Published in final edited form as:
Bioessays. 2007 June ; 29(6): 558–565. doi:10.1002/bies.20582.

Mutational activation of ErbB family receptor tyrosine kinases: insights into mechanisms of signal transduction and tumorigenesis

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Summary

Signaling by the Epidermal Growth Factor Receptor (EGFR) and related ErbB family receptor tyrosine kinases can be deregulated in human malignancies as the result of mutations in the genes that encode these receptors. The recent identification of EGFR mutations that correlate with sensitivity and resistance to EGFR tyrosine kinase inhibitors in lung and colon tumors has renewed interest in such activating mutations. Here we review current models for ligand stimulation of receptor dimerization and for activation of receptor signaling by receptor dimerization. In the context of these models, we discuss ErbB receptor mutations that affect ligand binding and those that cause constitutive receptor phosphorylation and signaling as a result of constitutive receptor dimerization. We discuss mutations in the cytoplasmic regions that affect enzymatic activity, substrate specificity and coupling to effectors and downstream signaling pathways. Finally, we discuss how emergent mechanisms of ErbB receptor mutational activation could impact the search for clinically relevant ErbB receptor mutations.

Introduction

The ErbB family of receptor tyrosine kinases includes the Epidermal Growth Factor Receptor (EGFR/ErbB1), ErbB2 (HER2/Neu), ErbB3 (HER3), and ErbB4 (HER4). Members of the Epidermal Growth Factor (EGF) family of peptide hormones serve as agonists for these receptors and include EGF, transforming growth factor alpha (TGFα), betacellulin, amphiregulin, epiregulin, epigen, heparin-binding EGF-like growth factor and members of the neuregulin (heregulin) subfamily of growth factors.(1) EGF hormones and ErbB receptors play critical roles in mammalian development and homeostasis and deregulated signaling by ErbB family receptors contributes to tumorigenesis and tumor progression; indeed, both EGFR and ErbB2 are validated targets for cancer chemotherapeutic agents.(1–3) Consequently, there is much interest in the mechanisms by which ErbB receptor signaling can be deregulated, particularly activating mutations of ErbB receptors. Here we will review the current model for ligand-induced ErbB receptor signaling. Within that context, we will also review activating mutations of ErbB family receptors. Activating mutations that have been identified in tumor specimens will be discussed. Activating mutations that have been generated in the laboratory but not yet identified in tumor specimens will also be discussed. This latter set of mutations

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may serve as the impetus for future attempts to identify activating mutations in tumor specimens.

Mechanisms of ErbB receptor signaling

ErbB family receptors consist of an extracellular region that contains two ligand-binding domains, an extracellular juxtamembrane region, a hydrophobic transmembrane domain, a cytoplasmic tyrosine kinase domain and cytoplasmic tyrosine residues that serve as sites for receptor phosphorylation (Fig. 1). Ligand binding results in receptor dimerization, phosphorylation across receptor dimers on tyrosine residues, recruitment of signaling molecules to the phosphorylated tyrosine residues, and coupling to downstream effectors and biological responses.(1) New insights into the mechanisms of ligand-induced ErbB receptor signaling have emerged from structural data. Fig. 2 indicates that the extracellular region of EGFR, ErbB3, and ErbB4 appears to exist in two distinct conformations.(4–7) The closed, inactive conformation features intramolecular interactions between extracellular subregions II and IV (Fig. 2A). These prevent the extension of the receptor dimerization domains present in subregions II and IV and also prevent the juxtapositioning of subregions I and III to form a ligand-binding pocket.

The open, active conformation lacks the intramolecular interactions between subregions II and IV (Fig. 2B). Thus, subregions II and IV extend away from the rest of the molecule and enable receptor dimerization via intermolecular contacts that involve subregions II and IV (Fig. 2C). Moreover, in the open conformation subregions I and III form a ligand-binding pocket that permits interactions between a single ligand molecule and subregions I and III (Fig. 2B).

In the absence of ligand binding, the extracellular region of EGFR, ErbB3 and ErbB4 appears to exist in equilibrium between the closed and open conformations. This equilibrium appears to favor the closed conformation; in the absence of ligand binding, it appears that approximately 95% of EGFR exists in the closed conformation. Given that only the open conformation appears to be capable of receptor dimerization, the preference of the receptor for the closed conformation seems to account for the absence of ligand-independent receptor signaling at typical physiologic receptor expression densities.(4,8)

