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Research Progress of Fluorescent Probe Imaging Technology in the Diagnosis and Treatment of Breast Cancer

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Abstract: As the most common malignant tumor among women worldwide, breast cancer requires early and accurate diagnosis, metastasis monitoring, and effective treatment to improve patient prognosis. Current diagnostic approaches mainly include imaging examinations (e.g., ultrasound, magnetic resonance imaging (MRI), and mammography) as well as pathological examination, all of which have certain limitations. Thus, it is crucial to continuously explore novel, rapid, accurate, and minimally invasive diagnostic methods for breast cancer. Fluorescent probe imaging technology has garnered extensive attention in medical research due to its advantages of high tissue penetration, high sensitivity, non-invasiveness, and real-time visualization. Nanostructured probes, which possess excellent biocompatibility and remarkable physicochemical properties (including superior optical performance), have been extensively studied and reported in the field of breast cancer diagnosis, demonstrating broad application prospects.

Keywords: Fluorescence Imaging; Probe; Breast Cancer; Diagnosis; Imaging.

1. Introduction

The global annual incidence of breast cancer has exceeded 2.2 million cases. Among these cases, triple-negative breast cancer (TNBC) lacks specific therapeutic targets, with a 5-year survival rate of only 77%, which is significantly lower than that of other subtypes[1]. This severe epidemiological situation highlights the urgency and great clinical significance of developing precise diagnosis and treatment strategies for breast cancer.

Traditional diagnosis and treatment models have numerous limitations. In early screening, mammography and ultrasound exhibit a detection rate of less than 50% for small lesions (with a diameter of <5 mm) and struggle to accurately distinguish benign from malignant lesions. In the treatment phase, systemic chemotherapy causes severe side effects and high drug resistance due to the lack of targeting, while single local therapy is prone to leading to recurrence and metastasis. In surgical treatment, margin assessment in breast-conserving surgery (BCS) relies on frozen-section pathological examination, with a false-negative rate as high as 20%–70%; this results in approximately 20% of patients requiring reoperation, which increases their physical and psychological distress as well as economic burden [2]. In addition, the delayed detection of breast cancer metastases, especially bone and lung metastases, is one of the main causes of poor patient prognosis.

Against this background, fluorescent probe imaging technology, with unique advantages such as high sensitivity, real-time visual feedback, and no radiation exposure, can achieve tumor-specific recognition and signal amplification through molecular design. It can provide precise support throughout the entire process of early screening, intraoperative navigation, and targeted therapeutic monitoring, thereby driving the transformation of breast cancer diagnosis and treatment from empirical medicine to precision medicine [3]. In recent years, with the interdisciplinary integration of nanotechnology, molecular biology, and materials science, fluorescent probes have made breakthrough progress in response mechanisms, targeting strategies, multimodal integration, and metastasis detection. Their core advantage lies in converting tumor-related molecular information into visual signals, providing a new approach to addressing clinical pain points [4,5].

2. Application of Fluorescent Probes in Breast Cancer Diagnosis

The prognosis of breast cancer is closely associated with the timing of diagnosis. The 5-year survival rate of patients with stage I breast cancer can reach 95%, whereas that of patients with stage IV disease is only 27%. However, traditional early screening methods have obvious limitations: mammography has a high missed diagnosis rate for dense breast tissue; ultrasound results are highly operator-dependent; and pathological biopsy, as an invasive procedure, not only fails to achieve real-time monitoring but also carries the risks of bleeding, infection, and other complications [6]. More critically, the early detection of breast cancer metastases (including bone, lung, and lymph node metastases) directly affects patient prognosis. Nevertheless, traditional imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) exhibit low detection rates for micrometastases with a diameter of <2 mm, accompanied by radiation exposure and long examination times [7]. Therefore, there is an urgent clinical demand for non-invasive, highly sensitive, and highly specific diagnostic techniques that can cover the entire process from early screening to metastasis monitoring.

