

The epidermal growth factor receptor family in breast cancer

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Abstract: The human epidermal growth factor receptor (HER) family comprises four homologous members: EGFR, HER-2, HER-3, and HER-4. The activation of these receptors triggers a complex series of signal transduction pathways which affect pivotal tumorigenic processes. The deregulation of HER signaling is seen in several human malignancies. HER-2 is now recognized as a key oncogene in breast cancer pathogenesis. Assessment of HER-2 status is of central importance in the prognosis of breast cancer patients. In the light of clinical data suggesting that HER-2 can also be useful as a predictive marker both for trastuzumab and chemotherapy, standardized determination of the HER-2 status in tumors has become more important. Moreover, current data provide evidence for the significance of HER-3 and HER-4 alterations in breast carcinogenesis. Because of the complex interactions among the HER receptors, it is likely that the effect on cell proliferation and tumor growth depends on receptor trans-signaling and thus, the evaluation of the combined expression pattern of all family members is of particular interest. This review presents the current evidence highlighting the role of the family as a whole panel and an update on the role of HER-3 and HER-4 receptors in breast cancer. Moreover, we provide updated data regarding the prognostic value of HER family members giving emphasis to novel methods for the determination of their status, such as real-time polymerase chain reaction. In addition, we review recent therapeutic approaches aimed at targeting the HER family in breast cancer patients.

Keywords: epidermal growth factor, receptor, breast cancer

Introduction

The human epidermal growth factor receptor (HER) family consists of four homologous members: ErbB-1 (epidermal growth factor [EGF] receptor [EGFR] or HER-1), ErbB-2 (HER-2) for which no ligand has been described so far, ErbB-3 (HER-3), which is characterized by its impaired kinase activity, and ErbB-4 (HER-4). All family members are transmembrane glycoproteins consisting of an extracellular ligand-binding domain, a hydrophobic transmembrane region, and a cytoplasmic section containing the tyrosine kinase domain and a carboxy-terminal region with tyrosine autophosphorylation sites. Despite their structural homology, HER receptors differ in their ligand specificities. Two main ligand classes have been recognized so far: the splice variants of neuregulins (NRGs) which bind exclusively to HER-3 and/or HER-4 and different EGF-related proteins (Harris et al 2003). Binding of specific ligands to the extracellular domain allows for receptor homo- or heterodimerisation through conformational changes resulting in activation of the cytoplasmic catalytic function, which leads to receptor autophosphorylation on tyrosine residues. This autophosphorylation triggers a complex series of signal transduction pathways such as phosphatidylinositol 3-kinase (PI3-K)-Akt, Ras-Raf-MEK-mitogen-activated protein kinase (MAPK)-dependent pathway, phospholipase C-protein kinase

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C (PLC–PKC), and janus kinase/signal transducer and activator of transcription (JAK/STAT). These pathways affect essential tumorigenic processes such as proliferation, differentiation, migration, inhibition of apoptosis, and enhanced survival (Mosesson and Yarden 2004; Krause and Van Etten 2005). Signaling diversity depends not only on the presence of specific receptors, but also on the characteristics of individual ligands. The HER family is characterized by a functional interdependency among its members, in terms of activity (Figure 1).

HER-2 and breast cancer

There is an extensive literature on the role of the HER family in breast cancer (Gullick and Srinivasan 1998) and particularly that of HER-2 which is considered a key oncogene in breast carcinogenesis. The extracellular domain of HER-2 is unique in that it is locked constitutively in a conformation resembling the ligand-bound states of the extracellular regions of the other HER receptors. As a ligand orphan receptor, HER-2 preferentially forms heterodimers with other family members. HER-2 is known to be the preferred heterodimerisation partner for EGFR, HER-3, and HER-4 (Graus-Porta et al 1997) and plays an important role in triggering signal transduction pathways. Moreover, heterodimers containing HER-2 are more mitogenic than others (Citri et al 2003). The transforming functions of HER-2 and its fundamental role in breast cancer pathogenesis are now well established (Moasser 2007; Ursini-Siegel et al 2007). In the majority of cases, HER-2 overexpression is a consequence of amplification at the DNA level.

Overexpression or amplification of HER-2 occurs in 15% to 30% of breast carcinomas and is considered to confer a more aggressive biology and an unfavorable impact on the course of the disease (Slamon et al 1987, 1989; Rilke et al 1991; Ross and Flether 1998). HER-2 overexpression is associated with estrogen receptor (ER) and progesterone receptor (PR) negativity, high histological grade, high rates of cell proliferation and lymph node involvement (Rilke et al 1991; Gusterson et al 1992; Lebeau et al 2003). Moreover, it is correlated with disease aggressiveness, increased rates of recurrence and poorer survival in node-positive breast cancer patients, whereas the prognostic significance in patients with node-negative tumors remains somewhat controversial (Borg et al 1990; Winstanley et al 1991; Paterson et al 1991; Clark and McGuire 1991; Toikkanen et al 1992; Marsigliante et al 1993; Hartmann et al 1994; Quenel et al 1995; Mitchell and Press 1999).

