ErbB-4: mechanism of action and biology

Graham Carpenter*

Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232-0146, USA

Received 9 December 2002, revised version received 17 December 2002

Abstract

The most recently described member of the ErbB receptor tyrosine kinase family is ErbB-4. In general, the structure of this receptor and its mechanism of action is similar to that described for ErbB-1. However, significantly less is known about ErbB-4 and there are several novel aspects to its structure, mechanism of action, and biology. This includes the spectrum of ligands that activate ErbB-4, the presence of functionally distinct isoforms, a proteolytic processing pathway to the nucleus, and the capacity to induce a spectrum of cellular responses such as mitogenesis, differentiation, growth inhibition, and survival.

© 2003 Elsevier Science (USA). All rights reserved.

Introduction: receptor identification, structure, isoforms, and gene localization

Employing a strategy of homology cloning, ErbB-4 was cloned from a human mammary carcinoma cell line and its cDNA sequence determined [1]. That data showed ErbB-4 to be related in sequence to other ErbB receptors and to be organized in a similar fashion (Fig. 1). A single transmembrane domain separates equal sized ecto- and cytoplasmic domains. Within the ectodomain is a cleaved signal sequence and two cysteine-rich regions (domains II and IV), typical of ErbB receptors; nearly all of the 50 ectodomain cysteine residues of ErbB-1 are conserved in ErbB-4. By analogy with ErbB-1, it seems likely that domain III mediates growth factor binding. Between domain IV and the transmembrane domain, ErbB-4 has a comparatively longer stalk region, which may make it uniquely sensitive, within this receptor family, to ectodomain cleavage (see Receptor Trafficking, below).

The cytoplasmic domain contains a juxtamembrane region, a tyrosine kinase domain, and a carboxyterminal domain—all typical of ErbB receptors. ErbB-4 tyrosine phosphorylation sites have not been mapped, but within other receptors in this family these sites are located in the carboxy-terminal domain. The tyrosine autophosphorylation residues in ErbB-1 that have been identified are all conserved in ErbB-4. While the ErbB-3 kinase domain has mutations that significantly attenuate its kinase activity, these changes are not present in the ErbB-4 kinase domain. ErbB-4 is an active kinase in the absence of a coreceptor, such as ErbB-2, which is required for activation of ErbB-3 as a signaling component.

An interesting feature of the ErbB-4 sequence is a carboxy-terminal sequence TVV, which constitutes a PDZ domain recognition motif. ErbB-2 is reported to contain an internal motif that recognizes PDZ domains. The function of this ErbB-4 motif in receptor proteolytic processing and association with PDZ domain-containing proteins is described below.

The human gene for ErbB-4 has localized to chromosome 2 in the q33.3–34 region [2]. Hence, each member of the ErbB receptor family is located on a different chromosome.

An interesting feature of the ErbB-4 receptor is the presence of isoforms, generated by mRNA splicing, that suggest functional differences. One isoform contains an altered sequence within the ErbB-4 receptor stalk region [3]. The originally cloned ErbB-4 isoform is designated Jm-a and the altered isoform is designated Jm-b. The different sequences of Jm-a and Jm-b in the stalk region are depicted in Fig. 1. The changes do not affect ligand binding or...
tyrosine kinase activity, but do influence the sensitivity of the ErbB-4 ectodomain to shedding from the cell surface.

A second isoform contains sequence changes within the ErbB-4 cytoplasmic domain and influences a receptor docking site for phosphatidylinositol-3 kinase (PI-3 kinase) [4]. The originally described ErbB-4 sequence [1] has a binding site for this signal transducer; however, this site is deleted in an isoform designated CYT-2. The presence of the PI-3 kinase site is referred to as the CYT-1 isoform. These changes are depicted in Fig. 1.

The occurrence of these ecto- and cytoplasmic domain changes suggests that potentially four isoforms of ErbB-4 may exist. However, it has not been formally shown that an ErbB-4 receptor exists that contains the Jm-b ectodomain sequence together with the CYT-2 cytoplasmic domain sequence. Additional information on these isoforms has been reviewed elsewhere [5] and is described below.

