Comparison of Her-2/Neu Gene Amplification or Expression between IHC and FISH in BC

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ABSTRACT

This study aimed to analyze and compare the application of FISH and IHC in BC tissue Her-2 / neu gene amplification or expression. For this purpose, 110 patients with BC were selected. FISH and IHC were performed on the BC tissues that were surgically removed, and the results were compared and analyzed. The results showed that in 110 BC tissues, the expression of HER-2 protein was (+++) 25 cases (22.73%); (++) 44 cases (40%); (+) 26 cases (23.64%); (1) 15 cases (13.64%). There were 42 cases of HER-2 gene amplification in 110 BC tissues, and 68 cases had no amplification. IHC test positive (+++) is consistent with FISH positive coincidence rate, IHC test negative (+/-) is consistent with FISH negative coincidence rate, IHC test suspicious positive (++) compared with the FISH result, the difference is statistically significant. However, the total coincidence rate between IHC test results and FISH test results was 89.29 (25/28), and the two test methods were positively correlated. Generally, IHC positive and negative expressions are in good agreement with FISH test results. The suspected positive expression of IHC is inconsistent with the FISH test result, suggesting that the IHC test is suspicious, and the positive specimens need to be tested by FISH.

Introduction

The clinical treatment of BC follows the principle of early detection and early diagnosis. It has multiple pathogeneses, of which 20% to 30% are related to Her-2 / neu gene amplification (1, 2). As an important factor in the treatment of BC, Her-2/neu overexpression or gene amplification is directly related to the patient's hormones, chemotherapeutic effects and prognosis, and has important significance for clinical treatment (3). Currently, the immunohistochemistry (IHC) method or fluorescence in situ hybridization (FISH) method is generally used to detect Her-2 / neu (4). However, there is still considerable disagreement about the reasonable interpretation and application of the inconsistency between IHC and FISH test results in clinical practice. In particular, the multiplicity of chromosome 17 has a great influence on the FISH test results (5, 6).

In recent years, with the improvement of medical level, tremendous progress has been made in the field of medical cancer research, but BC is still a major health problem and is currently the primary task of biomedical research (7). According to statistics, approximately 500,000 people worldwide die from metastatic BC each year (8). Her-2 / neu is an established treatment target for BC and one of the most targeted proteins in BC treatment (9, 10). There is no globally accepted method to determine its status. This gene encodes a homologous to epidermal growth factor receptor (11, 12).

Fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) are methods to evaluate fluorescence in situ hybridization (13). IHC is currently a common method for clinically detecting. At present, IHC is often used as the first detection method for evaluating the initial screening of Her-2 / neu state (14, 15). However, the IHC method has certain limitations, mainly in the preparation of tissue processing, formaldehyde fixation, and paraffin-embedded specimen preparation, which will negatively affect the antigen. Even after the repair. Sometimes it is difficult to restore the original

expression state (16, 17). Many studies have shown that those BC patients whose IHC test Her-2 / neu has a result of (+++) show a high degree of consistency with the FISH test Her-2 / neu gene, which can be used as a basis for guiding treatment (18-20).

FISH methods in HER-2 gene detection, the differences between the two detection methods can be analyzed to improve the standard of the HER-2 laboratory test, improve the accuracy of the test results, and strive to provide a clinical A simple, cheap, accurate and rapid detection method. Therefore, this paper analyzes and compares the fluorescence amplification to detect Her-2 / neu gene amplification in BC tissues by analyzing 110 cases of BC patients undergoing modified radical mastectomy. The results showed that the positive coincidence rate of the IHC test (+++) was consistent with FISH, the negative coincidence rate of the IHC test (+ / 1) and FISH was consistent, and the consistency of the IHC test was suspicious positive (+ +) and FISH was more difference. However, the total coincidence rate of IHC and FISH test results was 89.29 (25/28), and the two test methods were positively correlated.

Materials and methods
BC Specimen

The BC samples selected in this study were based on the following criteria: (1) The molecular identification of HER-2 / neu gene amplification and overexpression was performed on the specimens in advance using solid matrix blotting techniques (21, 22). (2) Amplification and expression analysis showed consistency between the amplification state and the expression state.

