A new anti-ErbB2 strategy in the treatment of cancer: Prevention of ligand-dependent ErbB2 receptor heterodimerization

Preventing ligand-dependent ErbB2 receptor heterodimers by an anti-ErbB2 monoclonal antibody shuts down receptor signaling and has potent antitumor activity even in tumors that express low levels of ErbB2, a finding that could result in a larger number of patients benefiting from anti-ErbB2 therapies.

The ErbB2 receptor (also known as HER2/*neu*) is a member of the epidermal growth factor receptor family that, in addition to ErbB2, includes the ErbB1 (or Epidermal Growth Factor Receptor), ErbB3, and ErbB4 receptors. The binding of the EGF family of ligands to ErbB receptors induces receptor activation by both receptor homodimerization and heterodimerization, thus generating a complex array of combinatorial signals. This horizontal network of interactions is crucial to the ErbB signaling pathway, since ErbB3 is devoid of intrinsic kinase activity and ErbB2 is a ligand-less receptor. Therefore, in isolation neither ErbB3 nor ErbB2 have the capacity to initiate downstream signaling. An exception to the rule is that ErbB2 can spontaneously form active ligand-less homodimers in cells overexpressing ErbB2 (for review see Yarden and Sliwkowski, 2001).

The normal function of this family of receptors and their ligands is to mediate cell-cell interactions in organogenesis and adulthood. Deregulation of the tightly regulated ErB receptor signaling pathways leads frequently to malignant transformation. In a variety of tumor types, ErbB pathways become activated by several mechanisms including overproduction of ligands, overexpression of receptors, or constitutive activation of

