ACADEMIC PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Experimental Cell Research

Experimental Cell Research 284 (2003) 54–65

www.elsevier.com/locate/yexcr

The deaf and the dumb: the biology of ErbB-2 and ErbB-3

Ami Citri, Kochupurakkal Bose Skaria, and Yosef Yarden*

Department of Biological Regulation, The Weizmann Institute of Science, Rehovot 76100, Israel

Received 10 December 2002, revised version received 17 December 2002

Abstract

ErbB-2 (also called HER2/neu) and ErbB-3 are closely related to the epidermal growth factor receptor (EGFR/ErbB-1), but unlike EGFR, ErbB-2 is a ligandless receptor, whereas ErbB-3 lacks tyrosine kinase activity. Hence, both ErbB-2 and ErbB-3 are active only in the context of ErbB heterodimers, and ErbB-2 · ErbB-3 heterodimers, which are driven by neuregulin ligands, are the most prevalent and potent complexes. These stringently controlled heterodimers are repeatedly employed throughout embryonic development and dictate the establishment of several cell lineages through mesenchyme-epithelial inductive processes and the interactions of neurons with muscle, glia, and Schwann cells. Likewise, the potent combination of signaling pathways engaged by the heterodimers drives an aggressive phenotype of tumors of secretory epithelia, including breast and lung cancers. This review highlights recent structural insights into the mechanism of ligand-induced heterodimer formation, and concentrates on signaling pathways employed by ErbB-2 and ErbB-3 in normal and in malignant cells.
© 2003 Elsevier Science (USA). All rights reserved.

Introduction

The ErbB-2 · ErbB-3 heterodimer constitutes the pinnacle of ErbB receptor evolution, demonstrating the capacity of evolution to form an extremely potent signaling module from a pair of singly inactive individual proteins. The diversification of the ErbB family during evolution, from one receptor/ligand in worms, through one receptor/multiple ligands in flies, to four receptors and multiple ligands in mammals, has created a network capable of precise signaling in a widely divergent fashion [1]. Thus, through utilization of defined receptor pairs, activated by specific ligands, a graded signaling potency can be obtained, leading to a precise cellular outcome. An additional level of signaling diversity is obtained through differential activation of distinct signaling molecules downstream of each receptor. The capacity to form precise signaling is best exemplified by the ErbB-2 · ErbB-3 dimer. ErbB-3 is an impaired kinase due to substitutions in critical residues in its catalytic domain [2], and thus can signal only in the context of a receptor heterodimer. In addition, it is now clear that ErbB-2 is devoid of an activating ligand [3] and can act only in the context of a heterodimer with a ligand-bound receptor. In stark contrast to their apparent disabilities, this receptor pair forms the most potent signaling module of the ErbB-receptor family in terms of cell growth and transformation [4,5]. That the most potent signaling module is formed by partners that are incapable of productively signaling in isolation suggests that evolutionary forces formed these mechanisms as a measure to tightly control the output of the network. As will be described below, the basis for the potency of signaling by the ligand-activated ErbB-2 · ErbB-3 heterodimer lies in the fact that this dimer has the capacity to signal very potently, both through the Ras-Erk pathway for proliferation, and through the phosphatidylinositol-3′-kinase (PI3K)-Akt pathway for survival. In addition, this receptor dimer evades downregulation mechanisms, leading to prolonged signaling [6,7].

ErbB-2 and ErbB-3 as determinants of cell lineages

Organ morphogenesis is controlled, at least in part, by multiple polypeptide factors that transmit signals between neighboring cells. The ErbB-receptor family plays a pivotal role in cell lineage determination in a variety of tissues, including mesenchyme-epithelial inductive processes in epithelial organs (reviewed by Burden and Yarden [8]).

* Corresponding author. Fax: +972-8-9342488.
E-mail address: yosef.yarden@weizmann.ac.il (Y. Yarden).

0014-4827/03/$ – see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0014-4827(02)00101-5

ErbB-2 and ErbB-3, as well as ErbB-1, are expressed in most epithelial cell layers, and mesenchymal cells are a rich source of ErbB-ligands, both neuregulins (NRGs) and epidermal growth factor (EGF)-like ligands. During development of the human fetus, ErbB-2 is found in the nervous system, developing bone, muscle, skin, heart, lungs, and intestinal epithelium [9]. Likewise, ErbB-3 is found in cells of the gastrointestinal, reproductive, and urinary tracts, as well as the skin, endocrine, and nervous systems [10].

Cardiac development

The roles of ErbB-2 and ErbB-3 in development are best delineated by the phenotypes of mice in which the *erbB-2* and *erbB-3* genes have been inactivated, as well as the phenotype of *nrg-1*$^{-/-}$ mice. Essentially, the cardiac phenotypes of NRG-1 null mice and ErbB-2 null mice appear similar, and represent the sum of the changes observed in ErbB-3 and ErbB-4 null mice [11]. The most striking phenotype of ErbB-2 null mice is early death at mid-gestation (E10.5) due to malformation of trabeculae in the heart [12], a phenotype shared with ErbB-4 and NRG-1-defective mice [13,14]. NRG-1 is expressed in the endocardium, an endothelial ventricular lining, while ErbB-2 and ErbB-4 are expressed in the myocardium, which is the underlying muscular portion of the ventricle and atrium. Mice expressing a kinase-defective mutant of ErbB-2 instead of wild-type receptor die at mid-gestation and express the same spectrum of embryonic defects seen in ErbB-2-defective mice, demonstrating that the catalytic activity of ErbB-2 is essential for its function in embryogenesis [15]. While the process of heart trabeculation does not appear to require ErbB-3, this receptor is essential for normal cardiac development [16]. Expression of ErbB-3 is restricted to the mesenchyme of the endocardial cushion, which develops into the heart valves. In *erbB-3*$^{-/-}$ mouse embryos the trabeculation occurs in a delayed but otherwise normal fashion, but their atrioventricular valves are rudimentary and thinned, leading to death at E13.5. NRG-1 plays a crucial role in this inductive process [17], but ErbB-2 does not appear to be involved, since it is not expressed in the embryonic endocardial cushion. Targeted inactivation of ErbB-2 in ventricular cardiomyocytes led to a severe dilated cardiomyopathy, causing cardiac dysfunction by the second postnatal month, suggesting that ErbB-2 signaling is essential for adult heart function [18,19]. The cardiac phenotypes expressed by mice carrying mutations in *erbB-2* and *erbB-3* demonstrate the basic principle underlying the function of the encoded receptors, i.e., heterodimerization is essential for induction of receptor's function.

Glial and neuronal cell development

In addition to their roles in synapse formation and neuronal development, NRGs appear to act as major regulators in the development of myelinating cells in the peripheral (Schwann cells) and central (oligodendrocytes) nervous systems [20]. Mice individually mutant for *erbB-2, erbB-3,* or *nrg-1* display a failure in neural crest development, leading to impaired formation of the sympathetic nervous system [21]. The action of NRG-1, secreted by neurons, is essential for both the proliferation and the maturation of Schwann cells and oligodendrocytes. Accordingly, ErbB-3 is expressed in the responding myelinating cells, and wherever investigated, the effect of NRG-1 appears to be mediated by ErbB-3, acting in the context of ErbB-1 or ErbB-2. While NRG-1 is important throughout oligodendrocyte differentiation, using complex transgenic systems it has been established that ErbB-2 is important for late oligodendrocyte differentiation, and for the development of myelin [22,23]. In addition, it has also been demonstrated that a heterodimer of ErbB-2 and ErbB-3 is the active receptor in Schwann cell differentiation [24,25]. Consistent with observations made in vitro with cultured cells, in *erbB-3*-defective mice Schwann cells fail to develop, and most sensory and motor neurons subsequently die (reviewed by Davies [26]).

Mammary gland development

The mammary gland is one of the few organs in which major morphogenetic changes take place after birth. Two phases of morphogenesis occur, i.e., during puberty, and during pregnancy. In contrast to their ligands, which are expressed in defined time windows, all ErbB receptors are expressed throughout most developmental stages of the mammary gland (reviewed by Troyer and Lee [27]). In organ cultures of mammary glands, NRG-1 stimulated lobuloalveolar budding and the production of milk proteins [28]. In addition, branching morphogenesis and lobulo-alveolar differentiation of the mammary gland could be abolished by blocking expression of endogenous NRG. However, in transgenic animals with targeted expression of NRG-1 in the mammary gland, persistence of terminal end buds was observed, suggesting that NRG-1 inhibits signals that normally lead to the terminal differentiation of these structures [29]. Consistent with a role for ErbB-2 as a coreceptor, transgenic mice expressing a dominant-negative ErbB-2 in the mammary gland display normal ductal growth, but have defective lobuloalveoli and reduced milk protein secretion [30]. Due to coexpression of all ErbBs and many of their ligands, more experimental models will be needed to resolve the exact role of ErbB-2 · ErbB-3 heterodimers in mammary development.

Formation of ErbB-2 · ErbB-3 heterodimers: structural insights

While it is clear that dimerization of ErbB proteins is crucial for signaling (reviewed by Heldin and Ostman [31]), the underlying mechanism remained elusive until very recently. Three published structures of the extracellular domains of ErbB family receptors [32–34] have recently pro-

vided a framework for understanding the large amount of data that have accumulated over the years with respect to ligand binding and receptor activation. ErbB receptors share a high degree of primary sequence homology. Four subdomains have been identified in the extracellular domain; subdomains I (L1) and subdomain III (L2) mediate ligand binding [35,36], while according to the recently published structures, the cysteine-rich subdomains II (S1) and IV (S2) play a role in receptor dimerization. Most previous lines of evidence relate to the interaction of EGF with ErbB-1 (reviewed by Groenen et al. [37]). For example, several lines of evidence concluded that EGF binds ErbB-1 with a 1:1 stoichiometry, perhaps through a cleft formed by subdomains L1, L2, and S1 [38]. In addition, EGF binding to an isolated extracellular domain has been associated with a conformational change [39], but the exact mechanism of dimerization remained unknown until very recently.

