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ERBB3/HER3 and ERBB2/HER2 Duet in Mammary Development and Breast Cancer

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Abstract

ERBB3/HER3 is one of the four members of the epidermal growth factor receptor (ERBB) family. It is activated by binding to ligands Neuregulin-1 and Neuregulin-2. Since ERBB3 lacks intrinsic kinase activity, signal transduction occurs through formation of heterodimers with EGFR, ERBB2, and ERBB4. ERBB3 is a signaling specialist since it has six binding sites for the p85 SH2 adapter subunit of phosphoinositide 3' kinases. These lipid kinases coordinate regulation of metabolism, cell size, proliferation, survival, and angiogenesis. Not surprisingly, ERBB3 signaling has been linked to cancer etiology and progression. In breast cancer, the partnership of ERBB2 and ERBB3 may be crucial for the aggressive properties of cancers with *ERBB2* amplification, and may contribute to pre-existing and acquired resistance to therapy. This partnership creates opportunities for improving efficacy of ERBB-targeted pharmaceuticals, by interfering with coupling of ERBB2 to ERBB3 through dimerization inhibitors, and by use of therapeutic compounds that target AKT-dependent pathways activated through ERBB3. Additional therapeutic opportunities may be identified through better understanding of how ERBBs are regulated and deployed in normal mammary gland processes. Work using mouse models has identified the main processes regulated by each of the four ERBBs, which has practical implications in understanding breast cancer etiology, and eventual development of better prognostic, predictive, and therapeutic tools.

Keywords

ErbB2/HER2; ErbB3/HER3; mammary; PI3 Kinase; Akt; breast cancer

ERBBs in Breast Cancer

Antibodies and small molecule drugs that inhibit members of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (RTKs) are among the most important new cancer therapies (1). The EGFR (*ERBB* or *HER* gene) family consists of four closely related RTKs. The founding member, the EGFR, is commonly expressed in epithelial and mesenchymal cells, where it is important in many processes involving growth regulation. The remaining members of this family (ERBB2/HER2, ERBB3/HER3, ERBB4/HER4) are involved in numerous processes in development and maintenance of the nervous system and the cardiovascular system in addition to their functions in epithelia and mesenchymal cells.

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Studies in rodent models indicate that each of these four receptors has unique functions in mammary development, from early patterning of the mammary gland on up to terminal differentiation during lactation (2, 3).

At least three of the four receptors, EGFR, ERBB2, and ERBB3, have important positive roles in human mammary carcinogenesis. *EGFR* and *ERBB2* are commonly amplified in breast cancer, with consequent overexpression of the receptors. The high rate of success of the ERBB2-targeted antibody drug Herceptin/Trastuzumab in treatment of patients with *ERBB2* amplification validates the importance of this receptor in sustaining breast cancer (4, 5). The US FDA has now approved the use of a dual small molecule EGFR/ERBB2 kinase inhibitor, Tykerb/lapatinib (in combination with capecitabine), for salvage therapy of patients with metastatic breast cancer who failed on Herceptin. The EGFR is overexpressed, usually without amplification, in basal mammary carcinomas. These are difficult to treat, and it has been suggested that clinical trials with EGFR-targeted therapies are warranted (6). The involvement of *ERBB4* in breast cancer is less certain, since clinical data are contradictory (7). This may be explained by the diversity of responses induced in mammary epithelium by different isoforms created by alternative splicing and by proteolysis (8, 9).

Regulation of ErbBs

ERBBs are regulated directly by ligand binding. EGFR, ERBB3, and ERBB4 are each activated by binding a subset of eleven EGF-related growth factors (10). Early work on the ERBBs revealed that they function as a tightly interconnected network. This is because they are often co-expressed, and form active heteromers after ligand binding (10–13). Indeed, both ERBB2 and ERBB3 rely on heteromeric activation, since there are no conventional ligands able to bind ERBB2, and since ERBB3 has no intrinsic kinase activity. The extracellular domains of EGFR, ERBB3, and ERBB4 are normally in equilibrium between auto-inhibited and open conformations that are related by a substantial conformational shift (14, 15). Intramolecular ligand binding to domains I and III clamps the receptors in an open conformation exposing domain II, which then nucleates receptor-receptor contacts that foster receptor dimerization. Additional inter-receptor contacts in juxtamembrane, transmembrane, and kinase domains further stabilize the dimer. Close proximity of two receptors facilitates cross-phosphorylation of the kinases, which is aided by conformational changes in an asymmetric dimer formed by the kinase domains (16).