With high sensitivity and specificity, fluorescent probes enable the visualization of tumor-related signals at the cellular, tissue, and in vivo levels, providing multi-dimensional evidence for breast cancer diagnosis. In early screening, in

vitro detection platforms based on fluorescent probes allow for the precise capture and quantitative detection of tumor markers or circulating tumor cells. In in vivo imaging, the rapid development of second near-infrared (NIR-II) and multimodal probes has effectively overcome the limit of tissue penetration depth of traditional imaging, enabling high-resolution imaging of deep tumors and tiny metastases [8,9].

To meet the demand for non-invasive early diagnosis of breast cancer, numerous studies have developed high-sensitivity detection platforms using fluorescent probes with highly specific molecular recognition capabilities, which can rapidly detect breast cancer cells, circulating tumor cells, tumor markers, and key target genes. These platforms are well-adapted to clinical early screening and molecular typing. For human epidermal growth factor receptor 2 (HER2)-positive breast cancer, an important subtype of breast cancer, researchers have designed a novel HER2-targeted cyclic peptide positron emission tomography (PET) probe via computer-aided design. Labeled with 68Ga, this probe specifically binds to HER2-positive breast cancer cells, with cellular uptake significantly higher in positive cells than in negative cells. It enables the non-invasive and accurate detection of HER2 expression, providing a new technical tool for breast cancer molecular typing [10]. A fluorescent probe system constructed based on DNA walker and branched hybridization chain reaction achieves highly sensitive and specific detection of the breast cancer target gene PIK3CA. The isothermal enzyme-free signal amplification greatly improves detection sensitivity, making it suitable for rapid high-throughput screening of clinical samples [11]. Label-free surface-enhanced Raman spectroscopy (SERS) fluorescent probes can rapidly distinguish breast cancer patients from healthy subjects by analyzing differences in serum metabolic profiles, realizing non-invasive liquid biopsy and offering a convenient and efficient method for large-scale population early screening [12].

In the field of in vivo imaging, fluorescent probes, relying on the excellent tissue penetration of the NIR and NIR-II optical windows combined with tumor-specific targeting or tumor microenvironment-responsive mechanisms, enable the precise localization and visual imaging of primary lesions, small lesions, and metastases. The novel KIAA1363-targeted NIR fluorescent probe Cy-JW exhibits rapid in vivo clearance and high affinity for all breast cancer subtypes. In tumor-bearing animal models, it achieves a tumor-to-background ratio (TBR) greater than 5, clearly delineating tumor boundaries and enabling precise imaging of breast cancer lesions of different subtypes [13]. The triphenylamine-based aggregation-induced emission probe AIE-BC overcomes the photobleaching and severe background interference of traditional fluorescent probes. With excellent photostability and biocompatibility, it selectively accumulates in breast cancer cells and tumor tissues, enabling real-time and long-term in vitro and in vivo imaging [14].

To address the clinical diagnostic challenge of metastases and small lesions, the nitroreductase (NTR)-activatable NIR fluorescent probe DHM-NO2 specifically responds to the tumor hypoxic microenvironment. It can accurately identify orthotopic breast cancer and effectively distinguish tumor cells from normal cells, with a detection limit as low as 25.6 pg/mL, providing a key tool for the early detection of hypoxia-related metastases [15]. The mitochondria-targeted hydrogen sulfide (H_2S) fluorescent probe 152T, optimized by biotin modification, enables real-time imaging of mitochondrial H_2S in breast cancer and rapid localization of tumor tissue, being particularly suitable for the early identification of small lesions [16]. As an important target for triple-negative breast cancer (TNBC), Trop2-targeted PET probes allow the non-invasive and accurate detection of Trop2 expression and effective identification of metastases, solving the clinical problem of missing diagnostic targets for TNBC [17].

In early therapeutic efficacy monitoring, fluorescent probe imaging technology overcomes the lag of traditional evaluation methods and enables real-time, non-invasive, and early monitoring of treatment response, providing critical evidence for the timely clinical adjustment of therapeutic regimens. For cyclin-dependent kinase 4/6 (CDK4/6) inhibitor therapy in hormone receptor (HR)-positive, HER2-negative breast cancer, the novel NIR-II fluorescent probe HSA-ICi specifically binds to the CDK4/6–cyclin D complex. A significant decrease in the tumor fluorescence signal can be detected as early as one week after the initiation of treatment, which is earlier than MRI changes and tumor volume reduction, realizing the early and accurate evaluation of targeted therapeutic efficacy [18]. A β-galactosidase-activatable fluorescent probe enables the visual monitoring of chemotherapy-induced cellular senescence in breast cancer, dynamically reflecting chemotherapeutic effects and providing a dynamic reference for the optimization of individualized treatment [19].

