The ErbB receptor tyrosine family as signal integrators

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Abstract

ErbB receptor tyrosine kinases (RTKs) and their ligands have important roles in normal development and in human cancer. Among the ErbB receptors only ErbB2 has no direct ligand; however, ErbB2 acts as a co-receptor for the other family members, promoting high affinity ligand binding and enhancement of ligand-induced biological responses. These characteristics demonstrate the central role of ErbB2 in the receptor family, which likely explains why it is involved in the development of many human malignancies, including breast cancer. ErbB RTKs also function as signal integrators, cross-regulating different classes of membrane receptors including receptors of the cytokine family. Cross-regulation of ErbB RTKs and cytokines receptors represents another mechanism for controlling and enhancing tumor cell proliferation.

Introduction

The epidermal growth factor (EGF) or ErbB family of type I receptor tyrosine kinases (RTKs) has four members: EGF receptor, also termed ErbB1/HER1, ErbB2/Neu/HER2, ErbB3/HER3 and ErbB4/HER4. We will refer to them, henceforth, as the ErbB receptors. All family members have in common an extracellular ligand-binding domain, a single membrane-spanning region and a cytoplasmic protein tyrosine kinase domain. A family of ligands, the EGF-related peptide growth factors, bind the extracellular domain of ErbB receptors leading to the formation of receptor homo- and heterodimers. Dimerization consequently stimulates the intrinsic tyrosine kinase activity of the receptors and triggers autophosphorylation/transphosphorylation of specific tyrosine residues within the cytoplasmic domain. These phosphorylated residues serve as docking sites for signaling molecules involved in the regulation of intracellular signaling cascades. Ultimately, downstream effects on gene expression determine the biological response to receptor activation.

ErbB receptors are expressed in a variety of tissues of epithelial, mesenchymal and neuronal origin where they play fundamental roles in development, proliferation and differentiation. Moreover, deregulated expression of ErbB receptors, in particular ErbB1 and ErbB2, has been implicated in the malignancy of numerous types of human tumors, including breast cancer.

The EGF-related peptide growth factors

ErbB receptors are activated by ligands, known as the EGF-related peptide growth factors (reviewed in Peles & Yarden 1993, Riese & Stern 1998). There are numerous ErbB-specific ligands, summarized in Fig. 1, each with an EGF-like domain which is sufficient to confer binding specificity. These include EGF, amphiregulin (AR) and transforming growth factor-α (TGFα), which bind specifically to ErbB1, and betacellulin (BTC), heparin-binding EGF (HB-EGF) and epi-regulin (EPR), which exhibit dual specificity in that they bind both ErbB1 and ErbB4. The neuregulins (NRG) comprise the third ligand family. NRG-1 and NRG-2 (Chang et al. 1997) both bind ErbB3 and ErbB4, whereas NRG-3 (Zhang et al. 1997) and NRG-4 (Harari et al. 1999) bind ErbB4 but not ErbB3. Despite the abundance of ligands identified for these three ErbB receptors, no direct ligand for ErbB2 has yet been discovered. However, increasing evidence suggests that the primary function of ErbB2 is as a co-receptor.

ErbB2 plays a central role in the ErbB family of receptors

Binding of the EGF-related peptide growth factors to their respective receptor induces receptor dimers and heterodimers.
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Heterodimerization is accomplished by the bivalent nature of the ligands (Groenen et al. 1994, Lemmon et al. 1997, Tzahar et al. 1997), which allows high affinity binding to a primary receptor and recruitment of the second partner. Importantly, the second partner is preferentially ErbB2 (Graus-Porta et al. 1997, Tzahar et al. 1997), which acts as a co-receptor increasing the affinity of ligand binding to the dimeric receptor complex (Karunagaran et al. 1996, Jones et al. 1999).

In order to explore the role of ErbB2 in more detail, we have down-regulated its function using an intracellularly expressed ErbB2-specific single chain antibody or Fv domain (scFv5R). Targeting of scFv5R to the endoplasmic reticulum causes retention of ErbB2 in this compartment leading to loss of receptor function (Beerli et al. 1994). This approach has yielded valuable information, revealing the central role of ErbB2 in this receptor family. Our results show that ErbB2 plays an important function in the potentiation of ligand-induced intracellular signaling (Beerli et al. 1995, Graus-Porta et al. 1995) and biological activity (Spencer et al. 2000), characteristics of ErbB2 which provide an explanation for its high oncogenic potential.

