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The epidermal growth factor receptor family: Biology driving targeted therapeutics

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Abstract

The epidermal growth factor family of receptor tyrosine kinases (ErbBs) plays essential roles in regulating cell proliferation, survival, differentiation and migration. The ErbB receptors carry out both redundant and restricted functions in mammalian development and in the maintenance of tissues in the adult mammal. Loss of regulation of the ErbB receptors underlies many human diseases, most notably cancer. Our understanding of the function and complex regulation of these receptors has fueled the development of targeted therapeutic agents for human malignancies in the last 15 years. Here we review the biology of ErbB receptors, including their structure, signaling, regulation, and roles in development and disease, then briefly touch on their increasing roles as targets for cancer therapy.

Keywords

Epidermal growth factor receptor; receptor tyrosine kinase; ErbB; HER2; HER3; HER4; EGFR

Introduction

Growth factors are essential for the development, growth and homeostasis of multicellular organisms. Acting through cell surface receptors, growth factors are required for cell-cell communications underlying embryonic tissue induction, fate determination, cell survival, apoptosis, tissue specialization and cell migration. Growth factor receptors transduce extracellular signals through the activation of intracellular messengers or directly through receptor translocation to the nucleus. Of the receptor tyrosine kinases (RTKs), the epidermal growth factor (EGF) family of RTKs, also called ErbB or HER receptors, is one of the most extensively studied for its role in development, physiology, and human cancer.

Ancestral gene duplication and functional specialization has allowed this family of receptors to take on diverse functions in the development and maintenance of specific tissue types. Downstream signal selectivity, signal amplification and receptor regulation is enhanced by unique structural features of individual EGF receptor (EGFR) family members on a common structural backbone. Combinatorial effects also increase the diversity of signals possible through different dimerization partners and temporospatially restricted protein expression. A multitude of EGFR family ligands have both redundant and non-redundant functions during development further enhancing these combinatorial effects. In addition, ligand-independent transactivation of EGF family receptors adds yet another layer of signaling complexity as

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the receptors and downstream pathways are recruited for use by other signaling pathways such G-protein, Wnt, integrin and other growth factor pathways. mRNA splice variants of both receptors and ligandsadd to this diversity. The great complexity of the system creates multiple targets for the development of therapeutic agents to treat human malignancies and potentially other human illnesses.

Receptor overview

The epidermal growth factor RTK family consists of four members: EGFR (ErbB1, HER1), ErbB2 (HER2, *neu* in rodents), ErbB3 (HER3) and ErbB4 (HER4). These structurally related receptors are single chain transmembrane glycoproteins consisting of an extracellular ligand-binding ectodomain, a transmembrane domain, a short juxtamembrane section, a tyrosine kinase domain and a tyrosine-containing C-terminal tail (Fig. 1A). Binding of soluble ligand to the ectodomain of the receptor promotes homo- and heterodimer formation between receptors. Receptor dimerization is essential for activation of the intracellular tyrosine kinase domain and phosphorylation of the C-terminal tail [1]. Phosphotyrosine residues then activate, either directly or through adaptor proteins, downstream components of signaling pathways including Ras/MAPK, PLCγ1/PKC, PI(3)kinase/Akt, and STAT pathways [2].

EGFR and ErbB4 can be thought of as fully functional receptors with the ability to both bind ligands and autophosphorylate C-terminal tails through functional intracellular tyrosine kinase domains. ErbB2, however, is unique in that it has no known ligand but is the preferred dimerization partner for other EGFRs [3,4]. ErbB3 is also unique as it has no intrinsic tyrosine kinase activity but can transduce signals through interaction with kinase active receptors, namely EGFR, ErbB2, and ErbB4 [4]. Although all ErbB receptors have been localized to the nucleus, ErbB4 is notable for a convincing ability to directly transduce extracellular signals to the nucleus through liberation of the intracellular domain by a ligand-dependent dual protease cleavage of the receptor [5].

Expansion of the number and specialization of ErbB receptors during evolution has been paralleled by differential binding of receptors to specific subsets of ligands. ErbB ligands begin as cell membrane anchored proteins that are proteolytically processed to release soluble molecules [6]. Ligand specificity, redundancy, processing and variable tissue expression patterns add to the signaling diversity of the EGF pathway. EGF [7,8], transforming growth factor-α (TGF-α) [9,10], and amphiregulin [11] uniquely bind EGFR. ErbB3 binds neuregulin-1 [12-16] and neuregulin-2 [17-20] and uniquely binds Neuroglycan C [21]. ErbB4 is also able to bind neuregulin-1 and neuregulin-2 and uniquely binds neuregulin-3 [22], neuregulin-4 [23], and tomoregulin [24] as well. Both EGFR and ErbB4 bind heparin-binding EGF-like growth factor (HB-EGF) [25], betacellulin [26,27], epiregulin [28,29] and epigen [30] (Table 1).

Receptor structure

Crystallographic studies of the EGFR family members has provided further insight into the biology of the receptors and helped elucidate the structural basis for differences in receptor function. The ligand-binding ectodomain is composed of four subdomains termed L1 (leucine-rich repeats 1), CR1(cysteine-rich 1), L2 and CR2 (alternatively I – IV). CR1 contains a β-hairpin loop essential to receptor function. Crystal structures of ErbB3 and ErbB4 show that, in the inactive, ligand-free form, the ectodomain takes on a “tethered” conformation in which the beta-hairpin loop of CR1 interacts with CR2 sequestering the dimerization loop [31,32]. Structures of the EGFR ectodomain with EGF or TGF-α demonstrate that binding of ligand to the L1 and L2 domains leads to a conformational change in which the receptor takes on an extended form that exposes this dimerization loop

and allows for interaction of receptor ectodomains. A 2:2 complex is formed by this interaction in which the ligands face outward from the interaction face of the two ectodomains [33,34]. Consistent with the inability to identify a ligand for ErbB2, the crystal structure of a truncated ErbB2 ectodomain reveals that the receptor is locked in the extended conformation poised to interact with other receptors [35]. The extended conformation abolishes the potential ligand-binding site by fixing domains L1 and L2 in close proximity.