Earlier lines of evidence

Mutagenesis of both NRG-1 [40] and EGF [36] concluded that these ligands bind their receptors in a bivalent manner. On the other hand, biophysical analyses of soluble ErbB-1 suggested dominance of a 2:2 ligand:receptor configuration [41]. Last, fluorescence imaging microscopy suggested that ErbB-1 exists in a predimerized state, but ligand binding induces a rotational rearrangement of the monomeric subunits [42]. This view is consistent with single molecule imaging of ErbB-1, which concluded that EGF first binds to predimerized receptors, and then a second EGF molecule binds to the 1:2 complex [43].

Insights from crystal structures

The crystal structures of ligand-bound ErbB-1 confirmed both bivalent ligand binding and a final 2:2 complex [33,34], consistent with the implications of the structure of a nonliganded ErbB-3 [32]. In the crystal structures of ErbB-1 and ErbB-3, L1 and L2 have a β-helical fold, while the S1 and S2 subdomains have an extended structure held together by disulfide bonds. The S1 subdomain traverses along one face of the β-helix of the L1 domain with a large interface conferring rigidity to the juxtaposition of the L1-S1 domains. Two structures of ErbB-1 have been described, one bound to EGF [33] and the other bound to transforming growth factor-α (cTGFα) [34]. In both cases, bivalent ligand binding to the L1 and L2 domains is observed and the ligand holds these domains in a rigid conformation. A long β-hairpin, termed the "dimerization loop" extends out of S1 and was found to be the primary mediator of dimerization of two monomeric ErbB-1 molecules. In the dimer, the two ligand-bound monomers approach each other back-to-back and the dimerization loop of one receptor extends deep into the dimer partner. The tip of the loop contacts residues in the L1 and L2 domains, contributing to dimer stabilization.

The role of the S2 domain

The published structures of ErbB-1 did not resolve the structure of S2. However, short cyclic peptides identical in sequence to the C-terminus of the S2 domain of ErbB-2 were found to bind the receptor as well as inhibit receptor signaling [44], suggesting that the S2 domain may be involved in dimerization. In addition, S2 has been previously demonstrated to reduce ligand binding affinity, suggestive of an intramolecular inhibitory interaction [45]. In the structure of the unliganded ErbB-3, an intramolecular interaction between the dimerization loop (from S1) and a β-hairpin protruding out from the S2 domains holds the receptor L domains far apart in an open conformation [32]. Taken together with the conformation of the ligand-bound ErbB-1, these lines of evidence raise the possibility that unengaged ErbB-1 and ErbB-4 may exist in a similar locked conformation on the cell surface. According to this model, ligand binding to domains L1 and L2 induces rotation of the rigid L1-S1 domains with respect to the L2 domain around the S1-L2 linker, and tightly bridges the L1 and L2 domains. Consequently, this conformational change exposes the dimerization loop of the receptor, rendering monomeric, ligand-bound receptors amenable to dimerization.

Dimers containing ErbB-2

Although ErbB-2 binds no known ligand, when recruited into heterodimers it increases ligand binding affinity [46,47]. It is also the favored receptor for heterodimerization [48,49]. Interestingly, in the structure of ErbB-2, unlike the other receptors in the family, a strong interaction between L1 and L2 domains was observed, mimicking the ligand-bound form in the ErbB-1 structure (A.W. Burgess, personal communication). This interaction involves regions corresponding to ligand-binding sites in the L1 and L2 domains of ErbB-1, rendering ErbB-2 incapable of binding ligands. The consequence of the L1-L2 interaction in ErbB-2 is a constitutively extended conformation of the dimerization loop. Hence, the promiscuous behavior of ErbB-2 and its inability to bind EGF-like ligands seem inherent to its structure. Fig. 1A presents a model for the formation of ErbB-2 · ErbB-3 heterodimers. This model was conceived based on the known structures of ErbB-3 and ErbB-1, in combination with previously described lines of evidence. Accordingly, the ligandless ErbB-2 is predisposed for dimerization because its dimerization loop is preextended. On the other hand, ligand binding to ErbB-3 releases a locked conformation and extends the dimerization loop. Finally, within the dimer, both S1 and S2 domains of each receptor form two distinct interfaces, which stabilize the heterodimer. An alternative view would suggest that a preformed heterodimer assumes a twisted active conformation upon binding of a ligand [42].

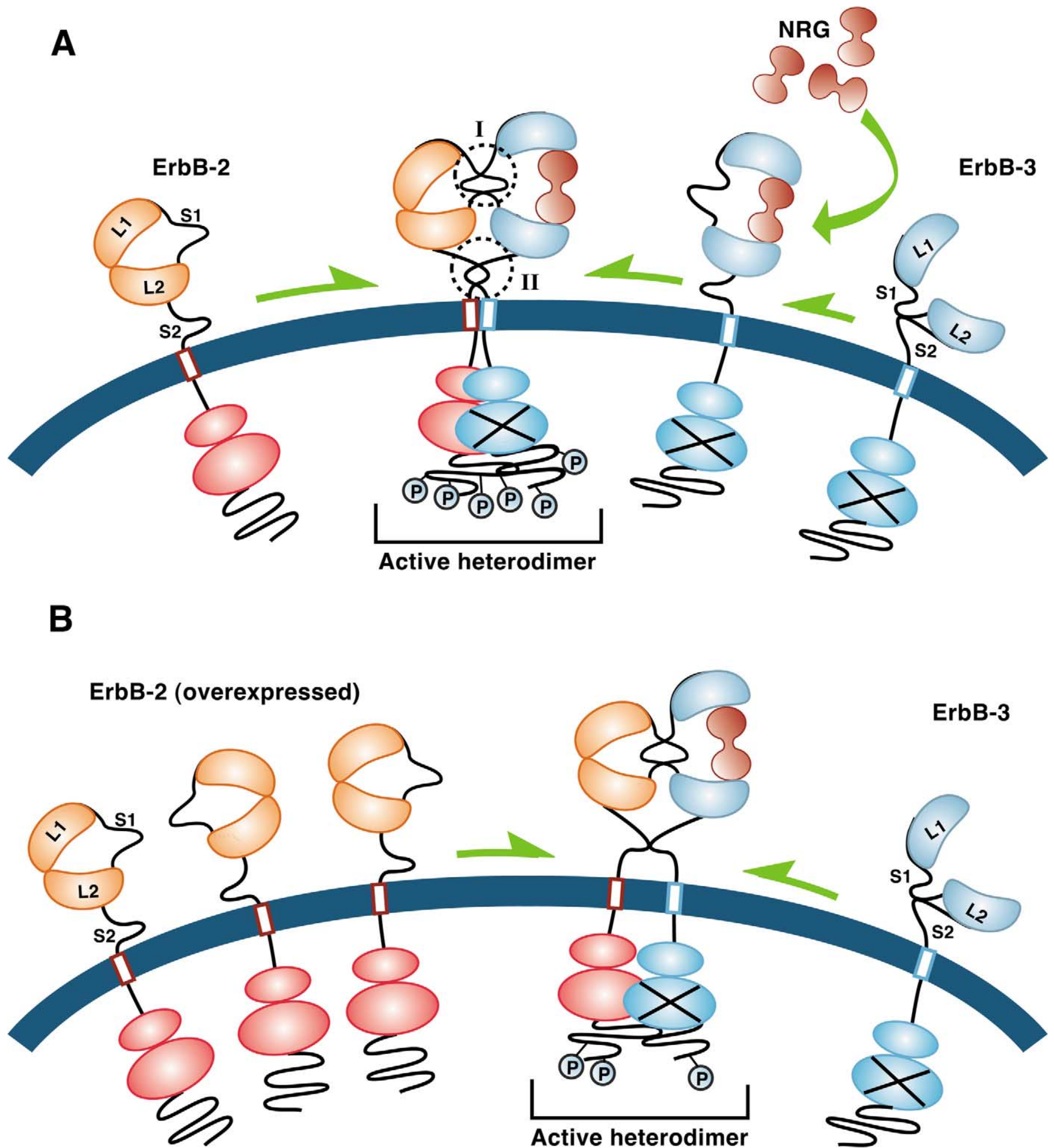

Fig. 1. Schematic representation of ligand-induced receptor heterodimers. The proposed mechanism of neuregulin-induced ErbB-2 · ErbB-3 heterodimers is depicted. (A) The extracellular domains are represented by two cysteine-rich domains (S1 and S2) and two cysteine-free, ligand-binding domains (L1 and L2). ErbB-3 (blue) exists on the cell surface in an autoinhibited conformation resulting from the interactions between the S1 and S2 domains. Bivalent binding of neuregulin (NRG, represented here as a red dumbbell) to the L1 and L2 domains of ErbB-3 rearranges the conformation of the extracellular domain, leading to protrusion of the S1 dimerization loop. In the case of ErbB-2, the intramolecular interaction between L1 and L2 results in a preextended conformation of the S1 dimerization loop. Dimerization between a ligand-bound ErbB-3 and an ErbB-2 molecule is mediated primarily by the dimerization loop (dotted circle I), with additional possible contributions from the loop in the S2 domain (dotted circle II). Additional stabilizing interactions between the transmembrane and kinase domains may also play a role. While the simplest scenario is depicted, receptor trimers and tetramers have also been proposed. (B) Overexpression of ErbB-2 at the cell surface may spontaneously recruit an autoinhibited ErbB-3 into heterodimers. The formed dimers may assume the ligand-induced conformation, resulting in weak but prolonged receptor activation. Alternatively, spontaneous homodimers formed upon overexpression of ErbB-2 cannot be excluded.

