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ARTICLE

HER-2, TOP2A and Chromosome 17 Alterations in Breast Cancer

Asli Rehber BESER,[1] Sitki TUZLALI,[2] Deniz GUZEY,[3] Semra DOLEK GULER,[4] Seniha HACIHANEFIOGLU,[5] Nejat DALAY[1]

[1]Istanbul University Oncology Institute, Department of Basic Oncology, [2]Istanbul University Istanbul Medical Faculty, Department of Pathology, [3]Vakif Gureba Hospital, Department of Surgery, [4]Istanbul University Oncology Institute, Department of Clinical Oncology, [5]Istanbul University Cerrahpasa Medical Faculty, Department of Medical Biology

HER-2 amplification is a biomarker for identifying patients who respond to trastuzumab and has been evaluated as a factor predicting the response to anthracyclines. The relationship between HER-2 and response to anthracycline therapy may also be the result of the close localization of TOP2A on 17q. It has been a matter of debate whether these two genes, HER-2 and TOP2A, behave separately on different amplicons or act together thus making it possible to predict the TOP2A status from the HER-2 status. In this study TOP2A, HER-2 and chromosome 17 aneusomy were investigated by fluorescent in situ hybridization (FISH) in 50 consecutive breast cancer patients. HER-2 amplification was detected in 11 patients (22%) and TOP2A changes were seen in 6 patients (12%); two amplifications and two deletions were observed in HER-2-amplified cases and two deletions in HER-2-nonamplified cases. Three of the TOP2A-deleted cases had polysomy 17. HER-2 copy number was higher than the TOP2A copy number in one patient with co-amplification. Polysomy was observed in 9 cases (18%) and monosomy in 6 cases (12%). Aneusomy was the sole anomaly in 11 patients (22%). We conclude that the TOP2A status cannot be predicted from the HER-2 status and evaluation of the TOP2A status only in patients with HER-2 overexpression may lead to missing cases with TOP2A deletion with possible resistance to therapy. Other factors modulating topo IIα activity may also affect the response to therapy. Studies evaluating different parameters that can modulate topo IIα activity and the response to the drugs targeting the enzyme are necessary. (Pathology Oncology Research Vol 13, No 3, 180–185)

Key words: TOP2A, HER-2, breast cancer

Introduction

Breast cancer is the most commonly diagnosed cancer in women in the US[12] and is the leading cause of death in the US and Europe among women aged between 40-59.[15] HER-2 is the most important oncogene associated with breast cancer. The HER-2 gene is located on chromosome 17q12-21 and codes for a 185-kD transmembrane protein of the ERBB membrane tyrosine kinase receptor family. The HER-2 gene is amplified in 15-30% of breast tumors,[18,33] and amplification has been associated with adverse outcome.[30] HER-2 amplification has been evaluated as a factor for predicting the response to different forms of chemotherapy[18] and has gained further importance as a biomarker for identifying patients who respond to trastuzumab,[33] a monoclonal antibody against the extracellular portion of the protein.[31] The most appealing relationship between HER-2 levels and chemotherapy has been observed for anthracyclines.[18] Studies have shown that patients with a high HER-2 expression benefit more from adjuvant therapy with anthracyclines.[7,28,34] However, subsequent studies conducted not only in the adjuvant but also in neoadjuvant and metastatic settings have not confirmed this relationship.[10,29,38] Therefore, it has been suggested that other genes on the 17q12-21 locus modulating the response to therapy could play a role in breast cancer.[19] Among these, TOP2A, the gene encoding the a isozyme of the human topoisomerase II received most attention. Topoisomerases are ATP-dependent endonucleases and

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Correspondence: Prof. Dr. Nejat DALAY, Istanbul University Oncology Institute, 34093 Capa, Istanbul, Turkey. Tel: 90212 4142434, fax: 90212 5348078, e-mail: ndalay@yahoo.com

ligases of vital importance to the cell and are responsible for transcription, recombination, replication, chromosome condensation and segregation during cell division and for preservation of the chromosome structure.[22] A class of cancer chemotherapeutics, the topoisomerase inhibitors stabilize the cleavable complexes formed by topoisomerases to cause DNA breaks and result in cell death.[22] The sensitivity of tumor cell lines to topo IIα inhibitors is associated with topo IIα expression.[24] Correlation between TOP2A amplification or deletion and response to anthracyclines has also been shown.[21] It has been proposed that the relationship between HER-2 and response to anthracycline therapy may be a secondary event resulting from the close localization of the two genes.[7,18] It has been a matter of debate whether HER-2 and TOP2A genes behave separately in different amplicons or act together thus making it possible to predict the TOP2A status from the HER-2 status. Aneusomy of chromosome 17, harboring these two genes, is an early and frequent event in breast cancer[11] and has been correlated with prognosis.[37] It has been suggested that this change may provide a marker when observed in benign breast cancer tissue.[11] This study was planned to evaluate the HER-2 and TOP2A gene copy numbers and aneusomy of chromosome 17 in breast cancer cases by fluorescent in situ hybridization.