Ligand binding to the ErbB ectodomain and receptor dimerization induces conformational changes in the intracellular tyrosine kinase domain that leads to receptor autophosphorylation. Crystal structures for the intracellular tyrosine kinase domain in a dimeric complex have been solved and delineate the mechanism by which this occurs. Similar to the cyclinA/CDK2 complex, the tyrosine kinase domain has a bilobed structure with binding of ATP between these two lobes. Activation of the RTK appears to require interaction between the N-lobe of one tyrosine kinase domain with C-lobe of its partner [36] (Fig. 1B).

Signaling pathways

Multiple signal transduction pathways lie downstream of activated EGFRs and have been reviewed elsewhere [2,37] (Fig. 2B). EGFR family members activate the Ras/MAPK, PI(3)K/Akt, PLCγ1/PKC, STAT and recently the Par6-atypical PKC pathways. Tyrosine phosphorylation of EGFR creates binding sites for Grb2 and Src homology 2 (Shc2), which activate the Ras/Raf/MAPK pathway through son-of sevenless (Sos) stimulating cell proliferation [38-40]. Other ErbB receptors also activate the Ras/MAPK pathway. The PI(3)K/Akt pathway is important for cell survival. High-level activation of this pathway through direct binding of the p85 subunit of PI(3)K to activated receptor occurs with ErbB3 and ErbB4 as these receptors contain p85-binding sites [41]. EGFR may also weakly activate the PI(3)K pathway through the adaptor protein Gab-1 [42]. EGFR is also able to activate PLCγ, leading to protein kinase C activation followed by c-Jun and MAPK activation regulating cell proliferation [43]. STAT3 plays an important role in maintaining epithelial cell polarity and adhesion. STAT3 binding to activated EGFR leads to STAT3 dimerization and translocation into the nucleus to regulate gene transcription [44]. Similarly STAT5 can bind to both EGFR and ErbB4 [41]. The complex of Par6-Par3-atypical PKC is important in epithelial organization. The complex interacts with activated ErbB2, causing dissociation of Par3 from the complex and resulting in loss of apical-basal polarity. Cells activated in this manner also display increased cell proliferation and survival, although the mechanisms underlying all these changes remain to be determined [45].

Receptor cross-talk

Cross-talk between ErbB RTKs and ErbB-independent signaling pathways occurs through multiple different mechanisms and facilitates combinatorial activation of signaling pathways to expand the diversity of cellular responses. ErbB cross-talk is also a mechanism by which human cancers escape from the inhibitory effects of tyrosine kinase inhibitors (TKIs, Fig. 2A) [46,47]. Activation of G protein-coupled receptors by ligands such as endothelin-1, bombesin, thrombin, lysophosphatidic acid, and angiotensin-II can transactivate EGFR through stimulation of ADAM family cell surface metalloproteinases, leading to cleavage of membrane-bound EGF family precursors like HB-EGF [48-52]. Growth hormone stimulation of Janus tyrosine kinase 2 (Jak2) leads to phosphorylation and activation of EGFR [53]. Similarly, prolactin secreted by breast cancer cells can act in an autocrine manner to stimulate Jak2 and activate ErbB2/HER2/neu [54]. The Frizzled ligands Wnt1 and Wnt5a are able to indirectly transactivate EGFR and activate the Ras/MAPK pathway in

mammary epithelial cells apparently by stimulating metalloproteinase-dependent cleavage of membrane-bound EGFR ligands [55].

The non-RTK c-Src is able to enhance EGFR signaling through C-terminal phosphorylation of the receptor [56]. Activation of G protein-coupled receptors is one avenue through which the c-Src tyrosine kinase may become activated to transactivate the ErbB receptors. c-Src is also implicated in the transactivation of ErbB2 by integrins. Integrins are cell-matrix adhesion molecules capable of activating intracellular signaling pathways and are thought to play a role in local cancer spread, invasion and metastasis. α6β4 integrin induces translational up-regulation of ErbB2 and ErbB3. Increased translation of ErbB3 promotes ErbB2/ErbB3 heterodimerization, thereby stimulating PI(3)K activity in carcinoma cells [57]. α6β4 integrin also increases ErbB2 translation, leading to increased EGFR phosphorylation and activation of the Ras/MAPK pathway [58]. Further, ErbB2 promotes phosphorylation of the α6β4 integrin signaling domain by c-Src. Subsequent formation of an ErbB2-Src-α6β4 integrin complex leads to ErbB2 kinase domain phosphorylation and increased activation of the receptor. Downstream activation of STAT3 and c-Jun may then promote mammary carcinogenesis through regulation of epithelial adhesion and cell proliferation, respectively [59].

Nuclear translocation of ErbB members

In addition to signaling from the cell membrane, ErbB family members may move directly to the nucleus to influence gene transcription. Nuclear localization of ErbB proteins is potentially relevant to human disease, as breast cancers expressing high levels of nuclear EGFR have a worse prognosis [60]. In addition, nuclear localization of ErbBs up-regulates many genes believed important in cancer biology and progression, including cyclinD1, B-myb, cyclooxygenase-2, and the iNOS/NO pathway [61].

Full-length EGFR, ErbB2, and ErbB3 as well as truncated constitutively active ErbB2 and truncated C-terminal ErbB4 (s80, E4ICD) have been found in the nucleus of cells [62-70]. EGFR has been proposed as a transcription factor as localization to the nucleus is associated with increased CyclinD1 expression [64]. Nuclear EGFR has also been shown to participate with STAT3 and E2F1 transcription factors in iNOS/NO pathway activation and B-myb expression, respectively [71,72]. Targeting to the nucleus seems to require three clusters of basic amino acids in the juxtamembrane domain of all ErbB family members that share homology with known nuclear localization sequences [73].

How these large transmembrane proteins are able to free themselves from early endosomes to enter the nucleus is unclear. Interaction with importin-β, Nup358 and CRM1 may facilitate import of receptors to the nucleus [66]. The early endosomal marker EEA1, ErbB2 and importin-γ form a complex that may help target early endosomes directly to the nuclear envelope for receptor translocation to the nucleus. Others have suggested that ErbB family members may be extracted from the nuclear membrane by the Sec61 translocon in a manner similar to the endoplasmic reticulum-associated degradation system [61,74].