In summary, by designing targeted fluorescent probes for different clinical needs, more precise and personalized diagnostic methods can be provided for breast cancer.

3. Application of Fluorescent Probes in Non-Surgical Treatment of Breast Cancer

Traditional breast cancer treatment faces numerous challenges: chemotherapeutic drugs lack targeting, leading to systemic toxicity; chemotherapy remains the main treatment for triple-negative breast cancer (TNBC), yet severe side effects and high drug resistance persist. Single treatment modalities (e.g., chemotherapy or photothermal therapy alone) are prone to inducing drug resistance, with a high risk of recurrence and metastasis. Moreover, the lack of real-time therapeutic monitoring makes it difficult for clinicians to adjust treatment regimens promptly [1,20]. Under these circumstances, the clinical demand for integrated “precision targeting, synergistic enhancement, and therapeutic monitoring” is increasingly urgent. As a core tool for theranostic integration, fluorescent probes enable targeted drug delivery, synergistic integration of multiple therapies, and real-time visualization of treatment effects, providing a new strategy for improving therapeutic efficacy and reducing side effects [21,22].

In recent years, the therapeutic application of fluorescent probes has evolved from single targeted drug delivery to multimodal synergistic therapy. By integrating photothermal therapy (PTT), photodynamic therapy (PDT), chemotherapy, and immunotherapy, these intelligent probes achieve “1+1>2” synergistic effects, while using fluorescence imaging to monitor treatment response in real time, thus providing a scientific basis for the dynamic adjustment of individualized treatment regimens [5].

In targeted therapy, multifunctional fluorescent probes show significant clinical potential. In targeted drug delivery

and chemotherapeutic sensitization, fluorescent probes serve as ideal drug carriers. Through active targeting modification, they achieve specific enrichment of chemotherapeutic agents at tumor sites, effectively solving the problems of poor targeting and high systemic toxicity associated with traditional chemotherapy. The cancer cell membrane-modified MnO_2@DOX nanoprobe MDC exhibits excellent homologous targeting ability, precisely delivering doxorubicin to breast cancer cells while monitoring in vivo drug distribution via MRI. Cellular experiments confirm its significant antitumor effect, realizing the integration of targeted delivery and imaging monitoring [23]. A succinylated D-type neuropeptide-integrated iron-based probe specifically targets breast cancer cell mitochondria through Y1 receptor-mediated endocytosis. SIRT5-mediated desuccinylation disrupts mitochondrial function and cellular homeostasis, ultimately inducing tumor cell apoptosis while synchronously achieving targeted therapy and real-time imaging [24]. A chemical probe with a 1,3,5-triazine and 2-aminopyrimidine skeleton precisely targets the 5-HT7-FOXM1 signaling axis in TNBC, exhibiting specific antitumor activity and providing a novel targeted therapeutic molecule for refractory triple-negative breast cancer [25].

In phototherapy and chemodynamic synergistic therapy, fluorescent probes, based on their photoresponsive and tumor microenvironment-responsive properties, achieve synergistic enhancement of PTT, PDT, chemodynamic therapy (CDT), and ferroptosis, constructing minimally invasive and highly efficient novel therapeutic models. An NIR-II fluorescence/photoacoustic dual-modal probe optimized with a heavy-atom structure achieves a photothermal conversion efficiency of 61.0%, integrating NIR-II imaging and PTT. In vivo experiments confirm effective tumor ablation, realizing imaging-guided precise photothermal therapy [26]. A pH-responsive manganese-based nanoprobe BSA-MnO_2@CPT specifically responds to the acidic tumor microenvironment, synchronously achieving synergistic chemotherapy and ferroptosis, and real-time monitoring of treatment progress via T1-weighted MRI, which significantly inhibits 4T1 breast cancer cell proliferation [27]. A nucleus-targeted theranostic probe 1HA4CD generates high-concentration ROS upon laser irradiation, precisely inducing DNA double-strand breaks in tumor cell nuclei and inhibiting damage repair, effectively overcoming breast cancer chemoresistance and achieving efficient precise treatment under NIR imaging guidance [28].

