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Review

The diverse signaling network of EGFR, HER2, HER3 and HER4 tyrosine kinase receptors and the consequences for therapeutic approaches

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Summary. The HER family of receptor tyrosine kinase couples binding of extracellular growth factor ligands to intracellular signal transduction pathways, contributing in this fashion to the ability of the cell to respond correctly to its environment. The HER family and its ligands are critically involved in the carcinogenesis of the mammary gland. Abnormal function of the members of HER family resulting in receptor hyper-activation (due to gene amplification, protein overexpression or abnormal transcriptional regulation) has been linked with breast cancer prognosis. It is also extensively studied as the predictive factor and target for therapy. There are clinical indications supporting the concept that none of the receptors: EGFR, HER2, HER3 and HER4 can be considered as the stand-alone receptor in breast cancer development and clinical course of the disease. There is a growing body of evidence that cooperation between them contributes to more aggressive tumor phenotype and influences the response to therapy. This underlines the importance of quantification of all HER family members and indicates the urgent need for implementation of methods that can efficiently and reliably examine four HER receptors as a whole panel in breast cancer patients.

Key words: HER family of tyrosine kinase receptors, Signaling network, Therapeutic approaches

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Introduction

The HER family of receptor tyrosine kinase couples binding of extracellular growth factor ligands to intracellular signal transduction pathways, contributing in this fashion to the ability of the cell to respond correctly to its environment. The HER family has evolved from a single ligand - receptor combination in *Caenorhabditis elegans* (Aroian et al., 1990), through one receptor and four ligands found in *Drosphila melanogaster* (Freeman, 1998), to complex networks made of four HER receptors and multiple EGF-related ligands in vertebrates (Tzahar and Yarden, 1998; Yarden, 2001; Marmor et al., 2004). The HER family in humans is made of four members: EGFR (ErbB-1/HER1), HER2 (ErbB-2), HER3 (ErbB-3) and HER4 (ErbB-4), which are activated upon ligand-induced receptor dimerization. Consequently numerous HER homo- and heterodimers are formed, suggesting that HER receptor family has evolved to provide a high degree of signaling diversity. Signaling diversity in humans is generated by the multi-layered signal-transduction network made of more than 30 ligands and four receptors that can form 10 dimeric combinations, multiple adaptor proteins, cascades of enzymes, second messengers and transcription factors (Fig. 1). Activation of the network may result in the variety of cellular responses, including proliferation, differentiation, cell motility and survival. The cellular outcome of the activation depends on the complement of signaling pathways that are induced, as well as their magnitude and duration, which in turn are determined by the composition of the receptor pair and the identity of the ligand. Multiple regulatory processes such as receptor heterodimerization and downregulation modulate signal transduction in the HER network (Yarden, 2001; Sweeney and Carraway, 2004; Wiley, 2003; Marmor et al., 2004).

HER-receptors - signaling diversity by dimer formation

Formation of homo- and heterodimeric HER receptor complexes represents a way for signal diversification. Dimer formation is driven by the higher stability of the complex formed between two receptors and ligand compared with monomeric receptor (Rubin and Yarden, 2001). Various dimeric pairs depend on the concentrations of receptors, the concentrations of particular ligands in the environment and some intrinsic degree of dimer selectivity (Pinkas-Kramarski et al., 1996; Tzahar et al., 1997). Further complexity to the signaling network is added due to the existence of an oncogenic receptor that enhances and stabilizes dimerization but has no ligand (HER2) (Klapper et al., 1999), and a receptor that can recruit novel proteins, but lacks kinase activity (HER3) (Guy et al., 1994).

It has been demonstrated that HER heterodimers are more potent in signal transduction than homodimers. Heterodimerization provides additional phosphotyrosine residues for the recruitment of effector proteins, as well as inducing distinct patterns of receptor phosphorylation and downstream signaling. Additionally, the attenuation of signaling through receptor endocytosis and subsequent lysosomal degradation differs between receptor dimers (Lenefrink et al., 1998; Olayioye et al., 2000). Heterodimerization follows a strict hierarchical principle with HER2 representing the preferred dimerization partner of all other HER receptors (Tzahar et al., 1996; Graus-Porta et al., 1997). HER2 containing heterodimers display increased potency due to the relatively slow rate of ligand dissociation and slow rate of receptor internalization. Thus, signaling by HER2 containing dimers is prolonged and results in enhanced activation of signaling pathways that activate biological

