Original Article

HER-2 Tissue Expression Correlated with Serum Levels in Breast Cancer Patients

(breast cancer / HER-2 / extracellular domain)

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Abstract. We explored the relationship between circulating HER-2 extracellular domain and tissue HER-2 status in a group of 42 postmenopausal breast cancer patients. All patients were examined before adjuvant chemotherapy or other adjuvant treatment. Serum levels were measured by BAYER Advia Centaur System, Golden, CO (the cut-off level was in our conditions considered at 12 ng/ml). Tissue expression was assayed with the DAKO HercepTest, North America, Inc, Carpinteria, CA. Our findings that serum levels are in consonance with tissue expression could be important in metastatic breast cancer, when it is impossible to get a new tumour sample and establish the actual HER-2 status, which may be different from the primary tumour. Although we know that serum HER-2 concentration cannot be substituted for IHC or FISH, we have observed a statistically significant correlation between serum level concentration and tissue HER-2 status.

Introduction

HER-2 gene (c-*erbB-2*) amplification is (with age, stage of disease, tumour grade and expression of hormonal receptors) one of the most important prognostic and predictive factors in breast cancer patients. Its increased amplification is a sign of rather poor prognosis and worse response to some cytostatics or hormonal treatment; on the other hand, it presents a target structure for specific biological treatment with trastuzumab. The benefit of this particular therapy has been sufficiently well confirmed in metastatic breast cancer (Cobleigh et al., 1999; Harries and Smith, 2002; Vogel et al., 2002); there is also evidence of significantly improved therapeutical results in adjuvant setting as shown in the published results of studies NSABP B31, NCCTG 9831, BCIRG 006, HERA (Baselga et al., 2006).

HER-2 biology

The *HER-2* gene was discovered in 1981 in cancerogen-induced rat tumour (Hynes and Stern, 1994). It is expressed in small quantities on the plasma membrane of normal human cells, its size being 185 000 Da.

HER-2 carries information for the transmembrane tyrosine kinase receptor classifiable as a member of the family of other epidermal growth factor receptors (EGFR family). Unlike other receptors of this group, there is no activation after the binding of a growth-stimulating ligand, an event which in other HER receptors triggers heterodimerization and a cascade of signals resulting in the gene's activation (Kraus et al., 1989; Plowman et al., 1993).

In terms of the binding characteristics of their receptors, epidermal growth factors come in four subgroups – HER-1, HER-2, HER-3 a HER-4. The ligands for HER-1, HER-3 and HER-4 are known: transforming growth factor-α, amphiregulin, β-cellulin, heregulin and HER-1 itself (Slamon et al., 2001). Often heterodimerized along with other HER receptors, HER-2 has an increased affinity for diverse ligands without them directly. There is scope for transactivation between two receptors, e.g., heterodimerization with other members of the HER group, or homodimerization of two HER-2 molecules (Gebhardt et al., 1998).

The family of HER receptors is shown in Fig. 1.

HER-2 amplification leads to increased tumorigenicity, invasiveness, increased metastatic potential, and altered susceptibility to hormonal and cytostatic drugs.

Increased expression is seen in approximately 30% of invasive breast cancer, with the gene amplified in 95% of that (Drebin et al., 1998). Overexpression may be as-

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Abbreviations: ECD – extracellular structures of the HER-2 domain, EGFR – epidermal growth factor receptor, ER – oestrogen receptor, FISH – fluorescence *in situ* hybridization, IHC – immunohistochemical methods, PR – progesterone receptor.