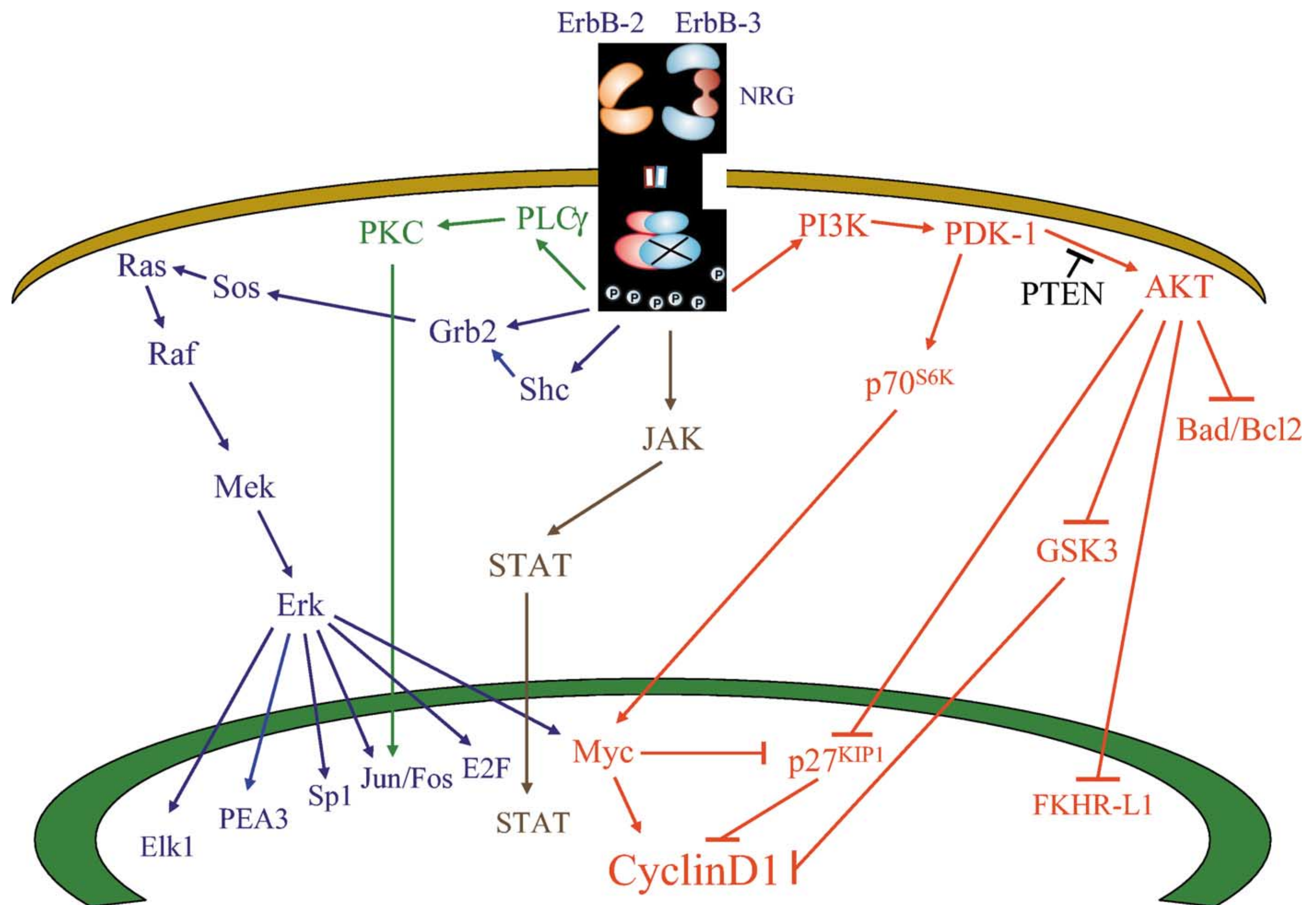

Fig. 2. The major signaling pathways stimulated by ligand-activated ErbB-2 · ErbB-3 heterodimers. Ligand-induced formation of the ErbB-2 · ErbB-3 heterodimer (see Fig. 1) at the cell surface leads to activation of several major pathways of signal transduction. This process results in enhanced cell survival and mitogenicity, and its deregulation can lead to tumorigenesis. Erk activation by the Ras-Raf pathway leads to cell proliferation through the activation of a number of nuclear targets, including Elk1, PEA3, Sp1, AP1, and the c-Myc oncoprotein, which is a major transcription factor and regulator of cell cycle progression. Another pathway is the P13K-Akt pathway, activation of which results in enhanced antiapoptotic and prosurvival signals, through inhibition of the proapoptotic proteins Bad, GSK3, and the transcription factor FKHR-L1. In addition, the PLCγ and the JAK-STAT pathways are indicated, with their resulting enhancement of transcription leading to cell proliferation. A major player acting downstream of ErbB-2 · ErbB-3 is cyclin D1. As indicated, a number of pathways lead from the receptors to enhanced activation of cyclin D1, thereby promoting cell cycle progression. Note that the outcome of activation of these different signaling pathways depends on the cellular context, and can vary from proliferation to differentiation, migration, and even induction of apoptosis.

Dimerization driven by receptor overexpression?

Amplification of the *erbB-1* and *erbB-2* genes is a common theme in epithelial cancers, and breast cancer patients whose tumors overexpress ErbB-2 better benefit from treatment with anti-ErbB-2 antibodies (reviewed by Yarden and Sliwkowski [1]). In model systems, overexpression of ErbB-1 is oncogenic but only in the presence of a ligand, whereas overexpression of ErbB-2 is transforming even in the absence of a ligand [50,51]. Likewise, although ErbB-2 bearing an activating point mutation is transforming in the absence of a ligand [52], the presence of another member of the ErbB family seems essential [53]. Hence, activation of ErbB-2 may not occur through formation of its own homodimers. Instead, ErbB-2-containing heterodimers may form when ErbB-2 is overexpressed. The model presented in Fig. 1B explains how heterodimerization of ErbB-2 with ErbB-3 may occur upon overexpression of ErbB-2, and a similar mechanism may underlie the oncogenic potential of coexpressed ErbB-1 and ErbB-2 [54].

Signal transduction by ErbB-2 · ErbB-3 dimers

Cancers do not necessarily arise as a linear result of an increased rate of cellular proliferation, but rather the balance between cell division and apoptosis is the crucial factor [55]. This principle is demonstrated by the oncogenic potential of the ErbB-2 · ErbB-3 dimer; this receptor signals through both the mitogen-activated protein kinase (MAPK) pathway, which drives cell proliferation and additional processes, and through the PI3K/Akt pathway, which primarily drives cellular survival and antiapoptotic signals (Fig. 2).

The ErbB-2 · ErbB-3 complex is the most active ErbB dimer

Comparative analyses of individual homodimers and heterodimers of ErbB proteins introduced into naive, ErbB-free cells, revealed that ErbB-3 is signaling defective, whereas ErbB-2 cannot be stimulated by any known ligand [4,56,57]. Nevertheless, ErbB-2 can enhance and prolong signaling by many EGF-like ligands [46]. In addition, within the hierarchical dimerization network of ErbB receptors, ErbB-2 represents the preferred heterodimerization partner of all other ErbB receptors, and the preferred dimerization partner of ErbB-2 is ErbB-3 [49]. The remarkable signaling potency of ErbB-2 · ErbB-3 dimers is the outcome of several features outlined below:

Slow rate of ligand dissociation

ErbB-2 enhances the affinity of the direct receptors for their ligands [47,49,58,59]. This effect seems to be shared by all EGF-like ligands and may reflect the promiscuous behavior of ErbB-2 as a heterodimerization partner [3]. While ligand affinity is determined by both the rate of association with the receptor, as well as by the rate of complex dissociation, only the latter parameter is modulated by ErbB-2 [46].

Relaxed specificity to EGF-like ligands

When ErbB-2 joins ErbB-3 it not only confers higher binding affinity, but also widens the spectrum of ligand binding toward neuregulins and EGF-like ligands [60,61]. This attribute is not a simple outcome of increased affinity, because some ligands, e.g., EGF and betacellulin, gain recognition, while others, e.g., TGFα, do not. Although the underlying mechanism remains unknown, it seems that this activity is due to an intrinsic capability of ErbB-2, because ErbB-2 · ErbB-4 heterodimers demonstrate a similar phenomenon [62].

Evasion of endocytosis

The major pathway leading to inactivation of signals emanating from ligand-activated growth factor receptors is an endocytic process that sorts active receptors to degradation in lysosomes (reviewed by Waterman and Yarden [63]). In the case of ErbB-1 this process is robustly regulated by an E3 ubiquitin ligase, called c-Cbl, that binds a specific phosphotyrosine of ErbB-1, thereby enhancing receptor ubiquitination and subsequent sorting to endocytosis and degradation [64–68]. In contrast, ligand-induced endocytosis of other ErbBs is slower [4,6], probably because these receptors are only weakly coupled to c-Cbl [48,69,70]. Consequently, ligands of ErbB-3 undergo relatively slow degradation [71], whereas ErbB-3 itself evades downregulation and undergoes recycling to the plasma membrane [72]. Defective endocytosis and enhanced recycling characterize not only the ErbB-2 · ErbB-3 dimer, but also other ErbB-2-containing heterodimers [73–75].

