Minireview

The ErbB signaling network in embryogenesis and oncogenesis: signal diversification through combinatorial ligand-receptor interactions

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Abstract Ligand-induced activation of receptor tyrosine kinases (RTK) results in the initiation of diverse cellular pathways, including proliferation, differentiation and cell migration. The ErbB family of RTKs represents a model for signal diversification through the formation of homo- and heterodimeric receptor complexes. Each dimeric receptor complex will initiate a distinct signaling pathway by recruiting a different set of Src homology 2- (SH2-) containing effector proteins. Further complexity is added due to the existence of an oncogenic receptor that enhances and stabilizes dimerization but has no ligand (ErbB-2), and a receptor that can recruit novel SH-2-containing proteins, but is itself devoid of kinase activity (ErbB-3). The resulting signaling network has important implications for embryonic development and malignant transformation.

Key words: Growth factor; Receptor; Tyrosine kinase; Signal transduction; ErbB

1. Introduction

The flow of information from the extracellular environment into the cell is at the core of a functional biological system. Receptor tyrosine kinases (RTK) are primary mediators of many of these signals and thus determine the fate of the cell: growth, differentiation, migration or death. RTKs are cell-surface allosteric enzymes consisting of a single transmembrane domain separating an intracellular kinase domain from an extracellular ligand-binding domain. Ligand binding induces receptor homo- or heterodimerization that is essential for activation of the tyrosine kinase and subsequent recruitment of target proteins, which initiate a complex signaling cascade [1,2]. The ErbB family of RTKs consists of four receptors: ErbB-1, (also called epidermal growth factor receptor (EGFR)), ErbB-2 (also called HER2 or Neu), ErbB-3 and ErbB-4. Due to extensive receptor–receptor interactions, the ErbB family constitutes a signaling network whose potential for diversification of biological messages is enormous (Fig. 1). Three layers of diversity generation may be distinguished. First, two groups of ligands, all sharing an EGF-like motif, exist. These are six direct ligands of ErbB-1 and two families of neuregulins (also called Neu differentiation factors, NDFs, or heregulins). Second, each of the many ligands has a different preference for stabilizing distinct receptor dimers, probably due to ligand bivalency. Third, because each receptor dimer has a different double set of tyrosine autophosphorylation sites, which serve as docking sites for specific SH2-containing proteins, each ligand-induced receptor dimer funnels its signal through a unique set of signaling pathways. Further complexity is added to this system by the casting of the family: namely, the existence of a receptor that enhances and stabilizes dimerization but apparently has no ligand (ErbB-2) [3] and a receptor that can recruit novel SH2-containing proteins, but by itself is devoid of kinase activity (ErbB-3) [4].

2. EGF-like ligands and their ErbB receptors

Six mammalian ligands that bind to ErbB-1 have been characterized, including epidermal growth factor (EGF), transforming growth factor-α (TGFα), amphiregulin, heparin-binding EGF-like growth factor, betacellulin (reviewed in [5]), and epiregulin [6]. The binding affinity of the EGF-like ligands to ErbB-1 differs as is their potency to induce signaling. In addition, each of the ErbB-1 ligands has a distinct expression pattern during development and in adult tissues, illustrating the many roles played by ErbB-1. The ligands for ErbB-3 and ErbB-4, neuregulins (NRG), are predominantly expressed in parenchymal organs and in the embryonic central and peripheral nervous systems [7,8]. The different NRG isoforms are the products of alternative splicing of a single gene. They are synthesized as large transmembrane precursors whose extracellular N-terminal domains contain a juxtamembrane EGF-like module and various other motifs, depending on the isoform. The most important region of the EGF-like and NRG ligands is probably the shared EGF-like domain, since it is sufficient for binding and receptor activation. This region is 45–55 amino acids long and includes six cysteine residues which interact covalently to form three loops.

