PERGAMON

European Journal of Cancer 37 (2001) S3–S8

European Journal of Cancer

www.ejconline.com

The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities

Y. Yarden*

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

Received 7 March 2001; accepted 26 June 2001

Abstract

Growth factors and their transmembrane receptor tyrosine kinases play important roles in cell proliferation, survival, migration and differentiation. One group of growth factors, comprising epidermal growth factor (EGF)-like proteins and neuregulins, stimulates cells to divide by activating members of the EGF receptor (EGFR) family, which consists of the EGFR itself and the receptors known as HER2–4. This highly conserved signalling module plays a fundamental role in the morphogenesis of a diverse spectrum of organisms, ranging from humans to nematodes, and has also been implicated in the development and growth of many types of human tumour cells. In humans, more than 30 ligands and the EGFR family of four receptors lie at the head of a complex, multi-layered signal-transduction network. Different activated receptor–ligand complexes vary in both the strength and type of cellular responses that they induce. Analysis of the multiple processes that modulate EGFR signal transduction, such as receptor heterodimerisation and endocytosis, has revealed new therapeutic opportunities and elucidated mechanisms contributing to the efficacy of existing anticancer treatments.

Keywords: Downregulation (physiology); Endocytosis; Neoplasms; Proto-oncogene proteins; Receptor; Epidermal growth factor; ErbB-2; Signal transduction

1. Introduction

The epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases lies at the head of a complex signal transduction cascade that modulates cell proliferation, survival, adhesion, migration and differentiation. While growth-factor-induced EGFR signalling is essential for many normal morphogenic processes and involved in numerous additional cellular responses, the aberrant activity of members of this receptor family has been shown to play a key role in the development and growth of tumour cells. This review highlights the complexity of the highly conserved EGFR signalling module, its central role in a diverse array of biological processes and the multiple mechanisms that modulate the strength and duration of EGFR signalling.

The EGFR family comprises four distinct receptors: EGFR/ErbB-1, HER2/ErbB-2, HER3/ErbB-3 and HER4/ErbB-4. These transmembrane receptors are composed of an extracellular ligand-binding domain and a cytoplasmic region with enzymatic activity [1]. This structure enables signals to be transmitted across the plasma membrane where they activate gene expression and ultimately induce cellular responses such as proliferation. The signal-transducing tyrosine kinase activity of the EGFR and related receptors is inactive when the receptors are in isolation. A number of different ligands, including EGF-like molecules, transforming growth factor (TGF)-α and neuregulins, activate the receptor by binding to the extracellular domain and inducing the formation of receptor homodimers or heterodimers (Fig. 1). Tyrosine residues on one receptor are presumably cross-phosphorylated by the other member of the receptor pair and then form docking sites for signalling complexes composed of cytoplasmic enzymes and adapter proteins. The subsequent dissociation of these signalling complexes releases activated effector and adapter proteins into the cytoplasm where they stimulate many different signal transduction cascades, such as the mitogen-activated protein kinase (MAPK) pathway, phosphoinositol kinase, the anti-apoptotic kinase Akt and several transcriptional regulators. Finally, the EGFR signal is inactivated pri-

* Tel.: +972-8934-3974; fax: +972-8934-2488.
E-mail address: yosef.yarden@weizmann.ac.il (Y. Yarden).