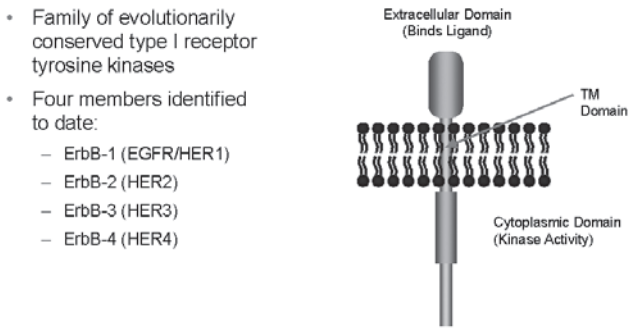

Fig. 1. ErbB Family of Tyrosine Kinase Receptors.

sociated with alternative mRNA linking and, subsequently, with changes in the intra- and extracellular structures of the HER-2 domain (ECD). The actual function of the extracellular domain has yet to be amply elucidated; it may well stimulate cell motility and proliferation in interaction with some proteins or in connection with other EGFRs.

HER-2 overexpression is a tell-tale sign of poor prognosis, as a rule associated with other negative prognostic signs – negativity of oestrogen and progesterone receptors, high S-phase fraction, axillary lymph node positivity, p53 mutation, and high nuclear grade.

Quite a few studies have been published showing reduced breast cancer cell susceptibility to anthracyclines and tamoxifen (Cobleigh et al., 1999; Harries and Smith, 2002; Mazouni et al., 2007); however, the problem has yet to be fully addressed.

The synthesis of trastuzumab, a humanized antibody against HER-2, is a turning point in the treatment of breast cancer as a generalized pathology and, to judge by the latest clinical studies, in adjuvant application. Adjuvant use can bring improvement of as much as 50% (Baselga et al., 2006). Its therapeutic exploitation is an example of target-specific biological treatment.

HER-2 testing

As part of histological diagnostics, HER-2 is routinely studied in sections of paraffin blocks of tissue using immunohistochemical methods (IHC). Rated as standard IHC approach is the DAKO HercepTest, North America, Inc, Carpinteria, CA, which assays the gene expression semi-quantitatively by means of a specific rabbit serum antibody. The intensity of staining and the stained cells count are compared with a standard scale and rated as 0, 1+, 2+, and 3+. Data interpretation may be influenced by the investigator's subjective view. Other IHC tests rate the expression intensity in per cent as poor, moderate and strong – with the risk of even greater subjective error. The value 3+ is regarded as positive for launching treatment with the specific antibody. Prior to the start of therapy, positivity is usually determined with the fluorescence *in situ* hybridization (FISH) method, especially where the IHC value is 2+, standing for not quite certain positivity.

Measuring *HER-2* gene amplification in the tissue, the FISH method usually makes use of formalin-fixed paraffin blocks. In either of the two technologies – Vysis FISH or Oncor FISH – the tests are regarded as highly sensitive and reliable. However, opinion still differs as to which method of testing – FISH or IHC – has a greater prognostic and predictive validity (Slamon et al., 1997).

Determination of the circulating extracellular domains of the receptor or their part in the serum is another line of HER-2 testing. This "soluble" receptor can be assayed with the ELISA method. Increased values of soluble HER-2 are associated with its overexpression. The ECD can bind anti-HER-2 antibodies, thereby reducing the therapeutic effect. Some authors (Carney et al., 2004; Schippinger et al., 2004) suggest a connection of its level with the number of cancer cells in the patient's body. Others (Hayes et al., 2001; El-Sawy et al., 2002; Fornier et al., 2005; Olsen et al., 2007) study its serum concentrations throughout the cure, correlating them with the therapeutic effect.

Our study was designed to ascertain the rate of correlation between immunohistochemically determined HER-2 tissue expression and the pre-treatment level of circulating extracellular domain in the serum of postmenopausal women with breast cancer.