The extracellular ligand binding domains of the ErbB proteins are relatively conserved among members of the family, despite the fact that the receptors bind different ligands. The cytoplasmic domains contain the tyrosine kinase catalytic sequences and C-terminal autophosphorylation sites which determine the down-stream effector molecules that will be recruited upon phosphorylation. The tyrosine kinase domains of ErbB proteins are highly conserved, except for the ErbB-3 tyrosine kinase domain that shows the least homology. These differences include residues that are critically conserved throughout the whole family of protein kinases, thus rendering ErbB-3 almost devoid of any kinase activity, although it is able to bind ATP [9].

3. Network function in embryonic development

Targeted inactivation of components of the ErbB signaling

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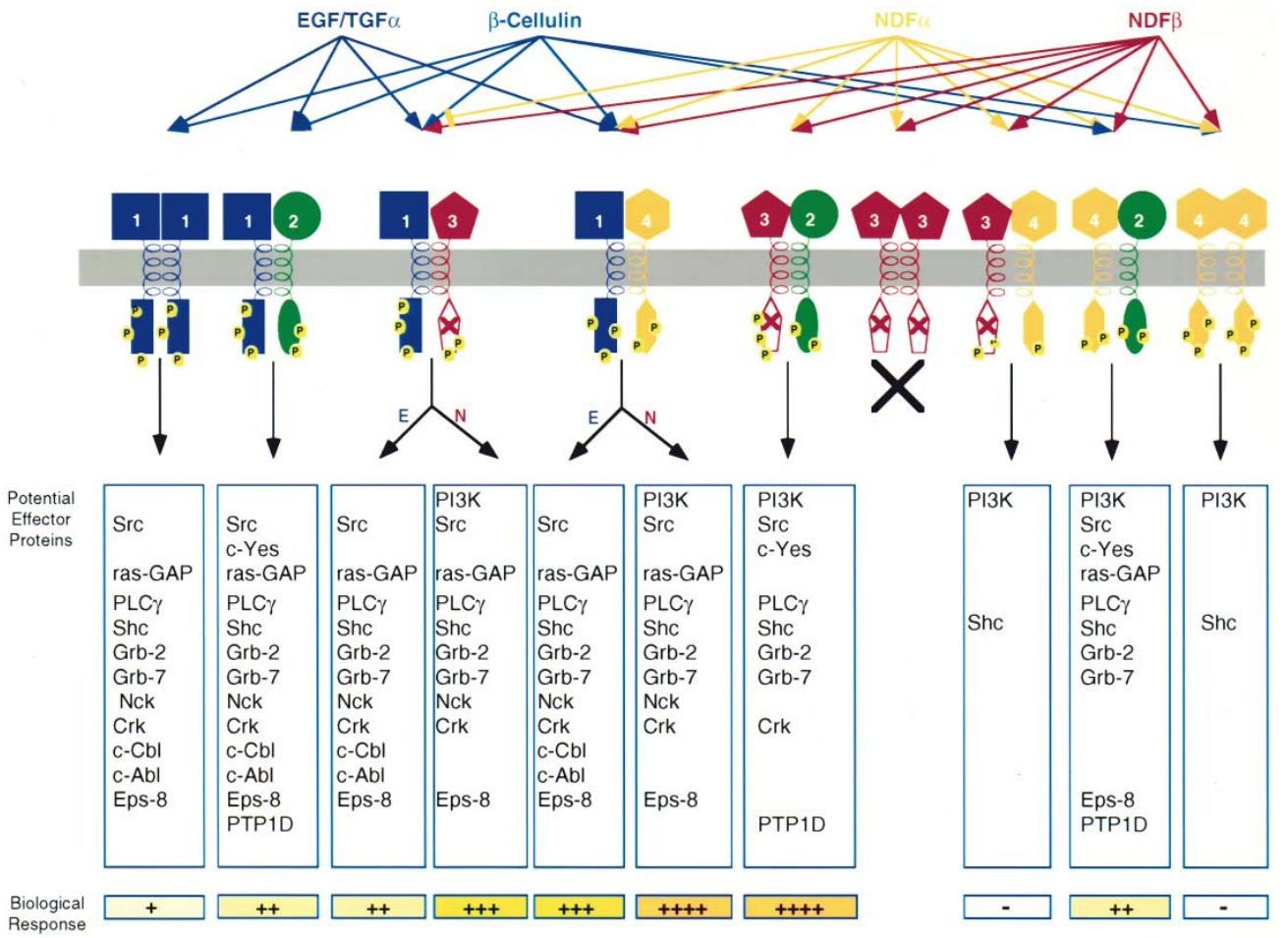

