HER-2/neu and Topoisomerase IIα Gene Amplification and Protein Expression in Invasive Breast Carcinomas

Chromogenic In Situ Hybridization and Immunohistochemical Analyses

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Abstract

We studied HER-2/neu (HER-2) and topoisomerase IIα (topo2a) amplification (using chromogenic in situ hybridization) and overexpression (immunohistochemical analysis) in 113 invasive breast carcinomas. A gene copy number/chromosome 17 copy number ratio of 2.0 or higher indicated amplification. A topo2a/chromosome 17 ratio of less than 0.8 indicated gene deletion. HER-2 overexpression was scored according to standard HercepTest guidelines (DAKO, Carpinteria, CA). Overexpression of topo2a was identified when nuclear staining was found in more than 5% of tumor cells. Of 113 tumors, 104 were analyzed successfully for HER-2 and topo2a amplification. Of the 104, 64 showed HER-2 amplification; 25 of these (39%) also showed topo2a amplification. No amplification was found in 40 tumors. Deletion of topo2a was seen in 7 (11%) of 64 HER-2–amplified tumors and 2 (5%) of 40 nonamplified tumors. Of 25 tumors with topo2a amplification, 18 (72%) overexpressed topo2a. Only 3 (4%) of 79 tumors without topo2a amplification overexpressed topo2a. Amplification of topo2a is associated with HER-2 amplification but not vice versa. Amplification of topo2a resulted in protein overexpression in 72% of tumors, but topo2a overexpression rarely occurred without gene amplification. Identification of topo2a and HER-2 status might have therapeutic and prognostic implications.

HER-2/neu (HER-2; c-erb-b2), a proto-oncogene located at chromosome band 17q21, is overexpressed or amplified in 15% to 25% of invasive breast carcinomas.1 Overexpression or amplification of HER-2 generally is associated with a poor prognosis.2-4 In patients with metastatic breast cancer, HER-2 status is an essential factor in selecting patients for treatment with trastuzumab (Herceptin, Genentech, South San Francisco, CA), a recombinant humanized monoclonal antibody against the HER-2 protein.5,6 Assessment of HER-2 status also is important for predicting response to other specific chemotherapy regimens.7,8

The topoisomerase IIα (topo2a) gene, located in the same chromosomal region, 17q12-17q21,9 encodes a 170-kd cell cycle–dependent topo2a protein. The protein peaks at the G2/M phase of the cell cycle and declines to a minimum level at the end of mitosis.10 The protein acts as an enzyme and resolves topological problems arising during various processes of DNA metabolism, including transcription, recombination, replication, and chromosome segregation during cell division.11

The relationship between HER-2 and topo2a genes seems to be more complex than just sharing the same chromosomal region. HER-2 and topo2a have been reported to be coamplified in a significant number of breast cancers.12 Tumors with amplification or overexpression of HER-2 have been shown to respond more favorably to anthracycline-based chemotherapy.13,14 The anthracyclines act as topo2a inhibitors. It is conceivable that the favorable response seen in this subset of HER-2–amplified tumors actually might be due to blockage of topo2a functions in tumors with topo2a amplification.

The present study was undertaken not only to assess the relationship between HER-2 amplification and topo2a amplification...
or deletion but also to define the relationship between topo2a gene status and protein expression.

Materials and Methods

Case Selection and Tissue Microarray Construction

We selected 113 invasive breast carcinomas with sufficient tumor present in a single tissue block for the study. Because topo2a amplification has so far been reported only in HER-2–amplified tumors, an effort was made to select tumors that had already tested positive for HER-2 amplification. Therefore, case selection was not random or consecutive. Tissue microarrays (TMAs) were created using 0.6-mm tissue cores as previously described. Four cores from different areas of the tumor in a single tissue block were sampled. An H&E-stained section was evaluated for the presence of invasive breast carcinoma, and the area to be used for creation of the TMA was marked on the slide and the donor block.

Chromogenic In Situ Hybridization

Chromogenic in situ hybridization (CISH) for HER-2 and topo2a was performed according to the manufacturer’s instructions (Zymed Laboratories, South San Francisco, CA). Briefly, the TMA sections were incubated at 55°C overnight. The slides were deparaffinized in xylene and graded ethanol. Heat pretreatment was carried out in the pretreatment buffer at 98°C to 100°C for 15 minutes. The tissue was digested with pepsin for 10 minutes at room temperature. After application of the SpotLight digoxigenin-labeled HER-2 or topo2a probe (Zymed), the slides were coveredslipped and edges sealed with rubber cement. The slides were heated at 95°C for 5 minutes followed by overnight incubation at 37°C using a moisturized chamber. A posthybridization wash was performed the next day and followed by immunodetection using the CISH polymer detection kit (Zymed).

