Applied Cancer Research. 2011;31(4):138-42.

REVIEW

Is polysomy 17 an important phenomenon to predict treatment with trastuzumab in breast cancer?

Beatriz Nunes Schiavon[1], José Vassallo[2], Rafael Malagoli Rocha[3]

Abstract

Objectives: Polysomy has been reported in 8 to 68% of invasive breast carcinomas. Polysomy 17 is frequently found in breast cancer and may complicate the interpretation of HER2 testing results. Abnormalities of chromosome 17 can lead to discrepant interpretations of FISH data. This study aimed to review the impact of polysomy 17 on HER2 testing and studied its clinicopathologic significance in relation to HER2 gene amplification and predicted treatment with trastuzumab. **Material and Methods:** A literature review was performed on polysomy 17 to clarify the significance of chromosome 17 polysomy in invasive breast cancer and show how the increase of CEP17 copy number is currently assessed for novel polysomy 17 testing techniques. **Conclusions:** Polysomy 17 tumors cannot be distinguished from HER2-negative tumors by standard pathologic criteria, including tumor grade and hormone receptor status. The literature indicates that HER2-directed therapy does not add benefit to cytotoxic chemotherapy in metastatic HER2 FISH-negative patients with polysomy 17; however, there is still controversy concerning clinical responses to trastuzumab in those specific cases. Accordingly, more studies with chromosome 17 polysomy and FISH negative are required.

Keywords: breast neoplasms, fluorescence, her2, in situ hybridization, polysome, trastuzumab.

INTRODUCTION

HER2 gene amplification is responsible for protein overexpression in approximately 90% of breast carcinomas. Overexpression of the protein leads to increased cell proliferation. HER2 overexpression is an established adverse prognostic factor in breast cancer, and in both node-positive and node-negative breast cancer, it is associated with a poor prognosis. Several studies have found a correlation between HER2 overexpression and a shorter disease-free period and shorter overall survival[1], besides being associated with aggressive disease, poor response to hormonal therapy and increased response to anthracycline based chemotherapy[2].

HER2 protein is expressed normally at low levels in a variety of epithelial cell types, including breast duct epithelium, and has an important role in normal cellular proliferation. It is overexpressed in 25 to 30%[3,4] of invasive breast cancers as part of the process of malignant transformation and tumor progression. HER2 protein overexpression most commonly results from gene amplification[5].

Chromosome 17 is one of the smallest and the second most densely gene-loaded human chromosome[6]. It is rearranged in at least 30% of breast cancers. Chromosome 17p is mainly involved in genetic losses, some of them possibly focal and targeting potential tumor suppressor genes (eg, *TP53*), whereas 17q is targeted by complex combinations of gains, amplifications, and losses affecting multiple loci[7]. Abnormalities of chromosome 17 can lead to discrepant interpretations of FISH data, depending on which criterion is used. The potential for such misinterpretations is significant, given that polysomy 17 is relatively common in breast carcinomas, although the reported frequency of this finding varies in the literature. An increased number of CEP17 signals may represent a focal gain in the centromeric region of chromosome 17 rather than a true polysomy 17[8]. According to the recently updated ASCO/CAP guidelines, patients with breast carcinomas displaying either a 3+ score by immunohistochemistry (IHC) or a HER2/CEP17 ratio greater than 2.2 in dual-color FISH or more than six HER2 gene copies in single-color FISH and chromogenic *in situ* hybridization (CISH) are eligible for HER2-tailored therapies[9].

Tumors showing an increased copy number of both the HER2 gene and the chromosome 17 centromere, but with a ratio of < 2 (or < 1.8, according to the ASCO/CAP recommendations), are considered polysomic. Chromo-

[1] MSc Student; Department of Anatomic Pathology, Hospital A.C. Camargo, São Paulo, Brazil.
[2] MD, PhD; Department of Anatomic Pathology, Hospital A.C. Camargo, São Paulo, Brazil. Laboratory of Investigative and Molecular Pathology, CIPED, Faculty of Medical Sciences, State University of Campinas, Unicamp, Campinas, Brazil.
[3] MSc, PhD; Department of Anatomic Pathology, Hospital A.C. Camargo, São Paulo, Brazil.