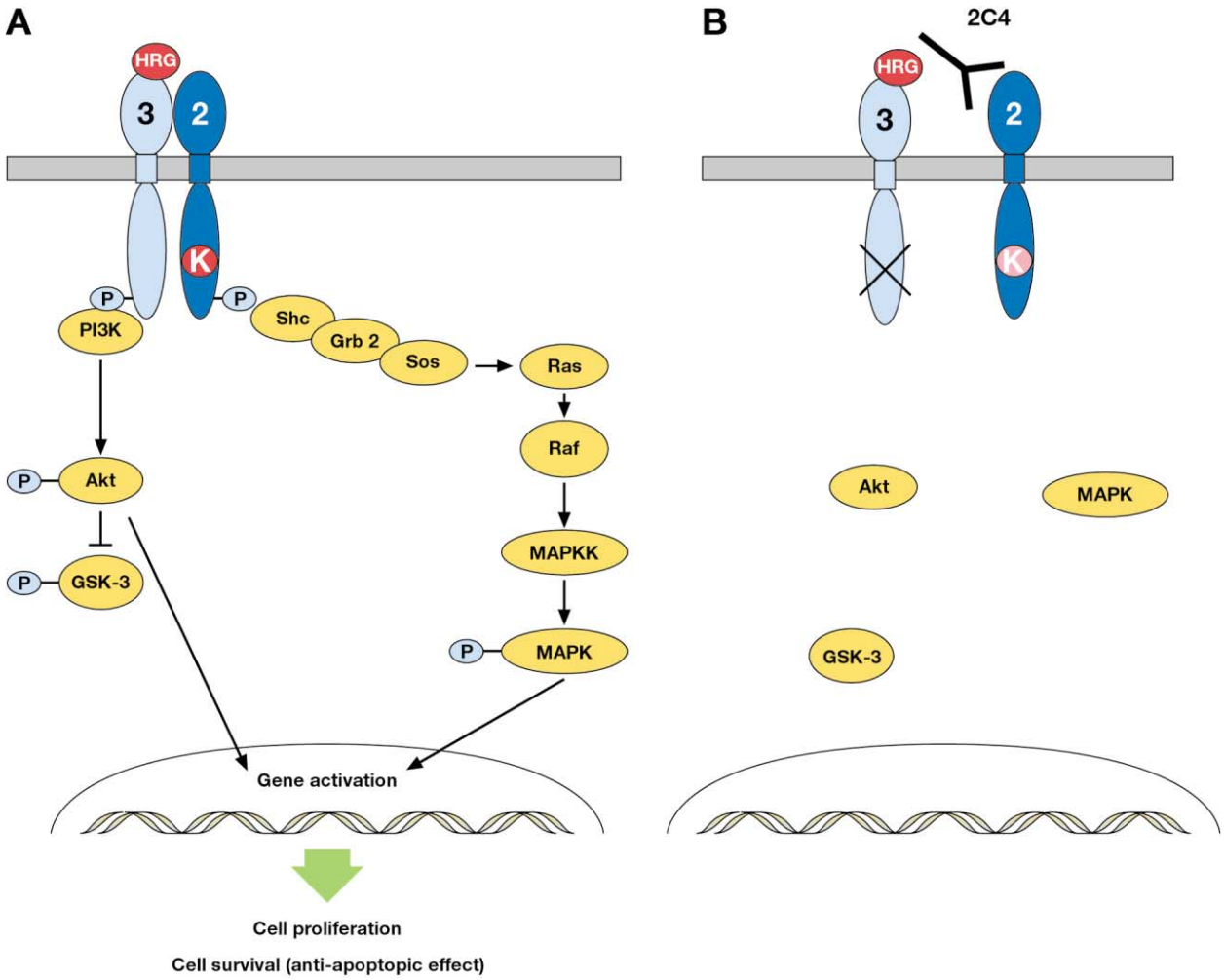

Figure 1. Prevention of ErbB2 receptor signaling by the monoclonal antibody 2C4

A: In unperturbed conditions, ErbB2 is activated by ligand-induced heterodimerization with other ErbB receptors, which in turn results in activation of the MAPK and PI3kinase/Akt pathways. **B:** The monoclonal antibody 2C4 binds to the extracellular domain of ErbB2 and sterically blocks the association of ErbB2 with other ErbB family members. As a result, it prevents downstream receptor signaling.

receptors. In particular, the ErbB2 receptor is amplified in 30% of breast cancers and its overexpression correlates with a worse prognosis (Slamon et al., 1987). The critical role of ErbB receptors in the development of solid tumors has made these receptors attractive targets for pharmacological intervention. Strategies being developed include (1) monoclonal antibodies directed at the easily accessible extracellular domain of these receptors and (2) small molecules that compete with ATP for binding to the ATP site in the receptor tyrosine kinase domain and that abrogate the receptor's catalytic activity, its receptor autophosphorylation, and its engagement with signal transducers.

The concept of targeting ErbB receptors as an anticancer strategy has already been validated in the clinic. The first agent of this new class of compounds approved for clinical use has been Herceptin®, a humanized monoclonal antibody directed at the ErbB2 receptor that has antitumor activity (Vogel et al., 2002) and that results in improved survival in patients with advanced breast cancer that have ErbB2 amplification (Slamon et al., 2001). Herceptin exerts its antitumor activity by several mechanisms including receptor downmodulation, prevention of cleavage of the receptor's extracellular domain (which leads to receptor constitutive activation), and by recruiting host's immune effector cells (Baselga et al., 2001). However, even in ErbB2-overexpressing breast tumors, Herceptin has antitumor activity in only up to one-third of patients, and it does not have any activity against tumors expressing lower levels of ErbB2. Although it is likely that other coexisting mechanisms may be responsible for the malignant growth of these tumors (and this could potentially explain their lack of response to Herceptin), it is also possible that Herceptin may not be interfering successfully with ErbB2 signaling. In fact, Herceptin is not capable of inhibiting signaling by ligand-induced ErbB2-containing heterodimers, a clearly important mechanism of receptor activation.

In this issue of *Cancer Cell*, Agus, Sliwkowski, and colleagues (Agus et al., 2002) report that the anti-erbB2 monoclonal antibody 2C4 that binds to a different epitope than Herceptin in the receptor's extracellular domain sterically blocks the association of ErbB2 with other ErbB family members. As a consequence, it prevents ligand-dependent ErbB2 signaling in both low- and high-ErbB2-expressing tumor cell lines (Figure 1). The antitumor effects of 2C4 both in vitro and in vivo are remarkable in a variety of breast and prostate carcinoma cell lines. The findings in prostate carcinoma cells lines are particularly interesting, since there is growing evidence that ErbB2 activation by ErbB ligands produced in the stroma of the tumor may play an important role in the progression to androgen-independent prostate tumors (Feldman and Feldman, 2001). In addition, 2C4 may be active and should be tested in other ErbB2-expressing tumor types such as non-small cell lung cancer, ovarian carcinoma, and bladder cancer, to name a few.