Fig. 1. Schematic representation of the ErbB signaling network. Three layers of diversity generation are proposed: multiple ligands with specificity to distinct receptors, nine receptor combinations, and various sets of cytoplasmic signaling proteins (most of them containing SH2 domains). The plasma membrane is shown as a gray horizontal bar and the various ErbB proteins as bilobular structures with helical transmembrane domains. The defective kinase of ErbB-3 is crossed. Receptor phosphorylation sites are shown by encircled P letters. The various signaling proteins underlying each receptor dimer are listed in vertical boxes. Receptor heterodimers that can be induced by either EGF (E) or NDF/neuregulin (N) are presented with two boxes. Note that only some of the known ErbB ligands are represented and they differ in their dimer recruitment abilities.

network, and expression patterns of ErbB receptors and their ligands highlighted the importance of short-range ligand-receptor interactions especially in mid-gestation inductive processes. Apparently, the network is involved primarily in two types of interactions: mesenchyme-epithel crosstalk and neuronal effects on target cells, including muscle, astroglia, oligodendrocytes and Schwann cells [10]. NRG is synthesized by mesenchymal or neuronal cells which influence the differentiation, proliferation and migration of adjacent epithelial or non-neuronal cells, respectively. An essential role in mid-gestation was indicated by embryonic lethality of ErbB-2-, ErbB-4- and NRG-deficient mice at around day 10 post-fertilization due to aberrant cardiac development [11–14]. Moreover, the non-redundant part played by the NRG receptors and ErbB-2 emphasizes that the functional complex is a heterodimer. The trabeculae, a finger-like extension of the ventricular myocardium fails to develop, and thus the mutant heart is characterized by irregular beat, an enlarged common ventricle and reduced blood flow. Since ErbB-4 is expressed in the underlying muscular portion of the ventricle and atrium (myocardium) and NRG is expressed in the endothelial ventricular lining, it seems that NRG activates trabeculea formation by the ErbB-4-expressing myocardium, thereby initiating ventricular differentiation. In addition to cardiac disorders, ErbB-4-deficient mice displayed severe defects in the development of the cranial sensory ganglia following migration from the neural crest, thus suggesting a unique role for ErbB-4.

Targeting of the *erbB-1* gene demonstrated a pivotal role during epithelial cell development, consistent with expression profiles of ErbB-1, EGF and TGFα in lung epithelium and in the gastrointestinal tract [15–17]. Mutant mice displayed impaired epithelial development in several organs, resulting in different phenotypes ranging from peri-implantation death to live progeny suffering from abnormalities in multiple organs, depending on the genetic background. Knockout of one the ErbB-1 ligands, namely TGFα, suggested that each ligand plays a distinct role during development. Thus, TGFα-disrupted mice displayed only part of the defects observed in ErbB-1 null mice, namely eye abnormalities and derangement of hair follicles [18,19].

