Annu. Rev. Pharmacol. Toxicol. 2004. 44:195–217
doi: 10.1146/annurev.pharmtox.44.101802.121440

First published online as a Review in Advance on August 14, 2003

ERBB RECEPTORS: Directing Key Signaling Networks Throughout Life

Thomas Holbro and Nancy E. Hynes
Friedrich Miescher Institute for Biomedical Research, 4002 Basel, Switzerland; email: holbro@fmi.ch, hynes@fmi.ch

Key Words EGFR, ErbB2, signaling pathways, cancer therapy

■ **Abstract** The epidermal growth factor (EGF)-related peptides bind the ErbB receptors, inducing the formation of different homo- and heterodimers. Receptor dimerization promotes activation of the intrinsic kinase, leading to phosphorylation of specific tyrosines located in the ErbB's cytoplasmic region. These phosphorylated residues serve as docking sites for a variety of signaling molecules whose recruitment stimulates intracellular signaling cascades, which ultimately control diverse genetic programs. Particular ligand-receptor complexes have essential roles in embryonic development as well as in the adult. Finally, ErbB receptors are being pursued as therapeutic targets because aberrant ErbB activity has been observed in many human cancers. In this review, we discuss these data in more detail, illustrating the importance of tightly regulated ErbB signaling throughout life.

INTRODUCTION

Cells in complex organisms are embedded in a continuous flow of information coming from both their external environment and neighboring cells. The correct interpretation and integration of all these signals is crucial for homeostasis, survival, and adaptation of the single cell, as well as of the organism itself. During evolution, various cellular tools have been developed to accomplish this challenge; membrane-localized receptors are one important example. Of these, receptors with tyrosine kinase activity form a large family, able to translate the information from a peptide ligand into an intracellular message, which ultimately controls gene expression and thus biological outcome.

The subclass I of the receptor tyrosine kinase (RTK) superfamily is formed by the ErbB or epidermal growth factor (EGF) receptors and includes four members: EGFR/ErbB1/HER1, ErbB2/Neu/HER2, ErbB3/HER3, and ErbB4/HER4. Henceforth, we refer to them as the ErbB receptors. All members have in common an extracellular ligand-binding region, a single membrane-spanning region, and a cytoplasmic protein tyrosine kinase–containing domain. Under normal physiological

conditions, activation of the ErbB receptors is controlled by spatial and temporal expression of their ligands, members of the EGF-related peptide growth factor family (1). Ligand binding to ErbB receptors induces formation of different homo- and heterodimers, and, as a consequence, the intrinsic kinase domain is activated, which results in phosphorylation on specific tyrosine residues within the cytoplasmic tail of the receptor. These phosphorylated residues serve as docking sites for a variety of signaling molecules whose recruitment leads to the activation of intracellular pathways (2, 3).

Reflecting the complexity of the organisms, the ErbB family has evolved from a minimal single ligand-receptor combination to a complex system comprising four receptors and a large number of ligands. In the nematode *Caenorhabditis elegans*, only one homologue of EGFR, LET-23, and one ligand, LIN-3, are known. A well-characterized function of this pathway is cell fate specification during vulva development, where the six vulval precursor cells express LET-23 and respond to the paracrine LIN-3 signal originating from the anchor cell (4). In the fruitfly *Drosophila melanogaster*, the ErbB system has evolved to five ligands with one receptor. Activation of the ErbB homologue DER leads to a variety of cell fates during oogenesis and embryogenesis, as well as proliferation/differentiation of imaginal discs (5). Binding of Spitz, Gurken, and Keren (6), all homologues of transforming growth factor-α (TGF-α), but also of Vein, a neuregulin (NRG)-like ligand, leads to activation of DER. By contrast, Argos inhibits DER activation by competing with the activating ligands. Interestingly, no such inhibitory molecule has been described in higher organisms, including mammals.

In higher vertebrates, four ErbB receptors bind a multitude of ligands, inducing formation of various homo- and heterodimers, thus providing for a potentially high degree of signal diversity. Expression of the ErbB receptors is seen in a variety of tissues throughout development where, as described below, they play essential roles during embryogenesis by controlling cell proliferation and differentiation. Moreover, aberrant ErbB signaling is involved in the progression of human malignancies. Cancer patients whose tumors have alterations in ErbB1 or ErbB2 tend to have a more aggressive disease, associated with parameters predicting a poor clinical outcome (7, 8). Based upon these clinical findings, ErbB receptors have become appealing therapeutic targets. Over the past few years, several approaches, including targeting with antagonistic antibodies or small-molecule tyrosine kinase inhibitors, have been developed and are currently under intense clinical investigation (9, 10).

In this review, our emphasis is not only on how ErbB receptors signal and control developmental processes under normal physiological conditions but also on the role of deregulated signaling and cancer promotion. Finally, we discuss clinical results with ErbB inhibitors in the context of our ever-increasing knowledge of the normal role of these receptors in embryonic development and in the adult.

ErbB SIGNALING: FROM THE OUTSIDE OF A CELL TO ITS NUCLEUS

ErbB receptor-mediated signal transmission from the outside to the inside of the cell encompasses several steps. The first step of ErbB signaling is binding of the EGF-like peptide ligands to specific receptors, which induces their conformational change and dimerization. The transmembrane ErbB receptors then pass the information from the cell membrane to the nucleus, where changes in gene expression allow the cell to adapt to the new situation. This signal flow occurs via phosphorylation cascades, starting from modifications of the receptors themselves and ending at the level of specific transcription factors. A simplified scheme of ErbB signaling is illustrated in Figure 1.

The EGF-Related Ligand Family

The EGF-related peptide growth factors, whose binding is responsible for activating the ErbBs, are produced as transmembrane precursors; their ectodomains are processed by proteolysis, leading to shedding of soluble growth factors (11). Interestingly, during evolution, these proteolytic events appear to be mediated through different enzyme classes, i.e., while the proligand principle is conserved, the processing mechanisms are not. In mammals, several studies have identified the zinc-dependent disintegrin-like and metalloproteinase-containing proteins, the ADAMs, as the proteases that cleave ErbB proligands (12). In contrast, in the fruitfly *Drosophila*, Rhomboids, which are seven-pass transmembrane serine proteases that are insensitive to metalloproteinase inhibitors, are responsible for cleavage of the three membrane-spanning, TGF-α-homologue precursors (13).

In mammals, each ErbB ligand contains an EGF-like domain that confers binding specificity [see also (2)]. Affinity toward one or more ErbB receptor allows them to be divided into three groups. The first group includes EGF, TGF-α, and amphiregulin (AR), which bind specifically to ErbB1; the second group includes betacellulin (BTC), heparin-binding EGF (HB-EGF), and epiregulin (EPR), which exhibit dual specificity in that they bind ErbB1 and ErbB4; and the third group, composed of the neuregulins (NRGs), forms two subgroups based upon their capacity to bind ErbB3 and ErbB4 (NRG-1 and NRG-2) or only ErbB4 (NRG-3 and NRG-4). Alternative splicing further increases the variability of NRGs. Intriguingly, despite the large number of identified ligands, no direct high-affinity ligand for ErbB2 has been described. New findings based upon structural studies have brought clarity to this issue.

ErbB Receptor Dimerization

Recent publications describing the crystal structure of ErbB1, ErbB2, and ErbB3's extracellular region, consisting of four domains (I–IV), have brought new insights

into some long unanswered questions concerning the process of ligand-induced receptor dimerization. The structures of ErbB1 bound to EGF (14) or TGF-α (15) have confirmed the importance of domains I and III in ligand binding. Moreover, these studies revealed a direct receptor-receptor interaction in which a protruding arm from domain II on one ErbB1 molecule directly contacts the second ErbB1 molecule, and vice versa. Interestingly, in the dimer formed by two 1:1 ligand:receptor complexes, the two ligands are distant from each other and bind only a single receptor, i.e., they are monomeric. Thus, ErbB receptor activation appears to occur by a novel mechanism because the ligands for many other transmembrane receptors directly mediate dimerization (16). For example, growth hormone (GH) or erythropoietin (EPO) are bivalent, and therefore, one ligand binds and brings together two receptors. Platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) form homodimers, thus bridging two of their respective receptors. Finally, the fibroblast growth factors (FGFs) bind only one receptor but are linked together through simultaneous interaction with heparin.

The structure of inactive ErbB1 is characterized by an intramolecular interaction between domains II and IV involving the same "dimerization arm" responsible for receptor-receptor interaction. This maintains ErbB1 in a "closed" conformation in which the ligand-binding domains I and III are held apart (17). A similar structure has been described for the "nonactivated" ErbB3 (18). These results imply that substantial domain rearrangements occur upon ligand binding, resulting in an "open" receptor conformation.

