A new therapeutic antibody masks ErbB2 to its partners

In cancer cells, the ErbB2 receptor tyrosine kinase can be activated in two ways: by overexpression or by ligand-mediated stimulation of another ErbB receptor. The ErbB2-targeting antibody trastuzumab (Herceptin) is used for treatment of metastatic breast cancer patients whose tumors overexpress ErbB2. A new structural study in this issue of *Cancer Cell* reveals how targeting ErbB2 with another antibody, pertuzumab (Omnitarg), prevents ligand-induced dimerization of ErbB2 with the other ErbB receptors. Pertuzumab's novel mode of action might offer additional therapeutic opportunities for treatment of tumors expressing ligand-activated ErbB2.

There are four ErbB receptor family members: ErbB1/EGF receptor, ErbB2, ErbB3, and ErbB4. Under normal physiological conditions, the activation of the ErbB receptors is controlled by spatial and temporal expression of their ligands, EGF-related peptides. Ligand binding induces the formation of multiple combinations of ErbB receptor homo- and heterodimers, resulting in activation of the cytoplasmic kinase domain. This in turn promotes the phosphorylation of specific tyrosine residues, leading to the stimulation of multiple signal transduction pathways (reviewed in Yarden and Sliwkowski, 2001). ErbB2 is ligandless; it functions as a coreceptor and is actually the preferred partner for the other three ErbB family members (Graus-Porta et al., 1997). In a broad spectrum of carcinomas, including breast, ovarian, gastric, and bladder, gene amplification leads to ErbB2 overexpression, which has been proposed to cause spontaneous dimerization and activation in the absence of ligand. Experimental results showing that high levels of ErbB2 transform cultured cells as well as clinical studies revealing that patients whose tumors overexpress ErbB2 tend to have a poor prognosis implied to many in the cancer field that ErbB2 would be especially suitable as a therapeutic target. While various approaches have been taken to attain this goal, targeting ErbB2's extracellular domain with the recombinant antibody trastuzumab (Herceptin) has achieved the most prominence. Trastuzumab is used for the treatment of ErbB2-overexpressing metastatic breast cancer patients. The crystal structure of ErbB2 complexed with another antibody, pertuzumab (Omnitarg), described in this issue of *Cancer Cell* (Franklin et al., 2004), sug-

