HER2 overexpression in various tumor types, focusing on its relationship to the development of invasive breast cancer

S. Ménard, P. Casalini, M. Campiglio, S. Pupa, R. Agresti & E. Tagliaabue

The Molecular Targeting Unit, Istituto Nazionale Tumori, Milan, Italy

Summary

To date, poor standardization in HER2 status evaluation has precluded reliable comparison of overexpression rates in different tumors. However, standardized methodologies have been introduced recently for these analyses, and have identified frequencies of 51%, 44%, 26% and 25% in Wilm’s tumor, bladder, pancreatic and breast carcinoma, respectively. Other tumors tested had frequencies below 20%. The frequency was greater than that predicted by gene amplification data in some tumor types, which may indicate overexpression due to gene deregulation, rather than gene amplification. Analysis of a large retrospective series of breast carcinomas demonstrated an association between HER2 positivity and a number of other prognostic markers. Together, these variables identify a subset of tumors with poor prognosis and early relapse post-surgery. HER2 expression is relatively stable, with 95% concordance between the HER2 status of primary and metastatic lesions. However, contralateral tumors are unrestricted with regard to HER2 status. Preliminary data indicate that the HER2 status of a hormone receptor-positive tumor may fluctuate according to the menstrual cycle. It is anticipated that the emerging wealth of standardized data for HER2 status will help to elucidate the role of HER2 in tumor progression.

Key words: breast cancer, gene regulation, HER2, Herceptin, immunohistochemical, trastuzumab

Introduction

The human epidermal growth factor receptor-2 (HER2) has been studied in a wide variety of human carcinomas and increased levels of expression and/or gene amplification have been identified in a number of different tumors [1–6]. Analysis of the literature, however, reveals a wide variation in HER2 levels within a single tumor type. This variation, most probably attributable to the lack of standardization in the methodologies used to assess HER2 status, precludes the use of the literature data to construct a reliable scale of overexpression.

The method most frequently employed for assessment of HER2 status is detection of the HER2-receptor protein by immunohistochemistry (IHC). Much of the published data have been derived from assays employing a variety of polyclonal and monoclonal antibodies (MAbs) reacting with HER2, which differ in terms of binding affinity, epitope specificity and/or cross-reactivity with non-HER2 proteins [7]. Variability is also introduced by varying the sensitivity of different detection methods, subjective assessment and the lack of a uniform scoring system to interpret HER2 staining patterns. Further details of the testing methodologies used in HER2-positive patients are provided elsewhere in this supplement [8].

Detection of gene amplification, by fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) methodologies, avoids some of the problems associated with IHC. Studies performing IHC and FISH on the same tissue samples have demonstrated good concordance between the results [9]. However, the use of double staining with IHC and FISH has identified some cells that are HER2 positive by immunostaining, but negative by FISH [10]. This rare discrepancy may arise if the HER2 gene is not amplified in cells that overexpress HER2 protein [11, 12], and has been attributed to transcriptional or post-transcriptional deregulation [2].

The FDA has recently approved a new IHC kit for selection of patients for trastuzumab (Herceptin) therapy. This kit, incorporating the A465 MAb (Dako), uses an arbitrary classification system to grade the degree of membrane staining from 0 to 3+. Evaluation of the kit has identified an increased sensitivity compared with antibodies used traditionally for HER2 analysis [13, 14]. Two kits for measurement of HER2 by FISH have also been approved, although interestingly for two different clinical applications — prognosis and prediction of response to therapy. The increasing use of standardized methodologies for evaluation of HER2 status is likely to generate reliable data and help to elucidate the role of HER2 in tumor progression.
Frequency of HER2 overexpression in different tumors

Evaluation of a large series of tumor types by a single pathologist using standardized IHC methodology, demonstrated a wide variation in the HER2 overexpression rates of different tumor types (Table 1). Interestingly, the highest expression rate was seen in Wilm's tumor (51%), which had not been tested previously. Bladder cancer also had a high frequency of overexpression (44%); this result was similar to that obtained using the standardized methodology in a different center (41%) and corresponded with the middle of the range in the published literature. This correspondence was also true for the overexpression rate in breast cancer (25%). The determined frequency of HER2 overexpression in pancreatic cancer differed in the two data series, indicating variation in the face of standardized methodology. Interestingly, at both centers the reported frequency for overexpression was below the rates indicated in the published literature. This trend was observed with the other tumors examined; the results obtained with the standardized IHC were below or at the lower end of the range reported in the literature. However, frequencies in some tumor types were greater than would be predicted by gene-amplification data [15-17]; this may indicate overexpression due to gene deregulation, rather than gene amplification.