HER-2 overexpression is also regarded as a predictive marker for reduced responsiveness to tamoxifen therapy (Tovey et al 2005; Kirkegaard et al 2007), although this is still an unresolved issue. The predictive value of HER-2 expression regarding response to chemotherapy is also still controversial, although numerous trials have supported an interaction between HER-2 expression and chemotherapy activity (Muss et al 1994; Mass 2000; Petit et al 2001; Zhang et al 2003; Moliterni et al 2003). It has been suggested that HER-2 overexpression or amplification in breast cancer predicts greater sensitivity to anthracycline-containing chemotherapy (De Placido et al 1995; Paik et al 1998, 2000; Ravdin et al 1998; Di Leo et al 1999, 2001, 2002; De Laurentiis et al 2001; Moliterni et al 2003; Pritchard et al 2006; Gennari et al 2008) and resistance to CMF regimen (TLBC 1988, 1989; Mansour et al 1989; Allred et al 1992; Gusterson et al 1992). HER-2 may also identify patients who are likely to benefit from higher doses of adjuvant chemotherapy (Wood et al 1994; Thor et al 1998; Arnould et al 2003; Bonnetterre et al 2003; Rodenhuis et al 2003; Del Mastro et al 2004; Dressler et al 2005). The association with response to taxane-based chemotherapy is unclear, as results have been conflicting (Konecny et al 2004; Gonzalez-Angulo et al 2004; Kostopoulos et al 2006; Hayes et al 2007).

HER-2 status determination

Assessment of HER-2 status is of crucial importance in the management of patients with breast cancer. In view of the clinical data suggesting that HER-2 can be useful as a predictive marker both for trastuzumab and chemotherapy, standardized determination of HER-2 status in tumors has become more important. However, while the clinical benefit of assessing HER-2 status in breast carcinomas is now accepted, there is no consensus on the ideal diagnostic method to use for this purpose. HER-2 can be analyzed at the DNA-, the mRNA- or the protein level. Various techniques are available, each with benefits and disadvantages (Dowsett et al 2000).

For practical reasons, immunohistochemistry (IHC) using an anti-HER-2 antibody is currently the method of choice for HER-2 testing. IHC is a rapid, simple and convenient technique, readily available as a standard method in a routine clinical service laboratory. Moreover, IHC is a relatively inexpensive assay which can be easily used on archival formalin-fixed paraffin-embedded (FFPE) tissues. Consequently, the majority of reports published on the clinical significance of HER-2 expression have used IHC to determine HER-2 status. However, the major drawbacks of IHC