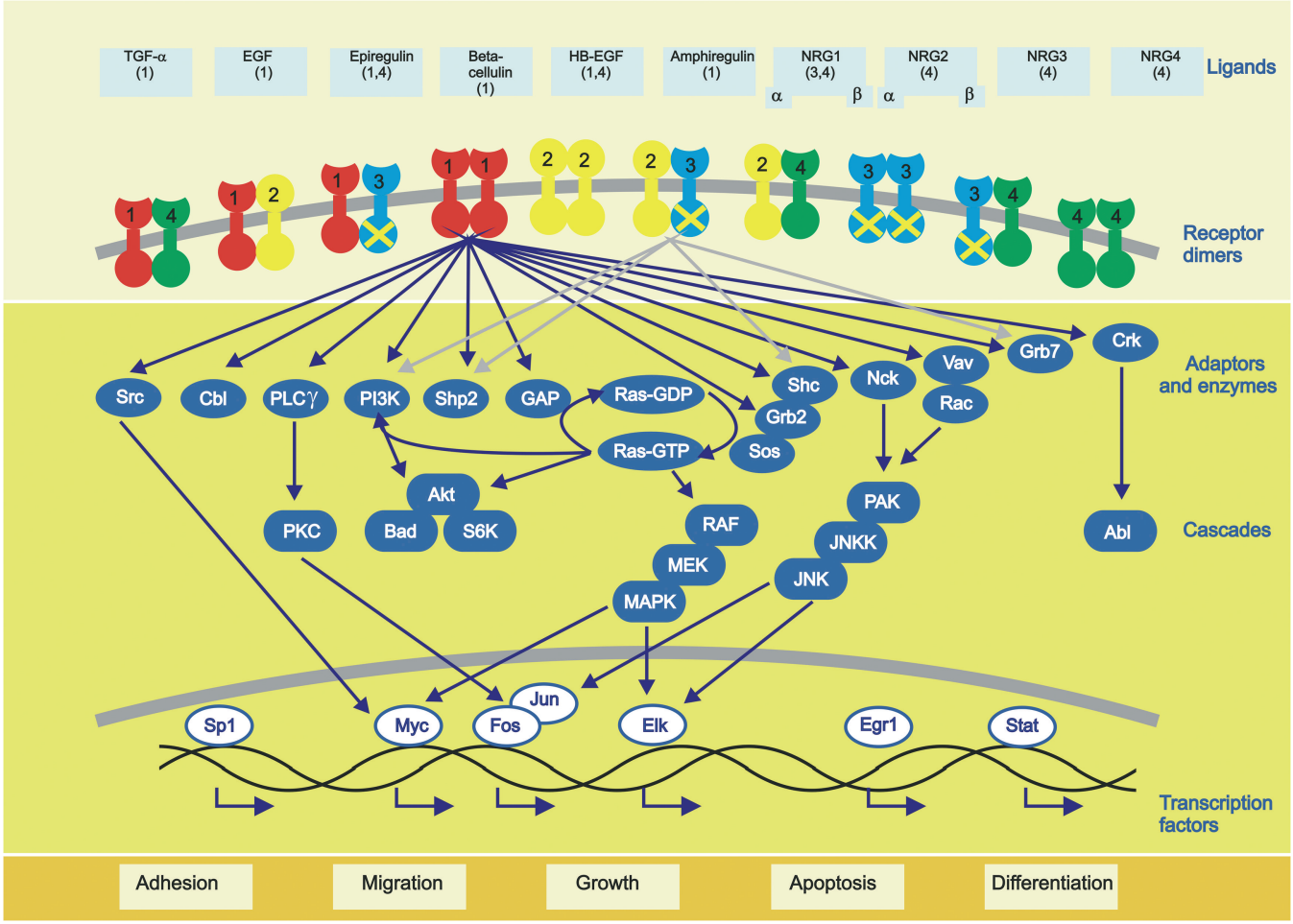

Fig. 1. HER signaling network. The HER signaling network is highly complex and consists of several layers. Ligands and receptors that form dimeric combinations lie at the head of this signaling network. Following receptor dimerization cascades of adaptor proteins, enzymes, second messengers and transcription factors are activated. This results in a variety of cellular responses, including cell growth, migration, apoptosis, adhesion and differentiation.

responses such as proliferation, morphological differentiation and migration/invasion. The most potent heterodimer is HER2/HER3, while HER3 containing homodimers are completely inactive due to impaired kinase activity of HER3 (Pinkas-Kramarski et al., 1996).

HER heterodimerization is a means not only for signal amplification but also for signal diversification. The subsets of adaptor proteins recruited to an activated receptor are defined by the pattern of phosphorylated tyrosine residues in the C-terminus of the receptor. Receptor phosphorylation may be, however, modulated by the dimerization partner (Olayioye et al., 1998). Thus, the signal elicited by a receptor heterodimer is not simply a sum of the signaling properties of the individual dimerization partners but is rather due to the unique properties acquired by heterodimer. HER receptor heterodimers, therefore, play significant roles in the number of developmental and proliferation processes, roles that cannot be performed by homodimers (Olayioye et al., 2000).

