UNTANGLING THE ErbB SIGNALLING NETWORK

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When epidermal growth factor and its relatives bind the ErbB family of receptors, they trigger a rich network of signalling pathways, culminating in responses ranging from cell division to death, motility to adhesion. The network is often dysregulated in cancer and lends credence to the mantra that molecular understanding yields clinical benefit: over 25,000 women with breast cancer have now been treated with trastuzumab (Herceptin®), a recombinant antibody designed to block the receptor ErbB2. Likewise, small-molecule enzyme inhibitors and monoclonal antibodies to ErbB1 are in advanced phases of clinical testing. What can this pathway teach us about translating basic science into clinical use?

MESENCHYME
Immature connective tissue that consists of cells embedded in extracellular matrix.

NEUREGULINS
EGF-like ligands whose primary receptor is ErbB3 and/or ErbB4. Four types of neuregulin are known.

STROMA
Supporting connective tissue in which a glandular or other epithelium is embedded.

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ErbBs are typical receptor tyrosine kinases that were implicated in cancer in the early 1980s when the avian erythroblastosis tumour virus was found to encode an aberrant form of the human epidermal growth factor (EGF) receptor (also known as ErbB1, HER or EGFR). Since then, the ErbB family has grown to four, and we are beginning to appreciate that the normal function of ErbBs and their ligands is to mediate cell–cell interactions in organogenesis and adulthood (reviewed in REF. 1).

In the epithelium, the basolateral location of ErbBs enables them to mediate signals between the MESENCHYME and the epithelium for cell growth[2]. The mesenchyme serves as a storehouse for many ligands including NEUREGULINS (NRGs), which bind ErbB3 and ErbB4. ErbB2 (also known as HER2) is a more potent oncoprotein than the other ErbBs, but no known ligand binds it with high affinity. It was first discovered as a rodent carcinogen-induced oncogene that encodes a variant of ErbB2 with a mutation that makes its tyrosine kinase constitutively active. ErbB2 is a shared co-receptor for several STROMAL ligands. Blocking the action of ErbB2 might thus inhibit a myriad of mitogenic pathways affecting ErbB-expressing tumour cells[3]. Although several strategies are being developed, Herceptin® — a HUMANIZED MONOCLONAL ANTIBODY to ErbB2 — has been the first to reach widespread clinical use, in particular for the treatment of metastatic breast cancer[4,5].

A layered signalling network

The components of the ErbB signalling pathway are evolutionarily ancient (BOX 1), and at first glance resemble a simple growth factor signalling pathway: ligand binding to a monomeric receptor tyrosine kinase activates the cytoplasmic catalytic function by promoting receptor dimerization and self-phosphorylation on tyrosine residues. The latter serve as docking sites for various ADAPTOR PROTEINS or enzymes, which simultaneously initiate many signalling cascades to produce a physiological outcome (FIG. 1). In higher eukaryotes, the simple linear pathway has evolved into a richly interactive, multilayered network, in which combinatorial expression and activation of components permits context-specific biological responses throughout development and adulthood.

The input layer. This comprises the ligands (EGF family of growth factors) and their receptors — the ErbBs (FIG. 1). All high-affinity ErbB ligands have an EGF-LIKE DOMAIN and three disulphide-bonded intramolecular loops. This receptor-binding domain is usually part of a large transmembrane precursor containing other structural motifs such as IMMUNOGLOBULIN-LIKE DOMAINS, heparin-binding sites and glycosylated linkers. Expression and processing of the precursor are highly regulated. For example, transformation by active Ras, or exposure to steroid hormones[6] leads to increased expres-

Box 1 | Evolution of the ErbB signalling network

Both the nematode *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster* have primordial linear versions of the ErbB signalling pathway. In higher organisms, this has evolved into a complex network, probably because an interconnected layered structure can confer selective gains in terms of adaptation, tolerance to mutations and signal diversification[91]. The main functional features of the ErbB module were defined in invertebrates: ErbB regulates the fate of diverse cell lineages in different developmental stages through short-range paracrine interactions.

***C. elegans* and *Drosophila* each contain a single ErbB homologue; however, the only EGF-like ligand of *C. elegans*, called Lin-3, is replaced by four ligands in *Drosophila*. Vulva development is a well-characterized function of the Lin-3 signalling pathway: the six vulva precursor cells (VPCs) respond to an inductive signal from a gonadal anchor cell, which is thought to secrete Lin-3. Lin-3 binds the juxtaposed receptor on one of the VPCs and instructs it to undergo several cell cycles and develop concomitantly a more differentiated phenotype. The Lin-3 pathway functions in other inductive morphogenic events; loss-of-function mutations in the receptor result not only in a vulvaless phenotype, but also in sterility, abnormal male tail development and death[92].**

The *Drosophila* EGF receptor (DER) is used repeatedly in several stages of development, including oogenesis, embryogenesis, and wing and eye development. Likewise, differentiation of the DER-expressing tendon cell is regulated by the myotube-derived NRG-like ligand, Vein[93]. Gurken, a homologue of transforming growth factor-α (TGF-α), functions primarily in the oocyte. Activation of another ligand, Spitz, which is anchored to the cell surface, requires proteolytic cleavage[94]. By contrast, Argos, a secreted DER ligand, is unique in that it negatively acts on receptor signalling[95].

sion of several ErbB ligands, and cleavage of ligand precursors by a METALLOPROTEINASE can be stimulated by activation of other receptors, such as G-protein-coupled receptors[7] (FIG. 2).

An important issue relates to the multiplicity and possible redundancy of ErbB ligands. This issue is particularly relevant to the many NRGs and their splice variants. Studies in cultured cells and initial attempts to address this issue in animals suggest that ErbB ligands have non-overlapping functions. For example, ligands such as EGF and NRG4, which bind to ErbB1 and ErbB4, respectively, have narrow specificity, whereas others such as epiregulin, NRG1β and betacellulin bind to two distinct primary receptors[8]. Overexpression of ErbB2, which biases heterodimer formation, can broaden ligand specificity (FIG. 1, dotted lines), and ligands that are better at recruiting this co-receptor can reduce the binding of less effective ligands. In addition, splice variants of NRGs and various ligand–receptor complexes also differ in their ability to recruit a partner receptor[9–11], which affects their potency and kinetics of signalling.

The four ErbBs share an overall structure of two cysteine-rich regions in their extracellular region, and a kinase domain flanked by a carboxy-terminal tail with tyrosine autophosphorylation sites. With few exceptions (for example, haematopoietic cells), ErbB proteins are expressed in cells of MESODERMAL and ECTODERMAL origins.