**Receptor ligands**

Ligands, which bind to ErbB-4 with high affinity and specificity and which provoke receptor activation and signaling, are divided into two groups, i.e., the neuregulins, also termed heregulins, and certain members of the epidermal growth factor (EGF) family of ErbB-1 ligands (Fig. 2). For consistency, the term neuregulin is used exclusively in this review. There are four neuregulin genes, denoted 1, 2, 3, and 4, and the product of each is capable of recognizing ErbB-4 in a biologically productive manner. Neuregulins 1 and 2 also exist in a number of splicing isoforms, but it is not clear how each isoform associates with ErbB-4. Evidence of the capacity of each neuregulin gene product to activate ErbB-4 has been published for neuregulin-1 [6–8], neuregulin-2 [9,10], neuregulin-3 [11], and neuregulin-4 [12]. Among these, neuregulins 1 and 2 also recognize ErbB-3 as a high affinity binding site, but neuregulins 3 and 4 are reported to interact only with ErbB-4. None of the neuregulins recognize ErbB-1 or ErbB-2 directly and with high affinity. A direct comparison of the capacity of various neuregulins to activate ErbB-4 confirmed that neuregulins 3 and 4 activate ErbB-4, but not ErbB-3 [13]. This study also showed that neuregulin-2α failed to activate ErbB-4, while neuregulin-2β was a potent activator. It does appear, however, that neuregulins 3 and 4 have a lower potency for ErbB-4 activation compared to neuregulins 1β and 2β.

There are seven different gene products that are known to act as high affinity ligands for ErbB-1 and a subset of these are also agonists for ErbB-4. These include betacellulin [14], heparin binding-EGF (HB-EGF) [15], and epi-regulin [16–18]. Other ErbB-1 agonists (EGF, transforming...
growth factor-α, and amphiregulin) have been tested for ErbB-4 interaction with none detected. The newest ErbB-1 agonist, epigen, has not yet been tested for ErbB-4 interaction.

There are three additional cloned growth factors that are reported to activate ErbB-4 autophosphorylation. These are designated Don-1 [19], tomoregulin [20], and NTAK [21]. Their potencies seem relatively low and they remain less well characterized as ErbB-4 agonists. Don-1 and NTAK seem to be related to the neuregulin family, while tomoregulin is closer to the EGF family of ligands. Finally, decorin, an extracellular proteoglycan, is reported to associate with ErbB-4 and provoke its activation [22].

The existence of multiple ligands for ErbB-4 raises the issue of whether these ligands provoke different biological responses or the utilization of different signal transduction pathways. In regard to the latter point, one study has reported overlapping but distinct patterns of ErbB-4 autophosphorylation following the addition of different agonists [23]. Since relatively little is known about downstream signaling following the addition of different agonists, it is unclear if the observed differences translate to changes in signaling specificity. Also, comparative studies indicate that biological responses, such as cell survival, are not promoted equally by the various ErbB-4 ligands—at least in the cell assays employed. Last, the presence or absence of ErbB-2 can influence these outcomes significantly and has not been surveyed in a rigorous manner for all ErbB-4 agonists.

Receptor activation and signaling

Following ligand binding, the ErbB-4 receptor is activated by a process common to other ErbB receptors, i.e., dimerization and autophosphorylation (Fig. 2). Initial studies showed that, unlike ErbB-3, ligand binding provoked autophosphorylation of ErbB-4 in a cellular environment devoid of other ErbB receptors [1,6–12]. In this regard ErbB-4 is analogous to ErbB-1. However, numerous studies have reported that heterodimerization of ErbB-4 with ErbB-2 forms a higher affinity binding site, enhances the level of autophosphorylation, and can significantly modify the biological response to ErbB-4 ligands [13,17,18,24–32,147,148]. In contrast, two studies have reported that ErbB-2 does not modify the activation of ErbB-4 by growth factors [33,34].

The mechanism of ErbB-4 homodimerization or heterodimerization has not been studied at the structural level. It has been reported that the isolated ErbB-4 ectodomain homodimerizes in the presence of its ligand [31]. This report also demonstrated the ligand-induced heterodimerization of ErbB-4 and ErbB-2 isolated ectodomains. Another study has described the capacity of isolated ErbB-4 transmembrane domain to dimerize [35].