**ErbB signaling potential.** The subsets of phosphotyrosine-binding signaling molecules (referred to as SH2- and PTB-binding proteins) recruited to an activated receptor are defined by the pattern of phosphorylated tyrosine residues in the C-terminus of each receptor. We speculated that signal diversification arises at one level by differential transphosphorylation of a given receptor in a distinct ErbB dimer. In order to approach this experimentally, NIH3T3 fibroblasts expressing defined combinations of ErbB receptors have been developed and used to examine the ability of an individual ErbB receptor to couple to specific signaling molecules in the context of distinct receptor dimers. More specifically, ErbB1 signaling was examined after activation either by EGF in the context of ErbB1 homodimers or by NRG-1 in the context of a ErbB1/ErbB4 heterodimer (Fig. 2). In both cases ErbB1 coupled to the adaptor protein Shc, but only when activated by EGF was it able to interact with Grb2 and Cbl. Compared with the rapid internalization of EGF-activated ErbB1, NRG-1-activated ErbB1 showed delayed internalization characteristics (Olayioye et al. 1998). Tryptic phosphopeptide analyses clearly showed that there are ligand-dependent differences in the patterns of ErbB1 phosphorylation (Olayioye et al. 1998) which could provide a biochemical explanation for the observed differences in recruitment of Grb2 and Cbl. Interestingly, Cbl has recently been identified as a RING finger domain-containing E3 ubiquitin protein ligase which

**ErbB receptors acquire distinct signaling properties dependent upon their dimerization partner**

The ability of ErbB ligands to induce not only receptor homodimers but also heterodimers expands and diversifies ErbB signaling potential. The subsets of phosphotyrosine-binding signaling molecules (referred to as SH2- and PTB-binding proteins) recruited to an activated receptor are defined by the pattern of phosphorylated tyrosine residues in the C-terminus of each receptor. We speculated that signal diversification arises at one level by differential transphosphorylation of a given receptor in a distinct ErbB dimer. In order to approach this experimentally, NIH3T3 fibroblasts expressing defined combinations of ErbB receptors have been developed and used to examine the ability of an individual ErbB receptor to couple to specific signaling molecules in the context of distinct receptor dimers. More specifically, ErbB1 signaling was examined after activation either by EGF in the context of ErbB1 homodimers or by NRG-1 in the context of a ErbB1/ErbB4 heterodimer (Fig. 2). In both cases ErbB1 coupled to the adaptor protein Shc, but only when activated by EGF was it able to interact with Grb2 and Cbl. Compared with the rapid internalization of EGF-activated ErbB1, NRG-1-activated ErbB1 showed delayed internalization characteristics (Olayioye et al. 1998). Tryptic phosphopeptide analyses clearly showed that there are ligand-dependent differences in the patterns of ErbB1 phosphorylation (Olayioye et al. 1998) which could provide a biochemical explanation for the observed differences in recruitment of Grb2 and Cbl. Interestingly, Cbl has recently been identified as a RING finger domain-containing E3 ubiquitin protein ligase which
is required for ErbB1 ubiquitylation and targeting of the receptor to the lysosomal compartment (Levkowitz et al. 1999). Thus, the difference in internalization kinetics observed for EGF- compared with NRG-1-activated ErbB1 might be explained by Cbl binding to the former but not to the latter. Our results show that the coupling of a given receptor to specific intracellular signaling proteins is modulated by the dimerization partner and may indeed originate from differential receptor phosphorylation.

**Receptor heterodimers enhance and diversify the signal potential of the ErbB network**

The ErbB family has evolved from a single ligand/receptor combination in *Caenorhabditis elegans* (Aroian et al. 1990), through *Drosophila* with one receptor and four ligands (Wasserman & Freeman 1997), to vertebrates where four ErbB receptors bind multiple EGF-related ligands (reviewed in Stein & Staros 2000). Consequently, numerous ErbB homo- and heterodimer combinations are possible in vertebrates, suggesting that the ErbB receptor family has evolved to provide a high degree of signaling diversity, which may have been necessary for the evolutionary development of metazoans.

Phenotypes of ErbB receptor knock-out mice are the most striking proof of the power of receptor heterodimers. Mice individually null for ErbB2, ErbB4 and NRG-1 each demnstrate a lack of trabeculae formation in the heart (Gassmann et al. 1995, Lee et al. 1995, Meyer & Birchmeier 1995), showing that an important signaling moiety in the myocardium is the ErbB2/ErbB4 heterodimer stimulated in a paracrine fashion by NRG-1. The essential contribution of this receptor collaboration to heart development is clearly observed in ErbB2 null mice, where NRG-1-induced ErbB4 dimers cannot replace the function of the ErbB2/ErbB4 heterodimer.