The structure of ErbB2's extracellular region revealed a major difference in comparison to ErbB1 and ErbB3 (19, 20). Indeed, ErbB2 was found in a fixed conformation similar to that of ligand-activated ErbB1, i.e., the domain II-IV interaction was absent and the dimerization loop was exposed. This structure not only explains why ErbB2 has no ligand, but also predicts that the receptor is, in fact, unable to bind EGF-related peptides because in the "open" conformation, domains I and III are so close that it would be impossible for a ligand to fit (20). These findings help explain the role of ErbB2 as the preferred partner for the other ligand-bound ErbBs (21, 22). However, despite the exposed "dimerization arm," ErbB2 failed to form homodimers in the crystal. This might be due to electrostatic repulsion; in contrast to ErbB1, ErbB2's domain II loop, as well as the pocket into which the loop tip docks, are electronegative (20). Furthermore, additional receptor-receptor interactions, mediated through extra— as well as intracellular regions, are known to contribute to dimer stabilization (23–25).

These structural data, as well as the absence of a direct ErbB2 ligand, imply that its heterodimerization with a second, ligand-binding ErbB receptor is necessary for ErbB2 activation (26). A second family member, ErbB3, has a similar requirement, but for another reason. ErbB3 has impaired kinase activity owing to substitutions in critical residues in the kinase domain itself (27). Hence, ErbB3 only becomes phosphorylated and functions as a signaling entity when dimerized with another

ErbB receptor. Thus, neither ErbB2 nor ErbB3 are able to form signaling units in isolation. Nevertheless, overexpression, especially of ErbB2, appears to force the equilibrium toward spontaneous homodimer formation, which leads to receptor activation in the absence of ligands. This is the situation observed in a variety of human cancers, as is discussed below.

Intracellular Signaling

Regulation of the nature, duration, and potency of the intracellular signaling pathways activated by ErbB receptors is very complex. The ligand, the receptor composition on a cell, the availability of intracellular docking molecules, as well as negative effectors downregulating the signal are some key parameters that determine the final outcome of ErbB activation.

An important function of the ligands is to control formation of defined ErbB dimers through selective binding to their target receptor, which then can dimerize with itself or other ErbB family members. In addition, ligand binding influences which receptor tyrosines become phosphorylated, thus the type of signaling molecules enrolled (28, 29). The binding of the cytoplasmic mediators, which include adaptor proteins or enzymes, occurs through their Src-homology 2 (SH2)- or phosphotyrosine-binding (PTB)-domains (30), whereas the recruitment specificity is determined by the amino acids surrounding the autophosphorylation site on the receptor itself. Analyses of the phosphotyrosine context in the cytoplasmic regions of the different ErbB receptors have shown that signaling redundancy as well as specificity occurs. The Shc- and/or Grb2-activated mitogen-activated protein kinase (MAPK) pathway is a common target downstream of all receptors (2). Similarly, the phosphatidylinosithol-3-kinase (PI-3K) pathway is directly or indirectly activated by most ErbBs (31). However, signaling extent is dependent upon the receptor because, e.g., ErbB3 couples particularly well to this pathway because of its multiple $p85^{PI\text{-}3K}$ docking sites (32, 33), whereas ErbB4 directly recruits $p85^{PI\text{-}3K}$ only if distinct isoforms are available (34). Other cytoplasmic docking proteins appear to be recruited by specific ErbB receptors and less exploited by others (35). These include the adaptors Crk, Nck, Eps15, Dok-R, the phospholipase Cγ (PLCγ), GTPase-activating proteins (GAP), the protein tyrosine phosphatase SHP-1, the intracellular tyrosine kinase Src (36), or the Cbl E3 ubiquitin protein ligase (37).

In addition to the particular pathways activated, the duration of signaling is another key factor in defining the biological output. In the case of ErbB1, but interestingly not for the other three ErbBs, internalization and targeting to the endosomal compartment is the main process that downregulates signaling (38). Further complexity is achieved by the specific ligand bound to ErbB1 because different ligand-receptor dissociation rates determine whether the receptor will be recycled or degraded in a Cbl-mediated manner (39, 40). Moreover, dimerization of ErbB1 with ErbB2 reduces its internalization rate (41), which may explain the signaling power and biological potency of the heterodimer (42, 43).

Stimulation of a spectrum of cytoplasmic pathways translates the signal to the nucleus where a plethora of transcription factors undergo activity changes, initiating waves of transcription programs. Numerous transcription factors downstream of ErbBs have been described; these include c-jun (44), c-fos and c-myc (45, 46), NF-κB (47, 48), zinc-finger transcription factors such as Sp1 (49), Ets family members (50), forkhead transcription factors (51), and signal transducer and activator of transcription (Stat) (52). Through these transcriptional programs, the ErbB signaling network controls a wide palette of biological outcomes, including cell proliferation, differentiation, survival, adhesion, and migration (53).

ErbBs, a Component in a Broader Signaling Network

Transregulation between different classes of membrane receptors provides for even more signaling diversity. ErbBs are good examples of signal integrators because they are the target of signaling events emanating from other receptor classes (Figure 2). ErbB transactivation is characterized by rapid receptor tyrosine phosphorylation and subsequent stimulation of signaling pathways. Basically, two mechanisms can be considered: Either ErbB receptors are phosphorylated by other kinases or ErbB receptors autophosphorylate as a consequence of increased kinase activity.

The first mechanism is characterized by a "passive" behavior of the ErbBs; the receptors act as scaffolds and activation of their kinase domain is not necessary for downstream signaling (Figure 2*a*). Binding of growth hormone (GH) (54) or prolactin (Prl) (55) to their receptors activates the constitutively associated Janus tyrosine kinase (Jak) 2, which phosphorylates the cytoplasmic domain of ErbB1 or ErbB2. In both cases, MAPK activity is dependent upon ErbB phosphorylation. Moreover, the cytoplasmic tyrosine kinase Src phosphorylates various residues on ErbB1, leading to increased receptor signaling (56).

The second mechanism of ErbB transactivation, namely, the ability of different receptors to indirectly stimulate the intrinsic kinase activity, has been intensively studied for G-protein coupled receptor (GPCR) agonists, such as endothelin-1 (ET-1), thrombin, bombesin, and lysophosphatidic acid (LPA) (57, 58). Mechanistically, this process involves rapid stimulation of metalloproteinases followed by cleavage of an EGF-like ligand precursor, e.g., pro-HB-EGF (59), whose binding activates ErbB1 and downstream signaling pathways, ultimately leading to a biological outcome (60). Considering the multitude of GPCRs and their wide pattern of cellular expression, a broader importance for this mechanism is well conceivable. This could easily be tested by measuring the phosphotyrosine content of individual ErbBs after addition of GPCR ligands. Recently, the ADAMs have been shown to play pivotal roles in proligand cleavage following treatment with GPCR agonists (12, 61, 62). The intracellular messenger that transmits the signal from the G-proteins to these metalloproteinases remains to be identified (Figure 2*b*). Finally, our laboratory has recently shown that Wnt binding to frizzled (Fz) receptors also stimulates ErbB1's activity through a metalloproteinase-mediated increase in ligand availability, which results in MAPK activation and induction of cyclin D1 expression (63).

THE ErbBs DURING MOUSE EMBRYOGENESIS

The importance of ErbB signaling during early mouse development is evident from gene targeting studies. Indeed, ablation of individual ErbB loci results in embryonic or perinatal lethality.

Inactivation of ErbB1 leads to a variety of phenotypes, which are dependent upon the mouse genetic background (64). Mice surviving until birth die soon afterward, suffering from defects in a broad range of organs, including skin, lung, the gastrointestinal tract, and some regions of the brain (64–66). In contrast to these severe phenotypes, germline mutations of genes encoding ErbB1 ligands show a lower penetrance. This suggests that different ligands have tissue-specific roles during development and/or that there might be compensation by other ligands in some organs. For example, mice deficient in TGF-α show similar defects in skin, hair, and eyes (67), but, in contrast to ErbB1 mutants, show no signs of neurodegeneration.

ErbB2 targeting has shown that it functions as an ErbB3/ErbB4 coreceptor, which is necessary for transmitting NRG-1 signals. Although the ErbB2/ErbB4 heterodimer acts principally in the heart, ErbB2/ErbB3 function is required for peripheral nervous system development. Initial evidence for the importance of heterodimer signaling was revealed by the analysis of mice null for ErbB2, ErbB4, or NRG-1. Each mutant dies at E10.5, displaying absence of trabeculae in the heart ventricle (68–70). Thus, the paracrine signaling of endocardium-produced NRG-1 to the ErbB2/ErbB4 heterodimer present on the apposed myocardium is essential for embryonic heart development.

Rescue of the cardiac phenotype by myocardium-specific ErbB2 expression in mice with an otherwise ErbB2-null background revealed the central role of this receptor in the peripheral nervous system (71, 72). Here, it is the ErbB2/ErbB3 heterodimer that transmits the NRG-1 signal. Loss of heterodimer signaling by targeting any of these components leads to hypoplasia of the sympathetic ganglion chain, loss of a portion of cranial sensory ganglia, and defective Schwann cell development (70, 73). Interestingly, all these neuronal tissues arise from cells of neural crest origin (74), which lose their migratory ability in mice with a defective ErbB2/ErbB3 signaling system (75).