These findings are the basis for the ongoing clinical development of this compound and 2C4 in an initial (phase I) study has been found to be safe without dose-limiting toxicities, and efficacy (phase II) studies are to be begin soon in a variety of tumor types. As 2C4 is being studied in the clinic, several questions arise. First, it will be important to identify the patients that may benefit from 2C4 therapy. Will it be required to demonstrate the expression of ErbB2 or, even more complicated, the presence of ligand-induced heterodimers in the cell surface of these tumors? Will it be possible to use as pharmacodynamic markers of sensitivity to 2C4 the inhibition of some of the key downstream receptors' signal transduction pathways such as MAPK and Akt? Another important issue is whether this antibody, devoid of any immune-mediated antitumor activity, will offer any additional advantage to the orally bioavailable small-molecular-weight ErbB tyrosine kinase inhibitors that are in late stages of clinical development. These tyrosine kinase inhibitors can induce inactive ErbB1 homodimers and ErB2-containing heterodimers (Arteaga et al., 1997) and impair ligand-induced ErbB2 transactivation. Further, because of the great homology in the kinase domain between the different ErbB receptors, some ATP-competitive small molecules can block the catalytic activity of more than one receptor (reviewed in Fry, 2000), a property not shared by 2C4, Herceptin, or anti-ErbB1 receptors due to their specificity of binding to a particular receptor extracellular domain epitope. Whether this capacity to prevent kinase activation of more that one receptor will result in greater antitumor activity or, on the other hand, in unacceptable toxicity, is unknown at this time.

In summary, preventing receptor heterodimerization appears to be a promising novel approach to the therapy of ErbB2-expressing tumors, and the monoclonal antibody 2C4 does this successfully. This observation suggests that in the emerging area of anti-ErbB2 therapies, there is hope beyond Herceptin. There are many ways to attack a receptor's function and 2C4—and the small ErbB tyrosine kinase inhibitors—are valid new additions to our anti-erbB receptors armamentarium. The availability of agents with nonoverlapping mechanisms of action directed at the same receptor implies that selection of therapy may not be any longer based solely on expression of the target but rather on functional assays that predict the best antireceptor strategy for a given tumor. It may also allow for sequential anti-ErbB2 therapies, since acquired resistance to one of these agents may not result in resistance to another agent in a similar fashion as it occurs with hormonal agents in the therapy of breast cancer. Finally, it also provides an opportunity to explore combining these agents in a full-scale anti-ErbB2 war by inducing receptor downregulation and an antireceptor immune response (Herceptin) by isolating ErbB2 from its transactivating ErbB partners (2C4) and by eliminating any remaining receptor catalytic activity (small tyrosine kinase inhibitors).

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Selected reading

Agus, D.B., Akita, R.W., Fox, W.D., Lewis, G.D., Higgins, B., Pisacane, P.I., Lofgren, J.A., Tindell, C., Evans, D.P., Maiese, K., et al. (2002). Cancer Cell *2*, this issue, 127–137.

Arteaga, C.L., Ramsey, T.T., Shawver, L.K., and Guyer, C.A. (1997). J. Biol. Chem. *272*, 23247–23254.

Baselga, J., Albanell, J., Molina, M.A., and Arribas, J. (2001). Semin. Oncol. *28 (5 Suppl 16)*, 4–11.

Feldman, B.J., and Feldman, D. (2001). Nat. Rev. Cancer *1*, 34–45.

Fry, D. (2000). Anticancer Drug Des. *15*, 3–16.

Slamon, D.J., Clark, G.M., Wong, S.G., Levin, W.J., Ullrich, A., and McGuire, W.L. (1987). Science *235*, 177–182.

Slamon, D.J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., et al. (2001). N. Engl. J. Med. *344*, 783–792.

Vogel, C.L., Cobleigh, M.A., Tripathy, D., Gutheil, J.C., Harris, L.N., Fehrenbacher, L., Slamon, D.J., Murphy, M., Novotny, W.F., Shak, S., et al. (2002). J. Clin. Oncol. *20*, 719–726.

Yarden, Y., and Sliwkowski, M. (2001). Nat. Rev. Mol. Cell Biol. *2*, 127–137.

Fas function and tumor progression: Use it and lose it

Recent studies have provided evidence that Fas and FasL interactions are important in the control of malignant disease and that changes in the level of Fas expression can determine immune escape and therapeutic responses.