Materials and methods

Patients

Fifty consecutive patients between 27-80 years of age (mean age 54±12.78 years) with primary breast cancer operated at Istanbul University Istanbul Medical Faculty and Vakif Gureba Hospital between January 2004 and February 2005, were included in the study. The study was approved by the Istanbul Medical Faculty Ethics Committee and written informed consent was taken from the patients.

FISH

Tissues obtained were fixed in buffered formalin and embedded in paraffin. Four-to-five-mm sections were cut from the paraffin blocks. Invasive parts of the tumor tissue were confirmed by a pathologist on a consecutive hematoxylin-eosin-stained section. Sections were placed on poly-lysine-coated slides (Menzel-Glazer). FISH was performed with the three-color LSI TOP2A spectrum orange, LSI HER-2 spectrum green and Cep 17 spectrum aqua probe set (Vysis, Inc., Downers Groove, IL) and deparaffinization was carried out using the Vysis paraffin pretreatment kit according to the manufacturer's instructions. The slides were heated at 56°C overnight and deparaffinized in xylene three times for ten min each. After immersion in 100% ethanol twice for 5 min, sections were dried on a 45°C hotplate and treated with 0.2 M HCl for 20 minutes, washed first in distilled water and then in wash buffer (Vysis, Inc.) for 3 min each and incubated for 30 min at 80°C in the pretreatment buffer. Slides were then washed for 5 min each as described above. Enzymatic digestion was carried out in protease buffer (Vysis, Inc.) for 10 min at 37°C. The slides were washed twice, dried on a 45°C hotplate, fixed in 10% buffered formalin for 10 min at room temperature, washed twice for 5 minutes each and dried again on the 45°C hotplate. Formamide denaturation was performed for 5 min in a 72°C water bath. Slides were dehydrated in graded dilutions (70%, 85% and 100%) of ethanol for one min each and were dried on the 45°C hotplate. 5-10 µl of the probe was applied to the slides depending on the amount of tissue to be hybridized, covered and was sealed with rubber cement. Hybridization was performed overnight in a humidified hybridization chamber at 37°C. The rubber cement was removed and the coverslip was let to float off in 2x SSC, 0.3% NP40 at room temperature. Post-hybridization washing was done using 2x SSC, 0.3% NP40 at 72°C for 2 min. Slides were air-dried in an upright position in the dark, counterstained with DAPI (Vysis, Inc.) and covered.

FISH was evaluated using the Olympus BX 51 epifluorescence microscope equipped with a 100 arc mercury lamp. 60x and 100x oil immersion objectives were used in signal enumeration. FITC, spectrum orange and aqua and DAPI single bypass filters were used to score the number of signals. Images were captured with a CCD camera (Zeiss Axiocam) and processed using the Metasystems Isis software (Altlussheim, Germany). Signals from 60 non-overlapping intact nuclei from at least three different regions of the tumor were evaluated and the mean number signal/cell ratio was determined. HER-2:Cep 17, TOP2A:Cep17 and Cep 17:number of cells ratios were calculated. HER-2 was considered amplified when the ratio was ≥2, while TOP2A was considered amplified when it was ≥1.5[9] and deleted when <0.8. Cep 17 levels between 1.35 and 1.85 were recorded as disomic, those <1.35 as monosomic and >1.86 as polysomic.[37]

Results

HER-2 and TOP2A copy numbers were within the normal ranges and chromosome 17 was disomic in 26 patients, while 48% of the patient population had one or more abnormalities in terms of copy number for the three loci studied. HER-2 amplification was detected in 11 patients (22%). Of these, two had polysomy 17, but HER-2 was still found to be amplified when HER-2 number was corrected for chromosome 17 centromere number (*Fig. 1a*).