Ligand binding stabilizes the EGFR extracellular region in the open conformation, thereby facilitating receptor dimerization and receptor signaling.(4,8) As depicted in Fig. 3, the mechanism by which receptor dimerization causes receptor signaling has been suggested by the structure of the EGFR kinase domain.(9) The EGFR tyrosine kinase domain is presumed to exist in equilibrium between catalytically active and inactive forms. This equilibrium favors the inactive form. However, formation of an asymmetrical dimer of kinase domains appears to stabilize the kinase domain of one of the receptor monomers in the active conformation. This proposed mechanism is analogous to the one by which cyclins stabilize cyclin-dependent kinases in the catalytically active conformation.(9) The asymmetrical dimer of receptor cytoplasmic domains is predicted to enable the presentation of tyrosine residues of one receptor monomer to the kinase domain of the other receptor monomer, thereby permitting tyrosine phosphorylation.(9) This model postulates that ErbB receptor tyrosine phosphorylation occurs in *trans* across a receptor dimer. Abundant experimental data support this model.(10–13)

The ErbB2 extracellular region exists in a conformation that does not appear to be suitable for binding EGF family peptide hormones.(14) However, this conformation does feature extension of the receptor dimerization arm found in extracellular subregion II. This suggests that ErbB2 is capable of ligand-independent dimerization and signaling. Indeed, despite the fact that ErbB2 does not bind any EGF family hormone, it homodimerizes at elevated expression levels and heterodimerizes with other ErbB family receptors in the presence of ligands for these receptors.(1,14)

Activating mutations of the extracellular region

The proposed models for ligand-induced ErbB receptor signaling suggest a variety of mechanisms by which ErbB receptor mutations could deregulate receptor signaling. Indeed, three different classes of activating mutations reside in the receptor extracellular region.

Ligand binding mutants

Ligand-binding mutants exhibit deregulated receptor signaling as a result of increased ligand-binding affinity. This may be manifested as increased affinity for a ligand that normally already binds with high affinity, resulting in increased sensitivity to that ligand. This may also be manifested as increased affinity for a ligand that typically fails to bind to the receptor, resulting in receptor signaling in tissues that typically do not display receptor signaling in response to that ligand. An example of the latter class is the artificial EGFR S418F mutant (also referred to as S442F, Table 1). EGFR Ser418 lieswithin the ligand-binding motif of extracellular subregion III (Fig. 1). The EGF family hormone neuregulin2 beta (NRG2β) does not bind to EGFR with high affinity and is not a potent agonist of EGFR signaling.(15–18) However, the S418F mutation increases the affinity of NRG2β for EGFR by more than an order of magnitude and increases the potency of NRG2β stimulation of EGFR tyrosine phosphorylation by a commensurate amount.(15) Thus, the S418F mutation and analogous EGFR mutations may result in stimulation of EGFR signaling by EGF family ligands in contexts in which these ligands do not typically stimulate signaling by wild-type EGFR. ErbB receptor mutants analogous to the EGFR S418F mutant have yet to be identified in tumors.

Conformational equilibrium mutants

Conformational equilibrium mutants alter the balance between the closed and open receptor extracellular region conformations to favor the open conformation. The resulting increase in the concentration of the receptor in the open conformation is typically manifested as increased ligand-dependent and -independent signaling.

Recall that interactions between EGFR extracellular subregions II and IV are required to maintain the EGFR extracellular region in the closed conformation (Fig. 2A). Disruption of these interactions would be predicted to shift the EGFR conformational equilibrium to favor the open conformation (Fig. 2B), resulting in increased ligand binding, receptor dimerization (Fig. 2C) and receptor signaling. Mutational activation of ErbB4 and EGFR appears to support this prediction. A soluble recombinant protein consisting of the entire ErbB4 extracellular domain (secErbB4) displays modest binding of the high-affinity ErbB4 ligand neuregulin1 beta (NRG1β). In contrast, deletion of a large portion of extracellular subregion IV from the recombinant ErbB4 extracellular domain (secErbB4 ΔG549-R649) results in a marked increase in NRG1β binding (Table 1).(19) Similarly, deletion of 10 residues from EGFR extracellular subregion IV (ΔV575-W584) increases EGF and TGFα binding (Table 1).(4) Presumably these artificial ErbB4 and EGFR mutants lack the interactions between subregions II and IV (Fig. 2A), thereby increasing ligand binding and receptor signaling by destabilizing the closed form of the receptor (Fig. 2A) and shifting the conformational equilibrium to favor the open form of the receptor (Figs 2B, C). Neither of these mutants has been found in human tumors.