Activation of a receptor tyrosine kinase initiates biochemical signals that initiate growth responses. The initial step in these signaling events is receptor association with cellular proteins, which in some cases results in the tyrosine phosphorylation of these proteins. To date a few of these receptor proximal signaling molecules have been identified, i.e., Shc, the p85 subunit of PI-3 kinase, GrbB2, GrbB7 JAK, and STAT [4,15,23,27,28,36–40,145,146]. ErbB-4 interaction with Shc and GrbB2 would predict activation of Ras and mitogenic signals, while the p85 subunit of PI-3 kinase would lead to elevated levels of phosphatidylinositol-3,4,5-trisphosphate and the activation of cell survival pathways. Activation of the JAK/STAT pathway would lead to changes in gene expression. The role of GrbB7 in downstream signal pathways and cellular responses is not clear, though it does associate with several activated receptor tyrosine kinases. To date no signaling molecule or pathway has been identified as novel to ErbB-4 activation.

Receptor trafficking

While ErbB-1 and most growth factor receptor tyrosine kinases are rapidly internalized through clathrin-coated pits following ligand binding, ErbB-4 is internalized very slowly after addition of its ligand [41]. That some ligand: ErbB-4 complexes are, in fact, sorted to lysosomes is indicated by the appearance of low molecular weight degradation products of the ligand [42]. ErbB-4 internalization is sufficiently slow that mechanisms other than the clathrin-coated pit need to be considered.

The failure of activated ErbB-4 to rapidly enter the endocytic pathway, considered to be a desensitization mechanism, led to the investigation of alternative desensitization mechanisms. These studies demonstrated that the ErbB-4 ectodomain was shed as a fragment of 120 kDa from the cell surface [43] and that this shedding can be stimulated by 12-0-tetradecanoylphorbol-13-acetate (TPA) [44] or by neuregulin [45] (Fig. 3). The shedding activity was identified as a metalloprotease on the basis of inhibitor sensitivity [43] and subsequently shown to be attributable to TACE (tumor necrosis factor-alpha converting enzyme) [46], a member of the transmembrane ADAM metallopeptase family also designated ADAM17. Whether TACE actually executes this cleavage event is not clear, but it is required for the ectodomain shedding.

Cell surface shedding activity releases the ErbB-4 ectodomain fragment into the media and leaves an 80-kDa fragment, representing the transmembrane and cytoplasmic domains, associated with the cell [44]. The potential function of the ectodomain fragment is unclear, but the 80-kDa fragment is an active tyrosine kinase at least in vitro [43].

As previously noted, ectodomain cleavage is stimulatable by TPA or neuregulin, but it should be mentioned that in the absence of these agents there is a basal level of cleavage in most all cells [43]. Control of this basal cleavage is not understood mechanistically. However, the TPA and heregulin mechanisms seem distinct in the following
ways [45]. TPA-dependent cleavage is blocked by an inhibitor of protein kinase C, but heregulin-mediated cleavage is not. Also, neuregulin cleavage is associated with ligand-dependent translocation to a detergent-insoluble fraction (see below) and receptor internalization, but TPA cleavage is not (Fig. 3). It seems plausible to suggest that TPA may mediate activation of TACE and thereby provoke cleavage of a number of cell surface substrates, while neuregulin mediates the colocalization of ErbB-4 and TACE in a detergent-insoluble fraction or internalized compartment and thereby stimulates cleavage.

Further examination has shown that the 80-kDa ErbB-4 fragment is processed by a second membrane-localized protease activity, γ-secretase, which typically cleaves a transmembrane protein within the transmembrane domain [47,48] (Fig. 3). This activity liberates the ErbB-4 cytoplasmic domain from the plasma membrane and into the cytosol. Subsequently this fragment translocates to the nucleus, but its function in the nucleus is not known. The means by which the 80-kDa fragment is recognized by γ-secretase is unclear, but may require the PDZ domain recognition sequence located its carboxy-terminus [49]. This recognition sequence has been shown to facilitate a constitutive interaction of ErbB-4 with the multi-PDZ domain containing protein PSD-95 [50–52], which may enhance clustering ErbB-4 molecules [51,52] and/or facilitate cleavage by γ-secretase [49].

