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# Advances in Molecular Diagnosis of Breast Cancer

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**Abstract:** ***Breast cancer is a highly heterogeneous malignancy. The continuous development and progress of molecular diagnostic technology has been applied in the diagnosis and treatment of breast cancer, which highlights the great potential of individualized precision diagnosis and treatment. Molecular diagnosis relies on experimental techniques of molecular biology, and plays an important role in the study of tumor pathogenesis, diagnosis, treatment and prognosis. Early detection, early diagnosis, early treatment and individualized diagnosis and treatment plan can effectively reduce the mortality of breast cancer patients and play an important role in improving patient prognosis. Molecular diagnosis has become an indispensable part of cancer diagnosis, prognosis and treatment prediction. With the rapid development of molecular biology, the molecular diagnosis of breast cancer has reached a new level.***

**Keywords:** Breast cancer, Molecular diagnosis, FISH, NGS.

## 1. Breast Cancer and Molecular Diagnosis

According to the statistical data released by the International Agency for Research on Cancer in 2020, the global number of new cancer cases reached a total of 19.29 million, with breast cancer accounting for 2.26 million new cases [1]. Breast cancer is also a common cause of cancer-related deaths among women [1], and has become the most common type of cancer [2]. In women under 45 years old, breast cancer ranks first in the mortality rate of malignant tumors [3]. Women with risk factors, such as a family history of breast cancer, early menarche, late age at first birth, and a history of chest radiation, can often detect lumps in the breast area through early self-examination. Upon consultation, it is recommended to undergo routine breast cancer screening. The common screening methods include mammography and breast ultrasound. However, due to individual differences among patients, factors such as the size and nature of the lump, as well as the phase of the menstrual cycle during the examination, can all affect the results, and may even lead to certain false-negative outcomes [4]. In addition, due to the high heterogeneity of breast cancer and the differences in molecular subtypes, the treatment of breast cancer is increasingly trending towards individualized precision therapy. Continuous research on the molecular subtyping of breast cancer by scholars has promoted our understanding of the disease. Treatments targeting specific molecules have emerged in an endless stream, providing breast cancer patients with more treatment options. Moreover, there is a growing emphasis on physical examinations, particularly among young women who are increasingly concerned about breast diseases during check-ups. With the continuous development of detection methods, early screening methods for breast cancer are flourishing. The purpose of screening is to detect lesions as early as possible and to receive standardized treatment under the professional advice of specialists. The prerequisite for early detection of breast cancer is the advancement and development of screening methods and technologies. Individualized precision diagnostic and treatment plans can effectively reduce the mortality rate of breast cancer patients and significantly improve patient outcomes [5].

Molecular diagnostics, accompanied by the progress in molecular biology, is increasingly recommended by scholars for the early screening and diagnosis of cancer. It involves the detection of changes in the structure, epigenetic specificity, and expression patterns of DNA, RNA, or proteins within patients using related instruments and equipment [6], this is done to determine the expression status of tumor-related molecules, thereby inferring the risk of cancer onset. Molecular diagnostics relies on molecular biology experimental techniques and plays a crucial role in the study of tumor etiology, pathogenesis, diagnosis, treatment, and prognosis. In the molecular diagnosis of breast cancer, the application of fluorescence in situ hybridization (FISH) technology, next-generation sequencing, gene chip arrays, and liquid biopsy techniques has significantly advanced the diagnosis and treatment of breast cancer. Particularly, the application of liquid biopsy technology has introduced new methods for the treatment monitoring of metastatic breast cancer, allowing for better observation of patients' treatment responses, guiding medication use, and assessing prognosis. With the progress of the times and the improvement of people's living standards, new demands are being placed on the healthcare industry. There is an increasing pursuit of individualized diagnostic and treatment models, and modern medicine is gradually entering the era of precision medicine. In the rapidly evolving era of precision medicine, molecular diagnostics has become an indispensable and important component of cancer diagnosis, prognosis, and treatment prediction. With the rapid development of molecular biology, the level of molecular diagnosis for breast cancer has also reached a new height.

