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Original Article

EphA2 as a new target for breast cancer and its potential clinical application

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Abstract: Purpose: The aim of this research was to study the expression of EphA2 to assess its suitability as a new breast cancer target. Methods: Immunohistochemistry (IHC) was used to detect EphA2 protein expression in pathology tissue samples from 250 cases of breast cancer, and the expression of EphA2 mRNA was detected by in situ hybridization (ISH). Breast cancer cells were isolated and cultured. The expression of EphA2 in the cells was detected by the indirect immunofluorescence assay (IFA), and the expression of EphA2 in breast cancer was analysed. Results: EphA2 protein and mRNA were mainly expressed in tumor cells and vascular endothelial cells. EphA2 protein was expressed in 187 cases, with a positive rate of 74.80%, whereas EphA2 mRNA was expressed in 209 cases, with a positive rate of 83.60%. EphA2 protein and mRNA expression were correlated with lymph node metastasis, clinical stage, and breast cancer histologic grade ($P<0.05$). In addition, the positive expression rates of EphA2 protein and EphA2 mRNA were correlated ($P<0.05$). EphA2 was barely expressed in normal breast cells but highly expressed in breast cancer cells. Conclusion: EphA2 is highly expressed in breast cancer tissues and has the potential to be a new breast cancer target, providing a preliminary basis for the development of new targeted drugs for breast cancer and the construction of fluorescent-targeted tracers for fluorescence-guided mastoscopic breast-conserving surgery.

Keywords: Breast cancer, EphA2, target

Introduction

Breast cancer is the most common type of cancer in women, and the numbers of new breast cancer cases and related deaths increase each year. For those with malignant tumors, curative effects for breast cancer are relatively good, but the overall survival rate is still unsatisfactory. At present, the positive expression of estrogen receptor (ER) and progesterone receptor (PR) allows patients to benefit from endocrine therapy, and the overexpression and amplification of human epidermal growth factor receptor 2 (HER-2) augments the tumor's response to trastuzumab and lapatinib, which enhance the prognosis and treatment of breast cancer. However, the disease-free survival span of some patients remains short, indicating the need for better clinical markers. EphA2 is specifically expressed in the neovascular system, and increasing evidence indicates that high levels of EphA2 promote all aspects of the malignant cell phenotype, including cell growth, migration and invasion, angiogenesis, and cancer cell survival [1-3]. Because EphA2 is highly expressed in a variety of malignant tumors and plays an important role in processes such as tumor cell proliferation, invasion and metastasis, and angiogenesis, it has the potential to be a new target for breast cancer. Because of modern surgical techniques, the amount of resection needed to treat breast cancer is decreasing, and the application of mastoscopy has also enabled minimally invasive surgery. Modern breast surgery research is pursuing the development of individualization, minimal invasion, precision, function protection, and physical and psychological recovery. For mastoscopic breast-conserving surgery, accurate determination of the surgical margin is crucial. However, current routine examinations have various limitations. Therefore, the construction

Table 1. Characteristics of 250 pathologic specimens

Clinicopathologic characteristic	Total (n=250)
Age	
≤45 years old	88
>45 years old	162
Tumor size	
<5 cm	153
≥5 cm	97
Histologic type	
Invasive ductal carcinoma	216
Other types	34
TNM stage	
0-I stage	25
II stage	118
III-IIV stage	107
Lymphatic metastasis	
+	162
-	88
Histologic grade	
I	33
II	148
III	69

of a fluorescent-targeted tracer for real-time visualisation of breast cancer tumors would be highly beneficial. This study was designed to investigate the expression of EphA2, to assess its suitability as a female breast cancer target, and to provide a preliminary basis for the future construction of fluorescent-targeted tracers for use in fluorescence-guided mastoscopic breast-conserving surgery.