Coupling to potent signaling pathways

Within a ligand-occupied ErbB-2 · ErbB-3 dimer, transphosphorylation takes place and as a result, several phosphotyrosine residues located in the carboxyl-terminus of each receptor undergo phosphorylation [5]. Remarkably, a single site of ErbB-2, which recruits Shc and couples to the MAPK pathway, is sufficient for cell transformation [76], and multiple sites of ErbB-3 are able to recruit PI3K [77], thereby activating the Akt pathway. Normally ErbB-3 is restrained through its lack of kinase activity, which is compensated by heterodimerization. The strong signaling potential of this receptor is exemplified by an artificial fusion protein containing the kinase domain of ErbB-1 and the carboxyl-terminal tail of ErbB-3 [7]. This chimera is extremely mitogenic because it strongly couples to PI3K, avoids c-Cbl and endocytosis, and transmits prolonged signals through the Shc-MAPK pathway. In conclusion, several mechanisms allow ErbB-2 and ErbB-3 to escape normal constraints, and their combined dimer is characterized by ligand promiscuity and potent signaling.

Signaling pathways activated by ErbB-2 and ErbB-3

Several major pathways are stimulated upon activation of ErbB-2 and ErbB-3. These are MAPK [76], PI3K [77,78], phospholipase-Cγ (PLCγ; [79,80]), protein kinase C, and the Janus kinase (Jak-STAT; [81]). Remarkably, it appears that the heterodimers avoid coupling to Grb2 and the Ras-specific GTPase-activating protein (Ras-GAP; [82]), effectors that can also negatively regulate mitogenic signals [83,84].

MAPK pathway

Stimulation of Erk occurs upon ligand-induced activation of a receptor dimer, which binds Grb2 through a phosphorylated tyrosine-based consensus site, or indirectly, through interaction with Shc (reviewed by Marshall [85]). Grb2 is associated with Sos, a guanine nucleotide exchange factor specific for Ras, and Sos activates Ras by exchanging GDP for GTP. In the GTPase active state, Ras interacts with Raf and stimulates a linear kinase cascade culminating in activation of Erk/MAPK. Erk phosphorylates a variety of cytoplasmic and membranal substrates, and is rapidly translocated to the nucleus, where it activates a number of transcription factors including Sp1, PEA3, E2F, Elk1, and the AP1 transcription factor formed by Jun and Fos.

PI3K/AKT

Activation of PI3K occurs through binding of the regulatory p85 subunit of the lipid kinase to a phosphotyrosine consensus site on the receptor, leading to allosteric activation of the p110 catalytic subunit. p110 activation produces phosphatidylinositol-3,4,5-trisphosphate [PtdIns(3,4,5)P_3] from PtdIns(4,5)P_2 within seconds, and delayed production of PtdIns(3,4)P_2 through the action of 5′-inositol phosphatases. The effects of polyphosphinositides in the cell are

mediated through the action of two lipid-binding domains, the FYVE domain, which binds to PtdIns(3)P, and the PH domain, which binds to PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3. The PH domain-containing proteins PDK-1 (reviewed by Tokev and Newton [86]) and Akt/PKB are key mediators of PI3K signaling, and both are essential for the transforming effects of PI3K (reviewed by Blume-Jensen and Hunter [87]). Upon production of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 following activation of PI3K by the ErbB-2·ErbB-3 receptor dimer, Akt is recruited to the plasma membrane by its PH domain, and is phosphorylated by PDK-1. Akt phosphorylation causes its activation and translocation to the nucleus, where it acts upon its targets, which are either regulators of apoptosis or of cell growth (reviewed by Meier and Hemmings [88] and Cantley [89]). The tumor suppressor PTEN is a lipid phosphatase, which dephosphorylates the 3′-OH position of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, thereby reverting the activity of PI3K, and downregulating the activity of PDK-1 and Akt.

PLCγ

Activation of PLCγ by ErbB-2, rather than by ErbB-3 [82], occurs through its SH2-mediated recruitment to phosphorylation-dependent docking sites on ErbB-2, as well as recruitment through its PH domain to the plasma membrane. In its phosphorylated active form, PLCγ hydrolyzes PIP_2 (PI4,5P_2; phosphatidylinositol 4,5 biphosphate) into IP_3 (inositol 1,4,5-triphosphate), and diacylglycerol. IP_3 activates the release of calcium from intracellular stores, and thereby activates calcium/calmodulin-dependent kinases, as well as additional pathways, and it collaborates with diacylglycerol to stimulate protein kinase C (reviewed by Karin and Hunter [90] and Hunter [91]).

Cell adhesion molecules

CD44, a surface glycoprotein implicated in cell adhesion and motility, has been found to complex with ErbB-2 in ovarian and Schwann cells. CD44 coimmunoprecipitates with ErbB-2 and ErbB-3, and may potentiate the response to NRG-1 by facilitating receptor heterodimerization [92,93]. An additional positive modulator is the MUC4 sialomucin, which forms a complex with ErbB-2 in a number of tissues. MUC4 has been suggested to act as a modulator of the signaling activity of ErbB-2, inducing specific phosphorylation of ErbB-2, and potentiating NRG signaling [94].

Cyclin-dependent kinases and cell cycle regulation

Hyperactivated signaling through ErbB receptors results in deregulation of the cell cycle homeostatic machinery and upregulation of complexes containing cyclin D and cyclin-dependent kinases (CDKs), resulting in enhanced proliferation and malignant transformation. Cyclin D1 is a central effector of signaling by ErbB-2 and ErbB-3, and has been implicated as a major player in breast cancer acting to promote cell cycle progression, through activation of its catalytic partners CDK4 and CDK6 (reviewed by Harari and Yarden [95]). Both MAPK and PI3K can modulate the activity of cyclin D1 downstream of ErbB-2 [96]. The MAPK pathway has been implicated in transcriptional upregulation of cyclin D1 through the Sp1, AP1, and E2F transcription factors [97,98], and posttranslational stabilization of cyclin D1 can be conferred by its phosphorylation by Akt [99]. Additional key regulators of CDK function are $p27^{KIP1}$ and $p21^{Waf1}$, previously implicated as CDK inhibitors, but currently suggested to produce both activating and inhibiting functions, depending on their expression levels. Thus, ErbB-2/Neu-induced, cyclin D1-dependent transformation is accelerated in p27-haplo-insufficient mammary epithelial cells, but impaired in p27 null cells [100], and ErbB-2 and ErbB-3 function together to stimulate mitogenic signaling networks by Akt- and c-Myc-dependent sequestration of $p27^{KIP1}$, leading to deregulation of the G1-S cell cycle transition [101].

Negative regulators

RALT is a feedback inhibitor of ErbB-2 signaling whose expression is induced upon stimulation of the MAPK pathway [102]. RALT binds to the kinase domain of ErbB-2, and inhibits Erk activation and cellular transformation driven by ErbB-2 [103]. A different mode of ErbB-2 inhibition is exemplified by Herstatin, an alternatively spliced form consisting of a segment of the extracellular domain of ErbB-2 fused to a novel carboxyl-terminus. Herstatin binds to ErbB-2 and inhibits heterodimerization and activation of ErbB-3 [104]. A similar type of naturally secreted protein consisting of the extracellular domain of the receptor has been described for ErbB-3. This secreted protein (p85-s) inhibits NRG-stimulated activation of ErbB-2, ErbB-3, and ErbB-4 through sequestration of the ligand [105]. An additional mode of regulation of ErbB-2 is mediated by the action of the heat shock proteins Hsp90 and Hsp70, and their associated E3-ubiquitin ligase CHIP, which acts to promote the ubiquitylation of ErbB-2, and its subsequent degradation [106,107]. This mechanism of cellular regulation bears significant potential for pharmaceutical intervention in ErbB-2-dependent tumors.

Clinical implications of the cooperation between ErbB-2 and ErbB-3

Several lines of evidence derived from animal models and in vitro systems imply that ErbB-1 can transform naive cells only when one of its ligands, primarily TGFα, is available [50,108]. Consistent with this notion, analyses of human tumors from gastric, breast, pancreatic, and other origins indicated that autocrine loops underlie poor prognosis of the relevant ErbB-1-overexpressing tumors (reviewed by Salomon et al. [109]). Another critical partner of ErbB-1 is ErbB-2, as their coexpression drives oncogenesis in model systems [54,57]. In analogy to ErbB-1, when singly expressed, ErbB-3 is nonmitogenic [4], but together with

ErbB-2 and a neuregulin it transmits not only potent mitogenic signals [4,56,57], but also signals for tumorigenic growth [5,57,110]. As discussed earlier, whether or not ErbB-2 can transform cells when singly expressed is yet unclear; while the catalytic activity of this receptor is relatively high, even in the absence of a stimulating ligand [111], a transforming mutant, whose catalytic activity is constitutively elevated [112], loses its oncogenic potential when expressed in an ErbB null cellular environment [53].

ErbB-2 is overexpressed in a large proportion of breast and ovarian tumors (20–30%), primarily due to gene amplification [113,114]. ErbB-2 appears not to be expressed in benign tumors before the onset of malignant disease [115], but overexpression is maintained in metastatic lesions. The prognostic significance of ErbB-2 overexpresion in human cancer has been extensively reviewed [116–118], and therefore we limit our discussion to the available clinical data related to coexpression of ErbB-2 and neuregulin receptors. Expression of the high affinity neuregulin receptor ErbB-4 is relatively variable in carcinomas, and it may associate with a differentiated phenotype and better prognosis of breast tumors [119]. In contrast, coexpression of ErbB-2 with ErbB-4 in childhood medulloblastoma predicts poor prognosis [120]. Significantly, when singly analyzed in brain tumors, expression of neither receptor was predictive. Coexpression of ErbB-2 with the low affinity neuregulin receptor ErbB-3 may be similarly relevant to epithelial tumors. However, detection of such an association is potentially blurred by the following two factors: First, ErbB-3 is expressed in the majority of tumors of the breast, skin, ovary, and gastrointestinal tract [121–124]. Second, the respective gene shows no amplification or rearrangements. Nevertheless, along with reports that failed detecting association between ErbB-3 and clinical outcome, several studies associated ErbB-3 expression with pathological parameters. Examples include advanced non-small cell lung carcinomas in which high ErbB-3 predicted shorter patient survival [125], early invasive ovarian lesions [126], hepatocellular carcinomas [127], oral squamous cell carcinomas [124], and bladder cancers in which coexpression correlated with patient survival [128].