That this proteolytic processing of ErbB-4 also constitutes a signaling pathway is indicated by the fact that γ-secretase inhibition prevents the capacity of an ErbB-4 agonist to provoke cell death under serum-free conditions [47,49]. Also, the nuclear localization of ErbB-4 has been noted, using immunohistochemistry, in certain tissues [53–55].

Two studies have described the association of ErbB-4 with detergent-resistant membrane microdomains and the influence of the cognate growth factor on this localization. In cardiac myocytes the majority of ErbB-4 is present in caveolin-enriched microdomains, most likely caveolae, and association of ErbB-4 and caveolin-3 was detected by cosedimentation [56]. Addition of neuregulin to myocytes promoted the rapid exit of some ErbB-4 from this specialized microdomain to the general (detergent-soluble) plasma membrane faction. Interestingly, ErbB-2 was also localized to the caveolin microdomain, but did not change location following the addition of neuregulin.

In a mammary carcinoma cell line, the addition of neuregulin promoted the migration of ErbB-4 [45] and ErbB-2 [57], but not ErbB-3, into a detergent-resistant membrane microdomain. Within the context of this microdomain both ErbB-4 and ErbB-2 were hypertyrosine phosphorylated. While the nature of this detergent-resistant fraction is unclear, it most likely resembles a lipid raft and may be analogous to microdomains found in cells that do not express caveolin and hence do not exhibit caveolae. The significance of these microdomain structures for ErbB-4 trafficking or signaling remains to be defined.

Other reports have indicated that ErbB-4 may associate with other cell surface molecules that could influence its trafficking or signaling properties. These include MUC1, a
large heavily glycosylated transmembrane molecule that associates with all four ErbB receptors [58], and CD44 [59], a transmembrane proteoglycan that has a role in cell adhesion to the extracellular matrix.

The above data suggest that ErbB-4 may be found in association with several other transmembrane proteins depending on cell type and the presence or absence of cognate growth factors. In many cases the quantitative aspects of these associations as well as their biological significance is unclear. Recently, a cytosolic protein termed Nrdp1 (neuregulin receptor degradation protein-1) was isolated on the basis of its interaction with ErbB-3 [60]. When the protein was overexpressed, the cellular levels of ErbB-3 and ErbB-4, but not ErbB-1 or -2, were reduced. A subsequent study has shown that Nrdp1 is a ubiquitin ligase [61]. Interestingly, the 80-kDa fragment produced from ErbB-4 by metalloprotease activity is ubiquitinated and proteosome inhibitors elevate the level of this fragment in cells [43].

**Growth responses in experimental systems**

Numerous articles have reported the influence of neuregulin/nergulin on the growth of cell lines that endogenously express ErbB-4. However, in nearly all of these cell lines ErbB-3 is also expressed and hence it is difficult to discern whether the cell response is mediated by ErbB-3 or ErbB-4 or both. For this reason these articles are not reviewed herein. Investigators have employed a few cell systems to experimentally evaluate growth responses mediated directly by ErbB-4 and in some cases by its coexpression with ErbB-2.

3T3 cell lines that do not normally express ErbB receptors have been used extensively as recipient cells to evaluate growth effects mediated by transfected ErbB receptors. In particular, this cell system is used to evaluate mitogenic responses and transforming activity. Expression of ErbB-4 in these cells has established that growth factor activation of ErbB-4 in the absence of other ErbB receptors provokes a significant increase in cell proliferation [15,26,62] and that either the CYT-1 or the CYT-2 isoforms of ErbB-4 can mediate this activity [63]. Hence, this response seems independent of ErbB-4 association with ErbB-2 or its capacity to activate (CYT-1) or not activate (CYT-2) PI-3 kinase. It is reported that while neuregulin activation of ErbB-4 increases cell proliferation, activation of ErbB-4 by HB-EGF does not [15]. Also, this study demonstrated that activation of ErbB-4 by either HB-EGF or neuregulin stimulated chemotaxis. This response was dependent on PI-3 kinase activation, as it was provoked by the CYT-1 ErbB-4 isoform, but not the CYT-2 isoform [63]. When 3T3 cells are starved for serum, cell death occurs slowly. Expression of the CYT-1 ErbB-4 isoform, but not the CYT-2 isoform, together with neuregulin, was able to prevent cell death in a cell survival response [63]. Hence, these studies demonstrate that ErbB-4 signaling for chemotaxis and cell survival requires activation of PI-3 kinase.

