Mechanism of action of anti-HER2 monoclonal antibodies

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Summary
The search for new methods of treating cancer, combined with advances in our understanding of carcinogenesis, molecular biology and technology, has resulted in the development of novel biologic agents with proven clinical efficacy. One such agent is trastuzumab (Herceptin), a humanized monoclonal antibody that targets the human epidermal growth factor receptor-2 (HER2). HER2 is a member of a family of receptors that interact with each other and various ligands to stimulate various intracellular signal transduction pathways involved in cell growth control. HER2 is overexpressed in 20%-30% of women with breast cancer and is associated with aggressive tumor characteristics and poor prognosis. Trastuzumab is the first humanized monoclonal antibody to be approved for therapeutic use and the first oncogene-targeted treatment with proven survival benefit in women with HER2-positive metastatic breast cancer. However, its mechanism of action has not been fully characterized and appears to be complex. This paper reviews current knowledge of the mechanism of action of trastuzumab, including HER2 protein downregulation, prevention of HER2-containing heterodimer formation, initiation of G1 arrest and induction of p27, prevention of HER2 cleavage, inhibition of angiogenesis, and induction of immune mechanisms. The significance of these mechanisms for selection of concomitant chemotherapy is also considered.

Key words: HER2, mechanism of action, monoclonal antibodies, signal transduction, Herceptin, trastuzumab

Introduction
Our approach to the management of cancer has developed considerably over the past 40 years. In the case of breast cancer, this resulted in the switch from radical mastectomy to breast-conserving therapy, which have equivalent rates of disease control [1], and the introduction of adjuvant chemotherapy and hormonal therapy, which reduce the incidence of recurrence and death [2-4]. New chemotherapeutic agents and regimens have further improved the outcome for patients with breast cancer. However, there is a demonstrable need for more effective agents with fewer and less-severe side effects: patients with metastatic breast cancer treated with traditional regimens such as cyclophosphamide–methotrexate–5-fluorouracil (5-FU) and 5-FU–doxorubicin–cyclophosphamide have 10-year disease-free survival rates as low as 3% [5].

The recognition that traditional regimens fail to produce long-term survival in many patients with breast cancer has been the stimulus for the development of many new chemotherapeutic agents, including taxanes, and new dosing schedules and formulations that attempt to maximize efficacy while minimizing toxicity (reviewed in Fornier et al. [6]) [7]. However, one of the most important recent developments in cancer therapy is our improved understanding of the factors and pathways that control cell growth and differentiation, and how dysregulation or abnormalities of these produce cancer. This understanding has in turn led to the identification of many new potential targets for therapy [8]. Simultaneously, improvements in technology have produced powerful methods of producing novel agents that can specifically target cellular factors or pathways, for example, the ability to produce monoclonal antibodies (MAbs) [9].

A number of biologic and immunologic approaches to the treatment of breast cancer are now in various stages of clinical development (for a review see Hortobagyi et al. [8]). One such novel approach that is currently approved for the treatment of breast cancer is the humanized anti-HER2 MAb trastuzumab (Herceptin) [10]. Trastuzumab is the product of research into the effects of the human epidermal growth factor receptor-2 (HER2) on breast cancer development and prognosis. The HER2 gene is a proto-oncogene located on chromosome 17 [11, 12] that encodes a 185kDa transmembrane tyrosine kinase receptor [11, 13]. Slamon et al. [14] demonstrated that amplification of the HER2 gene and overexpression of the encoded receptor, observed in approximately 20%-30% of women with breast cancer, correlate with decreased median survival duration in breast cancer; women with tumors with HER2 amplification/overexpression survive a median of three years whereas those in whom HER2 levels are normal survive a median of six to seven years. Further studies suggested that HER2 amplification/overexpression has a role in the pathogenesis of breast cancer [15].
Subsequently, Benz et al. [16] and Chazin et al. [17] demonstrated that transfecting the HER2 gene into human breast tumor cell lines increases DNA synthesis by 50%–75%, cell growth rate by 30%–50%, growth in soft agar by 225%, tumorigenicity by up to 400% and metastatic potential by 220%. Furthermore, the growth of HER2-overexpressing tumor cells, but not of cell lines expressing normal HER2 levels, has been shown to be inhibited by anti-HER2 MAbs [18–21]. This finding indicated that anti-HER2 MAbs had potential in the treatment of breast cancer, and ultimately led to the development of the humanized anti-HER2 MAb trastuzumab from the murine anti-HER2 MAb 4D5 [22].

