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HER2-Positive Breast Carcinomas with Co-amplification or Gain of Chromosome 17 Centromere Locus: Report of Three Cases and an Impact on HER2 Testing

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Recently we experienced three cases of human epidermal growth factor receptor 2 (*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

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 with co-amplification or gain of chromosome 17 centromere (*CEP17*). The *CEP17* copy number affects the HER2 test result when HER2 status is determined by *HER2*/*CEP17* ratio according to the American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP) recommendation.[4] However the ASCO/CAP guidelines did not define cases with increased *CEP17* signals. Therefore, we need to recognize the presence of cases with aberrant copy number alteration of *CEP17* and how to handle these cases in clinical samples.

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

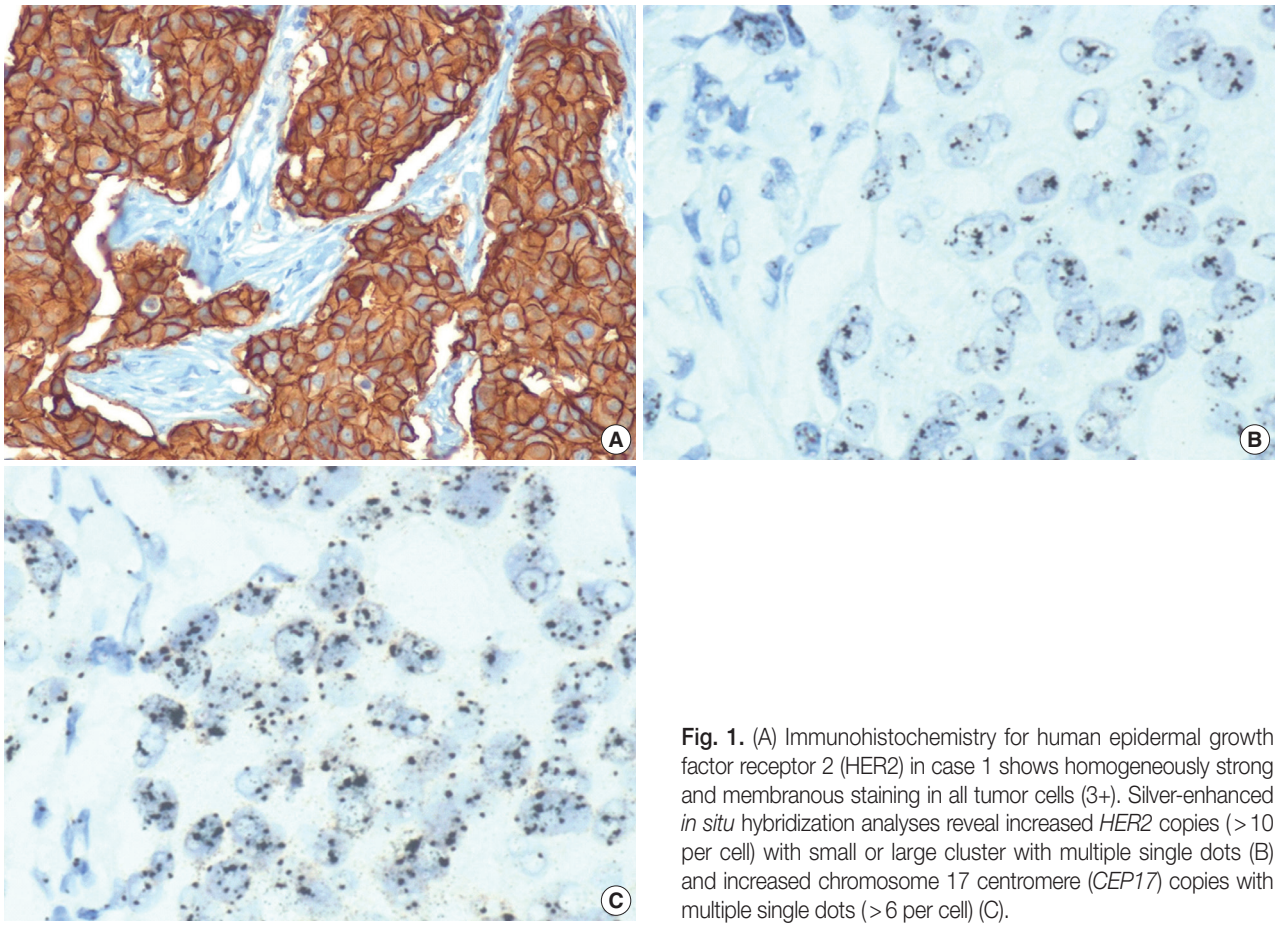

Fig. 1. (A) Immunohistochemistry for human epidermal growth factor receptor 2 (HER2) in case 1 shows homogeneously strong and membranous staining in all tumor cells (3+). Silver-enhanced *in situ* hybridization analyses reveal increased *HER2* copies (>10 per cell) with small or large cluster with multiple single dots (B) and increased chromosome 17 centromere (*CEP17*) copies with multiple single dots (>6 per cell) (C).