The expression data of 98 cases can be obtained by two or more detection methods. The expression data of the remaining 19 cases can only be obtained by one detection method (23, 24).

BC specimens with known HER-2 / neu gene copies and expression levels were evaluated as archival paraffin-embedded tissue specimens in this study. Arrange the specimens in two paraffin-embedded multi-tumor specimen blocks. 32 Histopathology of all specimens retained in the multiple specimen block was reviewed, and only specimens containing BC cells were studied. 110 cases that met all of the above criteria were included in this study, of which 43 cases of BC showed

amplification and overexpression, while 67 cases of BC did not show amplification and overexpression. The multiple tumor specimens blocking method allows the use of a small amount of reagents, and more importantly, ensures that each BC is equally exposed to all reagents in the assay system.

FISH Gene Amplification Using HER-2 / neu and Chromosome 17 Centromere Probes

This method eliminates the increase in gene copy number due to aneuploidy or tracheotomy alone. Because the HER-2 / neu gene is located on the chromosome, the alpha (pericentromeric) DNA probe on chromosome 17 was selected as an internal control for chromosome aneuploidy (25, 26).

IHC Testing

IHC was used in this experiment, and rabbit anti-human HE-2 polyclonal antibody was purchased from Wuhan Boshide Company. The working concentration of the primary antibody is 1: 100, and the secondary antibody is l: 200. The specimens were uniformly tested for IHC Streptomyces avidin-peroxidase linkage method (SP). The SP 3,3 dioxybenzidine (DAB) kits were purchased from Beijing Zhongshan Jinqiao Company, according to the kit instructions.

FISH Testing

FISH is a type of new technology used for the diagnosis of BC in the case of centromere 17 and HER-2 gene amplification (18). It is more accurate than IHC and is considered the gold standard for HER-2 gene monitoring. The experimental steps are as follows: (1) Extract the nucleus and extract the nucleus from the paraffin-embedded tissue. Perform HE staining on the embedded tissue; determine the dense part of the tumor cells under the microscope, paraffin section, the thickness is subject to 20 m. (2) Fabrication of nuclear array, using cell array technology to arrange blood cells of HER-2 negative specimens in ICH detection in the same slice. (3) FISH, the cell array slides were immersed in fixing liquid for 1 h, taken out and dried at 25°C; placed in pH-6.0 citrate buffer and heated in the microwave for 10 min; placed in $2 \times$ SSC solution at 37°C, 15min; properly digested in 0.4% pepsin; dehydrated with 70, 85, and 100% ethanol for 2min; placed in an oven at 45-50°C to evaporate ethanol; add 10μHER-2 /

neuDNA probe; sealed with sealing glue, 37°C Incubate for 6-15 hours; wash the probe and stain with 4,6-diamidino-2-phenylindole dihydrochloride (4,6-diamidino2-phenylindole, DAPI).

Criteria for Positive Results

The cell membrane showed a clear tan or tan as positive. HER-2 is localized in the cell membrane or cytoplasm and is characterized according to the percentage of positive cells. Three researchers interpreted the results and observed at least 10 cells in at least 10 high-power fields (400 times) in each slice. Using the DAKO HerceptTest scoring system: (1) means no cell staining or less than 10% weaker membrane staining; (+) means 10% weaker staining of the tumor cell membrane; (++) means 10% of the tumor cell membrane is completely weak or medium Staining; (+++) is strong staining of tumor cell membranes greater than 10%. According to the proportion of positive cells in the observed cells, semi-quantitative grading is performed: positive reactions are less than 10% (one), and 10% to 30% are (+). > 30% ～ 50% is (+ +),> 50% is (+ + +). Negative expression is counted as (1), (+) and (++), and (+ + +) is recorded as positive expression.

Statistical Processing

SPSS13.0 statistical software was used for analysis. The relationship between HER-2 and clinicopathological indicators was compared. The line x list was used for the y2 test, with d = 0.05 as the test level and P <0.05 as the difference was statistically significant.

