Trastuzumab (Herceptin), a Humanized Anti-HER2 Receptor Monoclonal Antibody, Inhibits Basal and Activated HER2 Ectodomain Cleavage in Breast Cancer Cells

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ABSTRACT

HER2 is a ligand-less tyrosine kinase receptor of the ErbB family that is frequently overexpressed in breast cancer. It undergoes proteolytic cleavage that results in the release of the extracellular domain and the production of a truncated membrane-bound fragment, p95. We show that HER2 shedding is activated by 4-aminophenylmercuric acetate (APMA), a well-known matrix metalloprotease activator, in HER2-overexpressing breast cancer cells. The HER2 p95 fragment, which appears after APMA-induced cleavage, is phosphorylated. We analyzed 24 human breast cancer specimens, and a phosphorylated Mr 95,000 HER2 band could be detected in some of them, which indicated that the truncated receptor is also present in vivo. The activation of HER2 shedding by APMA in cells was blocked with batimastat, a broad-spectrum metalloprotease inhibitor. Trastuzumab (Herceptin; Genentech, San Francisco, CA), a humanized monoclonal antibody directed at the HER2 ectodomain, which has been shown to be active in patients with HER2-overexpressing breast cancer, inhibited basal and induced HER2 cleavage and, as a consequence, the generation of phosphorylated p95. This inhibitory effect of trastuzumab was not shared by 2C4, an antibody against a different epitope of the HER2 ectodomain. The inhibition of basal and APMA-induced cleavage of HER2 by trastuzumab preceded antibody-induced receptor down-modulation, which indicated that the effect of trastuzumab on cleavage was not attributable to a decrease in cell-surface HER2 induced by trastuzumab. We propose that the inhibition of HER2 cleavage and prevention of the production of an active truncated HER2 fragment represent a novel mechanism of action of trastuzumab.

INTRODUCTION

The HER (or ErbB) family of transmembrane tyrosine kinase receptors is composed of four members, HER1 to HER4 (1). HER2, a ligand-less Mr 185,000 receptor encoded by the neu proto-oncogene, is overexpressed in 25–30% of human breast cancer and has been associated with enhanced tumor aggressiveness and a high risk of relapse and death (2). Recent evidence indicates that HER2 amplifies the signal provided by other receptors of the ErbB family by heterodimerizing with them (3). The important biological role of HER2 in the signaling network that drives epithelial cell proliferation and transformation, together with its extracellular accessibility and its overexpression in some human tumors led to considering HER2 as an appropriate target for tumor-specific therapies. Several MAbs directed against HER2 ectodomain that specifically inhibit the growth of tumor cell lines overexpressing HER2 have been developed. One of them, 4D5 (4), was humanized and the resulting antibody was termed trastuzumab (Herceptin; Ref. 5). Trastuzumab has antitumor activity against HER2-positive human breast tumor cells in laboratory models (6) and is active for the treatment of women with HER2-overexpressing breast cancers (7–9). On the basis of trastuzumab clinical efficacy, this antibody was approved in 1998 for clinical use for HER2 overexpressing metastatic breast cancer. Trastuzumab seems to exert its antitumor effects by several mechanisms that are not yet completely understood (10). In HER2 overexpressing cells, trastuzumab markedly down-regulates HER2 expression by accelerating receptor endocytosis and degradation (11) and inhibits cell cycle progression by inducing the formation of p27Kip1/Cdk2 complexes (10, 12). Trastuzumab also induces antibody-dependent cell-mediated cytotoxicity against the HER2 expressing tumor cells in animal models. This process is regulated by antibody receptors FcγRIII and FcγRIIB on myeloid cells (10, 13). Other additional mechanisms that have been proposed include suppression by trastuzumab of angiogenesis (14) and metastasis (10).

