

## CCR Translations

Commentary on Sperinde et al., p. 4226

## HER2 Fragmentation and Breast Cancer Stratification

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HER2 is a tyrosine kinase receptor whose overexpression in breast cancers correlates with poor prognosis. A subset of HER2-positive tumors also expresses a series of HER2 fragments, collectively known as p95HER2. These fragments are emerging as a valuable biomarker for this subset of patients, who have a particularly poor prognosis. *Clin Cancer Res*; 16(16); 4071–3. ©2010 AACR.

In this issue of *Clinical Cancer Research*, Sperinde and colleagues (1) report the generation and characterization of a monoclonal antibody that specifically recognizes one of the p95HER2 carboxy-terminal fragments of HER2. HER2 is expressed at excessive levels in 20% to 30% of breast cancers because of gene amplification. It is one of the most reliable biomarkers identified to date; the overexpression of HER2 is a hallmark of an aggressive form of the disease (2). Two types of agents are currently available for targeted treatment of HER2-positive tumors: monoclonal antibodies against the extracellular domain of HER2 (e.g., trastuzumab) and small molecule tyrosine kinase inhibitors (e.g., lapatinib). In addition to the full-length receptor, 20% to 40% of HER2-positive tumors also express a series of carboxy-terminal fragments of HER2 known as p95HER2 or HER2 carboxy-terminal fragments. Previous publications have indicated that these truncated forms of HER2 represent an independent biomarker because, compared with tumors expressing only full-length HER2, those expressing p95HER2 have worse prognosis and higher likelihood to metastasize to lymph nodes (3). In addition, the p95HER2 status might become a decisive factor when choosing between different therapeutic regimens, because p95HER2-positive tumors are resistant to trastuzumab (4) but respond to lapatinib at least as effectively as p95HER2-negative tumors (5).

To date, the main difficulty in conducting more clinical studies on the role of p95HER2 in breast cancer progression and confirming its value as a biomarker has been the lack of a simple and robust test to detect these truncated forms of HER2. Sperinde and colleagues (1) identified an elegant and timely solution to this difficulty by generating a monoclonal antibody that specifically recognizes the most active of the p95HER2 fragments.

The p95HER2 fragments are generated through at least two independent mechanisms. Cleavage of full-length HER2 by metalloproteases (6, 7) releases nearly the whole extracellular domain of HER2, leaving behind an active transmembrane fragment of 95 to 100 kDa with fewer than a dozen extracellular amino acids (Fig. 1; ref. 8). This type of proteolytic cleavage, known as ectodomain shedding, is frequently followed by a second cleavage carried out by the gamma-secretase proteolytic complex, which releases the intracellular domain (Fig. 1). However, the cleavage of the 95 to 100 kDa p95HER2 fragment has not been documented to date, and in any event, the resulting intracellular fragment is inactive (9).

A different mechanism, initiation of translation of the messenger RNA encoding HER2 from internal AUG codons, generates a transmembrane fragment with an extracellular domain of 42 amino acids and an apparent molecular size of 100 to 115 kDa as well as an inactive intracellular fragment of 90 to 95 kDa (Fig. 1; ref. 10). The only difference between the 95 to 100 and the 100 to 115 kDa fragments, a stretch of approximately 30 amino acids, makes a remarkable difference. This small region contains several cysteine residues that support constitutive homodimerization and, hence, hyperactivation of the fragment generated by alternative initiation of translation (Fig. 1; ref. 9). Perhaps the most compelling evidence for the hyperactivity of the 100 to 115 kDa fragment, referred to as p95HER2 by Sperinde and colleagues (1) for simplicity, is its ability to generate tumors when expressed in the mammary gland of transgenic mice. The tumors driven by p95HER2 are far more aggressive and metastatic than those driven by full-length HER2 (9).

