Original Paper

Diagnostic Value of ER, PR, FR and HER-2-Targeted Molecular Probes for Magnetic Resonance Imaging in Patients with Breast Cancer

Yin-Hua Jin^a Qi-Feng Hua^a Jian-Jun Zheng^a Xue-Hua Ma^b Tian-Xiang Chen^b
Shun Zhang^c Bin Chen^a Qi Dai^a Xiao-Hui Zhang^a

^aDepartment of Radiology, Ningbo No. 2 Hospital, Ningbo, ^bNingbo Institute of Materials Technology & Engineering, Chinese Academy of Sciences, Ningbo, ^cThe Stem cells and Regenerative Medicine Laboratory, Ningbo No. 2 Hospital, Ningbo, China

Key Words

Magnetic resonance imaging • Molecular probe • Estrogen receptor • Progesterone receptor • Folate receptor • Human epidermal growth factor receptor-2 • Breast cancer

Abstract

Background/Aims: Smart molecular probes are required in the application of Magnetic resonance imaging (MRI) for biochemical and clinical research. This study aims to investigate the diagnostic values of estrogen receptor (ER), progesterone receptor (PR), folate receptor (FR) and human epidermal growth factor receptor 2 (HER-2)-targeted molecular probes in the MRI diagnosis of breast cancer. **Methods:** Initially, a total of 508 female breast cancer patients were selected for breast cancer subtype classification by immunohistochemistry. Subsequently, the tumor size, lymph node metastasis, and histological grade of different breast cancer subtypes were compared. Molecular probes of Ab-ER-USPIO, Ab-PR-USPIO, Ab-FR-USPIO and Ab-HER-2-USPIO were constructed and screened. The specific binding of molecular probes to breast cancer cells was detected both *in vitro* and *in vivo* by Prussian blue staining and MRI using T1 and T2 weighted images. Finally, *in vivo* toxicity of Ab-HER-2-USPIO was analyzed using hematoxylin and eosin staining. **Results:** We identified the following subtypes of breast cancer: Luminal A (ER-positive, FR-positive, HER-2-negative), Luminal B (ER-positive, FR-positive, HER-2-positive), HER-2 overexpression (ER-negative, FR-negative, HER-2-positive), and triple-negative breast cancer (ER-negative, FR-negative, HER-2-negative). Featuring favorable *in vitro* biocompatibility and low *in vivo* toxicity, Ab-HER-2-USPIO can specifically bind to breast cancer cells BT47 and SKBR3, thus enhancing the quality of T1 weighted MRI images. **Conclusion:** The results indicate that HER-2-targeted MRI molecular probes may be used in the clinical diagnosis of breast cancer and facilitate the development of promising strategies for breast cancer treatments.

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Dr. Jian-Jun Zheng

Department of Radiology, Ningbo No. 2 Hospital
No. 41, Northwest Road, Haishu District, Ningbo 315010, Zhejiang Province (China)
Tel. +86-0574-83870280, E-Mail zhengjianjun280@163.com

Introduction

As a heterogeneous and hormone-dependent disease, breast cancer is one of the three most common cancers worldwide and is a leading cause of cancer-related death in women [1-3]. The incidence of breast cancer is substantially higher in postmenopausal females [4]. Each year, over one million new cases of breast cancer are diagnosed worldwide, which lead to over 400,000 deaths annually [5, 6]. As a strong risk factor of breast cancer, obesity has been shown to exert an adverse effect on the prognosis in breast cancer patients [7]. In postmenopausal women, adipose tissue is the primary source of estrogen, a key factor related to the tumorigenesis of breast cancer [4]. Although multi-modality treatments like targeted therapy, radiation therapy, and chemotherapy are highly useful in cancer treatments, they are limited by the risk of cardiac toxicity, especially when they are used in combination [8]. Patients undergoing these treatments may also experience various symptoms, which can reduce life quality and impair the normal functions of the body [9]. Therefore, it is necessary to have a better understanding of breast cancer to improve its diagnosis and treatment.