HER receptors in cancerogenesis

There is a wealth of clinical data demonstrating the importance of HER receptors, particularly EGFR and HER2, in the development and malignancy of human cancer (Holbro and Hynes, 2004).

Cancer development is a multistep process starting from a local benign hyperplasia and ending with an invasive tumor able to metastasize to other organs. During this process cells acquire new properties, which are necessary for the full malignant phenotype. During malignant process cells gain proliferative potential and divide continuously due to the circumvention of contact inhibition and cell cycle checkpoints, which normally would induce apoptosis. Moreover, for growth beyond a certain size, the primary tumor must ameliorate its supply of nutrients and oxygen through new vessel formation. The final step involves tumor cells leaving the site of primary growth and forming metastases. This step requires that the cells gain several new features including the ability to migrate and invade distant tissues (Hanahan and Weinberg, 2000). HER receptors have been shown to play a role in each of these processes including cell proliferation, apoptosis inhibition, angiogenesis, migration and invasion (Holbro et al., 2003b).

Cell proliferation

Cancer cells are characterized by their ability to evade normal growth inhibitory signals and to proliferate

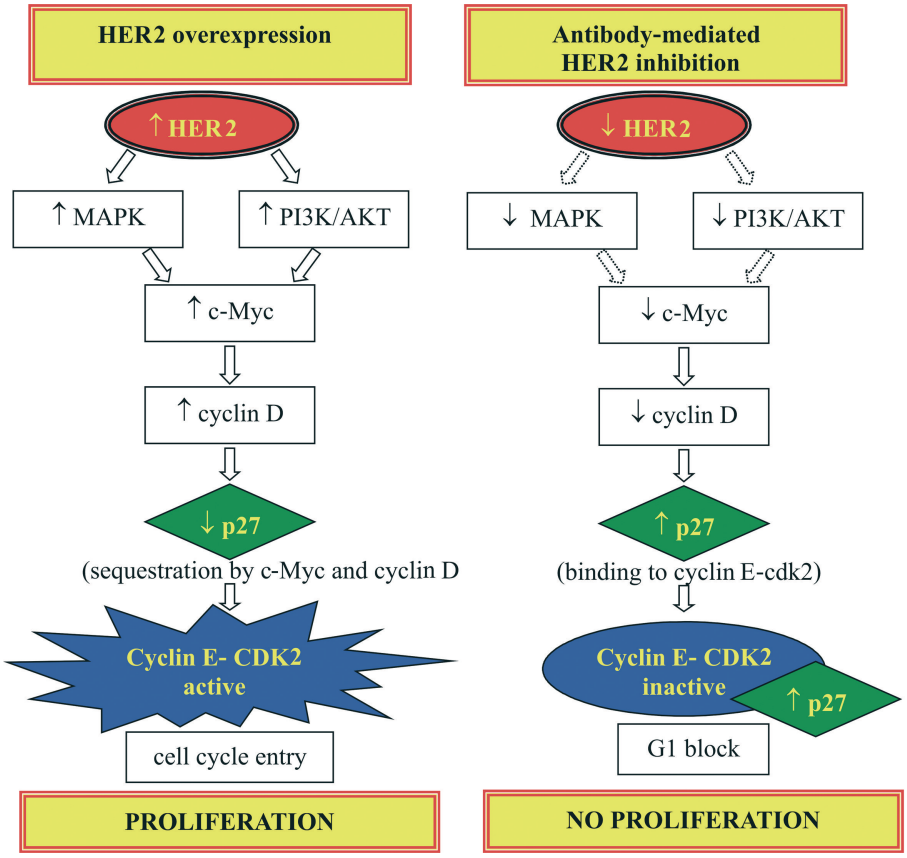

Fig. 2. HER2-mediated cell cycle regulation. HER2 overexpression leads to potentiation of cyclin E-CDK2 activity through upregulation of p27 sequestration proteins (c-Myc and cyclin D). HER2 inhibition results in decreased level of p27 sequestration proteins, inactivation of cyclin E-CDK2 by p27 binding and G1 block.

