Poor prognostic significance of unamplified chromosome 17 polysomy in invasive breast carcinoma

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The human epidermal growth factor receptor 2 (*HER2*) oncoprotein is overexpressed in about 20% of breast cancers, with *HER2* gene amplification responsible for protein overexpression in the vast majority of patients. A subset of breast cancers have chromosome 17 aneusomy, due to either 17 monosomy (a single copy of chromosome 17) or polysomy (increased copy numbers of chromosome 17). Although *HER2* overexpression is an established adverse prognostic factor in breast cancer, the role of unamplified chromosome 17 polysomy is uncertain and there is a paucity of literature on the correlation of chromosome 17 aneusomy with important prognostic and predictive pathologic factors in invasive breast carcinoma. Furthermore, while patients showing *HER2* amplification with or without polysomy 17 are treated with trastuzumab with or without other chemotherapy, treatment of patients with unamplified chromosome 17 polysomy is not well defined. Currently most of these patients are treated similar to patients with neither amplification nor 17 polysomy. The aim of this study was to compare some prognostic and predictive factors in invasive breast carcinoma in patients with unamplified chromosome 17 polysomy with that seen in cases with *HER2* gene amplification and those with neither amplification or polysomy. We found that invasive breast carcinomas with unamplified chromosome 17 polysomy are associated with several adverse prognostic indicators such as a higher nuclear grade, mitotic activity, Nottingham score, histologic grade, tumor stage, and greater estrogen receptor negativity with a trend towards the amplified group, in contrast to patients with neither amplification or polysomy. Although most patients with unamplified 17 polysomy have a 2+ equivocal score on immunohistochemistry, a minority has a 3+ positive score. An increased adverse role for unamplified polysomy along with 3+ protein expression in some patients supports the idea that these patients should be considered for therapy with trastuzumab and/or anthracyclines.

Modern Pathology (2009) 22, 1044–1048; doi:10.1038/modpathol.2009.61; published online 24 April 2009

Keywords: breast carcinoma; chromosome 17 polysomy; *HER2*

The role of human epidermal growth factor receptor 2 (*HER2*) oncogene was first reported in breast cancers in 1987.¹ The *HER2* oncogene is a member of the human epidermal growth factor receptor family and is located on the long arm of chromosome 17 (17q12–21.32).² The *HER2* protein is a 185 kDa growth factor receptor with tyrosine kinase activity and is expressed on the cell surface.^{3,4} The *HER2* oncoprotein is overexpressed in about 20% of breast cancers. *HER2* gene amplification is respon-

sible for protein overexpression in approximately 90% of breast carcinomas.^{5,6} Overexpression of the protein leads to increased cell proliferation. *HER2* overexpression is an established adverse prognostic factor in breast cancer. *HER2* overexpression in both node-positive and node-negative breast cancer is associated with a poor prognosis, and several studies have found a correlation between *HER2* overexpression and a shorter disease-free period and shorter overall survival.^{7–9}

The overexpressed protein is the therapeutic target for treatment with humanized monoclonal antibody, trastuzumab.^{10,11} Trastuzumab has been demonstrated to be effective in the treatment of breast carcinoma either as a single agent or in combination with traditional chemotherapy.¹² *HER2* overexpression is also a negative predictor for

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 Received 30 January 2009; revised 27 March 2009; accepted 29 March 2009; published online 24 April 2009

response to alkylating agents and a positive predictor for response to anthracyclines. However, a subset of breast cancers have chromosome 17 aneusomy, because of either 17 monosomy (a single copy of chromosome 17) or polysomy (increased copy numbers of chromosome 17). Polysomy for chromosome 17 may lead to increased *HER2* gene dosage and these cases may or may not be associated with *HER2* gene amplification. The incidence of chromosome 17 polysomy reported in the literature ranges from 5–50%.^{13–16} Although there are several studies correlating polysomy/aneusomy of chromosome 17 with immunohistochemistry for *HER2* amplification, there is a paucity of literature on the correlation of chromosome 17 aneusomy with important prognostic and predictive pathologic parameters in invasive breast carcinoma.

The aim of this study was to evaluate prognostic and predictive factors in invasive breast carcinoma in patients with unamplified chromosome 17 polysomy compared to patients with *HER2* amplification and those with neither amplification nor polysomy.

Materials and methods

We evaluated cases of invasive breast carcinoma analyzed by fluorescence *in situ* hybridization (FISH) from 1/1/2005 to 6/30/2007. FISH was performed utilizing the federal drug administration approved Pathvysion *HER2* DNA probe kit from Abbott/Molecular. The manufacturer's instructions were followed with no modifications. Sections were viewed using a fluorescence-equipped Zeiss Axio-plan microscope. Polysomy was defined as the presence of ≥ 3.0 *CEP17* copies/cell and *HER2* amplification was defined by a *HER2/CEP17* copy ratio > 2.2 . Three groups consisting of Normal-no polysomy and no amplification, 36 cases (N), 17 polysomy without amplification, 44 cases (P), and *HER2* amplification without polysomy, 35 cases (A), were correlated with Nottingham score, tubule formation, nuclear grade, mitotic activity, histologic grade, lymph vascular invasion, lymph node status, tumor stage, estrogen receptor and progesterone receptor negativity. Statistical analysis was performed using the statistical software SAS version 9.2 and a Z-test of two proportions was performed.