Molecular imaging (MI), an emerging field of novel research, aims to advance our understanding of key cellular and molecular events, including pathologic and normal processes, using noninvasive *in vivo* probes [10]. As a modality of MI, magnetic resonance imaging (MRI) plays a critical role in the treatment of cancers from multiple perspectives. In particular, hybrid nanoparticles or targeted MRI probes are being developed to facilitate the diagnosis and treatment of tumor [11, 12]. In addition, MI is helpful in clarifying the effects of drug actions at the preclinical and clinical stages of drug development [13].

Estrogen receptor (ER) regulates the proliferation of both neoplastic and normal breast epithelial, as well as the proliferation of breast cancer cells [14, 15]. Progesterone modulates the transcription of target genes by interacting with intracellular progesterone receptor (PR), which is a member of the nuclear receptor superfamily of transcription factors [16]. A previous study has demonstrated the role of progesterone in promoting the functional recovery and decreasing the lesion volume of breast cancer [17]. Human epidermal growth factor receptor 2 (HER-2) is regarded as a critical marker to evaluate the efficacy of targeted therapy and to predict the prognosis of breast cancer [18]. HER-2 is overexpressed in 30% of breast cancer patients and acts as an independent indicator for poor prognosis of breast cancer [19]. Folate receptor (FR) is highly expressed in a variety of malignancies and serves as a valuable therapeutic target in many types of cancers, including breast cancer and ovarian cancer. The quantification of functional FRs has promoted the clinical development of folate-targeted cancer therapies [20-22]. In this study, ER, PR, FR and HER-2 were selected as probe targets to evaluate their values in the diagnosis of breast cancer by MRI.

Materials and Methods

Ethical statements

This study was carried out upon the approval of the Ethics Committee of Ningbo No. 2 Hospital. Written informed consent was obtained from all patients. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and the Declaration of Helsinki.

Study subjects

This study enrolled 508 female breast cancer patients with a mean age of (44.40 ± 7.22) years who were admitted to Ningbo No. 2 Hospital from January 2014 to January 2016. All patients were pathologically diagnosed with invasive breast cancer, and were examined by magnetic resonance diffusion-weighted imaging (MR-DWI) before surgery. The inclusion criteria for this study were: 1) patients pathologically diagnosed with breast cancer who had complete medical records; 2) patients with no history of breast implants, mastitis, radiotherapy, chemotherapy, or breast surgeries; 3) patients undergoing adjunctive

treatments recommended by the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology [23]; 4) patients voluntarily participating in this study and having signed the form of written informed consent. The exclusive criteria of this study included: 1) patients without no pathological diagnosis; 2) patients with a gap of over one month between the time of MR-DWI examination and the time of surgery; 3) patients suffering from recrudescence and/or metastasis of breast cancer after treatment; 4) patients with a history of mental disease or failed to cooperate in the study; 5) patients in the period of pregnancy or lactation.

Immunohistochemistry

Immunohistochemistry was used in this study to detect the levels of ER, PR and FR in collected samples of tumor tissues. The judgment standard of HER-2 was in accordance with Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer [24]. Specimens of breast cancer tissues were sent to the Department of Pathology within 30 min of harvesting for evaluation. During the immunohistochemistry, the specimens were first cut into pieces of 5 mm in thickness, fixed for 6 h - 48 h in 10% neutral buffered formalin using around 10 times the volume of the specimens, embedded in paraffin, and subsequently sliced into thin sections. The monoclonal antibodies for ER (BM0345, BOSTER Biological Technology Ltd., Wuhan, China), PR (PB0077, Shanghai Haling Biotechnology Co., Ltd., Shanghai, China), FR (ab221543, Abcam, MA, USA), and HER-2 (TL-503, Beijing T&L Biological Technology Co., Ltd., Beijing, China) were used in conjunction with a Leica BOND-MAX Automated immunohistochemistry staining system. The positive signal of ER and PR products was localized in the nucleus. The samples with over 10% cancer cells expressing the hormone receptor markers were regarded as ER- and PR-positive [25]. According to the expression of ER, PR and HER-2 in the tumor tissues, breast cancer was divided into four different molecular subtypes, including Luminal A (ER-positive and/or PR-positive, HER-2-negative), Luminal B (ER-positive and/or PR-positive, HER-2-positive), HER-2 overexpression (ER-negative, PR-negative, HER-2-positive), and triple-negative breast cancer (TNBC; ER-negative, PR-negative, HER-2-negative).

