Retrospective analysis of the association between human epidermal growth factor receptor 2 amplification and chromosome enumeration probe 17 status in patients with breast cancer

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Abstract. The aim of the present study was to identify potential human epidermal growth factor receptor 2 (HER2) amplification, according to American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) 2013 HER2 testing guidelines, in patients previously determined not to possess HER2 amplification, in accordance with previous 2007 guidelines. Potential discrepancies may arise from chromosome enumeration probe 17 (CEP17) amplification, deletion, polysomy or monosomy. HER2, CEP17, tumor protein p53 (TP53) and retinoic acid receptor α (RARA) genes from 67 patient specimens with suspected amplification, polysomy or monosomy of CEP17 were analyzed using fluorescence in situ hybridization. HER2 status was interpreted using 2007 and 2013 ASCO HER2 test guidelines as well as the reference genes TP53 and RARA. According to ASCO/CAP2007 HER2 guidelines, 20 patients exhibited HER2 amplification (29.85%), 41 were without HER2 amplification (including 25 with polysomy, 15 with monosomy and 1 with suspected monosomy plus co-amplification of HER2 and CEP17) and the remaining 6 patients were equivocal. Using ASCO/CAP 2013 HER2 guidelines, 49 patients exhibited HER2 amplification (73.1%). The 29-patient increase included 6 originally at equivocal levels but now demonstrating amplification, 22 originally with polysomy but now revealing co-amplification, and 1 with suspected monosomy plus co-amplification of HER2 and CEP17. According to TP53 and RARA, HER2 was amplified in 43 patients (64.1%). Using the revised guidelines, HER2, originally identified as amplified in 6 patients, was not amplified following the introduction of TP53 and RARA control genes. Among these 6, 4 possessed normal TP53 and RARA. The incidence of co-amplification of HER2 and CEP17 was 1.4% (21/1,518). RARA and TP53 are suitable control genes to evaluate HER2 status.

Introduction

The human epidermal growth factor receptor 2 gene (HER2) is located on chromosome 17q12. In 1987, Slamon et al (1) proposed that the amplification of HER2 was associated with breast cancer prognosis. Subsequently, HER2 has been revealed to be amplified, or HER2 protein overexpressed, in between 20 and 30% of patients with breast cancer. These patients are generally diagnosed with high-grade cancer with increased rates of cell proliferation and a tendency to metastasize to the lymph nodes. Prognosis of these patients is markedly poorer compared with patients with breast cancer who do not overexpress HER2 (2-4). Herceptin/trastuzumab combined with chemotherapy may improve the quality of life of patients with HER2-positive breast cancer and prolong their disease-free survival time. Although a limited number have been described, occasional side effects of Herceptin treatment do occur, including cardiac toxicity that may weaken cardiac contractility, leading to cardiac insufficiency (5-9). On this basis, HER2 status is an important marker for selecting suitable therapy.

The HER2 test guidelines set out by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) were updated in 2013 from the previous 2007 version; the evaluation standards of immunohistochemistry (IHC) and in situ hybridization (ISH) test results were revised in these guidelines (10,11). In China, HER2 IHC is extensively applied as a preliminary screen, whereas ISH is primarily considered a confirmatory test for HER2 gene amplification, with the most common ISH test involving double-probe fluorescence (FISH). Distinctions between the 2013 and 2007 ASCO/CAP evaluation standards of double-probe FISH results are as follows: i) The threshold value of HER2 amplification was adjusted to be ≥2.0 (≥2.2 in the 2007 version); ii) in the 2013 version, HER2 amplification...
was also defined as HER2/CEP17 <2.0 with mean HER2 copies/nucleus ≥6.0, or HER2/CEP17 ≥2.0 with mean HER2 copies/nucleus <4.0. In the 2007 version, these values were considered to represent non-amplification (HER2/CEP17 <1.8) for patients identified with simultaneous HER2 and chromosome 17 centromere locus amplification. However, in the 2013 version HER2 is considered to be amplified in these patients and, therefore, these patients should be considered for HER2-targeted therapy. The aim of the present study was to evaluate the patients that did not exhibit HER2 amplification by 2007 standards, but with potential HER2 amplification by 2013 guidelines.

The selection of control genes for investigations using double probes is important. A control gene was selected for chromosome 17 to exclude influences of chromosome 17 polysomy in cancer cells. A second control gene was selected that is sufficiently distant from HER2 so as to remain stable when HER2 is amplified. On the basis of double-probe FISH studies by Troxell et al (12) and Varga et al (13), chromosome enumeration probe 17 (CEP17), tumor protein p53 (TP53) and retinoic acid receptor (RARA) were selected as controls for HER2.