Examination of the intracellular and extracellular domains of the ErbBs provides a satisfying explanation as to why a horizontal network of interactions is crucial to the ErbB signalling pathway: ErbB3 is devoid of intrinsic kinase activity[12], whereas ErbB2 seems to have no direct ligand[13]. Therefore, in isolation neither ErbB2 nor ErbB3 can support linear signalling (FIG. 3). Most inter-receptor interactions are mediated by ligands, and ErbB2-containing heterodimers are formed preferentially[14,15]. Nevertheless, overexpression of a specific receptor can bias dimer formation, especially in the case of ErbB2, whose homodimers can spontaneously form in ErbB2-overexpressing cells. Many cancers of epithelial origin have an amplification of the ErbB2 gene, which pushes the equilibrium towards ErbB2 homodimer and heterodimer formation. By contrast, ErbB4, whose expression pattern is relatively limited, has several isoforms that differ in their juxtamembrane and carboxyl termini, resulting in differences in the recruitment of phosphatidylinositol-3-OH kinase (PI(3)K)[16], which activates cell-survival pathways.

Signal-processing layers. The specificity and potency of intracellular signals are determined by positive and negative effectors of ErbB proteins, as well as by the identity of the ligand, oligomer composition and specific structural determinants of the receptors. The main determinant, however, is the vast array of phosphotyrosine-binding proteins that associate with the tail of each ErbB molecule after engagement into dimeric complexes (FIG. 1). Which sites are autophosphorylated, and hence which signalling proteins are engaged, are determined by the identity of the ligand as well as by the heterodimer partner[17]. The Ras- and Shc-activated mitogen-activated protein kinase (MAPK) pathway is an invariable target of all ErbB ligands, and the PI(3)K-activated AKT PATHWAY and p70S6K/p85S6K pathway are downstream of most active ErbB dimers. The potency and kinetics of PI(3)K activation differ, however, probably because PI(3)K couples directly with ErbB3 and ErbB4, but indirectly with ErbB1 and ErbB2 (REF. 18).

Simultaneous activation of linear cascades, such as the MAPK pathway, the STRESS-ACTIVATED PROTEIN KINASE cascade, protein kinase C (PKC) and the Akt pathway translates in the nucleus into distinct transcriptional programmes. These involve not only the proto-oncogenes *fos*, *jun* and *myc*, but also a family of zinc-finger-containing transcription factors that includes Sp1 and Egr1, as well as Ets family members such as GA-binding protein (GABP)[19]. Despite sharing some pathways, each receptor is coupled with a distinct set of signalling proteins. For example, unlike ErbB1, the kinase-defective ErbB3 cannot interact with the adaptor protein and UBIQUITIN LIGASE c-Cbl, the adaptor protein Grb2, the second-messenger-generating enzyme phospholipase Cγ or the Ras-specific GTPase-activating protein (GAP)[20], but it can associate with the adaptors Shc and Grb7 (FIG. 1).In addition to combinatorial interactions, an important determinant of signalling outcome is variation in the kinetics of specific pathways. The principal process that turns off signalling by the ErbB network is ligand-mediated receptor endocytosis, and the kinetics of this process also depend heavily on receptor composition (BOX 2).

The output layer. The output of the ErbB network ranges from cell division and migration (both associated with tumorigenesis) to adhesion, differentiation and

HUMANIZED MONOCLONAL ANTIBODY
An antibody, usually from a rodent, engineered to contain mainly human sequences. This process reduces the immune response to the antibody in humans.

ADAPTOR PROTEINS
Proteins that augment cellular responses by recruiting other proteins to a complex. They usually contain several protein–protein interaction domains.

EGF-LIKE DOMAIN
A motif with ~50 amino acids, including six cysteine residues and a mainly β-sheet structure, found in all ErbB-binding growth factors and in extracellular matrix proteins.

IMMUNOGLOBULIN-LIKE DOMAIN
A protein domain composed of two β-pleated sheets held together by a disulphide bond.

METALLOPROTEINASES
Proteinases that have a metal ion at their active sites.

