Advanced diagnosis technologies for HER2 breast cancer markers

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Abstract. Every year, the number of persons diagnosed with cancer depressive. As a result, cancer diagnosis is extremely crucial. Malignant tumor markers have become an important aspect of clinical tumor assessment, and different cancer cells have different markers. With the advancement of molecular biology technology, it is now possible to use it to diagnose, monitor, assess prognosis, forecast metastasis, and predict recurrence risk in malignant cancers. Breast cancer is the most frequent cancer among women, and the number of women diagnosed with it has been higher in many cancers. Histiocytic tumor markers in breast cancer include ER and PR, HER2,BRCA1 and BRCA2. Breast cancer can be categorized and treated using several markers, such as functional subtype HER-2 positive, and triple negative. As a result, precisely detecting the subtype of breast cancer is critical because it allows patients to receive the most effective treatment. HER2 is found in 30% of individuals diagnosed, with a poor prognosis and a significant recurrence rate. FISH, IHC, CISH, Dual ISH, and NGS are all common methods for detecting HER2 breast cancer. In this review, some of the most common HER2 breast cancer diagnostic procedures are summarized.

Keywords: HER2, breast cancer, markers, FISH, IHC, CISH, NGS and Dual ISH.

1. Backgroud

On a global scale, breast cancer remains a public-health issue, and with a rising number of breast cancer patients, more early diagnosis and therapy to increase breast cancer survival is critical. The basics diagnosis of breast cancer, such as MRI, biopsy and Breast ultrasonography and molybdenum target examination.[1, 2] The earliest tumor markers were found in the 1840s by researchers. More than 200 species have been identified so far, and they are presently used in clinical cancer diagnosis.[2] At the gene level, molecular tumor markers provide a crucial foundation for population census, early diagnosis, and disease monitoring in malignant tumors. [1, 3]Tumor indicators include carbohydrates, hormones, receptors, enzymes or metabolite proteins, oncogenes and tumor suppressor genes, and associated products. Based on some genetic changes, studying the changes in DNA that contribute to cancer growth can shed a lot of insight on cancer because most malignancies are heritable. Understanding how mutations in these genes affect cancer cell proliferation, invasion, and metastasis[3]. Due to the steady accumulation of genetic defects, breast cancer is also a heritable illness. Point mutations, chromosome amplification, deletion, rearrangement, translocation, and duplication are all examples of genetic disorders. About 20% of breast cancers are hereditary, and mutations in the BRCA1 and BRCA2 genes cause half of hereditary breast cancers[1, 3]. Because most breast cancers are sporadic, they are eventually produced by the accumulation of genetic alterations in a large number of somatic cells. Breast cancer can be loosely split into three types based on its molecular classification: functional subtype, HER2, and triple negative.[1] Classification of molecules shown in table 1.

Table 1 Breast cancer classification of molecules

Breast cancer diagnosis markers				
	HER2	ER	PR	Ki-67
HER2 negative	+	-	-	
HER2 positive	+	+		
Luminal A	-	+	High expression	Low expression
Luminal B	-	+	Low expression or -	High expression
Triple negative	-	-	-	

Note:"+" means positive; "-" means negative; HER2,ER,PR, and Ki-67 are breast cancer biomarks, based on these four biomarkers, breast cancer can be categorized into five subgroups.

Breast cancer diagnosis has altered dramatically during the previous 20 to 30 years. Continuous development in diagnosis, from imaging to genomic identification, and the deepening of the breast cancer marker, resulting in a better understanding of breast cancer treatment and prognosis [3-7]. Breast cancer patients' diagnosis and treatment, such as cancer diagnosis, prognosis, and monitoring of minimal residual cancer cells, are increasingly influenced by molecular diagnosis. In terms of the biomarker's potential to predict disease-specific behavior and treatment response, the ideal tumor marker is disease-specific and clinically valid[5, 6]. Chromosomal translocations, other chromosomal rearrangements, gene amplification, copy number abnormalities, point mutations, single nucleotide polymorphisms, gene expression variations, and epigenetic modifications are all examples of markers[5-7]. The genetic underpinning of tumor initiation and progression is gene mutation. Oncogenes are a group of genes that drive cells to become malignant. Oncogenes are genes that are required for cell growth and development in normal cells. These genes can cause unlimited cell proliferation and malignant transformation if their expression timing, expression place, expression amount, and expression product structure are all incorrect. When an oncogene is mutated or overexpressed, it can lead to uncontrolled cell proliferation and, eventually, cancer[6, 7]. Many oncogenes are mutations of proto-oncogenes, which are a kind of gene that regulates cell proliferation and differentiation. A total of 340 human oncogenes (families) have been discovered. Such as HER-2 gene, it's a proto-oncogene that causes malignancies such as breast, stomach, and lung tumors when it's overexpressed. The HER2 gene is found on chromosome 17 of the human genome[5-7].