In the present study, a retrospective analysis was performed to review HER2 FISH-analyzed cases and to compare the 2007 and 2013 ASCO/CAP guidelines. Alterations in HER2 status following the introduction of novel control genes were also determined. In addition, the effect of amplification or deletion, or polysomy of CEP17 in screening patients for targeted therapy was investigated.

Patients and methods

Samples. Specimens from 1518 patients with breast cancer were previously analyzed by HER2 FISH between February 2011 and January 2015; samples were collected from 15 hospitals, including The First Affiliated Hospital of Chongqing Medical University, The Second Affiliated Hospital of Chongqing Medical University, Yongchuan Hospital Chongqing Medical University, The Hospital of Traditional Chinese Medicine of Chongqing, The Fifth People's Hospital of Chongqing, The Ninth People's Hospital of Chongqing, The People's Hospital of Chongqing Rongchong, The Centre's Hospital of Chongqing Jiangjin, The People's Hospital of Chongqing Bishan, The People's Hospital of Chongqing Changshou, The People's Hospital of Chongqing Huchuan, The People's Hospital of Chongqing Qijiang, The People's Hospital of Chongqing Tongliang, The Centre's Hospital of Chongqing Fuling. FISH was performed for patients exhibiting medium to strong HER2 IHC levels prior to Herceptin administration, according to the ASCO/CAP 2013 criteria (11). From this FISH analysis, 67 specimens with suspected amplification, polysomy and monosomy of CEP17 were selected for inclusion in the present study. This retrospective study was approved by the Chongqing Medical University ethics committee.

FISH. Paraffin-embedded tissue samples (from the 67 selected patients) were fixed in 10% neutral buffered formalin at room temperature for between 24 and 48 h, and were sectioned at a thickness of 4 μm. Hematoxylin and eosin staining for 5-10 min at room temperature was performed to label infiltrating carcinomas, and observation with an Olympus BX41 microscope (magnification, x40). FISH for HER2, CEP17, TP53 and RARA was performed on paraffin sections according to the manufacturer's instructions (each individual probe of HER2, CEP17, TP53 and RARA and solid tumor FISH testing protocol were obtained from Beijing GP Medical Technologies, Ltd.; China Medical Technologies Inc., Beijing, China). Information about marker probes is presented in Table I. Fluorescence signal observation, photography and analysis were performed using an Olympus BX51 fluorescence microscope (magnification, x100) and FISH software (version 2.0; Beijing GP Medical Technologies, Ltd.; China Medical Technologies Inc.). HER2 status was interpreted according to the 2007 and 2013 ASCO/CAP HER2 test guidelines as well as the control genes, TP53 and RARA.

Table I. Labeled probes on chromosome 17.

| Gene                        | Color | Marker site |
|-----------------------------|-------|-------------|
| Human epidermal growth factor receptor 2 | Red   | 17q11.2-q12 |
| Chromosome enumeration probe 17 | Green | 17p11.1-q11.1 |
| Tumor protein p53            | Green | 17p13.1     |
| Retinoic acid receptor α     | Red   | 17q21.1     |

Results

FISH for CEP17 and HER2, as well as TP53 and RARA was performed on 67 samples. According to ASCO/CAP 2007 guidelines, 20 patients exhibited HER2 amplification (29.85%; 16 with CEP17 monosomy and 4 with partial CEP17 deletion), which was consistent with HER2/CEP17 ≥2.0 (Table II). On this basis, HER2 was concluded to be amplified. A total of 6 patients were revealed to be equivocal for HER2/CEP17 (4 patients with 2.2 > HER2/CEP17 >2.0 and 2 patients with 1.8 < HER2/CEP17 <2.0). A total of 41 patients did not experience HER2 amplification, including 25 with polysomy (6 with CEP17 and HER2 cluster-amplification and 19 with CEP17 and HER2 punctiform-amplification), 15 with monosomy and 1 with suspected monosomy plus co-amplification of HER2 and CEP17.