Results

Of 365 cases analyzed by FISH in the laboratory, polysomy 17 was detected in 83 patients (23%), of which 61 patients had unamplified polysomy (17%).

The distribution of Nottingham score and the histological grade in the different groups studied are shown in Tables 1 and 2. The most frequently occurring Nottingham score in group N was 6 compared with a score of 8 in P and scores of both 8 and 9 in A. For a score of 8, the difference between

Table 1 Distribution of Nottingham score in N, P, and A groups

Score	N (%)	P (%)	A (%)
4	10	0	0
5	15	9	0
6	39	15	12
7	14	25	20
8	11	37	34
9	11	14	34

Table 2 Distribution of histologic grade in N, P, and A groups

Histologic grade	N (%)	P (%)	A (%)
1	25	10	0
2	54	46	31
3	21	44	69

N and both P and A was significant ($P < 0.01$), whereas the difference between P and A was not significant. For a score of 9, group A had a significantly higher frequency compared with both N and P ($P < 0.05$). Histological grade 2 was the most frequent in group N, grade 3 most frequent in group A, whereas grades 2 and 3 were equally distributed in group P. Grade 3 was significantly higher in frequency in both P and A groups compared with N ($P < 0.05$). Furthermore, A had a greater frequency of cases with grade 3 compared with P ($P = 0.02$). A tubule score of 3 was most frequent in all three groups and the differences were not significant (data not shown). Distribution of nuclear grade is shown in Table 3 and mitotic score in Table 4. Nuclear grade 2 was most frequent in group N, compared with nuclear grade 3 in groups P and A ($P < 0.005$). Although the frequency of nuclear grade 3 in group A was higher than that seen in group P, the difference was not statistically significant. Mitotic score 1 was most frequent in group N. Mitotic score of 2 was significantly higher in both A and P compared with N ($P < 0.01$). Frequency of mitotic score 3 was higher in group A compared with group P, but the difference was not statistically significant.

Tumor stage 2 was seen in 18% of N, 31% of P and 30% of A. The difference between the three groups was not statistically significant. Lymph vascular invasion was noted on hematoxylin and eosin sections in 17% of N, 19% of P, and 34% of A. Although lymph vascular invasion was higher in A compared with both N and P, the difference was not statistically significant. Positive lymph nodes were detected in 38%, 42%, and 45% of N, P, and A respectively, and the differences were not statistically significant.

The percentage of estrogen receptor and progesterone receptor negativity is given in Table 5. Estrogen receptor negativity was seen more in P and A compared with N ($P < 0.05$). Cases with *HER2* amplification had a significantly greater frequency

of estrogen receptor negativity than those with unamplified polysomy ($P=0.05$). The difference in progesterone receptor negativity was not significant between the three groups.

Table 6 summarizes the key findings detailed in Tables 1–5.

About 13% of cases of unamplified polysomy 17 in our laboratory (eight cases) have 3+ *HER2* immunohistochemistry. Although the number of patients with 3+ immunohistochemistry and unamplified polysomy 17 is too small to form conclusions, this group had a greater percentage of patients with more adverse prognostic factors. Of these eight cases, seven had histologic grade 3, three had lymph vascular invasion, five had tumor stage 2 or higher, and two patients had subsequent local recurrences. One patient with 3+ immunohistochemistry and unamplified polysomy 17 had lymph

node and brain metastases at presentation. To the best of our knowledge, local recurrences or distant metastases were not seen in our patients with unamplified polysomy 17 who were 0–2+ by immunohistochemistry.

Discussion

We have demonstrated that invasive breast carcinoma with unamplified chromosome 17 polysomy is associated with several adverse prognostic indicators such as a higher nuclear grade, mitotic activity, Nottingham score, histologic grade, tumor stage, and greater estrogen receptor negativity with a trend towards the amplified group, in contrast to patients with neither amplification nor polysomy. Nodal involvement showed no significant correlation in the groups. 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 immunohistochemistry and FISH for *HER2* in cases with high polysomy than in cases with low polysomy.^{17–19}

There are only a few other studies that have examined prognostic and/or predictive factors in chromosome 17 aneusomy or polysomy. Watters *et al.*²⁰ found aneusomy 17 to be associated with grade 3 carcinoma ($P=0.008$), estrogen receptor negativity ($P=0.0032$) and a Nottingham prognostic index of greater than 5.4 ($P=0.039$), but was not associated with survival by univariate analysis.