2. The basic funamental of BP neural network HER2 breast cancer

2.1. HER2 gene

Proto-oncogenes can be activated in a variety of ways, leading in gene expression or overexpression, which can lead to cell cancer. Oncogenes have a variety of activation mechanisms and routes, which can be categorized into four categories[7].

1) A single point mutation; 2) Translocation of chromosomes; 3) Amplification of genes (gene amplification); 4) Induction of the virus and insertion of the promoter.[7]

HER2 is a member of EGFR family, HER2 gene is a proto-oncogene human epidermal growth factor receptor 2(chromosome 17 gene overexpression), which is an important prognostic factor for breast cancer patients[5, 7]. Other family members include EGFR or HER1, HER3 and HER4. The EGFR family proto-oncogene signal pathway as shown in fig1[6]. The outcome of receptor dimerization is phosphorylation of tyrosine residues within the cytoplasmic domain, which leads to the beginning of a number of signaling pathways involved in cellular proliferation, transcription, motility, and apoptosis suppression [5-7]. If HER2 overexpression causes cancers such as breast cancer, lung cancer, and stomach cancer, it should be investigated. This is a significant prognostic factor for people with breast cancer. When HER-2 gene amplification is positive or overexpressed, and breast cancer patients will have special clinical characteristics and biological behavior[6, 7]. And amplification or overexpression of the Human Epidermal Receptor 2 (HER2) oncogene is found in

18 to 30% of initial invasive breast cancers. Not only is HER2 a gold standard for detecting breast cancer, but it's also the basis for a number of molecular her2-targeting therapies, such as such as trastuzumab, pertuzumab, trastuzumab emtansine (T-DM1) and HER2-TKI[8]. We can gain a decent knowledge of a patient's illness microenvironment using these detection approaches[7, 8].