Trastuzumab has proven anti-tumor effects in vitro and in animal models [23, 24], but most importantly produces significant survival benefit both alone and in combination with various chemotherapeutic agents in women with HER2-positive metastatic breast cancer [25–27].

Despite the clinical efficacy of trastuzumab, the mechanism(s) of action of this and other anti-HER2 MAbs is not yet completely understood. However, the HER2 protein has certain features that may explain why it appears to have an important role in cancer pathogenesis and how anti-HER2 MAbs exert their effects.

HER2 is a member of a family of four closely related receptors (HER1, HER2, HER3 and HER4) that exist in various combinations in many tissues [28, 29]. These receptors form heterodimers that interact with a variety of ligands to stimulate intracellular signaling pathways [28]. The precise combination of receptors in a heterodimer and the ligand with which the heterodimer interacts determine which of a variety of signaling pathways is stimulated, resulting in complex control of cell growth [30, 31]. The presence of HER2 in a heterodimer appears to increase its affinity for ligand binding and decrease the rate of internalization of the heterodimer-ligand complex [32, 33]. Thus, intracellular signaling from such complexes may be of longer duration than signaling from other heterodimer complexes. Furthermore, HER2 homodimers appear to be constitutively active [34]. When the HER2 gene is amplified, cell membrane levels of the HER2 protein increase by up to 100-fold [35], resulting in HER2 homodimerization and constitutive activation.

Based on these observations and experiments in vitro and in animal models, a number of possible mechanisms of action for anti-HER2 MAbs have been proposed, and many or all of these are likely to contribute to the activity of trastuzumab. Such mechanisms include HER2 protein downregulation, prevention of HER2-containing heterodimer formation, initiation of G1 arrest, induction of p27, prevention of HER2 cleavage, inhibition of angiogenesis, and induction of a host immune response. The evidence for each of these mechanisms is reviewed and their impact on the activity of combination therapies including trastuzumab and various chemotherapeutic agents considered.

Using tumor cell lines transfected with the HER2 gene, Drebin et al. [18] and Hudziak et al. [19] have demonstrated that anti-HER2 MAbs reduce the amount of HER2 protein expressed on the cell surface (Figure 1). This MAb-induced downregulation of HER2 is a result of accelerated endocytic degradation [36, 37] and is associated with reversion of the transformed phenotype produced as a result of HER2 transfection [18].

The mechanism by which anti-HER2 MAbs induce HER2 downregulation from the cell surface is unclear, although it is shared by other anti-growth factor receptor antibodies such as antibodies against the EGFR [38]. At this point it is unclear whether HER2 downregulation is associated with a specific state of receptor phosphorylation. MAb binding to HER2 has been shown both to induce [36, 39] and inhibit [40] tyrosine auto-phosphorylation of the receptor. Interestingly, Stancovski et al. [41] have shown that although different anti-HER2 MAbs increase HER2 turnover, they have different effects on HER2 phosphorylation in vitro and that these effects do not correlate with anti-tumor activity in vivo.

Kumar et al. [40] demonstrated that anti-HER2 MAbs reduce HER2 phosphorylation to a greater extent than they downregulate cell-surface HER2 levels. They suggest that this could be due to interference with the phosphorylation induced by ligand binding. However, it is also possible that anti-HER2 MAbs produce decreases in HER2 levels, decreasing homodimer numbers and thus phosphorylation levels. Phosphorylation levels would decrease faster than HER2 levels because HER2 homodimer numbers are likely to reduce in proportion to HER2 density rather than absolute numbers.

Based on these and other data, Slawkowska et al. [42] have concluded that anti-HER2 MAbs induce HER2 homodimerization and autophosphorylation, but that this does not result in stimulation of downstream pathways. Instead, HER2 is downregulated from the cell surface, perhaps via a receptor-feedback mechanism. The presence of decreased amounts of HER2 does not result in stimulation of downstream pathways, but instead, HER2 is downregulated from the cell surface, perhaps via a receptor-feedback mechanism. The presence of decreased amounts of HER2 does not result in stimulation of downstream pathways, but instead, HER2 is downregulated from the cell surface, perhaps via a receptor-feedback mechanism.
Prevention of heterodimer formation

Anti-HER2 MAbs have been shown to interfere with the stability of HER2–HER3 and HER2–HER4 heterodimers, accelerating ligand dissociation [39, 43]. Formation of heterodimers between HER2 and other members of the HER family is important for the complex control of intracellular signaling and cell growth [30, 31]. The precise HER proteins involved in a heterodimer determine ligand binding, intracellular peptide binding, receptor phosphorylation and internalization rates [44]. Furthermore, HER3 and HER4 preferentially heterodimerize with HER2 and their activity is impaired when HER2 is not present in a cell [45]. Thus, interfering with HER2 heterodimerization is likely to have significant effects on cell growth characteristics.