Various experimental parameters of the transformation process are measurable in 3T3 cells. The most common assay with ErbB receptors is the focus forming assay—a measure of low density growth capacity. When ErbB-4 was expressed by itself, the data show no [26] or moderate [32,64] focus-forming activity in the presence of exogenous neuregulin. This cellular response to ErbB-4 activation was increased when ErbB-2 was coexpressed [26,32]. Similar results were obtained when colony formation in soft agar, a measure of anchorage-independent growth, was employed [65]. Expression of ErbB-4 by itself did not mediate colony formation in the presence of neuregulin; however, coexpression of ErbB-2 and ErbB-4 did provoke colony forming activity that was dependent on the presence of neuregulin. This is consistent with another report in which a constitutively active mutant of ErbB-4, dimerized by mutagenesis to Cys in the ectodomain, was unable to form colonies in soft agar, while a similar ErbB-2 mutant did form colonies [66]. Some investigators have evaluated ErbB receptor growth stimulating capacity by transfection of the receptors into the hematopoietic cell lines 32D (myeloid) or BaF3 (lymphoblastoid). These cells do not exogenously express any ErbB family members and require interleukin-3 (IL-3) for survival and proliferation. In this system one can measure cell survival or proliferation in the absence of IL-3. These studies [14,17,18,24,27,28,67] agree that by itself ErbB-4 has a weak capacity to support cell survival in the presence of its cognate ligand. However, when coexpressed with ErbB-2, most studies show that ErbB-4 more effectively mediates cell survival in a manner that is dependent on the presence of an ErbB-4 ligand. The picture that emerges is that ErbB-4 can mediate a proliferative signal, but its potential to do so is significantly enhanced by the presence of ErbB-2.

There is evidence based on other systems that ErbB-4 can provoke cell differentiation responses. In neuronal PC12 cells the expression and activation of ErbB-4 increases neurite extension and induces the expression of GAP-43, a neuronal differentiation marker [68]. In this cell system ErbB-4 is added by transfection and the cells endogenously express the other three ErbB receptors. However, neuregulin only induces differentiation in the cells that express ErbB-4. Neuregulin activation of ErbB-4 also protects these cells from apoptotic stimuli by a mechanism that requires activation of PI-3 kinase and cell survival pathways [69,70]. Studies of breast cancer cell lines have shown that ErbB-4 is necessary to mediate antiproliferative and differentiation responses provoked by neuregulin [71]. In these cells, suppression of ErbB-2 expression did not alter the ErbB-4-provoked response, indicating that homodimers of ErbB-4 were sufficient for these nonmitogenic responses.

**Roles in normal and tumor tissues**

A survey of ErbB receptor expression in a large number of adult and fetal human tissues showed that ErbB-4 is
ubiquitously expressed [72]. Expression is highest in brain and heart, but significant levels are present in the epithelia of skin, gastrointestinal, urinary, reproductive, and respiratory tracts, along with skeletal muscle, circulatory, endocrine, and nervous systems. Also, a variety of human cancers express ErbB-4, although squamous carcinomas seem relatively devoid of this receptor. The authors concluded with the general impression that ErbB-4 expression in normal and tumor tissue is skewed toward the differentiated compartments. If so, this view is consistent with the observation that endogenous ErbB-4 expression in cell lines is relatively uncommon. In the following sections, the expression and function, where addressed, are reviewed on a site-by-site basis.

Mammary tissue

Three studies have examined ErbB-4 expression in normal mammary tissue by western blotting [73] or immunohistochemistry [72,74]. The studies argue that ErbB-4 is highest during pregnancy and occurs primarily in the ductal epithelium, especially at the terminal ducts or end buds. Expression at lower levels is detected in nulliparous animals and during lactation and involution. Using polymerase chain reaction analysis, expression of mRNA for both the CYT-1 and CYT-2 isoforms of ErbB-4 have been identified in normal mammary tissue [75].