ERBBs are also activated through ligand-independent mechanisms (17). The receptors can be activated by irradiation, resulting in receptor crosslinking, and by heterologous hormone-response systems including other RTKs, notably MET, cytokine receptors (growth hormone receptor, gp130), and by G protein coupled receptors (GPCR). The mechanisms involved may include direct activation through formation of physical complexes, conformational, or phosphorylation changes, and indirect activation via activation of coupling signaling molecules including Src. Some GPCR agonists activate cell surface metalloproteinases that in turn release active HB-EGF (Heparin-binding EGF-like growth factor) from its inactive tethered pro-form, and other indirect agonists may work by suppressing phosphatase activity. Finally, activation or modulation of ERBBs by mucin complexes are unconventional, but physiogically important, forms of regulation (18, 19). ERBB activities are further modulated

through a series of binding proteins that affect subcellular routing and stability, reviewed (20).

The close integration of the ERBB network within individual cells has important practical implications for cancer therapies targeting these receptors. Since the receptors are somewhat promiscuous in their ability to form heterodimers, ligand binding to one receptor results in activation of the others (10). Receptor interactions can alter the timing and subcellular localization of post-activation events, most notably with ERBB2 forestalling downregulation of activated EGFR (21). Likewise, interference with the catalytic activity of one receptor is likely to indirectly reduce activation of the partner receptors. In contrast, downregulation of one receptor, as may occur with antibody drugs or with transcriptional modulators, will result in reassortment of homo- and heteromeric pools of the other receptors, which will in turn affect the constellation of intracellular signals generated. De novo production of agonists for non-targeted receptors may provide a route for acquired resistance to ERBB-directed therapeis (22). Since each receptor has unique signaling outputs, these interactions may have undesired or desired consequences for the cancer patient.

ErbB Signaling

Each of the four ERBBs has common as well idiosyncratic signaling outputs, with EGFR and ERBB2 classically coupling to RAS-MAP kinase pathways, PLC-γ/PKC pathways, SHP-2, Stats, Src-dependent pathways, and others (23). ERBB4 is linked to common pathways, but the JM-A spliced isoforms have sites that enable successive cleavage by metalloproteinases and γ-secretase. This dual cleavage releases soluble and constitutively active intracellular domains that relocate to the nucleus (24, 25). The soluble isoforms have been linked to diverse mitochondrial and nuclear functions, including nuclear translocation of STAT5, activation of proapoptotic pathways, and modulation of transcription through formation of complexes with transcription factors (Estrogen Receptor [ER]), co-activators, and co-repressors (26–32). Nuclear forms of EGFR and ErbB2 also have important functions, but ErbB4 is unique in its ability to be efficiently cleaved through γ-secretase processing. An additional splice choice that determines whether one exon is skipped further diversifies ErbB4 signaling (8, 33).

ERBB3 and PI3K Pathways

ERBB3 functions most effectively as a ligand binding receptor for neuregulins NRG-1 and NRG-2. ERBB3 is an extraordinary example of signaling specialization by a RTK, since it harbors six binding sites for the p85 SH2 adapter subunit of phosphoinositide (3’) kinases (PI3K) (34). The concentration of p85 binding sites means that the signaling output of active ERBB3 is dominated by activation of the PI3K cascade that results in production of 3’ phosphosoinositides. These facilitate tethering and activation of PH-domain effectors to membranes, including the pleiotropic protein kinase Akt family (reviewed, (35)). The PI3K system is important in integrating regulation of cell size/organismal growth with cell survival pathways. The system feeds out through a cascade eventually leading to phosphorylation of transcription factors, to enhanced protein translation through the TSC2 to TOR system, and through negative regulation of growth inhibitory pathways via

