ErbB2: From an EGFR Relative to a Central Target for Cancer Therapy

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See related article by Berger et al., Cancer Res 1988;48:1238–43.

Shortly after the discovery of the ErbB2/HER2–encoding gene, identified in the mid-1980s based on homology with the avian viral oncogene v-ErbB/EGFR (reviewed in ref. 1), publications describing ERBB2 amplification in a significant proportion of breast tumors appeared (2, 3). Indeed, ERBB2 amplification was the first consistent genetic alteration discovered in breast cancer. Moreover, data supporting the clinical relevance of ErbB2 were presented in these articles; patients with the chromosome 17q12–21 ERBB2 amplicon had dramatically elevated levels of the receptor, which was associated with an increased risk of early relapse and death. These articles, together with one from the Nussle laboratory showing that a high percentage of ductal carcinoma in situ had ErbB2 overexpression (4), paved the way for development of anticancer agents targeting ErbB2. Since then, a wealth of clinical data implicating ErbB2, not only in breast, but also in gastric, ovarian, endometrial, colorectal, and other cancer types have been published. Importantly, based on these first studies in breast cancer, ErbB2 was considered to be an excellent therapeutic target. Many laboratories in academia and in pharmaceutical companies contributed to developing ErbB2 inhibitors. Currently, there are four FDA-approved therapeutics targeting ErbB2: the humanized antibodies trastuzumab, and pertuzumab, the kinase inhibitor polymeric inhibitor conjugate (reviewed in ref. 5). The field of ErbB2 biology has exploded in the almost 30 years since the publications describing ErbB2 overexpression in breast cancer. In this short commentary, it is impossible to discuss all the important and exciting articles that have appeared during this period. I decided to discuss work that contributed to deciphering the role of ErbB2 signaling in cancer and in normal physiology, as well as important structural studies on the ErbB receptors. The clinical success of ErbB2-targeted therapies will also be mentioned and some reflections on the future of ErbB2 targeting will end the commentary.

The ErbB Receptor-Ligand Network

EGFR, ErbB2, as well as ErbB3 and ErbB4, which were discovered using homology cloning a few years after ErbB2, make up the subtype 1 of the receptor tyrosine kinase (RTK) family. Like other members of this superfamily, ErbB6s are regulated by ligand binding. In 1962, Cohen isolated the first ligand, EGF, well before its receptor was cloned. With the exception of TGFα, which was discovered by Sporn and Roberts in 1982, the other five EGFR ligands, as well as the neuregulins/hergulins, which bind ErbB3 and ErbB4, were discovered after ErbB2. All ligands have a characteristic pattern of six conserved cysteine residues forming three intramolecular disulfides, so-called EGF-like domains (reviewed in refs. 6, 7). Ligand binding to the ectodomain stabilizes receptor dimers and leads to activation of their intracellular kinase domain. This simple statement does not do justice to our current understanding of ErbB structures and how ligand binding impacts on kinase domain activation (reviewed in refs. 7–9). It is important to briefly describe these beautiful studies as they helped tremendously to decipher how therapeutics targeting the ErbB receptors function, and why they do not always live up to expectations.

EGFR ectodomain structures were published in 2002–2003 by the laboratories of Yokoyama, Garrett and Ward, as well as Ferguson and Lemmon (reviewed in ref. 8). The kinase domain structure was first published in 2002 by the Eigenbrot laboratory (reviewed in ref. 10). These studies revealed the singularity of the EGFR family. On the one hand, dimerization is mediated only by receptor–receptor contacts; the ligands are not involved, which is unique among the RTKs. In three of the unliganded receptors, ErbB2 being the exception, the ectodomain is “tethered” and the domain II dimerization arm is buried by intramolecular interactions with domain IV. Upon ligand binding, a dramatic conformational change occurs, which exposes the dimerization arm in domain II, leading to an “extended” structure that interacts with the arm of a second receptor. The intracellular kinase domain of the ligand-bound receptor is allosterically activated by asymmetric dimerization, meaning that the C-terminal lobe of the activator kinase interacts with the N-terminal lobe of the receiver kinase, which results in tyrosine phosphorylation of the relatively long carboxy-terminal tails of the receptors. Each ErbB receptor has a unique pattern of tyrosine phosphorylated residues that recruit SH2- or PTB-containing proteins, leading to stimulation of intracellular signaling pathways (reviewed in ref. 11).

ErbB2 Is the Preferred Dimerization Partner

Despite the intense efforts by many laboratories to isolate an ErbB2 ligand, none of the 11 EGF-related ligands were found to directly bind the receptor, even though ErbB2 becomes heavily tyrosine phosphorylated in response to all the ligands. How does the receptor get activated? An answer to this conundrum came from the X-ray structural studies. The ectodomain structure of ErbB2 was published by the laboratories of Leahey, and Garrett and Ward, at about the same time as the EGFR structure. Intriguingly, ErbB2 was found to be constitutively in an extended conformation with an exposed domain II, similar to that of ligand-bound EGFR (reviewed in ref. 9). An important conclusion from these studies is that ErbB2’s structure is not compatible with binding any of the EGF family ligands. Excitingly, this also leads to the speculation that ErbB2 is primed and ready to interact with the other ligand-bound receptors, one reason why it does not require an activating ligand.