The full-length HER2 receptor (p185) undergoes a slow proteolytic cleavage in HER2-overexpressing tumor cells in culture, and the resulting Mr 110,000 receptor ECD can be detected in the conditioned medium (15–17). Proteolytic cleavage also generates a Mr 95,000 NH2-terminally truncated membrane-associated fragment with in vitro kinase activity (18). HER2 ECD can be detected in the serum of breast cancer patients (19–22), and HER2 p95 has been described as present in some breast tumors (18), which indicates that HER2 ECD shedding actually occurs in vivo. This process may be of clinical importance, because high serum levels of HER2 ECD correlate with a poor prognosis and decreased responsiveness to endocrine therapy and chemotherapy in patients with advanced breast cancer (19–22). The potentially enhanced signaling activity of HER2 p95 offers a possible explanation for this finding. This view is suggested by studies showing that an engineered deletion of HER2 ECD increases the tyrosine kinase activity and transforming efficiency of the resulting truncated protein (23, 24). In this respect, it is interesting to note that, in the only study published thus far on p95 expression in breast cancer, the presence of this receptor fragment was associated with the presence of lymph-node metastasis (18).

The ectodomain of many cell membrane proteins, including growth factor receptors, can be released from the cell surface by a general shedding system activatable by protein kinase C (25–27). The proteolytic component(s) of the system are metalloproteinases of the so-called ADAM family of disintegrins and metalloproteases, such as TACE. HER4 is the only member of the ErbB family that has been demonstrated to be shed by this system. One of its two isoforms, named JM-a, undergoes ectodomain cleavage in a process dependent on TACE that can be activated by phorbol esters (28). The resulting membrane-anchored cytoplasmic domain retains tyrosine kinase activity and is degraded by the proteasome activity (29). The shedding of the rest of the receptors of the HER family is not so well characterized. However, in a previous report (30), we have demonstrated that HER2 cleavage is inhibited by the broad-spectrum metalloproteinase inhibitors EDTA, TAPI-2 and BB-94, but it is not significantly activated by protein kinase C. Further evidence that HER2 cleavage is not mediated by TACE is our finding that the tissue inhibitor of...
metalloproteases TIMP-1, a matrix metalloproteinase inhibitor that does not inhibit TACE (31), prevents HER2 shedding (30).

The study presented here aimed to further characterize HER2 shedding, and specially to determine the possible effects of trastuzumab on this process. We found that APMA, a mercurial compound used to activate matrix metalloproteases in vitro, induced shedding of HER2, which resulted in the generation of a phosphorylated p95 intracellular-bound receptor fragment. Trastuzumab was able to effectively block basal and induced HER2 cleavage, and this property was not shared by 2C4, another antibody against the HER2 ectodomain. Finally, analysis of human breast tumors revealed the presence of some of them of a phosphorylated HER2 fragment with a Mr ~95,000, which indicated that a truncated phosphorylated receptor is also present in vivo. Our results suggest that the inhibition of HER2 cleavage by trastuzumab could contribute to the antitumor properties of this antibody.

MATERIALS AND METHODS

Materials. APMA was purchased from Sigma Chemical Co. (St. Louis, MO). Pervanadate was prepared according to Codony-Servat (30). Trastuzumab and antibody 2C4 were kindly provided by Genentech, Inc. (South San Francisco, CA) and BB-94 by British Biotech Pharmaceuticals, Ltd. (Oxford, United Kingdom).

Tumor Cell Lines. The two breast adenocarcinoma cell lines used in this study, BT-474 and SK-BR-3, were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in DMEM/F12 supplemented with 10% fetal bovine serum, 2 mM glutamine, and, in the case of BT-474, 10% fetal bovine serum, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glutamine and, in the case of BT-474, 2 mM glu.

RESULTS

APMA Induces HER2 Shedding. Our previous data indicated that HER2 cleavage is catalyzed by a metalloproteinase (30). To find a specific way to induce receptor shedding, we assayed the effects of APMA, a well-known matrix metalloproteinase activator (32). Two HER2-overexpressing breast cancer cell lines were used, BT-474 and SK-BR-3. HER2 cleavage was analyzed by Western blot and by metabolic labeling followed by immunoprecipitation of cell lysates and medium; both techniques gave the same qualitative results. Exposure of cells to 0.25–1 mM APMA induced cleavage of HER2 p185, leading to the appearance of HER2 ECD in the culture medium and HER2 p95 in cells. This effect was time-dependent, reaching its peak after a 15-min incubation (Fig. 1A). APMA concentrations lower than 0.1 mM did not induce detectable HER2 cleavage after a 15-min incubation, whereas the effect of concentrations higher than 0.25 mM was almost dose-independent (Fig. 1B). The truncated p95 receptor was undetectable under basal conditions or at APMA concentrations that did not induce HER2 shedding, probably because of the small amounts of p95 present under these conditions. The APMA concentrations that were needed to activate HER2 shedding were similar to those used in vitro for matrix metalloproteinase activation, which usually ranged around 1 mM (33, 34).