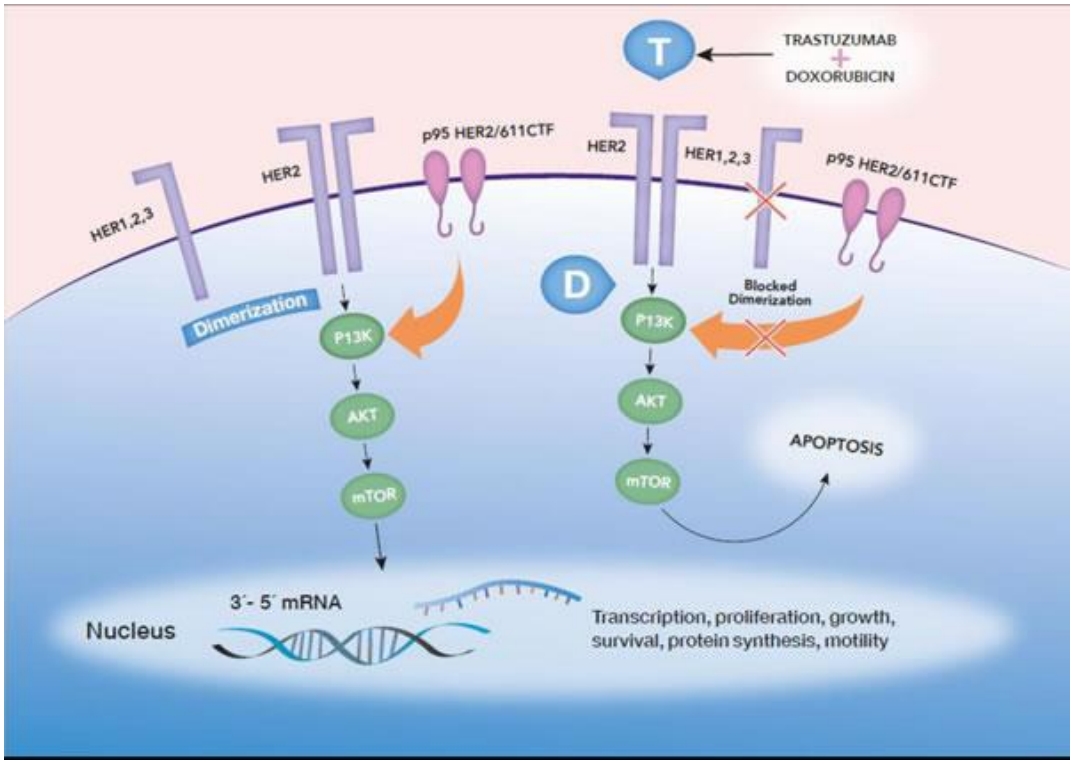


Figure1 HER2 proto-oncogene signal pathway[6].

HER2 is a proto-oncogene member of EGFR family, and it has the potential to cause cancer. Also, create a signaling system that promotes cancer cell proliferation.

2.2. HER2 breast cancer detection technique

HER2 is found in 30 percent of breast cancers. Because HER2 has a worse prognosis and a higher recurrence rate than the other two types, it is critical to detect it correctly. HER2 (17Q12) is a chromosome 17 gene amplification [7-9]. In situ hybridization is a type of nucleic acid molecular hybridization that uses histochemistry and molecular biotechnology to locate nucleic acids. Through hybridization, it detects and locates the presence of a specific target DNA or RNA directly in tissues, cell smears, cultured cell slivers, or metaphase chromosomes using tagged DNA sequences as probes[7, 8]. Because of HER2 gene amplification, FISH (fluorescence in situ hybridization) and CISH(colorimetric in situ hybridization) techniques can be used to detect HER2 gene amplification in breast cancer[9-14]. FISH can be used not only to detect gene amplification or deletion quantitatively, but also to analyze gene rearrangement qualitatively. FISH is method for visualizing specific locations on a chromosome by fluorescent complementary probe. And the aim of FISH is detection of chromosomal abnormalities[12, 13]. CISH is a new method testing HER2 gene amplification after FISH, because of the brilliant and clear high signal visible under conventional light microscopy, CISH does not result in high background and poor signal owing to partial sealing, and large fragment probes can get particularly strong signal strength. The most often utilized FDA-approved assays are IHC and FISH[14]. IHC offers several advantages over FISH in terms of speed, simplicity, and cost, whereas FISH is more expensive and takes longer. FISH is more trustworthy than IHC, which is a semi-quantitative method with the drawback of inconsistencies in findings due to changes in laboratory conditions, due to its quantitative character (Immunohistochemistry), is an unique staining technique used on fresh or frozen breast cancer tissue retrieved during a biopsy[6, 7,

9-15]. IHC is used to see if cancer cells have HER2 receptors or hormone receptors on their surface. This knowledge is crucial when it comes to treatment planning. Next-generation sequencing is another option (NGS). GNS is a high-throughput DNA sequencing method that can sequence hundreds of thousands to millions of DNA molecules in parallel at the same time. Currently, tumor NGS detection can be used for somatic mutation analysis.[16] Dual ISH（HER2 Gene Test Inform Dual ISH) is a novel genetic test that was authorized by the US Food and Drug Administration (FDA) today to assist medical practitioners in determining if women with breast cancer are HER2-positive and hence suited for breast cancer therapy[16, 17].

3. Current detection of HER2 breast cancer

Gene diagnosis is a method for determining the structure of DNA and RNA, as well as gene expression levels, using biological molecular technology to diagnose diseases[16]. The following are the characteristics of gene diagnosis: It detects gene mutation and expression information with high specificity by targeting specific genes. Signal amplification is a feature of molecular hybridization and PCR technology, and it may be used to diagnose trace materials with high sensitivity[16, 18]. It can also be utilized for prenatal diagnosis and group screening of the fetus before clinical signs. Samples were simple to obtain and were not limited by ontogenetic phases or tissue-specific gene expression. HER2 gene is found on the long arm of chromosome 17 and codes for a 185-kDa glycoprotein that belongs to the type 1 growth factor receptor family. When the ligand binds to the HER2/neu protein, it dimers and the intracellular domains are transphosphorylated. Here are some HER2 gene detection methods[4, 16, 19].