TOP2A changes were observed in 6 patients; two were amplifications and four were deletions. Both amplifications were observed in cases with HER-2 amplification

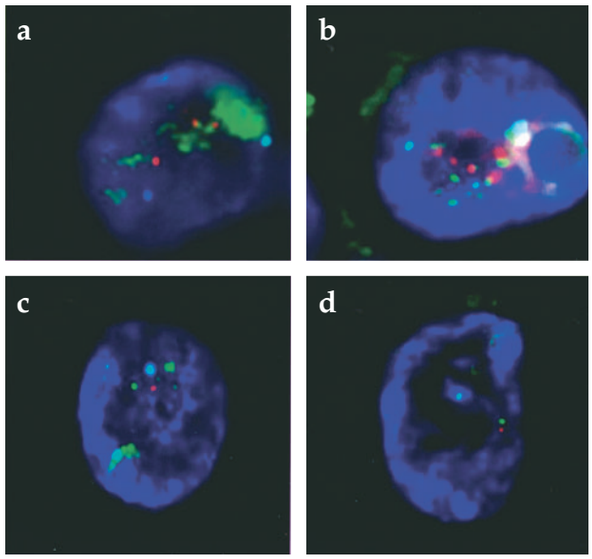

Figure 1. ***(a)*** *Polysomic cell with three copies of TOP2A (red) accompanied by polysomy (blue) and HER-2 amplification (green).* ***(b)*** *HER-2 and TOP2A amplification.* ***(c)*** *Cell with three copies of chromosome 17, HER-2 amplification and TOP2A deletion.* ***(d)*** *Monosomy 17.*

(*Fig. 1b*), HER-2 copy number being higher than the TOP2A copy number in one patient. Two cases with TOP2A deletion were also in the HER-2-amplified group (*Fig. 1c*). In the other two patients with TOP2A deletion HER-2 copy number was within the normal range. Polysomy 17 was observed in three of the four TOP2A-deleted cases (*Fig. 2*). TOP2A aberrations were found to be associated with higher nuclear grade ($p<0.01$) and progesterone receptor negativity ($p=0.025$) (data not shown).

Abnormalities in chromosome 17 copy number were detected in 15 patients: polysomy in 9 (*Fig. 2*) and monosomy in 6 cases (*Fig. 1d & Fig 3*). In 11 of these patients aneusomy 17 (5 polysomies and 6 monosomies) was the sole anomaly. The results are summarized in *Table 1*.

Discussion

HER-2 amplification was observed in 11 patients (22%) in our patient population This is in accordance with the reports in the literature.[20,33] The remaining 39 patients had normal copies of the gene.

Of the 11 patients with HER-2 amplification 4 (36.4%) had accompanying changes in the TOP2A locus; two amplifications (18% of the HER-2-amplified cases) and two deletions (18%). Deletions reported previously have been in the range of 16-42% and amplifications in 25-50% of HER-2-amplified tumors.[14,16] The number of coamplifications observed in our study are lower than those reported in the literature.[7,20,21] However, the number of HER-2-amplified cases are too low for conclusion. In our study TOP2A gene copy number was evaluated in consecutive breast cancer cases and not in specimens previously confirmed by IHC or FISH to be HER-2-overexpressing or -amplified. The differences in the literature have also been related to the differences in the study material and probes.[16]

A higher HER-2 copy number than that of TOP2A was observed in one of the HER-2/TOP2A co-amplified cases in this study. This phenomenon has also been reported previously.[14,16,20] No TOP2A amplifications were seen in patients without HER-2 amplification. It has been suggested that TOP2A and HER-2 might act separately on different amplicons and that deletion and amplification do not need to be correlated.[20] Subsequently, it has been shown that TOP2A and HER-2 are located in the same amplicon although they can be amplified at different levels.[16] Although the number of patients was low in this study, the

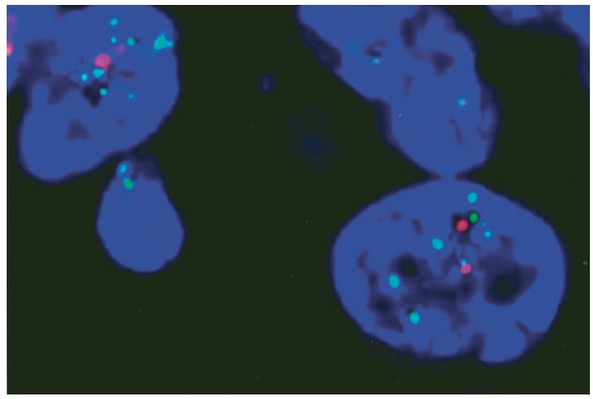

Figure 2. *A case with polysomy 17 (blue) and TOP2A deletion (red). HER-2 (green) number is increased accompanied by polysomy 17, but not the green signals are in focus at the same time.*

