Human epidermal growth factor receptor 2 (HER2)-positive breast carcinomas are associated with poor prognosis and sensitivity to anthracycline-based chemotherapy and HER2-targeted therapy such as trastuzumab.\(^1,2\) HER2 testing should be performed in patients with invasive breast carcinomas at the time of initial diagnosis and metastasis,\(^3\) and accurate measurement of HER2 status in tumor specimens is very important to patients, clinicians and pathologists. Since a fluorescence in situ hybridization (FISH)-based test was approved as the first diagnostic HER2 test by the United States Food and Drug Administration (FDA) in 1997, immunohistochemistry (IHC)-based diagnostic tests and bright field in situ hybridization techniques, such as chromogenic in situ hybridization (CISH) and silver-enhanced in situ hybridization (SISH) have been introduced to evaluate HER2 status. In Korea, IHC has been widely used as the primary test in most pathology laboratories and cases with equivocal IHC results have been retested by FISH or SISH. Recently we experienced three cases of HER2-amplified invasive breast carcinomas associated with co-amplification or gain of chromosome 17 centromere (CEP17) in silver-enhanced in situ hybridization (SISH) analysis. These cases revealed 2+ or 3+ staining for HER2 immunohistochemistry and >6 HER2 copies per cell on SISH analyses. However, the calculated HER2/CEP17 ratios were low (<2.2) and did not fit within the HER2-positive category. We interpreted those cases as HER2-positive tumors based on the number of HER2 copies per cell. There is a potential for misinterpretation of SISH analysis in cases showing increased CEP17 copy number, based on the criterion used for HER2 positivity (HER2 copies >6 per cell vs HER2/CEP17 ratio >2.2). We recommend reporting raw SISH or fluorescence in situ hybridization data, including number of cells counted, average numbers of HER2 and CEP17 signals, and the calculated HER2/CEP17 ratio to prevent underreporting of HER2 amplification.

Key Words: Breast neoplasms; HER2; Amplification; Chromosome 17; Polysomy

Case Reports

Case 1

A 66-year-old woman was diagnosed as having invasive ductal carcinoma, not otherwise specified (NOS), in the right breast by core needle biopsy. The patient underwent breast conserving surgery and axillary lymph node dissection. The tumor was 1.9 cm in size and metastasized to 26 out of 31 regional axillary nodes (pT1pN3M0). The histologic grade was III. It was estrogen receptor (ER)-positive (Allred score [AS] 5), progesterone receptor (PR)-negative and HER2 IHC 3+ (Fig. 1A). SISH was
performed with INFORM HER2 DNA and chromosome 17 (CEP17) probes (Ventana Medical Systems, Tucson, AZ, USA) on two sequential sections as described previously. CEP17 signals were visualized as small clusters with multiple single dots (>6 per cell) and HER2 signals as small or large clusters with multiple single dots (Fig. 1B, C). HER2/CEP17 ratio was <2.2 but HER2 copy number per cell was >10. We interpreted this case as a HER2-positive tumor, because HER2 IHC was 3+ and HER2 copies per cell were more than 6 although HER2/CEP17 ratio was within the normal limit.

Case 2

A 49-year-old woman received a right modified radical mastectomy with sentinel lymph node biopsy after being diagnosed as having ductal carcinoma in situ by core needle biopsy. The pathologic diagnosis for the right breast tumor was invasive ductal carcinoma, NOS with extensive intraductal component. The invasive tumor was 0.7 cm in size and grade III. Sentinel lymph node was negative for tumor metastasis. The tumor cells were negative for ER and PR, but positive (3+) for HER2 IHC (Fig. 2A). On SISH analysis, both HER2 and CEP17 signals were uncountable due to diffuse amplification and displayed multiple overlapping clusters (Fig. 2B, C). We interpreted this case as a HER2-positive tumor based on HER2 IHC result (3+) and the number of HER2 copies per cell (>20 per cell), even though HER2/CEP17 ratio was within the normal limit.

Case 3

A 35-year-old woman underwent a left modified radical mastectomy and axillary lymph node dissection due to breast cancer which was proven by core needle biopsy at local clinic. Histologic type of the tumor was invasive ductal carcinoma, NOS and the tumor size was 3.4 cm. The tumor showed lymphovascular invasion and high (III) histologic grade. Lymph node metastasis was found in one out of 15 regional lymph nodes. The tumor cells were positive for ER (AS7), PR (AS4) and equivocal for HER2 (2+) IHC (Fig. 3A). On SISH analysis, both HER2 and CEP17 displayed multiple gene copies. The average copy...
Co-amplification of HER2 and CEP17

number per cell was 7.2 for HER2 and 4.9 for CEP17 (Fig. 3B, C). The SISH ratio was 1.5. We interpreted this case as a HER2-positive tumor based on the number of HER2 copies per cell (7.2 per cell).