However, the EGFRvIII mutant, which is frequently found in human glioblastomas, appears in many respects to be analogous to the aforementioned artificial ErbB4 and EGFR mutants. The EGFRvIII mutant lacks all of extracellular subregion I and most of subregion II and displays increased ligand-independent signaling, The current model for ErbB receptor signaling predicts that the loss of intramolecular interactions between subregions II and IV (Fig. 2A) in the EGFRvIII mutant causes increased ligand-independent receptor signaling by shifting the conformational equilibrium to favor the open form of the receptor (Fig. 2B). Thus,

the EGFRvIII mutant is functionally equivalent to the liganded wild-type EGFR.(20) It should be noted that there has been some controversy concerning the signaling mechanism for the EGFRvIII mutant. There has been a report that EGFRvIII fails to dimerize and signals via a noncanonical mechanism.(21) However, subsequent reports indicate that EGFRvIII homodimerizes(22) and heterodimerizes with wild-type EGFR.(23)

Point mutations that are predicted to shift the equilibrium between the open and closed conformations of the EGFR extracellular domain also appear to increase ligand binding and receptor signaling. Indeed, simultaneous substitution of alanine residues for Asp563, His566, and Lys569 (D563A/H566A/K568A, Table 1) in the segment of EGFR extracellular subregion IV (Fig. 1) that appears to be responsible for intramolecular interactions with subregion II increases EGF and TGFα binding.(4) By disrupting the interactions of subregion II with subregion IV, these mutations shift the extracellular domain conformational equilibrium of this mutant away from the closed form (Fig. 2A) in favor of the open form (Fig. 2B). This shift in equilibrium would account for the increased ligand binding displayed by this mutant. However, it is not known whether this mutant displays the increase in ligand-independent EGFR dimerization and signaling activity that would be the expected effect of such a shift in the conformational equilibrium.

Point mutations of EGFR extracellular subregions II and IV have been found in glioblastoma samples (Table 1), suggesting that mutations that alter the equilibrium between the open and closed conformations may contribute to deregulated EGFR signaling in these tumors. For example, the T239P (T263P) and A265V/D/T (A289V/D/T) mutations in EGFR extracellular subregion II (Fig. 1) may disrupt the interactions between subregions I and II or II and IV (Fig. 2A), thereby shifting the EGFR conformational equilibrium to favor the open, dimeric conformation (Figs 2B, 2C).(24) Likewise, the P572L (P596L) and G574V (G598V) mutations in EGFR extracellular subregion IV may disrupt the interactions between subregions II and IV and shift the conformational equilibrium to favor the open, dimeric conformation.(24) These hypotheses are particularly attractive given that deletion of amino acids 575 through 584 of EGFR extracellular subregion IV causes a dramatic increase in EGF binding, indicative of a shift in the conformational equilibrium to favor the open, dimeric conformation.(4) In fact, the T239P, A265V and G574V mutations display ligand-independent (constitutive) receptor signaling and coupling to malignant growth transformation, indicating that these mutations do indeed shift the conformational equilibrium to favor the open, dimeric receptor conformation (Table 1).(24) Finally, the existence of these EGFR extracellular domain mutations in glioblastoma samples is the best argument to date that the scope of sequencing efforts to identify activating point mutations in ErbB receptors in human tumor samples should extend beyond the kinase domain.

The EGFR S418F mutant (Table 1) displays ligand-independent EGFR coupling to PI3 kinase activity, Akt phosphorylation, and cell survival.(15) Because this mutation lies in the predicted ligand-binding segment of EGFR extracellular subregion III (Fig. 1), it seems plausible to postulate that this mutation mimics ligand binding by stabilizing the EGFR extracellular region in the open conformation (Fig. 2B). It should be noted that the steady-state expression of the EGFR S418F mutant is markedly lower than that of wild-type EGFR. This suggests that the S418F mutation does indeed confer ligand-independent signaling and turnover.

The v-ErbB oncogene

The v-ErbB oncogene of the avian erythroblastosis virus encodes a homolog of the avian EGFR protein. However, the v-ErbB mutant (Table 1) lacks almost the entire extracellular region and possesses constitutive signaling activity.(25) It has long been postulated that ErbB receptor extracellular domains contain elements that inhibit receptor dimerization and signaling and that removal of these extracellular elements permits constitutive dimerization and signaling.

(26) A corollary of this postulate is that ErbB receptor transmembrane and cytoplasmic domains are sufficient for receptor dimerization and signaling. As will be discussed later, the transmembrane domain of ErbB receptors can mediate receptor dimerization. Furthermore, as was discussed earlier, the cytoplasmic tyrosine kinase domain of ErbB receptors can mediate asymmetric kinase domain dimerization. Apparently these two sites of interaction are sufficient for v-ErbB dimerization and signaling in the absence of positive or negative influences of the extracellular region.