## 2. Research Progress and Significant Findings in Breast Cancer Molecular Diagnostic Techniques

### 2.1 Fluorescence in Situ Hybridization, FISH

The FISH method involves hybridizing nucleic acid probes that have been directly or indirectly labeled with fluorophores to the nucleic acid target sequence in the sample to be tested, according to the principle of base complementary pairing. Subsequently, the gene status on the chromosomes within the

cell nucleus is observed and analyzed using a fluorescence microscope. The development of molecular techniques such as FISH has had a significant impact on tumor gene detection. The amplification of the human epidermal growth factor receptor 2 (HER-2) gene can lead to the occurrence and progression of breast cancer, as well as the metastasis of breast cancer and the invasion of surrounding tissues, and even distant metastasis, such as bone, brain, and liver metastases. With the increasing importance of driver genes such as HER-2, clearly determining the status of the HER-2 gene is an important principle in the molecular typing of breast cancer. The amplification of the HER-2 gene is found in about 20-30% of people diagnosed with breast cancer, making it crucial to determine the expression level of the HER-2 gene in the current diagnosis and treatment of breast cancer [7], At the same time, by assessing the HER-2 expression status, the molecular typing of breast cancer can be distinguished to guide targeted therapy and further judge the prognosis of related patients, achieving individualized diagnosis and treatment of breast cancer in order to realize precision medicine. For the detection of HER-2, immunohistochemistry (IHC) and FISH are two methods approved by the FDA for HER-2 determination [8].

The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) have developed guidelines for assessing HER-2 status based on HER-2 protein expression levels measured by immunohistochemistry (IHC) and HER-2 gene amplification levels [9]. In addition, the 2022 edition of the Breast Cancer CSCO Guidelines also suggests that for IHC results showing IHC 2+, which is considered indeterminate for HER-2, further HER-2 gene status testing should be conducted using the ISH method [10]. The HER-2 testing strategy is crucial for the subsequent treatment of breast cancer patients. The application of FISH technology in the field of breast cancer molecular diagnostics has further clarified the HER-2 status, and accurate detection of HER-2 expression and gene amplification is of great help in the application of HER-2-specific treatments and in assessing the treatment response of breast cancer patients [11], providing a clearer reference for screening patients who may benefit from HER-2-positive targeted therapy. Anna Krakowiak and others compared the similarities and differences in HER2 results between IHC and FISH in breast cancer detection and concluded that cases with IHC2+ should undergo additional testing using FISH [12]. Makoto Kamari and others studied the prognostic effect of HER-2 gene amplification measured by FISH in breast cancer, using FISH to examine HER-2 gene amplification and IHC to check HER-2 protein, estrogen receptor (ER), and progesterone receptor (PR) expression in 62 cases of advanced breast cancer. The results indicated that in breast cancer, detecting HER2 gene amplification by FISH is preferable compared to HER-2 protein expression measured by IHC [13].

**2.2 Gene Chip Technology**

The sequencing principle of gene chips is based on hybridization sequencing, which is a method for determining nucleic acid sequences by hybridizing with a set of nucleic acid probes of known sequences. Gene chip technology can detect the differences in gene expression profiles of thousands of genes in the same tissue at once. Applying this technology to the diagnosis and treatment of breast cancer, it is possible to screen out genes related to the occurrence and development of breast cancer by detecting the expression differences between breast cancer tissue and normal breast tissue, thus contributing to clarifying the pathogenesis, treatment, and prevention of breast cancer [14]. Mu Zhigang and others retrieved gene chip data related to early-onset breast cancer samples and non-early-onset breast cancer samples from the public gene chip database (GEO) at the National Center for Biotechnology Information, and based on bioinformatics, they screened for differentially expressed genes in early-onset breast cancer. They found that there are differences in gene expression profiles between early-onset breast cancer and non-early-onset breast cancer, among which LIPE and PLIN1 may be key genes in the occurrence and development of early-onset breast cancer [15]. Liu Jingling and others used bioinformatics methods to study the potential target genes, signaling pathways, and molecular mechanisms mediated by cell division cycle-related proteins (CDCAs) in breast cancer. They downloaded gene chip data from normal breast tissue and breast cancer samples from the GEO and TCGA databases and found that CDCAs are upregulated in breast cancer tissue, and the expression level of CDCAs significantly affects the prognosis of breast cancer patients. Compared to other CDCAs, CDCA 2/3/5/8 may be suitable targets for targeted therapy in breast cancer patients [16].