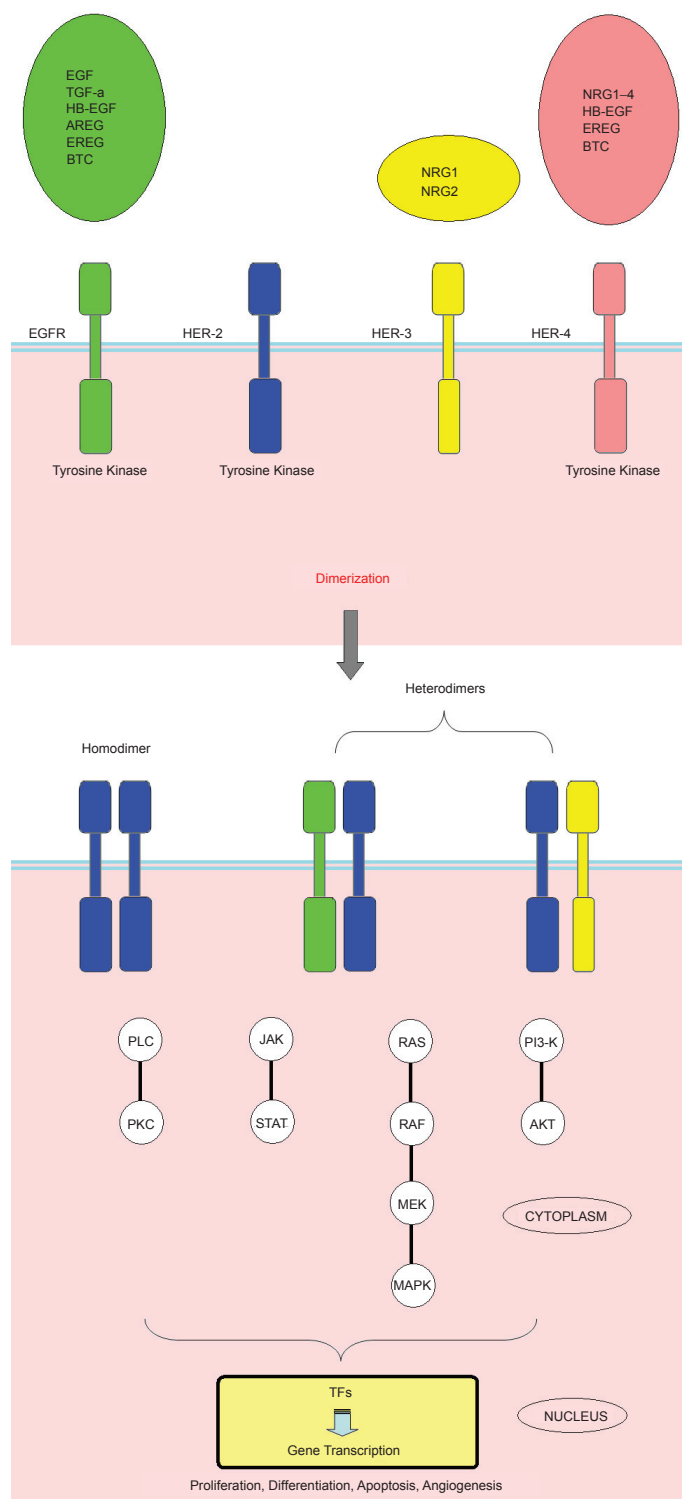


Figure 1 HER receptors and their ligands. Despite their structural homology, HER receptors differ in their ligand specificities. Some of these ligands bind exclusively to EGFR, such as EGF, TGF- α , and AREG, or bind exclusively to HER-4, such as NRG3 and NRG4. Others have a dual specificity. So far, no ligand has been described for HER-2, whereas HER-3 is characterized by impaired kinase activity. HER receptors achieve activation by forming ligand-bound homo-and/or heterodimeric receptor complexes. Ten possible dimers can be formed (Only a few examples of dimers are presented here). HER-2 is known to be the preferred heterodimerisation partner for EGFR, HER-3 and HER-4. The HER-2/HER-3 heterodimer constitutes the most mitogenic dimer in the family. The HER complexes signal from the cell surface to the nucleus through numerous downstream pathways such as phosphatidylinositol 3-kinase (PI3-K)-Akt, Ras-Raf-MEK-MAPK-dependent pathway, PLC-PKC, and JAK/STAT. These signaling cascades eventually transmit their signal to TFs, which affect the transcription of target genes, regulating critical tumorigenic processes including proliferation, differentiation, apoptosis, angiogenesis, and migration.

Abbreviations: EGF, epidermal growth factor; TGF- α , transforming growth factor- α ; HB-EGF, heparin-binding EGF-like ligand; AREG, amphiregulin; EREG, Epregrulin; BTC, Betacellulin; NRG, neuregulin; PI3-K, phosphatidylinositol 3-kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/extracellular signal-regulated kinase (Erk) kinase; PLC, phospholipase C; PKC, protein kinase C; JAK, janus kinase; STAT, signal transducer and activator of transcription; TFs, transcription factors.

are that the results are not quantitative, whereas substantial inter-observer variations have been reported (Thomson et al 2001). The interpretation of IHC results is subjective and prone to inter-observer variability, requiring experienced pathologists. In addition, the interpretation of the findings is considerably influenced by several technical factors, such as the use of antibodies with variable sensitivities and specificities, and different fixative protocols or staining procedures (Press et al 1994). While this discrepancy is improved by the use of standardized IHC tests (such as the HercepTest), it is generally recommended that (2+) HER-2 immunostaining requires further validation by fluorescence in situ hybridization (FISH) analysis (Bartlett et al 2003; Dowsett et al 2003; Ellis et al 2004).

FISH is a reliable, sensitive and highly specific technique for assessing HER-2 gene amplification (Kjeldsen et al 2002), a change that appears to be correlated with strong protein expression (Jacobs et al 1999). In contrast to IHC, FISH can give a more objective and reproducible estimation of HER-2 status. The result is quantitative, as it not only determines whether amplification is present, but also the degree of amplification. However, the technique is expensive and time consuming to perform. Moreover, FISH requires specialized expertise and a fluorescence microscopy facility and thus, it is currently available only in a minority of pathology laboratories. FISH is now being challenged by the chromogenic in situ hybridization (CISH) technique. CISH is similar to FISH, except that it uses a peroxidase reaction instead of a fluorescent dye, which allows evaluation in an ordinary light microscope (Isola et al 2004; Laakso et al 2006).

Despite efforts to standardise these methods, considerable intra-laboratory and inter-laboratory variability of the results still exist. A number of studies indicate that approximately 20% of HER-2 assays performed at the treatment site's pathology department are incorrect when the same sample is reassessed in a high-volume central laboratory (Paik et al 2002; Roche et al 2002; Perez et al 2006). Therefore, improvement in reproducibility of the results between different laboratories is a high priority (Di Leo 2007; Wolff et al 2007).

Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) has been recently suggested as an alternative technique for detection and quantification of HER-2 status. RT-PCR produces quantitative and reproducible results. Moreover, it can be easily standardized, reduces inter-observer variability, and does not require experienced pathologists for interpretation. However, a disadvantage of this technique is the specific requirements

for handling of tissue specimens to preserve the integrity of RNA (Masuda et al 1999).

HER-2 analyses using real-time PCR

A number of studies have used quantitative real-time PCR for the assessment of HER-2 status (Bieche et al 1999; O'Malley et al 2001; Kim et al 2002; Mrhalova et al 2003; Königshoff et al 2003; Ginestier et al 2004; Schlemmer et al 2004; Suo et al 2004; Gjerdrum et al 2004; Vanden Bempt et al 2005; Esteva et al 2005; Benöhr et al 2005; Bossard et al 2005; Tse et al 2005; Vinatzer et al 2005; Kulka et al 2006; Ntoulia et al 2006; Labuhn et al 2006). These studies investigated the extent of concordance of IHC, FISH (or CISH), quantitative PCR, and in some cases quantitative RT-PCR. In the majority of them, a substantial degree of agreement among different methods has been demonstrated, with respect to HER-2 status determination.

A limited number of studies have evaluated the prognostic power of HER-2 using quantitative real-time PCR. In a retrospective study, which analyzed the expression of HER-2 by real-time RT-PCR and IHC in 131 breast carcinomas, HER-2 positive patients as determined by RT-PCR had worse outcome than the HER-2 negative group. This was evident in all cases as well as in the node-positive group (Potemski et al 2006). Vinatzer and colleagues (2005) assessed HER-2 status at the DNA, mRNA, and protein levels with IHC, FISH and quantitative real-time RT-PCR in 136 tumor samples from 85 breast cancer patients. HER-2 overexpression, as determined by quantitative RT-PCR, positively correlated with high tumor grade, positive lymph node status, ER and PR negativity, consistent with published IHC results. Regarding the prognostic significance of HER-2 status, all methods showed a significant correlation of HER-2 with disease-free survival (DFS) and overall survival (OS), except FISH alone. The authors concluded that quantitative RT-PCR seems to be clinically as useful in the assessment of HER-2 status as the current standard methods, yielding comparable prognostic information. Bergqvist and colleagues (2007) used quantitative real-time PCR (Q-PCR) and RNA expression profiles (RNA-EPs) to evaluate HER-2 status in relation to clinical outcome of breast cancer patients. The authors compared these techniques with IHC supplemented with FISH or CISH. Analyses of relapse-free survival (RFS) and OS on the basis of 5 and 10 years of follow-up indicated equivalent hazard ratios for all three methods. In contrast to IHC/CISH, both Q-PCR and RNA-EP analyses of HER-2 also yielded significant results regarding RFS and breast cancer-corrected survival after 10 years of follow-up. The results of this