HER2 status assessment by standardized IHC is currently being used to determine patient eligibility for treatment with trastuzumab, and results obtained from a pivotal clinical trial have indicated that patients with tumors scoring 3+ probably respond best to trastuzumab compared with lower scores [18, 19]. The frequency of HER2 overexpression (3+) in a variety of tumor types is shown in Table 2. The highest frequency of HER2 overexpression (3+) was demonstrated in inflammatory breast cancer (50%) with a frequency in other breast cancers of 18%. Other tumors examined had HER2 overexpression (3+) frequencies below 10%. Inflammatory breast cancer therefore represents an ideal target for trastuzumab therapy, as it combines a high frequency of HER2 overexpression with a local concentration of lymphocytes to mediate antibody-dependent cytotoxic events at tumor sites.

HER2 status of infiltrating ductal carcinomas

The biologic and pathologic features of breast carcinomas have been studied extensively in order to identify markers that might accurately predict clinical outcome of breast cancer patients [20]. When considered independently many factors, including HER2 overexpression [21], and p53 alteration [22], appear to yield relevant information about disease progression and/or response to therapy. However, many prognostic parameters identified in univariate analyses lose prognostic significance upon multivariate analysis, indicating their association with other factors with a greater prognostic power. Associations of HER2 overexpression with p53 alterations [23], grading and lymphoid infiltration [24] have previously been demonstrated in breast cancer. In order to determine whether HER2-positive carcinomas represent a particular subset of infiltrating ductal carcinomas, we studied two large, retrospective series of consecutive breast carcinoma patients. In addition to the two-by-two analysis, we conducted a multiple correspondence analysis (MCA), as preliminary studies have indicated a role for this analysis in the evaluation of multiple prognostic factors in breast cancer [25]. Data on 10 clinical and pathologic parameters and 12 biologic parameters were obtained from a total of 2000 patients with primary breast carcinoma. The MCA enabled graphic examination of associations between the categories and revealed two distinct clusters (Figure 1). HER2 overexpression was closely associated with other poor prognostic indicators including tumor grade III, the presence of necrosis and lymphocyte infiltration at the tumor site, a high number of mitoses, p53 positivity, bcl-2 negativity, hormone receptor negativity, and ductal histotype. There was no, or only a very weak, association between HER2 overexpression and tumor size, vascularity, presence of laminin receptors and the proteolytic enzyme cathepsin D, and overexpression of phosphatases.
Table 3. Phenotypic characteristics of two distinct subsets of breast carcinoma.

<table>
<thead>
<tr>
<th>Phenotype A (HER2 negative)</th>
<th>Phenotype B (HER2 positive)</th>
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<tbody>
<tr>
<td>Low grade</td>
<td>High grade</td>
</tr>
<tr>
<td>Few mitoses</td>
<td>Many mitoses</td>
</tr>
<tr>
<td>No necrosis</td>
<td>Necrosis</td>
</tr>
<tr>
<td>No lymphoid infiltration</td>
<td>Lymphoid infiltration</td>
</tr>
<tr>
<td>p53 negative</td>
<td>p53 positive</td>
</tr>
<tr>
<td>Progesterone-receptor positive</td>
<td>Progesterone-receptor negative</td>
</tr>
<tr>
<td>Bcl-2 positive</td>
<td>Bcl-2 negative</td>
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Each parameter was assigned a numerical value (weight) on the basis of its position relative to HER2 overexpression. Each tumor in the series was then classified according to the sum of the weights attributed to each parameter. The distribution of the tumors according to this index was bimodal (Figure 2). These data were compatible with the existence of two phenotypes associated with HER2 negativity and positivity, and designated phenotype A (HER2 negative) and phenotype B (HER2 positive), respectively (Table 3). Phenotype A was observed more frequently in patients presenting at 60 years or greater, whereas phenotype B was a frequent finding in young patients. The survival data indicate improved short-term survival with phenotype A compared with phenotype B (Figure 3), although extrapolation of the curves to 22 years' survival suggests that this advantage is not maintained. This trend is also seen with relapse following surgery (Figure 4); there is a relatively steady rate of relapse from two to 10 years with phenotype A, whereas phenotype B is associated with a peak incidence of relapse at two to five years and little probability of recurrence thereafter. There are therefore two subtypes of breast cancer, and the subtype associated with HER2 overexpression has a poor prognosis.

