobular units, with cytology and tissue structure of epithelial hyperplasia lesions along with the various types of the UDH, and are benign in most cases. ADH, FEA, and DCIS were recognized as tumor hyperplasia.

Usual ductal hyperplasia (UDH) in intraductal proliferative lesions are the most common, and are also known as intraductal hyperplasia, general type hyperplasia, epithelial hyperplasia, common intraductal hyperplasia, and benign intraductal proliferative lesions. According to

the 2003 WHO classification of breast tumor tissues, UDH is defined as a lesion in the central lumen with hyperplasia cells with water like distribution characteristics of benign ductal hyperplasia [19].

The structural characteristics of UDH include: ① irregular window; ② the window of the surrounding water samples; ③ arrangement; ④ the stretching or bending of the epithelial cells and the bridge; ⑤ folding; ⑥ the cell morphology; ⑦ the existence of a mature phenomenon. One of the most important characteristics of UDH is the presence of two or more than two kinds of cells, (epithelial cells, myoepithelial cells, and (or) apocrine gland cells) in the mixed form. Usually immunohistochemistry shows the presence of stem cells, intermediate cells, and mature glandular epithelium with mixed expression (CK5+, CAM5.2+), and non-uniform ER positive expression [20].

This study shows that the positive expression rate of JARID1B/KDM5B in UDH was 9.68% and was lower than that of FEA (41.18%), ADH (47.83%), DCIS (45.45%), and IDC (74.43%), with statistically significant differences ($P<0.05$), whereas between UDH and normal breast tissues, no difference in the expression was observed ($P>0.05$). Positive p16 expression in the UDH was 80.65%, higher than that DCIS (36.36%), and IDC (35.80%), with statistically significant differences ($P<0.05$), but no significant difference was observed in the expression of p16 between UDH and ADH (73.91%), and FEA (70.59%) ($P>0.05$).

Flat epithelial atypical hyperplasia (flat epithelial atypia (FEA)) was first proposed in the 2003 edition of WHO breast cancer pathology and genetics in the classification of disease, and was defined as a possible intraductal tumor lesion, with a single layer or 3-5 layers of mildly atypical cells replacing the original skin cell characteristics. Immunohistochemistry showed diffuse high expression in ER, no expression of Her2, CK5/6-CK8+. This study showed that the positive expression of JARID1B/KDM5B in FEA was 41.18% and higher than that in normal breast tissue (7.39%), UDH (9.68%), but was lower than IDC (74.43%), with significant differences ($P<0.05$), whereas no statistical differences were seen between FEA and ADH (47.83%), DCIS (45.45%) ($P>0.05$). The positive expression rate of p16 in FEA is 70.59%, and showed no significant difference between that of normal breast tissues (82.95%) ($P>0.05$), but was higher than that in DCIS (36.36%), IDC (35.80%), where the difference was statistically significant ($P<0.05$).

Atypical ductal hyperplasia (ADH) is a frequently detected precancerous lesion in the breast after the age of 40-50; autopsy studies detected moderate to severe hyperplasia in over 30% of women aged 45-54 years and ADH in was observed in 7% of women aged 20-54 years. ADH is a well-established precursor of breast cancer. Women with ADH have an approximately five-fold increased risk of developing breast cancer. In addition, gene expression profiling of ADH, ductal carcinoma in situ (DCIS), and invasive ductal carcinoma (IDC) showed that these three stages of breast cancer are highly similar at the transcriptional level, further suggesting that ADH is a precursor stage during breast cancer evolution [21]. At present, there is no consensus on whether to adopt quantitative criteria to distinguish between ADH and low level DCIS, and some researchers believe that the atypical cells must occupy 2 catheters for a diagnosis of low grade DCIS and <2 catheter for ADH [22].

ADH are said to show the growth patterns of limbal epithelial hyperplasia cavity type, CK5/6-CK8+. These results show that the positive expression of JARID1B/KDM5B in ADH (47.83%), was higher than that in normal breast tissue (7.39%) and UDH (9.68%), with a statistically significant difference ($P<0.05$) and was lower than that in the positive expression of IDC (74.43%), again with statistically significant difference ($P<0.05$) with no statistical difference between that of DCIS (45.45%). The positive expression of p16 in ADH (73.91%) was not significantly different from normal breast tissue (82.95%), FEA (70.59%), and UDH (80.65%) ($P>0.05$), but was higher than the positive expression in DCIS (36.36%), and IDC (35.80%), with a statistically significant difference ($P<0.05$).

DCIS are also known as ductal carcinoma in situ. The 2010 edition of WHO for breast cancer histological classification, following the 2003 edition of the content, defined DCIS as a tumor of intraductal lesions, characterized by ductal neoplastic epithelial cell proliferation, cells with mild to severe atypia, that do not break through

the catheter/tubular basement membrane, and show no interstitial infiltration. DCIS is classified in to 3 grades: low level, middle level, and high level of DCIS. The 2012 edition of the WHO classification of breast tumors placed more emphasis on nuclear grade: low level of nuclear, intermediate level nuclear, and high-level nuclear grade. This study collected 22 cases of DCIS, and because of the small number, the cases are not clearly separated. This study showed that JARID1B/KDM5B positive expression rate in DCIS was 45.45%, but showed no difference between DCIS (45.45%) and FEA (41.18%), and ADH (47.83%) ($P>0.05$), with a significant difference in its expression in normal tissue (7.39%) and UDH (9.68%) ($P<0.05$). Positive p16 expression in DCIS (36.36%) is lower than the expression in normal breast tissue (82.95%), UDH (80.65%), ADH (73.91%), FEA (70.95%), with a statistically significant difference ($P<0.05$).

IDC is an invasive breast cancer observed in the largest group of patients, and because of lack of adequate tumor characteristics, its tissue is not like lobular carcinoma or tubular carcinoma as special histological types, and so invasive ductal carcinoma is a type of tumor with significant heterogeneity. The positive expression of JARID1B/KDM5B in IDC (74.43%) was higher than in normal breast tissue (7.39%), UDH (9.68%), FEA (41.18%), ADH (47.83%), and DCIS (45.45%), with a statistically significant difference ($P<0.05$). The positive expression rate of P16 was (35.80%) and is lower than that of normal breast tissue (82.95%), UDH (80.65%), FEA (70.59%), and ADH (73.91%) with a statistically significant difference ($P<0.05$), and the positive expression rate in DCIS is 36.36%, and showed no significant difference ($P>0.05$).

Based on the analysis of expression patterns and the negative correlation of JARID1B/KDM5B and p16 in intraductal proliferative lesions of the breast and invasive ductal carcinoma, this study suggests that JARID1B/KDM5B and p16 may play a supportive role in the diagnosis and differential diagnosis of the disease in combination with other indicators. Thus, these markers may have certain application value for breast intraductal proliferative lesions and for the diagnosis of invasive ductal carcinoma.

Disclosure of conflict of interest

None.

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