Imbalanced rates of apoptosis have been proposed to create a platform that is necessary and sufficient for tumor formation (Green and Evan, 2002). The host environment can influence cancer outgrowth by altering tumor gene expression, resulting in tumor proliferation and suppression of the endogenous apoptotic program. Fas and Fas ligand (FasL) are an interacting, extracellular proapoptotic receptor/ligand pair (reviewed in Nagata, 1999). Trimerization of membrane bound Fas with FasL causes recruitment of the FADD adaptor protein and procaspase-8, the key initiator caspase in the death receptor pathway. Procaspase-8 is activated by induced proximity and further activates downstream caspases and initiates cleavage of critical apoptotic substrates. Active caspase-8 also engages the intrinsic mitochondrial pathway of apoptosis through the cleavage of Bid, which translocates to the mitochondria and promotes release of cytochrome *c*.

The role of Fas-induced apoptosis in the maintenance of immune homeostasis is well established (Nagata, 1999). More recently, Fas-induced apoptosis has been implicated in the control of tumor progression and chemotherapeutic drug-induced death. Functional Fas is highly expressed on a variety of nonmalignant tissues, while Fas loss-of-function commonly accompanies the malignant phenotype. Multiple molecular mechanisms underlie Fas loss-of-function in cancer including downregulation of transmembrane Fas by promoter methylation (reviewed in Owen-Schaub et al., 2000), transcriptional repression (Ivanov et al., 2001), histone acetylation (Maecker et al., 2000), and alternative mRNA splicing to produce soluble Fas protein lacking a transmembrane anchor (reviewed in Owen-Schaub et al., 2000). Overexpression of the degenerate caspase homolog c-FLIP (Bullani et al., 2001) and inactivating Fas mutations (reviewed in Owen-Schaub et al., 2000) have also been shown to contribute to Fas loss-of-function in nonhematopoietic cancers. In several cancer types, Fas loss-of-function has been shown to track with an aggressive disease presentation and decreased patient survival. In experimental animal models (reviewed in Owen-Schaub et al., 2000), disruption of Fas has been shown to result in enhanced tumor development while Fas restoration has been shown to delay primary tumor outgrowth. The acquired ability to spread and metastasize represents the most intractable feature of cancer. Recent studies have implicated Fas and FasL interactions in the control of distant metastases (Owen-Schaub et al., 1998) as well as in the development of chemotherapeutic resistance in some cell types (reviewed in Johnstone et al., 2002). These observations suggest that Fas is a frequent target for inactivation during oncogenesis and that Fas-induced apoptosis plays a crucial role in the biology and response of malignant disease.

Both transmembrane and cleaved FasL can induce Fas clustering and initiate apoptotic cell death (Nagata, 1999). Although some nonhematopoietic tissues (retinal pigment epithelial cells and lung epithelial cells, for example) display FasL, expression is most prominent in bone marrow-derived immune cells including activated lymphocytes, neutrophils, natural killer (NK) cells, and macrophages. Conceivably, $FasL^+$ immune effectors could inhibit Fas^+ tumor survival by direct

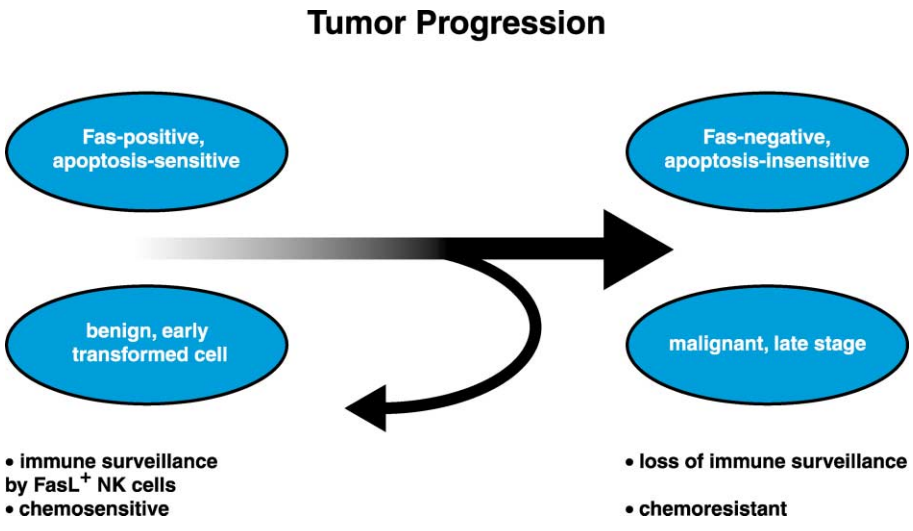

Figure 1. Model for Fas loss-of-function in tumor progression

This model is supported by the findings of Maecker et al. (2002) that Fas is important for NK cell-mediated immune surveillance and chemosensitivity.