Send correspondence to:
Rafael Malagoli Rocha.
Department of Anatomic Pathology, Hospital A.C. Camargo.
Rua Antonio Prudente, nº 211. São Paulo - SP. Brazil. CEP: 01509-900.
Tel: +55 (11) 6397-8917.
E-mail: rafael.malagoli@gmail.com

Submitted: 13/04/2012.
Aproved: 24/04/2012.

some 17 polysomy (defined as at least one more copy of chromosome 17 than normal) in the absence of HER2 gene amplification is sometimes associated with 2+ IHC staining but rarely with 3+, indicating that such polysomy has little influence on HER2 gene expression in breast carcinoma and no apparent influence on the clinical presentation or behavior in HER2-negative tumors. Patients with polysomic breast cancer are not candidates for targeted interventions unless their tumors exhibit a positive (3+) immunohistochemical assay. They are not considered eligible for the trials of adjuvant trastuzumab, neither are they deemed eligible for ongoing trials comparing different HER2-targeted therapies[10].

Polysomy has been reported in 8 to 68% of invasive breast carcinomas, since different authors have used different criteria to define it[11]. Polysomy of Chr 17, as defined by dual-color FISH, is observed in approximately 8% of all breast cancer specimens, mostly among cases with four to six HER2 gene copies (the so-called "equivocal range"). Polysomy is cytogenetically defined as the occurrence in a nucleus of extra copies of one or more individual chromosomes, and increases in copy number due to polysomy do not have the same biological impact as those caused by gene amplification[9].

Polysomy 17 will lead to increased HER2 gene copy number, but it is mechanistically distinct from HER2 gene amplification. The two aberrations can coexist, and it is uncertain whether they are linked or are independent. Polysomy of other chromosomes is rarely measured simultaneously[12].

Polysomy 17 and HER2 expression

The mechanisms for HER2 expression in non-amplified tumors scored 2+ by IHC are unclear and may involve increased gene dosage by chromosome 17 polysomy. Sauer and colleagues[13] found that an abnormal number of copies of chromosome 17 have a low impact on HER2 gene and its expression, but more studies are necessary to confirm these results[5]. Polysomy 17 may lead to increased HER2 gene dosage and these cases may or may not be associated with HER2 gene amplification[1]. Salido et al.[5] observed polysomy 17 in non-amplified cases more frequently than in FISH amplified cases (15% versus 10%), suggesting that the mechanism for amplification of HER2 gene is independent of polysomy.

There is some heterogeneity for these adverse prognostic indicators in the group of patients with unamplified polysomy 17. Differences in the level of polysomy and in the number of HER2 copies/cell thereof could contribute to this heterogeneity. Authors have reported higher HER2 copy numbers and greater frequency of discordance between IHC and FISH for HER2 in cases with high polysomy than in cases with low polysomy[1].

Zhu et al.[14] speculated that cases with HER2 amplification but without overexpression may be caused by (1) the failure of IHC detection possibly owing to invalid antibody and technical problems, (2) discrepancies in the results reported by different evaluators, (3) the early stage of gene amplification or transcription before translation of mRNA. On the other hand, for those tumors with HER2 overexpression but without gene amplification, it might be because of an increased chromosome 17 copy number, resulting in the increased total number of HER2 copies per tumor cell.

Some studies showed that polysomy 17 alone may not significantly contribute to the variation in HER2 copy number and HER2 protein overexpression[14,15].