My own laboratory showed that ErbB2 is the preferred partner of the other receptors using a novel technology to remove ErbB2
from the plasma membrane in tumor cells expressing the four ErbBs (12). Of note, this was in 1997, before the advent of siRNA technology that can now be used to downregulate any protein of choice. We showed that following intracellular expression of an ErbB2-directed single-chain antibody (scFv) that was targeted to the endoplasmic reticulum, the engineered cells retained ErbB2 in the endoplasmic reticulum, thereby preventing its appearance at the plasma membrane. After treatment of control and scFv-expressing tumor cells with EGFR or ErbB3/ErbB4 ligands, we measured activation of individual receptors and the resultant stimulation of intracellular signaling pathways. The results allowed us to make two important conclusions: first, in the absence of ErbB2, ligand-induced signaling was less robust and less prolonged, and second, ErbB2 was the preferred heterodimerization partner for the other ligand-activated ErbB receptors; only when ErbB2 was retained intracellularly could we detect an interaction between EGFR and ErbB3 (12).

Heterodimers Are Special

In addition to EGFR and ErbB4 homodimers, there is good evidence that all potential ErbB heterodimers form signaling complexes (reviewed in refs. 13, 14). Heterodimerization allows the receptors to diversify their signaling potential. Indeed, a particular ErbB receptor acquires distinct signaling properties depending upon its dimerization partner (12–15), which is likely due to differential tyrosine phosphorylation in their C-termini (see, e.g., ref. 15).

ErbB3 is unique among the ErbBs. On the one hand, it has been considered to be kinase impaired as there are amino acid substitutions in two sites that are conserved in all kinases. However, the Lemmon laboratory recently showed that the kinase domain of ErbB3 has about 1,000-fold less activity than that of EGFR, but they proposed that in the context of a heterodimer, this may be sufficient for transphosphorylation of, for example, ErbB2 (16). On the other hand, ErbB3 is extremely adept at activating the PI3K pathway as its carboxy terminal domain possesses six docking sites for the p85 adaptor subunit of PI3K, a characteristic that has turned out to be very important in tumor biology. To directly test the role of ErbB3 in cancer, we used a novel artificial zinc-finger protein (E3) that targeted the ERBB3 promoter, preventing its transcription. We showed that expressing E3 in ErbB2-overexpressing breast cancer cells blocked proliferation as efficiently as loss of ErbB2 (17). Moreover, expression of a constitutively active Akt rescued the proliferative block resulting from loss of ErbB2 or ErbB3, clearly showing that the ErbB2/ErbB3 dimer functions as an oncogenic unit to stimulate proliferation (17).

The importance of ErbB heterodimers in normal physiology is strikingly illustrated by genetic data. In 1995, the Hauser laboratory showed that ErbB2 is required for development of the heart. Embryos lacking the receptor die as the ventricular trabeculae are not properly formed. This phenotype is identical to what was described in mice lacking ErbB4 or its ligand NRG-1, which was published simultaneously by the Lemke and the C. Birchmeier laboratories, respectively. Thus, the NRG-1–induced ErbB2/ErbB4 heterodimer is essential for heart development; NRG-1–induced ErbB4 homodimers cannot substitute for ErbB2. In a comparable manner, loss of ErbB2 or ErbB3 has a similar impact on neuronal development (reviewed in ref. 18).

Trastuzumab and Breast Cancer Treatment

Overexpressed ErbB2 is constitutively phosphorylated in tumors and in cancer cell lines and targeting this receptor results in efficient inhibition of proliferation and downstream pathway activity. The first panel of mAbs targeting ErbB2 was isolated by the Ullrich laboratory at Genentech in 1989 (reviewed in ref. 1). Each mAb bound the ectodomain of the receptor, however, they had different effects when tested on ErbB2-overexpressing breast cancer cells. On the basis of the antiproliferative effects of mAb 4D5, it was chosen for further development. Trastuzumab, a recombinant humanized version of 4D5, was the first anti-ErbB2 drug approved, in 1998, for treatment of ErbB2-positive metastatic breast cancer, either alone or in combination with chemotherapy (19, 20). Treatment with trastuzumab was associated with a significantly longer time to disease progression, a higher response rate, a longer response duration, and improved overall survival. The mechanism underlying trastuzumab’s clinical efficacy is likely to be multifaceted and is still under discussion (21), although there is strong evidence pointing to an important role for antibody-dependent cellular cytotoxicity (22).