To further characterize the APMA-induced cleavage of HER2, we estimated the fraction of receptor that was cleaved in the presence of

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This assay was performed in metabolically labeled cells that were chased 2 h, a time long enough to allow newly synthesized HER2 to reach the cell surface (30) and then were treated with APMA as indicated (Fig. 1C). The results were quantitated using a PhosphorImager and showed that \( \geq 20\% \) of the immunoprecipitated units of HER2 at time 0 were immunoprecipitated from the conditioned medium at 30 min of APMA treatment. Similar results were seen in an independent experiment. Metabolic labeling was preferred to Western blotting to measure cleavage rate because Western blot detects total HER2, including forms of HER2 that are potentially nonaccessible to the active metalloprotease(s) (i.e., in the early secretory pathway or internalized and recycling receptor) and would result in an underestimation of HER2 cleavage. Indeed, the fraction of cleaved HER2 appeared to be less in Western blot assays (see Fig. 1, A and B). This apparent lower percentage compared with the \( \geq 20\% \) in metabolic labeling measurements suggests that HER2 cleavage occurs preferentially, or perhaps only, at the cell surface, as reported for most of the cleavable transmembrane proteins (Ref. 27; compare Fig. 1, C and D).

**BB-94 Inhibits APMA-induced HER2 Shedding.** To rule out the possibility that HER2 cleavage was a nonspecific consequence of the toxicity of APMA, the effects of BB-94, a hydroxamic acid-based metalloproteinase inhibitor (35), on HER2 shedding were analyzed. BB-94 was able to completely block basal HER2 cleavage, as previously reported (Ref. 30 and data not shown). In addition, BB-94 inhibited APMA-induced (Fig. 1D) HER2 cleavage in a dose-dependent manner. These results strongly support the conclusion that HER2 shedding was induced by APMA via the activation of a metalloproteinase inhibitable by BB-94.

**Trastuzumab Inhibits Basal and Induced HER2 Shedding.** Trastuzumab (Herceptin) is an anti-HER2 MAb that inhibits growth of HER2-overexpressing breast tumors (6). Because HER2 cleavage and ECD release have been associated with a more aggressive behavior in breast cancer, we decided to determine whether trastuzumab has an effect on HER2 shedding. We found that the addition of the MAb (10–100 nM) to the culture medium significantly reduced ECD appearance in as little as 30 min in BT-474 and SK-BR-3 cells (Fig. 2A). Another antibody directed against HER2 ectodomain, 2C4, did not significantly alter ECD release, even when used at concentrations as high as 500 nm, which demonstrated that the effect of trastuzumab on HER2 ECD cleavage is not a common property of all of the anti-HER2 ectodomain antibodies (Fig. 2B). Trastuzumab also inhibited APMA-induced HER2 shedding, being able to reduce both the release of ECD into the medium and the appearance of HER2 p95 in SK-BR-3 and BT-474 cells (Fig. 2C). This effect could be observed in as little as 15 min (Fig. 2C). Similar results were obtained in the case of HER2 cleavage induced by pervanadate (not shown).

Trastuzumab induces receptor down-modulation, reducing the amount of HER2 in the cell membrane accessible to proteolytic cleavage (11). To test whether down-modulation of HER2 could explain the effect of trastuzumab on HER2 shedding, we performed a series of experiments using biotin labeling and metabolic labeling to follow the levels of cell surface HER2 in BT-474 cells in the presence of trastuzumab. First, we found that the antibody at 10 and 100 nM had no significant effect on membrane HER2 after a 30-min incubation. Trypsin, used as a positive control, induced an almost complete disappearance of HER2 from the cell membrane (Fig. 3A). The inhibition of APMA-induced receptor shedding by trastuzumab could