CISH for chromosome 17 was performed on parallel TMA sections under conditions similar to those for HER-2 CISH except for the probe (SpotLight biotin-labeled chromosome 17 centromeric probe, Zymed) and the detection kit (CISH centromere detection kit, Zymed). CISH signals were counted with a light microscope using a 40x objective. The number of chromosome 17 signals, HER-2 and topo2a signals, and tumor nuclei scored were recorded for each core. At least 30 cells were counted per tissue core. Tumors were interpreted as amplified when the ratio of HER-2 or topo2a/chromosome 17 signals was 2.0 or more. The average ratio of different cores from the same tumor was used as the final score for determination of gene amplification status of that particular tumor. Although topo2a deletion might be evident when the topo2a gene copy number is less than the centromeric copy number, a ratio of less than 0.8 for topo2a/chromosome 17 signals was used as the cutoff value for topo2a deletion.

Several cores of normal breast tissue showing 2 signals per nucleus were used as internal control samples.

Immunohistochemical Analysis

TMA sections (4-5 µm thick) were stained by the standard method using antibodies against topo2a (monoclonal antibody, clone 3F6, 2nd Gen prediluted; citrate epitope retrieval; Zymed) and HER-2 (HercepTest, DAKO, Carpinteria, CA). Immunohistochemical staining was carried out according to the manufacturers’ instructions. All invasive carcinoma cells in all tissue cores were scored when interpreting immunohistochemical results; therefore, at least 500 cells for each tumor were scored for immunohistochemical analyses.

The HER-2 immunohistochemical results were interpreted as negative (0 or 1+) or positive (2+ or 3+) according to the standard criteria. The highest HER-2 immunohistochemical score from different cores of the same tumor was used as the final result for that tumor. For topo2a immunohistochemical results, a tumor was interpreted as positive if moderate to intense nuclear staining was detected in more than 5% of the tumor cells. A breast carcinoma consistently showing topo2a amplification and strong protein expression by immunohistochemical analysis was used as a positive control sample. Normal breast tissue was used in negative control samples.

Results

Of the 113 tumors, 104 were studied successfully for HER-2 and topo2a amplification. The reasons for failure were complete loss of tissue cores, fewer than 30 tumor cells available for scoring, and absence of HER-2, topo2a, or chromosome 17 signals in benign nuclei. The absence of signals probably resulted from underdigestion or overdigestion because tissue digestion for a particular tumor cannot be controlled on a TMA.

HER-2 amplification was detected in 64 tumors. Coamplification of HER-2 and topo2a was identified in 25 (39%) of the 64 tumors Table 1. Notable findings were that the copy numbers of HER-2 and topo2a often were different in the coamplified tumors Image 1 and HER-2 was amplified more often at a higher level than topo2a in the same tumor Table 2. topo2a deletion was seen in 7 (11%) of 64 tumors Image 2 (Table 1). No amplification of HER-2 or topo2a was identified in the remaining 40 tumors. However, 2 (5%) of these 40 showed topo2a deletion, whereas their HER-2 genes were not deleted (Table 1).

HER-2 status was concordant in 93 (89.4%) of 104 tumors in HER-2 gene amplification and protein overexpression,
which was consistent with the literature. Topo2a protein overexpression was identified in 18 (72%) of 25 topo2a-amplified tumors. The amplification ratios (topo2a/17) in these 18 tumors ranged from 2.1 to 6.7 (mean, 3.6). Of the 25 tumors, 7 (28%) did not show topo2a overexpression despite gene amplification. The amplification ratios in these 7 tumors ranged from 2.0 to 6.0 (mean, 3.5). In contrast, of the 79 tumors that did not show topo2a amplification, 76 (96%) also were negative for protein overexpression. Only 3 (4%) of these 79 tumors were positive for protein overexpression. All 3 tumors were highly proliferative with abundant mitoses (>30 mitoses per 10 high-power fields). This finding is consistent with the historic role of topo2a protein expression as a proliferation marker.10,11

**Discussion**

topo2a is a cell cycle–dependent enzyme protein, which peaks during the G2/M phase of the cell cycle and is reduced to a minimum level at the end of mitosis. However, in breast cancer, it seems to have a much broader role than just a proliferation marker. The topo2a gene is amplified in a significant number of breast cancers and also might be the target of topo2a inhibitors such as doxorubicin, used frequently to treat locally advanced breast cancer. Owing to its proximity to HER-2 on chromosome 17 and its coamplification with HER-2 in breast cancer, it originally was thought that both HER-2 and topo2a are present on the same amplicon. However, it later was shown that they are present on separate amplicons.16 This finding also is supported by the fact that in tumors with HER-2 and topo2a coamplification, HER-2 and topo2a often have different copy numbers. We also made this observation in the present study. The protein products of HER-2 and topo2a represent practical targets for trastuzumab and topo2a inhibitors, respectively, and are important clinically in the era of targeted therapy.

For the present study, we selected a large proportion of HER-2–amplified tumors to study topo2a amplification because studies reported to date12,15-17 have shown that topo2a amplification was found exclusively in breast carcinomas showing HER-2 amplification. Consistent with findings by other investigators, topo2a amplification was found in 39% (25/64) of HER-2–amplified tumors and was not detected in the absence of HER-2 amplification in our study.