Activating mutations of the juxtamembrane region

Substitution of a single cysteine residue for a residue in the extracellular juxtamembrane region of ErbB2 or ErbB4 can cause receptor homodimerization, presumably via an intermolecular disulfide bond between the novel cysteine residue of each of two mutant receptor molecules. Homodimerization can be accompanied by ligand-independent receptor tyrosine phosphorylation.(27–29) For example, the artificial ErbB4 Q646C, H647C and A648C mutants all display ligand-independent dimerization and tyrosine phosphorylation.(27,28) Likewise, a single amino acid substitution of a cysteine residue for residues 652 through 659 of the ErbB2 juxtamembrane domain results in constitutive ErbB2 dimerization.(29) However, only the ErbB4 Q646C and ErbB2 V656C and T657C mutants display constitutive coupling to signaling effectors and coupling to biological responses (Table 1).(28,29) This signaling specificity may be due to differences in the juxtapositioning or conformation of the receptor monomers within the receptor dimer, which may be manifested as differences in the sites of receptor phosphorylation or as differences in phosphorylation site availability for effector binding.

Analogous mutations in the juxtamembrane region of ErbB2 have been observed in tumors that arise in mice that carry the wild-type ErbB2 transgene under the transcriptional control of the promoter from the mouse mammary tumor virus.(30) These mutations either create or eliminate a cysteine residue and are predicted to create a novel intermolecular disulfide linkage between the juxtamembrane region of two ErbB2 molecules. Indeed, these mutants display increased tyrosine phosphorylation and transforming activity in fibroblasts.(30) However, there have been no reports of analogous ErbB juxtamembrane domain mutants in human tumor samples.

Activating mutations of the transmembrane region

The transmembrane region of wild-type ErbB2 self associates in artificial membranes, suggesting that the transmembrane region is sufficient to mediate receptor dimerization and signaling.(31) Substitution of a glutamate residue for Val664 in the transmembrane domain of ErbB2 (V664E, Table 1) has been observed in rat brain tumors.(32,33) This mutation increases ErbB2 dimerization affinity(34,35) and signaling and transforming activities.(36) Varying the site of glutamate substitution within the ErbB2 transmembrane region results in ErbB2 alleles with varying signaling and transforming activities, suggesting that ErbB2 signaling is sensitive to differential juxtapositioning of the ErbB2 monomers within the receptor dimer.(29,37,38) Artificial EGFR or ErbB4 transmembrane domain mutants that possess a glutamate residue substitution for the residue that corresponds to ErbB2 Val664 (V627E and I658E, Table 1) display constitutive signaling activity.(39,40) However to date there have been no reports of analogous ErbB transmembrane domain mutants in tumor samples.

Activating mutations of the cytoplasmic region

Kinase domain mutations

Kinase domain mutations in EGFR have recently been identified in a subset (~10–30%) of non-small cell lung carcinomas (NSCLC) and are correlated with a clinical response to

selective small molecule inhibitors of the EGFR kinase (Iressa; gefitnib and Tarceva; erlotinib). The recurrent L834R missense mutation (also referred to as L858R) and small in-frame deletions within the EGFR catalytic pocket (Table 1) are associated with increased sensitivity to gefitinib and erlotinib.(41) It has been proposed that these mutations cause increased EGFR signaling that selectively engages downstream survival pathways to which tumor cells become "addicted". Indeed, these mutants display increased kinase activity(9) and coupling to the phosphorylation of Akt and Stat5.(42) Consequently, disrupting EGFR signaling in these cells through the use of kinase inhibitors leads to cell death and tumor shrinkage.

The association of these EGFR kinase domain mutations with sensitivity to EGFR kinase inhibitors in NSCLC has led to numerous reports of EGFR kinase domain mutations in tumor samples as well as reports of correlations of EGFR kinase domain mutations with sensitivity to tyrosine kinase inhibitors. A comprehensive review of this literature is beyond the scope of this review. However, we refer readers to several excellent recent reviews of these topics. (43–46)

As discussed elsewhere, structural analyses suggest that the EGFR kinase domain exists in equilibrium between catalytically active and inactive conformations. This equilibrium favors the inactive conformer. Upon EGFR dimerization, two EGFR tyrosine kinase domain monomers appear to form an asymmetric dimer in which a catalytically inactive monomer stabilizes the other monomer in a catalytically active conformation through displacement of the kinase domain "activation loop". Dimerization also causes presentation of tyrosine residues on the catalytically inactive monomer to the kinase domain of the catalytically active monomer for phosphorylation. The L834R mutation and small in-frame deletions both appear to shift the conformational equilibrium to favor the active form, presumably accounting for the increased signaling displayed by these mutants.(9) Indeed, these mutants display ligand-independent coupling to biological responses.(47,48) This suggests a mechanism by which tumor cells can become "addicted" to these EGFR mutants.