0959-8049/01/$ - see front matter
PII: S0959-8049(01)00230-1

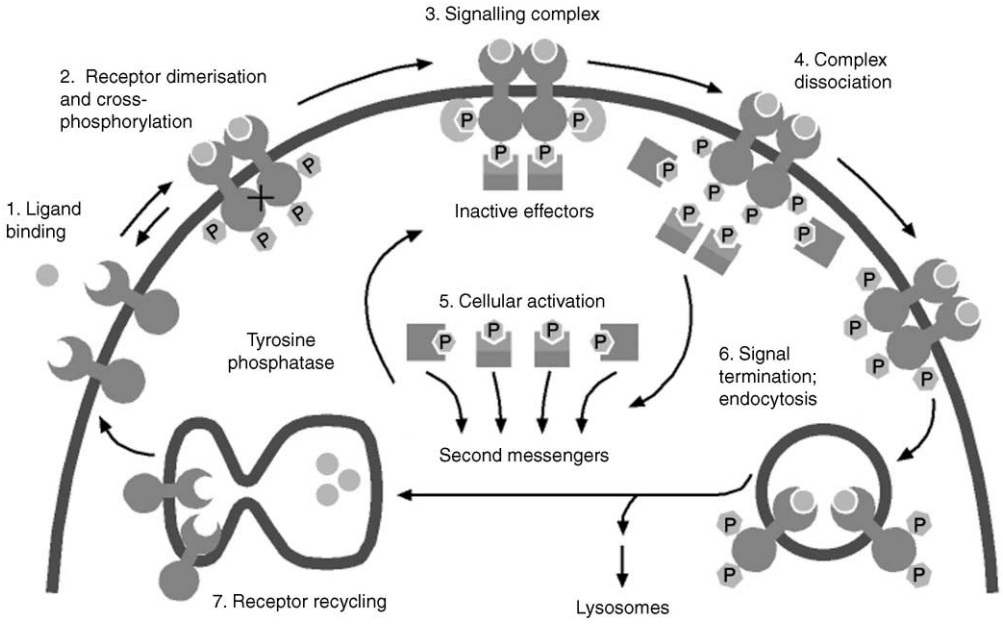

Fig. 1. The epidermal growth factor receptor (EGFR) signal transduction model. Ligand binding to receptors on the cell surface induces receptor dimerisation and cross-phosphorylation. A transient signalling complex composed of effector and adaptor proteins is then assembled. Dissociation of this complex leads to an enzymatic cascade culminating in gene activation and a cellular response. During signal termination, ligand–receptor complexes are internalised through clatherin-coated regions of the plasma membrane and then either degraded or recycled to the cell surface. Reprinted with permission from *Curr. Opin. Struct. Biol.*, 1991, **1**, 582–598 [3].

marily through endocytosis of the receptor–ligand complex. The contents of the resulting endosomes are then either degraded or recycled to the cell surface (reviewed in Ref. [2]).

In this signalling network, the major partner of EGFR is HER2 [4], and several mechanisms contribute to make HER2 heterodimeric signals particularly potent. First, activated heterodimeric complexes containing HER2 are more stable at the cell surface than are complexes containing other EGFR family members [5]. Although HER2 does not act as a receptor for EGF, it can decrease the rate of ligand dissociation from the cognate receptor, EGFR [6]. This results in stronger and more prolonged activation of the EGFR signalling network. Heterodimers containing HER2 also remain at the cell surface for a longer period of time, undergoing endocytosis at a lower rate than do EGFR homodimers. Furthermore, once the activated complex is internalised, HER2–EGFR heterodimers are targeted for recycling, while EGFR homodimers are destined for degradation. The recycling pathway returns receptors to the cell surface, ready for another cycle of activation and augments growth-factor signalling [5].

2. Morphogenesis—a conserved function of the EGFR signalling network

The EGFR signalling module has been highly conserved throughout the course of evolution. The pri-