**2.3 Next Generation Sequencing, NGS**

Following the completion of the Human Genome Project, cancer research has gradually focused on genome sequencing, with the aim of better understanding the genetic basis of tumor development and identifying actionable changes. NGS technology, also known as massively parallel sequencing, is a new sequencing method introduced in the last decade that can read billions of DNA bases simultaneously without the need to know the specific sequence in advance [17]. With the development of NGS technology, cancer research and treatment have entered the “genome era” [18]. The speed of DNA sequencing has increased by thousands of times, and the measurement cost has also decreased compared to the previous generation of sequencing technology, reducing the financial burden on patients. NGS can accelerate the early diagnosis of diseases and discover pharmacogenetic markers that assist in personalized treatment [19]. Over the past decade, NGS has become both cost-effective and reliable, capable of identifying functional DNA variants [20]. With the continuous development of molecular biology, tumor diagnosis has entered the era of precision medicine (PM). NGS can be divided into whole genome sequencing (WGS), whole exome sequencing (WES), transcriptome sequencing, epigenetic sequencing, and single-cell sequencing, among others [21]. n addition, NGS can also be applied to the analysis of the causes of resistance to chemotherapy or molecular targeted therapy.

NGS can be used to identify novel cancer mutations. Technologies such as NGS have helped researchers discover many new mutations in solid malignant tumors, such as breast cancer [22] and ovarian cancer [23]. Andrés E Quesada and others have found that in breast implant-associated anaplastic large cell lymphoma, NGS revealed a new STAT3-JAK2 fusion and other activating genetic changes within the

JAK-STAT pathway [24]. Additionally, NGS contributes to the precise diagnosis and prognosis assessment of tumors, emphasizes the necessity of targeted therapy for different types of tumors, and provides a reference for the selection of treatment methods for cancer patients [19]. Approximately 5-10% of breast cancers (BC) are hereditary, with mutations in the BRCA1 or BRCA2 genes accounting for about 30% of cases [25]. BRCA1 and BRCA2 encode tumor suppressor proteins that are essential for DNA repair and maintenance of genomic stability. For BC patients with a family history, Sanger sequencing is a traditional DNA sequencing method, but it is time-consuming and expensive. However, the emergence of NGS has brought a more effective, accessible, and definitive method, reducing time and cost [26], and has greatly improved the level of diagnosis and treatment for breast cancer.

### 2.4 Liquid Biopsy Technology

Due to the high heterogeneity of breast cancer, early symptoms are often subtle and varied, making early diagnosis and precision treatment a crucial aspect of individualized therapy for breast cancer at this stage. Existing detection methods, such as early mammography or ultrasound screening, have a certain margin of error. Ultrasound-guided needle biopsy is less compliant due to the invasiveness of the procedure. CT and MRI are not the first-line recommended tests for breast cancer screening, and they pose a significant economic burden for early screening patients. In recent years, the knowledge base of genomics has continuously expanded, gradually uncovering the complexity of malignant tumors. The approach to treating malignant tumors has begun to shift from tumor-type-oriented to gene-oriented, with individualized precision treatment based on the analysis of patients' tumor markers [27]. With the continuous development and progress in the field of molecular medicine, liquid biopsy technology has emerged. Over the past decade, the role of liquid biopsy in cancer treatment has become increasingly prominent, with clear clinical applications in lung cancer. In addition, the U. S. Food and Drug Administration has approved the Thera screen PIK3CA RGQ polymerase chain reaction assay as a companion diagnostic for detecting PIK3CA mutations in breast cancer, for use in tissue and liquid biopsies, highlighting the role of liquid biopsy in breast cancer treatment [28].