As mentioned above, ErbB receptors integrate signals from other networks, in some cases without the need of their intrinsic kinase activity. To rule out the possibility of a scaffold function during embryogenesis, mice with a kinase-dead ErbB2 "knock-in" into the *c-erbB2* locus were created. These animals die at midgestation with similar defects as null mutants, suggesting the necessity for the catalytic activity of ErbB2 during early development (76). Moreover, the essential role of a functioning ErbB2/ErbB3 heterodimer was demonstrated in an ex vivo system. Here, heart valve formation from the embryonic endocardial cushion, which involves an epithelial to mesenchymal transition, was severely impaired in ErbB2−/− and ErbB3−/− heart explant cultures, as well as wild-type explants treated with ErbB antagonists (77). In contrast, at least a partial scaffold function during embryonic development cannot be ruled out for ErbB1. In fact, the naturally

occurring *waved-2* mouse strain, characterized by a major reduction in the activity of the ErbB1 kinase, is completely viable and displays only a subset of the defects compared to ErbB1-null mice (78). Of course, it cannot be excluded that the residual receptor kinase activity is enough for normal development of some organs.

THE ROLES OF ErbBs IN THE ADULT

The fact that many null mutations in the ErbB signaling system are lethal has made it difficult to study the specific roles of these molecules later in development. An elegant way to circumvent this problem is to engineer conditional knockout mice and subsequently target the gene in a defined organ. To date, only conditional ErbB2 mice have been described. Another way to circumvent the early lethality problem is to use dominant-negative constructs under the control of tissue-specific promoters, which has been used for various ErbBs.

Mice with a conditional ErbB2 allele showing a perinatal decrease in receptor expression in the ventricular cardiomyocytes are viable. However, starting at approximately eight weeks, they develop severe dilated cardiomyopathy, resulting in reduced contractile function of the heart (79, 80). Other studies revealed the importance of ErbB2 in different tissues. Mice deficient for ErbB2 in skeletal muscles are viable, but lack muscle spindles and display defects in muscle regeneration (81). ErbB2 also regulates the formation of effective neuromuscular synapses and is essential for muscle spindles development (82). Moreover, mice with a conditional loss of ErbB2 in Schwann cells have abnormally thin myelin sheaths and display movement abnormalities and loss of motoneurons (83).

Homozygous newborn ErbB1−/− mice display immature development in several epithelial organs. For instance, they have thin skin, fewer hair follicles, and no hair outgrowth (65). In adult skin, ErbB1 is expressed in the basal, proliferative layer of the epidermis, and in the outer root sheath of hair follicles. The essential role of ErbB1 signaling in the skin was demonstrated by tissue-specific expression of a dominant-negative, truncated receptor. These mice are viable, but display defects in skin architecture and have abnormal hair follicle development and hair cycle (84). Although no tissue-specific ablation of ErbB1 has been reported, the broad expression of ErbB1 and its ligands in a variety of mature organs suggests that further roles will be uncovered.

The ErbB network has also been studied in the mammary gland, an organ that undergoes most of its development postnatally. This organ is particularly interesting because it is susceptible to ErbB-induced cancer. A number of studies have used gain-of-function and loss-of-function approaches to dissect the role of the ErbB receptors and their ligand during the mammary gland development. In this way, the importance of ErbB1 and its ligand AR for ductal growth and the contribution of ErbB2, ErbB4, and NRG-1α for lobulo-alveolar development and lactation have been shown (85).

ErbBs IN HUMAN CANCERS

Since the discovery that an oncogenic avian erythroblastosis retrovirus encoded a mutated homologue of ErbB1 (86), intense research has focused on the role of these receptors in cancer development. In many different cancer types, ErbB receptors, in particular ErbB1 and ErbB2, become constitutively activated as a result of autocrine ligand production, receptor overexpression, or mutation (summarized in Table 1). The wealth of pathological and clinical data makes it impossible to review the role of these receptors in all tumor types; thus, we list only some of the many examples.

In cancer cells, ErbB1 is activated by each of the mechanisms listed above. The most common mutation [type III, (87)], which is often found in glioblastomas (88), is a deletion in the extracellular region resulting in constitutive activation of the receptor. Overexpression of ErbB1 has been reported in squamous-cell carcinomas of head and neck (SCCHN), non-small cell lung cancer (NSCLC), and ovarian and other tumor types (89–92). Interestingly, in vitro studies, as well as the recently solved structure, suggest that ErbB1 overexpression is insufficient to cause receptor activation; ligand binding is still required (93). Accordingly, coexpression of a ligand, e.g., TGF-α, with ErbB1 is often seen in primary tumors (8, 94, 95). Early clinical reports using small numbers of patients with different types of cancers suggested that coexpression of ErbB1 and its ligands is associated with poor survival. A recent mega-analysis, summarizing more than 200 reports, strengthens these early studies and identified ErbB1 as a strong prognostic factor,

TABLE 1 The ErbB network in human cancer

Receptor	Activation	Cancer	References
ErbB1	Mutation (type III)	Glioma Breast, ovarian	(88) (138)
ErbB1	Overexpression	SCCHN Breast Ovarian Glioma	(89) (90) (92) (139)
ErbB1+ TGF-α	Autocrine loop	SCCHN NSCLC Lung, colon Breast	(94) (91, 95) (8) (140)
ErbB2	Overexpression	Breast Ovarian Stomach Bladder Salivary Lung	(100, 141) (97) (98, 142) (99) (143) (144)

associating its expression with reduced recurrence-free and overall survival in several cancer types, including SCCHN, ovarian, and others (96).

In contrast to ErbB1, no activating alterations of ErbB2 have been found in human tumors. Activation and constitutive signaling of ErbB2 is achieved through overexpression, often a consequence of gene amplification, which appears to induce spontaneous dimerization without need of a ligand. Overexpression of ErbB2 occurs in a broad spectrum of carcinomas, including breast, ovarian, gastric, bladder, and others (97–99). Particularly in breast cancer, ErbB2 overexpression is used as a marker associated with a poor patient prognosis and resistance to hormonal therapy (100).

There are only a few studies characterizing ErbB3 and ErbB4 in human cancers. To date, mutations have not been reported and, owing to the small number of analyzed tumors, no statistically significant correlation with prognosis can be made.

ErbB RECEPTORS AS TARGETS FOR CANCER THERAPY

The ErbB receptors have been intensely pursued as therapeutic targets owing to the large number of cancer patients whose tumors show aberrant ErbB expression, as well as the fact that these tumors display other clinical parameters associated with poor patient prognosis. Numerous antireceptor strategies have been taken. Considering in vitro approaches, these include (*a*) inhibiting transcription or translation by triplex-forming oligos, designer transcription factors, antisense approaches, or ribozymes (101–104); (*b*) preventing receptor cell surface localization with single-chain antibodies (46); or (*c*) affecting receptor stability with, e.g., geldanamycin (105). For clinical use, the most promising and advanced strategies include small chemical inhibitors that compete with ATP in the receptor kinase domain and antibodies that prevent ligand-binding, receptor activation and/or induce receptor internalization. Table 2 summarizes some of the compounds currently in clinical trials or, in the case of Herceptin, in wide therapeutic use. Interestingly, some of the small-molecule tyrosine kinase inhibitors (see Table 2) irreversibly block the kinase activity of the target ErbB receptor through direct binding to a specific cysteine in the ATP-pocket (106). Owing to space limitations, in the next sections, we focus only on some of the listed compounds, namely, the antibodies targeting ErbB1 (Cetuximab, C225, or Erbitux) or ErbB2 (Herceptin, Trastuzumab, or rhumMAb 4D5), as well as the most clinically advanced ErbB tyrosine kinase inhibitor (Iressa, ZD-1839, or Gefitinib).

Preclinical Studies: How do ErbB-Targeting Drugs Act?

Studies on in vitro culture systems and in animal models have shown the effectiveness of targeting ErbBs in blocking processes linked to tumor progression. The mechanistic understanding of targeting these receptors with a kinase inhibitor is

TABLE 2 ErbB-targeted cancer drugs in clinical use or development

Compound	Type	Company	Target	Status	Indication
Herceptin	Hum. mAb	Genentech/ Roche	ErbB2	Approved in several countries	Metastatic breast
Cetuximab	Chim. mAb	Imclone/ Merck/ Bristol-Myers Squibb	ErbB1	Phase III	SCCHN, colon
EMD 72000	Hum. mAb	Merck	ErbB1	Phase I/II	[a]
TheraCIM	Hum. mAb	YM Biosciences	ErbB1	Phase I/II	[a]
ABX-EGF	Hum. mAb	Abgenix	ErbB1	Phase I	[a]
Iressa	TKI	AstraZeneca	ErbB1	Phase III, approved in Japan	NSCLC
Tarceva (OSI-774)	TKI	OSI/ Genentech/ Roche	ErbB1	Phase III	Pancreas
PKI166	TKI	Novartis	ErbB1	Phase II, stopped[b]	[a]
CI-1033	TKI, irrev.	Pfizer	ErbB1, ErbB2	Phase II	[a]
GW2016	TKI	GlaxoSmith Kline	ErbB1, ErbB2	Phase I	[a]
EKB-569	TKI, irrev.	Wyeth	ErbB1	Phase I	[a]

Hum./Chim. mAb, fully humanized or human/mouse chimeric monoclonal antibody; TKI, small-molecule tyrosine kinase inhibitor; irrev., irreversible inhibitor

[a]In early clinical trial stages.