The EGFR T766M point mutation (also referred to as T790M) has been detected in ~50% of NSCLC tumors that initially responded to gefitinib or erlotinib but subsequently developed resistance to these agents (Table 1).(49) TheT766M mutation appears to be acquired during drug treatment. This mutation is analogous to the T315I mutation in BCR-Abl, the T674I mutation in PDGF receptor alpha and the T670I mutation of c-Kit, all of which are associated with the acquisition of resistance to small molecule tyrosine kinase inhibitors. The conserved threonine residue that is affected by these mutations is referred to as the "gatekeeper" site. It is situated deep within the catalytic pocket and substitution with a bulky amino acid appears to reduce drug binding yet preserves catalytic function of the tyrosine kinase domain. Indeed, relative to wild-type EGFR, the EGFR T766M mutant displays marked resistance to inhibition of tyrosine phosphorylation by gefitinib or erlotinib.(49) This model is supported by the observation that the T766M mutant retains sensitivity to the irreversible EGFR tyrosine kinase inhibitors CL-387,785 and HKI-272, which exhibit a much lower dissociation constant for EGFR than does gefitinib.(50) Thus, such irreversible inhibitors may represent an appropriate treatment for a subset of tumors that are initially responsive to gefitinib and erlotinib but subsequently become resistant to these agents. Interestingly, the T766M mutation has also been observed at a low frequency in a subset of untreated NSCLCs and esophageal tumors,(51) suggesting a potential oncogenic role in addition to its role in acquired drug resistance. Consistent with this possibility, it has recently been reported that in a family with evidence of inherited predisposition to NSCLC, the T766M mutation is present in the germline of affected individuals.(52)

Various mutations in the kinase domain of ErbB2 and ErbB4 have been identified in human tumor samples (Table 1).(53–55) Many of these mutations are located in or around the ATP-

binding region of the active site of the kinase domain, suggesting that these mutations cause increased receptor tyrosine kinase activity. Indeed, an insertion mutation in the ErbB2 kinase domain (Ins776YVMA, Table 1) found in NSCLCs displays increased tyrosine kinase activity, suppresses apoptosis and possesses transforming activity.(56) These data suggest that this mutation contributes to NSCLC tumorigenesis or malignant phenotypes. In contrast, little is known about the functional consequences of the ErbB4 kinase domain mutations or the roles that these ErbB4 mutants play in human malignancies.

Phosphorylation site mutations

Phosphorylation site mutations in EGFR and ErbB4 can alter receptor signaling. Phosphorylation of cytoplasmic tyrosine residues of ErbB receptors creates sites for docking cytoplasmic signaling proteins that contain SH2 or PTB domains and elimination of these sites of phosphorylation disrupts coupling to these proteins.(1,57) For example, EGFR phosphorylation on Tyr1045 permits c-Cbl binding to EGFR and EGFR ubiquitination and downregulation. The artificial Y1045F mutation (Table 1) abrogates c-Cbl binding to EGFR and EGFR ubiquitination. This results in decreased EGFR downregulation and increased EGFR signaling.(58) EGFR Tyr1068 and Tyr1086 are coupled to Cbl via Grb2 and the artificial Y1068F and Y1086F mutants (Table 1) display reduced EGFR ubiquitination and internalization.(59–61) However, the effects of Y1068F and Y1086F mutations on EGFR signaling are unknown. Moreover, EGFRY1045F, Y1068F and Y1086F mutations have not been detected in tumor specimens, nor have analogous mutations in other ErbB receptors been detected.

Substitution of phosphotyrosine mimics such as glutamate or aspartate for cytoplasmic ErbB receptor tyrosine residues may allow for constitutive ErbB receptor coupling to cytoplasmic signaling proteins and downstream events. Indeed, the artificial ErbB4 Y1056E mutation (Table 1) rescues signaling disrupted in the kinase-deficient ErbB4 K751M mutant and permits ErbB4 coupling to tumor suppression activity in prostate tumor cell lines.(62) However, given that ErbB4 Tyr1056 resides in a motif that is predicted to bind proteins that contain a WW domain, there are concerns that the Y1056E mutation does not function through increased binding of proteins that contain SH2 or PTB domains.(63–65) Furthermore, the ErbB4 Y1056E mutation and analogous mutations in ErbB4 or other ErbB family receptors have not been detected in tumor specimens.