We identified topo2a deletion in 11% (7/64) of HER-2–amplified tumors, a rate much lower than that reported by Jarvinen et al.12 This discrepancy might be due to the use of different probes, different techniques (CISH vs FISH), and slightly different criteria for determining amplification and deletion. The topo2a probe used in the present study might be more specific because it was created after removal of the repetitive sequences (subtraction probe technology, Zymed). The probe used by Jarvinen et al12 might have a lower hybridization and/or detection efficiency, leading to apparent
A tumor showing coamplification of HER-2/neu (HER-2) and topoisomerase IIα (topo2a) genes with protein overexpression. 

An HER-2/neu (HER-2)–amplified tumor showing topoisomerase IIα (topo2a) deletion. A, H&E, ×400. B, HER-2 overexpression (immunohistochemical score, 3+) (×400). C, Negative for topo2a by immunohistochemical analysis (×400). D, Chromogenic in situ hybridization for chromosome 17; average of 2 brown signals per nucleus (×400). E, HER-2 amplification. Large clusters of brown signals in each nucleus represent multiple copies of HER-2 (×400). F, Deletion of topo2a; average of 1 brown signal per nucleus represents deletion of 1 topo2a allele (×400).
false observation of gene deletion in some cases. In contrast, Coon et al.22 studied topo2a amplification and overexpression in locally advanced breast cancer and failed to detect any topo2a deletion; however, the number of cases was small. We also identified 2 tumors with topo2a deletion but without HER-2 amplification or deletion. The significance and mechanism of these deletions are unknown.

This study revealed some important and interesting findings. First, it confirmed that topo2a amplification occurs only in the presence of HER-2 amplification. One of the possible reasons is that because of its more telomeric location than HER-2, this secondary amplification of topo2a occurs through repeated breakage-fusion-bridge cycles.23,24 Our finding that in tumors with topo2a and HER-2 coamplification, topo2a amplification often is seen at a lower level than HER-2 seems to support this hypothesis.

Second, the relationship between topo2a gene amplification and protein expression was better elucidated. The criteria used for protein overexpression were relatively stringent and substantially reduced the potential for false-positive interpretation owing to the proliferative activity of the tumor cells. Our data demonstrated that topo2a overexpression rarely occurs in the absence of gene amplification, and when it occurs, it was only in highly proliferating tumors, suggesting that topo2a overexpression in such tumors might not be related directly to topo2a amplification. A study by Mueller et al.25 also showed that topo2a overexpression was found in high-grade breast carcinomas, even in the absence of topo2a amplification, and that topo2a expression strongly correlated with MIB-1 expression. A different topo2a monoclonal antibody was used, and no cutoff value for positive topo2a immunohistochemical staining was indicated by Mueller et al.25; therefore, a direct comparison of their results with ours might not be possible. Nevertheless, the antibody used by Mueller et al.25 appeared to be more suitable as a proliferation marker, whereas the antibody used in the present study apparently showed better correlation with topo2a status.

In the presence of topo2a gene, more than 70% (18/25 [72%]) of tumors showed protein overexpression. Several factors could contribute to the negative immunohistochemical results in tumors with topo2a amplification. One obvious explanation is the loss of antigenicity during tissue processing. Such an explanation might be applied to 1 of 7 such tumors in the present study; this tumor also showed HER-2 gene amplification with no protein overexpression. The remaining 6 tumors showed topo2a and HER-2 coamplification and HER-2 overexpression but no topo2a overexpression. Moreover, HER-2 overexpression was 3+ in all 6 tumors, which argued against loss of antigenicity.

Another possible reason for the disparity between amplification and overexpression might be the fact that topo2a is a cell cycle–regulated protein. Expression of topo2a is linked to the rate of cell proliferation and has been suggested as a proliferation marker.10,11,26 Its protein level fluctuates normally during the cell cycle without the contribution of gene amplification. It is, therefore, conceivable that factors other than gene amplification also participate in the regulation of topo2a expression. Whether topo2a-amplified tumors with or without topo2a overexpression would respond similarly to anthracycline-based therapy and whether tumors with HER-2 and topo2a coamplification would respond synergistically to combined anthracycline and trastuzumab therapy warrant further clinical investigation.

Amplification of topo2a occurs exclusively in the presence of HER-2 amplification and can be considered a surrogate marker for HER-2 gene amplification. In the majority of tumors, particularly tumors of relatively low proliferating activity, topo2a amplification correlates with topo2a overexpression (defined as >5% tumor cells positive for topo2a by immunohistochemical analysis). Determination of topo2a status in breast carcinomas would help identify a subset of patients who might have a response to topo2a inhibitors in addition to trastuzumab. Additional prospective clinical trials studying the effect of topo2a inhibitors in patients with topo2a amplified and overexpressed, amplified and not overexpressed, and nonamplified tumors would help further define the clinical significance of topo2a amplification, overexpression, or both.

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References