To assess the function of ErbB-4 in normal mammary development, a dominant-negative construct of ErbB-4 was expressed in the breast as a transgene under control of the MMTV promoter [40]. Mammary gland development was affected at mid-lactation when lobuloalveoli became condensed and deficient in lactation products—acid whey protein and α-lactalbumin. This result points to a critical role of ErbB-4 in the late differentiation of mammary gland function and are consistent with cell culture data showing a critical role for ErbB-4 in mammary cell differentiation [71].

Several groups have evaluated the expression of ErbB-4 in mammary carcinoma, particularly in view of the known high expression of ErbB-1 and ErbB-2 in this cancer [54,76–83]. These studies agree that ErbB-4 is not frequently overexpressed in breast carcinoma, as are ErbB-1 and ErbB-2, but is found at moderate to low levels. While ErbB-1 and ErbB-2 expression is correlated with tumors that are estrogen receptor negative and have a poor prognosis, the opposite is true for ErbB-4. Its expression correlates with the presence of estrogen receptors, a more differentiated tumor grade, and a more favorable prognosis. Interestingly, a significant percentage of breast tumors that express ErbB-4 demonstrate nuclear localization [54], perhaps as a result of ErbB-4 proteolysis and trafficking (see above). Also, both the CYT-1 and CYT-2 mRNA isoforms of ErbB-4 are found in mammary tumor specimens, as in normal mammary tissue [75], ErbB-4 is frequently expressed in Paget’s disease of the breast, which involves migration of neoplastic cells into the nipple [149]. It is suggested that neuregulin activation of ErbB-3 or ErbB-4 may mediate this migratory activity.

Other tumors

ErbB-4 expression has been noted in several other tumors in addition to mammary carcinoma [53,79,84–90]. These include carcinomas of the colon, prostate, lung, ovary, pancreas, endometrium, bronchus, cervix, stomach, and thyroid. Also, some astrocytomas and soft tissue sarcomas are reported to express ErbB-4. Expression of all known ErbB-4 isoforms (Jm-a, Jm-b, CYT-1, and CYT-2) have been tested in ovarian tumor specimens [90]. The Jm-a, CYT-1, and CYT-2 isoforms are expressed in nearly all samples, while the Jm-b isoform is not detected at significant levels.

Perhaps the most interesting and important tumors for ErbB-4 expression are found in pediatric brain tumors, i.e., medulloblastoma [91–93] and ependymoma [94]. In medulloblastomas, ErbB-4 is expressed in approximately 66% of the samples and is coexpressed with ErbB-2 in about 50% of the cases. These tumors also frequently express neuregulin. Patients with coexpression of ErbB-4 and ErbB-2 had the poorest prognosis compared to patients that expressed predominantly ErbB-4 or ErbB-2. Coexpression of ErbB-4, ErbB-2, and neuregulin had the greatest propensity to describe metastasis of the tumor. Also, Jm-a and Jm-b isoforms of ErbB-4 were detected in 53% and 28% of the tumor specimens.

In ependymoma, ErbB-4 expression is quite high, about 75% of the cases [94]. ErbB-2 expression is approximately 30%, while the expression of ErbB-1 or ErbB-3 is relatively low. Coexpression of ErbB-2 and ErbB-4 occurs in about 75% or more of these tumors and is correlated with a high proliferative index. Survival analysis indicates that patients who coexpress ErbB-4 and ErbB-2 had the poorest survival outcome. As with medulloblastomas, the Jm-a ErbB-4 isoform was frequently present while the Jm-b isoform was rarely detectable.

Heart development

The initial cloning studies of ErbB-4 also made clear that expression of this receptor was very high in the heart, skeletal muscle, and brain. Targeted disruption of the ErbB-4 gene in mice produces, in nullizygous animals, an embryonic lethal phenotype at approximately E10.5 [95]. In the embryonic heart, ErbB-4 is highly expressed in both the atrial and ventricular myocardium (muscle tissue), but is not detectable in the endocardium or epithelial lining. In the heart of ErbB-4 −/− embryos, muscle differentiation into trabeculae fails and this severely reduces blood flow. These
embryos also demonstrate impaired development in the central nervous system, which is discussed in the following section. Animals heterozygous for ErbB-4 are apparently normal.