Conclusions and outlook

Activating mutations in ErbB receptor tyrosine kinases can be found in a variety of locations throughout these proteins. While only a few of these classes of activating mutations have been found in tumor specimens, it is possible that future studies will reveal associations between the remaining classes of activating mutations and human malignancies or other pathological states. Indeed, the existence of activating mutations throughout the full length of ErbB receptors suggests that sequencing entire ErbB receptor cDNAs may be necessary to determine whether or not a given pathological state is associated with an activating mutation of that ErbB receptor. Given the emerging plethora of agents that target ErbB receptors and ligands, such studies may also reveal new opportunities for therapeutic intervention.

Abbreviations

EGFR, Epidermal Growth Factor Receptor; EGF, Epidermal Growth Factor; TGFα, transforming growth factor alpha; NRG2β, Neuregulin2 beta; NRG1β, Neuregulin1 beta; EGFRvIII, EGFR variant III; NSCLC, non-small cell lung carcinoma; STAT, signal transducer and activator of transcription; PTB, phosphotyrosine-binding; SH2, src-homology domain 2..

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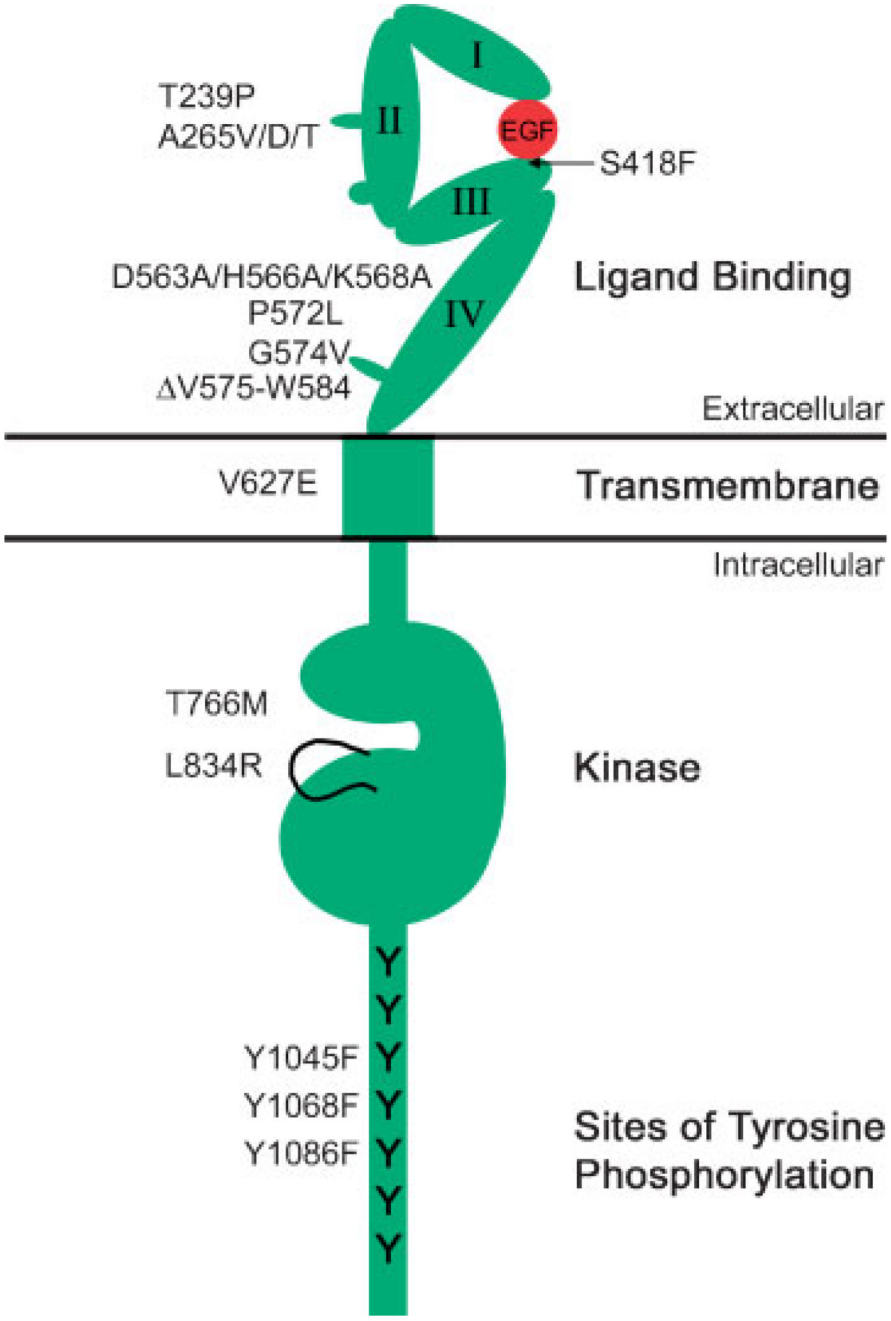

Figure 1.
Organization of a prototypical ErbB family receptor. Shown is a stylized depiction of the functional motifs. The extracellular domain is at the amino terminus and the cytoplasmic domain is at the carboxyl terminus. Examples of activating mutations in EGFR are listed.