in an autonomous fashion. The HER receptor activation plays a significant role in driving cancer cells through the cell cycle checkpoints. HER2 that is a master regulator of the HER network is especially important in this process. The HER2 overexpression has been shown to deregulate G1-S transition through modulation of the activity of G1 regulators: cyclin E - CDK2 complexes, c-Myc, cyclin D and the cyclin-dependent kinase inhibitor p27 (Lane et al., 2000; Neve et al., 2000, 2001). Cyclin E - CDK2 is responsible for cell cycle entry. It may be negatively regulated by different mechanisms including binding with the cyclin kinase inhibitor p27. Regulation of p27, in turn, involves a number of steps as p27 forms numerous complexes. When p27 is associated with c-Myc protein and cyclin D, cyclin E - CDK2 is active and initiates cell cycle entry. On the contrary, uncomplexed p27 is able to bind cyclin E - CDK2 and inhibit its activity (Lane et al., 2000; Neve et al., 2000). Hyper-activated signaling through the HER2 complexes results in increased levels of c-Myc protein and cyclin D, which results in sequestration of p27. Thus, HER2 overexpression leads to the potentiation of cyclin E - CDK2 activity and intensive cell proliferation (Fig. 2).

Antibody-mediated inhibition of HER2 results in the accumulation of cells in G1 block and inactivation of cyclin E - CDK2. This is because of the fact that inhibition of HER2 signaling leads to downregulation of MAPK and PI3K/AKT signaling pathways, which decreases levels of c-Myc and cyclin D. Low levels of p27 sequestration proteins contributes to p27 redistribution and cyclin E - CDK2 binding (Neve et al., 2001). Inactivation of the cyclin E - CDK2 complex is the direct reason for G1 block and cell proliferation inhibition (Fig. 2).

Cell survival

Avoiding cell death is an essential characteristic acquired during the malignant process. Cell death is regulated by two different mechanisms: extrinsic and intrinsic, both of which can be influenced by HER signaling. The extrinsic pathway is activated by external signals and is mediated by death receptors such as FAS or tumor necrosis factor receptor (TNFR). Activation of these receptors leads to apoptosis via caspase 8 cleavage (Schmitz et al., 2000; Igney and Krammer, 2002). The intrinsic apoptotic pathway is controlled by the relative levels and the localization of pro- and antiapoptotic members of the BCL family. It is characterized by cytochrome c release and activation of caspase 9 (Kroemer and Reed, 2000; Schulze-Bergkamen and Krammer, 2004). An effector of HER signaling, PI3K/AKT pathway, is especially important in mediating cell survival as the AKT substrates directly control apoptotic processes through blocking proapoptotic BCL family member BAD and caspase 9. AKT also phosphorylates targets that indirectly affect cell survival. Phosphorylation of transcription factors from the forkhead family inhibits expression of several genes that are critical for apoptosis such as FASL. Moreover, AKT leads to activation of NF-κB, transcription factor upregulating levels of prosurvival BCL-X_L and several inhibitors of apoptosis. Additionally, another HER effector protein, signal transducer and activator of transcription (STAT) has been associated with increased levels of the prosurvival BCL-X_L (Holbro et al., 2003b).

Angiogenesis

The process of angiogenesis begins early in tumor development and is essential for growth and metastasis. HER receptors have been implicated in the tumor cell production of proangiogenic factors, the most potent being VEGF (vascular endothelial growth factor) (Maity et al., 2000; Marmor et al., 2004).

Migration and invasion

The abilities to migrate and invade the surrounding basement membrane are essential for tumors to metastasize. The role of HER receptors in these processes is only beginning to emerge. It has been revealed that MAPK and PI3K pathways play an important role in cell migration (Holbro et al., 2003b). Neuregulin stimulation of HER3 and HER4 has been linked with invasion and acquiring of proteolytic activity by tumor cells. It may be due to the increased expression of matrix metalloproteinases: MMP-2, MMP-9 (Xu et al., 1997; Marmor et al., 2004) and serine protease - urokinase plasminogen activator (UPA) and its receptor (UPAR) (Mazumdar et al., 2001).

HER receptors in carcinogenesis of mammary gland

HER family and its ligands are critically involved in the carcinogenesis of the mammary gland (Gullick and Srinivasan, 1998; Holbro et al., 2003b). Abnormal function of the members of HER family resulting in receptor hyper-activation (due to gene amplification, protein overexpression or abnormal transcriptional regulation) has been linked with breast cancer prognosis. It is also extensively studied as the predictive factor and target for therapy (Ross and Fletcher, 1998, 1999; van de Vijver, 2001; Ross et al., 2003).

EGFR overexpression for the first time has been associated with poor prognosis in breast cancer by Sainsbury et al. (1985) and it has been confirmed by others (Klijn et al., 1992; Jardines et al., 1993; Railo et al., 1994; Torregrosa et al., 1997; Nicholson et al., 2001). Several studies showed a positive correlation of increased amounts of the receptor not only with shortened survival but failure of endocrine therapy in breast cancer as well (Bolufer et al., 1990; Nicholson et al., 1994). Additionally, ddPCR revealed that breast cancer patients with elevated or decreased gene dosages for EGFR are the high-risk subgroups for early onset of tumor progression (Brandt et al., 1995a,b).