phosphorylation of FOXO transcription factors and glycogen synthase kinase. Inhibition of pro-apoptotic BAD, caspase-9, and other targets tips the balance towards cell survival. Activation of HIF-1 alpha by Akt, and modulation of Rac and Rho by other PH domain proteins may promote other changes associated with tumor progression. With the importance of pro-growth and anti-apoptotic signaling in carcinogenesis, and increasing knowledge about gene mutations in specific cancers, it is now appreciated that signaling through PI3K-regulated pathways is a common feature of human cancer (36). In fact, activation of PI3K by mutations in the p110 catalytic subunit gene, or, indirectly through loss-of-function mutations in the PTEN phosphatase that hydrolyzes the 3' phosphate, are among the most prevalent mutations in human breast cancer. Moreover, bypass alterations promoting PI3K pathway activation are common in limiting effectiveness of RTK-targeted therapies.

ERBBs in Mammary Gland Development

Dysregulation of carcinogenic signaling systems does not completely divorce them from normal modes of regulation. For example, when *ERBB2* is amplified in breast cancer, the encoded receptor is overexpressed, but still able to be further activated through heteromerization by other ERBBs bound to their ligands, and still subject to many of the same downregulation mechanisms. Likewise, activated forms of EGFR common in non-small cell lung carcinoma retain some degree of regulation by EGF, but they are sensitized to EGF dose and may have altered connections to downstream signaling pathways (37). The extent to which normal regulation of carcinogenic ERBBs persists creates opportunities for therapies that modulate these mechanisms. And, since the fundamental regulatory framework remains, investigation of ERBB signaling systems in their normal tissue contexts has important implications for understanding cancer etiology, identification of new therapeutic targets, and anticipating modes of drug resistance.

In the mammary gland, ERBB activity and outputs is modulated globally by sex steroids, and modulated at the cellular level through interactions with other receptor systems including other RTKs, WNTs, steroid receptors, and steroid coreceptors. Interest in ERBBs in normal mammary gland development was prompted by early studies showing that EGF and TGF-α are potent growth regulators in rodent mammary cell and organ culture, and that slow-release pellets delivering these growth factors to the mouse mammary gland have potent effects on ductal outgrowth. More recent work has further explicated mammary functions of the ErbB receptor network by profiling ligand expression and through genetic and functional analysis of null and hypomorphic ErbBs (2, 3, 38).

The earliest phase in mammary development linked to ErbB function has been suggested by a microsatellite repeat polymorphism at the *scaramanga* (*ska*) locus which is associated with abnormal patterning resulting in variable number and position of mammary glands. The variation maps to introns in the *Nrg3* (Neuregulin-3) locus, and both *Nrg3* and the receptor ErbB4 are expressed in the presumptive mammary gland (39). Since *ErbB4* is not required for early embryonic mammary development (40), it is possible that NRG3 can act through other receptors, possibly ErbB3.

Aside from these intriguing studies of *ska*, there is considerable evidence supporting the importance of ErbBs and their ligands in postnatal mammary development. The most important transitions in this process occur at puberty, at which time systemic steroid hormones drive production and activation of several peptide growth factors including EGF family ligands, and maturation and terminal differentiation at pregnancy and lactation. ErbBs do not seem to be involved in remodeling associated with involution of the mammary gland induced by weaning (2).

Amphiregulin (AR) is among the most prevalent EGF agonists expressed in the mouse mammary gland at puberty (41). Single and combined knockouts of AR, TGF-α, and EGF have confirmed the importance of AR in this context (42). Complementary experiments have shown that EGFR is required for ductal elongation at puberty, and analysis of mammary gland chimerae with wild-type stroma and knockout epithelium and the converse revealed a limiting function for the EGFR in mammary stroma (43, 44). Since AR is mainly expressed in the epithelium, this work identifies AR as a mediator of epithelial to stromal communication.