[b]Development of PKI166 was interrupted due to high incidence of liver toxicity, 17% of patients showed Grade 3 elevated liver transaminases (145).

straightforward, involving direct inhibition of kinase activity, thus blocking signals originating from the receptors themselves. Treatment of cancer cell lines that have deregulated ErbB activity with Iressa results in a proliferative block and/or cell death (107, 108). In combination with cytotoxic drugs, the cytostatic effect of the compound is enhanced and apoptosis increased (107). Other processes linked to ErbB-mediated tumor progression, such as angiogenesis, are also efficiently blocked by Iressa treatment (109). Moreover, a variety of xenograft tumor models have been used to show that Iressa efficiently inhibits tumor growth and potentiates the effects of chemotherapeutic agents or radiation, resulting in tumor regression (107, 110).

Cetuximab is a human-mouse chimeric monoclonal antibody that binds to the extracellular region of ErbB1 and antagonizes ligand binding (111). In addition to preventing ligand-receptor interaction, there is also evidence that this antibody stimulates ErbB1 internalization (112). The efficacy of Cetuximab has been shown in vitro and in vivo. Treatment of a range of cancer cell lines with the antibody resulted in growth inhibition through upregulation of the cell cycle inhibitor $p27^{Kip1}$ (113, 114) and induction of apoptosis (115). Furthermore, in a number of in vivo model systems, Cetuximab has been shown to inhibit tumor growth (111, 116), to block angiogenesis (117), to prevent metastasis formation (117), and to increase sensitivity to chemotherapeutic agents (118).

Herceptin is a fully humanized monoclonal antibody that recognizes an epitope on the extracellular domain of ErbB2 (119). Cocrystallization of the extracellular region with Herceptin has further defined this interaction at the juxtamembrane domain (19). In contrast to Cetuximab, Herceptin's action is not to prevent ligand binding because there is no ErbB2 ligand, but rather to block receptor activity and downstream signaling pathways and to promote receptor internalization (120–122). Herceptin treatment of ErbB2 overexpressing tumor cell lines results in a proliferative block, reflected in a loss of positive cell cycle regulators, such as cyclins, and an increase in inhibitors, such as $p27^{Kip1}$ (119, 121, 122). The antitumor properties of Herceptin used alone and its increased efficacy when used in combination with cytotoxic agents have been confirmed using in vivo xenograft models (123). Furthermore, in vivo, Herceptin elicits antibody-mediated cytotoxicity through engagement of Fc receptors, and this process contributes to its antitumor activity (124).

Results from Clinical Trials

A number of advanced clinical trials using Herceptin, Cetuximab, or Iressa have been performed in the past few years, providing a wealth of information on efficacy and side effects, which, as we discuss below, may reflect normal organ-specific physiological roles of the receptors.

Herceptin, the only licensed ErbB2-specific drug, has been on the market since 1998 for treatment of ErbB2-overexpressing metastatic breast cancer. This subset of patients was chosen for treatment based upon the results from preclinical studies showing that Herceptin had antitumor activity only in ErbB2-overexpressing tumor cells but not in those with low levels of the receptor (119). Generally, Herceptin has been demonstrated to be safe, well tolerated, and without the side effects observed with conventional cytotoxic agents. Herceptin's clinical efficacy has been documented in two Phase III studies, showing that treatment with Herceptin as a single agent led to a good response in patients who had progressed after one or two chemotherapy regimes (125). Furthermore, Herceptin increased survival when added to first-line conventional chemotherapy (126). These studies have shown that when Herceptin was coadministered with anthracyclins or administered to patients who had undergone prior chemotherapy with these drugs, there was an increased

risk of cardiac side effects, including cardiac dysfunction (126, 127). In addition to metastatic breast cancer, Herceptin is currently in early clinical trials for other indications, including NSCLC (128).

The most prominent ErbB1-specific antibody, Cetuximab, has shown promising results in clinical trials with patients suffering SSCHN, NSCLC, or colorectal cancer. Though not completely humanized, Phase I and II studies have shown that it causes only a low human antichimera antibody (HACA) response; the most common side effects observed include skin rash and folliculitis (10). Cetuximab in combination with chemotherapeutic agents has had some promising clinical results, thus clearing the way for randomized advanced Phase III trials (129).

The small-molecule inhibitor Iressa has undergone single-agent Phase I trials in patients with various solid tumors. Although diarrhea was the marker for dose-limiting toxicity, only modest additional side effects were observed, including, as for Cetuximab, skin rash (130). Results from two Phase II trials have shown that Iressa used as a second- or third-line monotherapy in advanced NSCLC patients improved survival. Surprisingly, however, in two large Phase III trials involving NSCLC patients, Iressa gave no additional benefit compared to placebo when added to first-line standard chemotherapy (131). These contrasting results with Iressa underline the general importance of further studies on ErbB-targeted drugs, with the specific aim being to pinpoint those patients who might best benefit from a given therapy.

PERSPECTIVES

The successful treatment of cancer patients with the ErbB2 antagonist Herceptin is unquestionably an example speaking for the new generation of targeted cancer drugs. Instead of indiscriminately targeting cells with a high proliferative potential using standard chemotherapeutics, this antibody selectively targets a deregulated pathway, which is directly involved in tumor progression. Thus, in Herceptin-treated patients, severe side effects in organs such as the bone marrow have not been observed. The good tolerability, as well as positive clinical results in terms of patient survival, when administered alone or in combination with other agents, gives hope for the future of targeted therapeutics. Nevertheless, some clinical problems need addressing. Notably, only a fraction of patients treated with Herceptin actually respond, implying that ErbB2 overexpression is not the only parameter to consider prior to deciding for this therapeutic approach. Additional screening of tumors for ErbB2 activity may improve the predictive potential of overexpressed ErbB2. Indeed, analyses of ErbB2 phosphotyrosine content reflecting receptor activity have shown that only a subpopulation of ErbB2-overexpressing tumors is positive (132, 133). In the future, it will be important to see whether there is a correlation between phospho-ErbB2 status and therapeutic response.

There are also important questions remaining for basic research. As discussed above, under physiological conditions, ErbB2 needs a partner in order to signal.

This raises the question of whether, in tumors, ErbB2 overexpression can replace the normally essential dimerization partner. Our lab has shown that in ErbB2-overexpressing breast cancer cell lines, sensitivity to ErbB2-targeted therapy correlates with ErbB3 expression (133a), suggesting that ErbB2 activity alone is not sufficient to drive cancer cell proliferation. Further evidence pointing to the importance of ErbB2 heterodimers in tumor cells comes from xenograft studies, where it was shown that administration of the ErbB2-directed mAb 2C4, which prevents the receptor from heterodimerizing, results in potent antitumor effects, in some instances better than Herceptin (134). Because the structures of the extracellular regions of three ErbB receptors are now available, this may accelerate development of inhibitors designed to target specific epitopes. In this respect, it is conceivable that the antitumor effects of 2C4 may be due to binding the "dimerization arm," thus preventing ErbB2 from interacting with other ErbBs.

Another important issue to be considered is the side effects reported in the various clinical trials on ErbB-directed therapies. Basically, the increased risk of cardiac dysfunction in patients treated with Herceptin, as well as the skin rash and gastrointestinal disorders documented in Cetuximab and Iressa clinical studies, could be explained by the physiological function of the respective receptor in these organs. Indeed, as described above, ErbB2 has an important role in the postnatal heart (79), and mice with a natural ErbB1 mutation (*waved-2*) or with targeted alleles display severe skin defects (64, 78). Relating to this, analyses of skin biopsies from patients treated with Iressa have shown that ErbB1 phosphorylation, MAPK activation, and keratinocyte proliferation are in fact reduced, demonstrating the action of Iressa in this tissue (135). However, it is important to note that other observations suggest that the explanations for drug-induced side effects are likely to be complex. For example, the incidence of cardiac dysfunction in Herceptin-treated patients was lower in those receiving concomitant paclitaxel compared to those treated in combination with anthracyclines (127), suggesting that Herceptin-induced cardiotoxicity may occur only in combination with other heart "stress" factors, such as anthracyclines. This model is supported by in vitro experiments showing that adult rat myocytes treated with doxorubicin display changes in myofilament structures and that this myofibrillar disarray is increased by addition of an ErbB2-specific antibody (136). Fortunately, not all patients treated concomitantly with Herceptin and anthracyclines display cardiac dysfunction. Thus, prescreening patients to identify those in the "best response–lowest side effects" group would be ideal. In this regard, it has been reported that only a subset of breast cancer patients had specific myocardial uptake of radiolabeled Herceptin, and, remarkably, these same patients later developed signs of cardiotoxicity (137).

This short discussion should make it clear that continuing to increase our understanding of the mechanisms underlying physiological ErbB signaling, as well as the roles of these receptors during cancer progression, will not only provide explanations for side effects, but also may identify new diagnostic markers and therapeutic targets. Our ever-increasing knowledge on the important ErbB receptors is likely to improve the therapeutic index of drugs currently available or still

in development and should finally allow clinicians to select the best therapy for the individual patient.