HER2 amplification and/or overexpression has been

found in approximately 20-30% of human breast carcinomas (Slamon et al., 1987, 1989; Alimandi et al., 1995). It has been linked with breast cancer development, its progression and response to therapy. The original study published by Slamon in 1987 for the first time has linked *HER2* with poor prognosis in breast cancer. It was revealed that *HER2* amplification, determined by Southern blot, independently of the other prognostic factors, predicted time to disease relapse and overall survival in node-positive breast cancer patients (Slamon et al., 1987). Further studies confirmed these results and demonstrated that gene amplification correlates with HER2 receptor overexpression (Slamon et al., 1989). In breast cancers 90-95% of cases of *HER2* overexpression result from *HER2* gene amplification (Slamon et al., 1989; Pauletti et al., 1996). During the next years numerous studies on clinical significance of HER2 have been carried out. Forty seven of these trials, involving 15,248 patients, have been retrospectively analyzed by Ross and Fletcher (Ross and Fletcher, 1998). This analysis showed that HER2 status was an independent predictor of prognosis in 60% of these trials involving 67% of patients. Most of the large studies (more than 300 patients) included in this analysis confirmed the correlation between HER2 positive status and poor clinical-outcome in node-positive patients. Thus, it is generally accepted that there is a significant correlation between HER2 amplification/overexpression and poor prognosis in node-positive patients (Ross and Fletcher, 1998; Dowsett et al., 2000; van de Vijver, 2001). The relationship between *HER2* status and prognosis in node-negative patients is more controversial. Many studies have shown that there is a correlation between outcome and HER2 status in these patients (Seshadri et al., 1993; Press et al., 1997; Andrulis et al., 1998), although others have not (Bianchi et al., 1993). The majority of the data indicate that HER2 overexpression predicts the occurrence of metastases in these patients, but the clinical significance of this association has not been clarified (Cooke et al., 2001). HER2 status may be also useful in determining invasive potential in patients with ductal carcinoma in situ (DCIS) since HER2 overexpression is very common in high-grade, comedo-type DCIS (Dowsett et al., 2000).

There is a growing body of evidence that HER2 status may predict response to therapy, and therefore guide treatment decision-making (van de Vijver, 2001). Literature data indicate that HER2-positive tumors may be less responsive to hormonal therapy, mainly tamoxifen (Yamauchi et al., 1997; Jukkola et al., 2001; Hayes and Thor, 2002). Some studies have also indicated a reduced benefit from CMF (cyclophosphamide, methotrexate, 5-fluorouracil) therapy in patients with HER2 amplification/overexpression compared with patients without elevated levels of HER2 (Gusterson et al., 1992; Muss et al., 1994; Berns et al., 1995; Jukkola et al., 2001). In the case of anthracycline-based chemotherapy, there are reports that HER2-positive breast cancer patients exhibit an increased response to optimal anthracycline dosage (Muss et al., 1994; Budman et al., 1998). Similarly, it was reported that HER2-positive breast cancer patients are more likely to respond to taxane-containing combinations (Hayes and Thor, 2002).Generally, it is suggested that HER2-positive patients benefit more from adjuvant anthracycline-based treatment and taxanes than from CMF-like regimens (Piccart et al., 2000; Hayes and Thor., 2002). Anti-HER2 monoclonal antibody therapy with trastuzumab is licensed for the treatment of metastatic breast cancer and this is the only situation in which there is an absolute requirement for HER2 positive result (obtained with immunohistochemistry and/or FISH method) before implementation of the therapy (van de Vijver, 2001).

Despite the numerous studies devoted to the issue of clinical significance of HER2, the prospective value of *HER2* amplification/overexpression in the trials involving a sufficient number of patients to determine a statistically significant interaction have not been till now unequivocally proven. The number of controversial, sometimes conflicting data concerning prognostic and predictive value of HER2 is partially the result of various methods applied for HER2 status determination (Ross and Fletcher, 1998, 1999, 2003; Dowsett et al., 2000; Cooke et al., 2001; van de Vijver, 2001). It is of critical importance to standardize the methods and to apply common interpretation criteria to enable direct comparisons of results between laboratories. Appropriate assessment of HER2 status is even more important as it is essential for the selection of patients who are likely candidates for specific anti-HER2 therapies, such as trastuzumab.