performed with INFORM HER2 DNA and chromosome 17 (*CEP17*) probes (Ventana Medical Systems, Tucson, AZ, USA) on two sequential sections as described previously.[5] *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

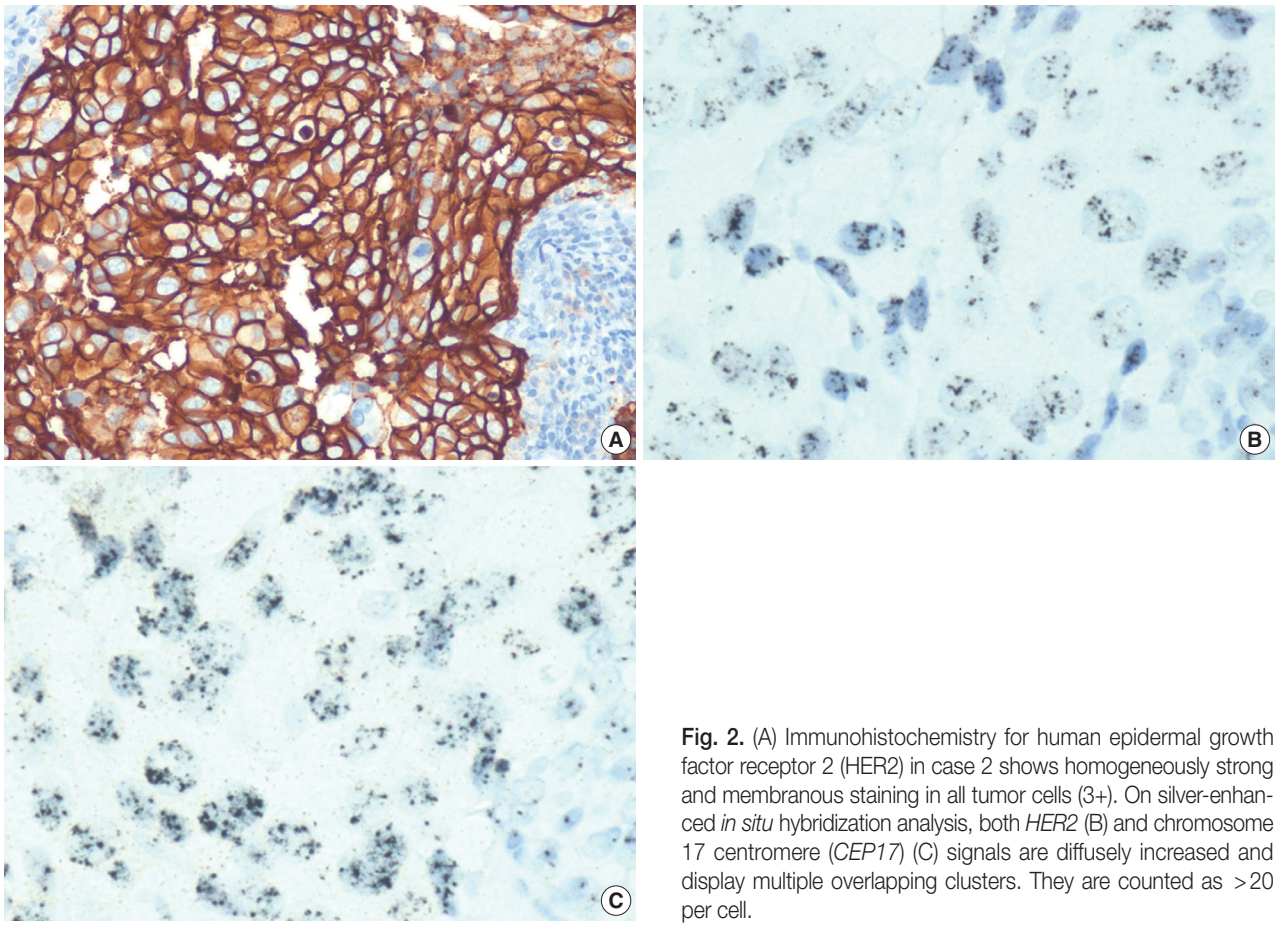

Fig. 2. (A) Immunohistochemistry for human epidermal growth factor receptor 2 (HER2) in case 2 shows homogeneously strong and membranous staining in all tumor cells (3+). On silver-enhanced *in situ* hybridization analysis, both *HER2* (B) and chromosome 17 centromere (*CEP17*) (C) signals are diffusely increased and display multiple overlapping clusters. They are counted as >20 per cell.