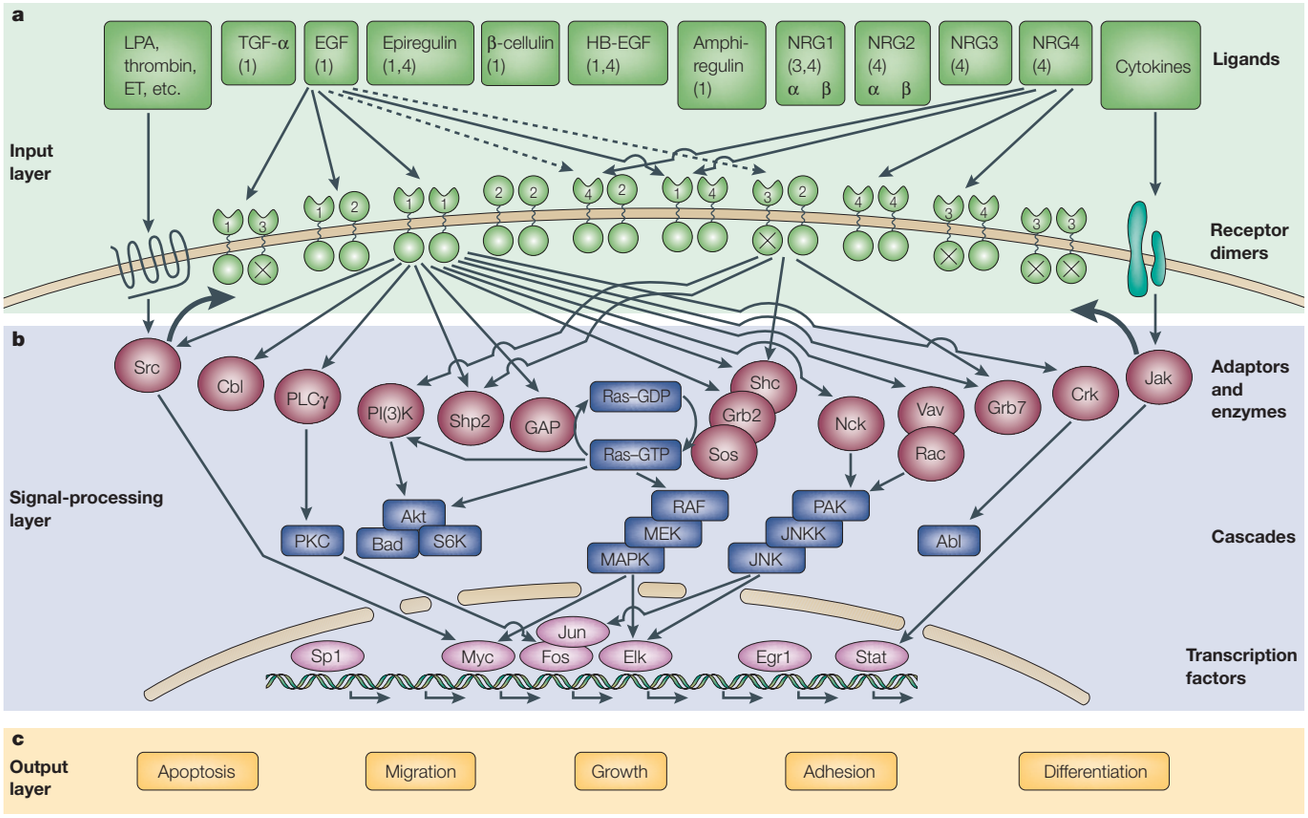


Figure 1 | **The ErbB signalling network. a** | Ligands and the ten dimeric receptor combinations comprise the input layer. Numbers in each ligand block indicate the respective high-affinity ErbB receptors[8]. For simplicity, specificities of receptor binding are shown only for epidermal growth factor (EGF) and neuregulin 4 (NRG4). ErbB2 binds no ligand with high affinity, and ErbB3 homodimers are catalytically inactive (crossed kinase domains). *Trans*-regulation by G-protein-coupled receptors (such as those for lysophosphatidic acid (LPA), thrombin and endothelin (ET)), and cytokine receptors is shown by wide arrows. **b** | Signalling to the adaptor/enzyme layer is shown only for two receptor dimers: the weakly mitogenic ErbB1 homodimer, and the relatively potent ErbB2–ErbB3 heterodimer. Only some of the pathways and transcription factors are represented in this layer. **c** | How they are translated to specific types of output is poorly understood at present. (Abl, a proto-oncogenic tyrosine kinase whose targets are poorly understood; Akt, a serine/threonine kinase that phosphorylates the anti-apoptotic protein Bad and the ribosomal S6 kinase (S6K); GAP, GTPase activating protein; HB-EGF, heparin-binding EGF; Jak, janus kinase; PKC, protein kinase C; PLCγ, phospholipase Cγ; Shp2, Src homology domain-2-containing protein tyrosine phosphatase 2; Stat, signal transducer and activator of transcription; RAF–MEK–MAPK and PAK–JNKK–JNK, two cascades of serine/threonine kinases that regulate the activity of a number of transcription factors.)

MESODERM
The middle germ layer of the developing embryo. It gives rise to the musculoskeletal, vascular and urinogenital systems, and to connective tissue (including that of the dermis).

ECTODERM
The outermost germ layer of the developing embryo. It gives rise to the epidermis and the nerves.

AKT PATHWAY
Akt (or protein kinase B) is a serine/threonine protein kinase activated by the phosphatidylinositol-3-OH kinase pathway that activates survival responses.

apoptosis (FIG. 1). Output depends on cellular context, as well as the specific ligand and ErbB dimer. This has been best shown in terms of mitogenic and transforming responses: homodimeric receptor combinations are less mitogenic and transforming than the corresponding heterodimeric combinations, and ErbB2-containing heterodimers are the most potent complexes[21–23] (FIG. 3).

Perhaps the best example of the ability of the ErbB module to tune mitogenic signalling is provided by the ErbB2–ErbB3 heterodimer: although neither ErbB2 nor ErbB3 alone can be activated by ligand, the heterodimer is the most transforming[24,25] and mitogenic[21] receptor complex. The ErbB2–ErbB3 heterodimer also increases cell motility on stimulation with a ligand[26]; but the other NRG receptor, ErbB4, which exists in several isoforms, has been associated with processes varying from cellular chemotaxis[27] to proliferation and differentiation[28].

A network of networks?

The ErbB network might integrate not only its own inputs but also heterologous signals, including hormones, neurotransmitters, lymphokines and stress inducers[29] (FIG. 1). Many of these *trans*-regulatory interactions are mediated by protein kinases that directly phosphorylate ErbBs, thereby affecting their kinase activity or endocytic transport[29]. The most extensively studied mechanism involves activation of G-protein-coupled receptors (GPCRs) by agonists such as lysophosphatidic acid (LPA), carbachol (which specifically activates muscarinic acetylcholine receptors) or thrombin (FIG. 2).

Experiments done with mutants and inhibitors of ErbBs imply that the mitogenic activity of some GPCR agonists requires transactivation of ErbB proteins. These agents increase tyrosine phosphorylation of ErbB1 and ErbB2, either by increasing their intrinsic

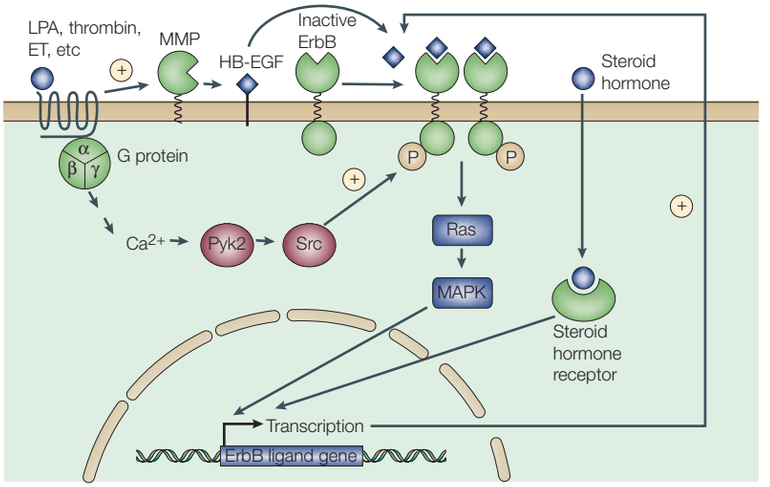


Figure 2 | **Crosstalk between the ErbB network and other signalling pathways.** G-protein-coupled receptors (GPCRs) such as those for lysophosphatidic acid (LPA), thrombin and endothelin (ET) can have positive effects on ErbB signalling through two mechanisms. First, through a poorly defined mechanism, they can activate matrix metalloproteinases (MMPs), which cleave membrane-tethered ErbB ligands (such as heparin-binding EGF-like factor, HB-EGF), thereby freeing them to bind to ErbBs. Second, GPCRs indirectly activate Src (perhaps via Pyk2), which phosphorylates the intracellular domains of ErbBs on tyrosine residues. Steroid hormones can have a positive effect on ErbB signalling by activating the transcription of genes encoding ErbB ligands. Finally, ErbB activation can activate a positive feedback loop through the Ras–MAPK (mitogen-activated protein kinase) pathway, which also activates transcription of ErbB ligand genes.