An example of a post-birth function of the ErbB network, which is obscured by lethality of gene-targeted mice, is provided by analyses of ErbB receptors in the developing mammary gland. ErbB-1 levels parallel the increase in DNA syn-

thesis in the mammary gland during pregnancy and decline immediately before the onset of lactation [20]. EGF treatment of mammary glands, both in vitro and in vivo, resulted in ductal and alveolar epithelial differentiation and in the suppression of the accumulation of milk fat droplets in the alveoli during mid- to late pregnancy, while overexpression of TGFα in mammary glands resulted in earlier alveolar development [21–23]. Thus, two different cell populations may function as targets of ErbB-1 ligands during distinct stages of mammary gland development. In vitro treatment of mammary glands with NRG induced formation of lobuloalveolar structures and increased appearance of milk-producing cells [24]. The NRG isoform α2 is highly expressed during the process of lobuloalveolar morphogenesis at pregnancy, and its levels markedly decrease during lactation and involution, while no expression is detected during the virginal period [25]. Interestingly, a switch between ErbB-3 and ErbB-4 expression was observed in the developing mammary gland, suggesting that the two receptors play different roles in mammary morphogenesis [26].

4. ErbB receptors and tumorigenesis

Overexpression of tyrosine kinase receptors has a deleterious effect on normal cell growth, leading to the induction of transformation [27]. The ErbB-1 receptor provided one of the first links between an activated oncogene and human tumor biology. Human ErbB-1 is highly homologous to the viral oncogene v-erbB, which is carried by an avian retrovirus. ErbB-1 is also overexpressed in a variety of human tumors and it may undergo oncogenic conversion by gene rearrangements, resulting in large amino-terminal deletions. For example, ErbB-1 overexpression is associated with non-small cell lung carcinoma and is correlated with high metastatic rate, poor differentiation and short patient survival time [28].

Amplification and overexpression of *erbB-2* have been reported for breast carcinoma, where high levels of *erbB-2* were correlated with poor prognosis in node-positive patients [29]. ErbB-2 is amplified and/or overexpressed in both non-invasive and invasive ductal breast carcinoma, reflecting its importance in the early as well as progressive stages of tumor development. In the rat, a single point mutation in the transmembrane domain of ErbB-2 results in a transforming ability, even at low expression levels, probably due to constitutive kinase activity. Analyses of other types of tumors indicated that overexpression of ErbB-2 may be present in most carcinomas, including lung adenocarcinomas and gastric and cervical carcinomas [30].

5. Receptor dimerization and signal diversification

Similar to other allosteric enzymatic systems, oligomerization of ErbB proteins is essential for their activation. Early work demonstrated that minimal oligomerization: namely, dimer formation, is sufficient for enzyme stimulation [31,32]. Furthermore, the extracellular ligand acts as an allosteric regulator of the cytoplasmic enzyme, simply by inducing receptor/enzyme oligomerization. Thus, the transmembrane topology of ErbB proteins allows an allosteric mechanism for signal transduction, that bypasses the need for vertical propagation of conformational changes across the plasma membrane. That dimerization is not limited to homodimer formation, but also includes heterodimerization of ErbB proteins, was shown by demonstrating that ErbB-2 can heterodimerize with the EGF- [33,34] and NRG-receptors [35]. The driving force for homo- as well as heterodimer formation is the higher stability of the ternary complex formed between a ligand and two receptors, as compared with a monomeric receptor. In other words, receptor dimers have higher ligand affinity when compared with the corresponding receptor monomers [36,37]. It was later demonstrated that at least nine different homo- and heterodimers of ErbB proteins exist but their formation displayed a distinct hierarchy [38]. In this network, ErbB-2 plays a major coordinatory role, as each liganded direct receptor appears to prefer ErbB-2 as its heterodimeric partner. This preference is further biased upon overexpression of ErbB-2, as seen in many types of human cancer cells. For two reasons, ErbB-2-containing heterodimers are characterized by extremely high signaling potency. First, due to the ability of ErbB-2 to remarkably reduce the rate of ligand dissociation, signaling by growth factors is prolonged and enhanced by this oncoprotein [39,3]. Second, because ErbB-2 can efficiently signal through MAP-kinases, its presence enhances mitogenic, and perhaps also other types of cellular signals [40]. Thus, ErbB-2 overexpression in tumor cells is thought to confer a selective advantage due to better utilization of stroma-derived EGF-like growth factors.