Materials and methods

Experimental specimens

From 2013 to 2018, a total of 250 postoperative pathologic specimens were acquired from female breast cancer patients at The First Affiliated Hospital of Dali University who had undergone radical mastectomies or modified radical mastectomies and had not been subjected to radiotherapy, chemotherapy, or any other special treatments before surgery. The patients were 26 to 74 years old: 88 patients were ≤45 years old, and 162 patients were >45 years old. The tumor sizes were <5 cm in 153 cases and ≥5 cm in 97 cases. There were 216 cases of invasive ductal carcinoma (IDC), with 34 cases being other pathologic types, including ductal carcinoma in situ (DCIS], mucinous carcinoma, or invasive lobular carcinoma (ILC). In addition, lymphatic metastasis had occurred in 162 cases but not in 88 cases. There were 25, 118, and 107 cases at TNM stages 0 to I, stage II, and stages III to IV, respectively, and 33, 148, and 69 cases corresponding to histologic grades I, II and III, respectively (**Table 1**). The 250 postoperative pathologic specimens were fixed with paraformaldehyde, embedded in paraffin, and subjected to serial sectioning at thickness of 4 mm.

Main reagents

The following kits and reagents were used in the study: Ready-to-Use Immunohistochemistry (IHC) UltraSensitiveTM SP Kit (Fuzhou Maixin Biotech. Co., Ltd., Fuzhou, China), rabbit anti-human EphA2 polyclonal antibody (Abcam, Cambridge, UK), Universal In Situ Hybridization (ISH) Detection Kit III (alkaline phosphatase, ALP) (Wuhan Boster Bio-Engineering Co., Ltd., Wuhan, China), and digoxigenin-labelled cRNA probe for human EphA2 (Fuzhou Maixin Biotech. Co., Ltd.). D-Hank's balanced salt solution, collagenase, pancreatin, phosphate-buffered saline (PBS), paraformaldehyde, Triton X-100, fetal bovine serum, rabbit anti-EphA2 antibody (Catalogue No. 34-7400), and FITC-labelled goat anti-rabbit Ig (G+M) antibody (Catalogue No. A16097) were obtained from ThermoFisher Scientific (Waltham, MA, USA).

Immunohistochemistry (IHC) and in situ hybridization (ISH) detection and interpretation

Pathologic sections from 250 breast cancer cases were subjected to IHC staining, 3, 3-diaminobenzidine (DAB) colour development, haematoxylin counterstaining, and neutral gum mounting before being observed under a light microscope. PBS was used in place of the primary antibody in the negative control. Positive EphA2 protein expression in breast cancer pathological specimens was indicated by brownish-yellow or brown staining of the cytoplasm and cell membranes. For each slice, five microscope fields at high power were selected, and the cells were counted. More than 500 cells were counted in each field. The proportion of stained tumor cells was used to distinguish between positive and negative expression. If the proportion of cells stained with DAB in each

of the five fields at high power was higher than 20%, they were considered to be positive for EphA2 protein expression. Otherwise, cells were considered negative for EphA2 protein expression. Subsequently, in situ hybridisation (ISH) was carried out on the sections of 250 breast cancer cases. After 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) color development, nuclear fast red counterstaining and neutral gum mounting, the sections were observed under a light microscope. Unlabelled probe hybridisation solution was used in place of the probe as the negative control. Purple or purple-blue staining of the cell membrane and cytoplasm indicated positive expression of EphA2 mRNA. The experimental results were interpreted by a team of two senior pathologists.

Isolation and culture of breast cancer cells

The normal and cancer breast tissue samples obtained were cleaned with D-Hank's balanced salt solution to remove surface blood stains, and tissue not required for culture was removed with surgical forceps. Afterwards, the tissues were re-cleaned and cut into several small pieces using a scalpel. The pieces were then put into a 5 ml centrifuge tube, and the required amount of buffer solution was added. The tissue pieces were cut repeatedly to about 1 mm in size with elbow ophthalmic scissors to form a paste. The paste was left to settle, and then the supernatant was drawn up with a pipette. An appropriate amount of buffer solution was added to wash the tissue again, and the mixture was passed through a 200-mesh screen to collect the filtrate. The tissue retained by the screen was digested with 0.25% collagenase, and the mixture was shaken at 37°C for 1 h. After digestion was complete, the fluid was passed through the screen, and the liquid containing cells was collected. The collected liquids were combined into a 50 ml centrifuge tube and centrifuged at 1000 rpm for 5 min. The supernatant was removed, and the cell number was adjusted to $2\text{-}5\times10^5$ cells/ml with culture medium. The mixture was then distributed into culture flasks and placed in an incubator with 5% CO_2 and cultured statically at 37°C. Generally, the primary cultured cells adhered to the bottom of the culture flask after 3 to 5 days and stretched to start growing. Then, new culture medium half the volume of the original culture medium was added, followed by further culture for 2 to 3 days before changing the medium. Normally, the bottom of the culture flask was completely covered after 7 to 14 days, which was a suitable time for subculturing. The repeated attachment method was applied to gradually remove the fibroblasts from the cells and purified normal breast and breast cancer cells were eventually obtained.