Construction of molecular probes

BIO-r-C (Beijing Oneder High-Tech Co., Ltd., Beijing, China) ultra-small superparamagnetic iron oxide (USPIO) (kernel: Fe_3SO_4 nanoparticle; surface modification: carboxyl; particle diameter: (10.3 ± 1.6) nm; Fe concentration: 3.5 mg/mL; pH value: neutral) was placed into a 2 mL Eppendorf (EP) tube. Fresh EDC/sulfo-NHS (0.5 mg of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and 1.4 mg of N-hydroxysulfo succinimide; 24510, Shanghai Haoyang Biotechnology Co., Ltd., Shanghai, China) was dissolved in 100 μL deionized water, and subsequently reacted with BIO-r-C at room temperature for 15 min. To identify activated BIO-r-C, the buffer was exchanged using a PD-10 exchange column with NaHCO_3 (0.1 M) as eluent. In the next step, the monoclonal antibodies of Ab-ER, Ab-PR, Ab-HER-2, and Ab-FR (each for 0.5 mg) were added respectively for coupling reaction. After 4 h of reaction, phosphate buffered saline (PBS) was added and incubated overnight for dialysis. After the coupling reaction, the reaction products were preserved in a refrigerator at 4°C and washed twice (5 min each) with sterile PBS before use.

Cell culture

Breast cancer cell lines BT474 (ER-positive, PR-positive, HER-2-high expression), SKBR3 (ER-negative, PR-negative, HER-2-high expression), ZR75B (ER-positive, PR-negative, HER-2-negative), MDAMB231 (ER-negative, PR-negative, HER-2-low expression) and a normal breast cell line were purchased from Shanghai Meixuan Biological Science and Technology Ltd. (Shanghai, China). The cell lines were cultured in a Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotics. The cells were harvested in the logarithmic phase by 3 min of digestion using 0.25% trypsin (1 mL). Subsequently, trypsin was neutralized by adding a serum-containing medium. The density of the cells was adjusted to 1×10^5 cells/mL. Subsequently, the cells were seeded into a 6-well plate with bottom covered by slide glass, and cultured for 24 h. After cell adherence, the cells were fixed by acetone for 30 min for subsequently experiments.

Prussian blue staining

Cells were harvested in the logarithmic phase by trypsin digestion and subsequently made into a single cell suspension. After cell counting, the cell density was adjusted to 5×10^4 cells/mL. The cells were then

added into a 24-well plate (1 mL per well) and cultured in an incubator at 37°C. When the cells reached over 80% confluence, the 24-well plate was taken out from the incubator and the medium was discarded. In the next step, Ab-HER-2-USPIO, bovine serum albumin (BSA), and BSA-USPIO (200 µL each) were added into each well and the plate was incubated in the refrigerator at 4°C for 2 h. Subsequently, the cells were washed twice with PBS and then fixed in 4% paraformaldehyde (300 µL) at room temperature for 30 min, and washed with PBS (500 µL/well) for 3 × 5 min. In the next step, 2% potassium ferrocyanide and 2% HCl were mixed (1 : 1), and 200 µL of the mixture were added into each well and incubated for 30 min, followed by 3 × 5 min PBS (500 µL/well) washing. A light microscope was then used to observe the samples and acquire images.

In vitro MRI

Ab-HER-2-USPIO samples containing different concentrations of Fe (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mM) were dispersed respectively in a 1% agarose solution, cooled and fixed. In the next step, BT474 and SKBR3 cells were seeded in a 6-well plate (1×10^5 cells/well) and incubated with Ab-HER-2-USPIO. After 24 h of culturing, the culture medium was collected and fresh culture media containing Ab-HER-2-USPIO of different Fe concentrations were added into the cells, followed by another 24 h of culturing. Subsequently, the medium was extracted, followed by twice PBS washing to remove the nanoparticles not absorbed by the cells. The cells were then detached from the plate bottom using trypsin/ethylenediamine tetraacetic acid (EDTA) digestion. After PBS washing, the cells were centrifuged at 1000 rpm/min for 5 min to collect the pellet, which was then resuspended in a 1% agarose solution, cooled, fixed, and evaluated by MRI.