When mouse mammary glands were reconstituted by transplantation of *ErbB2*-null embryonic mammary buds (45), and when MMTV-Cre induced recombination was used to disrupt *ErbB2* in the mammary epithelium (46), ductal outgrowth at puberty was transiently delayed. In the transplantation model, this was accompanied by structural abnormalities of the invasive terminal end buds (TEB) at the tips of the growing ducts, and comingling of cells with myoepithelial and luminal epithelial characteristics (45). EGFR and ErbB2 are the most prevalent ErbBs expressed at puberty (41, 44). So, it was surprising that ErbB2 was required in the epithelium, unlike the EGFR (43). ErbB2 requires a dimerization partner for ligand-regulated signaling. Despite the presence of EGFR in the epithelium, these results raised the possibility that another ErbB expressed in the epithelium is a functional partner for ErbB2 at puberty.

Some functional ErbB3 and/or ErbB4 is present at this juncture, since slow-release pellets containing NRG-1, which binds these receptors, induce ductal elongation at puberty (47). ErbB4 is not likely to be important, since *ErbB4* knockout mice show no defects in ductal elongation (40). However, there is evidence for the importance of ErbB3. One report, in which a transcriptional suppression scheme was used to reduce ErbB3 expression in the mammary epithelium, reduced TEB numbers, branching, and ductal density (48). Our laboratory has investigated the ability of transplanted embryonic mammary buds lacking ErbB3 to reconstitute cleared mammary fat pads (Jackson-Fisher et al., submitted for publication). The resulting mammary glands failed to completely fill the fat pad. This phenotype was maintained into adulthood and pregnancy, in contrast to *ErbB2* knockouts (45). TEBs were small and distorted, but the compartmentalization of myoepithelial and luminal cells was normal, unlike ErbB2 knockouts. Consistent with the importance of PI3K signaling activated by ErbB3, the ductal elongation defect is associated with increased caspase-3 cleavage and apoptosis, which we did not observe with loss of ErbB2.

In later stages of mouse mammary development, the ErbBs are most important in late pregnancy and lactation. Truncated dominant negative *ErbB2* (which may work through

heteromerization with all ErbBs) severely reduced late stages in formation of secretory alveoli, including expansion of alveoli and production of milk proteins (49). ErbB4 is required to sustain lactation, apparently because it is essential for maintaining activity of the mammary transcription factor Stat5a, and, possibly, secretion of milk constituents (40, 50, 51).

The potential to map carcinogenic functions of ErbBs onto their normal mammary functions reinforces the relevance of clarifying endogenous ErbB-dependent processes related to cancer. For example, the striking association of ErbB2 and ErbB3 with ductal proliferation and invasion of the mammary fat pad at puberty may be related to the frankly aggressive properties of tumors with highly active ERBB2 and ERBB3. The importance of ErbB4 in terminal differentiation, in contrast to association of the other ErbBs with growth and invasive processes, is consonant with findings in a number of clinical studies (but not all of them) that ERBB4 expression is associated with better prognosis (52–54). It is noteworthy, also, that *ERBB4* mRNA is associated with the luminal A subtype of breast cancer (55), which is associated with longer disease-free survival than other subtypes defined by transcription profiling. But, it is not yet clear how important ErbB4 is in this context. It has been suggested that the inconsistencies among clinical studies may be linked to the very different biological activities of full-length versus cleaved ErbB4 (25), and differential functions of spliced isoforms CYT1 and CYT2 (8, 33).

There are a number of challenges impeding full understanding of ErbB regulation in the mammary gland and other tissues. Functional redundancy among the receptors is a practical problem for genetic analyses, and has been partly circumvented through the use of dominant negative truncated receptor transgenes. A major problem is the difficulty in quantifying active growth factors. ERBB ligands are expressed as generally inactive propeptides, so expression of RNA and pro-hormones is necessary, but not sufficient for activity. The fully processed growth factors are active, but may be difficult to distinguish serologically from pro-peptides, and may diffuse away during tissue processing. Finally, the rate-limiting regulatory processes, which involve activation of metalloproteinases, are themselves poorly understood at the regulatory level, and they impinge on many biological targets in parallel. TACE/ADAM-17 seems to be the major regulator of AR cleavage in TEBs, but others may be involved (reviewed (3)).