Identification of breast cancer cells

The isolated and cultured normal breast and breast cancer cells were digested with pancreatin, counted, and inoculated in a 48-well plate. After the cells were cultured overnight, the expression of EphA2 was detected by IFA, and positive expression was indicated by green fluorescence. The procedure for IFA was as follows. The culture medium was discarded, and the cells were washed three times with PBS. Paraformaldehyde (4.0%) was added for 10 min to fix the cells, and then the cells were washed three times with PBS. The cells were permeabilised with 0.1% Triton X-100 for 10 min, and the cells were then sealed by incubation in PBS containing 10% foetal bovine serum at 37°C for 1 h. A 1:1000 dilution of rabbit anti-EphA2 primary antibody (Invitrogen, Catalogue No. 34-7400) was added, and the cells were incubated at 37°C for 1 h, followed by three washes with PBS. FITC-labelled goat anti-rabbit Ig (G+M) antibody (Thermo-Fisher, Catalogue No. A16097) was added, and the cells were incubated at 37°C for 1 h. After three PBS washes, we observed the cells with a fluorescence microscope and recorded the images.

Statistical methods

SPSS software (IBM, Armonk, NY, USA) was used to process the data, and the χ^2 test was used for sample rate comparison and correlation analysis. Differences were statistically significant if $P<0.05$.

Results

EphA2 protein and mRNA expression in pathological specimens of breast cancer tissue

Positive staining for the EphA2 protein was indicated when the membrane and cytoplasm of breast cancer and vascular endothelial cells were stained brownish-yellow or brown. Normal

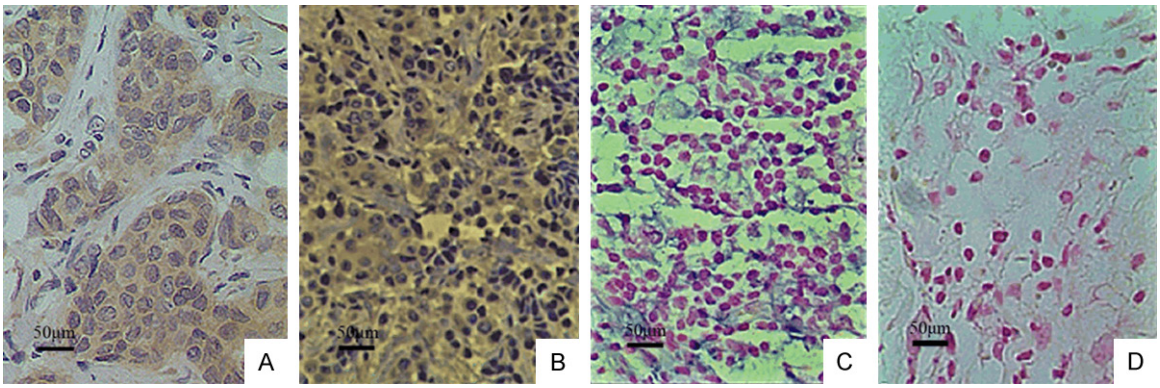

Figure 1. A. Positive expression of EphA2 protein in breast cancer (IHC×200); B. Negative control of EphA2 protein (IHC×200); C. Positive expression of EphA2 mRNA protein in breast cancer (ISH×200); D. Negative control of EphA2 mRNA (ISH×200).