Model establishment

A total of 30 BALB/c specific-pathogen free (SPF) female nude mice aging 5 - 6 weeks were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All mice were housed under SPF conditions in an animal room at Guangdong Medical Experimental Animal Center (Guangdong, China) equipped with an IVC system. The room temperature (20 - 25)°C, relative humidity (40 - 70)%, and ad libitum access to food and water were maintained thorough the experiment. SPF feed was made in Guangdong Medical Experimental Animal Center (Guangdong, China). The Walker-256 breast cancer cells were harvested in the logarithmic phase by trypsin digestion, washed twice with a Hank's balanced salt solution, stained by 1% trypan blue, and counted using a cell counter. The density of living cells was adjusted to 5×10^7 cells/mL. A total of 15 nude mice received subcutaneous inoculation (0.2 mL each) at left axilla. After tumor cell inoculation, the growth of tumor cells was observed daily. The other 15 nude mice received no intervention and served as the control.

In vivo MRI

The breast cancer-bearing mice were imaged on a 3.0-T Clinical Scanner for MRI to test the feasibility of using Ab-HER-2-USPIO during *in vivo* MRI imaging. The tumor-bearing mice were treated with intraperitoneal injection of 0.3% pentobarbital sodium and fixed in a prone position, with the bilateral breasts located naturally in the coil groove and the chest wall in contact with the coil. The scanning range included bilateral breast tissues, axilla and the corresponding horizontal anterior part of thorax with T1 and T2 weighted imaging on the MRI Apparatus. Subsequently, each mouse was given an intra-tumor injection of 100 µL PBS containing Ab-HER-2-USPIO (500 µg/mL). Two hours later, the mice were subjected to T1 and T2 weighted imaging again.

In vivo toxicity test

The mice injected with Walker-256 breast cancer cells were treated with tail vein injection of Ab-HER-2-USPIO (100 µL, 5 mg/mL). Normal healthy mice were used as control and were treated with tail vein injection of PBS (100 µL), once every four days. Their activity, such as eating and sleeping patterns, and body weight were observed for 14 days. During the observation, there was no death, weight loss or other abnormal behaviors. To further examine the *in vivo* toxicity of Ab-HER-2-USPIO, the mice were sacrificed on the 14th day. The organs, including heart, liver, spleen, lung, kidney, and small intestine, were collected, fixed in 4% formaldehyde, embedded in paraffin, sliced into sections, stained by hematoxylin and eosin, and observed under a Leica DMI3000 optical microscope (Leica, Germany).

Statistical analysis

SPSS 19.0 software (IBM Corp. Armonk, NY, USA) was used for data analysis. Measurement data were expressed as mean $\pm$ standard deviation, and numeration data were expressed as the number of cases or as percentages (%). Comparisons between two groups were conducted using *chi-square* tests. A *p* value of < 0.05 was considered as statistically significant.

Results

Clinical data of breast cancer patients

At first, we analyzed the general information of the 508 female breast cancer patients pathologically diagnosed with invasive breast cancer and undergoing MR-DWI before surgery. Among these patients, there were 116 cases of Luminal A, 176 cases of Luminal B, 96 cases of HER-2 overexpression, and 120 cases of TNBC. There was no significant difference among different breast cancer subtypes (all $p > 0.05$) in terms of age, tumor size, and lymph node metastasis ($p = 0.2741$; $p = 0.1041$; $p = 0.0503$). However, the histological grades of the patients were significantly different among different breast cancer subtypes. The percentage of grade I tumor tissues in Luminal A and Luminal B patients was obviously higher than that in the patients with HER-2 overexpression and TNBC, and the percentage of grade III tumor tissues in Luminal A and Luminal B patients was significantly lower than that in the HER-2 overexpression and TNBC patients ($p < 0.0001$) (Table 1).