ACKNOWLEDGMENTS

The laboratory is supported by the Novartis Forschungsstiftung Zweigniederlassung Friedrich Miescher Institute for Biomedical Research. T.H. was partially supported by grants from the Novartis Stiftung fuer Medizinisch-Biologische Forschung and the Krebsliga Beider Basel. Thanks to Dr. T. Schlange and Dr. M. Koziczak for critically reading the manuscript.

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

LITERATURE CITED

1. Riese DJ, Stern DF. 1998. Specificity within the EGF family/ErbB receptor family signaling network. *BioEssays* 20:41–48
2. Olayioye MA, Neve RM, Lane HA, Hynes NE. 2000. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J.* 19:3159–67
3. Yarden Y, Sliwkowski MX. 2001. Untangling the ErbB signalling network. *Nat. Rev. Mol. Cell Biol.* 2:127–37
4. Chang C, Sternberg PW. 1999. *C. elegans* vulval development as a model system to study the cancer biology of EGFR signaling. *Cancer Metastasis Rev.* 18:203–13
5. Schweitzer R, Shilo BZ. 1997. A thousand and one roles for the Drosophila EGF receptor. *Trends Genet.* 13:191–96
6. Reich A, Shilo BZ. 2002. Keren, a new ligand of the Drosophila epidermal growth factor receptor, undergoes two modes of cleavage. *EMBO J.* 21:4287–96
7. Hynes NE, Stern DF. 1994. The biology of erbB-2/neu/HER-2 and its role in cancer. *Biochim. Biophys. Acta* 1198:165–84
8. Salomon DS, Brandt R, Ciardiello F, Normanno N. 1995. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit. Rev. Oncol. Hematol.* 19:183–32
9. Mendelsohn J, Baselga J. 2000. The EGF receptor family as targets for cancer therapy. *Oncogene* 19:6550–65
10. de Bono JS, Rowinsky EK. 2002. The ErbB receptor family: a therapeutic target for cancer. *Trends Mol. Med.* 8:S19–26
11. Massague J, Pandiella A. 1993. Membrane-anchored growth factors. *Annu. Rev. Biochem.* 62:515–41
12. Seals DF, Courtneidge SA. 2003. The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev.* 17:7–30
13. Urban S, Lee JR, Freeman M. 2002. A family of Rhomboid intramembrane proteases activates all Drosophila membrane-tethered EGF ligands. *EMBO J.* 21:4277–86
14. Ogiso H, Ishitani R, Nureki O, Fukai S, Yamanaka M, et al. 2002. Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains. *Cell* 110:775–87
15. Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, et al. 2002. Crystal

structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha. *Cell* 110:763–73

16. Schlessinger J. 2000. Cell signaling by receptor tyrosine kinases. *Cell* 103:211–25
17. Ferguson KM, Berger MB, Mendrola JM, Cho HS, Leahy DJ, Lemmon MA. 2003. EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization. *Mol. Cell* 11: 507–17
18. Cho HS, Leahy DJ. 2002. Structure of the extracellular region of HER3 reveals an interdomain tether. *Science* 297:1330–33
19. Cho HS, Mason K, Ramyar KX, Stanley AM, Gabelli SB, et al. 2003. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* 421:756–60
20. Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, et al. 2003. The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. *Mol. Cell* 11:495–505
21. Tzahar E, Waterman H, Chen X, Levkowitz G, Karunagaran D, et al. 1996. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol. Cell Biol.* 16:5276–87
22. Graus-Porta D, Beerli RR, Daly JM, Hynes NE. 1997. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *EMBO J.* 16:1647–55
23. Qian XL, O'Rourke DM, Fei ZZ, Zhang HT, Kao CC, Greene MI. 1999. Domain-specific interactions between the p185(neu) and epidermal growth factor receptor kinases determine differential signaling outcomes. *J. Biol. Chem.* 274: 574–83
24. Ferguson KM, Darling PJ, Mohan MJ, Macatee TL, Lemmon MA. 2000. Extracellular domains drive homo- but not hetero-dimerization of erbB receptors. *EMBO J.* 19:4632–43
25. Penuel E, Akita RW, Sliwkowski MX. 2002. Identification of a region within the ErbB2/HER2 intracellular domain that is necessary for ligand-independent association. *J. Biol. Chem.* 277:28468–73
26. Klapper LN, Glathe S, Vaisman N, Hynes NE, Andrews GC, et al. 1999. The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. *Proc. Natl. Acad. Sci. USA* 96:4995–5000
27. Guy PM, Platko JV, Cantley LC, Cerione RA, Carraway KL III. 1994. Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity. *Proc. Natl. Acad. Sci. USA* 91:8132–36
28. Olayioye MA, Graus-Porta D, Beerli RR, Rohrer J, Gay B, Hynes NE. 1998. ErbB-1 and ErbB-2 acquire distinct signaling properties dependent upon their dimerization partner. *Mol. Cell Biol.* 18:5042–51
29. Sweeney C, Lai C, Riese DJ, Diamonti AJ, Cantley LC, Carraway KL III. 2000. Ligand discrimination in signaling through an ErbB4 receptor homodimer. *J. Biol. Chem.* 275:19803–7
30. Yaffe MB. 2002. Phosphotyrosine-binding domains in signal transduction. *Nat. Rev. Mol. Cell Biol.* 3:177–86
31. Soltoff SP, Cantley LC. 1996. p120cbl is a cytosolic adapter protein that associates with phosphoinositide 3-kinase in response to epidermal growth factor in PC12 and other cells. *J. Biol. Chem.* 271:563–67
32. Fedi P, Pierce JH, Di Fiore PP, Kraus MH. 1994. Efficient coupling with phosphatidylinositol 3-kinase, but not phospholipase C gamma or GTPase-activating protein, distinguishes ErbB-3 signaling from that of other ErbB/EGFR

family members. *Mol. Cell Biol.* 14:492–500

33. Prigent SA, Gullick WJ. 1994. Identification of c-erbB-3 binding sites for phosphatidylinositol 3′-kinase and SHC using an EGF receptor/c-erbB-3 chimera. *EMBO J.* 13:2831–41
34. Elenius K, Choi CJ, Paul S, Santiestevan E, Nishi E, Klagsbrun M. 1999. Characterization of a naturally occurring ErbB4 isoform that does not bind or activate phosphatidyl inositol 3-kinase. *Oncogene* 18:2607–15
35. Jorissen RN, Walker F, Pouliot N, Garrett TP, Ward CW, Burgess AW. 2003. Epidermal growth factor receptor: mechanisms of activation and signalling. *Exp. Cell Res.* 284:31–53
36. Tice DA, Biscardi JS, Nickles AL, Parsons SJ. 1999. Mechanism of biological synergy between cellular Src and epidermal growth factor receptor. *Proc. Natl. Acad. Sci. USA* 96:1415–20
37. Levkowitz G, Waterman H, Ettenberg SA, Katz M, Tsygankov AY, et al. 1999. Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol. Cell* 4:1029–40
38. Baulida J, Kraus MH, Alimandi M, Di Fiore PP, Carpenter G. 1996. All ErbB receptors other than the epidermal growth factor receptor are endocytosis impaired. *J. Biol. Chem.* 271:5251–57
39. Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, et al. 1998. c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev.* 12:3663–74
40. Waterman H, Sabanai I, Geiger B, Yarden Y. 1998. Alternative intracellular routing of ErbB receptors may determine signaling potency. *J. Biol. Chem.* 273:13819–27
41. Lenferink AE, Pinkas-Kramarski R, van de Poll ML, van Vugt MJ, Klapper LN, et al. 1998. Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers. *EMBO J.* 17:3385–97
42. Graus-Porta D, Beerli RR, Hynes NE. 1995. Single-chain antibody-mediated intracellular retention of ErbB-2 impairs Neu differentiation factor and epidermal growth factor signaling. *Mol. Cell Biol.* 15:1182–91
43. Alimandi M, Romano A, Curia MC, Muraro R, Fedi P, et al. 1995. Cooperative signaling of ErbB3 and ErbB2 in neoplastic transformation and human mammary carcinomas. *Oncogene* 10:1813–21
44. Quantin B, Breathnach R. 1988. Epidermal growth factor stimulates transcription of the c-jun proto-oncogene in rat fibroblasts. *Nature* 334:538–39
45. Cutry AF, Kinniburgh AJ, Krabak MJ, Hui SW, Wenner CE. 1989. Induction of c-fos and c-myc proto-oncogene expression by epidermal growth factor and transforming growth factor alpha is calcium-independent. *J. Biol. Chem.* 264:19700–5
46. Neve RM, Sutterluty H, Pullen N, Lane HA, Daly JM, et al. 2000. Effects of oncogenic ErbB2 on G1 cell cycle regulators in breast tumour cells. *Oncogene* 19:1647–56
47. Biswas DK, Cruz AP, Gansberger E, Pardee AB. 2000. Epidermal growth factor-induced nuclear factor kappa B activation: a major pathway of cell-cycle progression in estrogen-receptor negative breast cancer cells. *Proc. Natl. Acad. Sci. USA* 97:8542–47
48. Zhou BP, Hu MC, Miller SA, Yu Z, Xia W, et al. 2000. HER-2/neu blocks tumor necrosis factor-induced apoptosis via the Akt/NF-kappaB pathway. *J. Biol. Chem.* 275:8027–31
49. Alroy I, Soussan L, Seger R, Yarden Y. 1999. Neu differentiation factor stimulates phosphorylation and activation of the Sp1 transcription factor. *Mol. Cell Biol.* 19:1961–72