kinase activity[30] or by inhibiting an associated phosphatase activity. Signalling events downstream of ErbB1 are subsequently triggered, and this might account for the mitogenic potential of the heterologous agonists. Apparently, a cascade of tyrosine kinases links GPCRs such as the LPA receptor or the β-adrenergic receptor to ErbB1 and subsequently to MAPK. The cascade culminates in the stimulation of Src family kinases[31], which are recruited by either the calcium-regulated tyrosine kinase Pyk2 (REF. 32) or a GPCR-coupled kinase and an adaptor protein (for example, arrestin[33]). Another kinase that phosphorylates ErbB1 is the cytokine-regulated tyrosine kinase Jak2: on stimulation of adipocytes by growth hormone, Jak2 phosphorylates ErbB1, thus allowing MAPK activation even by a kinase-defective mutant of ErbB1 (REFS 34, 35).

Yet another cytokine, interleukin-6, elevates tyrosine phosphorylation of ErbB2 by increasing its intrinsic catalytic activity[36]. By contrast, factors that activate PKC, such as certain growth factors and hormones (for example, PDGF, LPA and EGF by itself), increase threonine and serine phosphorylation of ErbB1 and ErbB2, which decrease tyrosine phosphorylation and ligand binding affinity through a mechanism involving accelerated recycling of internalized receptors (BOX 2). These interconnections to other signalling modules help to integrate and coordinate cellular responses to extracellular stimuli.

Integrating developmental cues

The ErbB network is a key developmental signalling pathway throughout evolution. Its functions in worm and fly development are now well understood (BOX 1), but recent research using knockout and transgenic mice is beginning to clarify the functions of individual ErbBs and specific ligands in mammalian development.

ErbB1 and its ligands. Inactivation of ErbB1 impairs epithelial development in many organs, including those involved in tooth growth and eye opening[37–39]. Likewise, transgenic and *in vitro* studies implicate ErbB1 in promoting proliferation and differentiation of the epithelial component of skin, lung, pancreas and the gastrointestinal tract. These processes are probably regulated by growth factors from the local mesenchyme. Mice lacking expression of transforming growth factor-α (TGF-α) have abnormal skin, hair and eye development[40,41] but, in contrast with ErbB1-deficient mice, which undergo massive apoptosis in cortical and thalamic

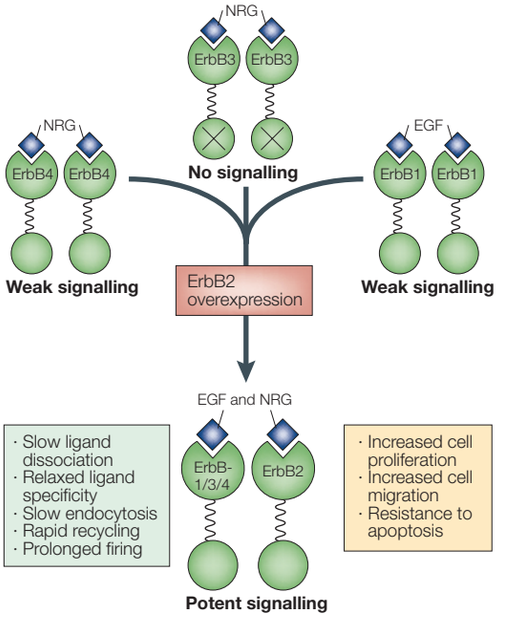


Figure 3 | **Signalling by ErbB homodimers in comparison with ErbB2-containing heterodimers.** Receptors are shown as two lobes connected by a transmembrane stretch. Binding of a ligand (EGF-like or NRG) to the extracellular lobe of ErbB1, ErbB3 (note inactive kinase, marked by a cross) or ErbB4 induces homodimer formation. When ErbB2 is overexpressed, heterodimers form preferentially. Unlike homodimers, which are either inactive (ErbB3 homodimers) or signal only weakly, ErbB2-containing heterodimers have attributes that prolong and enhance downstream signalling (green box) and their outputs (yellow box). Apparently, homodimers of ErbB2 are weaker signalling complexes than heterodimers containing ErbB2. (EGF, epidermal growth factor; NRG, neuregulin.)

STRESS-ACTIVATED PROTEIN KINASES
Members of the mitogen-activated protein kinase (MAPK) family that respond to stress. They include the Jun amino-terminal kinases (JNKs) and the p38 MAPKs.

UBIQUITIN LIGASES
Enzymes that catalyse the last stage of ubiquitylation, in which the small protein ubiquitin is transferred from a ubiquitin-conjugating enzyme (UBC or E2) to its target protein. They are also known as E3 enzymes.

GAPS
Proteins that inactivate small GTP-binding proteins, such as Ras family members, by increasing their rate of GTP hydrolysis.

Box 2 | **Turning off the ErbB response**

On ligand binding, ErbB1 molecules cluster over clathrin-coated regions of the plasma membrane, which invaginate to form endocytic vesicles. These mature to early and late endosomes, while gradually decreasing their internal pH and accumulating hydrolytic enzymes that lead to receptor degradation. Importantly, the other three ErbB proteins are endocytosis impaired and are more often recycled back to the cell surface[21,96]. Sorting to degradation is determined by the composition of the dimer: ErbB1 homodimers are targeted primarily to the lysosome; ErbB3 molecules are constitutively recycled[97]; and heterodimerization with ErbB2 decreases the rate of endocytosis and increases recycling of its partners[98,99]. Receptor internalization is determined by cytoplasmic motifs[100], but sorting in the early endosome seems to depend on the differential dissociation of ligand–ErbB complexes at mildly acidic pH. Complex dissociation leads to recycling, whereas continuous activation of tyrosine phosphorylation in the endosome leads to recruitment of c-Cbl, a ubiquitin ligase that preferentially binds to ErbB1 homodimers[101] and directs them to lysosomal degradation by tagging with polyubiquitin tracts[102].

brain regions[38], mice homozygous for a disrupted TGF-α gene show no brain abnormalities. So, the limited penetrance of TGF-α mutations and the confinement of the phenotype to the skin and eye suggest that each ErbB ligand has a distinct functional role and tissue specificity, analogous to the different roles played by each of the *Drosophila* EGF receptor ligands in insect development (BOX 2).