In immunotherapeutic sensitization and combination therapy, fluorescent probes significantly enhance immunotherapeutic efficacy by remodeling the tumor immune microenvironment and promoting immunogenic cell death, constructing multimodal synergistic antitumor systems. A cysteine/glutathione-depleting theranostic prodrug probe uses overexpressed antioxidants in the tumor microenvironment as activation signals to enhance type I PDT, synchronously achieving tumor imaging and precise treatment with greatly reduced damage to normal tissues [29]. A VEGFR2-targeted radiotheranostic probe 64Cu/177Lu-DOTA-diZD integrates precise PET imaging and radionuclide-targeted therapy, significantly prolonging the survival of TNBC tumor-bearing mice; its efficacy is further enhanced when combined with immunotherapy [30].

Through theranostic integration, fluorescent probe imaging technology achieves precise, visualized, and efficient non-surgical treatment, providing a novel solution for the individualized non-surgical management of breast cancer.

In summary, fluorescent probes can synergistically integrate therapeutic modalities such as photothermal therapy, photodynamic therapy, chemotherapy, and immunotherapy to achieve precise treatment of breast cancer and its metastases, showing broad application prospects.

4. Application of Fluorescent Probes in Surgical Treatment of Breast Cancer

Surgical resection is the core radical treatment for breast cancer. Accurate identification of tumor boundaries, complete lesion resection, precise recognition of metastatic lymph nodes, and protection of normal tissues are key to improving surgical outcomes and patient prognosis. Breast-conserving surgery (BCS) is the preferred surgical approach for early breast cancer, aiming to completely resect tumors while maximally preserving normal breast tissue and function. However, traditional margin assessment relies on intraoperative frozen-section pathology, which is time-consuming and has a false-negative rate of 20%–30%. This leads to 20%–70% of patients undergoing reoperation due to positive margins, significantly increasing trauma and medical burden [2]. Meanwhile, axillary lymph node dissection, an important procedure for breast cancer staging and treatment, cannot accurately distinguish metastatic from non-metastatic lymph nodes by conventional methods. Additionally, intraoperative injury to axillary lymphatic vessels easily causes severe complications such as postoperative lymphedema [31]. Therefore, new technologies enabling real-time and precise intraoperative navigation are urgently needed to reduce reoperation rates, protect normal tissue function, and improve overall patient prognosis.

Fluorescent probe imaging technology, with advantages of real-time visualization, high resolution, and convenient operation, achieves precise intraoperative localization of tumor boundaries, rapid margin assessment, real-time recognition of metastases, and effective protection of normal tissues. It comprehensively improves the accuracy and safety of breast cancer surgery and reduces postoperative recurrence and complications. In recent years, the application of fluorescence imaging in surgical margin evaluation, sentinel lymph node biopsy, and axillary tissue protection has become increasingly mature, with some technologies entering clinical practice and showing good translational prospects [32].

In the precise evaluation of tumor boundaries and surgical margins, fluorescent probes delineate tumor contours in real time, rapidly identify positive margins, and effectively solve the clinical problems of blurred tumor boundaries and delayed margin assessment in breast-conserving surgery. The novel KIAA1363-targeted NIR fluorescent probe Cy-JW is fully compatible with clinical intraoperative fluorescence imaging systems and clears rapidly in vivo, enabling precise delineation of surgical margins for all breast cancer subtypes and providing reliable navigation for complete and accurate tumor resection [13]. A peroxynitrite ($ONOO^-$)-responsive sprayable NIR fluorescent probe SWJT-43 can be directly sprayed onto surgical wounds, rapidly responding to high concentrations of $ONOO^-$ in the tumor microenvironment to instantly distinguish tumor from normal tissue, thus realizing real-time precise intraoperative navigation suitable for minimally invasive breast cancer resection [33]. The lipid droplet polarity fluorescent probe NA-LD rapidly distinguishes benign and malignant breast tissues based on differences in lipid droplet polarity, quantity, and size

between tumor and normal cells, replacing traditional frozen sections for rapid intraoperative pathological diagnosis and greatly improving diagnostic efficiency and accuracy [34].