Material and Methods

In the group of 42 postmenopausal women with early stage breast cancer were patients planned for adjuvant chemotherapy because of the presence of the risk factors, mainly women with positive axillary lymph nodes, high tumour grade, or with negative oestrogen (ER) and/or progesterone (PR) receptors. All of them were in stage I (N = 15) or stage II (N = 27) as it is shown in Table 1, mean age 58 years (48–76 years). Serum was collected as part of the staging tests within 2.5 to 4 weeks after the surgery, the average time being 3 weeks from the surgery, before chemotherapy was started. Tissue expression was assayed with the DAKO Company Herceptest and the intensity of staining was compared with the standard. To determine serum HER-2 levels we used fully automated, two-side sandwich immunoassay using direct chemiluminescent technology, the BAYER Advia Centaur System, Golden, CO. Tissue expression of ER and PR was assayed immunohistochemically with the DAKO murine antihuman antibody CLONE 1D5 and CLONE PgR 636. Positivity of both receptors was observed in 21 patients, negativity of both in 16 patients. The cut-off for positivity of ER and PR was 10%. All patients underwent chemotherapy with AC or AC-T regimen and in case of positivity of hormonal receptors sequentially hormonal treatment with tamoxifen or aromatase inhibitor.

Table 1. Distribution of ER and PR in relation to TNM (tumour, noduli, metastases)

TNM	Number of patients	ER+,PR+	ER+,PR-	ER-,PR+	ER-,PR-
T1,N0,M0	8	0	0	0	8
T1,N1,M0	14	11	0	0	3
T2,N0,M0	7	0	1	4	2
T2,N1,M0	10	8	0	0	2
T3,N0,M0	1	1	0	0	0
T3,N1,M0	2	1	0	0	1

Characteristics of the study group are shown in Table 1.

For statistical evaluations we used χ^2 test for 6 or 4 degrees of freedom, Spearman's non parametric test and Student's t-test.

Results

The expression of oestrogen and progesterone receptors correlated to the expression of HER-2 IHC-tested in tumour tissue is shown Table 2. It follows that 50% of the HER-2-positive patients also had negative ER and PR status. Statistical significance is high ($P < 0.01$)

A low degree of tumour differentiation (G) was observed as another risk factor associated with HER-2-positive tumours. HER-2 expression in correlation with tumour cell grade is summed up in Table 3. Our cohort is too small to confirm this ($P > 0.1$); however, one half of the 20 patients with HER-2 +++ had a low degree of grade – G3.

The values of HER-2 tissue expression and the soluble receptor quantity in ng/ml are summed up in Table 4.

As a rule, the value for serum HER-2 positivity, measured by ELISA using the Oncogene Science microtitre plate HER-2/neu assay, is adjusted to 15 ng/ml. In our case using the chemiluminescence method, no precise value was given; it was advisable to estimate it on the basis of the tests performed individually in each kit.

Table 2. HER-2 tissue expression related to ER, PR expression

HER-2 value	ER+,PR+	ER+,PR–	ER-PR+	ER-,PR–
0/+	2	1	0	1
++	15	0	1	2
+++	4	0	3	13

Table 3. HER-2 tissue expression related to cancer cell grade

HER-2 value	G1	G2	G3
0/+	2	1	1
++	5	9	4
+++	3	7	10

Regarding the results obtained, we took 12 ng/ml as that particular value. With respect to the recommended cut-off limit of 15 ng/ml, eight women of our cohort could be rated as positive, i.e., 40% of the HER-2 3+ patients. Given a value of 12 ng/ml there was a 100% congruence between the two modes of testing in our cohort. The levels of the soluble receptor found in HER-2 1+ a 2+ patients never reached such high values, since they were adequately lower as follows from Table 4. The lower level seen in our group may well have been due to the surgical removal of the tumour and to the gradually subsiding level of circulating ECD HER-2. Strong statistically significant difference was found between the HER-2-negative (0/+) group and HER-2-positive (+++) group calculated by Student's t-test, $P < 0.003$. Also the diference between HER-2-uncertain (++) and HER-2-positive (+++) was statistically significant – $P < 0.01$.