ErbB4 uses a mechanism unique to ErbB members to enter the nucleus. ErbB4 does not bind c-Cbl and does not internalize into endosomes, yet is able to directly influence gene transcription through targeting of its intracellular domain to the nucleus [5] (Fig. 2D). Two alternate splice forms of ErbB4 mRNA are translated: JM-b, which produces the canonical EGF family RTK, and JM-a, which produces an ErbB4 RTK that is susceptible to dual protease cleavage [75]. Stimulation of the JM-a splice variant of ErbB4 by ligand promotes proteolytic cleavage of ErbB4 by the ADAM17 metalloproteinase TACE at the extracellular surface. Presenilin/γ-secretase then cleaves ErbB4 in the transmembrane domain, which releases an active dimeric tyrosine kinase domain termed s80/E4ICD into the cytoplasm

[70]. s80 can then act as a chaperone for the WW domain YES-associated protein (YAP-1) as well as STAT5A [76,77]. In complex with s80, YAP-1 is translocated to the nucleus where the complex can activate transcription. This process is balanced by the WW domain protein WWOX that can sequester s80 in the cytosol [78]. Supporting the *in vivo* role for s80 in transcriptional regulation, s80 has been shown to form a complex with the adaptor protein TAB2 and the nuclear co-repressor N-CoR causing repression of genes involved in astrogenesis [79].

Signal attenuation

Unrestrained cell growth and survival underlies many human diseases and is the hallmark of cancer. Given the powerful ability of the ErbB receptors to stimulate cell proliferation, survival, differentiation and migration, tight regulatory control of the pathway is paramount to organism survival. Immediate negative regulation of signaling can occur through dephosphorylation and internalization of the activated receptors. Delayed signal attenuation through the expression of negative regulators and lysosomal degradation of receptors also helps keep ErbB signaling in check.

Tyrosine dephosphorylation of active ErbB receptors can occur through phosphatases, including density-enhanced phosphatase-1 (DEP1) [80] and protein tyrosine phosphatase PTP1B [81]. Dephosphorylation has the capacity to down-regulate activated receptors through abolishment of binding sites for signaling intermediates and adaptor proteins. Although PTP1B can dephosphorylate ErbB receptors, its relative contribution to down-regulation of ErbB receptors is questionable as PTP1B appears to promote oncogenesis in ErbB2-induced mammary tumorigenesis in mice, suggesting other targets for this phosphatase in regulating cell proliferation [82,83]. Internalization of activated receptors plays a central role in dampening signaling by targeting receptors for lysosomal degradation or in some cases promoting ligand dissociation (Fig. 2E). Receptor endocystosis may also play a role in targeting receptors to the cell nucleus. ErbB1 and ErbB2 can be found associated with caveolae in their unactivated state. Ligand binding and receptor dimerization may facilitate movement out of caveolae and is required for clathrin-mediated receptor internalization [84,85]. Binding of Grb2 to EGFR appears necessary for receptor internalization [86]. Activated, tyrosine phosphorylated EGFR uniquely binds the E3 ubiquitin ligase Cbl, which is capable of ubiquitinylating EGFR leading to lysosomal degradation of the receptor [87]. Cbl appears to be recruited to EGFR by Grb2, and this complex alone may be sufficient for endocytosis of the receptor [88]. Further complex formation with CIN85 and endophilin promotes receptor endocytosis into early endosomes [89]. EPS15 also associates with the clathrin adaptor protein AP-2 on receptor activation and may bind ubiquitinated EGFR through ubiquitin-interacting motifs (UIMs) to promote receptor endocytosis [90]. Parkin, an E3 ubiquitin ligase, promotes signaling through the PI(3)K/Akt pathway by activated EGFR by binding the UIM of EPS15 and limiting receptor endocytosis [91]. Surprisingly, endocytosis does not require activation of the EGFR tyrosine kinase domain, and rather appears dependent on receptor dimerization [84,85].

Once in early endosomes, unoccupied EGFR tends to quickly recycle back to the cell surface, whereas ligand-bound receptor recycles more slowly or is degraded in lysosomes [92]. As such, EGFR may become deactivated after ligand dissociation, promoting de-ubiquitination of the receptor and thus allowing for recycling of receptor back to the cell surface. Alternatively, recruitment of adaptors to ubiquitinated EGFR may target the receptor to lysosomes for degradation. Notably, targeting of receptors for lysosomal degradation may depend on the specific receptor-ligand pair. For instance, the association of TGF-α with EGFR is disrupted at endosomal pH allowing for recycling of ligand-free

receptors. EGF, however, has a higher affinity for EGFR at endosomal pH and the EGF-EGFR complex would be more likely to be targeted for lysosomal destruction [61,93,94].

Signal attenuation *via* lysosomal targeting of EGFR can be overcome by heterodimerization with ErbB2. Unlike other ErbB receptors that undergo endocytic recycling upon ligand-receptor internalization, ligand-mediated homodimerization and internalization of EGFR can target the ligand-receptor complex to the lysosomal compartment preferentially over recycling to the cell surface [95]. Overexpression of ErbB2 promotes heterodimerization with EGFR [96,97]. Notably, EGFR-ErbB2 heterodimers evade lysosomal degradation in favor of endocytic recycling of EGFR-ErbB2 to the cell surface, leading to increased signal duration and potency [94,98]. Therefore, ErbB2 overexpression leads to increased EGFR density on the cell surface and prolongs activation of downstream MAPK and c-Jun [96,99,100].

Activation of EGFR signaling leads to the transcription and translation of signal attenuators that dampen EGF signaling. Among these negative regulators is suppressor of cytokine signaling-5 (SOCS-5), which associates with EGFR and suppresses its mitogenic activity likely by promoting receptor degradation [101]. Others negative regulators induced by EGFR signaling include Sprouty-2, which modulates the Ras/MAPK pathway, LRIG-1, which enhances Cbl recruitment to EGFR and EGFR ubiquitinylation, and MIG-6/RALT, which binds directly to tyrosine kinase domain αI helix of EGFR allosterically inhibiting its catalytic activity [102,103]. Consistent with its proposed role in negatively regulating EGFR, mutation of LRIG-1 (LIG-1) in mice leads to psoriasiform skin lesions consistent with the proposed role of EGFR in psoriasis [104,105]. Notably, Mig-6 mouse mutants hyperactivate EGFR, leading to MAPK stimulation and the development of benign and malignant tumors of the stomach, colon, biliary tree and lung with enhanced susceptibility to carcinogen-induced skin cancer [106,107]. Mig-6 has also been found to be mutated in non-small cell lung cancer (NSCLC) cell lines and rarely in primary lung cancer, suggesting that MIG-6 may also play a role as a tumor suppressor gene in humans [107].