Neuregulins and their receptors. Like ErbB1 and its ligands involved in mesenchyme–epithelium interactions, the NRGs and their receptors are involved in the interaction between nerves and their target cells (for example, muscle, GLIA and SCHWANN CELLS), and are essential for cardiac and neural development. Mice defective in ErbB4, ErbB2 and NRG-1 die at embryonic day 10.5 from similar heart defects[1]. Endocardium-derived

Table 1 | **Expression of ErbBs and their ligands in cancer**

Molecule	Nature of dysregulation	Type of cancer	Notes	References
Ligands				
TGF-α	Overexpression	Prostate	Expressed by stroma in early, androgen-dependent prostate cancer and by tumours in advanced, androgen-independent cancer	52
	Overexpression	Pancreatic	Correlates with tumour size and decreased patient survival; may be due to overexpression of Ki-Ras, which also drives expression of HB-EGF and NRG1	108
	Overexpression	Lung, ovary, colon	Correlates with poor prognosis when co-expressed with ErbB1	51
NRG1	Overexpression	Mammary adeno-carcinomas	Necessary, but not sufficient for tumorigenesis in animal models	109
Receptors				
ErbB1	Overexpression	Head and neck, breast, bladder, prostate, kidney, non-small-cell lung cancer	Significant indicator for recurrence in operable breast tumours; associated with shorter disease-free and overall survival in advanced breast cancer; may serve as a prognostic marker for bladder, prostate, and non-small-cell lung cancers	110,111
	Overexpression	Glioma	Amplification occurs in 40% of gliomas; overexpression correlates with higher grade and reduced survival	35
	Mutation	Glioma, lung, ovary, breast	Deletion of part of the extracellular domain yields a constitutively active receptor	54
ErbB2	Overexpression	Breast, lung pancreas, colon oesophagus, endometrium, cervix	Overexpressed owing to gene amplification in 15–30% of invasive invasive ductal breast cancers Overexpression correlates with tumour size, spread of the tumour to lymph nodes, high grade, high percentage of S-phase cells, aneuploidy and lack of steroid hormone receptors	56
ErbB3	Expression	Breast, colon gastric, prostate, other carcinomas	Co-expression of ErbB2 with ErbB1 or ErbB3 in breast cancer improves predicting power	64,65
	Overexpression	Oral squamous cell cancer	Overexpression correlates with lymph node involvement and patient survival	112
ErbB4	Reduced expression	Breast, prostate	Correlates with a differentiated phenotype	66
	Expression	Childhood medullo-blastoma	Co-expression with ErbB2 has a prognostic value	67

(TGF-α, transforming growth factor-α; NRG1, neuregulin-1; HB-EGF, heparin-binding epidermal growth factor.)

GLIA
Supporting cells of the nervous system, including oligodendrocytes and astrocytes in the central nervous system, and Schwann cells in the peripheral nervous system. Glia surround neurons, providing mechanical and physical support, and electrical insulation between neurons.

SCHWANN CELLS
Cells that produce myelin and ensheath axons in the peripheral nervous system.

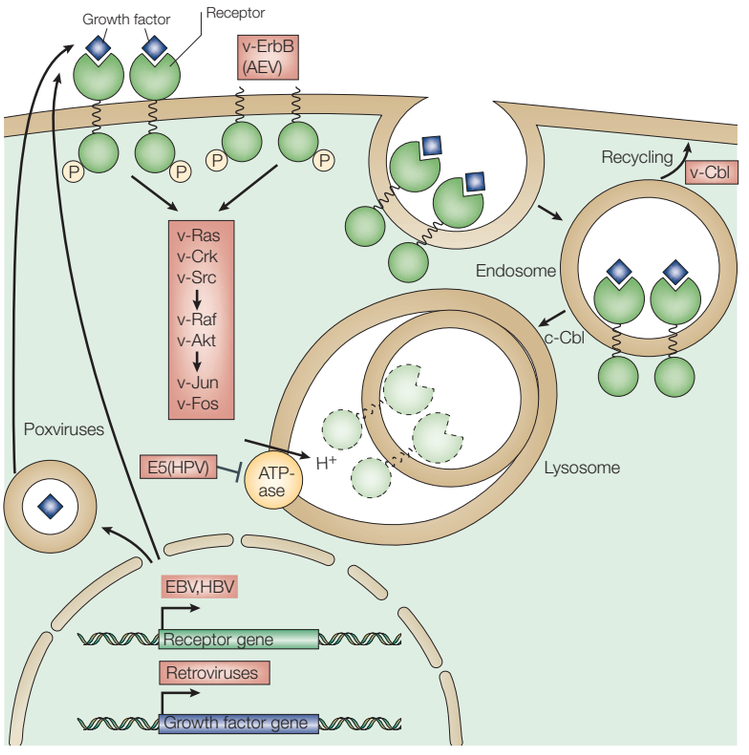
NRG1 stimulates an ErbB2–ErbB4 heterodimer on adjacent myocytes to initiate formation of the TRABECULAE. Surprisingly, the immunoglobulin domain and the cytoplasmic part of NRG1 — regions that are not involved in receptor binding — are essential for proper heart development[42,43]. ErbB3-deficient mice survive to embryonic day 13.5 and suffer from defective cardiac formation[44,45]. The alternative NRG-promoted heterodimer, ErbB2–ErbB3, is involved in different morphogenic events: mice lacking ErbB2, ErbB3 or NRG1 have a severely underdeveloped SYMPATHETIC GANGLION chain. This is probably caused by defective migration of neural progenitors from the NEURAL CREST[44].

The Schwann cell lineage is also controlled by the ErbB2–ErbB3 heterodimer. *In vitro* studies showed that NRG1 biases differentiation of neural crest progenitors towards a glial fate, and ErbB3-deficient mice showed partial lack of Schwann cells along peripheral and sensory neurons[45,46]. The ability of NRGs to control transcription of several ion channels underlies involvement of ErbBs in the neuromuscular junction[47]. NRGs elevate transcription of all subunits of the postsynaptic nicotinic acetylcholine receptor, but a nerve-derived splice variant seems to bias replacement of the γ-subunit with the ε-chain, which increases single-channel conductance. A similar subunit switch might occur at central synapses; NRG1β can markedly increase expression of the NR2C subunit of the *N*-methyl-D-aspartate receptor in slices of cerebellum[48].

