REVIEWS

Adv Clin Exp Med 2015, **24**, 5, 899–904
DOI: 10.17219/acem/27923

ISSN 1899–5276

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A Comparison of IHC and FISH Cytogenetic Methods in the Evaluation of HER2 Status in Breast Cancer

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

The *HER2* gene is responsible for the formation of the HER2 receptor on the surface of epithelial cells. Increased numbers of this receptor are associated with a worse prognosis in cancer. Increased numbers of copies of the *HER2* gene occur in about 20–30% of breast cancer patients, so determining HER2 receptor levels is important in the current diagnosis and treatment of breast cancer. One diagnostic technique is the immunohistochemical (IHC) method, which permits indirect measurement of overexpression of HER2 receptors, based on subjective determination of the intensity of the color reaction. Another technique is the use of fluorescent *in situ* hybridization (FISH), which permits the exact number of copies of the *HER2* gene to be specified. Based on the results of FISH tests, patients can be qualified for treatment with antibodies that partially block HER2 receptors. This treatment causes inhibition of tumor growth signals. Determining the HER2 status in breast cancer with the FISH method allows the further progress of the disease to be predicted, the right treatment to be chosen and the response to the treatment to be foreseen. Because of the widespread use of the FISH and IHC methods, comparing the advantages and disadvantages of these two methods seems to be relevant (**Adv Clin Exp Med 2015, 24, 5, 899–904**).

Key words: breast cancer, immunohistochemistry, HER-2/neu, fluorescence *in situ* hybridization.

Due to amplification of the *HER2* gene in 20–30% of patients with breast cancer [1], determination of HER2 status in those patients has a great importance in clinical practice [2]. *HER2* gene amplification correlates with negative predictors [3] and is associated with a shorter survival time, a higher percentage of recurrences of cancer and a lower response to chemotherapy and hormone therapy [4]. Jana et al. [5] state that overexpression of the HER2 protein is associated with a large tumor size and advanced tumor stage.

HER2 and *GRB7*: Genes from the 17q11-12 Amplicon with Predictive Value in Breast Cancer

Overexpression of the HER2 receptor is the result of the amplification of the gene located on chromosome 17 (17q11-12) [6]. There are, however, cases of patients with breast cancer in whom overexpression of HER2 receptor occurs without amplification of the *HER2* gene [7–9]. The opposite situation – when amplification of the *HER2* gene does not cause overexpression of the HER2 protein – also occurs. One of the mechanisms causing this inconsistency is incomplete amplification of a smaller region on chromosome 17q11-12, on which the GRB7 gene is located. Amplification of the *HER2* gene without overexpression of the HER2 protein is clinically important, because it prevents patients with breast cancer from benefitting from targeted HER2 therapy [10]. The GRB7 gene may amplify and cause GRB7 protein overexpression [11]. The *GRB7* gene plays an important role in the growth and migration of cancer. The GRB7 protein can interact with the HER2 protein (among other things) [12]. Amplification of the *GRB7* gene is associated with increased transcriptional activation and, irrespective of HER2 status, may have a high predictive value in the treatment of breast cancer [13].

Cytogenetic Methods

There are many methods for determining HER2 receptor overexpression, such as polymerase chain reaction (PCR), immunohistochemistry (IHC), fluorescent *in situ* hybridization (FISH), silver enhanced *in situ* hybridization (SISH) or chromogenic *in situ* hybridization (CISH). IHC and FISH are the two main methods in clinical practice for assessing *HER2* gene status [14].

Fluorescent *In Situ* Hybridization

Due to its sensitivity and specificity, FISH is regarded as the "gold standard". A decade ago, fluorescent microscopes equipped with the appropriate filters were quite expensive and not every laboratory could afford this equipment [14], but nowadays fluorescent microscopes are not unobtainable. McManus et al. [15] find FISH an objective and reliable method for measuring HER2 amplification. This method is used not only in the diagnosis of breast and stomach tumors [16], but also to monitor the response to treatment for these tumors [17].