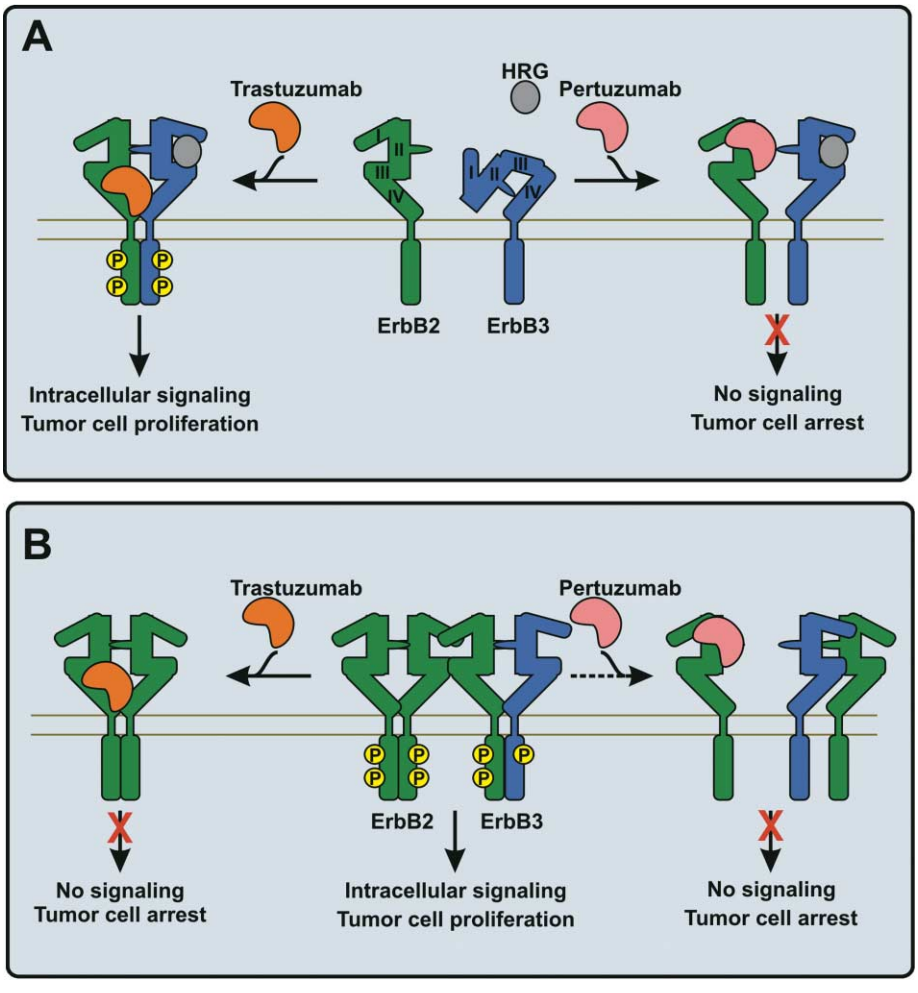

Figure 1. More than one way to inhibit ErbB2

A: In cells expressing low levels of ErbB2, HRG binding to ErbB3 induces a switch from the closed to the open state, exposing the domain II dimerization arm, which leads to the formation of ErbB2/ErbB3 heterodimers. Binding of pertuzumab to ErbB2's domain II prevents ligand-induced heterodimerization and intracellular signaling (right side). In contrast, binding of trastuzumab to domain IV of ErbB2 does not interfere with HRG-induced ErbB2/ErbB3 association and thus does not interrupt signaling activity and tumor cell proliferation (right side).

B: ErbB2-overexpressing cells show constitutive ErbB2 and ErbB3 signaling. In vivo, both antibodies have antitumor activity, which may reflect multiple mechanisms. Trastuzumab prevents ErbB2 shedding (Molina et al., 2001), induces downregulation of ErbB2 and ErbB3 signaling, and inhibits intracellular signaling (left side). While pertuzumab does not affect ErbB2 shedding, other in vivo antitumor activities are still unclear (indicated by the dotted line on the right side). It is possible that pertuzumab blocks the formation of ErbB2-containing homo- and/or heterodimers leading to tumor cell inhibition. N.B.: In ErbB2-overexpressing cells, the role of ErbB2's domain II dimerization arm in the formation of dimers has not yet been resolved.

gests that in the future trastuzumab might have to share the stage with pertuzumab. One might ask why we need two therapeutic antibodies that target the same receptor. The plethora of structural studies on ErbB receptors published in the past few years, together with the publication of Sliwkowski and colleagues in this issue, reveal how ErbB receptors are likely to dimerize and why it might be better to have two antibodies with different mechanisms of action in the therapeutic arsenal.

Publications describing the crystal structure of ErbB1, ErbB2, and ErbB3 extracellular regions (reviewed in Burgess et al., 2003) have allowed new insights into some intriguing questions concerning the process of ligand-induced receptor dimerization. Solving the structure of ErbB2 complexed with trastuzumab (Cho et al., 2003) and now with pertuzumab (Franklin et al., 2004) adds more information about domains of this receptor that are important for activity, both in the context of overexpression and ligand-induced activation. Furthermore, these structures potentially help us understand why there might be differences in clinical response to trastuzumab and pertuzumab.

The extracellular region of each ErbB receptor consists of four domains (I-IV) (Figure 1). Determination of the structure of ligand bound ErbB1 has confirmed the importance of domains I and III in peptide binding. Moreover, these studies revealed that there is a direct receptor-receptor interaction promoted by the domain II loop, the so-called dimerization arm. Interestingly, although each ligand simultaneously contacts two binding sites in the dimer, the ligand does not span the dimer; thus, unlike other transmembrane receptors, ErbB dimerization is entirely receptor mediated. The structure of ErbB2's extracellular region is dramatically different from that of ErbB1 and ErbB3. ErbB2 has a fixed conformation that resembles the ligand-activated state, i.e., the domain II-IV interaction is absent, and the dimerization loop in domain II is exposed. This might help explain why ErbB2 is the preferred partner for the other ligand-activated ErbBs (Graus-Porta et al., 1997). Furthermore, this structure explains why no ligand has been found, since it predicts that ErbB2's ligand binding site would be buried within the structure and not accessible for interaction.