The cancer connection

The potent cell proliferation signals generated by the ErbB network are used by cancer cells to fix oncogenic mutations by CLONAL EXPANSION. In addition, many types of oncogenic viruses exploit the ErbB network by manipulating its components (BOX 3). Human cancers use several mechanisms to activate the network at different layers. In many different cancer cell types, the ErbB pathway becomes hyperactivated by a range of mechanisms, including overproduction of ligands, overproduction of receptors, or constitutive activation of receptors (TABLE 1). It is extremely useful to know whether a

Box 3 | How do viruses harness the ErbB network?



Several transforming and non-transforming viruses constitutively elevate ErbB signalling by expressing an active component or by interfering with signalling shut-off. The hepatitis B virus (HBV), which is associated with hepatocellular carcinoma, upregulates transcription from the ErbB1 promoter[103]. Likewise, expression is deregulated by LMP1, a protein encoded by the Epstein–Barr virus (EBV), which is associated with several malignancies, including nasopharyngeal carcinoma[104]. Most members of the largest group of DNA viruses, poxviruses, encode EGF-like ligands, whose expression at sites of infection significantly increases pathogenicity[105]. RNA tumour viruses present the most divergent strategy to harness ErbB signalling: the avian erythroblastosis virus (AEV) encodes a truncated form of ErbB1 lacking most of the ectodomain and carrying many intracellular mutations. The oncoprotein v-ErbB forms ligand-independent covalent dimers at the cell surface[106]. Active mutants of various ErbB target proteins, including small GTP-binding proteins (v-Ras), adaptors (v-Crk), protein kinases (v-Src, v-Akt, v-Raf) and transcription factors (v-Jun, v-Fos), are encoded by oncogenes of different strains of retroviruses. In addition, the mouse Cas NS-1 retrovirus, which induces pre-B cell lymphomas and myeloid leukaemia, encodes a dominant active form of c-Cbl, a ubiquitin ligase that targets ErbB proteins to lysosomal degradation[102]. This interferes with receptor ubiquitylation and degradation, similar to the effect of E5, a product of the human papilloma virus (HPV) that inhibits ErbB1 degradation through inhibition of an endosomal proton-ATPase[107]. Both E5 and v-Cbl increase the rate of receptor recycling back to the cell surface.

TRABECULAE
Finger-like projections of cardiac muscle cells that form ridges in the ventricular wall.

SYMPATHETIC GANGLIA
Clusters of sympathetic neurons in which a glandular or other epithelium is embedded.

NEURAL CREST
A group of embryonic cells that separate from the embryonic neural plate and migrate, giving rise to the spinal and autonomic ganglia, peripheral glia, chromaffin cells, melanocytes and some haematopoietic cells.

CLONAL EXPANSION
Growth of a population of cells from a single precursor cell.

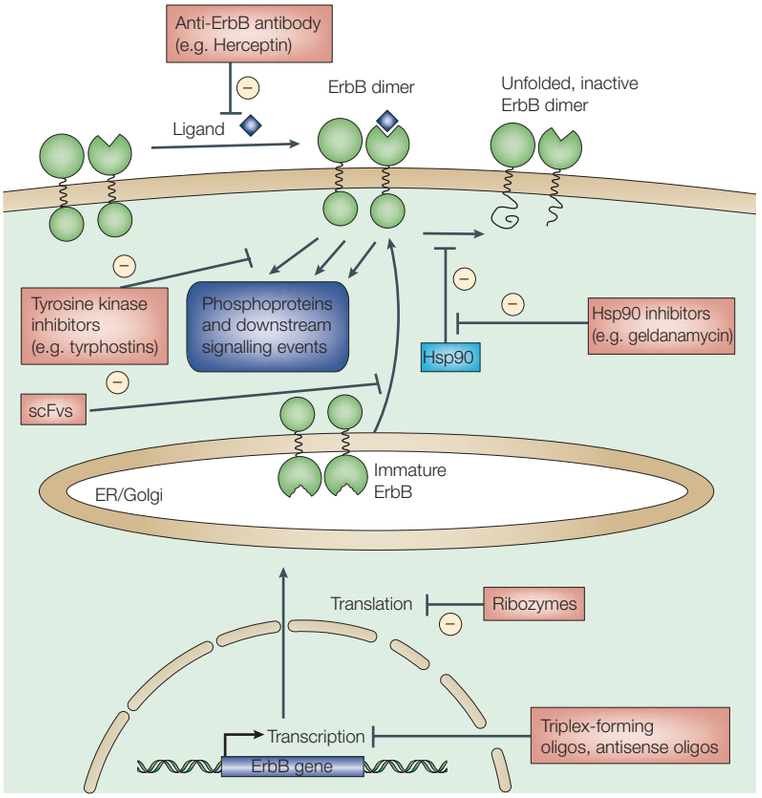


Figure 4 | **Therapeutic strategies for blocking the ErbB signalling network.** Anti-ErbB antibodies (such as Herceptin®, which binds ErbB2) block ligand binding and stimulate receptor internalization. Tyrosine kinase inhibitors such as tyrphostins block downstream signalling of the receptor–ligand complex, and Hsp90 inhibitors (for example, geldanamycin) prevent stabilization of ErbBs at the membrane. The active conformation of ErbB2 is maintained through interactions with a chaperone (Hsp90), and therefore chaperone antagonists inactivate the oncoprotein. It might also be possible to prevent ErbBs from reaching the cell surface, by blocking their transcription with triplex-forming oligonucleotides, their translation with antisense oligonucleotides or ribozymes, or their trafficking to the cell surface with intracellular single-chain Fv fragments of antibodies (scFvs). (ER, endoplasmic reticulum.)

particular tumour has an overactive ErbB pathway because of mutation, overexpression or amplification of a component of the ErbB pathway, as it can tell us what the patient's chance of survival is and with what drug they should be treated (FIG. 4).

Ligands. The relationship between ErbB ligand expression and tumorigenicity is complex: growth factors can be induced secondarily by a primary oncogene; either the stroma or the tumour can act as a ligand source; or the ligand can be expressed but unprocessed or sequestered in an inactive form[49].

Of all the ErbB ligands, the relevance of TGF-α to human cancer is best characterized. TGF-α and ErbB1 are co-expressed in several types of CARCINOMAS[50], and expression of TGF-α, particularly in lung, ovary and colon tumours co-expressing ErbB1, correlates with poor PROGNOSIS (reviewed in REF. 51). In prostate cancer, the pattern of expression of TGF-α seems to change as the disease progresses[52]. In early, ANDROGEN-DEPENDENT PROSTATE CANCER, TGF-α is expressed primarily in the tumour stroma, which suggests paracrine signalling. In advanced, androgen-independent disease, TGF-α is expressed by the tumour cells themselves, indicating AUTOCRINE signalling. Less information is available on other ligands (TABLE 1).