Liquid biopsy technology refers to the process of isolating and analyzing tumor-related substances such as tumor DNA, RNA, and tumor cells from body fluid samples of patients, including blood, urine, feces, and cerebrospinal fluid [29]. Through liquid biopsy technology, information such as tumor gene mutations, copy number changes of key genes, proteomics, and epigenetic changes can be obtained. The main circulating tumor markers detected by liquid biopsy technology include CTC, ctDNA, ctRNA, and exosomes, among others [4], playing a significant role in the early diagnosis, progression and metastasis, heterogeneity and drug resistance, and evaluation of prognosis and efficacy in breast cancer. As a relatively non-invasive detection method, liquid biopsy technology makes it easy to obtain continuous blood samples from patients during treatment, especially when the tumor metastasizes, particularly in locations where traditional biopsies are difficult to sample, such as breast cancer brain metastases and lung metastases, where the advantages of liquid biopsy become more pronounced. Previous studies have reported that liquid biopsy can be used to monitor the efficacy of breast cancer treatment and assess prognosis, further indicating the direction of clinical treatment for breast cancer patients [27]. Po-Han Lin and others studied circulating tumor DNA as a predictive marker of recurrence in stage II-III breast cancer patients receiving neoadjuvant therapy, indicating that the presence of ctDNA after NAT is a reliable marker for predicting recurrence in stage II to III breast cancer patients [30]. ctDNA detection may be incorporated into multiple stages of breast cancer clinical management in the future, including diagnosis, molecular phenotype determination, treatment response monitoring, prognosis, and identification of drug resistance mutations [31]. Timothy Kwang Yong Tay and others reviewed the research on liquid biopsy in breast cancer and studied its clinical application potential in the fields of early diagnosis, prognosis, monitoring of treatment response, detection of minimal residual disease, and prediction of breast cancer progression or recurrence risk, especially in monitoring treatment response and predicting disease progression or recurrence. Exosomes are the new frontier of cancer liquid biopsy. Exosome circRNA can serve as diagnostic and prognostic biomarkers for cancer. Targeting exosomes may represent a new method for treating cancer by using them as cell-free therapies and drug delivery systems and inhibiting their biogenesis and distribution. Ali Vahabi and others described the encapsulated circRNA from tumor-derived exosomes and their potential role in cancer development as well as their potential as biomarkers and therapeutic methods [32]. Dong Ye and others found that exosome circRNA derived from the body fluids of cancer patients can regulate tumor proliferation, invasion, metastasis, and drug resistance. They can serve as effective biomarkers for non-invasive early diagnosis and prognostic evaluation of tumors and are ideal targets for early precision treatment intervention [33].

## 3. Future and Prospects of Breast Cancer Molecular Diagnostics

In recent years, the further application of technologies such as FISH, gene chips, NGS, and liquid biopsies in the field of breast cancer diagnosis and treatment has greatly enhanced the level of breast cancer diagnosis. With the continuous improvement of people's living standards, the awareness of early disease screening has also been increasing, especially for breast diseases. The trend of younger age in breast cancer diagnosis cases indicates that the level of early breast cancer diagnosis is continuously improving. This is partly due to the increased screening awareness of patients and partly due to the application of highly feasible molecular diagnostic technologies. The continuous development and progress of molecular diagnostic technologies, when applied in the diagnosis and treatment of breast cancer, further highlight the immense potential of individualized precision medicine. Molecular typing can assist in the precise classification and stratification treatment of breast cancer, and gene mutation testing has now become a companion diagnosis for targeted breast cancer therapy. In particular, the rapid development of NGS technology is leading a major transformation in the precision treatment of breast cancer, with an increasing number of tumor mutation-driven genes being uncovered. As

NGS becomes more widespread and the cost of testing decreases, individualized precision treatment plans are expected to become a reality [34]. With further research and continuous improvements in the technology for isolating tumor-derived materials, liquid biopsies may continue to play an even greater role in the clinical management of breast cancer [28]. The field of breast cancer molecular diagnostics is constantly seeing new research and results, which has significantly improved the diagnostic and treatment capabilities for breast cancer. However, there are still some issues in the application process, and it is expected that more scientific researchers will conduct further scientific studies to solve some of the remaining application issues in breast cancer molecular diagnostics.

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