Initiation of G1 arrest and induction of p27

It has been shown that anti-HER2 MAbs have antiproliferative activity [21] and their effect is cytostatic rather than cytotoxic [22]. Consistent with these observations, Sliwkowski et al. [42] have demonstrated that treatment of HER2-overexpressing breast cancer cells with MAb4D5 and the humanized MAb derived from it, trastuzumab, results in an increase in the percentage of cells in G0/G1 phase (increase from approximately 67% to 77%–78%). This is accompanied by a decrease in the percentage of cells in S phase. Non-HER2-overexpressing cells do not display growth arrest when treated with MAb 4D5 or trastuzumab.

Investigations into the basis for this effect have shown that known inhibitors of the cell cycle such as p27 and p130 are induced when HER2-overexpressing cells are exposed to trastuzumab [42]. Lane et al. [46] have recently demonstrated that p27 accumulation and association with cyclin-dependent kinase-2 (cdk2) correlates with cdk2 inactivation and G1 arrest in HER2-overexpressing breast cancer cells exposed to anti-HER2 MAb4D5. Furthermore, trastuzumab may produce cytoxic effects by sensitizing HER2-overexpressing cells to tumor necrosis factor-α, part of the host-defense mechanism against tumors [42, 47].

Prevention of HER2 cleavage

The extracellular domain (ECD) HER2 can be proteolytically cleaved from the cell membrane and circulates in the serum [48, 49]. The transmembrane and intracellular portions of HER2 remain associated with the cell membrane. Elevated HER2 ECD levels correlate with poor prognosis and decreased responsiveness to hormone therapy and chemotherapy [50, 51].

Codony-Servat et al. [52] have recently demonstrated that HER2 cleavage is enhanced by pervanadate and inhibited by broad-spectrum matrix metalloproteinase inhibitors, suggesting that HER2 ECD shedding is regulated by tyrosine phosphorylation and involves protease activity. HER2 ECD shedding appears to be a constitutive process that occurs in a proportion of all breast cancers. HER2 ECD shedding is believed to result in constitutive activation of the remaining membrane-associated HER2 domains, known as p95, leading to increased signal transduction (Figure 2) [53, 54]. Cell membrane-associated p95 levels are increased in cells that overexpress the intact HER2 receptor [55], indicating that this type of stimulation of signal transduction is likely to be increased in such cells.

Initial studies suggested that HER2 ECD at high levels may bind to trastuzumab and inhibit its activity in vitro [56]. This led to the suggestion that the use of trastuzumab combined with inhibitors of HER2 ECD cleavage may be useful. However, it now appears that trastuzumab inhibits HER2 cleavage from HER2-overexpressing cells. Codony et al. [57] have demonstrated that HER2-overexpressing breast cancer cell lines treated with trastuzumab at doses up to 30 nM show down-regulation of both HER2 ECD and intact cell membrane-associated HER2 levels. However, HER2 ECD levels decrease prior to decreases in HER2 levels and decrease to a greater extent (Figure 3), indicating that trastuzumab inhibits HER2 cleavage through an as yet unidentified mechanism. Maintenance of the intact
form of HER2 on the cell surface could decrease constitutive receptor activation and signal transduction, inhibiting cell growth.

Inhibition of angiogenesis

Studies have demonstrated that treating HER2-overexpressing breast cancer cells with MAb 4D5 inhibits vascular endothelial growth factor (VEGF) production [58]. As VEGF has an important role in angiogenesis [59], which is critical to tumor growth and survival [60], inhibition of angiogenesis may contribute to the antitumor activity of anti-HER2 MAbs.

Induction of host immune response

Evidence from in vitro studies indicates that trastuzumab efficiently induces antibody-dependent cellular cytotoxicity (ADCC) against HER2-positive breast cancer cells but not against cells that do not overexpress HER2, a phenomenon that has not been observed with the murine MAb 4D5 [22, 61–64]. It appears that ADCC is due primarily to interactions with CD16 on natural killer cells and monocytes, which have high trastuzumab-coated target cell killing potency [42, 63]. Sliwkowski et al. postulate that HER2-overexpressing cells are preferentially targeted for ADCC in the presence of trastuzumab due to the strong avidity of trastuzumab binding to HER2, the high level of HER2 expression on these cells, and their greater susceptibility to cytotoxic damage than low HER2-expressing cell lines.