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.[4] 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.[6] Several studies reported that patients with polysomy 17 without *HER2* amplification have outcomes similar to HER2-negative, chromosome 17 eusomic patients.[7-9]

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.[7,10] 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.*[11] 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

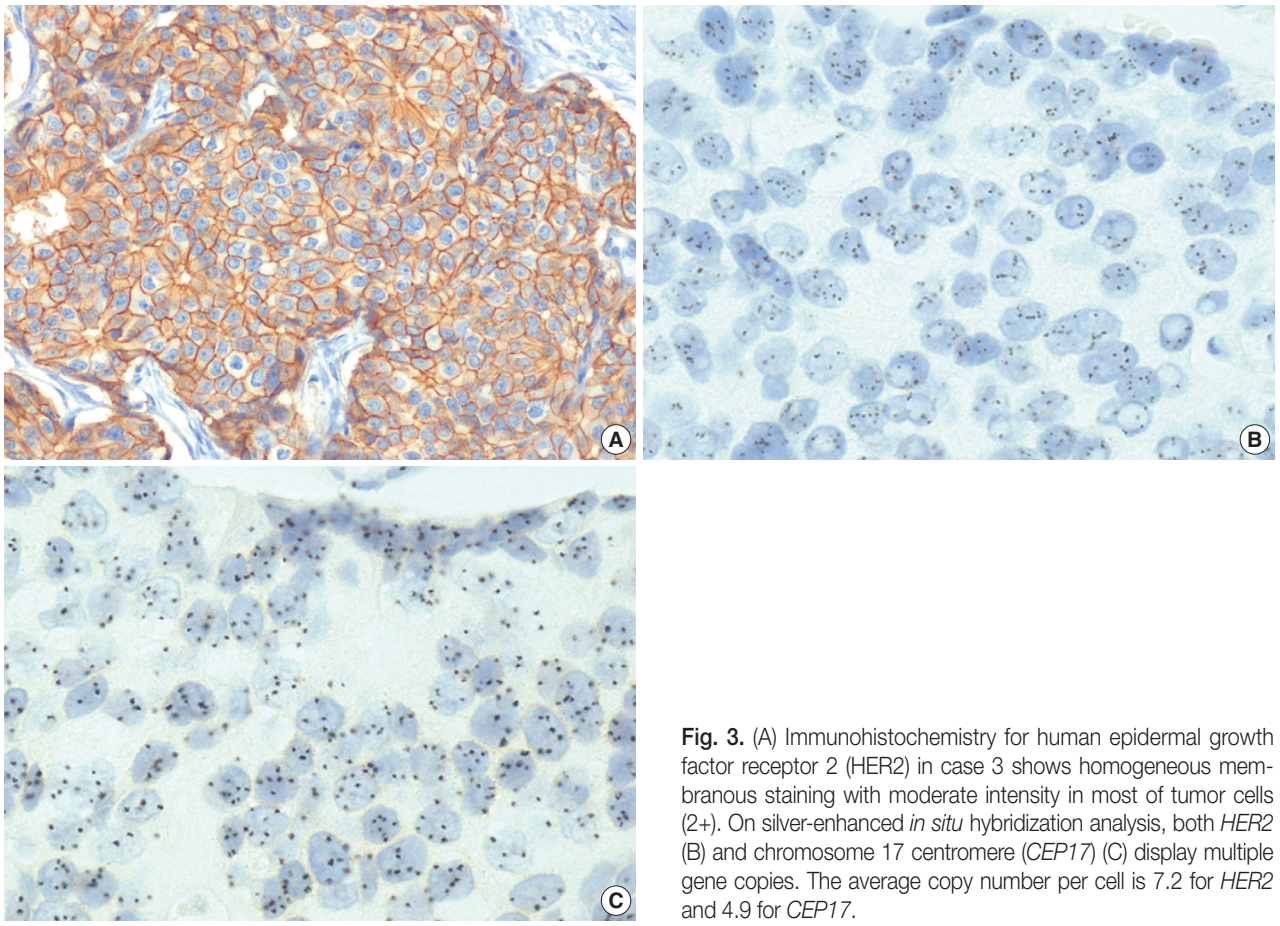

Fig. 3. (A) Immunohistochemistry for human epidermal growth factor receptor 2 (HER2) in case 3 shows homogeneous membranous staining with moderate intensity in most of tumor cells (2+). On silver-enhanced *in situ* hybridization analysis, both *HER2* (B) and chromosome 17 centromere (*CEP17*) (C) display multiple gene copies. The average copy number per cell is 7.2 for *HER2* and 4.9 for *CEP17*.

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.[12] This view is supported by a recent study by Perez *et al.*[13] 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[14] 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.*[15] 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.[15] 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 *CEP-17* 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.

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