study suggested that both the Q-PCR and RNA-EP assays are associated with high-quality HER-2 status determinations, and are of similar, or even superior, prognostic value compared with the current standard techniques.

EGFR and breast cancer

EGFR is overexpressed in several human tumors and is considered to initiate a variety of important steps during the malignant transformation (Nicholson et al 2001). In a review of 40 studies it was found that 45% of human breast carcinomas express EGFR (range 14% to 91%) (Klijn et al 1992). In contrast to HER-2, there are no widely accepted criteria for the determination of EGFR status. The prognostic significance of EGFR in breast cancer remains unclear (Chan et al 2006). EGFR has been correlated with ER/PR negativity (Fox et al 1994; Pawlowski et al 2000; Ferrero et al 2001; Tsutsui et al 2002; Bieche et al 2003; Bloom 2005). There may be an association between EGFR expression and high histological grade or lymph node involvement, although all studies are not in agreement (Fox et al 1994; Pawlowski et al 2000; Ferrero et al 2001; Witton et al 2003; Rampaul et al 2004, 2005; Ueda et al 2005). EGFR is generally considered a negative prognostic factor in breast cancer (Tsutsui et al 2002; Witton et al 2003) but up to now, no definitive association between EGFR expression and survival has been demonstrated.

The role of EGFR in HER-2 mediated transformation is not fully elucidated, so far. Transformation associated with the human HER-2 gene has been demonstrated, independently of the EGFR (Chazin et al 1992). On the other hand, experiments have provided evidence for a synergistic interaction of these receptors in cellular transformation and induction of mammary tumors (Kokai et al 1989; Muller et al 1996; DiGiovanna et al 1998). Moreover, interactions between EGFR and HER-2 with respect to the prognosis of breast cancer patients have been reported. Suo et al suggested that EGFR expression is likely to have a synergistic effect on the clinical influence of HER-2 expression (Suo et al 2002). In another study, Di Giovanna and colleagues (2005) showed that breast cancer patients whose tumors demonstrated co-overexpression of EGFR and HER-2 had the shortest survival.

HER-3 and breast cancer

The HER-3 gene is located on chromosome 12q13 and the encoded protein receptor binds to NRG isoforms. HER-3 overexpression has been documented in 20% to 30% of invasive breast carcinomas (Karamouzis et al 2007).

Moreover, HER-3 is frequently co-expressed with HER-2 (Naidu et al 1998; Bieche et al 2003; Witton et al 2003; Sassen et al 2008), suggesting a role of this receptor in HER-2 mediated carcinogenesis. HER-3 signaling relies on the formation of signaling-competent heterodimers with other members of the HER family. Ligand-bound or even ligand-independent HER-3 may form signaling complexes with HER-2. It has been demonstrated that the HER-2/HER-3 heterodimer constitutes the most mitogenic dimer in the HER family (Citri et al 2003). It seems that these two receptors cooperate synergistically in neoplastic transformation (Alimandi et al 1995). This hypothesis is supported by Holbro and colleagues (2003) who showed that HER-3 couples active HER-2 to the downstream signaling PI3-K/protein kinase B pathway. In another study, the activity of HER-3 decreased dramatically when the HER-2 receptor was blocked, suggesting that HER-2/HER-3 dimers are necessary for sustained signaling (Neve et al 2000). Liu and colleagues (2007) indicated that downregulation of HER-3 inhibits HER-2 mediated procarcinogenic activity via inactivation of the PI3-K/Akt pathway. Furthermore, HER-3 also contributes to HER-2 associated tamoxifen resistance. HER-2/HER-3 heterodimers signal through the PI3-K/Akt pathway, which is known to be activated in a wide range of cancers. HER-2 does not directly bind PI3-K and this function is mediated through HER-3, which has multiple tyrosine containing binding sites for p85, the regulatory subunit of PI3-K (Prigent and Gullick 1994; Soltoff et al 1994). On the other hand, it has been demonstrated that a naturally occurring secreted form of the human HER-3 receptor, p85-soluble ErbB3 (sErbB3), is a potent negative regulator of heregulin-stimulated HER-2, HER-3 and HER-4 activation (Lee et al 2001).

The prognostic value of HER-3 expression in breast cancer is poorly documented and the available data are still controversial (Lemoine et al 1992; Gasparini et al 1994; Quinn et al 1994; Travis et al 1996; Pawlowski et al 2000; Karamouzis et al 2007). Although overexpression of HER-3 has been linked to HER-2 positivity (Gasparini et al 1994) and lymph node involvement (Lemoine et al 1992; Bieche et al 2003), a definitive relationship with survival has not been established. In a study which evaluated HER family by IHC, patients with tumors that stained HER-3 strongly had significantly reduced survival (Witton et al 2003), whereas in a recently reported study, a negative impact of HER-3 gene amplification on DFS was demonstrated (Sassen et al 2008). In contrast, other studies have suggested a positive prognostic value of HER-3 receptor status. Quinn and colleagues (1994) showed that HER-3 overexpression was

positively, but not significantly, related to negative lymph node status and survival, whereas Knowlden and colleagues (1998) have demonstrated that increased HER-3 mRNA appears to be associated with the prognostically favorable ER phenotype. Moreover, Pawlowski and colleagues (2000) reported a univariate positive impact of HER-3 mRNA on survival. In a recent study, Lee and colleagues (2007) found that expression of HER-3 was correlated with positive ER and PR status and inversely correlated with histological grade. In the same study, HER-3 expression was associated with longer DFS.