The cardiac phenotype of ErbB-4−/−embryos is shared with that exhibited by embryos with targeted disruption of the ErbB-2 [96] or neuregulin-1 [97] genes. This indicates that during embryonic heart trabeculation, neuregulin must activate the ErbB-4/ErbB-2 heterodimer and that ErbB-4 homodimers are insufficient for this development sequelae.

Cardiac myocytes isolated from neonatal or adult animals express ErbB-4 and ErbB-2, but ErbB-3 is not detectable [98]. Hence, neuregulin biologic effects on these cells can be attributed to ErbB-4-dependent signaling. The stimulatory influence of neuregulin on the proliferation, hypertrophy, differentiation, and survival of these cells has been described [97,98,150].

**Nervous system**

ErbB-4 expression is widespread in various parts of the brain and nervous system [95,99–103], including the olfactory bulb [103–105] and retina [106]. This is reflected in the phenotype of knockout mice [95]. While the ErbB-4−/−mice die at embryonic day 10.5 due to abnormal development of the heart, axonal guidance is also impaired. In contrast to the cardiac defect, which is shared by mice nullizygous for ErbB-2 or neuregulin, misinervation of axons in the hindbrain of ErbB-4 mutant mice is not observed in ErbB-2 or neuregulin mutants [96,97]. Other studies of ErbB-4−/−embryos [107] or the expression of a dominant-negative ErbB-4 construct in primary cultures of neuronal cells [108] show additional ErbB-4 requirements for cell migration in the developing brain. The phenotypes observed in these studies include defects in pathfinding by cranial neural crest cells [107] and in the migration of cerebellar granule cells along radial glial fibers [108].

Cerebellar granule cells express significant levels of ErbB-4 and ErbB-2, but do not express ErbB-3 at detectable levels [109]. Hence, neuregulin influences on these cells are attributable to ErbB-4-dependent signaling. In granule cells neuregulin is reported to alter the subunit composition of the GABA and NMDA neurotransmitter receptors, which may alter the conductance properties of these receptors at synapses [109,110]. Also, ErbB-4 mediates increased expression of nitric oxide synthetase in these cells [111].

Several authors have noted expression of the ErbB-4 receptor at neuronal and neuromuscular synapses [50–52,112,113], where it is postulated to have a role in gene expression mediated by these junctions. However, the presence of ErbB-3 at these sites complicates this interpretation in terms of the exact role of ErbB-4.

Several groups have studied the influence of neuregulin on the proliferation, survival, and 2differentiation of cells in the oligodendrite lineage. The results are generally consistent in that neuregulin promotes proliferation and survival of oligodendrite precursors and decreases their differentiation into mature oligodendrites. In fact, neuregulin tends to enhance differentiation into other lineages. However, the data are difficult to interpret in terms of ErbB-4 as in some instances only ErbB-2 and ErbB-4 are expressed in the progenitors [114,115], while in other instances ErbB-3 is also present [116–118]. This could reflect the differing experimental conditions and reagents used.

Other studies have reported that ErbB-4 may be involved in neuronal plasticity [119] and that ErbB-4 expression is increased in neurons and microglia/macrophages at the site of closed head injury [120]. Last, a recent study of susceptibility to schizophrenia has identified neuregulin 1 as a candidate gene in humans [121]. This study also examined the behavioral phenotypes of mice heterozygous for neuregulin 1 or ErbB-4. The behavioral results are reported to overlap with that of mouse models for schizophrenia.

**Miscellaneous systems**

Understanding of the role of ErbB-4 in other tissues is only beginning, but given its widespread distribution in embryonic and adult tissues, functions in additional systems will not be surprising. These investigations include the role of ErbB-4 in hypothalamus and reproductive behavior [122], palatogenesis and its disorders [123], tooth development [124], chondrocyte biology [125], and pancreatic islet development [126,127]. Finally, two reports identify ErbB-4 on the outer surface of the blastocyst where it may have a role in implantation [151,152].

**Concluding remarks**

The author regrets that space limitation requires the omission of many studies indirectly related to the focus of this review and apologizes for the failure to cite any publication directly related to ErbB-4 function and biology. The interested reader is referred to other reviews for additional information and references. Many of these can be found in this issue of this journal. Other review articles and their focus are as follows: ErbB-4 [128], ErbB receptors [129–132], ErbB-4 isoforms [5], ErbB-4 in neuronal [133–136], or cardiac development [136,137], dimerization of ErbB receptors [138,139], neuregulin apoptosis [140], and ErbB receptors in mammary physiology and cancer [141–144].

