Review

Cancer Research

p95HER2 and Breast Cancer

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Abstract

A subtype of HER2-positive tumors with distinct biological and clinical features expresses a series of carboxy-terminal fragments collectively known as p95HER2. One of these fragments, named 100- to 115-kDa p95HER2 or 611-CTF, is hyperactive because of its ability to form homodimers maintained by intermolecular disulfide bonds. Despite lacking the majority of the extracellular domain, this HER2 fragment drives breast cancer progression *in vivo*. The recent availability of specific anti-p95 antibodies has confirmed previous results indicating that the expression of p95HER2 is predictive of poor prognosis and correlates with resistance to the treatment with trastuzumab, a therapeutic antibody directed against the extracellular domain of HER2. *Cancer Res; 71(5); 1515–9. ©2011 AACR.*

Introduction

One of the major challenges in breast cancer treatment stems from the fact that it is a heterogeneous disease. Analysis of gene expression profiles has shown the existence of at least 5 types of breast cancer with different biological properties (1). However, because gene expression profiles are not routinely done in the clinic, in practice, the classification of breast cancer depends in great part on the expression of 2 factors: estrogen receptor (ER), the nuclear receptor for the steroid hormone estrogen; and HER2 (also known as ErbB2, neu), a tyrosine kinase that belongs to the epidermal growth factor receptor (EGFR) family (2, 3). HER2 does not bind any ligand, and it is activated through the homo- or heterotypic interaction between its extracellular domain with that of other EGFR-like receptors. Within the homo- or heterodimers, the interactions between the intracellular domains of the receptors lead to the activation of the kinase domains and the subsequent transphosphorylation of tyrosine residues in the carboxy-terminal tails. The phosphotyrosines constitute docking sites for proteins that activate intracellular signaling pathways, including the mitogen-activated protein kinases (MAPK) and the phosphoinositide 3-kinase (PI3K)–activated Akt pathways. Ultimately, these signaling cascades control the expression of target genes that act coordinately to modify key aspects of cellular biology, including proliferation, migration, survival, and differentiation (4).

Underscoring the unique value of HER2 as a biomarker, one type of breast cancer is named after the tyrosine kinase receptor. HER2-positive cancers account for 20% to 30% of total breast cancers and are characterized by overexpression of the receptor due to gene amplification (2). In addition to being a reliable biomarker, HER2 is a validated therapeutic target. Trastuzumab, a monoclonal antibody against the extracellular domain of HER2, has contributed to the increase in survival rates of breast cancer patients observed during the last decades. However, this success has been hampered by the substantial proportion (~70%) of HER2-positive breast cancers that are either intrinsically resistant to treatment with trastuzumab or develop resistance after treatment (5). Lapatinib (tykerb), a tyrosine kinase inhibitor that targets HER2, has been developed recently as an alternative treatment for HER2-positive cancers (2). This review focuses on recent findings on a series of HER2 fragments, collectively known as p95HER2 or HER2 carboxy terminal fragments (CTF). p95HER2 may constitute a novel biomarker of an aggressive subtype of HER2-positive cancers with distinct biological and clinical features. In the near future, the presence of p95HER2 may determine the best therapeutic option for HER2-positive breast cancers.

Biology of p95HER2 Fragments

p95HER2 fragments arise through at least 2 different mechanisms: proteolytic shedding of the extracellular domain of the full-length receptor and translation of the mRNA encoding HER2 from internal initiation codons (6, 7). Shedding of the ectodomain of HER2 is likely carried out by the metalloprotease ADAM10 at a site proximal to the transmembrane domain, generating a 95- to 100-kDa p95HER2 membrane-anchored fragment (Fig. 1; refs. 8, 9). Translation of the mRNA encoding HER2 can be initiated from the AUG codon that gives rise to the full-length protein of 1,255 amino acids or, alternatively, from 2 internal initiation codons at positions 611 and 678 (codons numbered according to the full-length molecule), located upstream and downstream of the transmembrane domain, respectively. Alternative initiation of translation generates 2