50. O'Hagan RC, Hassell JA. 1998. The PEA3 Ets transcription factor is a downstream target of the HER2/Neu receptor tyrosine kinase. *Oncogene* 16:301–10
51. Jackson JG, Kreisberg JI, Koterba AP, Yee D, Brattain MG. 2000. Phosphorylation and nuclear exclusion of the forkhead transcription factor FKHR after epidermal growth factor treatment in human breast cancer cells. *Oncogene* 19:4574–81
52. Olayioye MA, Beuvink I, Horsch K, Daly JM, Hynes NE. 1999. ErbB receptor-induced activation of stat transcription factors is mediated by Src tyrosine kinases. *J. Biol. Chem.* 274:17209–18
53. Holbro T, Civenni G, Hynes NE. 2003. The ErbB receptors and their role in cancer progression. *Exp. Cell Res.* 284:99–110
54. Yamauchi T, Ueki K, Tobe K, Tamemoto H, Sekine N, et al. 1997. Tyrosine phosphorylation of the EGF receptor by the kinase Jak2 is induced by growth hormone. *Nature* 390:91–96
55. Yamauchi T, Yamauchi N, Ueki K, Sugiyama T, Waki H, et al. 2000. Constitutive tyrosine phosphorylation of ErbB-2 via Jak2 by autocrine secretion of prolactin in human breast cancer. *J. Biol. Chem.* 275:33937–44
56. Biscardi JS, Maa MC, Tice DA, Cox ME, Leu TH, Parsons SJ. 1999. c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and Tyr1101 is associated with modulation of receptor function. *J. Biol. Chem.* 274:8335–43
57. Carpenter G. 2000. EGF receptor transactivation mediated by the proteolytic production of EGF-like agonists. *Science STKE* 2000:E1
58. Gschwind A, Zwick E, Prenzel N, Leserer M, Ullrich A. 2001. Cell communication networks: epidermal growth factor receptor transactivation as the paradigm for interreceptor signal transmission. *Oncogene* 20:1594–600
59. Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, et al. 1999. EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* 402:884–88
60. Gschwind A, Prenzel N, Ullrich A. 2002. Lysophosphatidic acid-induced squamous cell carcinoma cell proliferation and motility involves epidermal growth factor receptor signal transactivation. *Cancer Res.* 62:6329–36
61. Asakura M, Kitakaze M, Takashima S, Liao Y, Ishikura F, et al. 2002. Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. *Nat. Med.* 8:35–40
62. Yan Y, Shirakabe K, Werb Z. 2002. The metalloprotease Kuzbanian (ADAM10) mediates the transactivation of EGF receptor by G protein-coupled receptors. *J. Cell Biol.* 158:221–26
63. Civenni G, Holbro T, Hynes NE. 2003. Wnt1 and Wnt5a induce cyclin D1 expression through ErbB1 transactivation in HC11 mammary epithelial cells. *EMBO Rep.* 4:166–71
64. Threadgill DW, Dlugosz AA, Hansen LA, Tennenbaum T, Lichti U, et al. 1995. Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science* 269:230–34
65. Miettinen PJ, Berger JE, Meneses J, Phung Y, Pedersen RA, et al. 1995. Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* 376:337–41
66. Sibilia M, Steinbach JP, Stingl L, Aguzzi A, Wagner EF. 1998. A strain-independent postnatal neurodegeneration in mice lacking the EGF receptor. *EMBO J.* 17:719–31
67. Mann GB, Fowler KJ, Gabriel A, Nice EC, Williams RL, Dunn AR. 1993. Mice with a null mutation of the TGF alpha

gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. *Cell* 73:249–61

68. Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C. 1995. Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature* 378:394–98
69. Gassmann M, Casagranda F, Orioli D, Simon H, Lai C, et al. 1995. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature* 378:390–94
70. Meyer D, Birchmeier C. 1995. Multiple essential functions of neuregulin in development. *Nature* 378:386–90
71. Morris JK, Lin W, Hauser C, Marchuk Y, Getman D, Lee KF. 1999. Rescue of the cardiac defect in ErbB2 mutant mice reveals essential roles of ErbB2 in peripheral nervous system development. *Neuron* 23:273–83
72. Woldeyesus MT, Britsch S, Riethmacher D, Xu L, Sonnenberg-Riethmacher E, et al. 1999. Peripheral nervous system defects in erbB2 mutants following genetic rescue of heart development. *Genes Dev.* 13:2538–48
73. Riethmacher D, Sonnenberg-Riethmacher E, Brinkmann V, Yamaai T, Lewin GR, Birchmeier C. 1997. Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. *Nature* 389: 725–30
74. Christiansen JH, Coles EG, Wilkinson DG. 2000. Molecular control of neural crest formation, migration and differentiation. *Curr. Opin. Cell Biol.* 12:719–24
75. Britsch S, Li L, Kirchhoff S, Theuring F, Brinkmann V, et al. 1998. The ErbB2 and ErbB3 receptors and their ligand, neuregulin-1, are essential for development of the sympathetic nervous system. *Genes Dev.* 12:1825–36
76. Chan R, Hardy WR, Laing MA, Hardy SE, Muller WJ. 2002. The catalytic activity of the ErbB-2 receptor tyrosine kinase is essential for embryonic development. *Mol. Cell Biol.* 22:1073–78
77. Camenisch TD, Schroeder JA, Bradley J, Klewer SE, McDonald JA. 2002. Heart-valve mesenchyme formation is dependent on hyaluronan-augmented activation of ErbB2-ErbB3 receptors. *Nat. Med.* 8:850–55
78. Luetteke NC, Phillips HK, Qiu TH, Copeland NG, Earp HS, et al. 1994. The mouse waved-2 phenotype results from a point mutation in the EGF receptor tyrosine kinase. *Genes Dev.* 8:399–13
79. Crone SA, Zhao YY, Fan L, Gu Y, Minamisawa S, et al. 2002. ErbB2 is essential in the prevention of dilated cardiomyopathy. *Nat. Med.* 8:459–65
80. Ozcelik C, Erdmann B, Pilz B, Wettschureck N, Britsch S, et al. 2002. Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy. *Proc. Natl. Acad. Sci. USA* 99:8880–85
81. Andrechek ER, Hardy WR, Girgis-Gabardo AA, Perry RL, Butler R, et al. 2002. ErbB2 is required for muscle spindle and myoblast cell survival. *Mol. Cell Biol.* 22:4714–22
82. Leu M, Bellmunt E, Schwander M, Farinas I, Brenner HR, Muller U. 2003. Erbb2 regulates neuromuscular synapse formation and is essential for muscle spindle development. *Development* 130:2291–301
83. Garratt AN, Voiculescu O, Topilko P, Charnay P, Birchmeier C. 2000. A dual role of ErbB2 in myelination and in expansion of the Schwann cell precursor pool. *J. Cell Biol.* 148:1035–46
84. Murillas R, Larcher F, Conti CJ, Santos M, Ullrich A, Jorcano JL. 1995. Expression of a dominant negative mutant of epidermal growth factor receptor in the epidermis of transgenic mice elicits striking alterations in hair follicle development and skin structure. *EMBO J.* 14:5216–23
85. Stern DF. 2003. ErbBs in mammary