Unlike homodimers whose biological activities are relatively weak, heterodimers appear to be more potent. This is best exemplified by the ability of each ErbB protein to transform a normal fibroblast into a cancer cell: co-expression of two ErbB proteins, either ErbB-1 and ErbB-2, or each of the two NRG receptors together with ErbB-2 or ErbB-1, drives cellular transformation more efficiently than by each singly expressed protein [41,42]. In model cellular systems whose growth depends on an interleukin, co-expression of two ErbB proteins confers a superior proliferative effect, consistent with the synergistic transforming potential [43,4]. A graded range of mitogenic signals is thus formed in which homodimers of the kinase-defective receptor, ErbB-3, are completely inactive and heterodimers between ErbB-3 and ErbB-2 are the most mitogenic. The ErbB-2/ErbB-3 heterodimer exemplifies the role of heterodimer formation, not only in signal diversification but also in achieving better control; formation of the most potent combination requires both a ligand for ErbB-3, namely NRG, and a heterodimerizing partner, ErbB-2.

Does each receptor dimer recruit one unique signaling protein that allows selection of a distinct signaling pathway? Although such a model is attractive when trying to explain differential signaling by the various receptor combinations, it appears to be incorrect. Like their invertebrate homologs in *Drosophila* and in *C. elegans*, all ErbB proteins apparently utilize the Ras–Raf–MAP–kinase as a major signaling route. Nevertheless, each receptor complex may select a distinct set of signaling proteins that collectively specify its unique cellular signature (Fig. 1). Examples include Cbl, a protooncogenic adaptor protein, that is recruited by all ErbB-1-containing receptor complexes, but not by other dimers [44], and the relatively strong association between ErbB-3 and phosphatidylinositol 3′-kinase [45]. Nevertheless, the sets of signaling molecules identified so far as recruited to specific receptors are largely overlapping.

6. Ligand multiplicity and the mechanism of receptor dimerization

Important questions that are currently open include possible functional redundancy of the multiple ErbB ligands, especially those sharing receptor specificity. Another question relates to the mechanism by which ligand binding promotes dimerization of identical or different receptors. Recent experimental observations imply that the two questions may have a common answer. Analysis of NRG signaling indicated that α- and β-isoforms of this family share an ability to form heterodimers of ErbB-3 with ErbB-2, but only β-isoforms are capable of stabilizing an ErbB-3/ErbB-1 heterodimer [46]. Indirect evidence suggest that the many ErbB-1-specific ligands also differ in their abilities to recruit different receptor heterodimers [47]. While the mechanism by which each ligand drives formation of specific heterodimers may involve a conformation-induced opening of a cryptic dimerization site, our most recent results support a simpler model: namely, NRG molecules appear to contain two receptor binding sites so that ligand bivalency may be the sole driving force for dimer formation. Apparently, the two binding sites of NRG differ. Whereas the N-terminal site has high affinity and selectively binds to both ErbB-4 and ErbB-3, the C-terminally located site has low affinity and broad receptor selectivity. Thus, it is the interaction with this site that allows selection of the heterodimeric receptor partner. Future analyses may identify similar broad-specificity sites in other EGF-like ligands, and also determine their relative order of receptor selectivity, which in the case of NRGs appears to be ErbB-2 > ErbB-3/4 > ErbB-1.

7. Perspectives

The ErbB family is able to enhance signal diversification through formation of different homo- and heterodimeric interactions. The formation of a specific receptor complex and signaling through a selected pathway is most likely governed by the available ligands and the receptors expressed by the cell. The resulting enormous signal diversity appears needed to specify the many cell lineages of the mammalian nervous and epithelial systems. Consistent with this possibility, no network is found in lower organisms, but the ErbB family is represented in these organisms by a single receptor. It is worthwhile to note that most RTKs belong to small families of homologous receptors that maintain functional interactions. Therefore, lessons learned in the ErbB family may be relevant to the mechanism by which other growth factors and their receptors transmit intercellular signals.

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