In sentinel lymph node and metastasis identification, fluorescent probe imaging enables real-time precise localization of sentinel lymph nodes and rapid judgment of metastatic status, effectively avoiding excessive dissection and tumor residue. NIR-II fluorescent probes, with deeper tissue penetration and higher imaging signal-to-noise ratio, show significant advantages in breast cancer sentinel lymph node imaging. Through dual-color fluorescence imaging and ratiometric fluorescence analysis, they achieve real-time non-invasive diagnosis of sentinel lymph node metastasis with an accuracy rate of 92.8%, which is far superior to traditional methods, with convenient and time-saving detection [35]. The mitochondria-targeted triple synergistic H_2S fluorescent probe SCy-H_2S features an ultra-high signal-to-noise ratio, precisely identifying metastatic breast cancer and providing key support for image-guided complete resection of metastases to minimize tumor residue [36].

In the standardization and clinical translation of surgical navigation, fluorescent probe imaging is gradually moving toward standardized clinical application as an important support for improving breast cancer surgical outcomes. The clinically classic NIR fluorescent dye indocyanine green (ICG) has been standardized in breast cancer intraoperative navigation and sentinel lymph node biopsy. Systematic reviews confirm its sensitivity of 93.3%–100% and specificity of 60%–96% in surgical margin assessment, effectively improving negative margin rates [37]. The standardized axillary reverse mapping technique based on ICG fluorescence imaging accurately identifies and protects axillary lymphatic vessels, significantly reducing surgical complications such as lymphedema and improving postoperative quality of life.

In summary, fluorescent probe imaging technology demonstrates unique value in assisting breast cancer surgery. With the increasing maturity of its applications in margin assessment, sentinel lymph node biopsy, and axillary tissue protection, fluorescence imaging is expected to be widely used clinically in the near future, bringing more precise and safer surgical treatment to breast cancer patients.

5. Conclusion

Fluorescent probe imaging technology, with unique advantages including high tissue penetration and real-time visualization, has emerged as a core technology for breaking through the bottlenecks in precise breast cancer diagnosis and treatment, offering a novel approach to addressing clinical pain points of traditional management, such as low early diagnostic sensitivity, poor therapeutic targeting, and blurred intraoperative boundary identification. This technology covers the entire process of breast cancer diagnosis, treatment, and intraoperative navigation. In terms of diagnosis, relying on high-sensitivity in vitro detection platforms and NIR-II/multimodal probes, it achieves non-invasive early screening and submillimeter-precise monitoring of micrometastases in sites such as bone and lung, thereby greatly improving early detection rates. In the treatment field, it has evolved from single targeted drug delivery to theranostic integration with multimodal synergy of photothermal therapy, chemotherapy, and immunotherapy, providing new therapeutic strategies especially for refractory subtypes such as triple-negative breast cancer (TNBC). In intraoperative navigation, fluorescent probes real-time display tumor boundaries and lymph node locations, significantly improving the accuracy of margin assessment in breast-conserving surgery (BCS), reducing reoperation rates, and achieving precise protection of axillary lymphatic tissue to decrease surgical complications.

At present, this technology has entered the stage of clinical translation. Classic probes such as indocyanine green (ICG) have been standardized, and several novel probes have completed preclinical validation or first-in-human trials. However, core challenges remain, including insufficient biocompatibility, limited targeting due to tumor heterogeneity, difficulty in large-scale preparation, and poor compatibility with imaging equipment. Researchers are striving to achieve breakthroughs in probe structure design, preparation technology, pharmacokinetic optimization, and clinical standardized validation.

Nevertheless, we believe that fluorescent probe imaging technology will develop toward precise intelligent responsive probes, integrated multimodality, and interdisciplinary integration with artificial intelligence. It will focus on overcoming the detection of metastases and minimal residual disease, deeply integrate with immunotherapy and gene therapy, and is expected to achieve full-cycle precision diagnosis and treatment of breast cancer. Ultimately, it will become a core pillar of breast cancer precision medicine, providing patients with personalized whole-process management to further improve clinical outcomes.

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