The literature data concerning clinical significance of HER3 in breast cancer bring confusing information. Along with the reports that failed to detect association of HER3 and clinical outcome, there are studies that associated HER3 with pathological parameters. The group of Lemoine (Lemoine et al., 1992) found that high HER3 expression was positively associated with the presence of lymph node metastases, whereas the group of Quinn failed to confirm this observation (Quinn et al., 1994) nor did Travis and associates (Travis et al., 1996). Neither groups demonstrated the relationship between HER3 expression and patient survival nor did the others (Gasparini et al., 1994). The work of Naidu suggested that the overexpression of HER3 could play an important role in tumor progression from non-invasive to invasive form. It was also revealed that strong HER3 immunoreactivity occurred in a high percentage of estrogen-receptor (ER) negative and lymph node positive tumors (Naidu et al., 1998). On the contrary, Knowlden demonstrated that increased HER3 expression was associated with the prognostically-favorable ER-positive phenotype (Knowlden et al., 1998).

Several groups have evaluated the expression of HER4 in breast cancer, particularly in view of the known high expression of EGFR and HER2 in this cancer (Bacus et al., 1996; Gullick et al., 1998; Kew et al.,

2000). These studies agree that HER4 is not frequently overexpressed in breast cancer, as are EGFR and HER2, but is found at moderate to low levels (Bacus et al., 1996; Gullick and Srinivasan, 1998; Kew et al., 2000; Witton et al., 2003; Hudelist et al., 2003). There is still controversy regarding the role of HER4 in breast cancer. The majority of published data associate HER4 overexpression with good prognosis and longer survival (Suo et al., 2002; Witton et al., 2003). HER4 overexpression has been linked with prognostically-favorable features such as the presence of estrogen receptors and more differentiated tumor grade (Bacus et al., 1996; Knowlden et al., 1998; Vogt et al., 1998; Suo et al., 2002). However, some studies showed no association with survival (Kew et al., 2000), while others describe HER4 as an adverse prognostic marker (Lodge et al., 2003).

HER directed therapies

The aberrant function of the HER signaling network in the wide spectrum of epithelial cancers has provided a rationale for targeting this signaling network with novel treatment approaches designed to specifically inhibit HER signaling (Arteaga, 2003). The localization of HER receptors at the cell surface makes them easy, particularly attractive targets. As they are expressed only in low levels in normal tissue, this permits a suitable therapeutic window to minimize damage to normal cells (Rubin and Yarden, 2001). Several anti-receptor therapeutic strategies, mostly anti-EGFR, anti-HER2 or against both of them, are currently under development. They include anti-EGFR and anti-HER2 monoclonal antibodies, tyrosine kinase inhibitors, gene therapy and immunotoxins.

Immunological therapies

Humanized monoclonal antibodies directed against the receptor's ligand-binding extracellular domain are the immunological strategy that stands ahead in its clinical development. The underlying mechanisms that mediate the antitumor effects of anti-HER monoclonal antibodies are not completely understood, but there are several proposed mechanisms. It has been identified that these antibodies block binding of receptor-activating ligands and can induce receptor endocytosis and downregulation (Arteaga, 2003).

Monoclonal antibodies directed against HER2 are currently the most promising approach. Recombinant humanized anti-HER2 antibodies, named trastuzumab (Herceptin; Genentech, San Francisco, CA, USA) may be used to inhibit multiple signal transduction pathways associated with HER2 receptor tyrosine kinase and thus suppress the malignant phenotype of cancer cells overexpressing the protein (Baselga et al., 1996). Trastuzumab has been effective in phase II and III clinical trials of women with HER2-positive metastatic breast cancer either as the first-line therapy or in patients who failed or relapsed after previous extensive treatment with chemotherapy (Baselga et al., 1996, 1998; Pegram et al., 1998; Pegram and Slamon, 2000). Recently, it has been shown that anti-HER2 antibodies enhance the antitumor activity of some chemotherapeutic agents such as paclitaxel and doxorubicin (Baselga et al., 1998), cisplatin or carboplatin (Pegram et al., 1998; Pegram and Slamon, 1999). Among many anti-EGFR antibodies that are under development most utilized are the IMC-C225 humanized monoclonal antibodies (cetuximab, Im-Clone Systems Incorporated, Somerville, NJ) (Arteaga, 2003). Early trial results indicate a high efficacy of this strategy, the good safety profile and benefit in combination therapies with radiation and chemotherapy. There are ongoing phase II and III studies evaluating IMC-C225 therapeutic modality in the tumors of head and neck, colon, pancreas and non-small cell lung cancer (Kim et al., 2001).