Polysomy 17 related to HER2 targeted therapy

Not only does HER2 overexpression predict response to certain chemotherapeutic agents, such as anthracyclines or paclitaxel, it is also considered to be a strong predictive marker for clinical benefit from HER2--targeted therapy (trastuzumab) in the metastatic setting and, more recently, also in the adjuvant setting. Although tumors not expressing HER2 have virtually no chance of responding to trastuzumab, moderate or even high levels of expression are not always associated with therapeutic success. Moreover, treating patients with breast cancer with trastuzumab is expensive and not without risk, because serious cardiac toxicity has been observed in approximately 1 to 4% of patients[16].

By contrast, a score 3+ on IHC was not found in tumors displaying polysomy 17 in the absence of HER2 gene amplification. It is important to realize that polysomy 17 has a substantial impact on the interpretation of HER2 testing results, especially in those patients with an equivocal HER2 status on IHC (score 2+)[16]. Bartlett et al.[17] suggested a possible association between CEP17 amplification and response to anthracyclins. Hofmann et al.[2] reported that patients with breast cancer with polysomy 17 responded well to the therapy of trastuzumab.

Thus, the counterintuitive reported benefit of trastuzumab for patients with allegedly HER2-negative cancers might, at least in part, be explained by an incorrect classification of HER2-amplified breast cancers as chromosome 17 polysomic, on the basis of dual-probe *in situ* hybridization assays. These patients could actually have HER2-positive disease by absolute HER2 gene copy number that escapes confirmation by *in situ* hybridization with a dual-probe assay due to an increased number of CEP17 signals, indicating CEP17 gain or amplification and not true chromosome 17 polysomy. On this basis, there is no plausible biological explanation why the extra CEP17 copies would block the effect of the extra HER2 gene copies. Hence, patients with increased copies of HER2 (more than six) are likely to respond to trastuzumab even if CEP17 are greater than two[7].

It is conceivable, however, that a subset of polysomy 17 tumors could respond to trastuzumab, for example, in tumors with high ploidy. If trastuzumab benefits any polysomy 17 tumors, factors other than HER2 expression will likely influence response, and the magnitude of benefit may be different than in HER2-positive tumors. Targeted therapy does not always work exactly as predicted. Truly HER2-negative tumors are unlikely to respond to HER2--directed therapy (although there is some recent disagreement), but only half of HER2-positive tumors respond to trastuzumab[12].

Patients such as those described in Tse et al.[18] study with falsely low HER2: chromosome 17 ratios based on CEP17 analysis alone (polysomy 17) have traditionally been excluded from clinical trials of and other HER2-targeted drugs. Although the response rate of these patients is unknown at this point, their demonstration of what appears to be true amplification of the HER2 gene in a large subset of patients excluded because of polysomy 17 may, in fact, benefit from HER2-targeted therapies.

The group of Hoffman et al.[2] showed that two "polysomic" patients that were FISH negative (but IHC 3+) responded to trastuzumab indicating that FISH analysis can lead to false negative results mainly based on CEP17 amplification[6].

Polysomy 17 assessed by IHC and *in situ* hybridization

To date, the effect of polysomy 17 on FISH or IHC results remains controversial[14]. Studies showed that increased CEP17 copy numbers, as detected by CEP17 FISH, CISH, or SISH, are often the result of CEP17 duplication or amplification, or gains or amplification in the pericentromeric region of chromosome 17. Therefore, FISH, CISH, or SISH assessment of CEP17 does not indicate polysomy 17 in most cases[7].

Hofmann et al.[2] suggested that tumors with more than 10 HER2 signals per cell should be regarded as FISH positive, regardless of the HER2/CEP17 ratio. Thus, the absolute number of HER2 signals should be considered in FISH negative by dual probe system/IHC 3+ tumors. Based on the experience of Zhu et al.[14] they would suggest that the HER2 amplification and chromosome 17 copy number should be recorded simultaneously in the pathologic report.

Novel polysomy 17 testing techniques

Testing issues, such as the impact of chromosome 17 polysomy and local versus central HER2 testing and emerging novel HER2 testing techniques, including messenger RNA (mRNA)-based testing by real time polymerase chain reaction (RT-PCR) and DNA microarray methods, HER2 receptor dimerization, phosphorylated HER2 receptors and HER2 status in circulating tumor cells are of current interest in the management of breast cancer[19].