Table II presents HER2 status according to various interpretation standards (ASCO/CAP 2007, ASCO/CAP 2013 and reference genes TP53 or RARA). According to ASCO/CAP 2013 guidelines, 49 patients were diagnosed with HER2 amplification (73%). The additional 29 patients who were not diagnosed with HER2 amplification according to the 2007 criteria included 6 patients originally at the equivocal level but now demonstrating amplification (4 patients with HER2/CEP17 ≥2.0 and 2 patients with 1.8 < HER2/CEP17 <2.0 but HER2 ≥6 signals/nucleus), 22 patients originally with polysomy but now exhibiting amplification (HER2/CEP17 <2, but HER2 ≥6 signals/nucleus) and 1 patient with suspected monosomy plus co-amplification of HER2 and CEP17 (HER2/CEP17 <2, but HER2 ≥6 signals/nucleus).
The introduction of TP53, RARA and CEP17 as control genes indicated that HER2 was amplified in 43 patients (64.3%). A total of 6 patients with HER2 amplification according to ASCO/CAP 2013 guidelines did not exhibit amplification following the introduction of TP53 and RARA control genes. Among these 6 patients, 4 exhibited normal TP53 and RARA, partial CEP17 deletion, HER2/CEP17 ≥ 2, but HER2/TP53 < 2, HER2/RARA < 2 and HER2 < 4 signals/nucleus, and the
remaining 2 patients demonstrated HER2 ≥6 signals/nucleus and HER2/CEP17 <2, but HER2/TP53 <2 and HER2/RARA <2, on which basis polysomy was defined. Of the 15 patients with monosomy, 3 patients exhibited normal TP53 and RARA, therefore the number of monosomic patients was 12.

Using TP53, RARA and CEP17 as control genes, the incidence of chromosome 17 polysomy in 1,518 patients was 0.2% (3/1,518) and the incidence of monosomy was 0.8% (12/1,518). The incidence of co-amplification of HER2 and CEP12 was 1.4% (21/1,518).

HER2 status was associated with the status of CEP17 and the reference genes. Fig. 1 demonstrates common HER2 and CEP17 status using FISH. Fig. 2 reveals co-amplification of HER2 and CEP17 polysomy. If only applying CEP17, HER2/CEP17 <2 and therefore HER2 was not amplified according to the 2007 ASCO/CAP version, but was amplified according to the 2013 version (HER2 ≥6 signals/nucleus). Fig. 3 reveals that chromosome 17 monosomy was accompanied by irregular HER2 and CEP17 status. Fig. 4 demonstrates CEP17 deletion by FISH. If only applying CEP17, HER2/CEP17 ≥2 and therefore HER2 was amplified according to the 2013 version of ASCO/CAP guidelines. However, FISH analysis of TP53 and RARA revealed HER2 to be normal.

**Discussion**

Samples without HER2 amplification according to the ASCO/CAP 2007 HER2 test guidelines may be classified as with HER2 amplification according to the revised 2013 HER2 test guidelines, particularly in contentious co-amplified specimens. This suggests that these patients may benefit from HER2-targeted medicine. Therefore, in the present study, FISH results from 1,518 patients were reviewed and 67 patients were identified with abnormal CEP17 signals, including suspicious co-amplification, deletion, polysomy and monosomy.

The incidence rate of co-amplification of HER2 and CEP17 was 1.4% (21/1,518), which demonstrates distinction from previous studies. Troxell et al (12) identified that 7/858 patients with cancer exhibited abnormal HER2 and CEP17 (6 with breast cancer and 1 with ovarian carcinoma); the incidence rate of CEP17 amplification was 0.8%, whereas no HER2 amplification was revealed in 3/7 patients. On this basis, the incidence rate of co-amplification was 0.47%. Varga et al (13) identified that 14 patients were diagnosed with co-amplification of >5,000 patients with breast cancer who underwent FISH analysis between 1999 and 2009, on the basis of which, the co-amplification incidence rate was 0.3%. Press et al (14) observed co-amplification in 2/2,600 patients with breast cancer, on the basis of which the co-amplification incidence rate was 0.08%. Gunn et al (15) selected 20 patients who exhibited unclear HER2 status following routine FISH and IHC investigations, and identified HER2 status through array-based comparative genomic hybridization (aCGH). Co-amplification of HER2 and CEP17 was observed in 3/20 patients, for which the co-amplification rate was 15% in patients suspected to be positive for HER2; there was a tendency for a false negative result if based only on the HER2/CEP17 ratio. Marchio et al (16) randomly selected...
18 patients (~8% of all cases) with a mean CEP17 ≥3 signals/nucleus to perform an aCGH test and identified that 17q containing the centromere locus was amplified in 11 patients, 17q excluding the centromere locus was amplified in 1 patient and was combined with true polysomy in 1 other patient, whereas amplification of only the centromere locus was identified in 5 patients. Therefore, the co-amplification incidence rate was 61.1% (11/18). On this basis, the overall co-amplification rate was 4.9%. Tse et al (17) selected 171 patients with a mean CEP17 signals/nucleus of >2.6 to analyze HER2 FISH results from 5,683 patients. Novel control genes were introduced into the interpretation standards, RARA and TP53. Following the introduction of these control genes, HER2 of 58 patients (43.9%) was defined to be amplified in 132 patients previously identified as non-amplified (on the 2007 ASCO/CAP criteria of HER2/CEP17). HER2 gene amplification was identified in 13/14 patients at the threshold value. The ratio of HER2/CEP17 was at the threshold value of 1.8-2.2 or HER2 gene copy 4.0-6.0. Additionally, HER2 status continued to be defined as amplified in 25 patients in whom amplification was classified previously. The results observed a limited number of patients with polysomy, and the co-amplification rate was 1.8% ([58+13+25]/5863). Egervari et al (18) investigated chromosome 17 polysomy and observed, using FISH, that 5/405 patients with breast cancer presented CEP17 ≥3 alongside HER2 amplification, on the basis of which the co-amplification incidence was 1.23%. At the same time, Egervari et al (18) proposed that a pseudomorph of chromosome 17 polysomy was induced by CEP17 centromere locus amplification and therefore the incidence of chromosome 17 polysomy may be less.