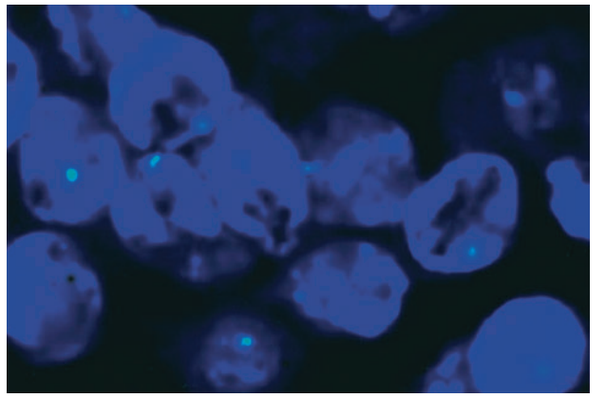

Figure 3. *A case with monosomy 17. Image taken with an aqua single pass filter*

Table 1. **HER-2, TOP2A and chromosome 17 copy number changes**

HER-2	*TOP2A*	*Monosomy 17*	*Polysomy 17*	*Disomy*
Amplified	Amplified	–	–	2
	Deleted	–	1	1
	No change in copy number	–	1	6
Normal	Amplified	–	–	–
	Deleted	–	2	–
	No change in copy number	6	5	26

pattern of amplifications and deletions were in accordance with the model proposed by Jacobson et al.[16]

Deletion of TOP2A was observed in two cases without HER-2 amplification (5%). In HER-2-nonamplified cases the number of TOP2A aberrations varies from no changes to 8%.[21,23] Because of the putative association between TOP2A aberrations and HER-2 amplification, there is a trend in recent studies to evaluate the TOP2A status in tumors with HER-2 amplification or overexpression as evaluated by IHC. Since this is likely to cause about 5-8% of cases in the HER-2-nonamplified group to be missed, it would be more appropriate to evaluate the TOP2A gene copy number status in all cases.

It has been proposed that chromosome 17 aneusomy can affect the expression level of HER-2 and thus may be used in the clinical assessment of the HER-2 status.[3,8,35,36] The response of polysomic, HER-2-nonamplified but IHC-positive tumors to trastuzumab therapy needs further evaluation.

Aneuploidy of chromosome 17, when detected in peripheral blood or bone marrow epithelial cells, may also provide a tool for the diagnosis and monitoring of patients.[11] In our study chromosome 17 aneuploidy was observed in 15 patients (30%). The range reported in the literature is wide and varies from 20% and to 93%.[11,27,35] Of the 15 aneusomic cases 9 (60%) were polysomic and 6 (40%) monosomic. The distribution in the literature varies from equal distribution[27] to 86% polysomic.[37] Different cut-off values, different sample types and study groups have been suggested to explain these variations. The frequency of the polysomic and monosomic cases was 18% and 12%, respectively. Again, values reported in the literature vary from 13% polysomy and 2% monosomy[32] to only 5% polysomy.[36] 22% of the patients had aneusomy as the sole anomaly. This is consistent with a previous study.[37]

In our study HER-2 amplification was present in only two of the 9 cases with polysomy 17. It has been proposed that the mechanism of HER-2 amplification is independent of that of polysomy. Polysomy 17 is observed more often in cases without HER-2 amplification.[32,37] Interestingly, three of the four cases found to be TOP2A deleted in this study were also polysomic for chromosome 17, which seems to be in accordance with the hypothesis that TOP2A deletion occurs before polysomy.[20]