Immunohistochemistry

The immunohistochemical method is cheaper, convenient to carry out and storage of the specimens is easier [18]. However, it is a semi-quantitative method [19], because it is based on a subjective determination of the intensity of the color reaction. There is still a debate about which method is the preferred one for determining *HER2* gene status.

A Comparison of the FISH and IHC Methods

Because of the widespread use of FISH and IHC methods, comparing the advantages and disadvantages of these two methods seems to be relevant. In a study comparing the FISH and IHC methods, Gould et al. [17] reported that FISH is increasingly considered to be the most accurate and predictive test for determining HER2 amplification and the response to the treatment of breast cancer. The results of several studies [20, 21] suggest that FISH has higher accuracy than IHC and is a better prognostic indicator in the case of high-risk breast cancers. In the study by Goud et al. [17], 90 breast cancer tissue samples were used to analyze the FISH and IHC methods. The authors suggest that HER2 status should be evaluated using FISH in all cases with IHC 2+. IHC 3+ results can be analyzed with FISH in order to exclude chromosome 17 polysomy, which can be misinterpreted as HER2 overexpression in when using the IHC method. The authors state that it is necessary to determine the cut-off values for HER2 amplification results with FISH against HER2 overexpression results with IHC, to make it possible to compare and calibrate the two methods [17]. The concordance rate of results obtained by the FISH and IHC methods is controversial [9]. Generally, comparative studies of these two methods show a high concordance of results. Overexpression of the HER2 protein may be found without amplification of the *HER2* gene, or *HER2* gene amplification can occur without overexpression of the HER2 protein [21]. Tubbs et al. [22] documented that there is a large discordance rate between FISH and IHC for all four IHC scores (0, 1+, 2+, 3+), and they suggest using only the FISH method in diagnostics. Similar studies were carried out by Singhai et al. [9], who analyzed 50 cases of breast cancer. The concordance of results was 82% of all cases of breast cancers. The authors concluded that the immunohistochemical method can be used primarily as a screening method to determine HER2 status. They also concluded that FISH can be an additional, complementary method to detect false negative IHC results, especially those with a high degree of malignancy. A study by Dolan and Snover [23] showed that the concordance rate between FISH and IHC results is 27.1%. Lan et al. [24] documented amplification of the *HER2* gene with the FISH method in only 96 out of 221 cases of breast cancer, and found that the concordance rate of the two methods is 44.4%. Also, on the basis of their study, Kuo et al. [25] reported a high degree of discordance between the FISH and IHC methods. According to other authors [26], FISH and IHC correlate well with each other when IHC results at level 2+ are in concordance with the results of FISH. Wand et al. [27] obtained a high rate of concordance: 98%. IHC negative and IHC 1+ results showed no gene amplification with the FISH method. IHC 3+ results showed gene amplification with FISH, while results of IHC 2+ showed a large discordance with the results of the FISH method [27]. The results of a study by Panjwani et al. [28] show that there is no statistically significant relationship between HER2 status and tumor size. A positive correlation occurs between the stage of the cancer and HER2 status, which is confirmed by the literature [29]. The research carried out by Panjwani et al. shows that 64.5% of tumors with a high degree of malignancy (grade 3 tumors) are correlated with *HER2* gene amplification.

Most studies (including the one by Panjwani et al.) generally present high concordance rate between IHC 3+ results and FISH results showing *HER2* gene amplification, and between negative IHC results and FISH results with no *HER2* gene amplification. The discordance between IHC and FISH methods is related mainly to IHC 2+ results. This discordance is an indicator of undetermined HER2 status [30]. Several studies show an approximately 20% discordance rate in HER2 status results between IHC and FISH [31]. In a study by Apple et al., the discordance rate is only 5% [32]. The possible reasons for discordant results are differences in pre-analytic factors such as binding time, or differences in laboratory processes and variances between interobservers in the evaluation of HER2 receptor status.