Heregulin (HRG) binding to ErbB3 induces ErbB2/ErbB3 dimerization, and pertuzumab has been shown to interfere with heterodimer formation and downstream signaling (Agus et al., 2002). The model of pertuzumab bound to ErbB2 presented by Franklin et al. provides structural data that explain these observations. Pertuzumab binds to ErbB2 near the center of domain II; binding is predicted to sterically block the region necessary for ErbB2 dimerization with other ErbBs. Pertuzumab does not block signaling to ErbB3 when tested with ErbB2 mutants in residues predicted to be important for this interaction, confirming the structural data. In striking contrast to pertuzumab, trastuzumab is unable to block the formation of ErbB2-ErbB3 dimers (Franklin et al., 2004; Agus et al., 2002), likely due to the fact that trastuzumab binds ErbB2 in a region not involved in dimerization (Cho et al., 2003) (Figure 1A).

Do the differences in ErbB2 binding have an impact on the antitumor activity of the antibodies? In vivo results show that they do. Pertuzumab but not trastuzumab inhibits the growth of tumors displaying low ErbB2 levels (Agus et al., 2002), presumably due to its ability to prevent autocrine- or paracrine-produced ligands from inducing ErbB2-containing heterodimers. Importantly, pertuzumab also prevents in vivo growth of tumors overexpressing ErbB2, the same tumors that are sensitive to trastuzumab (Figure 1B). So how is pertuzumab functioning to block ErbB2-overexpressing tumors? As experimentally shown for trastuzumab (Clynes et al., 2000), some of pertuzumab's in vivo antitumor activity is likely to be due to the ability of the immunoglobulin G1 Fc region to engage Fc γ receptors on immune effector cells. However, pertuzumab may have additional activities. On the one hand, it is possible that pertuzumab disrupts ErbB2-containing heterodimers, or higher-order receptor oligomers, that form in the absence of ligand. In fact, in some ErbB2-overexpressing tumor cells, the ErbB2/ErbB3 heterodimer functions as an oncogenic unit; ErbB3 couples ErbB2 to the phosphatidylinositol 3-kinase signaling pathway. It is currently not known how ErbB2-ErbB3 heterodimers are formed in the absence of a ligand; nevertheless, in vitro, trastuzumab downmodulates ErbB2, preventing ErbB3 transactivation and tumor cell proliferation (Holbro et al., 2003). As observed in cells with low ErbB2, pertuzumab might mask ErbB2 to ErbB3 in tumor cells overexpressing the receptor, something that awaits further study. On the other hand, one cannot exclude the possibility that pertuzumab also prevents the formation of ErbB2 homodimers. Unfortunately, because of the predicted steric clash (Franklin et al., 2004) or repulsive electrostatic charges (Garrett et al., 2003), ErbB2's extracellular domain does not homodimerize in solution. This has prevented the resolution of the structure that is presumably present in ErbB2-overexpressing tumors, precluding an understanding of pertuzumab's impact on this structure.

In the clinical setting, trastuzumab is used for the treatment of breast cancer patients with ErbB2-overexpressing tumors; however, not all patients respond to treatment. While other genetic defects may allow these tumors to escape from trastuzumab, another explanation may be related to its mode of action. Indeed, since trastuzumab does not prevent heterodimerization, the formation of ligand-induced ErbB2-containing heterodimers might still be possible, something that would be unlikely with pertuzumab. Thus, the addition of pertuzumab to the therapeutic arsenal should broaden the number of ErbB2-expressing tumors that can be rationally targeted by antibodies to include tumors with low levels of ligand-activated ErbB2.

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Thymidylate synthase as an oncogene?

A surprising finding in the report by Rahman et al. in this issue of *Cancer Cell* is that forced overexpression of human thymidylate synthase transforms immortalized murine cells into a malignant phenotype. We discuss the possibility that elevated levels of thymidylate synthase noted in some human malignancies may contribute to tumor progression and may also reflect increased levels of its transcriptional activator E2F-1.