Torrisi et al.[20] also found that unamplified polysomy was not associated with poor prognostic factors. There could be a number of reasons for adverse behavior in patients with unamplified polysomy 17, such as increased levels of HER2 protein because of increased gene dosage. Although some authors have shown overexpression of HER2 mRNA both with or without HER2 amplification, majority of published literature indicates that polysomy 17 does not have an effect on HER2 mRNA content[1,21].

In tumors with polysomy 17, HER2 mRNA is not substantially increased and when examining protein overexpression by immunohistochemistry, strong and complete membrane staining is not observed; by contrast, in those with HER2 amplification, HER2 mRNA and protein concentrations are significantly increased[7].

Polysomy 17, without HER2 gene amplification, does not seem to significantly increase HER2 mRNA. However, polysomy 17 alone may increase HER2 protein expression. Most studies find little IHC 3+ expression (although very high ploidy may be associated with IHC 3+). But IHC 2+ expression is common in polysomy 17 tumors lacking HER2 gene amplification, although no more than half of IHC 2+ tumors seem to have polysomy 17[12].

In addition to gene amplification and polysomy, other molecular mechanisms contribute to HER2 protein overexpression. The amount of HER2 may be regulated at the transcriptional level. Examples showing the importance of HER2 transcriptional regulation are from cell lines with an elevation of HER2 mRNA levels per gene copy[2].

Studies using array CGH on breast tumors suggest that polysomy of chromosome 17 is a rare event in breast cancer[9,22]. Some other studies obtained promising results with multiplex ligation-dependent probe amplification (MLPA) in comparison with ISH and evaluated this technique to simultaneously determine copy number changes of HER2 and TOP2A, a gene that has shown involvement in the response to anthracyclines by some groups[6].

DISCUSSION

Polysomy 17 tumors do not overexpress HER2 mRNA or protein, and they cannot be distinguished from HER2-negative tumors by standard pathologic criteria, including tumor grade and hormone receptor status. Vanden Bempt et al.[16] ask whether tumors with polysomy 17, but lacking HER2 gene amplification, should be considered HER2 negative or positive. The answer seems to be that they are functionally HER2 negative. Extra copies of the HER2 gene in a seemingly normal context on chromosome 17 are not associated with increased mRNA or protein, in contrast to what occurs with gene amplification. This question has not been directly studied, but polysomy 17 tumors' similarity to HER2-negative tumors suggests that they would be unlikely to respond to trastuzumab[12].

Because polysomy 17 on its own is not associated with HER2 overexpression and because it does not have the same clinicopathologic significance as true HER2 gene amplification, one may wonder whether polysomy 17 tumors benefit from HER2-targeted therapy such as trastuzumab, which targets the HER2 protein at the tumor cell membrane[16].

In the Krishnamurti et al.[1] study, the authors concluded that chromosome 17 polysomy appeared to be a major cause of clinical responses to trastuzumab in FISH--negative cases and they propose that patients who are HER2-positive, but FISH negative should be first retested by immunohistochemistry, and patients with positive results should be considered for trastuzumab therapy.

Thus, the HER2/CEP17 ratio may not be the best way to evaluate the HER2 status in all cases and the absolute HER2 gene copy number (whether increased through amplification or polysomy) may be the most important determinant for trastuzumab response for some patients[6].

Tse et al.[18] investigated CEP17 copy number, as well as several other genes on Chr 17 (SMS, RARA, and TP53) that are an effective way to determine the true HER2 amplification status in patients with polysomy 17 in relation to HER2 copy number. The authors concluded that true polysomy for Chr 17 is rare, that CEP17 copy number may not reflect true Chr 17 copy number, that the use of alternate Chr 17 genes may be a more effective means of determining true HER2 amplification status, and that by using these alternate reference genes, 45% of the cases previously scored as "nonamplified polysomy" are reclassified as "amplified". This would have important clinical significance, as a subset of patients currently being classified as "nonamplified" or "nonamplified polysomic" might in fact be amplified, and represent a group who would derive benefit from HER2-directed therapy.