Regulation of dimerization is another strategy for attenuating signaling (Fig. 2C). As above, ErbB2 is unique among the ErbB family of RTKs with an ectodomain that maintains an extended structure similar to the ligand-bound EGFR ectodomain. ErbB2 is also a potent activator of intracellular signaling upon heterodimer formation with other ligand-bound ErbB receptors. HSP90 binds to the N-lobe of the catalytic tyrosine kinase domain of ErbB2, apparently independent of cellular stress. HSP90 has been shown to stabilize ErbB2, likely by preventing ubiquitinylation and proteosomal degradation [108,109]. HSP90 also limits both tyrosine kinase activity and heterodimer formation thus restraining excessive EGF signaling [110]. HSP90 also appears to restrain Src-dependent activation of ErbB2 by phosphorylation of Tyr877 in the activation loop of the tyrosine kinase domain [111]. Inconsistent with the apparent role of HSP90 in limiting receptor phosphorylation and dimerization, high HSP90 levels in breast cancers have been associated with poor prognosis possibly by increasing levels of ErbB2 available for dimerization, although the precise mechanism remains to be determined [112].

ErbB members in mammalian development

Mice deficient in ErbB family members display a wide range of phenotypes consistent with roles for the receptors in cell differentiation, proliferation, migration, and survival (Table 2). Wide variation in the phenotypes of EGFR-deficient mice in different genetic backgrounds highlights the amount of redundancy and regulation within this system. EGFR-deficient mice die from preimplantation to 3 weeks postnatally depending on genetic background and show defects in cell proliferation, differentiation and migration in a wide range of tissues

including skin, central nervous system, intestines, lung, liver, kidneys, placenta, palate and facies [113-116]. Strain-independent neurodegeneration within the frontal cortex, olfactory bulbs, and thalamus occurs postnatally in EGFR-deficient mice as well [117,118].

ErbB2 is the common and preferred partner of other ErbB family members. ErbB2 plays a role in both the formation and maintenance of the ventricular myocardium. Mice lacking ErbB2 die at mid-gestation likely due to failure of cardiac trabeculae formation [119,120]. Conditional deletion of ErbB2 in the ventricular myocardium of mice leads to postnatal dilated cardiomyopathy and death [121,122]. In addition, mice deficient in ErbB2 show defects in cranial sensory ganglia likely due to defects in cranial neural crest [119,123]. ErbB2 plays a role in the terminal differentiation of oligodendrocyte precursors to mature oligodendrocytes in the spinal cord [124] as well as in the myelination of peripheral nerves by Schwann cells [125-127]. Rescue of the embryonic lethal cardiac defects of ErbB2-deficient mice by myocardium-restricted expression of wild-type ErbB2 results in mice with severe defects in Schwann cell migration in the peripheral nervous system with loss of sensory and motor neurons [125,126].

ErbB3-knockout mice show defective atrioventricular valve formation, leading to profound valvular regurgitation likely contributing to death around embryonic day (E) 13.5. ErbB3-null mice also develop neuropathies from defective myelination of peripheral nerves due to lack of Schwann cell precursors and therefore mature Schwann cells [128]. In addition, they have defects in formation of the cerebellum and cranial nerve ganglia [120].

Similar to ErbB2 mutant mice, ErbB4-deficient mice die mid-gestation (E10 – E11) with failure of trabeculation of the ventricular myocardium [129]. Also, conditional disruption of ErbB4 expression within the ventricular myocardium leads to dilated cardiomyopathy [130]. Unlike neuregulin-1 or ErbB2, ErbB4 mutant mice also have defective innervation of the hindbrain likely due to the loss of an inhibitory effect on axonal pathfinding by ErbB4 [129].

Mice lacking EGFR ligands show much less severe phenotypes than EGFR mutant mice, demonstrating the high degree of redundancy built into EGFR signaling. Mice lacking EGF, TGF-α or amphiregulin fail to recapitulate the full range of EGFR-knockout phenotypes. EGF- and amphiregulin-null mice do not have an easily identifiable phenotype [131,132]. Targeted and naturally occurring (*waved-1*) mutations of TGF-α in mice leads to defects in hair follicle and eye development, phenotypes similar to the spontaneous EGFR mutation of the *waved-2* mouse [133-135]. Similar to EGFR-null mice, TGF-α-knockout mice also show decreased forebrain neural progenitor cell proliferation, although this does not manifest in an obvious behavioral phenotype in the mice [136]. Female mice with combined deficiency of amphiregulin, EGF and TGF-α display diminished mammary gland ductal outgrowth during puberty with severe defects in mammopoeisis and lactogenesis [131]. Triple mutant mice deficient in EGF, TGF-α, and amphiregulin partially phenocopy EGFR-deficient mice, displaying decreased cell proliferation in the intestine leading to attenuated ileal villi and decreased mucin production [132].

Unlike the apparent redundancy in ligand signaling through EGFR, the role of ErbB receptors and ligands in cardiac development appears to have less redundancy. Neuregulin-1 binds both ErbB3 and ErbB4 [1]. ErbB3 and ErbB4 participate with ErbB2 in activating downstream signaling. Unlike the EGFR ligands above, targeted disruption of neuregulin-1 leads to failed cardiac trabeculation and cardiac cushion defects closely recapitulating the phenotypes ErbB2 and ErbB4 mutants. Neuregulin-1 is expressed in the endocardium and appears to act in a paracrine fashion through myocardium-restricted ErbB2 and ErbB4 to stimulate myocardial trabeculae formation [129,137]. Neuregulin-1-deficient mice also have deficient Schwann cell and cranial ganglia formation similar to those seen in ErbB2 and

ErbB3 mutant mice [120,125-128,137]. Similarly, deletion of HB-EGF, a ligand for both EGFR and ErbB4, leads to dilated cardiomyopathy similar to conditional mutant mice lacking ErbB2 or ErbB4 in the ventricular myocardium [138]. The cardiac phenocopies of neuregulin-1 and HB-EGF mutant mice with their cognate receptor mutants shows that, although ErbB signaling has used ligand redundancy in some processes, restricted ligand-receptor pairing has also occurred to aid the specification of defined tissues during mammalian development.