The expression of HER-2 in Luminal A, Luminal B and TNBC breast cancer

Subsequently, we performed immunohistochemistry to measure the expression of ER, PR, FR and HER-2 in the breast cancer tissues (Fig. 1). The positive signal of ER and PR products was located in the nucleus, whereas the positive signal of HER-2 product was observed on the cell membrane as brownish-yellow or brownish particles. The positive staining of FR was mainly located in the cytoplasm and some positive signals of FR could be observed on the cell membrane. In Luminal A breast cancer, the expression of ER, PR and FR was positive while the expression of HER-2 was negative. In Luminal B breast cancer, the expression of ER, FR, PR and HER-2 was all positive. HER-2 overexpression breast cancer showed negative ER and PR, yet positive HER-2 and FR. In TNBC, the expression of ER, PR and HER-2 was negative but the expression of FR was positive.

Fig. 1. Immunohistochemical images of HER-2 expression in Luminal A, Luminal B and TNBC breast cancer ($\times 100$). ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2; FR, folate receptor; TNBC, triple-negative breast cancer.

Table 1. Clinical data of patients with different molecular subtypes of breast cancer. Note: TNBC, triple-negative breast cancer; HER-2, human epidermal growth factor receptor 2

Item	Luminal A (n = 116)	Luminal B (n = 176)	HER-2 overexpression (n = 96)	TNBC (n = 120)	p
Age					
≤ 45 years	76 (65.52%)	100 (56.82%)	55 (57.29%)	64 (53.33%)	0.2741
> 45 years	40 (34.48%)	76 (43.18%)	41 (42.71%)	56 (46.67%)	
Tumor size					
≤ 2 cm	55 (47.41%)	63 (35.80%)	40 (41.67%)	49 (40.83%)	0.1041
2-5 cm	46 (39.66%)	101 (57.38%)	46 (47.92%)	56 (46.67%)	
≥ 5 cm	15 (12.93%)	12 (6.82%)	10 (10.41%)	15 (13.33%)	
Lymph node metastasis					
0	46 (45.69%)	76 (54.55%)	40 (41.67%)	47 (39.17%)	0.0503
≤ 3	26 (27.59%)	32 (27.27%)	32 (34.38%)	30 (25.00%)	
3 - 10	36 (10.34%)	54 (9.09%)	15 (12.50%)	27 (22.50%)	
≥ 10	8 (16.38%)	14 (9.09%)	9 (11.46%)	16 (13.33%)	
Histological grade					
Grade I	43 (37.07%)	67 (38.07%)	13 (13.54%)	27 (22.50%)	< 0.0001
Grade II	50 (43.10%)	84 (47.73%)	40 (41.67%)	45 (37.50%)	
Grade III	23 (19.83%)	25 (14.20%)	43 (44.79%)	48 (40.00%)	

Ab-HER-2-USPIO specifically bound to BT47 and SKBR3 breast tumor cells

Subsequently, binding experiments were carried out to detect the specificity of Ab-HER-2-USPIO probes. In the experiments, Ab-HER-2-USPIO probes were incubated with different cell lines of breast cancer (BT474, SKBR3, ZR75B, and MDAMB231) as well as normal breast cells (Fig. 2). Compared with the normal breast cells, a large number of blue-stained particles (i.e., iron particles) appeared around the BT47 and SKBR3 cells incubated with Ab-HER-2-USPIO, while the number of blue-stained particles in the ZR75B and MDAMB231 cells incubated with Ab-HER-2-USPIO was decreased. These results revealed that Ab-HER-2-USPIO could specifically bind to BT47 and SKBR3 cells, because HER-2 was highly expressed in BT47 and SKBR3 cells.

Ab-HER-2-USPIO enhanced T1 weighted images in vitro

When BT47 and SKBR3 cells were incubated with Ab-HER-2-USPIO molecular probes containing an increased concentration of Fe (Fig. 3, from right to left), the T1 weighted signal of Ab-HER-2-USPIO was enhanced during *in vitro* MRI, and the images became increasingly brighter. After co-culturing Ab-HER-2-USPIO with cells, the T1 weighted signal of samples was weaker than that of Ab-HER-2-USPIO alone, possibly due to incomplete uptake of Ab-HER-2-USPIO by the cells. However, the T2 weighted signal of Ab-HER-2-USPIO showed no change over the increased concentration of Fe. These results indicated that Ab-HER-2-USPIO could enhance T1 weighted images *in vitro* (Fig. 3).