Distinctions were observed in the incidence rates of co-amplification between the results of the present study and the aforementioned previous studies. A total of 22/1518 patients, analyzed using FISH in the present study, were observed to exhibit co-amplification, all of whom presented with medium to strong levels of HER2 IHC and excluded HER2 negative and weak specimens. If counting these negative or weak specimens, the incidence rate of co-amplification was ~0.55% (22/4016).

Currently, the definitions of polysomy and monosomy are as follows, polysomy occurs when an entire chromosome is duplicated one or more times, whereas monosomy is the result of complete deletion of a chromosome (11). With the inclusion of the control genes TP53 and RARA in the present study, the incidence rate of polysomy was ~0.2% (3/1518), suggesting that true polysomy was less common than what was previously observed in the literature. In cases where increased levels of polysomy are detected, it may have occurred due to CEP17 amplification, as suggested by Zeng et al (19), whereas decreases in polysomy incidence rate may be caused by the section thickness being less than the diameter of cells (20,21). Chromosome 17 polysomy may indicate poor efficacy of cytotoxic medicines, leading to tumor metastasis (22,23), on the basis of which Herceptin and/or anthracyclines may be more suitable. However, whether patients with breast cancer who exhibit chromosome 17 polysomy should receive Herceptin therapy is disputed. Moelans et al (24) recommended not using the term ‘polysomy 17’ when in actuality a ‘CEP17 copy number increase’ was meant. Hanna et al (25) suggested that mean HER2 copies/cell should replace the HER2/CEP17 ratio to evaluate HER2 status.

Currently, compared with polysomy, investigations into monosomy are rare. Following the inclusion of TP53 and RARA control genes in the present study, the number of patients with monosomy was decreased from 15 to 12. The 3 discrepant cases experienced CEP17 deletion rather than true monosomy, leading to HER2 false positives (HER2/CEP17 ≥2). Those patients with HER2 amplification induced by true monosomy were not sensitive to targeted therapy and prognosis was unsatisfactory (26).

In the present study, no TP53 or RARA amplification was identified in breast cancer cells. Therefore, TP53 and RARA may be considered as control genes of HER2, suitable for the diagnosis of suspected HER2 and CEP17 co-amplification. However, TP53 and RARA only represent part of, not the whole of, chromosome 17.

Previous studies indicate that gene sequencing may be carried out directly on chromosome 17 based on aCGH (16). Observation using aCGH of whether HER2 was amplified was the optimal method to evaluate gene status, which was expensive. It was reported that when chromosome 17 was in a complex gene status, whole gene tests were recommended as positive FISH results were consistent with results of aCGH tests (16).

In conclusion, HER2 was previously determined to not be amplified in 29 patients but was revealed, through retrospective analysis in the present study, to be amplified according to ASCO/CAP 2013 HER2 test guidelines. HER2 in 23 patients which had previously been judged to not be amplified, was revealed to be amplified following the inclusion of RARA and TP53 control genes. The distinction of HER2 status is important as it enables patients to receive targeted medicine. ASCO/CAP 2013 HER2 test guidelines are more accurate than 2007 guidelines. In addition, RARA and TP53 may be considered suitable control genes to evaluate HER2 status.