development. *Exp. Cell Res.* 284:89–98

86. Downward J, Yarden Y, Mayes E, Scrace G, Totty N, et al. 1984. Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. *Nature* 307:521–27
87. Voldborg BR, Damstrup L, Spang-Thomsen M, Poulsen HS. 1997. Epidermal growth factor receptor (EGFR) and EGFR mutations, function and possible role in clinical trials. *Ann. Oncol.* 8:1197–206
88. Ekstrand AJ, Sugawa N, James CD, Collins VP. 1992. Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. *Proc. Natl. Acad. Sci. USA* 89:4309–13
89. Grandis JR, Melhem MF, Barnes EL, Tweardy DJ. 1996. Quantitative immunohistochemical analysis of transforming growth factor-alpha and epidermal growth factor receptor in patients with squamous cell carcinoma of the head and neck. *Cancer* 78:1284–92
90. Klijn JG, Berns PM, Schmitz PI, Foekens JA. 1992. The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: a review on 5232 patients. *Endocr. Rev.* 13: 3–17
91. Rusch V, Klimstra D, Venkatraman E, Pisters PW, Langenfeld J, Dmitrovsky E. 1997. Overexpression of the epidermal growth factor receptor and its ligand transforming growth factor alpha is frequent in resectable non-small cell lung cancer but does not predict tumor progression. *Clin. Cancer Res.* 3:515–22
92. Bartlett JM, Langdon SP, Simpson BJ, Stewart M, Katsaros D, et al. 1996. The prognostic value of epidermal growth factor receptor mRNA expression in primary ovarian cancer. *Br. J. Cancer* 73:301–6
93. Di Fiore PP, Pierce JH, Fleming TP, Hazan R, Ullrich A, et al. 1987. Overexpression of the human EGF receptor confers an EGF-dependent transformed phenotype to NIH 3T3 cells. *Cell* 51: 1063–70
94. Grandis JR, Melhem MF, Gooding WE, Day R, Holst VA, et al. 1998. Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J. Natl. Cancer Inst.* 90:824–32
95. Hsieh ET, Shepherd FA, Tsao MS. 2000. Co-expression of epidermal growth factor receptor and transforming growth factor-alpha is independent of ras mutations in lung adenocarcinoma. *Lung Cancer* 29:151–57
96. Nicholson RI, Gee JM, Harper ME. 2001. EGFR and cancer prognosis. *Eur. J. Cancer* 37(Suppl. 4):S9–15
97. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, et al. 1989. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244:707–12
98. Lemoine NR, Jain S, Silvestre F, Lopes C, Hughes CM, et al. 1991. Amplification and overexpression of the EGF receptor and c-erbB-2 proto-oncogenes in human stomach cancer. *Br. J. Cancer* 64:79–83
99. Sauter G, Moch H, Moore D, Carroll P, Kerschmann R, et al. 1993. Heterogeneity of ErbB-2 gene amplification in bladder cancer. *Cancer Res.* 53:2199–203
100. Ross JS, Fletcher JA. 1998. The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. *Stem Cells* 16:413–28
101. Noonberg SB, Scott GK, Hunt CA, Hogan ME, Benz CC. 1994. Inhibition of transcription factor binding to the HER2 promoter by triplex-forming oligodeoxyribonucleotides. *Gene* 149:123–26
102. Beerli RR, Dreier B, Barbas CF III. 2000. Positive and negative regulation of

endogenous genes by designed transcription factors. *Proc. Natl. Acad. Sci. USA* 97:1495–500

103. Roh H, Pippin JA, Green DW, Boswell CB, Hirose CT, et al. 2000. HER2/neu antisense targeting of human breast carcinoma. *Oncogene* 19:6138–43
104. Juhl H, Downing SG, Wellstein A, Czubayko F. 1997. HER-2/neu is rate-limiting for ovarian cancer growth. Conditional depletion of HER-2/neu by ribozyme targeting. *J. Biol Chem.* 272:29482–86
105. Basso AD, Solit DB, Munster PN, Rosen N. 2002. Ansamycin antibiotics inhibit Akt activation and cyclin D expression in breast cancer cells that overexpress HER2. *Oncogene* 21:1159–66
106. Fry DW. 2003. Mechanism of action of erbB tyrosine kinase inhibitors. *Exp. Cell Res.* 284:131–39
107. Ciardiello F, Caputo R, Bianco R, Damiano V, Pomatico G, et al. 2000. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. *Clin. Cancer Res.* 6:2053–63
108. Moasser MM, Basso A, Averbuch SD, Rosen N. 2001. The tyrosine kinase inhibitor ZD1839 ("Iressa") inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. *Cancer Res.* 61:7184–88
109. Hirata A, Ogawa S, Kometani T, Kuwano T, Naito S, et al. 2002. ZD1839 (Iressa) induces antiangiogenic effects through inhibition of epidermal growth factor receptor tyrosine kinase. *Cancer Res.* 62:2554–60
110. Huang SM, Li J, Armstrong EA, Harari PM. 2002. Modulation of radiation response and tumor-induced angiogenesis after epidermal growth factor receptor inhibition by ZD1839 (Iressa). *Cancer Res.* 62:4300–6
111. Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. 1995. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin. Cancer Res.* 1:1311–18
112. Sunada H, Magun BE, Mendelsohn J, MacLeod CL. 1986. Monoclonal antibody against epidermal growth factor receptor is internalized without stimulating receptor phosphorylation. *Proc. Natl. Acad. Sci. USA* 83:3825–29
113. Wu X, Rubin M, Fan Z, DeBlasio T, Soos T, et al. 1996. Involvement of p27KIP1 in G1 arrest mediated by an anti-epidermal growth factor receptor monoclonal antibody. *Oncogene* 12:1397–403
114. Peng D, Fan Z, Lu Y, DeBlasio T, Scher H, Mendelsohn J. 1996. Anti-epidermal growth factor receptor monoclonal antibody 225 up-regulates p27KIP1 and induces G1 arrest in prostatic cancer cell line DU145. *Cancer Res.* 56:3666–69
115. Wu X, Fan Z, Masui H, Rosen N, Mendelsohn J. 1995. Apoptosis induced by an anti-epidermal growth factor receptor monoclonal antibody in a human colorectal carcinoma cell line and its delay by insulin. *J. Clin. Invest.* 95:1897–905
116. Prewett M, Rothman M, Waksal H, Feldman M, Bander NH, Hicklin DJ. 1998. Mouse-human chimeric anti-epidermal growth factor receptor antibody C225 inhibits the growth of human renal cell carcinoma xenografts in nude mice. *Clin. Cancer Res.* 4:2957–66
117. Perrotte P, Matsumoto T, Inoue K, Kuniyasu H, Eve BY, et al. 1999. Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice. *Clin. Cancer Res.* 5:257–65
118. Inoue K, Slaton JW, Perrotte P, Davis DW, Bruns CJ, et al. 2000. Paclitaxel enhances the effects of the anti-epidermal growth factor receptor monoclonal antibody ImClone C225 in mice with metastatic human bladder transitional

cell carcinoma. *Clin. Cancer Res.* 6: 4874–84

119. Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A. 1989. p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor. *Mol. Cell Biol.* 9:1165–72
120. Sliwkowski MX, Lofgren JA, Lewis GD, Hotaling TE, Fendly BM, Fox JA. 1999. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Semin. Oncol.* 26:60–70
121. Lane HA, Beuvink I, Motoyama AB, Daly JM, Neve RM, Hynes NE. 2000. ErbB2 potentiates breast tumor proliferation through modulation of p27(Kip1)-Cdk2 complex formation: receptor overexpression does not determine growth dependency. *Mol. Cell Biol.* 20:3210–23
122. Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S, Arteaga CL. 2002. Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt Is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. *Cancer Res.* 62:4132–41
123. Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J. 1998. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. *Cancer Res.* 58:2825–31
124. Clynes RA, Towers TL, Presta LG, Ravetch JV. 2000. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. *Nat. Med.* 6:443–46
125. Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, et al. 1999. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.* 17: 2639–48
126. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, et al. 2001. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* 344:783–92
127. Seidman A, Hudis C, Pierri MK, Shak S, Paton V, et al. 2002. Cardiac dysfunction in the trastuzumab clinical trials experience. *J. Clin. Oncol.* 20:1215–21
128. Azzoli CG, Krug LM, Miller VA, Kris MG, Mass R. 2002. Trastuzumab in the treatment of non-small cell lung cancer. *Semin. Oncol.* 29:59–65
129. Herbst RS, Hong WK. 2002. IMC-C225, an anti-epidermal growth factor receptor monoclonal antibody for treatment of head and neck cancer. *Semin. Oncol.* 29:18–30
130. Arteaga CL, Johnson DH. 2001. Tyrosine kinase inhibitors-ZD1839 (Iressa). *Curr. Opin. Oncol.* 13:491–98
131. Wilkinson E. 2002. Surprise phase III failure for ZD1839. *Lancet Oncol.* 3: 583
132. Thor AD, Liu S, Edgerton S, Moore D, Kasowitz KM, et al. 2000. Activation (tyrosine phosphorylation) of ErbB-2 (HER-2/neu): a study of incidence and correlation with outcome in breast cancer. *J. Clin. Oncol.* 18:3230–39
133. DiGiovanna MP, Chu P, Davison TL, Howe CL, Carter D, et al. 2002. Active signaling by HER-2/neu in a subpopulation of HER-2/neu-overexpressing ductal carcinoma in situ: clinicopathological correlates. *Cancer Res.* 62:6667–73

133a. Holbro T, Beerli RR, Maurer F, Koziczak M, Barbas CF 3rd, et al. 2003. The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. *Proc. Natl. Acad. Sci. USA* 100: 8933–38