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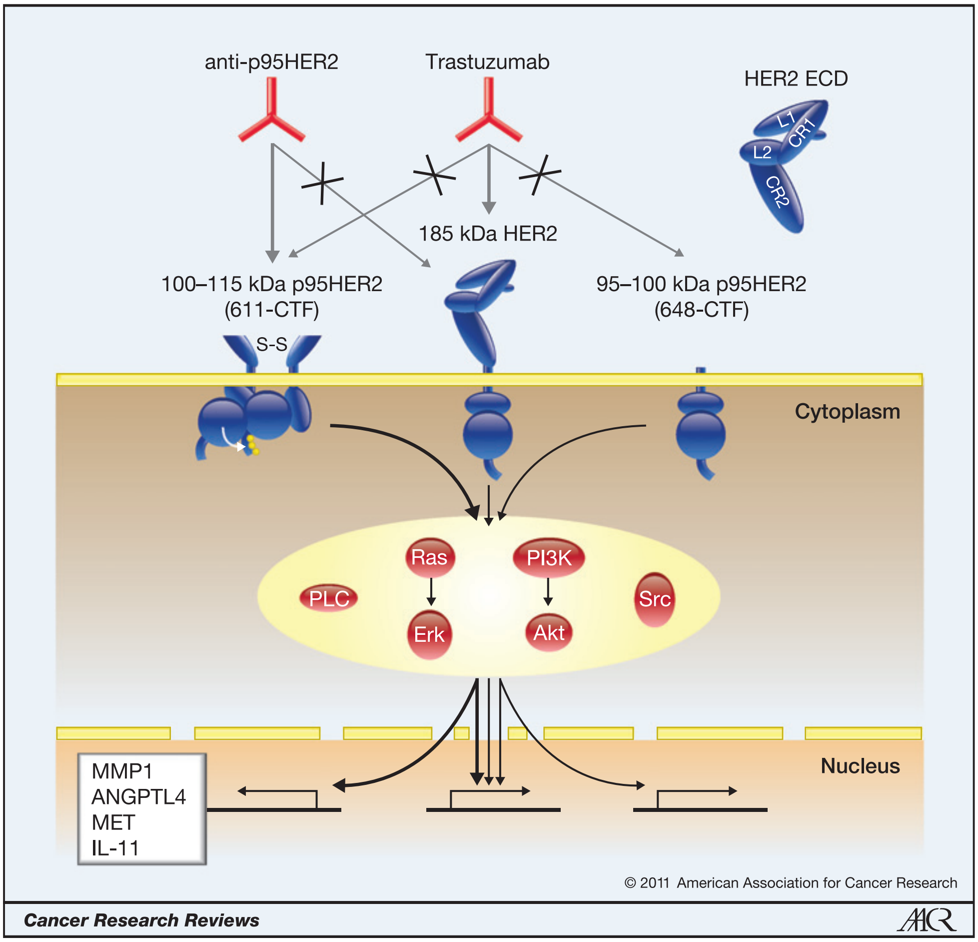

Figure 1. Blue, full-length HER2 (middle), the 100- to 115-kDa p95HER2 fragment generated by alternative initiation of translation from the AUG codon in position 611 (left) and the 95- to 100-kDa p95HER2 generated by proteolytic cleavage of full-length HER2 (right). Note that the names of different domains are marked in the soluble extracellular domain of HER2 (HER2 ECD). Top, the anti-p95HER2 antibodies recognize epitopes in 100- to 115-kDa p95HER2 that are masked in full-length HER2 and absent in 95- to 100-kDa p95HER2. Within the cytoplasm, the red globs represent selected components of signaling pathway activated by HER2 and p95HER2. Bottom, gene expression is regulated by the different HER2 forms. Note that a group of genes specifically regulated by 100- to 115-kDa p95HER2, such as *MMP1*, *ANGPTL4*, *MET*, and *IL-11*, have been causally involved in the metastatic progression.

p95HER2 fragments of 100 to 115 kDa and 90- to 95-kDa, also known as 611-CTF and 678-CTF, respectively. Despite lacking a signal peptide, the 100- to 115-kDa p95HER2 fragment is efficiently incorporated into the secretory pathway and transported to the plasma membrane (Fig. 1). The 90- to 95-kDa p95HER2 fragment can be found both in the cytoplasm and nucleus (10).

Pedersen and colleagues recently analyzed the activity of the individual p95HER2 fragments using a variety of approaches. They showed that the soluble intracellular 90-to 95-kDa p95HER2 fragment, despite having an intact kinase domain, was inactive (10). The membrane-anchored p95HER2 fragments were active, although they differed in their potency. Although the activity of the 95- to 100-kDa (648-CTF) fragment was comparable with that of the full-length receptor, expression of the 100- to 115-kDa (611-CTF) fragment led to a much more rapid and acute activation of different signaling cascades (10). As a result, expression of the 100- to 115-kDa p95HER2 fragment leads to the regulation of a specific set of genes not regulated by full-length HER2 (10). Several of these genes, such as *MMP1*, *ANGPTL4*, *MET*, *CD44*, *PLAUR*, *EPHA2*, *ITGA2*, *ITGFB*, *TGFA*, and *IL-11*, are causally involved in the metastatic progression (10). Furthermore, the hyperactive 100- to 115-kDa p95HER2 fragment induces cell migration much more efficiently than full-length HER2 through the phosphorylation of cortactin, a cytoskeleton-binding protein (11).