In conclusion, there are clinical indications supporting the concept emerging from in vitro studies that neither ErbB-2 nor ErbB-3 can be considered as stand-alone receptors. Future studies must also address the presence of neuregulins, because unlike ligands of EGFR, which seem to control autocrine loops in human cancers [129], neuregulins may form paracrine loops in breast [130] and prostate cancer [131]. Another variable is the occurrence in tumors of secreted ErbB-3 isoforms capable of neuregulin binding [105].

Perspectives

Peaking in the recent months, the past 15 years have been highly instructive as to the basic principles underlying the action of ErbB receptors, and in describing their relevance to tumorigenesis, thereby opening windows for therapeutic opportunities. Thus, the basic principle of receptor heterodimerization has taught us that the context in which a receptor functions is crucial for predicting the resulting signal. This context is beginning to be extended to the interaction of the ErbBs with receptors of other families and to cross-talk between signaling pathways (reviewed by Carpenter [132]). The recently resolved structures of the ectodomains of ErbB receptors will be instructive not only for understanding how ligands promote receptor dimers, but also help develop peptidergic and other ErbB blockers. It is interesting that a naturally occurring antagonistic ligand exists in flies (i.e., Argos [133]), but attempts to generate a similar blocker of mammalian ErbBs have failed so far.

Apart from spaciotemporal regulation of the expression of ligands, receptors, and downstream effectors, it is currently unclear how different neuregulins elicit unique responses, although they utilize similar receptor combinations. Differences in affinity of different ligands for the same receptor combination have been suggested to be a deciding factor in signal outcomes. Another possibility is the amplification of subtle differences in the conformations of the dimers induced by different ligands, when higher order oligomers of the receptors are formed [134]. Would low affinity viral ligands induce an open conformation of the dimerization loop, or do they exploit the small fraction of predimerized receptors by rearranging them to potentiate signaling [135]? If a ligand binds to only one receptor, how is the identity of the receptor dimer decided? What is the mechanism by which heterodimerization is preferred over homodimerization? And, how is the dimerization signal transferred through the membrane, leading to activation of the kinase domain? These and other questions will most likely await the resolution of the structure of a receptor heterodimer in the context of a ligand. Last, in terms of cancer therapy, our understanding of the basic mechanisms underlying ErbB-dependent tumorigenesis have led to a major focus on the receptors as targets for therapeutics. Given the perception of multilayered signaling through the ErbB family, this choice appears to be valid, but future targeting of additional components of the ErbB network is expected to increase clinical success in the future.

Acknowledgments

We thank Sarah Bacus, Jorma Isola, and Mark Sliwkowski for insightful discussions. Our laboratory is supported by grants from the National Cancer Institute, the U.S. Army, the European Commission, and the Israel Academy of Sciences and Humanities.

References

[1] Y. Yarden, M.X. Sliwkowski, Untangling the ErbB signalling network, Nat. Rev. Mol. Cell. Biol. 2 (2001) 127–137.

[2] P.M. Guy, J.V. Platko, L.C. Cantley, R.A. Cerione, K.L. Carraway 3rd, Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity, Proc. Natl. Acad. Sci. USA 91 (1994) 8132–8136.

[3] L.N. Klapper, S. Glathe, N. Vaisman, N.E. Hynes, G.C. Andrews, M. Sela, Y. Yarden, 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 (1999) 4995–5000.

[4] R. Pinkas-Kramarski, L. Soussan, H. Waterman, G. Levkowitz, I. Alroy, L. Klapper, S. Lavi, R. Seger, B.J. Ratzkin, M. Sela, Y. Yarden, Diversification of Neu differentiation factor and epidermal growth factor signaling by combinatorial receptor interactions, EMBO J. 15 (1996) 2452–2467.

[5] C. Wallasch, F.U. Weiss, G. Niederfellner, B. Jallal, W. Issing, A. Ullrich, Heregulin-dependent regulation of HER2/neu oncogenic signaling by heterodimerization with HER3, EMBO J. 14 (1995) 4267–4275.

[6] J. Baulida, M.H. Kraus, M. Alimandi, P.P. Di Fiore, G. Carpenter, All ErbB receptors other than the epidermal growth factor receptor are endocytosis impaired, J. Biol. Chem. 271 (1996) 5251–5257.

[7] H. Waterman, I. Alroy, S. Strano, R. Seger, Y. Yarden, The C-terminus of the kinase-defective neuregulin receptor ErbB-3 confers mitogenic superiority and dictates endocytic routing, EMBO J. 18 (1999) 3348–3358.

[8] S. Burden, Y. Yarden, Neuregulins and their receptors: a versatile signaling module in organogenesis and oncogenesis, Neuron 18 (1997) 847–855.

[9] L. Coussens, T.I. Yang Feng, Y.C. Liao, E. Chen, A. Gray, J. McGrath, P.H. Seeburg, T.A. Libermann, J. Schlessinger, U. Francke, A. Levinson, A. Ullrich, Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene, Science 230 (1985) 1132–1139.

[10] S.A. Prigent, N.R. Lemoine, C.M. Hughes, G.D. Plowman, C. Selden, W.J. Gullick, Expression of the c-erbB-3 protein in normal human adult and fetal tissues, Oncogene 7 (1992) 1273–1278.

[11] A.N. Garratt, S. Britsch, C. Birchmeier, Neuregulin, a factor with many functions in the life of a schwann cell, Bioessays 22 (2000) 987–996.

[12] K.F. Lee, H. Simon, H. Chen, B. Bates, M.C. Hung, C. Hauser, Requirement for neuregulin receptor erbB2 in neural and cardiac development, Nature 378 (1995) 394–398.

[13] M. Gassmann, F. Casagranda, D. Orioli, H. Simon, C. Lai, R. Klein, G. Lemke, Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor, Nature 378 (1995) 390–394.

[14] D. Meyer, C. Birchmeier, Multiple essential functions of neuregulin in development, Nature 378 (1995) 386–390.

[15] R. Chan, W.R. Hardy, M.A. Laing, S.E. Hardy, W.J. Muller, The catalytic activity of the ErbB-2 receptor tyrosine kinase is essential for embryonic development, Mol. Cell. Biol. 22 (2002) 1073–1078.

[16] S.L. Erickson, K.S. O'Shea, N. Ghaboosi, L. Loverro, G. Frantz, M. Bauer, L.H. Lu, M.W. Moore, ErbB3 is required for normal cerebellar and cardiac development: a comparison with ErbB2-and heregulin-deficient mice, Development 124 (1997) 4999–5011.

[17] D. Riethmacher, E. Sonnenberg-Riethmacher, V. Brinkmann, T. Yamaai, G.R. Lewin, C. Birchmeier, Severe neuropathies in mice with targeted mutations in the ErbB3 receptor, Nature 389 (1997) 725–730.

[18] S.A. Crone, Y.Y. Zhao, L. Fan, Y. Gu, S. Minamisawa, Y. Liu, K.L. Peterson, J. Chen, R. Kahn, G. Condorelli, J. Ross Jr., K.R. Chien, K.F. Lee, ErbB2 is essential in the prevention of dilated cardiomyopathy, Nat. Med. 8 (2002) 459–465.

[19] C. Ozcelik, B. Erdmann, B. Pilz, N. Wettschureck, S. Britsch, N. Hubner, K.R. Chien, C. Birchmeier, A.N. Garratt, Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy, Proc. Natl. Acad. Sci. USA 99 (2002) 8880–8885.

[20] K. Adlkofer, C. Lai, Role of neuregulins in glial cell development, Glia 29 (2000) 104–111.

[21] S. Britsch, L. Li, S. Kirchhoff, F. Theuring, V. Brinkmann, C. Birchmeier, D. Riethmacher, The ErbB2 and ErbB3 receptors and their ligand, neuregulin-1, are essential for development of the sympathetic nervous system, Genes Dev. 12 (1998) 1825–1836.

[22] J.K. Morris, W. Lin, C. Hauser, Y. Marchuk, D. Getman, K.F. Lee, Rescue of the cardiac defect in ErbB2 mutant mice reveals essential roles of ErbB2 in peripheral nervous system development, Neuron 23 (1999) 273–283.

[23] S.K. Park, R. Miller, I. Krane, T. Vartanian, The erbB2 gene is required for the development of terminally differentiated spinal cord oligodendrocytes, J. Cell Biol. 154 (2001) 1245–1258.

[24] J.B. Grinspan, M.A. Marchionni, M. Reeves, M. Coulaloglou, S.S. Scherer, Axonal interactions regulate Schwann cell apoptosis in developing peripheral nerve: neuregulin receptors and the role of neureguins, J. Neurosci. 16 (1996) 6107–6118.

[25] A.D.O. Levi, R.P. Bunge, J.A. Lofgren, L. Meima, F. Hefti, K. Nikolics, X.M. Sliwkowski, The influence of heregulins on human Schwann cell proliferation, J. Neurosci. 15 (1995) 1329–1340.

[26] A.M. Davies, Neuronal survival: early dependence on Schwann cells, Curr. Biol. 8 (1998) R15–R18.