Using fibroblasts expressing defined combinations of ErbB proteins, we have examined the ability of distinct receptor dimers to induce various signal transduction pathways (Olayioye et al. 1999). Importantly, as implied from the phenotype of the knock-out mice, our results showed directly that a heterodimer can acquire novel signaling properties that are not the sum of the activity of individual receptor dimers. Cells in which ErbB2 was activated by a dimerization inducing ErbB2 specific monoclonal antibodies (mAb) were compared with cells where ErbB4 dimers or ErbB2/ErbB4 heterodimers were activated by NRG-1. Each of these ErbB dimers induced elevated intracellular MAP kinase activity. In striking contrast, only the ErbB2/ErbB4 heterodimers stimulated activation of Stat5, a member of the signal transducer and activator of transcription (Stat) family (Fig. 3) (Olayioye et al. 1999). It will be interesting to explore the role of Stat5
An ErbB heterodimer is capable of acquiring novel signaling properties. ErbB2 homodimers were induced by mAb binding; ErbB2/ErbB4 heterodimers and ErbB4 homodimers were induced by NRG binding. All three ligand dimers activated the MAP kinase (MAPK) pathway; however, only ErbB2/ErbB4 heterodimers activate Stat5. P, phosphotyrosine.

downstream of the ErbB2/ErbB4 heterodimer considering the importance of this dimer in heart development.

The ErbB receptors and human cancer

Deregulated expression of ErbB receptors, in particular ErbB1 and ErbB2, has been implicated in the development and malignancy of numerous types of human cancers. The interested reader is referred to these reviews (Hynes & Stern 1994, Salomon et al. 1995, Tang & Lippman 1998). Overexpression of ErbB2 is observed in a significant proportion of breast and ovarian cancers, where it is associated with poor prognosis (Slamon et al. 1989). Overexpression of ErbB2 triggers ligand-independent activation of the kinase domain, apparently as a result of spontaneous dimer formation. Furthermore, many breast tumors display autocrine activation of ErbB1 due to expression of one or more ErbB1 ligands (Salomon et al. 1995). The ability of ErbB2 to potentiate ErbB1 signaling, as discussed above, very likely leads to an enhancement of the malignant process.

ErbB receptors as signal integrators

An emerging theme in the signal transduction area is cross-regulation of different classes of membrane receptors; ErbB RTKs are prime examples of signal integrators. This has been particularly well studied for the G-protein coupled receptors (GPCR), whose agonists stimulate MAP kinase via transactivation of ErbB1. For more detailed discussion of this cross-talk the interested reader is referred to some recent reviews (Carpenter 1999, Hackel et al. 1999, Luttrell et al. 1999). Cross-regulation between ErbB receptors and members of the cytokine receptor family has also been observed. Cytokine receptors are devoid of kinase activity and associate with members of the Janus tyrosine kinase (Jak) family to transduce signals. This cross-regulation results in either a positive or a negative influence on the target receptor. As an example of positive regulation, activation of the growth hormone (GH) receptor induces the MAP kinase pathway through ErbB1. More specifically, GH activates Jak2 which phosphorylates a tyrosine residue in the cytoplasmic domain of the ErbB1 leading to Grb2 association and stimulation of the MAP kinase pathway (Yamauchi et al. 1997).

Cross-regulation of ErbB receptors and cytokine receptors in mammary gland development and breast cancer

Receptor tyrosine kinases and cytokine receptors have also been shown to negatively regulate each other. If we consider the mammary gland specifically, there is a complex interaction between ErbB1 and prolactin (Prl) receptor (Fig. 4). On the one hand, Prl receptor activation has a negative
influence on ErbB1-induced signaling. In mammary cells continuously exposed to Prl, ErbB1 was found to be phosphorylated on specific threonine residues, a modification which had a negative effect upon its kinase activity leading to impaired EGF-induced biological responses (Quijane & Sheffield 1998). Conversely, it has been known for a number of years that EGF has a negative effect upon Prl receptor signaling (Hynes et al. 1990, Marte et al. 1995). Very recently, we have found that continuous exposure of mammary cells to EGF dampens the ability of the cells to

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**Figure 4** Cross regulation of ErbB1 and Prl receptor in mammary cells. Prl receptor activation stimulates a Ser/Thr (S/T) kinase which phosphorylates ErbB1 leading to down-regulation of its signaling potential (upper panel). ErbB1 activation induces expression of a PTP which inhibits Prl-induced Jak2 activation (lower panel). P, phosphotyrosine.
respond to Prl-induced signaling, due to EGF stimulation of a protein tyrosine phosphatase (PTP) which negatively regulates the Prl receptor-associated Jak2 kinase (K Horsch, M Schaller & N E Hynes, unpublished results).

It is interesting to consider the negative regulation mediated by Prl receptor on ErbB1 and vice versa in light of their roles in the development of the mammary gland. ErbB1 is expressed at all stages (Schroeder & Lee 1998, Darcy et al. 2000) and appears to have a particularly important role in proliferation of ductal epithelial cells since wa-2 mice, which harbor a mutation in the ErbB1 kinase domain, exhibit sparse ductal growth (Fowler et al. 1995). Prl receptor does not appear to have a major role in the proliferative phase, but plays an essential role in differentiation of the gland into a milk producing organ (Brisken et al. 1999). ErbB1 negative regulation of Prl signaling might reflect a general mechanism to prevent differentiation-inducing signals during the normal proliferative stage of development. Conversely, it is not surprising that ErbB1, a major growth promoter, is down-regulated during differentiation. Moreover, a loss of this mechanism might even play a role in tumor development.