ErbB1. Both overexpression and structural alterations of ErbB1 are frequent in human malignancies. However, *in vitro* studies suggest that overexpression of the normal receptor leads to transformation only in the presence of a ligand. Accordingly, expression of EGF-like ligands often accompanies ErbB1 overexpression in primary tumours. Overexpression of ErbB1 is a very frequent genetic alteration in brain tumours; amplification of the gene occurs in 40% of gliomas[53]. Overexpression is associated with higher grade, higher proliferation and reduced survival. In a significant proportion of tumours, GENE AMPLIFICATION is accompanied by rearrangements. The most common mutation (type III) deletes part of the extracellular domain[35], yielding a constitutively active receptor. Recent studies identified an identical alteration in carcinomas of the lung, ovary and breast, suggesting broader implications to human cancer[54].

ErbB2. Several types of cancers overexpress ErbB2 (reviewed in REF. 56). The association of ErbB2 expression with cancer is best studied in breast cancer, where protein is overexpressed owing to gene amplification in 15–30% of invasive DUCTAL BREAST CANCERS[55]. Overexpression correlates with tumour size, spread of the tumour to lymph nodes, high grade, high percentage of S-phase cells, ANEUPLOIDY and lack of steroid hormone receptors, implying that ErbB2 confers a strong proliferative advantage to tumour cells[56,57]. Paradoxically, a higher degree of ErbB2 overexpression is reported in early forms of breast cancer relative to more advanced invasive carcinomas, suggesting that alterations in ErbB2 alone are insufficient for breast tumour progression from a relatively benign to a more malignant phenotype[56].

The identification of ErbB2 amplifications by FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH; FIG. 5) has now been approved by the US Food and Drug Administration to pinpoint patients at high risk for recurrence and disease-related death with node-negative invasive breast cancer[56,58]. Efforts are also being made to correlate ErbB2 status with predictive value — in other words, do patients with ErbB2 amplifications benefit from particular types of therapy? Again FISH technology can identify patients who might benefit from more aggressive therapy[59]. Several studies have shown that ErbB2 overexpression is associated with resistance to anti-oestrogen therapy[60]. Most ErbB2-overexpressing tumours do not express the oestrogen and progesterone receptors, indicating inverse relationships between the steroid hormone axis and the ErbB network.

Clinically, this crosstalk might be critical: patients treated with an anti-oestrogen drug were found to have a worse outcome if their tumours overexpressed ErbB2

CARCINOMA
A malignant tumour of epithelial origin.

PROGNOSIS
The likely outcome or course of a disease.

ANDROGEN-DEPENDENT PROSTATE CANCER
An early form of prostate cancer that is responsive to androgens and anti-androgen therapy.

AUTOCRINE
Activation of cellular receptors by ligands produced by the same cell.

GENE AMPLIFICATION
A differential increase in a specific portion of the genome. Amplification is associated with neoplastic transformation and acquisition of drug resistance.

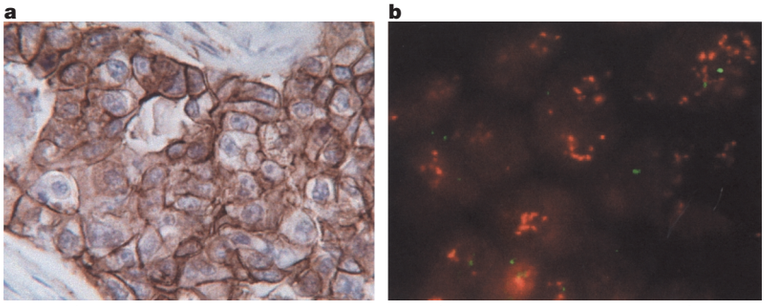


Figure 5 | **Molecular diagnosis of breast cancer. a** | Immunohistochemistry and **b** | fluorescence *in situ* hybridization (FISH) analysis of ErbB2 in human breast cancer. Immunohistochemistry was performed using HercepTest and FISH using a PathVysion ErbB2 DNA probe kit. The ErbB2 gene is seen as red fluorescence and the chromosome-17 centromeric α-satellite probe as green fluorescence. (Image courtesy of D. Eberhard, E. Huntzicker and B. Wright, Genentech, Inc.)

(REF. 61). On the one hand, *in vitro* studies indicate that overexpression of ErbB2 or NRG confers resistance to anti-oestrogens and renders cancer cells independent of oestrogen[62]. On the other hand, oestrogen suppresses transcription from the ErbB2 promoter, and specifically inhibits growth of ErbB2-overexpressing mammary cells[63]. Taken together, the molecular and clinical observations imply that the steroid and ErbB pathways are alternative, but functionally linked pathways that enhance cell proliferation (FIG. 2).

Neuregulin receptors. The catalytically inactive member of the ErbB family, ErbB3, is expressed in several cancers, but there is no evidence for gene amplification and overexpression is limited. However, a large recent study found that co-expression of ErbB2 with ErbB1 or ErbB3 in oral squamous-cell carcinoma was significant and it critically improved the predicting power[64], consistent with the non-autonomous role of ErbB3. Similarly, analysis of prostate cancer suggests the existence of a paracrine loop involving NRG1 and the ErbB2–ErbB3 heterodimer[65]. Some studies observed lower expression of ErbB4 in breast and prostate tumours relative to normal tissues, and an association with a relatively differentiated histological phenotype[66]. By contrast with epithelial tumours, childhood medulloblastomas often express ErbB4, whose co-expression with ErbB2 has a prognostic value[67], in line with the importance of receptor heterodimerization.

The network as a target for cancer therapy

The central role of the ErbB network in the development of solid tumours, its availability to extracellular manipulation, and detailed understanding of the underlying biochemistry have made the ErbB network an attractive target for pharmacological intervention (FIG. 4). Most efforts have concentrated on ErbB2 and ErbB1 owing to their increased expression in certain tumour cells relative to normal cells.

Immunological strategies. One approach — a humanized antibody to ErbB2 called Herceptin® — has been approved for clinical use, both alone and in combination with chemotherapeutic agents. In addition to downregulating surface ErbB2, Herceptin induces the cyclin-dependent kinase inhibitor $p27^{Kip1}$ and the Rb-related protein p130, which reduce the number of cells in S phase[68]. The recruitment and activation of immune effector cells to the ErbB2-overexpressing tumour might also contribute to Herceptin's mechanism of action[69].