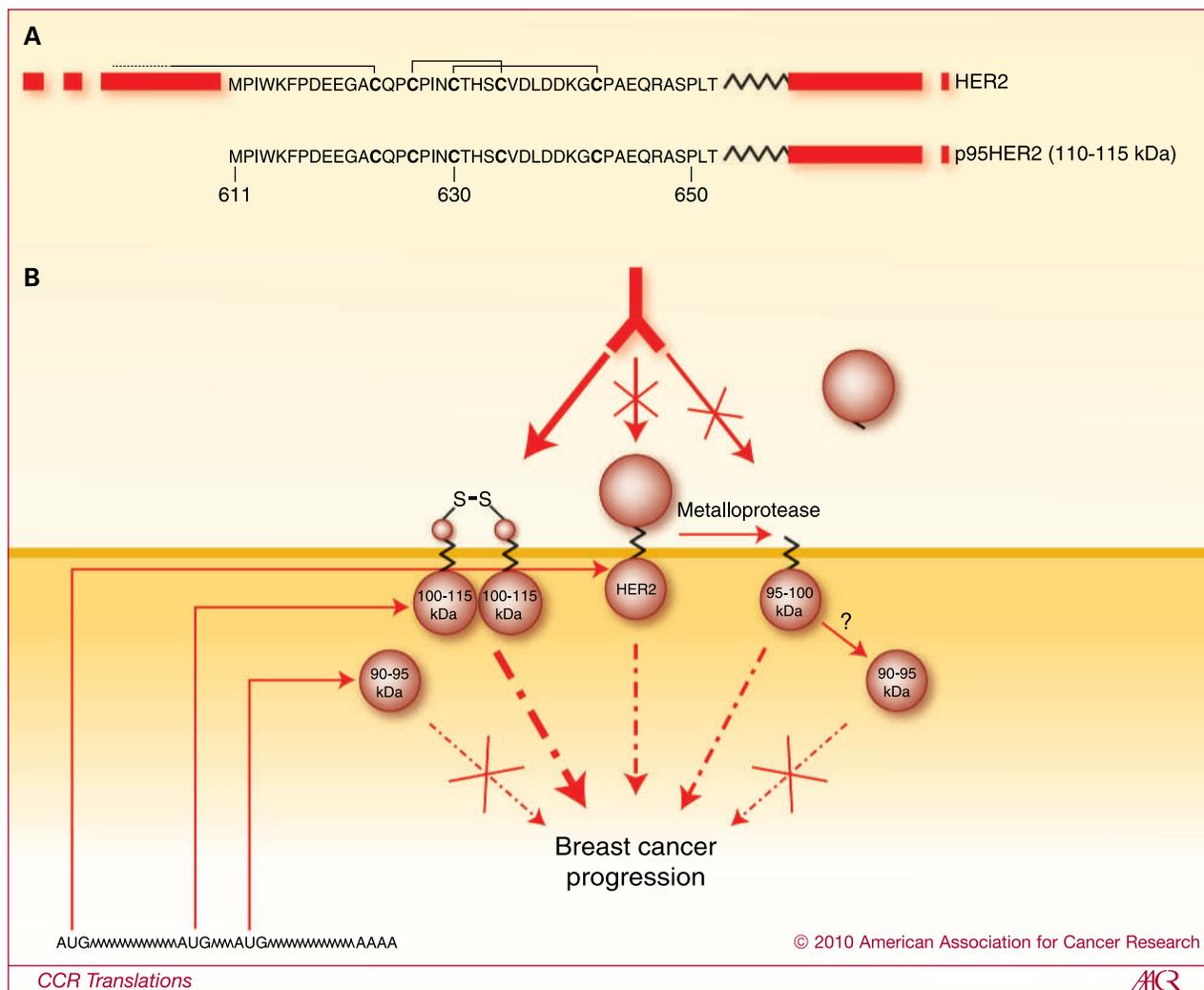
Within the full-length HER2 molecule, the cysteine-rich membrane-proximal region is highly structured and stabilized by intramolecular disulfide bonds (Fig. 1A; ref. 11). In contrast, the same region is likely unstructured in p95HER2, and some of the cysteines are known to establish intermolecular disulfide bonds (9). Thus, presumably, the tridimensional structure of the 42 extracellular juxta-membrane amino acids is different in HER2 and p95HER2 (Fig. 1A). This well-founded hypothesis prompted Sperinde and colleagues (1) to develop antibodies against peptides corresponding to the extracellular minidomain

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**Fig. 1.** A, primary sequence of the cysteine-rich, membrane-proximal regions of full-length HER2 (top) and p95HER2 fragment of 100 to 115 kDa (bottom). The intramolecular disulfide bonds established in the full-length receptor are marked. The numbers denote the position of the amino acids in full-length HER2. B, the intracellular soluble 90 to 95 kDa and the transmembrane 100 to 115 kDa p95HER2 fragments, as well as full-length HER2, are synthesized by initiation of translation of the mRNA encoding HER2 from different AUG codons. The proteolytic cleavage of full-length HER2 by metalloproteases generates a 90 to 95 kDa p95HER2 fragment that could be cleaved by gamma-secretase to generate a 90 to 95 kDa p95HER2 fragment. The 100 to 115 kDa p95HER2 fragment is constitutively hyperactive because of its ability to form dimers maintained by intermolecular disulfide bonds. The activity of the 95 to 100 kDa fragment is comparable to that of full-length HER2, whereas the soluble intracellular fragments are inactive (9). The monoclonal antibody generated by Sperinde and colleagues (1) recognizes an epitope that is exposed in the 100 to 115 kDa p95HER2 fragment but not in full-length HER2 and is absent in the 95 to 100 kDa p95HER2 fragments.

of p95HER2. Confirming the hypothesis, one of these antibodies recognized an epitope that is exposed in p95HER2 but not in full-length HER2.

Once validated, the anti-p95HER2 assay was used on formalin-fixed, paraffin-embedded samples from a cohort of patients with metastatic breast cancer treated with trastuzumab. They found that most of the HER2-positive tumors (approximately 80%) expressed detectable levels of p95HER2, although the range of expression was wide (1- to 20-fold relative to negative controls). The cutoff selected by the authors classified approximately 30% of the HER2-positive tumors as "high-p95HER2" tumors.