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References

1. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A and McGuire WL: Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235: 177-182, 1987.
2. Pauletti G, Dandekar S, Rong H, Ramos L, Peng H, Seshadri R and Slamon DJ: Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: A direct comparison of fluorescence in situ hybridization and immunohistochemistry. J Clin Oncol 18: 3651-3664, 2000.
3. Ross JS, Fletcher JA, Bloom KJ, Linette GP, Stec J, Clark E, Ayers M, Symmans WF, PusztaI L and Hortobagyi GN: HER-2/neu testing in breast cancer. Am J Clin Pathol 120 (Suppl): S33-S71, 2003.
4. Winston JS, Ramanaryanan J and Levine E: HER-2/neu evaluation in breast cancer: Are we there yet? Am J Clin Pathol 121 (Suppl): S33-S49, 2004.
11. Troxell ML, Cobleigh MA, Tripathy D, Guthiel JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, et al: Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 20: 719-726, 2002.

12. Cobleigh MA, Vida VL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shok S, Lieberman G and Slamon DJ: Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 17: 2639-2648, 1999.

13. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, et al: American Society of Clinical Oncology/Collage of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 25: 118-145, 2007.

14. Press MF: How Is Her-2/neu Status Established When Her-2/neu gene and chromosome 17 centromere are both amplified? Am J Clin Pathol 126: 673-674, 2006.

15. Gunn S, Yeh IT, Lytvak I, Tirtorahardjo B, Dzidic N, Zadeh S, Kim J, McCaskill C, Lim L, Gorre M and Mohammed M: Clinical array-based karyotyping of breast cancer with equivocal HER2 status resolves gene copy number and reveals chromosome 17 complexity. BMC Cancer 10: 396, 2010.

16. Marchiò C, Lambros MB, Gugliotta P, Di Cantogno L, Bott C, Pasini B, Tan DS, Mackay A, Fenwick K, Tamber N, et al: Does chromosome 17 centromere copy number predict polymyosity in breast cancer? A fluorescence in situ hybridization and microarray-based CGH analysis. J Pathol 219: 16-24, 2009.

17. Tse CH, Hwang HC, Goldstein LC, Kandalet PF, Wiley JC, Kussick SJ and Gown AM: Determining true HER2 gene status in breast cancers with polymyosity by using alternative chromosome 17 reference genes: Implications for Anti-HER2 targeted therapy. J Clin Oncol 29: 4168-4174, 2011.

18. Egervári K, Kosa C and Szollosi Z: Impact of chromosome 17 centromere region assessment on HER2 status reported in breast cancer. Pathol Res Pract 207: 468-471, 2011.

19. Zeng X, Liang ZY, Wu SF, Gao J, Zhou WX and Liu TH: HER2 status in breast cancer of Chinese women: A study of 1,170 cases using fluorescence in-situ hybridization. Zhonghua Bing Li Xue Za Zhi 37: 594-598, 2008 (In Chinese).

20. Orsaria M, Khelifa S, Buza N, Kamath A and Hui P: Chromosome 17 polymyosity: Correlation with histological parameters and HER2NEU gene amplification. J Clin Pathol 66: 1070-1075, 2013.

21. Jiang H, Bai X, Zhao T, Zhang C and Zhang X: Fluorescence in situ hybridization of chromosome polymyosity in breast cancer using thin tissue sections causes the loss of CEP17 and HER2 signals. J Pathol 209: 719-726, 2005.

22. Orsaria M, Khelifa S, Buza N, Kamath A and Hui P: Chromosome 17 polymyosity: Correlation with histological parameters and HER2NEU gene amplification. J Clin Pathol 66: 1070-1075, 2013.

23. Krishnamurti U, Hammers JL, Atem FD, Storto PD and Silverman JF: Poor prognostic significance of unamplified chromosome 17 polymyosity in invasive breast carcinoma. Mod Pathol 22: 1044-1048, 2009.

24. Moelans CB and van Diest PJ: CEP17 copy number increase does not indicate polymyosity. J Clin Pathol 67: 454-455, 2014.

25. Hanna WM, Rüschhoff J, Bilous M, Coudry RA, Dowsett M, Osamura RY, Renauld-Llorca F, van de Vijver M and Viale G: HER2 status resolves gene copy number and reveals chromosome 17 complexity. BMC Cancer 10: 396, 2010.

26. Ristos N, Casorso L, Redana S and Montemurro F: HER2 gene-amplified breast cancers with monosomy of chromosome 17 are poorly responsive to trastuzumab-based treatment. Oncol Rep 13: 305-309, 2005.