Tyrosine kinase inhibitors

Tyrosine kinase inhibitors are small molecules (approximate molecular weight 300-500 Da) that bear selective specificities towards the ATP binding sites of EGFR or HER2, resulting in the inhibition of proliferation of cells expressing the respective receptor (Arteaga, 2003). Several classes of tyrosine kinase inhibitors have been developed and two quinazoline-based compounds OSI 774 (erlotinib, Tarceva) and ZD1839 (gefitinib, Iressa), are now in the phase II/III clinical trials. Preclinical studies with these agents show that they are potent, reversible inhibitors that are highly selective for EGFR tyrosine kinase. Both agents have substantial antitumor activity against a range of human tumor xenografts, including lung, breast, head and neck, pancreas and ovary (Arteaga, 2003). In phase I studies ZD1839 (gefitinib) was active against non-small lung cancer across a broad range of doses and in randomized phase II response rates of 9-19% were reported (Baselga et al., 2002; Fukuoka et al., 2003). It was recently approved by FDA as third-line therapy for non-small cell lung cancer. Also irreversible EGFR inhibitors have been developed, as well as agents that inhibit both EGFR and HER2 (GW2016 and PKI-166), or all of the HER receptors (CI-1033) (Allen et al., 2002).

Gene therapy

Another strategy, used mainly to suppress HER2-induced transformation, is the inhibition of HER2 overexpression by modifications at gene level. Hung et al. have shown that the adenovirus type 5 early region 1A (E1A) gene product, delivered by cationic liposomes or adenovirus vector, inhibits transcription of the HER2 promoter (Hung and Lau, 1999). Deshane et al., in turn, have developed the method of selective oncogene "knock-out" using anti-HER2 intracellular single-chain (sFv) antibody gene transfection (Deshane et al., 1996). Selective expression of suicide genes driven by

regulatory regions of the HER2 promoter, anti HER2-targeted hammerhead ribozymes and antisense cDNA constructs are also applied to suppress tumorigenic potential of cells overexpressing HER2 (Arteaga, 2003).

Immunotoxins

Conjugates of monoclonal antibodies and toxins have been constructed using various anti-HER2 antibodies coupled to bacterial toxins such as a recombinant form of *Pseudomonas exotoxin* lacking its cell-binding activity. Several other agents have been similarly targeted, including ricin, doxorubicin and enzyme prodrugs, all presenting specific cell inhibitory effects. Not only anti-HER2 antibodies, but also ligand-directed proteins against HER have been examined as beneficial carriers (Kirschbaum and Yarden, 2000).

HER receptors interactions - clinical implications in tumor development and response to therapy

The literature data have reported the contribution of each of HER family member to the many processes linked to malignant development such as: proliferation, cell survival, angiogenesis, migration and invasion (Holbro et al., 2003b). Most clinicopathological analyses of HER receptors in breast cancer are related to the expression of single HER family members. Since heterodimerization between HER members is necessary for their activation, studies of all four receptors as the whole panel may shed light on some new features with clinical significance.

Cooperation between HER receptors has been observed in oncogenic transformation, both *in vitro*, in cultured cells and in primary human tumors. For example, HER3 expression increases HER2 mediated transformation and tumorigenic growth in NIH3T3 cell line (Alimandi et al., 1995; Wallasch et al., 1995). Also, NRG-1 induced transformation of fibroblasts by HER4 requires co-expression of either EGFR or HER2 (Zhang et al., 1996). Many human tumors that contain HER2 also exhibit autocrine stimulation of EGFR via expression of one of its numerous ligands. HER2 is co-expressed with HER4 in more than 50% of childhood medulloblastomas (Gilbertson et al., 1997). HER heterodimers cooperation during tumor development is confirmed by the observation that expression of HER3 is seen in many tumor types that overexpress HER2, including breast, bladder and melanomas (Lemoine et al., 1992; Rajkumar et al., 1996; Bodey et al., 1997). It has been suggested that there is selective pressure leading to co-expression of both receptors (Siegel et al., 1999). In fact, HER2/HER3 heterodimers function as an oncogenic unit, driving proliferation of breast cancer cells (Holbro et al., 2003a).

There is growing evidence that cells expressing multiple HER receptors are characterized by enhanced transforming properties and more aggressive phenotype. This is probably due to the diversity and signaling potency emanating from HER receptor combinations that can deregulate cellular proliferation associated with tumor progression (Arteaga, 2003). The concept that co-expression of members of the HER network confers a poor clinical outcome compared to tumors with a high level of only one HER receptor was confirmed by many authors. Suo and associates found that co-expression of EGFR and HER2 was associated with worse prognosis of breast cancer patients, while HER4 antagonized HER2 effect on clinical course of breast carcinoma (Suo et al., 2002). Similar results indicating that co-expression of EGFR, HER2 and HER3 was associated with reduced survival, while HER4 expression resulted in improved survival in breast cancer patients was found by the group of Witton (Witton et al., 2003). Recently, the same group demonstrated that tumors found to be positive for EGFR, HER2 and HER3 had significantly higher bromodeoxyuridine labeling indices in comparison to HER4-positive tumors, which were significantly correlated with low bromodeoxyuridine labeling values (Tovey et al., 2004). These results indicate that EGFR, HER2 and HER3 are associated with tumor proliferation, whereas HER4 is involved in a non-proliferative or even protective role (Tovey et al., 2004). Co-expression of EGFR, HER2 and HER3 was significantly associated with lymph node involvement and distant metastasis occurrence as well as shortened survival in squamous cell carcinoma (Xia et al., 1999). EGFR and HER2 overexpression occurring together in human lung adenocarcinoma has been linked with shortened survival (Tateishi et al., 1994).