Overall, then, ErbBs have multiple and recurring functions in mammary development. The importance of ErbBs in promoting proliferation, invasion, and differentiation of mammary tissue at major developmental junctions means that these receptors can be quite active at multiple stages of mammary development. It is likely that both expression and activity of the receptors in endogenous mammary epithelium sets the stage for selection of precancerous cells in which ERBB activity is excessive, for example with *ERBB2* amplification. Biological differences among EGFR-positive basal breast carcinomas, ERBB2-positive carcinomas, and ERBB4-positive luminal A carcinomas may mean that differential activity of ERBBs expands specific stem or transit cell compartments, or favors mammary differentiation in specific directions. The extent to which ERBB agonists and other peptide growth factors (notably FGFs and IGF-1) mediate estrogen-stimulated growth and invasion processes, the intersection of ERBB and ER-regulated signaling pathways, and the

possibility of direct interactions between ERBB2 and the ER means that therapeutic targeting of ERBBs may be beneficial to patients even in tumors that are largely driven by steroid hormones.

ERBB3 in Cancer

ERBB3 is unique in the ability to channel ErbB signaling to the PI3K/Akt signaling pathway, which undoubtedly favors tumor growth and progression. ERBB3 limits the impact of exclusively targeting ERBB1 or ERBB2, especially in the presence of ERBB3 ligands, and provides a route for acquired resistance to anti-cancer drugs that inhibit ERBBs or other receptors. For these reasons, also, targeting ERBB3 may prove to enhance the efficacy of other RTK inhibitors. Since ERBB3 is catalytically inactive, other tactics must be used for functional inhibition. For example, the antibody drug Pertuzumab/Omnitarg binds to the ERBB2 dimerization arm and prevents formation of heteromers with ERBB3, thereby severing the cross-activation pathway (56). Drugs targeting the ERBB3-activated PI3K pathway, including AKT's and mTOR, are under intensive study for treatment of many cancers and other proliferative disorders.

Cell culture and Animal Models

Early studies in which ERBBs were expressed in various combinations identified major differences in signaling by these receptors (10, 57, 58), and suggested that ErbB2 and ErbB3 is a potent signaling combination in cell growth and in mammary cell line tumorigenesis (22). Findings in mammary tissue extended these findings. In mouse models for breast cancer initiated by high level expression of ErbB2, overexpression and functional activation of ErbB3 commonly occurs (59). Xenografts from a MMTV-NeuT cell line with activated ErbB2 are inhibited by the ErbB-inhibitor gefitinib, which correlates with reduction in ErbB3 phosphorylation (60). And, *ERBB3* overexpression and activation was found to be common in human breast cancer. Some studies (61, 62) have identified an association of *ERBB3* overexpression with *ERBB2* overexpression in breast cancer. This may occur through gene amplification, transcriptional, or post-transcriptional mechanisms (59). In cells with high *ERBB2* and *ERBB3*, downregulation of ErbB3 with siRNA reduces phosphorylation of both receptors, implying mutual cooperation of the two receptors in maintenance of signaling (63). This may be mediated by ligand-dependent or independent heteromerization.

ERBB3 and ERBB ligands in Human Breast Tumors

ERBB2 amplification/overexpression is linked to an aggressive subset of breast cancer. These findings led to interest in the extent to which *ERBB3* overexpression predicts poor outcome, either alone, or in combination with other ERBBs. In a PCR-based study of 225 breast cancers (64), *ERBB2* amplification was most common (26%), and *ERBB3* amplification occurred in 10% of cases. Although high *ERBB3* was associated with low grade in this series, there was a non-random association with *ERBB2* amplification, especially in node-positive patients. Another RNA study associated *ERBB3* with poor prognosis (65). Overexpression of ERBB3 determined by immunohistochemistry (IHC),

often in conjunction with ERBB2, was associated with poor outcome (62). Sassen and coworkers evaluated all ERBBs by fluorescent *in situ* hybridization (FISH) and by IHC in tissue microarrays of 278 invasive breast cancers. *ERBB3* FISH (but not IHC) was associated with higher risk (66). *ERBB3* amplification is significant, especially since it implies selection during tumorigenesis. But, in *ERBB3* amplicons, there may be neighboring genes under selection as well.