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**Fig. 1.** Effects of APMA and BB-94 on HER2 shedding in BT-474 cells. A, kinetics of APMA-induced HER2 ECD secretion and HER2 p95 production. Cells growing in six-well plates were treated at 37°C with 1 mM APMA in 0.2 ml of serum-free medium, and cell lysates (20 μg of protein) and medium (50 μl) were analyzed by Western blotting. Control cells were kept for 20 min in serum-free medium in the absence of the mercurial compound. B, effects of different APMA concentrations on HER2 shedding. Cultures were treated with APMA for 15 min in 0.2 ml of serum-free medium, and cell lysates (20 μg of protein) and medium (50 μl) were analyzed by Western blot. C, BT474 cells were labeled for 3 h with 500 μCi/ml of \(^{35}\)S-translabeled in methionine- and cysteine-free medium. The label was monitored 2 h in complete medium. Then, 1 mM APMA was added for the indicated times. D, effects of BB-94 on APMA-induced HER2 cleavage. Cells were treated with APMA and/or BB-94 for 15 min in 0.3 ml of serum-free medium, and cell lysates (20 μg of protein) and medium (50 μl) were analyzed by Western blotting. BB-94 was added just before (~30 s to 1 min) APMA. Preincubation with BB-94 for longer times was not deemed necessary because protease inhibitors act immediately, and long exposures to them may result in nonspecific effects (26). kDa, M, in thousands.

**Fig. 2.** Effects of trastuzumab on HER2 shedding. A, effects of trastuzumab on basal HER2 shedding in BT-474 and SK-BR-3 cells. Cells were treated with trastuzumab in 0.3 ml of serum-free medium for 30 min. Media were concentrated to 50 μl. The entire volume in the case of SK-BR-3 and 25 μl for BT-474 were each subjected to Western blot. B, 2C4 does not inhibit basal HER2 shedding in BT-474 cells. Cultures were incubated with 2C4 or with trastuzumab for 2 h in 0.3 ml of serum-free medium, which was afterward concentrated to 50 μl and analyzed by Western blot. C, trastuzumab inhibits APMA-induced HER2 cleavage in BT-474 and SK-BR-3 cells. Serum-free medium containing APMA and/or trastuzumab was added to cultures; and after 15 min, cells were lysed and medium (50 μl) and lysates (100 μg in the case of SK-BR-3 and 20 μg for BT-474 cells) were subjected to Western blot analysis. Media were assayed with no prior concentration. Film exposure times were 5 min for SK-BR-3 medium, 1 min for BT-474 medium, and 8 min for lysates.
INHIBITION OF HER2 SHEDDING BY TRASTUZUMAB

Fig. 3. The effects of trastuzumab on HER2 shedding are not a consequence of receptor down-modulation. A, trypsin, but not trastuzumab, reduces plasma membrane HER2 levels after a 30 min-treatment in BT-474 cells. Cells were treated in serum-free medium for 30 min, and cell membrane proteins were biotinylated as described in “Materials and Methods.” After cell lysis, aliquots containing 150 μg of protein were precipitated with streptavidin-Sepharose and resuspended in 150 μl of SDS-PAGE loading buffer. Lysates samples containing 2.5 μg of protein and 15 μl aliquots of precipitates were subjected to Western blot analysis. B, trastuzumab inhibits HER2 cleavage well before inducing receptor down-modulation. BT-474 cells were cultured in serum-free medium in the absence or presence of trastuzumab. Media were collected, concentrated to 50 μg, and subjected to Western blotting. Cells were labeled with biotin and lysed, cell membrane proteins were precipitated with streptavidin-Sepharose as described above, and cells were resuspended in 150 μg of buffer. Lysates (2.5 μg) and precipitates (15 μl) were analyzed by Western blotting.

be seen as early as 10 min after initiation of the treatment (data not shown). At this time point, there was no evidence of any detectable amount of HER2 internalization induced by trastuzumab. A time course experiment in biotin-labeled cells demonstrated that trastuzumab did not significantly reduce membrane HER2 after as much as 6 h and showed an effect only after a 24-h incubation of the cells with the antibody (Fig. 3B), whereas the inhibition of ECD release was observed at all times tested. Similar results were seen in metabolic-labeling experiments (data not shown). Because ~20% of cell surface HER2 was cleaved by APMA, if the inhibition of HER2 cleavage by trastuzumab was dependent on receptor internalization, this level of cell surface HER2 down-regulation (~20%) should have been detected in the two experimental approaches that assayed cell surface HER2 or in prior work (11). Taken together, these results show that trastuzumab reduces HER2 shedding well before it induces any detectable decrease in cell surface HER2, which supports the observation that it has a direct inhibitory effect on HER2 shedding that is not mediated by receptor down-modulation.