HER-4 and breast cancer

The HER-4 gene is located on chromosome 2q33.3–34 and the encoded protein can be activated by both NRGs and some ligands of the EGF family. In contrast to the other HER receptors, the existing evidence suggests that HER-4 is characterized by antiproliferative activity (Sartor et al 2001; Naresh et al 2006). Moreover, HER-4 overexpression has been reported as a favorable prognostic factor in the literature. This positive effect is most likely associated with growth controlling and differentiation signaling. HER-4 is expressed in four isoforms, one of which, ErbB4 CYT-2, lacks a PI3-K binding site and thus is incapable of activating PI3-K signaling pathway (Kainulainen et al 2000). Other studies have indicated that NRG-activated HER-4 homodimers stimulate only the apoptosis-controlling PI3-K/Akt pathway and not cell proliferation (Yarden and Sliwkowski 2001). In cell line experiments, when HER-2 positive cancer cells were transfected to overexpress HER-4, a reduction in proliferation and an increase in apoptosis were observed (Sartor et al 2001), suggesting that HER-4 antagonizes HER-2 signaling activity (Barnes et al 2005). More recent studies have increased our knowledge regarding the HER-4 associated apoptosis (Naresh et al 2006). On the other hand, contrasting results of the prognostic significance of HER-4 have also been reported (Bieche et al 2003). Tang and colleagues (1999) have shown that ribozyme-mediated down-regulation of HER-4 in breast cancer cells inhibits their proliferation both in vitro and in vivo. These differences may be explained by the variable responses of HER-4 to its ligands, resulting in either proliferation or differentiation, and perhaps influenced by dimerisation with other HER-family members.

HER-4 expression has been associated with favorable prognostic factors (ER positivity, low histological grade) (Knowlden et al 1998; Tang et al 1999; Kew et al 2000; Suo et al 2002; Witton et al 2003; Zaczek et al 2008) and a more favorable outcome in breast cancer patients

(Pawlowski et al 2000; Suo et al 2002; Witton et al 2003; Aubele et al 2007). Suo et al (Suo et al 2002) suggested that HER-4 antagonizes the HER-2 effect on the patient clinical course and thus, integrating HER-4 status analysis into the diagnosis of breast cancer may also be of importance (Barnes et al 2005). In contrast, Bieche and colleagues (2003) suggested that HER-4 mRNA status might be a molecular marker of poor outcome in subsets of breast cancer patients.

Studies evaluating the HER family as a whole panel

Most clinicopathological studies have focused on the expression and/or gene amplification of individual HER family members. Consequently, the clinical outcome of breast cancer patients with regard to HER family as a whole panel remains largely unidentified. Because of the complex interactions among the HER receptors, it is likely that the effect on cell proliferation and tumor growth depends on receptor trans-signaling and thus, the evaluation of the combined expression pattern of all family members is of particular interest.

Few data are available on the expression pattern of all four HER receptors in large series of breast tumors. In a study (Witton et al 2003) which investigated the HER family by IHC in 220 breast carcinomas, patients whose tumors overexpressed EGFR, HER-2, or HER-3 had reduced survival ($P \leq 0.001$), whereas those whose tumors overexpressed HER-4 had increased survival ($P = 0.013$). In Cox's multiple regression analysis, EGFR, HER-2, HER-3 and HER-4 positivity, independently affected the survival. A recent study (Sassen et al 2008) evaluated the four members (EGFR, HER-2, HER-3, HER-4), both at the DNA and protein levels using FISH and IHC, in 278 patients. In this study, the negative impact of HER-2 amplification on patient DFS and OS was verified. Moreover, a univariate negative impact of HER-3 gene amplification on DFS was demonstrated ($P = 0.031$).

A number of studies have demonstrated strong correlations between HER mRNA copy numbers and HER protein levels, suggesting that HER family expression can reliably be assessed at the mRNA level (Knowlden et al 1998; Srinivasan et al 1998; Walker and Dearing 1999; Suo et al 2002). Data regarding the evaluation of all HER family members using RT-PCR are limited. Suo et al evaluated the HER family members using IHC and RT-PCR in 100 breast cancer patients. In this study, all the immunoreactive tumors were confirmed positive by RT-PCR. Statistical analysis revealed a significant