Table 2. Correlation between EphA2 protein expression in breast cancer specimens and clinicopathologic factors

Clinicopathologic factor	Expression of EphA2 protein		χ^2	*p* (n=250)
	+ (%)	- (%)		
Age				
≤45 years old	72 (81.8)	16 (18.2)	3.549	0.060
>45 years old	115 (71.0)	47 (29.0)		
Tumor size				
<5 cm	110 (71.9)	43 (28.1)	1.765	0.184
≥5 cm	77 (79.4)	20 (20.6)		
Histologic type				
Invasive ductal carcinoma	165 (76.4)	51 (23.6)	2.127	0.145
Other types	22 (64.7)	12 (35.3)		
TNM stage				
0-I stage	12 (48.0)	13 (52.0)	16.303	0.000
II stage	84 (71.2)	34 (28.8)		
III-IV stage	91 (85.0)	16 (15.0)		
Lymphatic metastasis				
+	137 (84.6)	25 (15.4)	23.296	0.000
-	50 (56.8)	38 (43.2)		
Histologic grade				
I	18 (54.5)	15 (45.5)	9.119	0.010
II	118 (79.7)	30 (20.3)		
III	51 (73.9)	18 (26.1)		

breast tissues were used as negative controls, and staining did not produce any colour. The positive expression of EphA2 mRNA was indicated by purple or purple-blue staining, and normal breast tissue, which was not stained, was used as the negative control (**Figure 1A-D**). Among the postoperative pathologic specimens from 250 breast cancer patients, 187 and 209 cases exhibited positive EphA2 protein expression and positive EphA2 mRNA expression, respectively; the corresponding positive rates were 74.80% and 83.60%, respectively.

Correlation between EphA2 protein and mRNA expression and clinicopathologic factors in breast cancer specimens

The positive expression rates of EphA2 protein and EphA2 mRNA in pathological breast cancer specimens did not correlate with patient age, tumor size, or histologic type (P>0.05), but they did correlate with lymphatic metastasis, clinical stage, and histologic grade (P<0.05) (**Tables 2** and **3**).

Correlation between EphA2 mRNA and EphA2 protein expression in breast cancer specimens

The rate of positive EphA2 protein expression correlated with that of the EphA2 mRNA (P< 0.05) (**Table 4**). Among the 250 specimens, the coincidence rate of positive expression of EphA2 mRNA and EphA2 protein was 87.20%, whereas that of negative expression was 27.00%.

Table 3. Correlation between EphA2 mRNA expression in breast cancer specimens and clinicopathologic factors

Clinicopathologic factor	Expression of EphA2 mRNA		χ^2	*p* (n=250)
	+ (%)	- (%)		
Age				
≤45 years old	76 (86.4)	12 (13.6)	0.757	0.384
>45 years old	133 (82.1)	29 (17.9)		
Tumor size				
<5 cm	127 (83.0)	26 (17.0)	0.101	0.750
≥5 cm	82 (84.5)	15 (15.5)		
Histologic type				
Invasive ductal carcinoma	182 (84.3)	34 (15.7)	0.503	0.478
Other pathological types	27 (79.4)	7 (20.6)		
TNM stage				
0-I stage	13 (52.0)	12 (48.0)	20.648	0.000
II stage	101 (85.6)	17 (14.4)		
III-IV stage	95 (88.8)	12 (11.2)		
Lymphatic metastasis				
+	145 (89.5)	17 (10.5)	11.709	0.001
-	64 (72.7)	24 (27.3)		
Histologic grade				
I	19 (57.6)	14 (42.4)	19.816	0.000
II	127 (85.8)	21 (14.2)		
III	63 (91.3)	6 (8.7)		

Table 4. Correlation between EphA2 mRNA and protein expression in breast cancer specimens

EphA2 protein	EphA2 mRNA		Total	χ^2	*p*
	+	-			
+	163	24	187	6.882	0.009
-	46	17	63		
Total	209	41	250		

Isolation and culture of breast cancer cells

In vivo observation with an inverted microscope showed that both normal breast cells and breast cancer cells grew well in culture. The breast cancer cells grew fast, took on fusiform, polygon, and other forms, and gathered into a nest structure (**Figure 2A**, **2B**).

Identification of breast cancer cells

IFA detection indicated that there was barely any EphA2 expression in normal breast cells, whereas EphA2 was highly expressed in breast cancer cells (**Figure 2C**, **2D**).