Ab-HER-2-USPIO enhanced T1 weighted images in vivo

As shown in Fig. 4, the T1 weighted images of the tumor area became brighter (labeled with red circles) at 2 h after the injection with Ab-HER-2-USPIO, while the signal intensity in T2 weighted images remained the same (labeled with blue circles). These results showed that Ab-HER-2-USPIO could enhance T1 weighted images *in vivo*, and hence could serve as a contrast agent for enhancing T1 weighted images during clinical tumor diagnosis.

Ab-HER-2-USPIO was associated with low in vivo toxicity

Finally, we performed HE staining to determine the toxicity of Ab-HER-2-USPIO in mice organs. The tissue sections of normal mice and tumor-bearing mice were shown in Fig. 5. Compared with those in normal mice, the organs of tumor-bearing mice showed no obvious lesions, such as tissue damage, inflammation, or necrosis. The results indicated that, at an injection dose of 25 mg/kg, Ab-HER-2-USPIO did not cause damages to the organs in the mice

Fig. 2. Ab-HER-2-USPIO specifically bound to the surface of BT47 and SKBR3 breast tumor cells ($\times 400$).

Fig. 3. Ab-HER-2-USPIO enhanced T1 weighted images *in vitro*. Panel A, T1 weighted images of MRI *in vitro*; Panel B, T2 weighted images of MRI *in vitro*; HER-2, human epidermal growth factor receptor 2; MRI, magnetic resonance imaging.

Fig. 4. Ab-HER-2-USPIO enhanced T1 weighted images *in vivo*.

Fig. 5. Ab-HER-2-USPIO exhibited minimal *in vivo* toxicity ($\times 400$). HER-2, human epidermal growth factor receptor 2.

within 14 days. Therefore, Ab-HER-2-USPIO not only showed better *in vitro* biocompatibility, but also displayed low *in vivo* toxicity, confirming its great potential in clinical applications.

Discussion

MRI allows the visualization of complex biochemical processes involved in disease states and normal physiology, in real-time, in living cells or tissues, and even in intact subjects [26]. MRI has become a promising tool in the early diagnosis of cancer. In addition, the application of contrast agents can further increase the detection performance of MRI [27]. The aim of this study was to evaluate the diagnostic values of ER, PR, and HER-2-targeting molecular probes in the MRI diagnosis of breast cancer, with the expectation to develop an innovative method for breast cancer diagnosis.

According to the expression of ER, PR and HER-2 in the tumor tissues, breast cancer can be divided into four different molecular subtypes, including Luminal A, Luminal B, HER-2 overexpression, and TNBC [28-31]. The pattern of ER and PR expression has been used in evaluating breast cancer [32]. First of all, ER is important for estrogen-dependent growth and can regulate the proliferation of both neoplastic breast and normal epithelial. In addition, the level of ER is regarded as a key factor determining the response to endocrine therapies and the prognosis in ER-positive breast cancer [14, 33]. Estrogen affects the breast cancer primarily by its expression in tumor tissues. ER α was shown to promote cell proliferation and angiogenesis [34], while ER β could lead to a poor prognosis of breast cancer by increasing endocrine therapy resistance [35]. In addition, progesterone plays a key role in the regulation of important reproductive functions and can exert its effects through both nuclear PRs and membrane PRs [36]. As an ER-regulated gene, PR expression is closely regulated by ERs [37, 38]. As a member of the erbB family of receptor tyrosine kinases, HER-2 is a target for antibody-based therapy in the treatment of breast cancer [39, 40]. A previous study has suggested that the expression of HER-2 can be used as an independent biomarker to predict breast cancer mortality and disease recurrence [41]. Higher expression of HER-2 has been related to a higher grade of breast cancer and elevated expression of ER and PR [42]. ER, PR and HER-2 have been used as druggable targets in ~30% of breast cancer patients due to their roles in promoting the genesis and development of breast cancers [43, 44]. In addition, the high expression of HER2 in breast cancer not only makes it a good therapeutic target, but also a target for imaging. In specific terms, HER2-targeted imaging could be used for the characterization and staging of breast cancer by measuring the expression of HER2 in tumor tissues [45]. For breast cancer patients showing overexpression of ERBB2 (formerly HER2 or HER2/neu), preoperative MRI may be most preferable because of the increased probability of concomitant diseases [46]. The diagnostic performance of MRI in breast cancer patients undergoing neoadjuvant chemotherapy (NAC) is associated with the profile of molecular biomarkers in these patients. The accuracy of MRI was poorer in patients carrying HR(+) tumors than that in patients carrying HER2(-) tumors. In addition, the accuracy of MRI was also worse in low-proliferation tumors than that in high-proliferation tumors [47]. Therefore, ER, PR, and HER-2 might serve as important targets for the clinical diagnosis and prognosis of breast cancer. Consistent with a previous study, the results of this study showed that ER, PR, and HER-2 could be used as MRI targets to detect and stage the breast cancer as