Another study (67) measured expression of ERBB ligands in 363 breast tumors by RT-PCR, and combined this analysis with measurements of receptor mRNAs. The vast majority of cancers expressed one or more of the ligands, and 80% expressed the ERBB3 ligand NRG-1. ERBB2 and ERBB3 were co-expressed in 59 of the cancers, and these showed some upregulation of several EGF family ligands. Of these, only EGF was a (positive) prognostic factor. Of course, the predictive value of ligand mRNA expression is uncertain at present, since at least some of the ligands must be activated by proteolysis. An inherent limitation of all of these studies is that gene amplification, ERBB3 expression, or ligand expression were studied, since none of these will precisely predict ERBB3 activity. This can be measured by analysis of ERBB3 phosphorylation, which poses some technical challenges from variable activity of phosphatases during sample preparation.

ERBB3 and Resistance to Cancer Therapies

Along with the biological impact of *ERBB3* signaling on ERBB2-amplified breast cancer, increasing evidence links active ERBB3 to resistance to breast cancer therapeutics targeted at ERBB2 and ER (reviewed, (68)). This may be relevant for predicting response to these agents, and also suggests new therapeutic avenues, e.g. use of agents like Pertuzumab and/or use of AKT and mTOR inhibitors. Since ERBB3 has no catalytic activity, gain-of-function mutations (beyond overexpression) are not likely to be important. Instead it appears that ERBB3 promotes drug resistance by enabling autocrine or paracrine ligands (NRG1 and NRG2) to activate catalytically competent RTKs, and through its capacity to channel signaling to PI3K/Akt signaling pathways. The former has been well demonstrated in cell culture systems, in which, for example, a Herceptin-sensitive cell line becomes resistant in the presence of NRG, provided that ERBB3 is still present (22) and studies of Gefitinib-resistance mediated by HRG (69). Finally, it is formally possible that ERBB3 affects response to ERBB inhibitors indirectly, through protection of ERBB2 kinase domain or extracellular domain in heterodimers from phosphatases or inhibitors, or by reducing formation of ERBB2 homodimers, or dimers with other receptors such as ERBB4 that may have protective value for patients.

ER Inhibitors

With the importance of ER and ERBBs as therapeutic targets in breast cancer, there is great interest in interactions between ERBBs and estrogen antagonists. In a parallel analysis of all four ERBBs in breast tumors (61), high ERBB2 and ERBB3 were each linked to earlier relapse on Tamoxifen (Tam). In progesterone receptor-positive patients, expression of either EGFR, ERBB2, or ERBB3 (but not ERBB4), was linked to early relapse on Tam. Downregulation of ERBB3 in MCF-7 cells rendered resistant to Tam by ERBB2

overexpression enhances Tam-induced apoptosis, implicating ERBB3 in Tam resistance (63). A study using a similar model implicated ERBB3 signaling in the paradoxical ability of fulvestrant to promote growth in the presence of estradiol, and the growth was inhibited by Pertuzumab (70). Potential mechanisms for functional interactions include modulation of ER by ERBB-activated MAPK and Akt pathways, phosphorylation of the ER coactivator AIB1 by receptors, which enhances estrogenic activities of Tam, and direct physical and functional interactions between ERBBs and ER.

Herceptin and other ERBB-targeted drugs

Genetic and functional studies of Herceptin responses support an important role for PI3K pathway signaling. ErbB-dependent ERBB3 to PI3K signaling persists in BT474 xenografts selected for Herceptin resistance (71), perhaps through upregulation of NRG and other agonists. In breast cancer, changes associated with upregulation of the pathway include gain-of-function mutations in *PIK3CA*, which encodes the catalytic subunit of the PI3K (very common) or Akt (less common), and *PTEN* loss-of-function mutation or low expression (reviewed, (36). *PTEN* mutations, which indirectly upregulate PI3K signaling cascades, confer Herceptin resistance (72). ERBB2-dependent activation of PI3K signaling through Src resulted in inactivation of PTEN and greater activity of the pathway. Herceptin inhibits ERBB2-dependent Src activation, resulting in enhanced PTEN activity and consequent downregulation of Akt signaling. *PTEN* mutations reduced the impact of Herceptin, but could, in principle, be partly overcome through enhanced ERBB3-dependent activation of PI3K. Another study (73) surveyed shRNA viruses targeting eight thousand genes for the ability to reduce Herceptin resistance of BT-474 cells, which have amplified *ERBB2*. *PTEN* shRNA was among the top hits, and patients treated with Herceptin with *PIK3CA* mutations or low PTEN expression relapsed sooner.