HER2 status in atypical ductal hyperplasia, ductal carcinoma in situ and invasive ductal carcinoma

A consistent observation has been the absence of HER2 overexpression in atypical ductal hyperplasia (ADH) [26], a monoclonal lesion that can effectively be considered to be a precursor for infiltrating ductal carcino-
removed from 200 pre-menopausal women were ana-
nalyses in hormone levels on HER2 expression, we eval-
uated HER2 levels during the menstrual cycle. Tumors
were analyzed and classified according to the time point in the
monthly menstrual cycle at which surgery was performed. In
hormone receptor-negative tumors, the frequency of
HER2 overexpression increased slightly during the pre-
menstrual phase, but did not reach statistical significance.
In hormone receptor-positive tumors, the frequency of
HER2 overexpression was significantly reduced in tu-
mors obtained during the luteal period, compared with
those obtained during the follicular phase (7% vs. 25%;
P = 0.05). These data would suggest that the HER2
classification of a tumor may vary according to the time
of surgery relative to the menstrual cycle. In order to
investigate this hypothesis further, we compared the
HER2 status of biopsies and surgical samples removed
from the same patient during follicular and luteal phases
of the menstrual cycle. In many cases, a negative HER2
status was demonstrated in both samples, but three of
the five patients with HER2-positive biopsy samples
obtained during the follicular phase had negative luteal
samples. These preliminary data require confirmation
but should be considered when making therapeutic
decisions. Furthermore, such data predict that hormone
levels in patients could be modified in order to increase
HER2 expression and optimize the use of targeted anti-
HER2 therapies, such as trastuzumab.

Discussion and conclusions

The emerging data on HER2 indicate that the available
assays for measurement of HER2 expression require
careful standardization in order to ensure the reliable
classification of HER2 status. Dependable assessment
of HER2 status in a range of different tumor types will,
hopefully, provide additional information about its role
as a prognostic indicator. Furthermore, accurate assess-
ment of HER2 status will enable more confident deci-
sions regarding treatment options, particularly those
pertaining to the use of therapies targeting the HER2
protein, such as trastuzumab.

HER2 is overexpressed in a wide range of tumors in
addition to breast carcinomas, albeit most commonly at
a lower frequency than breast carcinomas. The inci-
dence of HER2 overexpression in Wilms' tumor, how-
ever, was demonstrated to be 51%, over twice that
observed in breast cancer. A high incidence of HER2
overexpression was also reported in bladder cancer.
Bladder cancer overexpresses HER1 frequently, and
therefore has a great potential for the formation of
HER1–HER2 heterodimers. It will be interesting to see
whether such 'double-positive' tumors respond well to
trastuzumab therapy, or whether the major signaling
event is mediated through HER1 homodimers, with a
consequent poor response to trastuzumab.

Finally, although HER2 overexpression has been
demonstrated to be relatively stable, preliminary data
demonstrate some fluctuations according to the men-
strual status of the patient, particularly in cases where
the tumor co-expressed the hormone receptor. This
observation suggests that hormonal modification in a

Stability of HER2 expression

The stability of HER2 expression is an important con-
ideration, as therapy decisions may be based on the
results of HER2 analyses performed on samples ob-
tained at an earlier time point. Our results concur
with those of other centers and show that HER2 positivity is
relatively stable, with few differences between different
lesions from the same patient. Indeed, analysis of HER2
data for the primary tumor and relapse in 49 cases
revealed a 95% concordance, whereas there is no associ-
ation between HER2 status of primary and contralateral
tumors.

The promoter of the HER2 gene is hormone de-
pendent, and in vitro experiments have shown that HER2
expression in cell lines expressing the hormone receptor
can be modulated by addition of exogeneous estrogen
[32]. In order to examine the effect of physiologic fluctu-
atations in hormone levels on HER2 expression, we eval-
uated HER2 levels during the menstrual cycle. Tumors
removed from 200 pre-menopausal women were ana-
alyzed and classified according to the time point in the

Discussion and conclusions

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strual status of the patient, particularly in cases where
the tumor co-expressed the hormone receptor. This
observation suggests that hormonal modification in a
patient may be used to increase tumor HER2 expression,
and thus potentiate the therapeutic effect of HER2-
targeting therapies, such as trastuzumab.

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Correspondence to.
S. Ménard, MD
Molecular Targeting Unit
Department of Experimental Oncology
Istituto Nazionale Tumori
Via Venezian 1
20133 Milan
Italy
E-mail: menard@istitutotumori.mi.it