ErbB members in disease

The phenotypes of mice deficient in ErbB members demonstrate the extensive role these receptors play in the development and maintenance of many tissues in the mammal. Aberrant signaling through the EGF family of receptors is implicated in many human diseases. Psoriasis may be driven in part by unregulated activation of EGFR consistent with its aberrant expression throughout the interfollicular epidermis of psoriatic lesions. Mouse models also support this hypothesis with thinned epidermis in mice lacking EGFR, and psoriasiform lesions in mice lacking the EGFR inhibitor LRIG-1 [104,105]. *Mycobacterium leprae* can bind directly to ErbB2 to activate the receptor and MAPK signaling independent of dimerization with other ErbB receptors. Activation of the receptor leads to demyelination of nerves and would be expected to lead to the peripheral neuropathies characteristic on leprosy [139].

Neuregulin-1 has also been linked to the cerebral cortical illnesses Alzheimer's disease and schizophrenia. In the brains of patients with Alzheimer's disease, neuregulin-1 is up-regulated in neuritic plaques, suggesting a role for ErbB signaling in the disease [140]. Accumulation of unprocessed neuregulin-1 and defective myelination occurs within cells lacking β-amyloid cleaving enzyme-1 (BACE1), an enzyme thought important in the pathophysiology of the Alzheimer's disease [141]. Polymorphisms in human neuregulin-1 gene are also associated with psychotic features in some families with late onset Alzheimer's disease [142]. Similarly, neuregulin-1-ErbB4 signaling has also been implicated in the pathogenesis of schizophrenia based on association between neuregulin-1 polymorphisms and the disease in some populations [143-145]. Supporting this genetic association, increased neuregulin-1-dependent ErbB4 signaling is seen in the prefrontal cortex of the schizophrenic brain and is associated with decreased activity of the NMDA receptor, possibly contributing to the pathogenesis of schizophrenia [146].

Of the diseases in which EGF signaling is thought to play a role, cancer is the most studied and has led to the development of numerous strategies to target the receptors as well as effectors of ErbB signaling. Early evidence for the role of ErbB receptors as proto-oncogenes came from chemically induced neuroblastomas in rats. Neuroblastomas from these animals were shown to express a mutant ErbB2 homolog called *neu*. Since this discovery, a successful effort to generate mouse models of ErbB2-dependent breast cancer has informed our understanding of ErbB2/HER2/neu-dependent tumorigenesis [147]. Transgenic mice driving activated *neu* (*neu*-NT) expression within mammary epithelium under the mouse mammary tumor virus promoter (MMTV-neu-NT) rapidly develop mammary tumors within the entire mammary epithelium [148-150]. The rapidity of tumor development in theses mice suggested that activated *neu* alone might be sufficient to transform mammary epithelium without the need for second site mutations. Conditional expression of *neu*-NT in the mammary epithelium using a tetracycline regulatory element (MMTV-rTA;TetO-neu-NT) also led to multiple metastatic mammary tumors after administration of doxycycline [151]. Notably, withdrawal of doxycycline led to the regression of both primary mammary tumors and pulmonary metastases, supporting the notion that activated *neu* alone could promote and sustain mammary tumors. Over time,

however, mammary tumors recurred independent of *neu*, possibly due to the development of oncogenic mutations within the originally hyperproliferative epithelium. Up-regulation of the transcriptional repressor Snail promoted this *neu*-independent mammary tumor recurrence [152]. Therefore, activation of ErbB2 alone was sufficient to induce mammary tumors and maintain tumor growth and survival, while abolishment of ErbB2 stimulation within established mammary tumors was not sufficient to permanently eradicate these tumors.

Consistent with the initial identification of ErbB2-dependent malignancy in chemically induced neuroblastomas in the rat, mouse models of ErbB2-dependent mammary carcinogenesis and the oncogenic potential of retroviral EGFR homolog v-erb, the EGFR family of RTKs has now been implicated in a wide range of human malignancies (Table 3). Overexpression and mutation of ErbB family members and their ligands have been identified in cancers of the breast, lung, colon, stomach, pancreatic, ovary, brain, prostate, and kidney [153]. A massive effort has led to understanding of the mechanisms and mutations leading to unregulated ErbB signaling in human malignancies.

Overexpression of wild-type ErbB receptors is very common in a wide range of tumors and represents a common strategy of malignancies to activate pathways downstream of ErbB receptors, thereby promoting cell proliferation, survival, invasion and metastasis. Gene amplification of ErbB2 leads to receptor overexpression in 20 – 30 % of breast cancers and is associated with decreased survival and increased relapse rate [154]. About 40 % of primary glioblastoma multiforme (GBM) and 5 – 10 % of NSCLCs have gene amplification of EGFR [155–157].

Activating deletions and truncations within EGFR happen frequently in GBM and less commonly in carcinomas. N-terminal truncation (EGFRvI), deletion of exons 14 – 15 (EGFRvII), deletion of exons 2 – 7(EGFRvIII), deletion of exons 25 – 27 (EGFRvIV) and C-terminal truncation (EGFRvV) as well as other C-terminal duplications and truncations have been identified as contributing to carcinogenesis [157]. Of these mutations, EGFRvIII is the most common in GBM and also occurs in lung, breast, ovarian and prostate cancers [158-161]. Activating EGFR tyrosine kinase domain mutations have also been documented in 10 – 20 % of NSCLCs. These mutations cluster around exons 18 – 21, which define the ATP binding pocket of the tyrosine kinase domain. Three types of mutation have been defined: missense mutations causing L858R, G719X and L861Q, in frame deletions within exon 19, and insertions in exon 20 [155,162]. Most of these mutations are associated with adenocarcinomas, including bronchioloalveolar carcinoma, typically in non-smoking women of East Asian ancestry.

Targeted therapy

Given the wealth of information regarding the structure and function of ErbB receptors and their importance in human cancer, the ErbB RTKs have proven ideal targets for the rational design of anti-cancer agents. The development of such agents has paved the way for a new era in the cancer chemotherapeutics.

The mechanism of action of ErbB receptors depends heavily on the interactions of their extracellular domains (ECDs) with activating ligands and with the ECDs of their dimerization partners. The ECDs are accessible to monoclonal antibodies (mAbs) and their function can be disrupted by appropriately targeted antibodies. Three humanized mAbs targeting ErbB receptors have been approved for clinical use. These mAbs were developed before the crystal structures of the ErbB ECDs were solved. The rationale for their development was the hypothesis that they would interfere with ligand activation of their targets or possibly induce endocytosis and degradation.