Mechanisms of Resistance to ErbB-Targeted Therapy

Resistance to trastuzumab remains an important clinical problem. One mechanism by which the cancer cells escape trastuzumab treatment is by responding to ErbB ligands in the tumor milieu, produced in an autocrine or paracrine manner. As trastuzumab binds to domain IV of the receptor’s ectodomain, it neither blocks binding of ligands to domain II, nor ensuing activation of ErbB2-containing heterodimers; even in the presence of trastuzumab, these activated heterodimers drive proliferation (23). Pertuzumab is an ErbB2 antibody that binds domain II and prevents ligand-induced ErbB2/ErbB3 heterodimer activation. On the basis of its different mechanisms of action and clinical success, pertuzumab was approved in 2013 for use in combination with trastuzumab and docetaxel for neoadjuvant treatment of ErbB2-positive breast cancer patients in the setting of early breast cancer (http://www.cancer.gov/about-cancer/treatment/drugs/fda-pertuzumab).

Another mechanism contributing to trastuzumab resistance is PI3K activation. Using ErbB2-overexpressing tumor cells, Slawekowski and colleagues showed biochemically that trastuzumab causes destabilization of ligand-independent, constitutive ErbB2/ErbB3 complexes, which uncouples ErbB3 from ErbB2, leading to blockade of downstream PI3K/Akt signaling (24). However, in trastuzumab-treated cells with mutant PI3K, Akt activity remains high, suggesting that the mutant kinase might remain localized at the membrane, perhaps using its Ras-binding domain or potentially by coupling to another RTK. One way or another, PI3K continues to catalyze PI3P formation, Akt activation, and tumor cell proliferation. On the basis of this, and other preclinical data suggesting that ErbB2 independent, PI3K pathway activation might be a resistance mechanism to overcome clinical effects of trastuzumab, the BOLERO3 trial tested the addition of the mTOR inhibitor everolimus to trastuzumab and a chemotherapy, in a phase III clinical trial. There was a short but significant clinical benefit.
in patients receiving the mTOR inhibitor (25). Importantly, these results confirm in patients the preclinical data showing the importance of targeting the PI3K pathway.

The first ErbB-directed inhibitors were designed to target EGFR and/or ErbB2, however, ErbB3 has now emerged as an important therapeutic target in its own right. ErbB3 is not only a ‘slave’ to ErbB2, but has been implicated in various tumor types where it partners with other ErbBs, or with other members of the RTK family (reviewed in ref. 26). ErbB3 activity has also been implicated in resistance to targeted therapy. It is now commonly accepted that over the course of tumor development, feedback inhibition of signaling pathways is an essential mechanism that allows tumor cells to escape stress-induced death. ErbB3 is one of the important receptors targeted by such inhibition. Intriguingly, using specific inhibitors for the Erk or Akt pathways, it was discovered that these relieve a block that keeps ErbB3 transcription at low levels. Thus, in the presence of these pathway inhibitors, ErbB3’s expression is released from constraints, thereby contributing to escape from targeted inhibitors (27, 28). ErbB3 has also been shown to be upregulated and to drive PI3K pathway activity in the context of resistance emerging in response to treatment with lapatinib (reviewed in ref. 26), an FDA-approved drug that blocks ErbB2 kinase activity. Despite the fact that in the ErbB2/ErbB3 heterodimer ErbB2 is blocked by lapatinib, this is apparently not sufficient to prevent ErbB3 from activating the PI3K pathway. Indeed, lapatinib does not block ErbB3’s ‘weak’ kinase activity (16). Additional mechanisms that might contribute to trastuzumab resistance have been proposed (reviewed in refs. 5, 18, 29, 30).

The Future of ErbB2-Targeted Treatments

An important clinical goal is to manage metastatic disease at the level of circulating tumor cells (CTC). This would circumvent the difficulty of obtaining biopsies from metastasis. In other words, the problem of minimal residual disease is now being tackled at the level of single cells (31). Recently, it was reported that in some patients, overt distant metastases, and even CTCs, display higher ErbB2 levels than what was measured in the primary tumor. This has stimulated clinical trials aimed at investigating whether these patients, who had not previously been offered an ErbB2-targeted therapy, might benefit from this treatment. One example is the DETECT III trial in which CTCs from metastatic patients will be examined for ErbB2 levels and if positive, the patients will receive an ErbB2-directed therapy combined with standard-of-care chemotherapy or endocrine therapy. This trial as well as others on this topic is discussed in a recent review (32).

Finally, it should be obvious that the publications describing breast tumors with overexpressed ErbB2 opened up a new field in breast cancer research. All types of scientists: biochemists, molecular and structural biologists, geneticists, and oncologists, have contributed to our current knowledge of the ErbB receptor family. These studies are likely to continue for some time, because as we learn more, we will hopefully be able to help many more cancer patients with therapeutics targeting the ErbBs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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