Results and discussion
Gene Amplification Using HER-2/neu and Centromere Probes on Chromosome 17

In each of the 110 BC specimens, the average copy number of the HER-2 / neu gene and the average chroma of 17 centromere copies were determined in 60 tumor cell nuclei (Figure 1 (*Figure from www.baidu.com*)). HER-2 / neu-chromosome 17 sequence from 0.69 to 19.07; value greater than or equal to 2.0 indicates gene amplification. In this assay, 42 BCs were amplified, but 75 were not. After the results were revealed, the sensitivity was 95.4% and the specificity was 98.6% (Table 1). The HER-2 / neu-chromosome 17 central region ratios of two BCs

is known to have gene amplification are 1.98 and 1.89, respectively. Another BC known to lack gene amplification has an HER-2 / neu-chromo centromere ratio of about 4.06. 107 of 110 BCs were correctly classified, with an accuracy rate of 97.4%. The degree of agreement (kappa) is 0.9450.
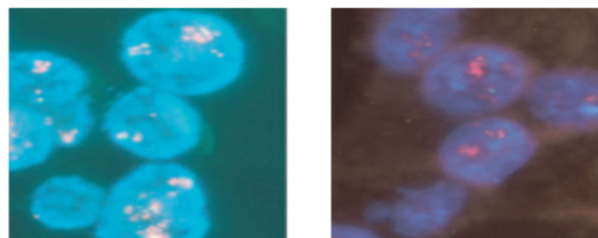

Figure 1. Vysis PathVysion FISH assay

Table 1. FISH assay results using 110 BCs with known HER-2/neu gene amplification and overexpression status

Assay	FISH, Vysis
No. Positive/No. Negative	42/75
Sensitivity	0.954
Specificity	0.986
Kappa	0.945
Accuracy	0.974

DAKO HercepTest is used for Immunoenzyme Staining to Detect HER-2/neu Protein

Immunostaining of HER-2/neu oncoprotein was done by HercepTest kit. 17 cases of BC were immunostained three times, 13 cases were immunostained twice, 5 cases were immunostained once, 82 cases were not performed Immunostaining. The staining result is shown in Figure 2(*Figure from www.baidu.com*). Known expression levels indicate that 30 of 43 overexpressed BCs and all 74 underexpressed BCs have been correctly classified. Sensitivity is 69.8%; specificity is 100%; accuracy is 88.9%. The degree of agreement (kappa) is 0.745(Figure 3). If only three types of BC are considered positive, the false-negative rate of the Hercep Test will be greatly improved.
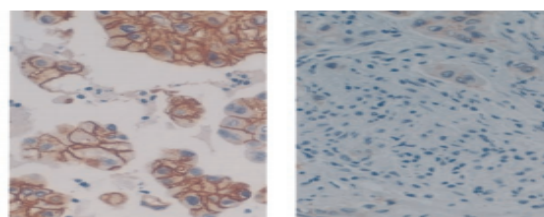

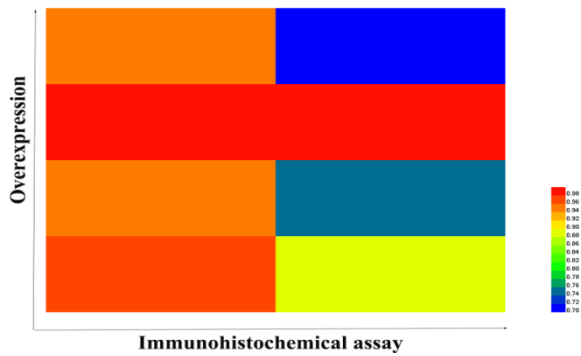**Figure 2.** DAKO HercepTest immunohistochemical assay

Figure 3. Immunohistochemical assay results using 110 BCs with known HER-2/neu gene amplification and overexpression status

Analysis of the Results of the Comparison of the Two Methods

In this group of experiments, the statistical results are shown in Figure 4, the coincidence rate of FISH after the IHC test (++ +) was 96% (24/25), and the coincidence rate of FISH after the IHC test (++) was 4.55% (2/44). The coincidence rate of the FISH test after the IHC test (+) was 96.15 (25/26), and the coincidence rate of the FISH test after IHC test (1) was 93.33 (14 / l5). That is, the positive coincidence rate of the IHC test (+ + +) is consistent with FISH, the negative coincidence rate of the IHC test (+ / a) is consistent with the negative coincidence of FISH, and the consistency of the IHC test suspicious positive (+ +) and FISH results are poor. However, the total coincidence rate of IHC and FISH test results was 89.29% (25/28), and the two test methods were positively correlated, as shown in Table 2.