HER2 p95 Is Tyrosine-phosphorylated in APMA-induced BT-474 Cells. The tyrosine phosphorylation of p95 was examined by treating BT-474 cells with APMA and blotting the lysates with an antibody against phosphorylated HER2. A phosphorylated band of M\textsubscript{r} 95,000 was detected in all of the tumors analyzed, always elevated in comparison to paired normal samples. Five of the tumors also showed a phosphorylated band of M\textsubscript{r} 95,000 (Fig. 5B). These tumors were those expressing the highest levels of p95.

DISCUSSION

The results presented here demonstrate that trastuzumab has a direct inhibitory effect on basal and activated HER2 proteolytic cleavage. We have found that treatment of HER2-overexpressing breast cancer cells with trastuzumab inhibits basal HER2 ECD release. In contrast, another MAb directed against HER2 ectodomain, 2C4, does not have any significant effect on HER2 shedding. This finding indicates that the effect of trastuzumab on HER2 ectodomain cleavage is not a common property shared by all anti-HER2 ectodomain MAbs. Trastuzumab inhibits HER2 shedding well before it induces any
decrease in cell surface receptor levels. Overall, these data indicate that trastuzumab has a direct inhibitory effect on HER2 shedding, probably acting by steric hindrance, blocking the cleavage site of the receptor. In this respect, it is particularly interesting to note that the epitope recognized by trastuzumab is near the HER2 transmembrane domain, where the cleavage site of the receptor is located, whereas the epitope for MAb 2C4 is very discontinuous in the amino acid sequence and the two antibodies do not cross-block (4). Our results provide the first evidence that an antibody directed against the ECD of a membrane protein can block the proteolytic cleavage of the protein.

To find a specific way to activate HER2 shedding and further study this process, we assayed the effects of the general metalloproteinase inhibitor APMA. APMA was found to induce HER2 shedding in breast cancer cells overexpressing this receptor in a time- and dose-dependent manner. The induction of HER2 cleavage by APMA could be completely blocked by BB-94, a broad-spectrum metalloprotease inhibitor. In a previous report (30), we had already demonstrated that BB-94 also blocks basal HER2 cleavage. Trastuzumab was also found to effectively block APMA-induced HER2 shedding and, therefore, HER2 ECD release and p95 generation. Hence, the antibody retains its ability to inhibit receptor cleavage even when the shedding machinery that acts on HER2 is up-regulated. The degree of effect of trastuzumab on released ECD versus the truncated intracellular fragment was difficult to compare because they are recognized by different antibodies. However, the production of cell-associated HER2 fragment was usually markedly reduced or suppressed concomitantly with the inhibition of ECD release. Further research is needed to identify possible physiological activators of HER2 shedding and to determine whether trastuzumab can also block their effects. In this respect, preliminary data suggest that heregulin, an ErbB ligand that is frequently expressed in breast tumors, stimulates HER2 cleavage (36).

Christianson et al. (18) have reported that the membrane-bound HER2 p95 fragment present in breast tumor cell lines in basal conditions is phosphorylated and has kinase activity. We found that the p95 truncated fragment generated on APMA-induced HER2 cleavage in tumor cells in culture is also phosphorylated. Because APMA is a cystein reactive agent that targets other proteins in addition to metalloproteases, the explanation for the increased phosphorylation of full-length HER2, present after APMA treatment, may possibly include the targeting and inactivation of phosphotyrosine phosphatases. Another possibility, which would not exclude the prior one, is that the phosphorylated cytoplasmic p95 HER2 fragment that arises after receptor cleavage may dimerize with the full-length receptor and contribute to the increased phosphorylation of HER2 that follows APMA treatment. The presence of trastuzumab in the culture medium inhibited the appearance of the HER2 p95 phosphorylated band, as a consequence of the prevention of receptor shedding. It is attractive to hypothesize that the inhibition of HER2 cleavage by trastuzumab may contribute to its therapeutic value by preventing the formation of the potentially deleterious intracellular HER2 p95 kinase fragment that arises from receptor cleavage.