Discussion

High EphA2 expression in breast cancer tissue leads to the occurrence and development of breast cancer

Many studies have proven that EphA2 is highly expressed in esophageal cancer, lung cancer, and other cancers, and its expression is consistent with cancer progression [4-6]. In this study, high expression of the EphA2 protein and EphA2 mRNA was observed in breast cancer tissues, with positive rates of 74.80% and 83.60%, respectively. EphA2 participates in regulating the growth and invasion of tumor cells and angiogenesis; however, the mechanisms underlying EphA2 overexpression in breast cancer remain unclear. Unstable cell-cell contact may reduce the ability of EphA2 to bind to its membrane-immobilised ligand, leading to reduced ligand-mediated degradation and overexpression of EphA2. Alternatively, EphA2 overexpression may be due to enhanced gene expression and/or improved protein stability [7, 8]. The mechanisms of EphA2 regulation in cancer cells have not yet been fully elucidated, but EphA2 is a direct transcriptional target of the Ras-MAPK pathway. Studies have shown that the presence of EphA2 alone can cause carcinogenesis [9]. Furthermore, elevated levels of EphA2 have been observed in invasive breast cancer-derived cell lines. The EphA2-EphrinA1 signaling axis is crucial for the malignant transformation of normal cells; the main downstream molecules of this signaling axis, including phosphatidylinositol 3-kinase (PI3K), Src family kinases, ρ and Rac1 GTPases, mitogen-activated protein kinases (MAPKs), integrin, and other oncogenic receptors (such as EGFR), regulate cell adhesion, cytoskeletal structure, and the development of cell proliferation/migration and angiogenesis. Elevated levels of EphA2 expression are also related to the inhibi-

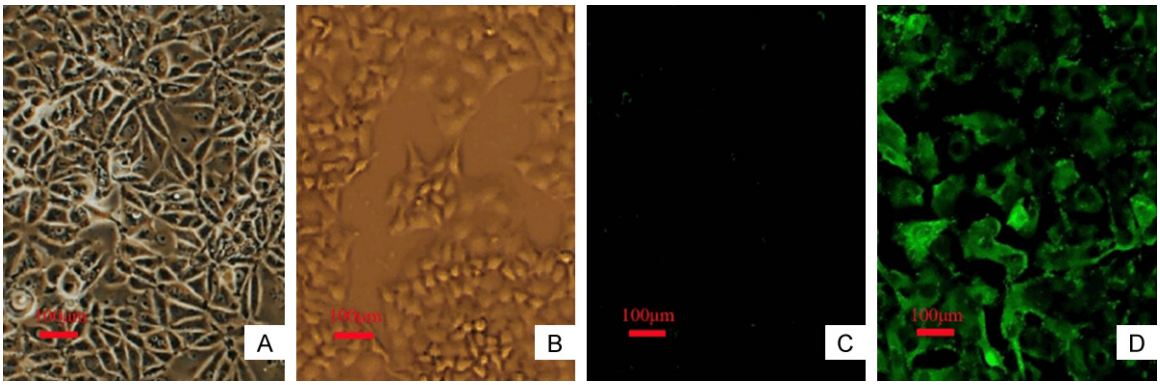

Figure 2. A. Normal breast cells (×100); B. Breast cancer cells (×100); C. EphA2 detection in normal breast cells (IFA×100); D. EphA2 detection in breast cancer cells (IFA×100).

tion of Ras and related protein kinase B (Akt) activities. During tumorigenesis, the normal EphA2-EphrinA1 signalling pathway is interrupted as a result of a loss of cell contact, leading to the overexpression of EphA2 and the transduction of oncogenic signals. The interruption of this signalling pathway is related to several key aspects of tumorigenesis, such as cytoskeleton regulation; cell adhesion, metastasis, and proliferation; and angiogenesis [10]. EphA2 can also influence cancer progression through its interaction with the Ras/ERK pathway because EphA2 has positive and negative regulatory effects on ERK [11]. Interactions between EphA2 and many proteins involved in cell migration, including Src, FAK, GTPase, and AKT, have also been observed. In addition, EphA2 facilitates tumor growth by promoting glutamine metabolism. Glutamine metabolism in breast tumors relies on glutamine uptake, mainly conducted by the neutral amino acid transporter SLC1A5, and the utilisation of glutamine by glutaminase (GLS) through conversion into glutamate metabolites. The HER-2-positive breast cancer subtype is highly dependent on glutamine metabolism, which may be caused by the upregulation of HER-2-dependent GLS and SLC1A5. In the HER-2-positive breast cancer model, EphA2 promotes glutamine metabolism, which facilitates tumor growth [12]. There is strong evidence for the idea that SLC1A5 is an important factor in breast cancer tumor growth. The high levels of EphA2 expression in breast cancer cells detected by IFA in this study further advance the hypothesis that EphA2 is involved in the occurrence and development of breast cancer.