134. Agus DB, Akita RW, Fox WD, Lewis GD, Higgins B, et al. 2002. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. *Cancer Cell* 2:127–37
135. Albanell J, Rojo F, Averbuch S, Feyereislova A, Mascaro JM, et al. 2002. Pharmacodynamic studies of the epidermal growth factor receptor inhibitor ZD1839 in skin from cancer patients: histopathologic and molecular consequences of receptor inhibition. *J. Clin. Oncol.* 20:110–24
136. Sawyer DB, Zuppinger C, Miller TA, Eppenberger HM, Suter TM. 2002. Modulation of anthracycline-induced myofibrillar disarray in rat ventricular myocytes by neuregulin-1beta and anti-erbB2: potential mechanism for trastuzumab-induced cardiotoxicity. *Circulation* 105:1551–54
137. Behr TM, Behe M, Wormann B. 2001. Trastuzumab and breast cancer. *N. Engl. J. Med.* 345:995–96
138. Moscatello DK, Holgado-Madruga M, Godwin AK, Ramirez G, Gunn G, et al. 1995. Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. *Cancer Res.* 55:5536–39
139. Watanabe K, Tachibana O, Sata K, Yonekawa Y, Kleihues P, Ohgaki H. 1996. Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. *Brain Pathol.* 6:217–23
140. Umekita Y, Ohi Y, Sagara Y, Yoshida H. 2000. Co-expression of epidermal growth factor receptor and transforming growth factor-alpha predicts worse prognosis in breast-cancer patients. *Int. J. Cancer* 89:484–87
141. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235:177–82
142. Falck VG, Gullick WJ. 1989. c-erbB-2 oncogene product staining in gastric adenocarcinoma. An immunohistochemical study. *J. Pathol.* 159:107–11
143. Stenman G, Sandros J, Nordkvist A, Mark J, Sahlin P. 1991. Expression of the ERBB2 protein in benign and malignant salivary gland tumors. *Genes Chromosomes Cancer* 3:128–35
144. Tateishi M, Ishida T, Mitsudomi T, Kaneko S, Sugimachi K. 1991. Prognostic value of c-erbB-2 protein expression in human lung adenocarcinoma and squamous cell carcinoma. *Eur. J. Cancer* 27:1372–75
145. Traxler P. 2003. Tyrosine kinases as targets in cancer therapy—successes and failures. *Expert Opin. Ther. Targets* 7:215–34

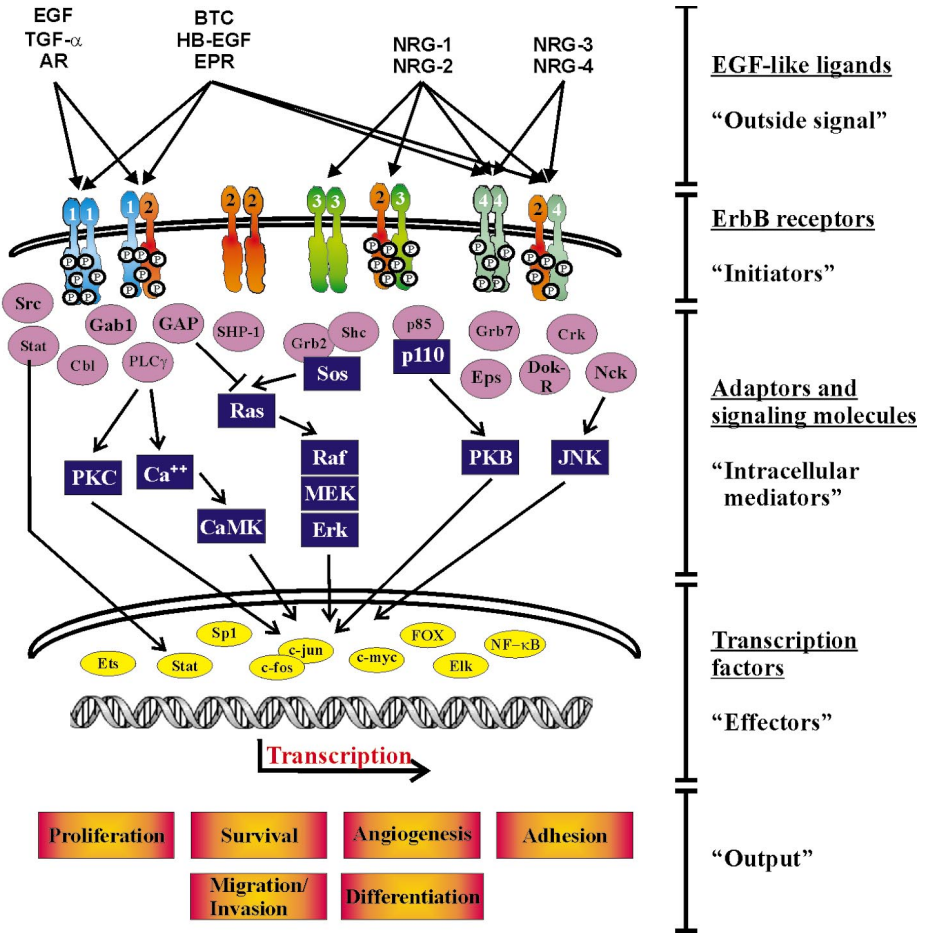

Figure 1 The ErbB signaling network. Ligands of the EGF family bind to their receptors causing the formation of different ErbB dimers. ErbB2, which has no direct ligand inducing homodimerization, needs a partner to acquire signaling potential (indicated by the *encircled P*). However, overexpression of ErbB2 in human tumors can circumvent this requirement (see text for a more detailed explanation). Moreover, ErbB3 homodimers cannot signal because the receptor has impaired kinase activity. Following receptor activation, various molecules (*violet*) with adaptor or enzymatic functions are directly recruited to the ErbBs. These then activate downstream signaling components (*dark blue*), which ultimately lead to changes in the activity of multiple nuclear transcription factors (*yellow*). For illustrative clarity, we have omitted many components in each of the signaling layers. FOX, forkhead transcription factor; Ets, members of this transcription factor family. For more details see the text.

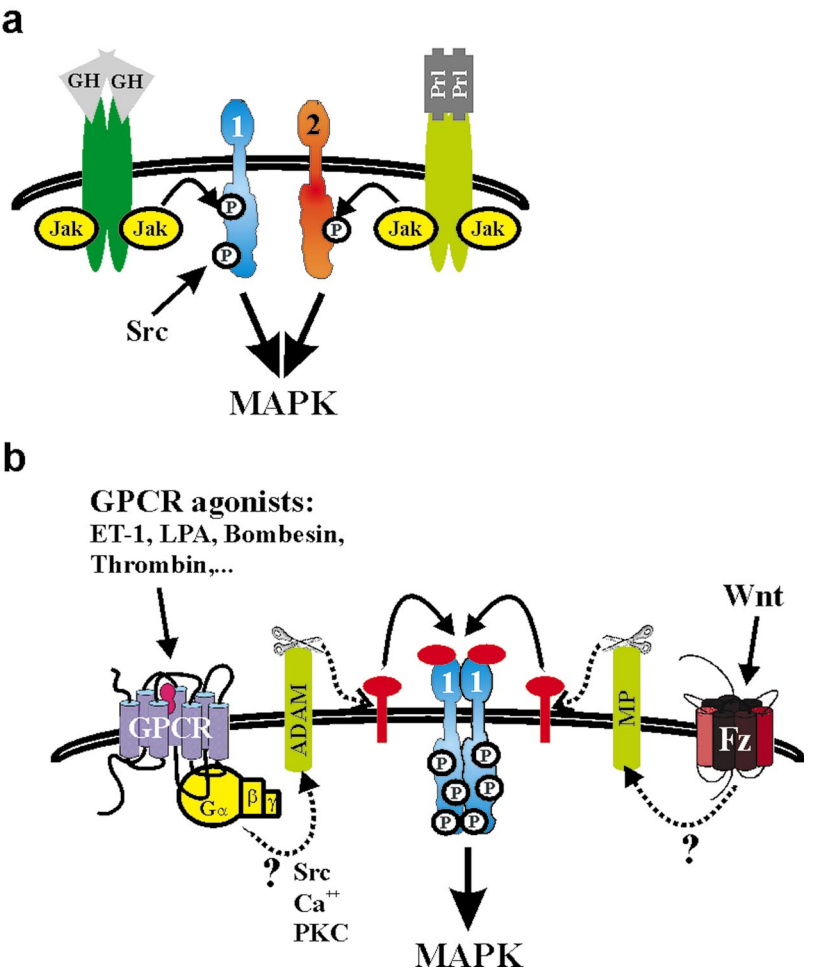

Figure 2 ErbB receptors as signal integrators. ErbB receptors can acquire signaling potential and activate the MAPK pathway upon exposure of cells to non-EGF-related peptides. The two known mechanisms of ErbB transactivation are shown. (*a*) Growth hormone (GH) or prolactin (Prl) treatment lead to ErbB1 or ErbB2 phosphorylation, respectively. This occurs through the direct action of Jak2, a tyrosine kinase that is constitutively bound to the cytokine receptors and activated following ligand binding. Activation of Src in response to various stimuli also results in ErbB1 phosphorylation. In these examples, no ErbB receptor kinase activity is required for MAPK activation. (*b*) Several GPCR agonists, as well as Wnt ligands, stimulate ErbB1 by increasing the availability of EGF-related peptides, which bind and activate the receptor. Although the ADAMs have been reported to catalyze cleavage of the proligands after GPCR stimulation, the nature of the metalloproteinase (MP) downstream of Wnt signaling is not yet known. See the text for more details.

Annual Review of Pharmacology and Toxicology
Volume 44, 2004

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