**Acknowledgments**

The author appreciates the efforts of Sue Carpenter in manuscript preparation and Lori Bennett in preparation of figures. Support of National Cancer Institute grant CA97456 is appreciated.
References


C.-Y. Ni, H. Yuan, G. Carpenter, Role of the ErbB-4 carboxyter-


J.M. Mendrola, M.B. Berger, M.C. King, M.A. Lemmon, The single

K. Feroz, E. Williams, D.J. Riese II, ErbB2 and ErbB3 do not


M. Vecchi, J. Baulida, G. Carpenter, Selective cleavage of the

J. Baulida, M.H. Kraus, M. Alimandi, P.P. Di Fiore, G. Carpenter,

L. Puricelli, C.J. Proietti, L. Labriola, M. Salatino, M.E. Balata,

J.A. Ghiso, R. Lupu, O.P. Pigman, E.H. Charru, E.B. de Kier


J. Baulida, G. Carpenter, Heregulin degradation in the absence of

F. Jones, T. Welte, X.-Y. Fu, D.F. Stern, ErbB4 signaling in the

J. Baulida, G. Carpenter, Constitutive proteolysis of the ErbB-4

M. Vecchi, J. Baulida, G. Carpenter, Selective cleavage of the

M. Vecchi, G. Carpenter, Constitutive proteolysis of the ErbB-4

C.-Y. Ni, M.P. Murphy, T.E. Golde, G. Carpenter, γ-Secretase

H.-I. Lee, K.-M. Jung, Y.Z. Huang, L.B. Bennett, J.S. Lee, L. Mei,

C.-Y. Ni, H. Yuan, G. Carpenter, Role of the ErbB-4 carboxyter-

R.A.G. Garcia, K. Vasudevan, A. Buonanno, The neuregulin recep-

R.G. García, K. Vasudevan, A. Buonanno, The neuregulin recep-


Y.Z. Huang, Q. Wang, S. Won, Z.G. Luo, W.C. Xiong, L. Mei,

R.A.G. Garcia, K. Vasudevan, A. Buonanno, The neuregulin recep-

Y. Srivivasan, E. Benton, F. McCormick, H. Thomas, W.J. Gullick,

R. Srivivasan, C.E. Gillett, D.M. Barnes, W.J. Gullick, Nuclear

M. Zhang, D. Ding, R. Salvi, Expression of heregulin and ErbB/Her

Y.-Y. Zhao, O. Feron, C. Dessy, X. Han, M.A. Marchionni, R.A.

Zhou, W., Carpenter, G. Heregulin-dependent translocation and

J.A. Schroeder, M.C. Thompson, M.M. Gardner, S.J. Gendler,

Transgenic MUC1 interacts with epidermal growth factor receptor

W. Zhou, G. Carpenter, Heregulin-dependent activation of ovarian
cancer cells depends on the relative levels of HER-2 and HER-3 expression,

V. Kainulainen, M. Sundvall, J.A. Mååtå, E. Santiesteban, M.
Klagsbrun, K. Elenius, A natural ErbB4 isoform that does not activate phosphoinositide 3-kinase mediates proliferation but not survival or chemotaxis, J. Biol. Chem. 275 (2000) 8641–8649.


[99] T. Lindholm, S. Cullheim, M. Deckner, T. Carlstedt, M. Risling, Expression of neuregulin and ErbB3 and ErbB4 after a traumatic lesion in the ventral funiculus of the spinal cord and in the intact primary olfactory system, Exp. Brain Res. 142 (2002) 81–90.


[102] O. Bermingham-McDonogh, K.L. McCabe, T.A. Reh, Effects of GGF/neuregulins on neuronal survival and neurite outgrowth cor-


[125] R.J. Fiddes, D.H. Campbell, P.W. Jones, S.P. Sivertsen, H. Sasaki, C. Wallasch, R.J. Daly, Analysis of Grb7 recruitment by heregulin-