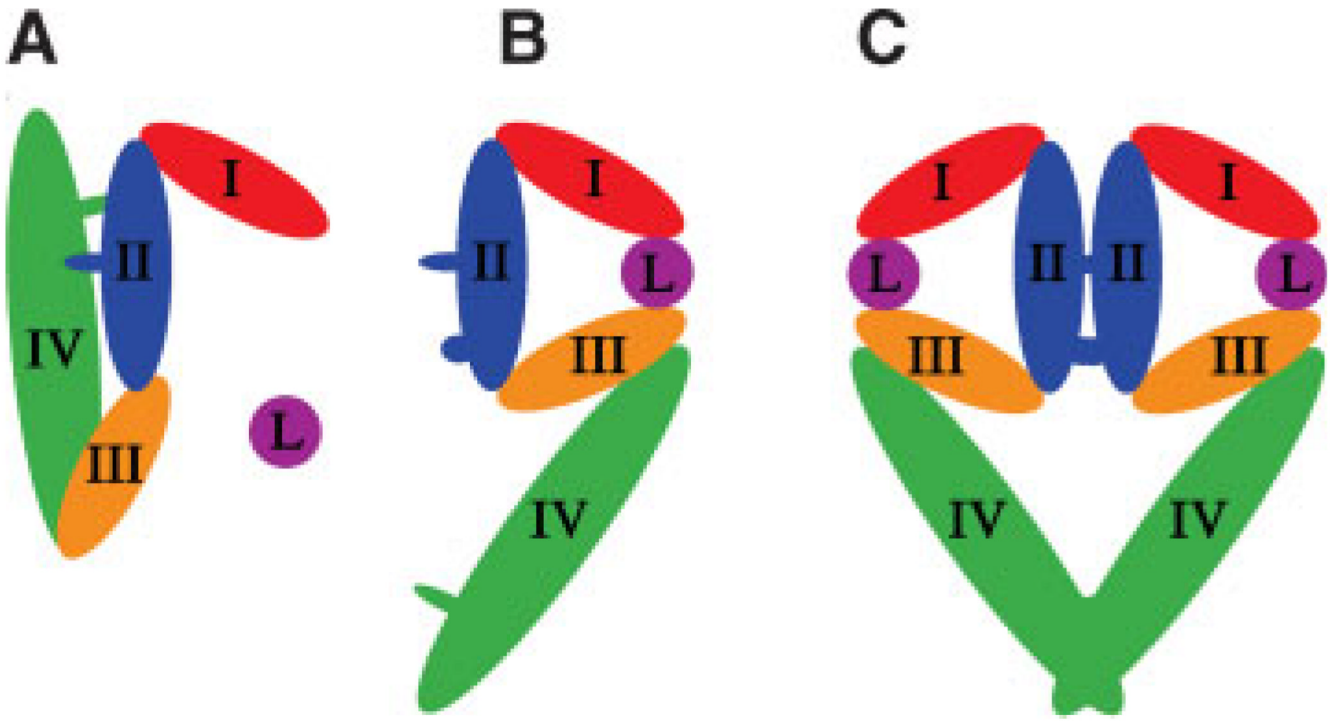

Figure 2.
Structure of the unliganded and liganded EGFR extracellular domain. Diagram showing the structure of the **A** unliganded EGFR extracellular domain, **B** EGFR extracellular domain in complex with EGF, and **C** dimerized liganded EGFR extracellular domains. Adapted from Dawson JP, Berger MB, Lin CC, Schlessinger J, Lemmon MA et al. 2005 Mol Cell Biol 25:7734–7742.