3.1. FISH technique

FISH(fluorescence In situ hybridization), a non-radiative in situ hybridization technique created on the basis of the original radiogenic in situ hybridization technology in the late 1980s, is a developing molecular cytogenetic approach. FISH (fluorescent in situ hybridization) is a cytogenetic technique for detecting and locating nucleic acids[9]. Fluorescent-labeled nucleic acid probes are only heterozygous with nucleic acids that are highly similar, and can be used to find genes on chromosomes or in molecular ecology to identify ribosomal RNA in different bacteria or archaea categories[9, 19]. Fluorescence in situ heterozygozygosis can also be used to check for normal fetal chromosomes. The probe DNA should be tagged with a fluorescent dye, and the denatured single strand should be hybridized with the fluorescent dye or nuclear target DNA using the base complementation principle, resulting in detectable hybridized double-stranded nucleic acid[14, 19]. Because DNA is ordered linearly along the dye's vertical axis, the results are examined and recorded using a fluorescence microscope. By finding particular genes on the dye and using standard radiolabeled in situ hybridization, the probe may directly hybridize with the dye[9, 14, 19]. Fast signal detection, excellent hybridization specificity, and multiple staining are all features of fluorescence in situ hybridization. HER2 amplification was studied using FISH[19]. In comparison to the chromosome 17 control, human breast cancer specimens hybridized with the HER2 gene probe (green) and the chromosome 17 central point probe (red) reveal a substantial increase in the HER2 gene copy number[14, 19]. The HER2 BC result of FISH shown in fig2.

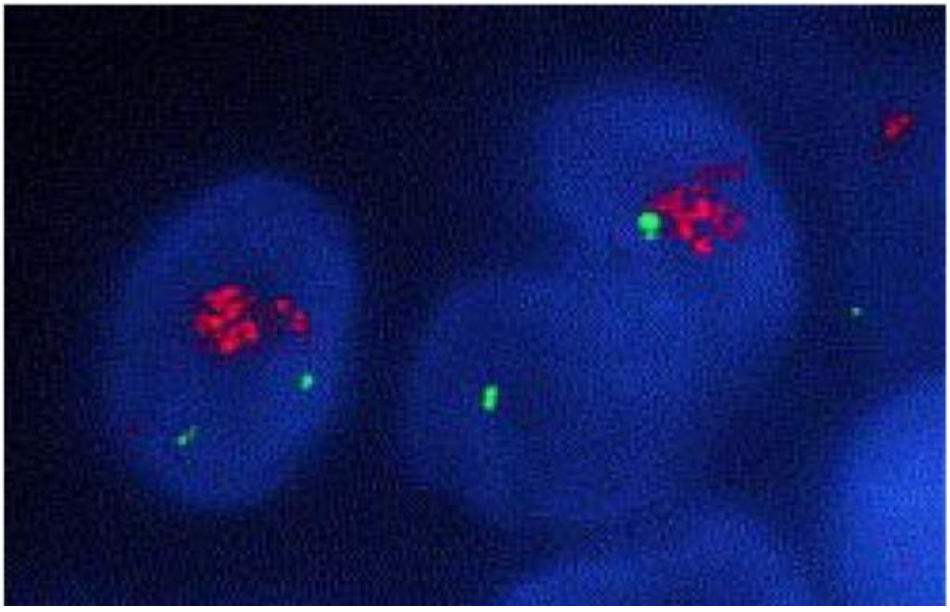


Figure 2 HER2 BC result of FISH[19].

Under a fluorescent microscope, HER breast cancer cells are green, as seen in the figure 2, indicating whether or not the patient is unwell.