The ECD of several membrane-anchored proteins, including some tyrosine-kinase receptors such as HER2, can be released as a soluble fragment by the action of a cell surface endoproteolytic system. Substantial evidence has demonstrated that the ectodomain of this class of receptors acts as a molecular “brake” of the intracellular kinase activity. Some retroviral receptor oncogenes code for oncoproteins that lack most of the ECD and give rise to constitutively active membrane-bound receptor fragments (37). Moreover, the in vitro deletion of the ectodomain of several receptor tyrosine kinases including HER2 (23, 24), confers transforming potential to the mutated receptor. In the case of the neurotrophin receptor TrkA, ectodomain cleavage generates a cell-associated fragment with increased phosphotyrosine content and probably higher catalytic activity (38). Finally, ectodomain shedding of HER4 results in the formation of a membrane-truncated fragment that has tyrosine kinase activity, is tyrosine phosphorylated, and may act as a membrane-localized docking molecule for signaling molecules with SH2 domains (29). Taken together, these data suggest that proteolytic cleavage of the ECD constitutes a ligand-independent mechanism for the activation of tyrosine kinase receptors via the generation of a cell-bound receptor fragment with constitutive kinase activity. This mechanism could be relevant in the case of HER2 for several reasons. First, HER2 does not seem to have a natural ligand; second, receptor cleavage has been shown by us and others to be a regulated process (30, 36); and third, presence of ECD in serum (15–17) and membrane-bound truncated receptor (18) has been shown in human breast cancer and is associated with a more aggressive behavior. The prevention of HER2 ECD shedding by trastuzumab and the subsequent reduction in the generation of the HER2 p95 membrane-bound fragment could, therefore, represent one of the mechanisms contributing to the antitumor effects of this antibody. Further research is needed to fully understand the biology of HER2 p95, and we are presently studying whether or not it can dimerize with HER2 p185 or other ErbB receptors, and we are determining its pathway of proteolytic degradation.

To analyze whether HER2 p95 is also present and phosphorylated in human breast cancer, 24 tumor samples and paired normal tissues were analyzed by Western blotting. A HER2 band Mr 95,000 was also apparent in tumor samples with the highest p95 levels. It is, therefore, possible that the truncated receptor is phosphorylated, and may act as a membrane-localized docking molecule for signaling molecules with SH2 domains (29). Taken together, these data suggest that proteolytic cleavage of the ECD constitutes a ligand-independent mechanism for the activation of tyrosine kinase receptors via the generation of a cell-bound receptor fragment with constitutive kinase activity. This mechanism could be relevant in the case of HER2 for several reasons. First, HER2 does not seem to have a natural ligand; second, receptor cleavage has been shown by us and others to be a regulated process (30, 36); and third, presence of ECD in serum (15–17) and membrane-bound truncated receptor (18) has been shown in human breast cancer and is associated with a more aggressive behavior. The prevention of HER2 ECD shedding by trastuzumab and the subsequent reduction in the generation of the HER2 p95 membrane-bound fragment could, therefore, represent one of the mechanisms contributing to the antitumor effects of this antibody. Further research is needed to fully understand the biology of HER2 p95, and we are presently studying whether or not it can dimerize with HER2 p185 or other ErbB receptors, and we are determining its pathway of proteolytic degradation.
breast cancer cells in culture by APMA via the activation of a metalloproteinase(s) that is inhibited by BB-94. A truncated membrane-bound phosphorylated HER2 p95 fragment appears on APMA-induced receptor cleavage. Trastuzumab, a MAb that is effective in the therapy of breast tumors that overexpress HER2, has a direct inhibitory effect on basal and APMA-induced HER2 shedding. In contrast, 2C4, another MAb against HER2 ectodomain, does not show any effect on HER2 cleavage. Finally, a p95 HER2 fragment is present and phosphorylated in some human breast cancer tumors, which indicates that HER2 cleavage and the subsequent generation of a truncated fragment with potential signaling activity also occurs in vivo. Our findings suggest that the inhibition of HER2 shedding by trastuzumab may be one of the mechanisms responsible for the antitumor effects of this MAb.

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REFERENCES


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