Tsukamoto *et al.*¹⁶ found lymph node metastasis to be significantly correlated with polysomy of chromosomes 1, 11, and 17. Progesterone receptor negativity was significantly higher in patients with polysomy of chromosomes 11 and 17 in their study ($P<0.05$). Takehisa *et al.*¹⁴ also found a statistical correlation with a high histology grade in cases with polysomy 17. Lu *et al.*²¹ found polysomy 17 with or without amplification to be associated with high

Table 3 Distribution of nuclear grade in N, P, and A groups

Nuclear grade	N (%)	P (%)	A (%)
1	19	9	0
2	55	30	26
3	26	61	74

Table 4 Distribution of mitotic score in N, P, and A groups

Mitotic score	N (%)	P (%)	A (%)
1	64	20	17
2	18	54	43
3	18	26	40

Table 5 ER and PR negativity N, P, and A groups

Hormone receptor negative	N (%)	P (%)	A (%)
ER negative	11	30	50
PR negative	24	35	34

Table 6 Summary of key findings in tables 1–5

Prognostic and predictive factors	N (%)	P (%)	A (%)	N vs P	P vs A
Nottingham score-8	11	37	34	$P=0.008$	—
Nottingham score-9	11	14	34	—	$P=0.033$
Nuclear grade-3	26	61	74	$P=0.002$	—
Mitotic score-2	18	54	43	$P=0.001$	—
Mitotic score-3	18	26	40	—	—
Histologic grade 3	20	44	69	$P=0.02$	$P=0.02$
T stage-2	18	31	30	—	—
Lymph vascular invasion present	17	19	34	—	—
Positive LN	38	42	45	—	—
ER negativity	11	30	50	$P=0.04$	$P=0.05$
PR negativity	24	35	34	—	—

— = not significant.

nuclear atypia and with lymphatic metastases. Salido *et al*²² found that although nodal involvement was significantly associated with polysomy 17 ($P=0.046$) and patients with polysomy 17 showed a non-statistical trend towards relapse ($P=0.181$), there was no statistically significant correlation between histologic grade, estrogen receptor, progesterone receptor status and polysomy 17. Torrisi *et al*.¹³ 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,²³ majority of published literature indicates that polysomy 17 does not have an effect on *HER2* mRNA content.^{17,24,25}

It is well known that cancer tumorigenesis is a complex process. In addition to overexpression secondary to *HER2* gene amplification, other molecular mechanisms can contribute to *HER2* protein overexpression. Regulation of *HER2* expression at the transcriptional level, alternative splicing with isoforms such as herstatin are some ways protein expression can be altered.²⁶ There may be amplification of other potentially oncogenic genes and this may facilitate the generation of other genetic aberrations.²⁷ For example, topoisomerase II- α (TOP2A) is an enzyme that represents a molecular target for anthracyclines. In fact the relationship of *HER2* to response to anthracyclines has been proposed as possibly being due to the close localization of *HER2* and *TOP2A* on 17q. It has been a subject of debate whether these two genes behave separately or act together.²⁸ In addition to alternate mechanisms at the nucleic acid level, recycling of the oncoprotein and altered protein degradation by changes in protein ubiquitination are other mechanisms that can result in *HER2* protein overexpression.²⁹ Magnifico *et al*.³⁰ in their study suggest that *HER2* overexpression in some *HER2* 2+ breast carcinomas is due to an accumulation of recycled oncoprotein on the cell surface induced by activation of protein kinase C- α . Alternate mechanisms occurring at the protein level would explain how *HER2* protein can be overexpressed without changes in *HER2* mRNA levels. Therefore, increased adverse parameters in patients with unamplified polysomy 17 could be secondary to activation of other oncogenes that have not yet been identified or at the level of the protein itself by alterations in its half-life.

Although we may not fully understand the reasons for the presence of increased adverse parameters in patients with unamplified polysomy 17, it is important to consider whether these patients should be treated differently. Currently patients with unamplified polysomy 17 are treated similar to patients with neither amplification or polysomy. Although most patients with unamplified polysomy

17 have a 2 + equivocal expression of *HER2* protein on immunohistochemistry, some patients have a 3 + positive score for overexpression on immunohistochemistry (13.0% in our laboratory, data not shown). Hofmann *et al*.³¹ reported a subset of patients (2/4) with unamplified 17 polysomy and 3+ *HER2* immunohistochemistry expression responding to treatment with trastuzumab. The authors in that study 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.

An increased adverse role for unamplified polysomy along with 3 + protein overexpression supports the idea that these patients should be considered for therapy with trastuzumab and/or anthracyclines. However, definitive recommendations can be made only after randomized clinical trials testing the efficacy of trastuzumab therapy in patients with unamplified polysomy.

Conflict of interest

The authors declare no conflict of interest.

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