Like ERBB3, IGF-1R is strongly linked to PI3K/Akt signaling pathways. IGF-1 treatment induces Herceptin resistance of breast cell lines engineered to express high level IGF1-R (74). Some evidence indicates that Herceptin resistance is associated with high level expression of IGF1-R (75). A surprising mechanistic possibility is the formation of heteromers between IGF1-R and ErbB2 (76, 77).

In BT474 cells and other ERBB2-dependent tumor lines treated with EGFR/ERBB2 kinase inhibitors, signaling through the AKT pathway was surprisingly persistent (78). This led to the discovery that ERBB3 is reactivated in the face of chronic suppression of EGFR and ERBB2 activity after 96 hours, ERBB2 is required for ERBB3 reactivation, suggesting that residual ERBB2 kinase activity is responsible. Effects on subcellular localization and accessibility of ERBB3 to phosphatases may be involved, in what the authors propose is a negative feedback mechanism that endows cancer cells with a buffering capacity to resist all but the most intensive kinase inhibitors. On the practical side, targeting the ERBB3 relocalization process and the PI3K pathway itself may augment treatment efficacy.

A remarkable series of experiments has revealed the potential for ERBB3 to potentiate signaling by heterologous receptor families (79). A lung carcinoma cell line driven by EGFR mutations was selected in vitro for resistance to the EGFR kinase inhibitor gefitinib. Array

analysis revealed the importance of *MET* amplification, which in turn induced gefitinib-resistant ERBB3 activation and signaling. Importantly, dual treatment with EGFR and MET inhibitors reduced ERBB3 signaling and led to apoptosis. Thus, a convoluted circuit involving three RTKs could be defeated once the major components were identified. In glioblastoma, MET signaling is induced by the EGFRvIII isoform, which is also expressed in breast cancer, and here, also, dual use of MET and EGFR inhibitors was advantageous (80). These and other studies suggest that cross-receptor interactions may be more common than previously appreciated, and suggest that parallel targeting of multiple receptors will be important.

Concluding Remarks

The importance of ERBBs in human cancer, and the early development of ERBB-inhibiting antibody and small molecule drugs has made this receptor family a prototype for implementation of RTK-targeted therapies. Despite encouraging results, the failure of Herceptin therapies for many *ERBB2*-amplified breast cancers, the difficulty in identifying the appropriate subset of patients with non-small cell lung carcinoma and colon carcinoma for treatment with ERBB inhibitors, and the eventual development of therapeutic resistance in cases where robust responses occur at first, all mean that there is a compelling need to further understand how these RTKs function in cancer, and how best to eliminate their activities. The tight integration of the ERBB signaling network reinforces the importance of dealing with these receptors as a suite, rather than individually, and the powerful coupling of ERBB3 to cancer processes makes this RTK especially important in this context. There is also an increasing awareness of the extent to which different RTK families support one another in cancer etiology and development of drug resistance, including the interactions of ERBBs with IGF1-R and MET discussed here. This reinforces the conclusion that most effective use of receptor and pathway-targeted pharmaceuticals will require robust methods for comprehensive profiling of activation of RTKs and their dependent pathways so that the appropriate single-agent and combination therapies can be identified.

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Abbreviations

EGFR	epidermal growth factor receptor
RTK	receptor tyrosine kinase
GPCR	G protein coupled receptor
ER	estrogen receptor
NRG	neuregulin
PI3K	phosphoinositide (3’) kinase
AR	amphiregulin

TEB	terminal end bud
IHC	immunohistochemistry
FISH	fluorescent in situ hybridization
Tam	Tamoxifen

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