The first mAb to enter clinical use was the ErbB2-targeting mAb trastuzumab (Herceptin®). Trastuzumab is widely used in the treatment of ErbB2-amplified breast cancer where it causes tumor regression in 11 – 26 % of patients with metastatic disease and increases disease-free survival when used with traditional chemotherapy in early stage disease [163-165]. Our understanding of ErbB2 signaling has evolved since the development of trastuzumab, and interference with ligand binding or the induction of receptor endocytosis and degradation are unlikely mechanisms of inhibition in the case of trastuzumab and ErbB2 [166]. The clinical anti-cancer activity of trastuzumab has generated efforts to determine its mechanism of action but no compelling mechanism has yet emerged. Trastuzumab binds to ErbB2 in the juxtamembrane region of the ECD but the functional significance of this binding activity is not currently known [167]. Our understanding of the biology of ErbB2 in mouse models has also been predictive of side effects of ErbB2 inhibition. Consistent with the cardiomyopathy seen in mice lacking myocardial ErbB2 [121,122], one complication of trastuzumab is the development of a reversible cardiomyopathy often with congestive heart failure in approximately 3 % of patients [168].

Since the structural basis for ErbB dimerization has been elucidated, a second ErbB2-targeting mAb, pertuzumab, has been developed that binds to the dimerization interface of the ErbB2 ECD [169]. Pertuzumab potentially interferes with ErbB2 dimerization providing a rational basis for inhibiting ErbB2-mediated oncogenic signaling. Clinical studies are ongoing to determine the anti-tumor activities of pertuzumab.

Two more mAbs, cetuximab (Erbitux®) and panitumumab (Vectibix®), target the ECD of EGFR and are approved for clinical use. These mAbs have activity against colorectal cancer [170]. While colorectal cancers are not known to harbor genomic abnormalities that activate EGFR, a number of models suggest that they may be driven by overactivity of wild-type EGFR through persistent autocrine loops [171]. Cetuximab is also active in the treatment of cancers of the head and neck, which frequently overexpress EGFR [172].

The tyrosine kinase function of ErbB members is essential for intracellular signaling and cell transformation. As such, inhibition of this enzymatic function provides a rational basis for another class of targeted therapies, tyrosine kinase inhibitors (TKIs). However, the catalytic kinase domain of ErbB proteins shares significant homology with many other members of the human kinome, which presents unique challenges in the development of agents that selectively target ErbB kinase functions. Compounds based on the quinazoline structure show remarkable selectivity for ErbB family members. Three such compounds, gefitinib (Iressa®), erlotinib (Tarceva®), and lapatinib (Tykerb®), have been approved for clinical use. These compounds competitively bind within the ATP binding region of the kinase domain of ErbB receptors, with gefitinib and erlotinib most active against EGFR and lapatinib equally active against EGFR and HER2 [173,174]. Through inhibition of the ErbB tyrosine kinase domain, they block the generation of downstream signals. Both ligand-dependent and -independent signaling can be potentially inhibited by these agents. Although TKIs are similar in that they bind within the ATP-binding region of the kinase domain, there are significant differences among them. The kinase domain of EGFR generally exists in an auto-inhibited inactive conformation that adopts an active conformation by asymmetric contacts made during receptor dimerization [36]. EGFR kinase domain mutations in lung cancers destabilize the inactive conformation thereby favoring the active state [175]. Erlotinib and gefitinib bind the active conformation of the kinase domain and are effective in suppressing the activity of wild-type and certain mutant EGFRs [173,175]. The binding characteristics of each TKI appear to vary with the specific EGFR mutation and in the future the choice of TKI may be tailored to the unique molecular features of individual tumors. Lapatinib binds the inactive conformation of EGFR with a particularly tight binding that affords it a particularly slow off-rate [174].

The TKIs gefitinib and erlotinib are highly active in the treatment of NSCLCs with activating mutations in the tyrosine kinase domain of EGFR. Five retrospective studies of gefitinib in the treatment of NSCLC patients with and without EGFR mutations showed response rates of 60 – 94 % in patients with mutations versus 9 – 13 % without mutations. The same studies showed significant survival increases from 6.6 – 13 months to 13 – 30.5 months in patient with EGFR mutations [176]. The TKI lapatinib also shows activity and is approved for use in the treatment of breast cancers with HER2 overexpression when used in combination with certain chemotherapies [177].

Other strategies for ErbB-directed chemotherapy have been proposed but have not yet realized clinical application. The molecular chaperone HSP90 stabilizes ErbB2 at the cell membrane and increased expression of HSP90 in breast cancer is associated with poor prognosis [108,112]. Inhibition of HSP90 with molecules such as geldanamycin leads to ErbB2 degradation and ultimately attenuation of signaling [109]. The *in vivo* utility of such an approach remains to be determined given the diverse functions of HSP90. The ADAM family metalloproteinases are important in the proteolytic release of EGF ligands from the cell membrane and have been shown to be responsible for TKI (gefitinib) resistance in NSCLC cells, likely by increasing the free ligand concentration for EGFR and ErbB3 activation. Targeting of ADAM17 in NSCLC cells with the ADAM selective inhibitor (INCG3619) restores gefitinib sensitivity and is a novel strategy for adjunctive therapy with TKIs [178].

Inhibition of cycooxygenase-2 and histone deacetylase has also been proposed targets to block downstream effects of ErbB activation. Although theoretically sound, the lack of specificity of these therapies could possibly lead to intolerable systemic side effects. In contrast, siRNA, ribozymes, and antisense technologies would be specific means to down-regulate oncogenic ErbB but have yet to realize clinical utility [37].

Resistance to targeted therapy

The promise of the impressive response rates to TKIs in tumors with EGFR mutations is balanced by an unfortunately high rate of resistance to TKI therapy after initial treatment. For instance, the majority of NSCLCs with EGFR mutation initially respond to TKIs but then become resistant to this class of drugs [176]. Understanding the mechanism by which cancer cells become resistant to EGFR family TKI therapy is important for developing future therapies to overcome these mechanisms of resistance.