3.2. CISH（Chromogenic in situ hybridization)

Chromogenic in situ hybridization (CISH) is a cytogenetic technique that combines immunohistochemistry (IHC) techniques' chromogenic signal detection approach with in situ hybridization[17]. Under a light microscope, the oncogene probe was labeled with digoxin or biotin, and the mRNA expression level of paraffin tissue was measured using a peroxidase or alkaline phosphatase reaction[19]. Because of the fluorescence half-life, it is advisable to do studies as soon as possible. If the probe is temporarily replaced by fluorescence labeled probes in each experiment, and then the hybrid is behind it, pay attention to the wet end of the box and set the slice level, not tilted, to avoid hybrid fluid loss[17, 19]. It would fail due to lack of organization and dryness. Degeneration of the hybridization probe to minimize fluorescence quenching, sample hybridization and post-hybridization treatment should be done in the dark. CISH is also affordable and can retain tissue slices for a long time because it does not require fluorescence microscopy, camera equipment, or analysis software. CISH is most typically employed in gene amplification testing, such as HER2 gene amplification detection in breast cancer. There were 15 signals per nucleus, the HER2 gene was recognized as unamplified CISH (FIG.2A)[17]. The 610 signal was found in more than 50% of the tumor nuclei, tumors were considered to have modest levels of CISH amplification (Figure 2C). Finally, tumors with more than 10 signals in the nucleus or more than 50% gene replication clusters were determined to have significant levels of HER2 amplification[15, 19].

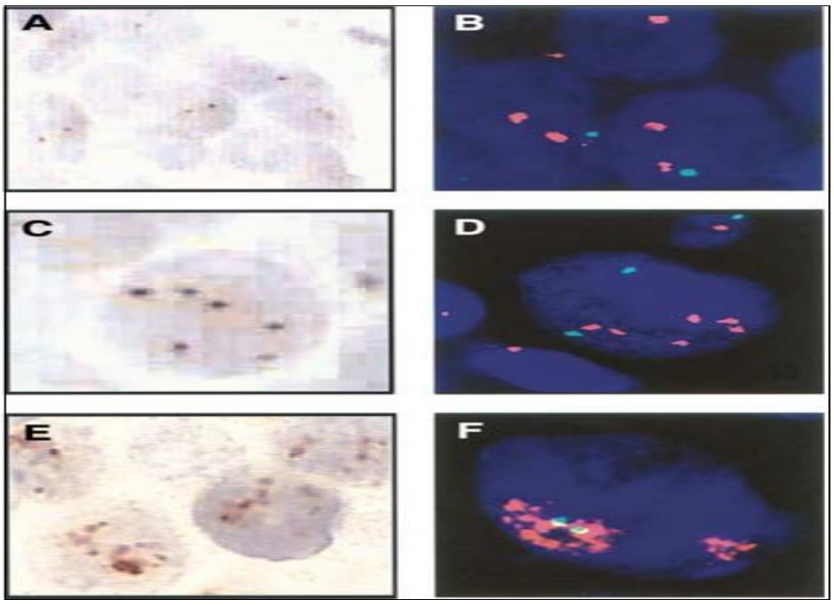


Figure 3 CISH and FISH HER2 breast cancer result[17].

A, C, E are result of CISH. B, D, F are result of FISH. CISH eliminates the need for direct observation using a fluorescence microscope, making it easier to use.

3.3. IHC (Immunohistochemistry)

Immunohistochemistry and immunocytochemistry work by forming a covalent bond between an enzyme and an antibody, marking the antibody with an enzyme, reusing specific enzyme catalysis on a substrate, generating an insoluble colored product, or using a range of electron density of particles on a normal cell surface under a microscope and an electron microscope, as well as different antigen compositions in the cell[11]. For investigations, the immunohistochemical indirect staining approach of immune enzyme is being employed. Antigen compounds (proteins, peptides, enzymes, hormones, pathogens, and receptors) may be identified at the cellular and subcellular levels with this method. Immunohistochemistry has a high specificity, sensitivity, and accuracy in localization, and it may be used to investigate morphology[15]. Tumor indicators include carbohydrates, hormones, receptors, enzymes or metabolite proteins, oncogenes and tumor suppressor genes, and associated products[9, 15]. Currently, immunohistochemical standardization is based on formalin-fixed paraffin-embedded tissue sections, with non-biotin enzyme polymer detection method of non-biotin enzyme polymer immune group detection system, which effectively prevents endogenous biotin interference, has high sensitivity, and is simple to use. It is the ideal staining procedure for immunohistochemistry standardization and is frequently used in immunohistochemistry laboratories. HER2 receptor protein on the surface of cancer cells is immunohistochemistry (IHC). When there are too many HER2 receptors on a cell, it receives too many signals telling it to grow and divide. The IHC test scores the amount of HER2 receptor protein on the surface of cells in a breast cancer tissue sample from 0 to 3+. If the score is 0 to 1+, it's called HER2 negative. If the score is 2 or higher, it is considered borderline. A score of 3 or above indicates HER2 positivity[9].