Topo IIα is thought to be responsible for the response to anthracyclines, and many studies have attempted to evaluate the correlation between its gene/protein levels and anthracycline response. It has been shown previously that *in vitro* sensitivity of cancer cells to topo II inhibitors is related to the expression level of topo IIα, cells with low topo IIα levels being less sensitive.[18] Relationship between response to therapy and TOP2A levels or topo IIα expression has been reported in different studies conducted in adjuvant, neoadjuvant or metastatic settings[5-7,23,25] but not in others.[17,29] Surprisingly, even a positive correlation between TOP2A deletion and anthracycline response, although statistically not significant, has been reported.[23] One of the reasons that can possibly explain these different results may be the discrepancy between the TOP2A gene copy number changes and the corresponding enzyme levels. In contrast to the correlation between HER-2 amplification and overexpression, TOP2A amplification is not always accompanied by protein overexpression as measured by IHC.[1,9,26] Another possible reason may be the relation between topo IIα expression levels and the proliferative state of the tumor. Levels may be low in cells which are not undergoing mitosis.[5] The efficacy of topo IIα inhibitors depends on the number of cleavable complexes and is correlated with the expression and activity of topo IIα.[22] Therefore, it is conceivable that other means of regulation such as phosphorylation, interactions with other proteins and proteasome-mediated degradation may affect the response to therapy.[22] HER-2 activation can increase the topo IIα enzyme activity directly, leading to an increase in the sensitivity to topo IIα inhibitors.[13] Drug resistance mechanisms can also affect the response to topo II-targeted therapy.[22] Anthracycline therapy may cause selective proliferation of TOP2A-deleted cells and produce resistant clones in tumors with intratumoral heterogeneity at the TOP2A locus.[18,25] In humans, a small part of the topo IIα pool exists as α/β heterodimers.[2,4] It has been proposed that the β isoform may exert a rescue effect in proliferating cells when topo IIα concentrations are low, it is mutated or pharmacologically inhibited,[22] indicating the possibility that TOP2A deletion may not cause the absolute loss of topo IIα activity. It is evident that there are many unknown factors affecting topo IIα function in cancer cells *in vivo*. Therefore, more studies are needed to reveal the relationship between TOP2A amplification, enzyme activity and response to anthracycline-based therapy.

In conclusion, our study, although conducted in a limited number of patients, shows that TOP2A status cannot be

predicted from the HER-2 status. Not all HER-2-amplified cells are TOP2A-amplified and some cells without HER-2 amplification can have TOP2A deletion. Therefore, evaluation of the TOP2A status in clinical trials comprising only patients with HER-2 overexpression may lead to missing TOP2A-deleted cases with possible resistance to therapy. Furthermore, various factors discussed above may also contribute to the conflicting results obtained during the clinical trials. Studies evaluating different parameters that can modulate topo IIα activity and the response to the drugs targeting the enzyme are still needed.