One mechanism underlying the development of resistance to ErbB-directed therapies is the activation of parallel or downstream pathways that allow autonomous tumor growth in the absence of direct signaling through ErbB receptors. Activating mutations in EGFR and less commonly ErbB2 occur in lung cancer and activate the Ras/MAPK pathway. However, mutational activation of KRAS is seen in lung cancers as well and can render these tumors resistant to ErbB receptor targeting TKIs [179]. Such a phenomenon is seen in approximately 14 % of lung adenocarcinomas [155,180]. NSCLCs containing KRAS mutations have a less than 4 % chance of responding to TKIs, whereas NSCLCs with EGFR mutation or increased copy number have a response rate of 60 – 94 % [155,176]. In a small subset (<3 %) of lung cancers BRAF is also mutated with alterations that would be predicted to activate the MAPK pathway [181,182].

Mutation of the TKI-binding site would be an expected mechanism of resistance in human cancer as such mutants would have a selection advantage in the setting of TKI therapy. In NSCLCs with mutant EGFR, a common exon 20 missense mutation (T790M) within the tyrosine kinase domain of mutant EGFR is responsible for TKI resistance in approximately

50 % of cases [183-185]. Also, point mutations in the ATP-binding pocket of HER-2/Neu may significantly impair the response of breast cancer cell lines to gefitinib [186].

EGFR mutant tumors can activate ErbB3 and subsequently the PI(3)K/Akt pathway to promote cell survival. Treatment of lung tumors with the TKI gefitinib down-regulates the PI(3)K/Akt pathway, leading to decreased cell proliferation and programmed cell death [187-189]. An increasing body of evidence highlights the central role of ErbB3 in the circuitries that mediate resistance to TKIs. MET is amplified in about 20 % of lung cancers with resistance to gefitinib or erlotinib, and is able to transactivate ErbB3 thereby promoting cell survival in gefitinib-resistant lung cancer cell lines [46]. Importantly, MET inhibition restores gefitinib sensitivity acting as a model for future therapies aimed at overcoming TKI resistance due to ErbB3 transactivation. Similarly, breast cancers containing amplified ErbB2 would be expected to respond to TKIs directed at ErbB2, but clinical studies show widespread resistance to TKI therapy in this disease [166]. This appears to be due to the failure of TKIs to effectively suppress ErbB3 signaling in these tumors due to Akt-driven negative feedback signaling loops [47]. Understanding the pathways important in ErbB3 transactivation in human malignancies may facilitate the development of therapies that limit resistance at the level of the receptor or inhibit ErbB3 transactivation. Understanding the complexities in the targeted therapy of ErbB proteins is critical to develop highly effective treatments for cancers driven by this oncogenic family of receptors [190].

The EGFR family members have roles in a broad spectrum of human diseases and are a paradigm for the translation of fundamental biological discoveries into therapeutics for human disease. Conversely, the attempt to clarify the mechanism of action of ErbB directed therapeutic agents has informed our understanding of the regulation of these receptors. Further understanding of the complexities of this pathway will undoubtedly lead to continued advances in cancer treatment and potentially the treatment of schizophrenia, neurodegenerative disease, psoriasis and other human illnesses.

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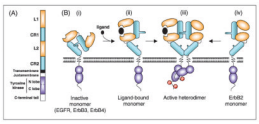

Figure 1.
Structure of ErbB receptors. (*A*) Linear representation of ErbB receptor domains. The extracellular N-terminal domain contains four subdomains. The leucine-rich subdomains L1 and L2 directly interact with ligand. The cysteine-rich subdomain CR1 contains the dimerization loop responsible for receptor-receptor interaction. A short transmembrane and juxtamembrane domain links the extracellular domain to bilobed tyrosine kinase domain and the C-terminal tail. (*B*) Schematic overview of the structural basis for ErbB receptor dimerization and activation. (i) In the ligand-free state, EGFR, ErbB3, and ErbB4 have a tethered conformation stabilized by interaction between of CR1 and CR2. (ii) Binding of ligand between L1 and L2 creates an extended conformation, which exposes the dimerization loop of CR1 allowing for receptor homo- and heterodimerization. (iii) Receptor dimerization apposes the tyrosine kinase N-lobe of one receptor with the C-lobe of its partner leading to C-terminal tyrosine phosphorylation, creating phosphotyrosine binding sites for binding of adaptors, signaling molecules and regulatory proteins. (iv) ErbB2 is unique in that it is fixed in the extended conformation ready to interact with other ErbB receptors.

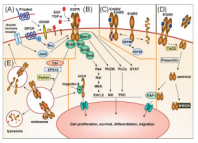

Figure 2.

ErbB receptor signaling and regulation. (*A*) Receptor activation. Activation of ErbB receptors by ligand occurs through paracrine and autocrine mechanisms. Ligands are membrane bound until processing by ADAM family metalloproteinases. Stimulation of ADAM metalloproteinase activity may come through activation of Frizzled by Wnt1 or Wnt5a or through activation of G protein-coupled receptors by ligands such as thrombin, endothelin, and lysophosphatidic acid. Alternatively, G protein-coupled receptor activation activates the soluble tyrosine kinase Src, which can phosphorylate EGFR. Activation of growth hormone receptors activates Jak2, which is able to phosphorylating EGFR as well. (*B*) Signal transduction to nucleus. Activation of ErbB receptors leads to activation of Ras/MAPK. PI(3)K/Akt, PLCγ/PKC, and STAT pathways. Tyrosine phosphorylated ErbB receptors bind adaptors such as Shc and Grb2 leading to Sos recruitment and Ras/MAPK pathway activation. EGFR requires the docking protein Gab-1 for activation of the PI(3)K/Akt pathway. Other ErbB receptors do not require Gab-1. Receptors themselves may also translocate to the nucleus to influence gene transcription. EGFR has a defined nuclear localization signal and interacts with importin-β in its transit to the nucleus. The cytosolic s80/E4ICD, the product of proteolytic release of the intracellular tyrosine kinase, translocates to the nucleus and has been shown to directly influence gene transcription. (*C*) Regulation of ErbB2 by HSP90. HSP90 stabilizes ErbB2 at the cell membrane and prevents unregulated heterodimer formation with other ErbBs such as the ‘kinase dead’ ErbB3. HSP90 also prevents degradation of ErbB2. (*D*) Sequential proteolysis of ErbB4. Two-step proteolysis of ErbB4 in the extracellular juxtamembrane region by TACE/ADAM17 followed by cleavage in the transmembrane domain by presenilin/γ-secretase liberates the intracellular portion of ErbB4 (s80/E4ICD), which can act as a nuclear chaperone for molecules such as YAP-1 and STAT5A. The WW-domain containing protein WWOX sequesters s80/E4ICD in the cytosol. (*E*) Ligand binding and dimerization of EGFR stimulates receptor endocytosis involving EPS15, which is inhibited by Parkin. The E3-ubiquitin ligase Cbl binds to phosphorylated EGFR leading to ubiquitinylation (Ub) and lysosomal targeting. Both de-ubiquitinated, ligand-free EGFR and EGFR/ErbB2 heterodimers preferentially recycle to the cell surface.