DISCUSSION

Before the era of trastuzumab, the first FDA-approved diagnostic test for HER2 status to measure breast cancer prognosis was a FISH-based assay consisting of only a probe for HER2 gene (Ventana Inform FISH test, Ventana Medical Systems). Since HER2 has become a biomarker to predict adjuvant trastuzumab benefit, FISH (PathVysion HER-2 DNA Probe Kit, Abbott Molecular, Abbott Park, IL, USA), CISH (Spot-Light HER2 CISH assay, Invitrogen, San Francisco, CA, USA), and SISH (INFORM HER2 Dual ISH DNA Probe Cocktail, Ventana Medical Systems) including probe for CEP17 as reference for HER2 gene were developed and approved by the FDA. In 2007, the ASCO/CAP guideline recommended that the cutoff for positive HER2 status is a HER2/CEP17 ratio > 2.2 or > 6 HER2 gene copy number per nucleus for a test without an internal control probe. The use of CEP17 as reference for HER2 evaluation is based on the fact that an increased HER2 gene copy number as a result of chromosome 17 polysomy may not have the same clinical significance as HER2 amplification. Several studies reported that patients with polysomy 17 without HER2 amplification have outcomes similar to HER2-negative, chromosome 17 eusomic patients.

Polysomy indicates that the number of a particular chromosome is greater than diploid. In several studies, chromosome 17 polysomy was defined as ≥ 3 CEP17 signals in FISH analyses and found in 12% of study population. However, a recent study demonstrated that an increased CEP17 signal on FISH is most likely due to the amplification or gain of the CEP17 region rather than a true chromosome 17 polysomy. Marchiò et al. reported that 17 of 18 polysomic cases by FISH represented gain of 17q with involvement of the centromere, 17q gain sparing the centromeric region, or amplification of the centromeric region and only one case was true chromosome 17 polysomy in
their study using microarray-based comparative genomic hybridization and FISH for HER2 (17q12), CEP17, Smith-Magenis syndrome (17p11.2) and retinoic acid receptor alpha (RARA, 17q21.2).

In our three cases, the increased CEP17 signals may represent amplification (case 1 and 2) or focal gain (case 3) in the centromeric region of chromosome 17 rather than true chromosome 17 polysomy. Regardless of the exact mechanism of increased CEP17 signals, the ASCO/CAP guideline (HER2/CEP17 ratio > 2.2 or HER2 copies > 6 per cell) for HER2-positive tumors are contradictory in our cases because discrepancies occurred in SISH results. The calculated HER2/CEP17 ratios were low (< 2.2) and did not fit within the HER2-positive category in our cases. If we report only the HER2/CEP17 ratio in SISH analysis, the result may deny targeted therapy in these patients even though HER2 copies are > 6 per cell. A consensus opinion of a number of breast cancer experts is that it is the HER2 copy number that is important, and the actual ratio is of lesser importance. This view is supported by a recent study by Perez et al. They reported the benefit of trastuzumab in patients with HER2-positive tumors (IHC 3+, FISH HER2/CEP17 ratio ≥ 2.0 or both) was independent of HER2/CEP17 ratio and CEP17 copy number in the N9831 adjuvant trastuzumab trial. Viale suggested accounting for the mean number of HER2 gene signals, irrespective of the number of CEP17 signals for the accurate assessment of HER2 status. Varga et al. reported a complex FISH pattern in 14 cases with HER2/CEP17 co-amplification by use of FISH with additional chromosome 17 probes (17p11.1-q11.1, 17p11.2-p12, tumor protein p53 [TP53] on 17p13.1, RARA on 17q21.1-3 and TOP2 on 17q21.3-22). They found an enormous discrepancy in the FISH results between the three participating institutions when applying ASCO/CAP guidelines (HER2/CEP17 ratio). Overall agreement on FISH results was 64% between the institutions. They suggested performing confirmatory HER2 IHC and recommended to report raw FISH or SISH data, including CEP17 and HER2 gene count, to prevent underreporting of HER2 amplification in those cases.

For one year, 352 SISH analyses of patients with invasive breast carcinomas were performed in our institution. Co-amplification of HER2/CEP17 or gain of CEP17 associated with HER2...
amplification was observed in three of these cases (0.9%). This very low incidence of co-amplification has been reported.

Even though the HER2/CEP17 ratio becomes nearly 1 in patients with co-amplified HER2 and CEP17, they should be correctly identified as patients with HER2-positive tumors and should have a chance to receive targeted therapy.

In conclusion, we report three cases of HER2-positive invasive breast carcinomas representing co-amplification or gain of CEP17 in SISH analysis. Based on which criterion used for HER2 positivity (HER2 copies > 6 per cell or HER2/CEP17 ratio > 2.2), there is a potential for misinterpretation of FISH or SISH analyses in cases showing increased CEP17 copy number. We recommend reporting raw FISH or SISH data, including number of cells counted, the average number of HER2 and CEP17 signals and the calculated HER2/CEP17 ratio to accurately identify patients eligible for targeted therapy.

REFERENCES