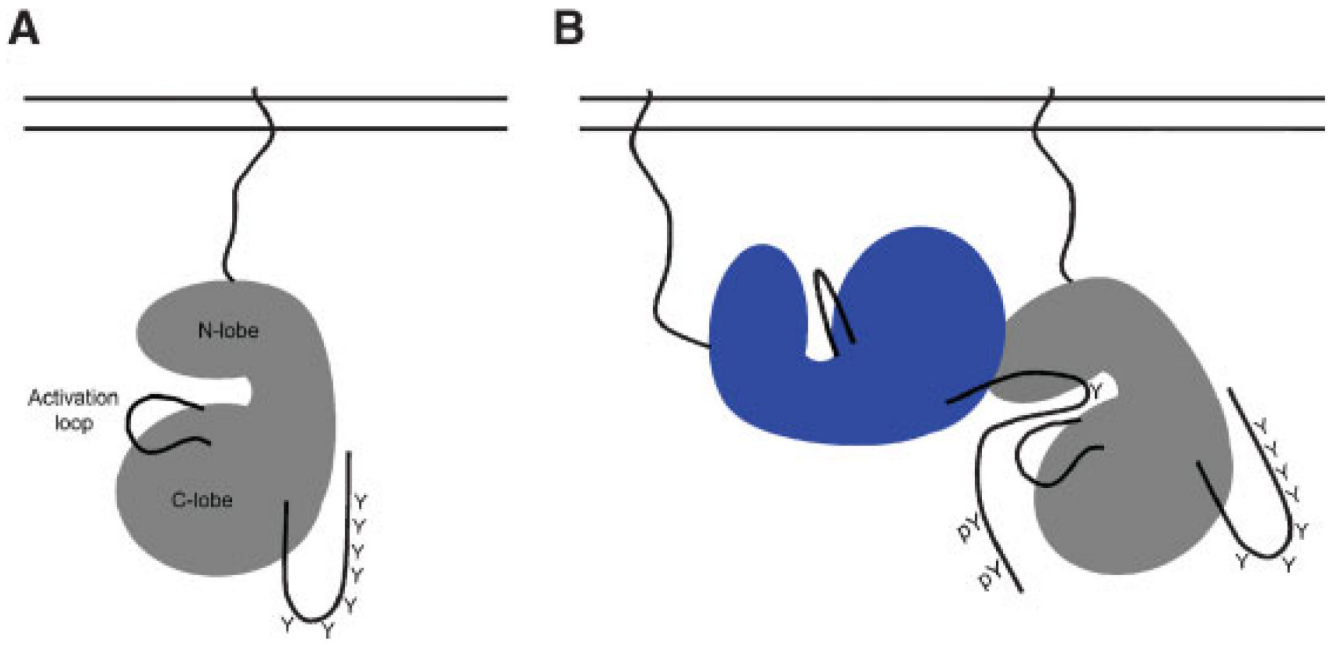

Figure 3.
Structure of the EGFR tyrosine kinase domain. Shown is a stylized depiction of the structure of the **A** monomeric tyrosine kinase domain and of the **B** dimeric kinase domain. Adapted from Zhang X, Gureasko J, Shen K, Cole PA, Kuriyan J. 2006 Cell 125: 1137–1149.

Table 1

EGFR activating mutations

Location	Mutation(s)	Source	Proposed Effect	Reference(s)
Extracellular Domain Subregions I & II	EGFRvIII	Glioblastomas	Shift in conformational equilibrium	20–23
Extracellular Domain Subregion II	EGFR T239P; A265V/D/T	Glioblastomas	Shift in conformational equilibrium	24
Extracellular Domain Subregion III	EGFR S418F	Artificial	Expanded ligand binding; shift in conformational equilibrium	15
Extracellular Domain Subregion IV	secErbB4 ΔG549-R649	Artificial	Shift in conformational equilibrium	19
”	EGFR ΔV575-W584	Artificial	Shift in conformational equilibrium	4
”	EGFR D563A/H566A/K568A	Artificial	Shift in conformational equilibrium	4
”	EGFR P572L; G574V	Glioblastomas	Shift in conformational equilibrium	24
Almost Entire Extracellular Domain	v-ErbB	Oncogenic Retrovirus	Loss of elements that prevent receptor dimerzation	25,26
Juxtamembrane Domain	ErbB4 Q646C	Artificial	Constitutive dimerization and signaling	27,28
”	ErbB2 V656C; T657C	Artificial	Constitutive dimerization and signaling	29
”	ErbB2 in frame deletions	Mouse mammary tumors	Constitutive dimerization and signaling	30
Transmembrane Domain	ErbB2 V664E	Rat neural tumors	Constitutive dimerization and signaling	32,33
”	EGFR V627E	Artificial	Constitutive dimerization and signaling	39
”	ErbB4 I658E	Artificial	Constitutive dimerization and signaling	40
Kinase Domain	EGFR L834R	NSCLC	Increased signaling activity	9,41–48
”	EGFR T766M	NSCLC	Resistance to kinase inhibitors	49–52
”	Various ErbB2 and ErbB4	Various Human Tumors	Unknown	53–56
”	ErbB2 Ins776YVMA	NSCLC	Increased signaling activity	56
Sites of Tyrosine Phophorylation	EGFR Y1045F; Y1068F; Y1086F	Artificial	Decreased internalization and degradation	58–61
”	ErbB4 Y1056E	Artificial	Phosphomimic; effector unknown	62