association between HER-2 expression and reduced DFS ($P=0.033$) and cancer-specific survival ($P=0.042$). HER-4 expression was correlated with a longer DFS ($P=0.049$) and cancer-specific survival ($P=0.044$). Co-expression of HER-2 and EGFR was associated with a worse prognosis (Suo et al 2002). Pawlowski and colleagues (2000) assessed the expression of the family with real-time RT-PCR, in a series of 365 breast cancers. HER-3 and HER-4 were positively correlated to each other and negatively correlated to EGFR. In RFS studies, Cox univariate analyses revealed prognostic value of HER-4 ($P=0.015$; risk ratio [RR], 0.65) which was retained in multivariate analyses ($P=0.035$; RR, 0.67). Regarding OS studies, univariate analyses demonstrated prognostic significance of EGFR ($P=0.026$; RR, 1.6), HER-3 ($P=0.0093$; RR, 0.58), and HER-4 ($P=0.0024$; RR, 0.52), whereas the expression of HER-2 was not a prognostic factor. In the multivariate analyses, none of these receptors maintained their prognostic value on OS. HER-4 was found to be an independent prognostic factor on RFS (Pawlowski et al 2000). Bieche and colleagues (2003) used a real-time quantitative RT-PCR assay to quantify HER family mRNA copy numbers in 130 breast tumors from patients with known long-term outcome. In this study, a positive correlation between HER-3 and HER-4 mRNA levels was found, together with a negative correlation between the expression of these two genes and that of EGFR. RFS was shorter among patients with HER-3-overexpressing tumors ($P=0.0092$) and longer among those with HER-4-underexpressing tumors ($P=0.0085$), relative to patients with normal expression of the respective genes. In contrast, RFS was not significantly influenced by EGFR or HER-2 mRNA status. Only HER-4 retained its prognostic significance in Cox multivariate regression analysis ($P=0.015$).

The existing data with respect to the expression of HER family members, particularly that of EGFR, HER-3, and HER-4, are extremely variable and thus, a comparison of the results from different studies is difficult. Most of those studies have evaluated the expression at the protein level, whereas the majority of them have not investigated the expression of all HER members simultaneously. Up to now, it is not clear whether the assessment of the prognostic value of the HER family at the DNA-, the mRNA- or the protein level yields comparable results. In a study which investigated the HER receptors using both IHC and RT-PCR, the authors used protein expression for the evaluation of relationships to clinicopathological parameters, considering that the biological influence of these factors is reflected by protein level (Suo et al 2002). Studies evaluating the HER receptors as

a whole panel at the protein level have confirmed the value of HER-2 as a negative prognostic factor (Suo et al 2002; Witton et al 2003; Sassen et al 2008). A similar finding was not demonstrated in studies which assessed the HER family at the mRNA level (Pawlowski et al 2000; Bieche et al 2003), although those which investigated the prognostic power of HER-2 only, using real-time RT-PCR, showed that this technique seems to be as useful as the current standard methods, yielding comparable correlations of HER-2 status with the patient outcome (Vinatzer et al 2005; Potemski et al 2006; Bergqvist et al 2007). Regarding the EGFR receptor, although a number of studies suggest a negative prognostic value (Pawlowski et al 2000; Witton et al 2003), others have failed to demonstrate its prognostic significance (Bieche et al 2003; Sassen et al 2008). Furthermore, most of the studies evaluating the HER family are in agreement regarding the negative prognostic value of HER-3 in breast cancer patients (Bieche et al 2003; Witton et al 2003; Sassen et al 2008). Likewise, the favorable impact of HER-4 on patient outcome has been demonstrated through the majority of the studies which assessed all family members simultaneously (Pawlowski et al 2000; Suo et al 2002; Witton et al 2003). Moreover, co-expression of HER receptors (EGFR/HER-2, HER-2/HER-3, HER-2/HER-4) is likely to have clinical importance, due to the possible synergistic or antagonistic effect among HER family members.

The results of studies evaluating the HER family demonstrate a complex expression pattern of HER receptors in breast cancer patients. Moreover, the available data provide evidence of an implication of HER-3 and HER-4 alterations in breast carcinogenesis. Thus, it is likely that HER-3 and HER-4 could have a role as prognostic markers and that their integration into the routine management of the disease would provide useful additional information. Taken together, the findings of the relevant studies indicate that the combined expression profile of the HER family, and not the isolated expression of individual members, is likely to be more important when assessing the prognosis of the patients. Therefore, it is possible that studies evaluating the HER receptors as a whole panel may shed light on the role of the HER family in breast carcinogenesis and open new directions in patient management.

Targeting the HER family

Based on the evidence implicating the HER family in breast cancer pathogenesis, numerous approaches aimed at targeting these receptors have been developed (Petrelli et al 2008). The dependency of HER-2 overexpressing breast tumors

on HER-2 activity has rendered this receptor an attractive target. A humanized monoclonal antibody directed against the HER-2 protein, trastuzumab (Herceptin), has demonstrated substantial efficacy in breast cancer and has been considered as a “therapeutic revolution” in the management of the disease. Clinical trials evaluating trastuzumab monotherapy in HER-2 positive metastatic breast cancer have indicated overall response rates ranging from 15% to 30% (Vogel et al 2002; Baselga et al 2005a). The pivotal phase III study showed that the addition of trastuzumab to first-line chemotherapy [either doxorubicin (or epirubicin) and cyclophosphamide or paclitaxel] was associated with a longer survival (median survival, 25.1 vs 20.3 months; $P = 0.01$) in patients with metastatic breast cancer and HER-2 overexpression (Slamon et al 2001). Furthermore, a recent phase II randomized trial which compared first-line trastuzumab plus docetaxel versus docetaxel alone in patients with HER-2 positive metastatic breast cancer, showed a survival advantage (median survival, 31.2 vs 22.7 months; $P = 0.0325$) from the addition of trastuzumab to chemotherapy (Marty et al 2005). Various nonrandomized studies have demonstrated the activity of trastuzumab in combination with the majority of chemotherapeutic agents used in the management of breast cancer.