Another issue demonstrating how HER receptors co-expression and cooperation can influence tumor behavior is cancer cells response to the trastuzumab treatment, which depends not only on HER2 amplification/overexpression. It has been found that antibody-induced inhibition of HER2 receptor activity does not necessarily predict cellular response to the antibody treatment (Lane et al., 2000). This phenomenon correlated with the fact that although all patients treated with trastuzumab did have tumors exhibiting HER2 overexpression, not all responded to treatment (Baselga et al., 1996; Pegram et al., 1998; Cobleigh et al., 1999). To elucidate the cause of non-response, the group of Neve (Neve et al., 2001) studied the effects of monoclonal antibody 4D5, the murine precursor of trastuzumab, on the two cell lines overexpressing HER2: responding to 4D5 BT-474 and non-responding MKN7. Cells from both lines were treated with 4D5 and then the activity of intracellular signaling proteins was measured. In BT-474 HER2/HER3 heterodimer plays a key role in maintaining high signaling activity. Treatment with 4D5 affected activity of both HER2 and HER3 receptors because HER3 is dependent upon HER2 for its activity. Therefore, there was a dramatic decrease in PKB and MAP kinase activity and inhibition of cell proliferation. MKN7 cells apart from HER2 have high levels of EGFR, which is unaffected by 4D5 treatment. Thus, 4D5 treatment impaired only signaling from HER2, but cells

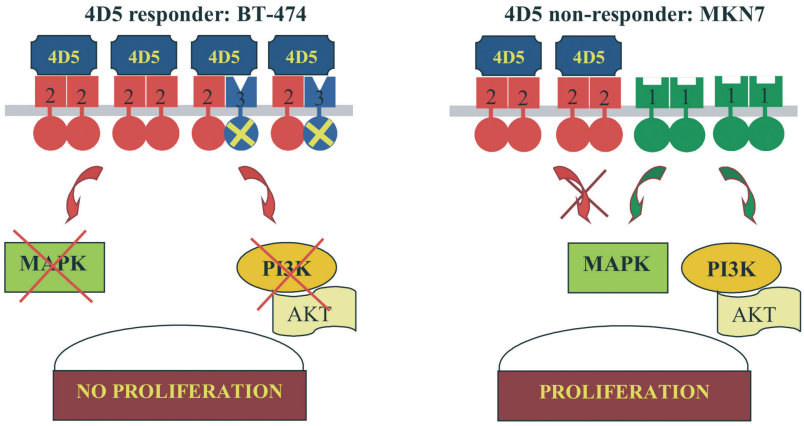

Fig. 3. Reversal of malignant growth by 4D5 – dependency on various HER receptors. In BT-474 HER2/HER3 heterodimer plays a key role in maintaining high signaling activity. Treatment with 4D5 affected activity of both HER2 and HER3 receptors because HER3 is dependent upon HER2 for its activity. Therefore, there was a dramatic decrease in PKB and MAP kinase activity and inhibition of cell proliferation. MKN7 cells apart from HER2 have high levels of EGFR, which is unaffected by 4D5 treatment. Thus, 4D5 treatment impaired only signaling from HER2, but cells could still proliferate (after (Neve et al., 2001); modified).

could still proliferate (Fig. 3).

This suggests that the response of a patient to trastuzumab may not only be dependent on overexpressed HER2, but may be influenced by other members of the HER family, which are expressed in the tumor cell. It seems that contribution of other HER receptors may influence tumor response to treatment and it should be taken into account for evaluations of HER2 as a target for tumor therapy.

In conclusion, there are clinical indications supporting the concept that none of the receptors: EGFR, HER2, HER3 and HER4 can be considered as the stand-alone receptor in breast cancer development and clinical course of the disease. There is a growing body of evidence that cooperation between them contributes to a more aggressive tumor phenotype and influences the response to the therapy. This underlines the importance of quantification of all HER family members and indicates the urgent need for implementation of methods that can efficiently and reliably examine four HER receptors as a whole panel in breast cancer patients.

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