Another factor leading to differences between FISH and IHC results is intratumoral heterogeneity, which is a result of using core needle biopsy to take samples [32]. Rossi et al. state that tumors with IHC 2+ and FISH negative results (with no *HER2* gene amplification), which are known as HER2 negative, have a worse prognosis than tumors with no or poor expression of *HER2* (IHC 0 or 1+). A HER2 negative result is determined on the basis of the diagnostic algorithm used to qualify patients for treatment with the anti-HER2 antibody [33].

HER2 is used as a biomarker in targeted immunotherapy with trastuzumab [35], a recombined humanized monoclonal antibody used with high clinical effectiveness in patients with amplification of the *HER2* gene or overexpression of the HER2 protein [36]. Many clinical trials show that FISH has a higher predictive value than IHC for response to treatment with transtuzumab [21, 35]. On the basis of the results of FISH tests, patients can be qualified for treatment with antibodies partially blocking HER2 receptors, which inhibit tumor growth signals. However, many laboratories use IHC as the first method of choice [10].

Trastuzumab binds with the extracellular domain of HER2 protein and blocks the proliferation of *HER2* gene-amplified cancer cells. It is also capable of inducing cellular toxicity against tumor cells [36]. In patients with HER2 protein overexpression, chemotherapy with trastuzumab slows the progression of the disease, leads to a higher rate of positive response to treatment and a significant reduction in mortality in comparison to chemotherapy without this drug [37]. Trastuzumab treatment is only recommended in cases where FISH results show amplification of the *HER2* gene or IHC results scored 3+ [9].

Other Methods Used in *HER2* Diagnostics

A relatively new method for measuring *HER2* gene amplification is quantitative real-time polymerase chain reaction (PCR PCR). It is characterized by potentially high sensitivity, specificity, reliability and low cost. High sensitivity means that even nanograms of low quality DNA can be detected and quantified [38]. RT PCR was used to determine *HER2* gene amplification by Chariyalertsak et al. [2]. They consider that the development of this method makes it possible to carry out more a precise quantitative analysis of gene amplification. The method is simple and quick to perform; it can be automated; and it can potentially be used as a routine test in breast cancer screening. An additional advantage of this method is that it doesn't require a specialist to interpret the results. RT PCR is viable competition for FISH [2].

Another method is immunohistochemiluminescence. It is similar to immunohistochemistry and uses chemiluminescence produced by electromagnetic radiation as the result of chemical reaction. Its intensity depends on the concentration of chemiluminescent reagent in the reaction [39]. This method has many advantages, such as high sensitivity, the stability of the reagents, and simplicity in practice. Additionally, the products are not degradable under the influence of light [40].

Summary

Overexpression of the HER2 protein may be found without the amplification of the HER2 gene, and *HER2* gene amplification can occur without overexpression of the HER2 protein. Amplification of the *HER2* gene without overexpression of the HER2 protein is clinically important, because patients with this type of breast cancer will not benefit from targeted HER2 therapy. The *GRB7* gene is located with the *HER2* gene on chromosome 17q11-12. Amplification of the *GRB7* gene is associated with increased transcriptional activation and, irrespective of HER2, may have high predictive value in the treatment of breast cancer.

The immunohistochemical method can be used primarily as a screening method, allowing HER2 status to be determined. The concordance rate differs from study to study, but research results generally present a high concordance rate between IHC 3+ results and FISH results showing *HER2* gene amplification, and between negative IHC results and FISH results with no *HER2* gene amplification. The discordance between IHC

and FISH methods is related mainly to IHC 2+ results. This discordance is an indicator of undetermined HER2 status, and because of that, HER2 status should be evaluated using FISH in all cases with IHC 2+.

Many clinical trials show that FISH has a higher predictive value for response to treatment with trastuzumab than IHC. Trastuzumab treatment is only recommended in cases where FISH results show amplification of the *HER2* gene or where IHC results indicate overexpression scored 3+.

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Conflict of interest: None declared

Received: 13.04.2014
Revised: 30.05.2014
Accepted: 27.06.2014