[27] K.L. Troyer, D.C. Lee, Regulation of mouse mammary gland development and tumorigenesis by the ERBB signaling network, J. Mammary Gland Biol. Neoplasia 6 (2001) 7–21.

[28] Y. Yang, E. Spitzer, D. Meyer, M. Sachs, C. Niemann, G. Hartmann, K.M. Weidner, C. Birchmeier, W. Birchmeier, Sequential requirement of hepatocyte growth factor and neuregulin in the morphogenesis and differentiation of the mammary gland, J. Cell Biol. 131 (1995) 215–226.

[29] I.M. Krane, P. Leder, NDF/heregulin induces persistence of terminal end buds and adenocarcinomas in the mammary glands of transgenic mice, Oncogene 12 (1996) 1781–1788.

[30] F.E. Jones, D.F. Stern, Expression of dominant-negative ErbB2 in the mammary gland of transgenic mice reveals a role in lobuloalveolar development and lactation, Oncogene 18 (1999) 3481–3490.

[31] C.-H. Heldin, A. Ostman, Ligand-induced dimerization of growth factor receptors: variations on the theme, Cytokine Growth Factor Rev. 7 (1996) 33–40.

[32] H.S. Cho, D.J. Leahy, Structure of the extracellular region of HER3 reveals an interdomain tether, Science 297 (2002) 1330–1333.

[33] T.P. Garrett, N.M. McKern, M. Lou, T.C. Elleman, T.E. Adams, G.O. Lovrecz, H.J. Zhu, F. Walker, M.J. Frenkel, P.A. Hoyne, R.N. Jorissen, E.C. Nice, A.W. Burgess, C.W. Ward, Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha, Cell 110 (2002) 763–773.

[34] H. Ogiso, R. Ishitani, O. Nureki, S. Fukai, M. Yamanaka, J.H. Kim, K. Saito, A. Sakamoto, M. Inoue, M. Shirouzu, S. Yokoyama, Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains, Cell 110 (2002) 775–787.

[35] I. Lax, F. Bellot, R. Howk, A. Ullrich, D. Givol, J. Schlessinger, Functional analysis of the ligand binding site of EGF-receptor utilizing chicken/human receptor molecules, EMBO J. 8 (1989) 421–427.

[36] A.E. Summerfield, A.K. Hudnall, T.J. Lukas, C.A. Guyer, J.V. Staros, Identification of residues of the epidermal growth factor receptor proximal to residue 45 of bound epidermal growth factor, J. Biol. Chem. 271 (1996) 19656–19659.

[37] L.C. Groenen, E.C. Nice, A.W. Burgess, Structure-function relationships for the EGF/TGF-α family of mitogens, Growth Factors 11 (1994) 235–257.

[38] R.N. Jorissen, V.C. Epa, H.R. Treutlein, T.P. Garrett, C.W. Ward, A.W. Burgess, Characterization of a comparative model of the extracellular domain of the epidermal growth factor receptor, Protein Sci. 9 (2000) 310–324.

[39] C. Greenfield, I. Hils, M.D. Waterfield, M. Federwisch, A. Wollmer, T.L. Blundell, N. McDonald, EGF binding induces conformational change in the external domain of its receptor, EMBO J. 8 (1989) 4115–4123.

[40] E. Tzahar, R. Pinkas Kramarski, J.D. Moyer, L.N. Klapper, I. Alroy, G. Levkowitz, M. Shelly, S. Henis, M. Eisenstein, B.J. Ratzkin, M. Sela, G.C. Andrews, Y. Yarden, Bivalence of EGF-like ligands drives the ErbB signaling network, EMBO J. 16 (1997) 4938–4950.

[41] M.A. Lemmon, Z. Bu, J.E. Ladbury, M. Zhou, D. Pinchasi, I. Lax, D.E. Engelman, J. Schlessinger, Two EGF molecules contribute additively to stabilization of the EGFR dimer, EMBO J. 16 (1997) 281–294.

[42] T.W. Gadella, T.M. Jovin, Oligomerization of epidermal growth factor receptors on A431 cells studied by time-resolved fluorescence imaging microscopy. A stereochemical model for tyrosine kinase receptor activation, J. Cell Biol. 129 (1995) 1543–1558.

[43] Y. Sako, S. Minoghchi, T. Yanagida, Single-molecule imaging of EGFR signalling on the surface of living cells, Nat. Cell Biol. 2 (2000) 168–172.

[44] A. Berezov, J. Chen, Q. Liu, H.T. Zhang, M.I. Greene, R. Murali, Disabling receptor ensembles with rationally designed interface peptidomimetics, J. Biol. Chem. 277 (2002) 28330–28339.

[45] T.C. Elleman, T. Domagala, N.M. McKern, M. Nerrie, B. Lonnqvist, T.E. Adams, J. Lewis, G.O. Lovrecz, P.A. Hoyne, K.M. Richards, G.J. Howlett, J. Rothacker, R.N. Jorissen, M. Lou, T.P. Garrett, A.W. Burgess, E.C. Nice, C.W. Ward, Identification of a determinant of epidermal growth factor receptor ligand-binding specificity using a truncated, high-affinity form of the ectodomain, Biochemistry 40 (2001) 8930–8939.

[46] D. Karunagaran, E. Tzahar, R.R. Beerli, X. Chen, D. Graus Porta, B.J. Ratzkin, R. Seger, N.E. Hynes, Y. Yarden, ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer, EMBO J. 15 (1996) 254–264.

[47] M.X. Sliwkowski, G. Schaefer, R.W. Akita, J.A. Lofgren, V.D. Fitzpatrick, A. Nuijens, B.M. Fendly, R.A. Cerione, R.L. Vandlen, K.L. Carraway, Coexpression of erbB2 and erbB3 proteins reconstitutes a high affinity receptor for heregulin, J. Biol. Chem. 269 (1994) 14661–14665.

[48] D. Graus Porta, R.R. Beerli, J.M. Daly, N.E. Hynes, ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling, EMBO J. 16 (1997) 1647–1655.

[49] E. Tzahar, H. Waterman, X. Chen, G. Levkowitz, D. Karunagaran, S. Lavi, B.J. Ratzkin, Y. Yarden, A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor, Mol. Cell. Biol. 16 (1996) 5276–5287.

[50] P.P. Di Fiore, J.H. Pierce, M.H. Kraus, O. Segatto, C.R. King, S.A. Aaronson, *erbB-2* is a potent oncogene when overexpressed in NIH/3T3 cells, Science 237 (1987) 178–182.

[51] R.M. Hudziak, J. Schlessinger, A. Ullrich, Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3T3 cells, Proc. Natl. Acad. Sci. USA 84 (1987) 7159–7163.

[52] C.I. Bargmann, M.C. Hung, R.A. Weinberg, Multiple independent activations of the *neu* oncogene by a point mutation altering the transmembrane domain of p185, Cell 45 (1986) 649–657.

[53] B.D. Cohen, P.A. Kiener, J.M. Green, L. Foy, H.P. Fell, K. Zhang, The relationship between human epidermal growth-like factor receptor expression and cellular transformation in NIH3T3 cells, J. Biol. Chem. 271 (1996) 30897–30903.

[54] Y. Kokai, J.N. Myers, T. Wada, V.I. Brown, C.M. LeVea, J.G. Davis, K. Dobashi, M.I. Greene, Synergistic interaction of p185c-*neu* and the EGF receptor leads to transformation of rodent fibroblasts, Cell 58 (1989) 287–292.

[55] J.C. Reed, Dysregulation of apoptosis in cancer, J. Clin. Oncol. 17 (1999) 2941–2953.

[56] D.J. Riese 2nd, T.M. van Raaij, G.D. Plowman, G.C. Andrews, D.F. Stern, The cellular response to neuregulins is governed by complex interactions of the erbB receptor family, Mol. Cell. Biol. 15 (1995) 5770–5776.

[57] K. Zhang, J. Sun, N. Liu, D. Wen, D. Chang, A. Thomason, S.K. Yoshinaga, Transformation of NIH 3T3 cells by HER3 or HER4 receptors requires the presence of HER1 or HER2, J. Biol. Chem. 271 (1996) 3884–3890.

[58] E. Peles, R. Ben-Levy, E. Tzahar, N. Liu, D. Wen, Y. Yarden, Cell-type specific interaction of Neu differentiation factor (NDF/heregulin) with Neu/HER-2 suggests complex ligand-receptor relationships, EMBO J. 12 (1993) 961–971.

[59] T. Wada, X. Qian, M.I. Greene, Intermolecular association of the $p185^{neu}$ protein and EGF receptor modulates EGF receptor function, Cell 61 (1990) 1339–1347.

[60] M. Alimandi, L.M. Wang, D. Bottaro, C.C. Lee, A. Kuo, M. Frankel, P. Fedi, C. Tang, M. Lippman, J.H. Pierce, Epidermal growth factor and betacellulin mediate signal transduction through co-expressed ErbB2 and ErbB3 receptors, EMBO J. 16 (1997) 5608–5617.

[61] R. Pinkas-Kramarski, A.E. Lenferink, S.S. Bacus, L. Lyass, M.L. van de Poll, L.N. Klapper, E. Tzahar, M. Sela, E.J. van Zoelen, Y. Yarden, The oncogenic ErbB-2/ErbB-3 heterodimer is a surrogate receptor of the epidermal growth factor and betacellulin, Oncogene 16 (1998) 1249–1258.

[62] L.M. Wang, A. Kuo, M. Alimandi, M.C. Very, C.C. Lee, V. Kapoor, N. Ellmore, X.H. Chen, J.H. Pierce, ErbB-2 expression increases the spectrum and potency of ligand-mediated signal transduction through ErbB-4, Proc. Natl. Acad. Sci. USA 95 (1998) 6809–6814.