Table 1

ErbB receptor-ligand binding specificity.

	EGF	TGF-α	Amphiregulin	HB-EGF	Betacellulin	Epigen	Epiregulin	Neuregulin-1	Neuregulin-2	Neuregulin-3	Neuregulin-4	Tomoregulin	Neuroglycan C
EGFR	+	+	+	+	+	+	+						
ErbB2													
ErbB3								+	+				+
ErbB4					+	+	+	+	+	+	+	+	

Table 2

EGF receptor family mouse mutant phenotypes.

Gene	Mutation	Phenotype	References
EGFR	Exon 2 targeted deletions; Exon1 targeted deletion	Strain-dependent phenotypes CF-1: Peri-implantation embryonic lethal: inner cell mass degeneration 129Sv: Mid-gestation embryonic lethal: placental/ spongiotrophoblast defect CD-1: Perinatal lethality: curly, fragile whiskers, disoriented hair follicles, open eyes, epidermal thinning, disordered colonic crypts, thickened hepatocyte cords, distorted hepatic sinusoids, cystic dilatation of renal collecting ducts with epithelial flattening, renal failure, cerebellar atrophy, decreased cerebral cellular migration, anterior cerebral cortical atrophy 129Sv×C57BL/6: Perinatal lethality: small placenta, rudimentary whiskers, open eyes, corneal thinning, epidermal thinning, failure of hairy coat development, lung hypoplasia 129Sv×Swiss Webster Black: Perinatal lethality: open eyes, short, curly whiskers, delayed external ear opening, epidermal thinning, decreased hair follicles, disturbed terminal differentiation of epidermis and hair follicles, immature intestinal and lingual epithelium, decreased proliferation of jejunal enterocytes, intestinal villi disintegration, immature lungs	[113-115]
	Targeted deletions	Strain-independent phenotypes Postnatal neurodegeneration within frontal cortex, thalamus, olfactory bulbs	[117,118]
	waved-2	Wavy whiskers and fur, eye defects	[135]
erbB2	Promoter+exon 1 targeted deletion; Juxtamembrane domain-LacZ fusion; Exon coding amino acids 256–304 targeted deletion; TM domain targeted deletion	Mid-gestation embryonic lethal Cardiac: Absent cardiac trabeculae Nervous system:Abnormal development of cranial neural crest derived neurons Reduced Schwann cell precursors along sensory and motor neuron projections Reduced neuronal precursors in sympathetic ganglion chain Increased dorsal root ganglion cell death Defasciculation and degeneration of motor nerves Impairment of junctional folds at neuromuscular junction Failure of oligodendrocyte terminal differentiation in spinal cord	[119,120, 123,124, 127]
	Cardiac rescue of erbB2$^{-/-}$ mice	Reduced Schwann cell precursors along spinal nerve roots and distal peripheral nerves Sensory and motor nerve degeneration Defasciculated sensory and motor nerves	[125,126]
	Ventricular cardiomyocyte specific deletion	Dilated cardiomyopathy Cardiomyocyte sensitivity to anthracycline toxicity	[121,122]

Gene	Mutation	Phenotype	References
	Myocyte specific deletion	Proprioceptive defect due to loss of muscle spindles Decreased myoblast survival	[191]
	Colonic epithelial and enteric nervous system deletion	Enteric neuron and glia loss postnatally Colonic distension	[192]
	Mammary epithelial deletion	Reduced ductal elongation and branching	[193]
erbB3	Exons containing CR2 domain targeted deletion; Exon containing amino acids 73–107 targeted deletion	Mid-gestation embryonic to perinatal lethal Cardiac: Thinned cardiac cushion Hypoplastic cardiac valves Nervous system: Lack of Schwann cell precursors Lack of Schwann cells: peripheral nerve axons, cranial ganglia and dorsal root ganglia Degeneration of motor and sensory nerves Hypoplasia of sympathetic chain Absent mandibular branch of trigeminal ganglion Reduced enteric ganglia Cerebellar defects: hypoplasia, misshapen cerebellar plate, absent vermis, axonal reduction, reduced Purkinje cells, malformed choroid plexus of fourth ventricle Absent adrenal chromaffin cells Intestinal: Thinning of stomach wall, pancreatic hypoplasia	[120,123, 128]
erbB4	Targeted deletion	Mid-gestation embryonic lethal Cardiac: Absent cardiac trabeculae, small endocardial cushion Nervous system: Near fusion cranial ganglia V with VII/VIII Mis-targeting of sensory and motor axons to and from hindbrain Aberrant cranial neural crest migration	[129,194, 195]
	Cardiac defect rescue	Failure of mammary gland lobuloalveolar development, impaired lactation Cranial nerve defects Increased cerebellar large interneurons	[196]
	Ventricular cardiomyocyte-specific deletion	Dilated cardiomyopathy	[130]

Table 3

Genomic level abnormalities in ErbB genes reported in human cancers.

Gene	Malignancy	Alteration	Frequency
EGFR	Breast cancer	Gene amplification	5%
	Glioblastoma multiforme	Extracellular domain deletions and mutations Gene amplification	40–60%
	Non-small cell lung cancer	Kinase domain mutations	5–10% non-East Asian 30–50% East Asian
	Others	Gene amplification	Uncommon
ErbB2/HER2	Breast cancer	Gene amplification	20–30%
	Non-small cell lung cancer	Kinase domain mutation	4%
ErbB3	None reported		
ErbB4	Multiple	Kinase domain mutations	1–2% East Asian

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