Discussion

Many authors have found an ELISA-detected correlation between the values of the circulating extracellular domain and IHC- and FISH-established tissue levels (Carney et al., 2004; Schippinger et al., 2004; Perez, 2006). Moreover, the assay of HER-2 expression in tissues is a one-off test over a specific length of time. Monitoring for ECD serum levels is a method easy to repeat and fit to keep measuring the value throughout the period of monitoring and provide a real-time assessment of the HER-2 status. Enzyme-linked immunosorbent assay methods used to measure the circulating HER-2 have shown the prevalence of elevated ECD levels to be approximately 18.1% in women with primary breast cancer and approximately 45.6% in women with metastatic breast cancer (Hayes et al., 2001). High concentrations

Table 4. Results – tissue expression and serum levels of HER-2

Tissue HER-2 value	Number of patients	HER2 (ng/ml) Serum levels
0/+	4	7,7–9,8
++	18	10,0–11,9
+++	20	12,0–26,8

of ECD HER-2 are associated with higher cancer aggressiveness and predict response to tratuzumab and anti-oestrogen therapies in advanced breast cancer.

According to other authors, even patients whose primary tests for tissue HER-2 and for serum levels of HER-2 came out negative, were found to have positive values on relapse of breast cancer (El-Sawy et al., 2002; Olsen et al., 2007).

Following up the levels of circulating extracellular domain throughout the course of the disease helped us find out that a clinically manifest relapse of the disease may be preceded by ECD release into the serum. This provides important information for managing the therapy of patients with metastatic breast cancer. Some authors consider ECD elevation as an early sign of cancer progression (Fornier et al., 2005).

Studying the dynamics of ECD serum values in the course of chemobiotherapy with trastuzumab, other authors reached the conclusion that subsiding ECD serum levels after the initial 2–3 cycles were a good response to treatment. In this way, it is possible to estimate the prospective effect very soon after the start of therapy. ECD values in excess of 15 ng/ml were found to have a similar predictive value. At levels lower than that, the therapeutic response was usually rather poor (Fornier et al., 2005; Mazouni at al., 2007).

In other studies the ECD level inversely correlated with the response to chemotherapy treatment, indicating that patients with elevated levels of ECD have a reduced susceptibility. With regard to the effect of serum HER-2 elevations on the response to hormone therapy the response rate was twice as high in patients with normal serum levels than in patients with elevated ECD (Hait, 2001; Salvadori et al., 2005). Despite this predictive value of ECD estimation, the prognostic significance was not fully confirmed.

Almost all of these studies evaluated blood markers in patients with advanced disease. Just a few (Isola et al., 1994; Kong et al., 2006) have reported influence of surgery in early breast cancer patient on serum levels of HER-2. Authors have reported results of 40 patients undergoing operation without any preoperative treatment. They compared blood samples before surgery, day 1, day 3 and day 5 after operation. They adopted manufacturer cut-off of 15 ng/ml. The baseline pre-surgical concentrations were significantly decreased in post-surgical samples in most of the patients. The most rapid decrease was measured on the first day and was followed with slight decrease on the following days. These findings can support our observation of lower levels of ECD HER-2 also in HER-2 IHC tissue-positive patients. We were not able to compare our findings with preoperative serum levels of HER-2.

The presence of ECD HER-2 in the circulation raises several possible problems:

First is the influence of ECD to binding trastuzmab and possible blocking its function. Some studies showed a lower effect of trastuzumab in patients with elevated ECD (Salvadori et al., 2005).

Second is a question of the release of ECD HER-2 into the circulation immediately after chemotherapy administration. Could it be used as a marker of early response to chemotherapy? If ECD is released into the blood after tumour destruction, an early rise can indicate a responding patient, and failure to see an increase might predict patients who would derive little benefit from the chemotherapy used.

These questions are open for future investigation.

The aim of this study was to determine whether tissue expression of HER-2 and its serum levels are in concordance or not. We have found a correlation between serum ECD levels and IHC-assayed values of tissue HER-2. While this method can hardly replace the well-tried IHC and FISH testing, it may have quite a role to play in predicting the patients' response to trastuzumab and, possibly, also in early detection of relapses.

As for metastatic cancer, the method could be useful to ascertain the actual state of HER-2 expression, and, in some cases when no tissue is available for the testing, it can help facilitate the choice of adequate therapy.

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