Both domestic and international experiments and clinical literature on the study of HER-2 gene expression generally have the problem of small sample size, and some of them have inconsistent or even contradictory phenomena (15, 27). It is largely limited to the personal experience and status of the observer (28). At the same time, there is no standard and objective basis for the test results. There is still great controversy about the choice of the HER-2 gene detection method, although the IHC test has a wide range of applications. However, there are obvious shortcomings, and the application of FISH has been delayed due to price issues (29, 30). For the comparison of the advantages and disadvantages of the IHC and FISH methods in the HER-2 gene detection, the differences between the two detection

methods can be analyzed to improve the standard of the HER-2 laboratory test, improve the accuracy of the test results, and strive to provide clinical A simple, cheap, accurate and rapid detection method.

Table 2. Analysis of the results of IHC and FISH comparison

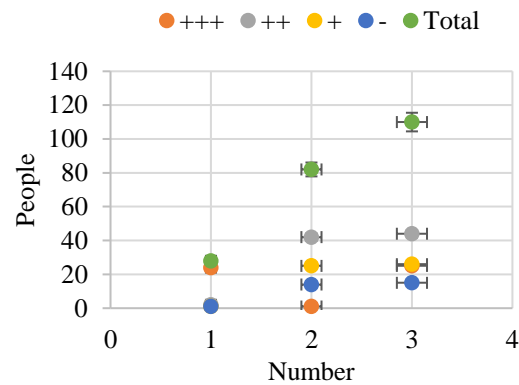| IHC test results	FISH test results		Total
	Positive	Negative	
+++	24	1	25
++	2	42	44
+	1	25	26
-	1	14	15
Total	28	82	110

Figure 4. Analysis of the results of IHC and FISH comparison

IHC is still the preferred test method for evaluating the status of HER-2 due to its advantages such as small quantity required for organised specimens, simple operation, low requirements, low price, short experimental process, easy storage of stained slides, and fast reading of slides (31). The main feature of FISH different from IHC is the quantitative interpretation of results, which avoids subjective errors and has a high degree of reliability and repeatability. Studies have shown that FISH has high false positives and false negatives in detecting HER-2 protein, and the effectiveness and accuracy of FISH is significantly higher than IHC, which can be widely used in clinical practice. HER-2 gene amplification is associated with positive axillary lymph nodes. Studies have shown that FISH and IHC have a high consistency in detecting the expression status of HER-2. Combined with the actual situation including cost and other limitations, on the basis of IHC as the initial screening, FISH is still recommended as a test for

HER-2 gene expansion. Increased standard methods. FISH technology to detect HER-2 gene expression in BC patients can provide a more reliable basis for clinical guidance of medication and prognosis evaluation. Studies have shown that IHC can be used as the preferred method for screening HER-2 in BC patients. When the expression of HER-2 protein is (1) or (++), the consistency of IHC and FISH detection is more; while for IHC Patients with (+) or (++) should undergo FISH testing to determine HER-2 gene expression. Studies have shown that FISH is more accurate than IHC in detecting HER-2 expression, and is particularly suitable for judging HER-2 expression in patients with IHC (++). There is a certain inconsistency in the expression of HER-2 between the primary and metastatic BC. The results of this study show that the positive coincidence rate of IHC test (+++) is consistent with the positive rate of FISH, and the negative IHC test (+ / a) Consistent with the negative coincidence rate of FISH, the suspected positive (+ +) IHC test is inconsistent with the FISH result. However, the total coincidence rate of IHC and FISH test results was 89.29 (25/28), and the two test methods were positively correlated ($X2 = 84.89$, $P <0.01$).

In summary, the positive and negative expressions of IHC are in good agreement with the FISH test results. Suspected positive expression of IHC, compared with FISH test results, the difference was statistically significant ($P <0.05$). Prompt IHC test suspected positive specimens need to be tested by FISH, and positive and negative specimens do not need to be retested.

Acknowledgements

Interest conflict

The authors declare no conflict of interest.

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