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References

1. *Bhargava R, Lal P, Chen B:* HER-2/neu and topoisomerase IIα gene amplification and protein expression in invasive breast carcinomas: chromogenic in situ hybridization and immunohistochemical analyses. Am J Clin Pathol 123:889-895, 2005
2. *Biersack H, Jensen S, Gromova I, Nielsen IS, Westergaard O:* Active heterodimers are formed from human topoisomerase II alpha and II beta isoforms. Proc Natl Acad Sci USA 93: 8288-8293, 1996
3. *Bose S, Mohammed M, Shintaku P, Rao PN:* Her-2/neu gene amplification in low to moderately expressing breast cancers: possible role of chromosome 17/Her-2/neu polysomy. Breast J 7:337-344, 2001
4. *Burden DA, Osheroff N:* Mechanism of action of eukaryotic topoisomerase II and drugs targeted to the enzyme. Biochim Biophys Acta 1400:139-154, 1999
5. *Cardoso F, Durbecq V, Larsimont D, Paesmans M, Leroy JY, Rouas G, Sotiriou C, Renard N, Richard V, Picaart MJ, Di Leo A:* Correlation between complete response to anthracycline-based chemotherapy and topoisomerase II alpha gene amplification and protein overexpression in locally advanced/metastatic breast cancer. Int J Oncol 24: 201-209, 2004
6. *Coon JS, Marcus E, Gupta-Burt S, Seelig S, Jacobson K, Chen S, Renta V, Fronda G, Preisler HD:* Amplification and overexpression of topoisomerase II predict response to anthracycline-based therapy in locally advanced breast cancer. Clin Cancer Res 8: 1061-1067, 2002
7. *Di Leo A, Gancberg D, Larsimont D, Tanner M, Jarvinen T, Rouas G, Dolci S, Leroy JY, Paesmans M, Isola J, Piccart MJ:* HER-2 amplification and topoisomerase II? gene aberrations as predictive markers in node-positive breast cancer patients randomly treated either with an anthracycline-based therapy or with cyclophosphamide, methotrexate, and 5-fluorouracil. Clin Cancer Res 8: 1107-1116, 2002
8. *Downs-Kelly E, Yoder BJ, Stoler M, Tubbs RR, Skacel M, Grogan T, Roche P, Hicks DG:* The influence of polysomy 17 on HER2 gene and protein expression in adenocarcinoma of the breast: A fluorescent in situ hybridization, immunohistochemical, and isotopic mRNA in situ hybridization study. Am J Surg Pathol 29:1221-1227, 2005
9. *Durbecq V, Desmed C, Paesmans M, Cardoso F, Di Leo A, Mano M, Rouas G, Leroy JY, Sotiriou C, Piccart M, Larsimont D:* Correlation between topoisomerase-IIalpha gene amplification and protein expression in HER-2 amplified breast cancer. Int J Oncol 25:1473-1479, 2004
10. *Faneyte IF, Chrama JO, Peterse JL, Remijse JL, Rodenhuis S, Van der Vijver MJ:* Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome. Br J Cancer 88: 406-412, 2003
11. *Fehm T, Morrison L, Saboorian H, Hynan L, Tucker T, Uhr J:* Patterns of aneusomy for three chromosomes in individual cells from breast cancer tumors. Breast Cancer Res Treat 75: 227-239, 2002
12. *Greenlee RT, Hill-Harmon MB, Murray T, Thun M:* Cancer Statistics 2001. CA Cancer J Clin 51:15-36, 2001
13. *Harris LN, Yang L, Liotcheva V, Pauli S, Iglehart JD, Colvin OM, Hsieh TS:* Induction of topoisomerase II activity after ERbB2 activation is associated with a differential response to breast cancer chemotherapy. Clin Cancer Res 7: 1497-1504, 2001
14. *Hicks DG, Yoder BJ, Pettay J, Swain E, Tarr S, Hartke M, Skacel M, Crowe JP, Budd GT,* Tubbs RR: The incidence of topoisomerase II-alpha genomic alterations in adenocarcinoma of the breast and their relationship to human epidermal growth factor receptor-2 gene amplification: a fluorescence in situ hybridization study. Hum Pathol 36:348-356, 2005
15. *Hoffken K:* The European Experience. J Clin Oncol 19: 112-117, 2001
16. *Jacobson KK, Morrison LE, Henderson BT, Blondin BA, Wilber KA, Legator MS, O'Hare A, Van Stedum SC, Proffitt JH, Seelig SA,* Coon JS Gene copy mapping of the ERBB2/TOP2A region in breast cancer. Genes Chromosomes Cancer 40:19-31, 2004
17. *Jarvinen TA, Holli K, Kuukasjarvi T, Isola JJ:* Predictive value of topoisomerase II alpha and other prognostic factors for epirubicin chemotherapy in advanced breast cancer. Br J Cancer 77: 2267-2273, 1998
18. *Järvinen TAH, Liu ET:* HER-2/neu and Topoisomerase IIá – simultaneous drug targets in cancer. Comb Chem High Throughput Screen 6: 79-99, 2003
19. *Jarvinen TAH, Liu ET:* Topoisomerase IIα gene (TOP2A) amplification and deletion in cancer – more common than anticipated. Cytopathology 4:309-313, 2003
20. *Jarvinen TAH, Tanner M, Barlund M, Borg A, Isola J:* Characterization of topoisomerase IIα gene amplification and deletion in breast cancer: Genes Chromosomes Cancer 26:142-150, 1999
21. *Jarvinen TAH, Tanner M, Rantanen V, Barlund M, Borg A, Grenman S, Isola J:* Amplification and deletion of topoisomerase IIα associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. Am J Pathol 156: 839-847, 2000
22. *Kellner U, Sehested M, Jensen PB, Gieseler F, Rudolph P:* Culprit and victim – DNA topoisomerase II. Lancet Oncol 3:235-243, 2002
23. *Knoop AS, Knudsen H, Balslev E, Rasmussen BB, Overgaard J, Nielsen KV, Schonau A, Gunnarsdottir K, Olsen KE, Mouridsen H, Ejlertsen B;* Danish Breast Cancer Cooperative Group: Retrospective analysis of topoisomerase IIα amplifications and deletions as predictive markers in primary breast cancer patients randomly assigned to cyclophosphamide, methotrexate, and fluorouracil or cyclophosphamide, epirubicin, and fluorouracil: Danish Breast Cancer Cooperative Group. J Clin Oncol 23:7483-7490, 2005
24. *MacGrogan G, Rudolph P, de Mascarel I, Maurica L, Durand M, Avril A, Dilhudy JM, Robert J, Mathoulin-Pelissier S, Picot V, Floquet A, Serankowski G, Coindre JM:* DNA topoisomerase II alpha expression and the response to primary chemotherapy in breast cancer. Br J Cancer 89: 666-667, 2003