[63] H. Waterman, Y. Yarden, Molecular mechanisms underlying endocytosis and sorting of ErbB receptor tyrosine kinases, FEBS Lett. 490 (2001) 142–152.

[64] G. Levkowitz, H. Waterman, E. Zamir, Z. Kam, S. Oved, W.Y. Langdon, L. Beguinot, B. Geiger, Y. Yarden, c-Cb1/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor , Genes Dev. 12 (1998) 3663–3674.

[65] G. Levkowitz, H. Waterman, S.A. Ettenberg, M. Katz, A.Y. Tsygankov, I. Alroy, S. Lavi, K. Iwai, Y. Reiss, A. Ciechanover, S. Lipkowitz, Y. Yarden, Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cb1/Sli-1, Mol. Cell. 4 (1999) 1029–1040.

[66] N.L. Lill, P. Douillard, R.A. Awwad, S.M.L. Ota, S. Lupher Miyake Jr., N. Meissner-Lula, V.W. Hsu, H. Band, The evolutionarily conserved N-terminal region of Cbl is sufficient to enhance down-regulation of the epidermal growth factor receptor, J. Biol. Chem. 275 (2000) 367–377.

[67] H. Waterman, G. Levkowitz, I. Alroy, Y. Yarden, The RING finger of c-Cb1 mediates desensitization of the epidermal growth factor receptor, J. Biol. Chem. 274 (1999) 22151–22154.

[68] M. Yokouchi, T. Kondo, A. Houghton, M. Bartkiewicz, W.C. Horne, H. Zhang, A. Yoshimura, R. Baron, Ligand-induced ubiquitination of the epidermal growth factor receptor involves the interaction of the c-Cbl RING finger and UbeH7, J. Biol. Chem. 274 (1999) 31707–31712.

[69] G. Levkowitz, L.N. Klapper, E. Tzahar, A. Freywald, M. Sela, Y. Yarden, Coupling of the c-Cbl protooncogene product to ErbB-1/EGF-receptor but not to other ErbB proteins, Oncogene 12 (1996) 1117–1125.

[70] S.K. Muthuswamy, M. Gilman, J.S. Brugge, Controlled dimerization of ErbB receptors provides evidence for differential signaling by homo- and heterodimers, Mol. Cell. Biol. 19 (1999) 6845–6857.

[71] J. Baulida, G. Carpenter, Heregulin degradation in the absence of rapid receptor-mediated internalization, Exp. Cell. Res. 232 (1997) 167–172.
[72] H. Waterman, I. Sabanai, B. Geiger, Y. Yarden, Alternative intracellular routing of ErbB receptors may determine signaling potency, J. Biol. Chem. 273 (1998) 13819–13827.
[73] A.E. Lenferink, R. Pinkas Kramarski, M.L. van de Poll, M.J. van Vugt, L.N. Klapper, E. Tzahar, H. Waterman, M. Sela, E.J. van Zoelen, Y. Yarden, Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers, EMBO J. 17 (1998) 3385–3397.
[74] A. Sorkin, P.P. Di Fiore, G. Carpenter, The carboxyl terminus of epidermal growth factor receptor/erbB-2 chimerae is internalization impaired, Oncogene 8 (1993) 3021–3028.
[75] R. Worthylake, L.K. Opresko, H.S. Wiley, ErbB-2 amplification inhibits down-regulation and induces constitutive activation of both ErbB-2 and epidermal growth factor receptors, J. Biol. Chem. 274 (1999) 8865–8874.
[76] R. Ben-Levy, H.F. Paterson, C.J. Marshall, Y. Yarden, A single autophosphorylation site confers oncogenicity to the Neu/ErbB-2 receptor and enables coupling to the MAP kinase pathway, EMBO J. 13 (1994) 3302–3311.
[77] S.A. Prigent, W.J. Gullick, Identification of c-erbB-3 binding sites for phosphatidylinositol 3′-kinase and SHC using an EGF receptor/c-erbB-3 chimera, EMBO J. 13 (1994) 2831–2841.
[78] E. Peles, R. Lamprecht, R. Ben-Levy, E. Tzahar, Y. Yarden, Regulated coupling of the Neu receptor to phosphatidylinositol 3′-kinase and its release by oncogenic activation, J. Biol. Chem. 267 (1992) 12266–12274.
[79] F. Fazioli, U.H. Kim, S.G. Rhee, C.J. Molloy, O. Segatto, P.P. Di Fiore, The erbB-2 mitogenic signaling pathway: tyrosine phosphorylation of phospholipase C-gamma and GTPase-activating protein does not correlate with erbB-2 mitogenic potency, Mol. Cell. Biol. 11 (1991) 2040–2048.
[80] E. Peles, R.B. Levy, E. Or, A. Ullrich, Y. Yarden, Oncogenic forms of the neu/HER2 tyrosine kinase are permanently coupled to phospholipase C gamma, EMBO J. 10 (1991) 2077–2086.
[81] J. Liu, J.A. Kern, Neuregulin-1 activates the JAK-STAT pathway and regulates lung epithelial cell proliferation, Am. J. Respir. Cell. Mol. Biol. 27 (2002) 306–313.
[82] P. Fedi, J.H. Pierce, P.P. di Fiore, M.H. Kraus, 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 (1994) 492–500.
[83] D. Bar-Sagi, A. Hall, Ras and Rho GTPases: a family reunion, Cell 103 (2000) 227–238.
[84] H. Waterman, M. Katz, C. Rubin, K. Shtiegman, S. Lavi, A. Elson, T. Jovin, Y. Yarden, A mutant EGF-receptor defective in ubiquitylation and endocytosis unveils a role for Grb2 in negative signaling, EMBO J. 21 (2002) 303–313.
[85] C.J. Marshall, MAP kinase kinase kinase, MAP kinase kinase and MAP kinase, Curr. Opin. Gen. Dev. 4 (1994) 82–89.
[86] A. Toker, A.C. Newton, Cellular signaling: pivoting around PDK-1, Cell 103 (2000) 185–188.
[87] P. Blume-Jensen, T. Hunter, Oncogenic kinase signaling, Nature 411 (2001) 355–365.
[88] R. Meier, B.A. Hemmings, Regulation of protein kinase B, J. Recept. Signal Transduct. Res. 19 (1999) 121–128.
[89] L.C. Cantley, The phosphoinositide 3-kinase pathway, Science 296 (2002) 1655–1657.
[90] M. Karin, T. Hunter, Transcriptional control by protein phosphorylation: signal transmission from the cell surface to the nucleus, Curr. Biol. 5 (1995) 747–757.
[91] T. Hunter, Signaling—2000 and beyond, Cell 100 (2000) 113–127.
[92] L.Y. Bourguignon, H. Zhu, A. Chu, N. Iida, L. Zhang, M.C. Hung, Interaction between the adhesion receptor, CD44, and the oncogene product, p185HER2, promotes human ovarian tumor cell activation, J. Biol. Chem. 272 (1997) 27913–27918.
[93] L.S. Sherman, T.A. Rizvi, S. Karyala, N. Ratner, CD44 enhances neuregulin signaling by Schwann cells, J. Cell. Biol. 150 (2000) 1071–1084.
[94] S. Jepson, M. Komatsu, B. Haq, M.E. Arango, D. Huang, C.A. Carraway, K.L. Carraway, Muc4/sialomucin complex, the intramembrane ErbB2 ligand, induces specific phosphorylation of ErbB2 and enhances expression of p27(kip), but does not activate mitogen-activated kinase or protein kinaseB/Akt pathways, Oncogene 21 (2002) 7524–7532.
[95] D. Harari, Y. Yarden, Molecular mechanisms underlying ErbB2/HER2 action in breast cancer, Oncogene 19 (2000) 6102–6114.
[96] A.E. Lenferink, D. Busse, W.M. Flanagan, F.M. Yakes, C.L. Arteaga, ErbB2/neu kinase modulates cellular p27(Kip1) and cyclin D1 through multiple signaling pathways, Cancer Res. 61 (2001) 6583–6591.
[97] R.J. Lee, C. Albanese, M. Fu, M. D'Amico, B. Lin, G. Watanabe, G.K. Haines 3rd, P.M. Siegel, M.C. Hung, Y. Yarden, J.M. Horowitz, W.J. Muller, R.G. Pestell, Cyclin D1 is required for transformation by activated Neu and is induced through an E2F-dependent signaling pathway, Mol. Cell. Biol. 20 (2000) 672–683.
[98] G.M. Wulf, A. Ryo, G.G. Wulf, S.W. Lee, T. Niu, V. Petkova, K.P. Lu, Pin1 is overexpressed in breast cancer and cooperates with Ras signaling in increasing the transcriptional activity of c-Jun towards cyclin D1, EMBO J. 20 (2001) 3459–3472.
[99] J.A. Diehl, M. Cheng, M.F. Roussel, C.J. Sherr, Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization, Genes Dev. 12 (1998) 3499–3511.
[100] R.S. Muraoka, A.E. Lenferink, B. Law, E. Hamilton, D.M. Brantley, L.R. Roebuck, C.L. Arteaga, ErbB2/Neu-induced, cyclin D1-dependent transformation is accelerated in p27-haploinsufficient mammary epithelial cells but impaired in p27-null cells, Mol. Cell. Biol. 22 (2002) 2204–2219.
[101] R.M. Neve, H. Sutterluty, N. Pullen, H.A. Lane, J.M. Daly, W. Krek, N.E. Hynes, Effects of oncogenic ErbB2 on G1 cell cycle regulators in breast tumour cells, Oncogene 19 (2000) 1647–1656.
[102] M. Fiorini, C. Ballaro, G. Sala, G. Falcone, S. Alema, O. Segatto, Expression of RALT, a feedback inhibitor of ErbB receptors, is subjected to an integrated transcriptional and post-translational control, Oncogene 21 (2002) 6530–6539.
[103] L. Fiorentino, C. Pertica, M. Fiorini, C. Talora, M. Crescenzi, L. Castellani, S. Alema, P. Benedetti, O. Segatto, Inhibition of ErbB-2 mitogenic and transforming activity by RALT, a mitogen-induced signal transducer which binds to the ErbB-2 kinase domain, Mol. Cell. Biol. 20 (2000) 7735–7750.
[104] N.G. Azios, F.J. Romero, M.C. Denton, J.K. Doherty, G.M. Clinton, Expression of herstatin, an autoinhibitor of HER-2/neu, inhibits transactivation of HER-3 by HER-2 and blocks EGF activation of the EGF receptor, Oncogene 20 (2001) 5199–5209.
[105] H. Lee, R.W. Akita, M.X. Sliwkowski, N.J. Maihle, A naturally occurring secreted human ErbB3 receptor isoform inhibits heregulin-stimulated activation of ErbB2, ErbB3, and ErbB4, Cancer Res. 61 (2001) 4467–4473.
[106] A. Citri, I. Alroy, S. Lavi, C. Rubin, W. Xu, N. Grammatikakis, C. Patterson, L. Neckers, D.W. Fry, Y. Yarden, Drug-induced ubiquitylation and degradation of ErbB receptor tyrosine kinases: implications for cancer therapy, EMBO. J. 21 (2002) 2407–2417.
[107] W. Xu, M. Marcu, X. Yuan, E. Mimnaugh, C. Patterson, L. Neckers, Chaperone-dependent E3 ubiquitin ligase CHIP mediates a degradative pathway for c-ErbB2/Neu, Proc. Natl. Acad. Sci. USA 99 (2002) 12847–12852.
[108] A. Rosenthal, P.B. Lindquist, T.S. Bringman, D.V. Goeddel, R. Derynck, Expression in rat fibroblasts of a human transforming growth factor-alpha cDNA results in transformation, Cell 46 (1986) 301–309.