EphA2 participates in breast cancer angiogenesis and promotes invasion and metastasis

In this study, we found that EphA2 was mainly expressed in breast cancer vascular endothelial cells, suggesting that it may participate in angiogenesis and promote the invasion and metastasis of breast cancer cells. The overexpression of EphA2 in tumors is significantly associated with high levels of angiogenesis markers, especially VEGF. With its ligand-independent activity, EphA2 has been found to be involved in multiple communication streams with other cellular molecular networks (including EGFR, FAK, and VEGF pathways) and synergises with these networks to stimulate the invasion and metastasis of tumor cells. EphA2 regulates tumor-related angiogenesis, which is necessary for tumor survival and growth, and this regulation occurs through the PI3K signalling pathway. The overexpression of EphA2 during breast tumorigenesis is also related to angiogenesis and microvessel density [2, 13]. This study found that the expression of EphA2 and EphA2 mRNA was correlated with lymph node metastasis, clinical stage, and histologic grade ($P<0.05$), suggesting that EphA2 may be related to the malignancy, progression, and metastasis of breast cancer. Malignant cells often display decreased cell adhesion, which is mediated by the loss of E-cadherin function, leading to a reduced chance of interaction between expressed receptors and ligands in adjacent cells. The phosphorylation of EphA2 and the location of cell contact points depend on the expression and function of E-cadherin; in cancer, these are the main causes of disrupted epithelial cell-cell contacts, leading to tumor

metastasis and invasion. In breast cancer cells, the downregulation of E-cadherin expression affects the growth and adhesion of tumor cells by influencing EphA2 [3]. Studies found that EphA2 Ser-897 phosphorylation is mainly expressed in grade IV human glioma specimens, particularly in growth factor-enriched and invasive cell areas; moreover, EphA2 Ser-897 phosphorylation expression was detected in tumors that had infiltrated adjacent normal brain tissue [14]. This supports the hypothesis that EphA2 promotes tumor cell migration. A direct consequence of EphA2 stimulation is the loss of extracellular matrix (ECM) attachment, which leads to tumor migration and invasion [15]. Matrix metalloproteinases (MMPs) play important roles in cancer invasion and metastasis, and their main effect is the degradation of the ECM and basement membranes. MMP-9 causes tumor metastasis by degrading denatured collagen and type IV collagen, both of which are major structural components of the ECM. In hepatocellular carcinomas, EphA2 and MMP-9 expression are positively correlated [9]. Brantley-Sieders et al. pointed out that EphA2 expression levels increase with degree of breast cancer malignancy [16]. Furthermore, studies have shown that relatively high expression levels of EphA2 mRNA are significantly associated with lower overall survival rates [17]. In HER-2-positive breast cancer patients, increased EphA2 mRNA levels are associated with a decrease in overall disease-free survival. In the breast cancer specimens analysed in this study, positive expression of the EphA2 protein was correlated with the expression of EphA2 mRNA ($P<0.05$). Among the 250 breast cancer specimens, the coincidence rate of positive EphA2 protein and EphA2 mRNA expression was 78.00%, whereas that of negative expression was 41.5%. The expression of EphA2 and its mRNA in breast cancer specimens was correlated, implying that many different transcriptional and post-translational modifications are involved in EphA2 regulation in breast cancer tissues. At present, dasatinib, which is used in the in vitro treatment of breast cancer cells, reduces the expression and phosphorylation of EphA2 and the activities of related kinases. A subset of EphA2 antibodies strongly responds to breast (MDA-231) cancer cells but not to normal immortalized breast (MCF-10A) cells, indicating that EphA2 has potential as a therapeutic target. The evidence described above, and our research findings, suggest that EphA2 is related to the occurrence, development, and metastasis and invasion of breast cancer and may be a new target for breast cancer.