Previous studies on p95HER2 have analyzed the presence of fragments ranging from 90 to 115 kDa via Western blot analysis. Samples were scored positive if any fragments within that range were detected at levels above a certain cutoff. Therefore, in principle, those studies did not distinguish between samples expressing only inactive p95HER2 fragments of 90 to 95 kDa and samples expressing only active p95HER2 fragments of 95 to 100 or 100 to 115 kDa, or any intermediate combination of these possibilities.

In contrast, the assay developed by Sperinde and colleagues (1) only detects the hyperactive 100 to 115 kDa

p95HER2 fragment generated by initiation of translation from methionine 611 (Fig. 1) or larger fragments. Therefore, a priori, one would expect that if the expression of the different p95HER2 fragments is not coordinated, the anti-p95HER2 assay should only find positive a subset of the samples detected as positive via Western blot analysis. However, the percentage of p95HER2 positivity described by Sperinde and colleagues (33%) is similar to that found via Western blot analysis (28%) (ref. 3). This finding indicates that despite the existence of independent mechanisms of generation, the expression of all the p95HER2 fragments is somehow coordinated, and only few if any samples express only the inactive p95HER2 fragments of 90 to 95 kDa. Confirming this conclusion, and supporting the robustness of the novel anti-p95HER2 assay, our group has recently developed a monoclonal antibody similar to the one described here. We also found correlation between expression of the p95HER2 fragments as assessed via Western blot analysis and positivity toward our anti-p95HER2 antibody in immunohistochemistry.<sup>4</sup>

The clinical data on p95HER2-positive tumors indicate that these tumors constitute a subgroup of HER2-positive tumors with distinct clinical features. As shown by Sperinde and colleagues (1), patients with high-p95HER2 tumors

had significantly shorter progression-free survival and overall survival compared with those with low-p95HER2 tumors. Because all patients were treated with trastuzumab, this result concurs with the previous study reporting the resistance of p95HER2-positive tumors to the treatment with this antibody (4). Furthermore, the high-p95HER2 tumors were twice as likely to metastasize to the lungs, and, although not statistically significant, a higher proportion had metastasized to the skin and the central nervous system (1). These observations are in full agreement with earlier publications showing that patients with p95HER2-positive tumors scored using Western blot analysis have reduced disease-free survival and higher likelihood to develop nodal metastasis (3).

In conclusion, the development of a specific anti-p95HER2 assay served as a powerful tool to validate earlier preclinical and clinical studies that had indicated that p95HER2 is, in addition to an apparent breast tumor driver, a useful biomarker to identify a subset of patients that may benefit from p95HER2-targeting therapy.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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<sup>4</sup> Parra-Palau JL, Pedersen K, Peg V, et al. A major role of 611-CTF, a carboxy-terminal fragment of HER2, in the downmodulation of the ER in HER2-positive breast cancers, submitted for publication.

#### References

1. Sperinde J, Jin X, Banerjee J, et al. Quantitation of p95HER2 in paraffin sections using a p95-specific antibody and correlation with outcome in a cohort of trastuzumab-treated breast cancer patients. *Clin Cancer Res* 2010;16:4226–35.
2. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;235:177–82.
3. Saez R, Molina MA, Ramsey EE, et al. p95HER-2 predicts worse outcome in patients with HER-2-positive breast cancer. *Clin Cancer Res* 2006;12:424–31.
4. Scaltriti M, Rojo F, Ocana A, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst* 2007;99:628–38.
5. Scaltriti M, Chandarlapaty S, Prudkin L, et al. Clinical benefit of lapatinib-based therapy in patients with human epidermal growth factor receptor 2-positive breast tumors coexpressing the truncated p95HER2 receptor. *Clin Cancer Res* 2010;16:2688–95.
6. Liu X, Fridman JS, Wang Q, et al. Selective inhibition of ADAM metalloproteases blocks HER-2 extracellular domain (ECD) cleavage and potentiates the anti-tumor effects of trastuzumab. *Cancer Biol Ther* 2006;5:648–56.
7. Xia W, Liu LH, Ho P, Spector NL. Truncated ErbB2 receptor (p95ErbB2) is regulated by heregulin through heterodimer formation with ErbB3 yet remains sensitive to the dual EGFR/ErbB2 kinase inhibitor GW572016. *Oncogene* 2004;23:646–53.
8. Yuan CX, Lasut AL, Wynn R, et al. Purification of Her-2 extracellular domain and identification of its cleavage site. *Protein Expr Purif* 2003;29:217–22.
9. Pedersen K, Angelini PD, Laos S, et al. A naturally occurring HER2 carboxy-terminal fragment promotes mammary tumor growth and metastasis. *Mol Cell Biol* 2009;29:3319–31.
10. Anido J, Scaltriti M, Bech Serra JJ, et al. Biosynthesis of tumorigenic HER2 C-terminal fragments by alternative initiation of translation. *EMBO J* 2006;25:3234–44.
11. Cho HS, Mason K, Ramyar KX, et al. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* 2003;421:756–60.

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