The efficacy of trastuzumab in patients with advanced disease prompted the evaluation of this monoclonal antibody in patients with HER-2 positive early breast cancer. Four randomized trials have been recently reported, showing that the addition of trastuzumab to adjuvant chemotherapy halves the risk of relapse (Romond et al 2005; Piccart-Gebhart et al 2005; Slamon et al 2005; Joensuu et al 2006). Moreover, in the joint analysis of two North-American trials, treatment with trastuzumab for 52 weeks, combined with paclitaxel after doxorubicin and cyclophosphamide, was associated with a 33 percent reduction in the risk of death ($P = 0.015$) among women with surgically removed HER-2 positive breast cancer (Romond et al 2005). Likewise, after a median follow-up of 2 years in the Herceptin Adjuvant (HERA) study which compared 1 or 2 years of trastuzumab treatment with observation alone after standard neoadjuvant or adjuvant chemotherapy, 1 year of treatment with trastuzumab was associated with a significant reduction in the risk of death ($P = 0.0115$) (Smith et al 2007). However, only interim analyses with relatively short follow-up have been reported so far and thus, important issues with respect to the cumulative toxicity and the optimal duration of use of trastuzumab in the adjuvant treatment of early breast cancer remain unclear.

The mechanisms of trastuzumab action have not been fully elucidated yet (Valabrega et al 2007). Accumulating data

indicate that the effect of trastuzumab on cancer cells may be due to the activation of antibody-dependent cellular cytotoxicity (ADCC) (Lewis et al 1993; Cooley et al 1999; Clynes et al 2000; Gennari et al 2004). Other possible mechanisms of action include inhibition of shedding of the extracellular HER-2 domain (Molina et al 2001), induction of HER-2 downregulation and degradation (Austin et al 2004; Valabrega et al 2005), inhibition of the PI3-K pathway (Delord et al 2005), inhibition of angiogenesis (Izumi et al 2002; Klos et al 2003) and G1 cell cycle arrest (Lane et al 2001).

Resistance to trastuzumab treatment may be either primary or secondary. It has been shown that only 15% to 30% of HER-2 overexpressing metastatic breast cancers responded to trastuzumab monotherapy (Vogel et al 2002; Baselga et al 2005a). In the majority of these cases the disease will progress, usually within one year (Slamon et al 2001). Even in the adjuvant setting, approximately 15% of patients eventually develop metastatic disease. Thus, both de novo and acquired resistance are significant problems in patients treated with trastuzumab (Bender and Nahta 2008). Although the development of resistance remains unclear, several hypotheses have been suggested. These include loss of the tumor-suppressor phosphatase with tensin homologue (PTEN) (Nagata et al 2004), activation of alternative signaling pathways such as insulin-like growth factor-I receptor (IGF-IR) pathway (Lu et al 2001), increased expression of ligands of the HER family receptors such as transforming growth factor- α (TGF- α) (Valabrega et al 2005) and receptor masking or epitope inaccessibility (Nagy et al 2005).

Another potential mechanism of resistance is the presence of multiple truncated forms of HER-2 and the effects of these forms on trastuzumab response (Nahta and Esteva 2007). HER-2 targeted monoclonal antibodies have been shown to bind to circulating HER-2 ECD, decreasing the level of antibodies available to bind to membrane-bound HER-2 (Zabrecky et al 1991). Moreover, the accumulation of truncated forms of the HER-2 receptor that lack the extracellular trastuzumab-binding domain represents another possible mechanism of resistance. Amino terminally truncated carboxyl terminal fragments of HER-2, collectively known as p95HER2 or C-terminal fragments, are frequently found in HER-2 overexpressing breast cancer cell lines and tumors (Molina et al 2002). These fragments result either from alternative translation start sites (Anido et al 2006) or through the proteolytic shedding of the extracellular domain of HER-2 (Christianson et al 1998). In a recent study

(Scaltriti et al 2007), breast cancer cells stably expressing p95HER2 were resistant to trastuzumab but remained sensitive to the antiproliferative effects of the tyrosine kinase inhibitor (TKI) lapatinib, both in vitro and in vivo. Furthermore, in a series of patients with HER-2 positive metastatic breast cancer treated with trastuzumab, the presence of p95HER2 was correlated with clinical resistance, whereas tumors expressing only the full-length receptor exhibited a high response rate (Scaltriti et al 2007).

Trastuzumab engages both activatory (fragment C receptor [Fc gamma R] IIIa; Fc gamma RIIa) and inhibitory (Fc gamma RIIb) antibody receptors. Fc gamma R polymorphisms may affect the ADCC of natural-killer cells/monocytes. Recently, Musolino and colleagues (2008) evaluated the role of Fc gamma RIIIa, Fc gamma RIIa, and Fc gamma RIIb polymorphisms in predicting activity of trastuzumab in patients with HER-2 positive metastatic breast cancer. In this study, the Fc gamma RIIIa 158 valine/valine (V/V) genotype, alone and in combination with the Fc gamma RIIa 131 histidine/histidine (H/H) genotype, was significantly associated with better response rate and progression-free survival to trastuzumab compared with other Fc gamma R genotypes. Moreover, ADCC analysis showed that 158 V/V and/or 131 H/H peripheral blood mononuclear cells (PBMCs) had a significantly higher trastuzumab-mediated cytotoxicity than PBMCs harboring other genotypes. This study supports the hypothesis that Fc gamma R polymorphisms play a role in trastuzumab-mediated ADCC and have predictive ability in patients with breast cancer treated with trastuzumab-based therapy.