EphA2 as a new target for breast cancer: providing a preliminary basis for fluorescence-guided mastoscopic breast-conserving surgery

Although breast-conserving surgery has reduced the amount of breast tissue removed during treatment, issues with obvious scarring and poor breast shape retention remain. Mastoscopic breast-conserving surgery has several advantages, including small or hidden incisions, good cosmetic effects, the facilitation of sufficient margins for tumor resection, and better precision [18, 19]. Moreover, by suspending the skin or building an air cavity, compression of the tumor can be avoided to achieve the principle of “no contact”, which is recommended in tumor surgery. Mastoscopic breast cancer surgery also has a lower risk of circulating tumor cell (CTC)-associated metastasis [20]. Mastoscopic precision and minimally invasive surgery are the overall aims of breast cancer surgery developments. Breast-conserving surgery requires a safe negative margin after complete resection of the primary lesion because a positive margin will lead to increased local recurrence and postoperative mortality rates. In addition, successful breast-conserving surgery should ensure a safe surgical margin to achieve certain cosmetic effects. Expanding the area of surgical resection not only fails to reduce the local recurrence rate and improve the overall survival rate but also results in much worse cosmetic outcomes. At present, there is no universal standard for a safe surgical margin width; therefore, the accurate determination of the surgical margin is a key issue in breast-conserving surgery. The currently used methods of molybdenum target x-rays and breast color Doppler ultrasound examination for preoperative positioning have low accuracy, whereas breast MRI is limited in its ability to provide real-time intraoperative guidance. Furthermore, mastoscopic surgery is more challenging for surgeons than open surgery. Therefore, the construction of a targeted fluorescent tracer that enables real-time fluorescence imaging of breast cancer tumors would be highly beneficial in helping doctors to accurately determine surgical margins. Optical

molecular imaging, mainly applied for intraoperative guidance and surgical navigation, allows the assessment of tumor resection margins to improve the negative margin rate. Fluorescence-guided surgery also improves the quality of tumor resection during surgery. Currently, there are a number of targeted tracers for various tumors under research and development. Rituximab is a specific humanized monoclonal antibody raised against CD20 molecules on the membrane of B lymphocytes in the lymph nodes, and a novel indocyanine green-rituximab-targeted fluorescent tracer has also been successfully used for imaging sentinel lymph nodes in breast cancer. Vascular Endothelial Growth Factor A (VEGFA) is overexpressed in locally advanced rectal cancer (LARC), and bevacizumab can target vascular VEGFA. A research team has confirmed the application potential of the near-infrared targeted fluorescent tracer bevacizumab-800cw [21], which can be used to target VEGFA in backtable fluorescence-guided imaging for margin evaluation in LARC patients, which may lead to more reliable intraoperative evaluations of rectal cancer resection margins in the future. NTSR1, a pancreatic cancer marker [22], is overexpressed in 75%-88% of pancreatic cancers and not expressed in healthy pancreas or during pancreatitis. Based on the structure of the NTSR1 agonist, a series of targeted fluorescent tracers were synthesized, which successfully delineated cancer cells in the surrounding tissues in the preclinical model of pancreatic cancer and facilitated precise tumor resection under fluorescence guidance. In this study, IFA showed that EphA2 was highly expressed in breast cancer tissue and barely expressed in normal breast tissue. IFA detection indicated that there was barely any EphA2 expression in normal breast cells, whereas there was high expression of EphA2 in breast cancer cells. Therefore, in the future, a fluorescent-targeted tracer could be constructed by coupling an anti-EphA2 antibody with a fluorescence imaging agent. Performing intraoperative fluorescence guidance for precise resectioning in mastoscopic breast-conserving surgery using optical molecular imaging is feasible.

In conclusion, EphA2 has excellent potential as a new breast cancer target. This research provides a preliminary basis for the construction of new targeted breast cancer drugs and fluorescent-targeted tracers for fluorescence-guided mastoscopic breast-conserving surgery.

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Disclosure of conflict of interest

None.

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