The short extracellular domain of the 100- to 115-kDa p95 HER2 fragment contains 5 cysteines. At least some of these cysteines establish intermolecular disulfide bonds. Therefore, the mechanism of activation of 100- to 115-kDa p95HER2 (611-CTF) consists in the constitutive generation of homodimers maintained by covalent bonds (Fig. 1; ref. 10). It should be noted that the 95- to 100-kDa p95HER2 proteolytic fragment cannot be activated through a similar mechanism because it only has 4 extracellular amino acids, none of them cysteines. Predictably, these 4 amino acids do not promote dimerization. The most likely explanation for the activity of the 95- to 100-kDa p95HER2 fragment is a low homotypic affinity of the transmembrane and intracellular domains, similar to that described for full-length HER2 (12). Supporting this conclusion, p95HER2 fragments have been shown to interact with the full-length HER2 receptor (13).

Undoubtedly, the most compelling evidence for the oncogenicity of the 100- to 115-kDa p95HER2 fragment comes from the characterization of transgenic mice. Expression of low levels of the 110- to 115-kDa p95HER2 fragment in the mammary gland leads to the progression of breast tumors far more aggressive and metastatic than those driven by high levels of full-length HER2 (10). Another remarkable difference between the tumors arising in the HER2 and 110- to 115-kDa p95HER2 transgenic mouse strains is their latency periods. The tumors induced by full-length HER2 and 110- to 115-kDa p95HER2 are first detectable, as an average, in ~30- and ~15-week-old mice, respectively. The relatively long latency period for the progression of tumors expressing full-length HER2 is required for the acquisition of activating mutations in the transgene. As a consequence, sequencing of transgenic HER2 from these tumors invariably shows the presence of genetic alterations (10, 14). These alterations include point mutations, deletions, and insertions, but, despite this variability, they share 2 common features: they are located in the juxtamembrane region of HER2 and they lead to an unbalanced number of cysteines (15). As a result, the mutated transgenes translate into aberrant forms of HER2 that constitute hyperactive dimers maintained by intermolecular disulfide bonds, a mechanism of activation identical to that of the 100- to 115-kDa p95HER2. These findings show that the mere overexpression of full-length HER2 is not sufficient to drive malignant transformation, at least in this animal model. Despite intense search, mutations similar to those observed in the transgene of the mouse model have not been found in human tumors (16). Thus, these mutations are a particularity of the transgenic mice, and it has been proposed that in humans HER2 overexpression is oncogenic *per se*. The expression of 110- to 115-kDa p95HER2 in a subgroup of HER2-positive breast cancers points to an alternative explanation. At least in these tumors, the expression of this hyperactive form of HER2, and not the mere overexpression of full-length form, may contribute to the malignant progression. Human breast cancer cells expressing even low levels of 100- to 115-kDa p95HER2 may have growth advantages similar to those of cells expressing the mutant transgene in the animal mouse model. Therefore, the presence of this fragment may explain, at least in part, the lack of HER2 mutations in the vast majority of human breast cancers.

p95HER2 as a Biomarker

An early study by Christianson and colleagues showed that the expression of p95HER2 in breast tumors correlated with metastasis to the lymph nodes (6). Several subsequent studies supported that p95HER2 may be used as a biomarker of an aggressive subtype of HER2-positive breast cancer (17, 18). This conclusion was followed up by investigations aimed to determine the effect of p95HER2 expression on anti-HER2 therapies. Retrospective studies showed that tumors expressing p95HER2 tend to be resistant to treatment with trastuzumab (19, 20) but do respond to lapatinib (21). The effectiveness of lapatinib on p95HER2-postive tumors is not surprising because the tyrosine kinase inhibitor also blocks the activity of the p95HER2 fragments (10). Therefore, tyrosine kinase inhibitors may be a good therapeutic approach to treat p95HER2-positive tumors. Because both the 95- to 100-kDa and the 100- to 115-kDa transmembrane p95HER2 fragments lack the epitope recognized by trastuzumab, an obvious explanation for the lack of response to the antibody in p95HER2-positive tumors is that expression of these fragments drives tumor growth even under treatment with trastuzumab. Experiments with cell lines expressing either full-length HER2 or p95HER2 have confirmed that trastuzumab is not effective on cells expressing the fragments. However, due to the lack of an appropriate experimental model, the ability of p95HER2 to confer resistance to trastuzumab in cells also expressing full-length HER2, the situation found in human breast cancers, has not been tested yet. Because the effectiveness of trastuzumab is not only based on its ability to interfere with HER2 signaling, but also on the immune response against cells targeted by the antibody, the sensitivity of cells expressing both full-length HER2 and p95HER2 should be tested in the future. Conceivably, these experiments would clarify whether the combination of lapatinib and trastuzumab is more effective than lapatinib alone in p95HER2-expressing tumors.