[109] D.S. Salomon, R. Brandt, F. Ciardiello, N. Normanno, Epidermal growth factor-related peptides and their receptors in human malignancies, Crit. Rev. Oncol. Hematol. 19 (1995) 183–232.

[110] M. Alimandi, A. Romano, M.C. Curia, R. Muraro, P. Fedi, S.A. Aaronson, P.P. Di Fiore, M.H. Kraus, Cooperative signaling of ErbB-3 and ErbB-2 in neoplastic transformation of human mammary carcinoma cells, Oncogene 15 (1995) 1813–1821.

[111] F. Lonardo, E. Di Marco, C.R. King, J.H. Pierce, O. Segatto, S.A. Di Aaronson, P.P. Fiore, The normal *erb*B-2 product is an atypical receptor-like tyrosine kinase with constitutive activity in the absence of ligand, New Biol. 2 (1995) 992–1003.

[112] Y. Yarden, Agonistic antibodies stimulate the kinase encoded by the neu protooncogene in living cells but the oncogenic mutant is constitutively active, Proc. Natl. Acad. Sci. USA 87 (1990) 2569–2573.

[113] D.J. Slamon, G.M. Clark, S.G. Wong, W.J. Levin, A. Ullrich, W.L. McGuire, Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene, Science 235 (1987) 177–182.

[114] D.J. Slamon, W. Godolphin, L.A. Jones, J.A. Holt, S.G. Wong, D.E. Keith, W.J. Levin, S.G. Stuart, J. Udove, A. Ullrich, M.F. Press, Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer, Science 244 (1989) 707–712.

[115] D.C. Allred, G.M. Clark, R. Molina, A.K. Tandon, S.J. Schnitt, K.W. Gilchrist, C.K. Osborne, D.C. Tormey, W.L. McGuire, Overexpression of HER-2/Neu and its relationship with other prognostic factors change during the progression of *in situ* to invasive breast cancer, Hum. Pathol. 23 (1992) 974–979.

[116] W.J. Gullick, R. Srinivasan, The type 1 growth factor receptor family: new ligands and receptors and their role in breast cancer, Breast Cancer Res. Treat. 52 (1998) 43–53.

[117] N.E. Hynes, D.F. Stern, The biology of erbB-2/neu/HER-2 and its role in cancer, Biochem. Biophys. Acta 1198 (1994) 165–184.

[118] L.N. Klapper, M.H. Kirschbaum, M. Sela, Y. Yarden, Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors, Adv. Cancer Res. 77 (2000) 25–79.

[119] Z. Suo, B. Risberg, M.G. Kalsson, K. Willman, A. Tierens, E. Skovlund, J.M. Nesland, EGFR family expression in breast carcinomas. c-erbB-2 and c-erbB-4 receptors have different effects on survival, J. Pathol. 196 (2002) 17–25.

[120] R.J. Gilbertson, R.H. Perry, P.J. Kelly, A.D. Pearson, J. Lunec, Prognostic significance of HER2 and HER4 coexpression in childhood medulloblastoma, Cancer Res. 57 (1997) 3272–3280.

[121] B. Bodey, B. Bodey Jr., A.M. Groger, J.V. Luck, S.E. Siegel, C.R. Taylor, H.E. Kaiser, Clinical and prognostic significance of the expression of the c-erbB-2 and c-erbB-3 oncoproteins in primary and metastatic malignant melanomas and breast carcinomas, Anticancer Res. 17 (1997) 1319–1330.

[122] N.R. Lemoine, D.M. Barnes, D.P. Hollywood, C.M. Hughes, P. Smith, E. Dublin, S.A. Prigent, W.J. Gullick, H.C. Hurst, Expression of the ERBB3 gene product in breast cancer, Br. J. Cancer 66 (1992) 1116–1121.

[123] C.A. Maurer, H. Friess, B. Kretschmann, A. Zimmermann, A. Stauffer, H.U. Baer, M. Kore, M.W. Buchler, Increased expression of erbB3 in colorectal cancer is associated with concomitant increase in the level of erbB2, Hum. Pathol. 29 (1998) 771–777.

[124] W. Xia, Y.K. Lau, H.Z. Zhang, F.Y. Xiao, D.A. Johnston, A.R. Liu, L. Li, R.L. Katz, M.C. Hung, Combination of EGFR, HER-2/neu, and HER- 3 is a stronger predictor for the outcome of oral squamous cell carcinoma than any individual family members, Clin. Cancer Res. 5 (1999) 4164–4174.

[125] E.S. Yi, D. Harclerode, M. Gondo, M. Stephenson, R.W. Brown, M. Younes, P.T. Cagle, High c-erbB-3 protein expression is associated with shorter survival in advanced non-small cell lung carcinomas, Mod. Pathol. 10 (1997) 142–148.

[126] B.J. Simpson, J. Weatherill, E.P. Miller, A.M. Lessells, S.P. Langdon, W.R. Miller, c-erbB-3 protein expression in ovarian tumours, Br. J. Cancer 71 (1995) 758–762.

[127] Y. Ito, T. Takeda, M. Sakon, M. Tsujimoto, S. Higashiyama, K. Noda, E. Miyoshi, M. Monden, N. Matsuura, Expression and clinical significance of erb-B receptor family in hepatocellular carcinoma, Br. J. Cancer 84 (2001) 1377–1383.

[128] N.H. Chow, S.H. Chan, T.S. Tzai, C.L. Ho, H.S. Liu, Expression profiles of ErbB family receptors and prognosis in primary transitional cell carcinoma of the urinary bladder, Clin. Cancer Res. 7 (2001) 1957–1962.

[129] R. Derynck, D.V. Goeddel, A. Ullrich, J.U. Gutterman, R.D. Williams, T.S. Bringman, W.H. Berger, Synthesis of messenger RNAs for transforming growth factors alpha and beta and the epidermal-growth-factor-receptor by human tumors, Cancer Res. 47 (1987) 707–712.

[130] A. deFazio, Y.E. Chiew, R.L. Sini, P.W. Janes, R.L. Sutherland, Expression of c-erbB receptors, heregulin and oestrogen receptor in human breast cell lines, Int. J. Cancer 87 (2000) 487–498.

[131] H. Friess, P. Berberat, M. Schilling, J. Kunz, M. Kore, M.W. Buchler, Pancreatic cancer: the potential clinical relevance of alterations in growth factors and their receptors, J. Mol. Med. 74 (1996) 35–42.

[132] G. Carpenter, Employment of the epidermal growth factor receptor in growth factor-independent signaling pathways, J. Cell Biol. 146 (1999) 697–702.

[133] R. Schweitzer, R. Howes, R. Smith, B.-Z. Shilo, M. Freeman, Inhibition of Drosophila EGF receptor activation by the secreted Argos protein, Nature 376 (1995) 699–702.

[134] P. Nagy, G. Vereb, Z. Sebestyen, G. Horvath, S.J. Lockett, S. Damjanovich, J.W. Park, T.M. Jovin, J. Szollosi, Lipid rafts and the local density of ErbB proteins influence the biological role of homo- and heteroassociations of ErbB2, J. Cell Sci. 115 (2002) 4251–4262.

[135] E. Tzahar, J.D. Moyer, H. Waterman, E.G. Barbacci, J. Bao, G. Levkowitz, M. Shelly, S. Strano, R. Pinkas-Kramarski, J.H. Pierce, G.C. Andrews, Y. Yarden, Pathogenic poxviruses reveal viral strategies to exploit the ErbB signaling network, EMBO J. 17 (1998) 5948–5963.