The Vanden Bempt et al.[16] study goes even further and states that polysomy 17 and HER2 gene amplification are two distinct genetic aberrations with different clinicopathologic significance in breast cancer. They show that polysomy 17 on its own does not result in HER2 overexpression at all, neither at the protein nor at the mRNA level. Also, Downey et al.[23] reported that Chr 17 polysomy in HER2-nonamplified adjuvant patients also failed to be associated with the benefit to trastuzumab, although this was an extremely small subset (n = 37), but initial studies of a HER2-directed monoclonal antibody, trastuzumab, showed that antitumor effect was limited to cell lines with HER2 overexpression. However, other studies have suggested that women whose breast cancers lack HER2 gene amplification but have Chr 17 polysomy may be responsive to HER2-directed therapies in the metastatic setting[23,24].

In fact, this review reports that the tumors showing polysomy are more histopathologically similar to HER2--negative tumors than to HER2 gene-amplified tumors, raising doubt that polysomy would be associated with a benefit from HER2-directed therapy. However, the available literature indicates that polysomy 17 per se does not have a significant effect on HER2 protein or mRNA levels. This supports the results from this analysis that HER2-directed therapy does not add benefit to cytotoxic chemotherapy in metastatic HER2 FISH-negative patients with polysomy[23]. Therefore, further studies with a large set of patients with chromosome 17 polysomy and FISH negative submitted to targeted therapy are required to clarify the inquires into the role of polysomy in breast cancer and at what stage of disease that therapy may be effective.

REFERENCES

1. Krishnamurti U, Hammers JL, Atem FD, Storto PD, Silverman JF. Poor prognostic significance of unamplified chromosome 17 polysomy in invasive breast carcinoma. Mod Pathol 2009;22:1044-8.
2. Hofmann M, Stoss O, Gaiser T, Kneitz H, Heinmöller P, Gutjahr T, et al. Central HER2 IHC and FISH analysis in a trastuzumab (Herceptin) phase II monotherapy study: assessment of test sensitivity and impact of chromosome 17 polysomy. J Clin Pathol 2008;61:89-94.
3. Ma Y, Lespagnard L, Durbecq V, Paesmans M, Desmedt C, Gomez--Galdon M, et al. Polysomy 17 in HER-2/neu status elaboration in breast cancer: effect on daily practice. Clin Cancer Res 2005;11:4393-9.
4. Salido M, Tusquets I, Corominas JM, Suarez M, Espinet B, Corzo C, et al. 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 2005;7:R267-73.
5. 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 2004;121:70-7.
6. Moelans CB, de Weger RA, van Diest PJ. Absence of chromosome 17 polysomy in breast cancer: analysis by CEP17 chromogenic in situ hybridization and multiplex ligation-dependent probe amplification. Breast Cancer Res Treat 2010;120:1-7.
7. Moelans CB, Reis-Filho JS, van Diest PJ. Implications of rarity of chromosome 17 polysomy in breast cancer. Lancet Oncol 2011;12:1087-9.
8. Vranic S, Teruya B, Repertinger S, Ulmer P, Hagenkord J, Gatalica Z. Assessment of HER2 gene status in breast carcinomas with polysomy of chromosome 17. Cancer 2011;117:48-53.
9. Marchiò C, Lambros MB, Gugliotta P, Di Cantogno LV, Botta C, Pasini B, et al. Does chromosome 17 centromere copy number predict polysomy in breast cancer? A fluorescence in situ hybridization and microarray-based CGH analysis. J Pathol 2009;219:16-24.
10. Viale G. Be precise! The need to consider the mechanisms for CEP17 copy number changes in breast cancer. J Pathol 2009;219:1-2.
11. Egervari K, Kosa C, Szollosi Z. Impact of chromosome 17 centromere region assessment on HER2 status reported in breast cancer. Pathol Res Pract 2011;207:468-71.
12. Rosenberg CL. Polysomy 17 and HER-2 amplification: true, true, and unrelated. J Clin Oncol 2008;26:4856-8.
13. Sauer T, Beraki K, Jebsen PW, Ormerod E, Naess O. Numerical aberrations of chromosome 17 in interphase cell nuclei of breast carcinoma cells: lack of correlation with abnormal expression of P53, neu and nm23 protein. APMIS 1998;106:921-7.
14. Zhu X, Lu Y, Lu H, Yang W, Tu X, Cai X, et al. Genetic alterations and protein expression of HER2 and chromosome 17 polysomy in breast cancer. Hum Pathol 2011;42:1499-504.
15. Wang S, Hossein Saboorian M, Frenkel EP, Haley BB, Siddiqui MT, Gokaslan S, et al. Aneusomy 17 in breast cancer: its role in HER-2/neu protein expression and implications for clinical assessment of HER-2/neu status. Mod Pathol 2002;15:137-45.