Alternative approaches to the use of naked monoclonal antibodies to ErbBs include making antibodies toxic to cancer cells by linking them to radionuclides, toxins or prodrugs. Active immunization with portions of ErbB2 is another promising approach[70]. Monoclonal antibodies directed to a mutant form of ErbB1 (EGFRvIII) found in gliomas and carcinomas inhibit brain tumours in a manner dependent on the Fc receptor[71]. Comparison of two tumour-inhibitory monoclonal antibodies to ErbB1 revealed that only one depends on immune mechanisms; the other acts primarily by altering receptor functions. The chimeric version of this antibody, C225, competes with ligand binding to ErbB1 and arrests cultured cells at G1 because of an elevation in $p27^{KIP1}$ (REF. 72). This therapeutic antibody is now in late-stage clinical testing in patients with colorectal or head and neck cancers.

Low molecular weight inhibitors. The discovery of naturally occurring compounds capable of inhibiting the ErbB network (for example, herbimycin, genistein and emodin) led to the synthesis of analogues specific to the nucleotide-binding sites of ErbB proteins or their putative chaperones, the 90-kDa heat-shock proteins (Hsp90)[73]. The chaperone might escort ErbB proteins from the endoplasmic reticulum to the plasma membrane, where it might stabilize the active conformation of the kinase. The crystal structures of related kinases were used to enhance selectivity of synthetic tyrosine kinase inhibitors to ErbBs[73].

Both reversible and irreversible inhibitors[74] capable of discriminating between ErbBs and other kinases have been developed. When applied *in vitro* and in animal models, the compounds variably inhibited cell growth with some specificity for ErbB1- and ErbB2-expressing cells. At least five of these compounds are now being tested in human clinical studies. Because some studies indicated that Ras and Src are essential for transformation by ErbB proteins, FARNESYL TRANSFERASE INHIBITORS, Src-specific TYRPHOSTINS, MAPK inhibitors and Akt inhibitors might also be therapetically effective in containing activated ErbB pathways[75].

Gene therapy. Strategies aimed at blocking transcription, translation or maturation of ErbB transcripts or proteins are candidates for gene therapy. Early studies have shown that the adenovirus type 5 early region 1A (E1A) gene product can block ErbB2 overexpression and suppress the tumorigenic potential of ErbB2-overexpressing ovarian cancer cells[76]. This method is now being tested in a phase I trial with ovarian cancer patients. Intracellular single chain antibodies (scFvs) directed to either ErbB1 or ErbB2 can effectively inhibit

DUCTAL BREAST CANCER
Cancer arising from the lining of the milk ducts, as opposed to the lobules of the breast (lobular breast cancer).

ANEUPLOIDY
An abnormal number of chromosomes caused by their inaccurate segregation during cell division.

FLUORESCENCE *IN SITU* HYBRIDIZATION
Visualizing a genetic marker on a chromosome by using a fluorescently labelled polynucleotide probe that hybridizes to a gene on a chromosome during metaphase.

FARNESYLTRANSFERASE INHIBITORS
Inhibitors that block the activity of Ras by preventing the addition of a farnesyl group that targets it to the plasma membrane.

TYRPHOSTINS
A type of tyrosine kinase inhibitor.

receptor transfer from the endoplasmic reticulum to the plasma membrane, and thereby reduce signalling[77].

A human protocol for the treatment of ErbB2-positive ovarian cancer with scFvs has been developed following demonstration of selectivity and phenotypic effects *in vitro*[78]. Triplex-forming oligonucleotides that bind to a purine-rich sequence in the ErbB2 promoter are potent and specific inhibitors of ErbB2 transcription in an *in vitro* assay[79]. Antisense oligonucleotides[80], various dominant-negative mutants of ErbBs[81] and specific ribozymes[82] show specificity and efficacy in blocking receptor expression in cultured cells, and therefore might also prove useful as therapeutic lead compounds.

Perspectives

Successful treatments have been or are being developed to target aberrant ErbB receptor signalling in cancer; however, the potential for exploiting this pathway is still in its infancy. Antagonizing ErbB signalling might be a useful strategy for treating proliferative diseases other than cancer. One such opportunity might be coronary atherosclerosis. The migration of vascular smooth muscle cells in the arterial intima contributes to this cardiovascular disorder, particularly restenosis. Activation of the thrombin receptor is required for smooth muscle cell migration and proliferation, and activation of this G-protein-coupled receptor depends on transactivation by ErbB1 in response to heparin-binding EGF. Blockade of ErbB1 activation might therefore aid in the treatment of this disorder[83].

Another opportunity for intervention by targeted ErbB therapy might be psoriasis[84]. In normal skin, ErbB1 expression is restricted to the basal layer whereas in psoriatic skin, ErbB1 and one of its ligands, amphiregulin, are highly expressed throughout the entire epidermal layer[85]. Inhibition of ErbB1 activation might help control the spread or recurrence of psoriatic lesions.

In contrast to inhibiting ErbB signalling, potential also exists for activating the pathway in clinically meaningful ways. For example, ErbB ligands might promote wound healing[86]. ErbB signalling is also involved in fetal lung development, and appropriate activation of these pathways might benefit premature infants[87]. Neuregulins, which are also known as glial growth factors, are potent mitogens for Schwann cells[88]. Activation of Schwann cells with NRG might help resolve peripheral nerve injuries or neuropathies[89].

In summary, the ErbB field has made significant strides since Stanley Cohen's initial observation that EGF induces precocious eyelid opening in neonatal mice[90]. Although many of the individual molecules involved in ErbB signalling have been characterized, a full understanding of how the network functions in homeostasis — or malfunctions in a number of diseases — requires further definition. Regardless, the interface between basic and translational science has been established, and exploiting the ErbB pathway will probably yield other meaningful advances in the very near future.

Links

DATABASE LINKS ErbB1 | NRGs | ErbB3 | EGF | epiregulin | NRG1β | betacellulin | PI(3)K | Shc | p70S6K | PKC | Akt | Grb7 | *fos* | *jun* | *myc* | zinc finger | Sp1 | Egr1 | GABP | Grb2 | phospholipase Cγ | Src | Pyk2 | arrestin | Jak2 | interleukin-6 | TGF-α | *Drosophila* EGF receptor | NRG1 | Herceptin | $p27^{Kip1}$ | Rb | p130 | C225 | Hsp90 | amphiregulin | Vein | Gurken | Spitz | Argos

FURTHER INFORMATION The tumour gene database

ENCYCLOPEDIA OF LIFE SCIENCES *C. elegans* vulval induction | *Drosophila* embryo: dorsal–ventral specification

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