Since a considerable proportion of patients do not respond to trastuzumab, the evaluation of additional molecular parameters such as alternate HER family members or the co-expression profile of HER receptors, is an ongoing challenge. Cetuximab is a chimeric monoclonal antibody that competitively binds to the extracellular domain of the EGFR. A randomized phase II study evaluated the combination of weekly irinotecan/carboplatin with or without cetuximab in patients with metastatic breast cancer (O'Shaughnessy et al 2007). The preliminary assessment showed that the addition of cetuximab to chemotherapy was associated with a higher response rate but also with greater toxicity. Clinical studies evaluating EGFR TKIs failed to demonstrate activity in metastatic cancer patients with disease refractory to chemotherapy (Roy and Perez 2006). Available data from phase II trials which investigated the EGFR TKI gefitinib in pretreated patients have shown limited efficacy (Albain et al 2002; Baselga et al 2005b; von Minckwitz

et al 2005). Furthermore, when gefitinib was combined with first-line chemotherapy, an additional benefit was not found (Fountzilias et al 2005). A combination of erlotinib with docetaxel as first-line treatment resulted in a response rate of 55% (Kaur et al 2006). However, the nonrandomized nature of this trial does not clarify the added benefit with respect to the efficacy of erlotinib. A number of studies have demonstrated underexpression of the EGFR receptor in breast tumors (Dittadi et al 1993; Robertson et al 1996; DeFazio et al 2000; Bieche et al 2003). Moreover, in another study a marked reduction of EGFR expression with breast cancer progression was found, and such a decrease of expression of the receptor was associated with resistance to gefitinib in vitro (Choong et al 2007). These findings might be an explanation for the low activity of EGFR TKIs in breast cancer and are likely to have implications in the design of further clinical trials targeting the HER family.

It has been suggested that the form of EGFR/HER-2 dimers might be important for breast cancer cell growth and thus, the inhibition of these receptors could possibly block cell proliferation (Jannot et al 1996). Recently, it has been found that the growth inhibitory activity of trastuzumab on HER-2 overexpressing breast cancer cells is significantly modulated by EGFR co-expression (Diermeier et al 2005). Therefore, it is likely that the optimization of treatments targeting the HER family requires to account for EGFR co-expression. Lapatinib is an oral dual TKI selective for inhibition of EGFR and HER-2. It shows synergy with trastuzumab, and has demonstrated clinical activity in trastuzumab-resistant tumors (Blackwell et al 2004). Recent data provide encouraging evidence of the effectiveness of lapatinib in advanced breast cancer and for its potential in patients with brain metastases (Gomez et al 2005; Geyer et al 2006; Cameron et al 2008). Several clinical studies exploring the activity of lapatinib in combination with chemotherapeutic agents, hormonal therapy and other targeted treatments are ongoing in advanced or in neo-adjuvant and adjuvant settings (Bilancia et al 2007). In contrast, dual targeting of EGFR and HER-2 using concomitant gefitinib and trastuzumab might be detrimental in breast cancer patients, due to a possible antagonistic effect between these agents (ECOG E1100 2003).

The relatively limited activity of TKIs in HER-2 overexpressing breast tumors is likely to be associated with a failure to inhibit HER-3 efficiently. Even though these agents block EGFR and HER-2 autophosphorylation, the transphosphorylation of HER-3 is only transiently inhibited, leading to PI3-K/Akt pathway resistance

(Sergina et al 2007). Therefore, the HER-3 receptor might also present a challenging target that could potentially overcome TKI resistance. However, the inhibition of HER-3 using current therapeutic approaches would be difficult since this receptor is catalytic kinase deficient and thus, not a direct target of TKIs (Hsieh and Moasser 2007). Moreover, another therapeutic approach is to inhibit simultaneously all members of the HER family using TKIs such as canertinib. Nevertheless, a number of studies have indicated that HER-4 antagonizes the effect of HER-2 on the clinical course of breast cancer (Suo et al 2002) and thus, the use of pan-HER targeted treatments could possibly attenuate the favorable effect of HER-4 on patient outcome.

Accumulating data suggest that ER and HER-2 have a bidirectional cross talk which leads to tamoxifen resistance or conversion of tamoxifen to an ER agonist (Pietras et al 1995; Shou et al 2004; Yang et al 2004). Increased expression of EGFR and HER-2 receptors might be associated with tamoxifen resistance (Schiff et al 2005; Massarweh et al 2008). In a recent study (Kirkegaard et al 2007), high amplified in breast cancer 1 (AIB1) expression in patients with HER-2 and HER-3 overexpressing tumors or tumors expressing one or more of EGFR, HER-2, or HER-3 was associated with an increased risk of relapse on tamoxifen. These findings indicate a cross-talk between ER-alpha and growth factor receptor pathways through changes in expression of specific coactivator proteins, such as AIB1. A number of studies have investigated the use of drugs against EGFR, in tamoxifen-resistant breast cancer (Nicholson et al 2002; Knowlden et al 2003; Nicholson et al 2004). Moreover, clinical trials have examined the inhibition of growth factor signaling as a therapeutic strategy in endocrine-resistant breast cancer patients (Robertson et al 2003; Marcom et al 2007).

In conclusion, the HER family represents an attractive area for the application of targeted therapies in breast cancer and considerable treatment advances have been made so far. However, the incorporation of targeted agents into the treatment of the disease has been associated with variable and in some cases unexpected results. HER-2 overexpression alone is probably inadequate to predict the impact of targeted agents on cell proliferation. Since trans-signaling is now considered an essential feature of HER family function, the role of lateral signaling partners such as HER-3 is increasingly recognized. Studies including a more comprehensive evaluation of all HER receptors and their ligands are required to elucidate how these different signaling pathways interact in breast carcinogenesis, providing a basis for the development of

targeted treatments with respect to individualized patient management.

Disclosure

The authors report no conflicts of interest in this work.

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