25. *Martin-Richard M, Munoz M, Albanell J, Colomo L, Bellet M, Rey MJ, Tabernero J, Alonso C, Cardesa A, Gascon P, Fernandez PL:* Serial topoisomerase II expression in primary breast cancer and response to neoadjuvant anthracycline-based chemotherapy. Oncology 66: 388-394, 2004
26. *Mueller RE, Parkes RK, Andrulis I, O'Malley FP:* Amplification of the TOP2A gene does not predict high levels of topoisomerase II alpha protein in human breast tumor samples. Genes Chromosomes Cancer 39:288-297, 2004
27. *Nakopoulou L, Giannopoulou I, Trafalis D, Gakiopoulou H, Keramopoulos A, Davaris P:* Evaluation of numeric alterations of chromosomes 1 and 17 by in situ hybridisation in invasive breast carcinoma with clinicopathologic parameters. Appl Immunohistochem Mol Morphol 10:20-28, 2002
28. *Paik S, Bryant J, Park C, Fisher B, Tan-Chiu E, Hyams D, Fisher ER, Lippman ME, Wickerham DL, Wolmark N:* erbB2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer. J Natl Cancer Inst 90: 1320-1327,1998
29. *Petit T, Wilt M, Velten M, Millon R, Rodier JF, Borel C, Mors R, Haegele P, Eber M, Ghnassia JP:* Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. Eur J Cancer 40: 205-211, 2004
30. *Ross JS, Fletcher JA:* HER-2/neu (c-erb-B2) gene and protein in breast cancer. Am J Clin Pathol 112(Suppl 1):53-67, 1999
31. *Ross JS, Fletcher JA, Bloom KJ, Linette GP, Stec J, Symmans WS, Pusztai L, Hortobagyi GN*. Targeted therapy in breast cancer: the HER-2/neu gene and protein. Mol Cell Proteomics 3: 379-398, 2004
32. *Salido M, Tusquets I, Corominas JM, Suarez M, Espinet B, Corzo C, Bellet M, Fabregat X, Serrano S, Solé F*: Polysomy of chromosome 17 in breast cancer tumors showing an overexpression of ERBB2: a study of 175 cases using fluorescence in situ hybridization and immunohistochemistry. Breast Cancer Res 7:267-273, 2005
33. *Tanner M, Jarvinen P, Isola J:* Amplification of HER-2/neu and topoisomerase IIα in primary and metastatic breast cancer. Cancer Res 61: 5345-5348, 2001
34. *Thor AD, Berry DA, Budman DR, Muss HB, Kute T, Henderson IC, Barcos M, Cirrincione C, Edgerton S, Allred C, Norton L, Liu ET:* ERbB-2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer. J Natl Cancer Inst 90: 1346-1360, 1998
35. *Varshney D, Zhou YY, Geller SA, Alsabeh R:* Determination of HER-2 status and chromosome 17 polysomy in breast carcinomas comparing HercepTest and PathVysion FISH assay. Am J Clin Pathol 121:70-77, 2004
36. *Wang S, Hossein Saboorian M, Frenkel EP, Haley BB, Siddiqui MT, Gokaslan S, Hynan L, Ashfaq R:* Aneusomy 17 in breast cancer: its role in HER-2/neu protein expression and implication for clinical assessment of HER-2/neu status. Mod Pathol 15:137-45, 2002
37. *Watters AD, Going JJ, Cooke TG, Bartlett JMS:* Chromosome 17 aneusomy is associated with poor prognostic factors in invasive breast carcinoma. Breast Cancer Res Treat 77: 109-114, 2003
38. *Zhang F, Yang Y, Smith T, Kau SW, McConathy JM, Esteva FJ, Kuerer HM, Symmans WF, Buzdar AU, Hortobagyi GN, Pusztai L:* Correlation between HER-2 expression and response to neoadjuvant chemotherapy with 5-fluorouracil, doxorubicin, and cyclophosphamide in patients with breast carcinoma. Cancer 97:1758-1765, 2003