In this issue of *Cancer Cell*, Rahman et al. present data showing that high levels of thymidylate synthase (TS), generated by the transduction of immortalized mouse cell lines with human TS cDNA or by induction via a tetracycline-responsive promoter, result in transformation. By the criteria of anchorage independence and tumor formation in nude mice, they show that a high level of TS is capable of transforming these cells to malignancy. Thus, TS may be considered a protooncogene in that it is a normal protein that functions as a positive regulator of growth and is converted to an oncogene by overexpression.

Does a high level of TS expression contribute to tumor growth in patients? Studies show that most metastatic colorectal cancers have high levels of TS mRNA and protein as compared to primary colorectal cancers and that primary colorectal cancers have higher levels of TS as compared to normal colonic tissue. Several studies also show that patients with metastatic colorectal cancer with tumors that have high levels of TS are less likely to respond to treatment with 5-fluorouracil than patients with lower levels of this enzyme (reviewed by Aschele et al., 2002; Bertino and Banerjee, 2003; vanTriest and Peters, 1999). Only 20%–30% of patients have what are considered low levels of this enzyme, possibly related to deletion of a portion of one of the alleles of chromosome 18, the location of the TS gene (Bardot et al., 1991). Of interest, high TS levels in the primary tumor have been associated with a poor outcome, regardless of treatment (Edler et al., 2002).

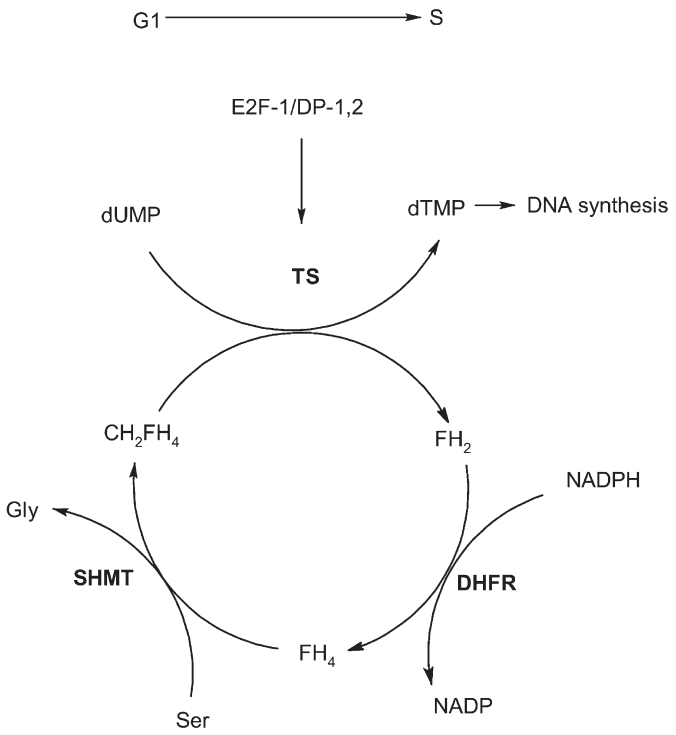

Figure 1. The thymidylate cycle

E2F-1 as the heterodimer E2F-1/DP-1 activates transcription of TS. The other two enzymes of the thymidylate cycle are dihydrofolate reductase (DHFR) and serine hydroxymethyltransferase (SHMT). FH_2, dihydrofolate; FH_4, tetrahydrofolate; CH_2FH_4, 5-10-methylene tetrahydrofolate.

The high levels of TS in some tumors have been attributed to one of two mechanisms: an increase in gene copies of TS (only 5%–7%) or increased transcriptional activation as a result of the transcription factor E2F-1 (Degregori et al., 1995). E2F-1 heterodimerizes with DP-1 and DP-2 and activates the transcription of genes encoding DNA synthetic enzymes, including TS (Banerjee et al., 1998). Interestingly, E2F-1 has itself been described as an oncogene or an oncogenic messenger. Indeed, a recent report indicates that most metastatic colorectal tumors overexpress E2F-1, associated with amplification of this gene, raising the possibility that TS is simply a player in malignant transformation attributed to E2F-1 (Iwamoto et al., 2004).

A second question that arises is whether or not the oncogenic activity that is attributed to TS is related to its enzyme activity. TS is a