3.4. NGS（Next-generation sequencing）

Tissue biopsy is a vital step in cancer diagnosis, but it's difficult to tell if the disease has spread, and the tissue can be difficult to obtain at times, so it's not particularly accurate in early cancer detection[20]. As a result, a more precise, sensitive, and specific detection method is required. Liquid biopsies are becoming more common as some cancer signs may be found in a person's blood. PCR is a simple, easy-to-use, and low-cost nucleic acid detection method[21]. However, because of the slow detection speed and restricted input, false positive and false negative findings are highly frequent. The introduction of a new technique, NGS, may be able to address some of PCR's drawbacks[21, 22].

Massively parallel sequencing, sometimes known as "next-generation" sequencing technology, is a type of high-throughput sequencing technology (MPS). Unlike standard Sanger (dideoxy) sequencing, this approach allows for the simultaneous sequencing of a huge number of nucleic acid molecules. In most cases, a sequencing process may generate at least 100 megabytes of sequencing data. High-throughput sequencing is a game-changing advancement over classical sequencing, allowing scientists to sequence hundreds of thousands to millions of DNA molecules at once[22].

3.5. Dual ISH

The in situ hybridization(ISH) evidence of HER2 receptor protein overexpression thus ISH can determine whether patients can receive molecular targeted therapy for HER2. The HER2 Gene Test (Inform Dual ISH) is a new genetic test that was approved by the US Food and Drug Administration (FDA) today to help doctors determine if women with breast cancer are HER2-positive and hence suitable for breast cancer therapy[23, 24]. The HER2 Dual ISH DNA Probe Cocktail is a lab test that calculates the number of copies of the HER2 gene in breast cancer tissue samples in order to assist clinicians in identifying individuals who should be treated with the medicine HERCEPTIN (transtuzumab)[4, 8]. It's a companion diagnostic, which is a sort of lab test. The FDA authorized Transtuzumab, the first single medication, in 1998, ushering forth a new era in the treatment of HER2 breast cancer[23, 24]. Chemotherapy was the mainstay of breast cancer treatment in the past, and the mechanism of chemotherapy medications was cytotoxicity, which meant that patients getting chemotherapy treatments had a lot of side effects. Transtuzumab, on the other hand, is gaining popularity as a first-line therapy for HER2-positive breast cancer, with little side effects[8, 24]. As a result, the precision testing provided by Dual ISH technology can cut down on diagnostic time, allowing clinicians to swiftly evaluate a patient's condition and suggest a treatment plan[24].

4. Conclusion

Fluorescence in situ hybridization (FSIH) has garnered a lot of attention in molecular cytogenetics because it offers a lot of advantages over traditional radiolabeled in situ hybridization, such as a quick detection signal, high specificity, and multiple staining[22]. However, The results must be seen using a fluorescent microscope and cannot be stored for an extended period of time.Various factors, such as specimen fixation, baking temperature, and poor protein digestion, can impact FISH results[24]. CISH is easier to use than FISH since it requires less expertise, and the findings can be seen with the naked eye and retained for a long time[15]. PCR is commonly used to detect genes, but false-positive or false-negative results can occur.The findings of the NGS will be accessible sooner[21]. The dual ISH created an upgraded assay for high-quality in-house testing using brightfield technology, so your lab can deliver results on the first read. Hence, it is possible to establish rapidly whether patients can be treated with medications like Transtuzumab[8, 24].

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