ErbB2 and Prl receptor cross-regulation has also been observed. It was recently shown in an ErbB2 overexpressing breast tumor cell line that Prl-mediated activation of Jak2 led to an enhancement of ErbB2 tyrosine phosphorylation. Moreover, blocking autocrine production of Prl in these tumor cells led to a decrease in the phosphotyrosine content of ErbB2 and a decrease in MAP kinase activity (Yamauchi et al. 2000). Intriguingly, an examination of approximately 70 primary breast tumors revealed that the vast majority co-expressed Prl and its receptor, and of these the ErbB2 overexpressing sub-group had the highest proliferative activity (Yamauchi et al. 2000). Both Prl receptor (Brisken et al. 1999) and ErbB2 (Jones & Stern 1999) play important roles during normal development of the mammary gland. It will be interesting to examine whether they biologically synergize during breast tumor development.

**ErbB1 cooperates with interleukin-6 (IL-6) in breast cancer cells**

IL-6 is a member of the IL-6-type cytokine family which comprises oncostatin M, leukemia inhibitory factor, ciliary neurotropic factor and IL-11. These peptides promote similar biological responses in various tissues and cells. This redundancy in biological actions can be explained at the molecular level because the different members of this cytokine family share signaling molecules. Indeed, IL-6-type cytokines

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**Figure 5** Cooperation between IL-6 receptor and ErbB1 in T47D breast cancer cells. ErbB1 is activated to low levels due to autocrine stimulation, providing a scaffold for signaling molecules such as Grb2, Gab1 and Shp-2. Following IL-6 stimulation, Jak1 is activated leading to the recruitment and phosphorylation of Shp-2 and Gab1 which, in turn, recruit signaling molecules which feed into the MAP kinase (MAPK) pathway. P, phosphotyrosine.
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bind to multimeric receptors comprising an α chain which confers ligand specificity and a signal transducing β subunit, gp130, which is common to all IL-6-type cytokines (Heinrich et al. 1998, Hirano 1998). IL-6-type cytokines have diverse actions on breast cancer cells, including changes in morphology, decreased cell–cell association and inhibition of proliferation (Tamm et al. 1989, Douglas et al. 1997, Badache & Hynes 2001). IL-6 and other family members are expressed in many primary breast tumors (Crichton et al. 1996, Robinson et al. 1998, Fontanini et al. 1999). Intriguingly, IL-6 expression is reduced in invasive breast carcinoma relative to normal mammary tissue and its expression level appears to be inversely associated with histological grade (Basolo et al. 1996, Fontanini et al. 1999). Although little is known about IL-6-induced signal transduction in breast cancer, these observations suggest that this cytokine might be involved in growth regulation of cancer cells.

We have investigated IL-6-type cytokine-induced signaling in the T47D breast carcinoma cell line and have observed that these cytokines inhibit proliferation but increase cell migration (Badache & Hynes 2001). The two biological effects are mediated by independent pathways involving Stat3 activity for the former and MAP kinase activation for the latter. Furthermore, we have observed that IL-6-induced MAP kinase activation depends upon the intactness of an ErbB1 autocrine loop. The PTP Shp-2 and the multisubstrate docking molecule Gab1, which are constitutively associated with ErbB1 in T47D cells and recruited to the gp130 transducing subunit where they are tyrosine phosphorylated upon IL-6 treatment, appear to play pivotal roles in the mechanism by which the combined actions of IL-6 and ErbB1 autocrine activity promote a synergistic increase in MAP kinase activity (Fig. 5). Most breast tumors coexpress ErbB1 and one of its ligands, along with IL-6 signaling components. Our results suggest that in primary tumors, activation of both ErbB receptor tyrosine kinases and cytokine receptors might synergize to potentially activate intracellular signaling pathways. Moreover, our results imply that ErbB1, even when expressed at low levels, can play an important role in tumor cell biology and should therefore be considered as a potential therapeutic target.

Our view on the mechanism underlying the contribution of ErbB receptors to cancer development has expanded in recent years. We have known for some time that constitutive ErbB activation resulting either from autocrine ligand production or from overexpression contributes to uncontrolled intracellular signaling. It is now becoming clear that the ErbB RTKs also function as signal integrators, cross-regulating different classes of membrane receptors including those in the cytokine family. As we discuss here, cross-regulation also leads to enhanced tumor cell proliferation by mechanisms which are now starting to be understood.

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