The early studies on the relevance of p95HER2 as a biomarker, although encouraging, were carried out with few breast cancer samples due to the technical difficulties of analyzing the levels of p95HER2 in clinically relevant samples. These technical difficulties have been solved in a timely fashion, independently but simultaneously, by 2 groups. Sperinde and colleagues (23) and Parra-Palau and colleagues (26) generated monoclonal antibodies that specifically recognize 100- to 115-kDa p95HER2. This step was possible because of the different tridimensional structures of the extracellular juxtamembrane region in the fragment and in full-length HER2. In the full-length receptor, this region is highly structured and maintained by several intramolecular disulfide bonds (22). In contrast, the same region is likely to be unstructured in 100- to 115-kDa p95HER2, and at least some of the cysteines establish intermolecular disulfide bonds. As a result, the *N* terminus of 100- to 115-kDa p95HER2 contains epitopes that are not accessible in full-length HER2 (20, 23, 24). The specific antibodies were able to detect 100- to 115-kDa p95 HER2 in formalin-fixed paraffin-embedded samples, by far, the most frequent type of sample available for clinical studies. Stratification of breast cancer patients according to the levels of expression of 100- to 115-kDa p95HER2, as judged by immunohistochemistry with the specific antibodies, confirms that, indeed, tumors positive for this HER2 fragment constitute a subgroup of HER2-positive breast cancers with distinct biological and clinical features. Previous reports had shown that although nearly 80% of HER2-negative breast tumors are positive for ER, only ~50% of HER2-positive breast tumors are ER positive (25). Analysis of ER expression in a cohort of HER2-positive tumors classified as 100- to 115-kDa p95HER2-positive and -negative subgroups showed a remarkable connection between expressions of the ER and the HER2 fragment. Although the frequency of ER positivity in HER2-positive and 100- to 115-kDa p95HER2-negative tumors is similar to that reported for HER2-negative tumors, the number of ER-positive tumors in the 100- to 115-kDa p95HER2-positive subgroup was very low (~30%; ref. 23). Using similar anti-p95HER2–specific antibodies, Sperinde and colleagues showed that the 100- to 115-kDa p95HER2-positive tumors were twice as likely to metastasize to lungs (26). Furthermore, patients with 100- to 115-kDa p95HER2-positive tumors had significantly shorter progression-free survival and overall survival compared with patients who express only the full-length receptor (26). Because all patients in the cohort analyzed had been treated with trastuzumab, this result concurs with the previously described resistance of p95HER2-positive tumors to treatment with this antibody (19).

In addition to the expression of p95HER2, several mechanisms of resistance to trastuzumab have been proposed. These include increased signaling from other HER family receptors or from insulin-like growth factor 1R (IGF-1R; recently reviewed in ref. 5) and overactivation of the PI3K pathway (27). Although possible relationships between the different mechanisms of resistance to trastuzumab have not been specifically analyzed, expression of 100- to 115-kDa p95HER2 induces the overexpression of different components of HER signaling, including receptors and ligands such as EGFR and TGF α (10). Therefore, it is possible that, in addition to being a HER2 isoform that cannot be targeted by trastuzumab, 100- to 115-kDa p95HER2 induces resistance to trastuzumab by upregulating the expression of ligands and receptors that may confer resistance to the antibody. The PI3K signaling pathway is overactivated in 70% of breast cancers (28). There seems to be an apparent link between the PI3K pathway and the response to anti-ErbB receptor therapies, including trastuzumab. For example, aberrant activation of the PI3K pathway reduces the response to trastuzumab and lapatinib (27, 29, 30). Despite the fact that the HER2/HER3 dimer is a potent activator of the PI3K pathway (4), 100- to 115-kDa p95HER2 has been shown to activate this pathway far more effectively than full-length HER2, even in HER3-positive cells (10). Therefore, p95HER2 may sustain tumor growth in a manner analogous to mutations of the PI3K. Foreseeably, the specific antibodies against 100- to 115-kDa p95HER2 will allow studies on a large number of samples that will definitively establish the value of p95HER2 as a biomarker and will clarify its relationship with other mechanisms of resistance to anti-HER2 therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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