16. Vanden Bempt I, Van Loo P, Drijkoningen M, Neven P, Smeets A, Christiaens MR, et al. Polysomy 17 in breast cancer: clinicopathologic significance and impact on HER-2 testing. J Clin Oncol 2008;26:4869-74.
17. Bartlett JM, Desmedt C, Munro A, O'Malley FP, Larsimont D, di Leo A, et al. Chromosome 17 polysomy: a unifying hypothesis underlying benefit from adjuvant anthracyclines?. Cancer Res 2009;69(Suppl):6059.
18. Tse CH, Hwang HC, Goldstein LC, Kandalaft PL, Wiley JC, Kussick SJ, et al. Determining true HER2 gene status in breast cancers with polysomy by using alternative chromosome 17 reference genes: implications for anti-HER2 targeted therapy. J Clin Oncol 2011;29:4168-74.
19. Ross JS. Breast cancer biomarkers and HER2 testing after 10 years of anti-HER2 therapy. Drug News Perspect 2009;22:93-106.
20. Torrisi R, Rotmensz N, Bagnardi V, Viale G, Curto BD, Dell'orto P, et al. HER2 status in early breast cancer: relevance of cell staining patterns, gene amplification and polysomy 17. Eur J Cancer 2007;43:2339-44.
21. Dal Lago L, Durbecq V, Desmedt C, Salgado R, Verjat T, Lespagnard L, et al. Correction for chromosome-17 is critical for the determination of true Her-2/neu gene amplification status in breast cancer. Mol Cancer Ther 2006;5:2572-9.
22. Yeh IT, Martin MA, Robetorye RS, Bolla AR, McCaskill C, Shah RK, et al. Clinical validation of an array CGH test for HER2 status in breast cancer reveals that polysomy 17 is a rare event. Mod Pathol 2009;22:1169-75.
23. Downey L, Livingston RB, Koehler M, Arbushites M, Williams L, Santiago A, et al. Chromosome 17 polysomy without human epidermal growth factor receptor 2 amplification does not predict response to lapatinib plus paclitaxel compared with paclitaxel in metastatic breast cancer. Clin Cancer Res 2010;16:1281-8.
24. Kaufman PA, Broadwater G, Lezon-Geyda K, Dressler LG, Berry D, Friedman P, et al. CALGB 150002: correlation of HER2 and chromosome 17 (ch17) copy number with trastuzumab (T) efficacy in CALGB 9840, paclitaxel (P) with or without trastuzumab in HER2+ and HER2- metastatic breast cancer (MBC) [abstract]. J Clin Oncol 2007;25(20 Suppl):1009. [Presented at 2007 ASCO Annual Meeting, 2007 June 1-5, Chicago(II).