Trastuzumab may also induce complement-mediated tumor cell lysis. Complement activation with subsequent deposition of complement components on tumor tissue is known to occur in animal models [65, 66]. However, some tumor cells use defense mechanisms, particularly membrane complement regulatory protein (mCRP) production, to protect against complement-mediated lysis. Jurianz et al. [67] have recently shown that trastuzumab activates human complement in vitro, but that HER2-positive human breast cancer cells express mCRPs in response. Complement activation was proportional to the level of HER2 overexpression. In this study, neutralization of mCRPs enhanced lysis of breast cancer cells targeted with trastuzumab, although efficient complement-mediated cytotoxicity was not observed. Jurianz et al. concluded that complement activation may contribute to the potent in vivo anti-tumor activity of trastuzumab.

Anti-HER2 MAbs and chemotherapeutic agents

Various anti-HER2 MAbs, including TAb 250 [61] and 4D5 [68], have been shown to have synergistic anti-tumor effects when used in combination with cisplatin in vivo. These effects are specific to HER2-overexpressing cells and for anti-HER2 MAbs. The mechanism for this pharmacologic synergy is believed to be that anti-HER2 MAbs decrease DNA repair, perhaps through inhibition of signal transduction pathways, increasing the cytotoxic effects of cisplatin-induced DNA damage [68].

Pegram et al. [69] have extended this type of analysis to other chemotherapeutic drugs. This has shown that trastuzumab is synergistic in combination with cisplatin, etoposide and thiostepa, additive with anthracyclines, paclitaxel, methotrexate and vinblastine, and less than additive with 5-FU. These researchers discuss possible mechanisms for these effects and conclude that synergy is most likely to be due to interference with DNA repair because cisplatin, etoposide and thiostepa all cause DNA damage. Additive effects are probably due to the agents having independent mechanisms of action. Finally, the less than additive effects with 5-FU, an anti-metabolite, could be due to the changes in cell cycle distribution of HER2-overexpressing cells caused by trastuzumab.

The interaction between trastuzumab and doxorubicin and paclitaxel has been examined more closely, because these agents are used frequently to treat breast cancer [24]. The data show that trastuzumab in combination with doxorubicin or paclitaxel decreases tumor volume more than any agent alone (Figure 4). When complete tumor regression rates were examined, trastuzumab plus doxorubicin produced significantly greater effects than control (trastuzumab + doxorubicin, 33%; control, 6.4%; P = 0.01), whereas the tumor regression rate with trastuzumab in combination with paclitaxel was significantly greater than that with paclitaxel alone (59% vs. 17%, P < 0.04). These results reflect the clinical benefit observed in the pivotal trial examining the combination of trastuzumab plus chemotherapy [25, 27].

As a possible explanation for this observation, HER2 overexpression renders cells resistant to paclitaxel. This is supported by data showing that HER2-overexpressing cells are less likely to respond to paclitaxel than HER2-normal cells, but that this resistance can be overcome by the addition of a transcriptional repressor of
HER2 [70]. Furthermore, molecular characterization of the effects of HER2 overexpression indicates that paclitaxel resistance may be caused by upregulation of p21, which inhibits p34 kinase, part of a pathway essential to the apoptotic effects of paclitaxel [71]. Downregulation of HER2 overexpression by trastuzumab inhibits p21 formation, thus enabling paclitaxel to induce apoptosis via the p34 kinase pathway.

Conclusions

Anti-HER2 MAbs, including trastuzumab, which is available for clinical use, appear to have a complex mechanism of action. This is not surprising given the complex interactions between HER2 and the other members of the HER family, the various ligands that interact with these receptors, and the many signal transduction pathways that appear to be activated as a result of these interactions. Current data suggest that trastuzumab acts via HER2 protein downregulation, prevention of HER2-containing heterodimer formation, initiation of G1 arrest and induction of p27, prevention of HER2 cleavage, inhibition of angiogenesis, and induction of immune mechanisms. These mechanisms are important to consider when designing trials of trastuzumab in combination with chemotherapeutic agents, because while some of the effects of trastuzumab may potentiate the effects of these agents, others may reduce their effect. The necessity of such considerations has been supported by the results of clinical trials, in which the clinical effects of trastuzumab reflect preclinical observations.

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