well as to monitor the outcome of treatments [13]. In addition, in a report on tumor-targeted PEG-PLGA nanopolymerosomes (NPs), which were encapsulated by quantum dot (QD) and doxorubicin (DOX) for organ fluorescence microscopy imaging and chemotherapy in breast cancer, folate-targeted DOX-QD NPs were preferentially accumulated in 4T1 and MCF-7 cells. In addition, folate-targeted DOX-QD NPs showed higher cytotoxicity compared with non-targeting NPs and the free form of the drug. Moreover, FR-targeted QD-encapsulated NPs were mainly accumulated at tumor sites in tumor-bearing mice [20].

The topical application of molecular probes has several advantages in surgeries, such as the small amounts of required molecular probes and the reduction of systemic toxicity [48]. However, smart molecular probes are required to use MRI in biochemical and clinical research [49]. Our study confirmed that Ab-HER-2-USPIO could specifically bind to BT47 and SKBR3 cells, possibly due to the high expression of HER-2 in these two cell lines [50, 51]. A previous study demonstrated that the overexpression of HER-2 in human breast cancer xenografts significantly increased the viability of hypoxic tumor cells and overall tumor [52]. Intratumoral heterogeneity of HER-2 amplification has been shown to occur with variable frequencies in breast cancer [53]. The amplification or overexpression of HER-2 in 20% to 25% of breast cancer cases also led to elevated HER-2 activation [54], which might facilitate the specific binding of Ab-HER-2-USPIO to breast cancer cells.

A previous study has reported that T1 weighted images in MRI can provide accurate measurement to assess novel risk factors for breast cancer [55, 56]. The MRI of BT47 and SKBR3 breast cancer cells showed that Ab-HER-2-USPIO could effectively enhance the T1 weighted images *in vivo*, and hence may contribute to the early diagnosis of breast cancer. In addition, Ab-HER-2-USPIO was associated with favorable *in vitro* biocompatibility and low *in vivo* toxicity, making it a popular agent to achieve MRI enhancement during *in vivo* investigation of neuroinflammation [57, 58]. Although previous data indicate that HER-2-targeted MRI molecular probes may be used in the clinical diagnosis of breast cancer and facilitate the development of promising strategies for breast cancer treatments [46, 47], in our study, we further observed Ab-HER-2-USPIO can specifically bind to breast cancer cells BT47 and SKBR3, thus enhancing the quality of T1 weighted MRI images; also, we found that Ab-HER-2-USPIO not only possessed better *in vitro* biocompatibility, but also showed low *in vivo* toxicity, identifying its great potential in clinical applications.

In conclusion, as a molecular probe targeting HER-2, Ab-HER-2-USPIO is of a significant diagnostic value in the early diagnosis of breast cancer by MRI. Additionally, a better understanding on the pathology of breast cancer achieved by using better molecular probes will help to improve the therapies for breast cancer patients. Nevertheless, more research is required to verify the results of this study.

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Disclosure Statement

The authors declare to have no competing interests.

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