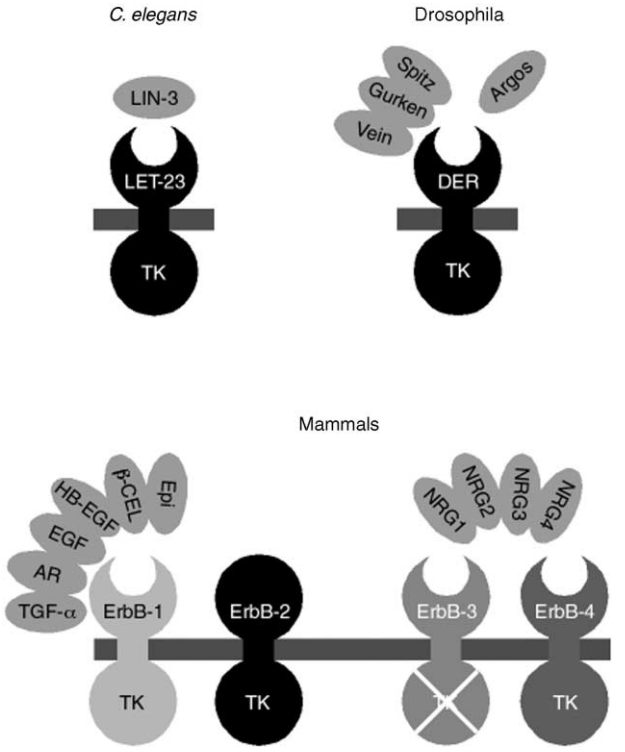

Fig. 2. Evolution of the epidermal growth factor receptor (EGFR)–HER module. During the course of evolution the EGFR signalling module grew in complexity from the single receptor and ligand found in *C. elegans* to the multiple receptors and growth factors expressed in mammals. ErbB-1 and ErbB-2 are alternative names for EGFR and HER2, respectively. DER, drosophila EGF receptor; NRG, neuregulin; AR, amphiregulin; EPI, epiregulin; β-cel, β-cellulin; HB-EGF, heparin-binding-EGF.

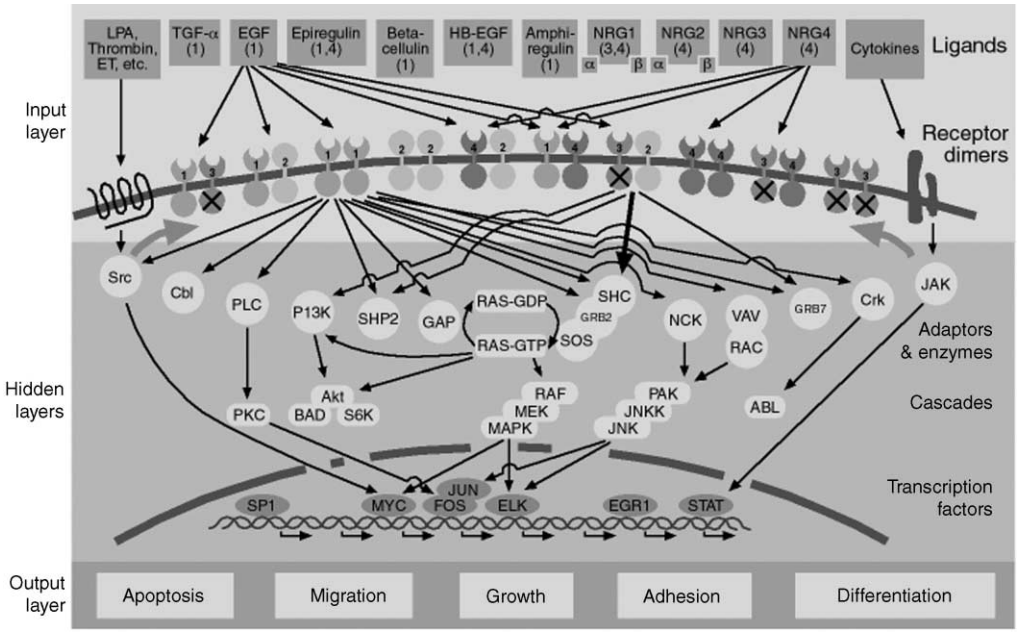

Fig. 3. The epidermal growth factor receptor (EGFR) signalling network. The EGFR signalling network is highly complex and consists of several layers. The input layer contains receptors and ligands, while multiple hidden layers of enzymes, adaptor proteins, second messengers and transcription factors lie beneath the cell surface. The output layer includes a variety of cellular responses. In most cases, the end result of EGFR activation is stimulation of cell growth. 1, EGFR/ErbB-1; 2, HER2/ErbB-2; 3, HER3/ErbB-3; 4, HER4/ErbB-4; LPA, lysophosphatidic acid; ET, endothelin. Reprinted by permission from *Nat Rev Mol Cell Biol*; **2**, 129–132, 2001 Macmillan Magazines Ltd [12].

mordial signalling unit found in the nematode *Caenorhabditis elegans* consists of a single EGF-like ligand known as LIN-3 and one receptor protein called LET-23 [7,8] (Fig. 2). In this organism, the EGFR network plays a central developmental role, determining the fate of several types of cells. The first function identified for this ancient signalling module was vulval induction, which occurs when the LIN-3 ligand secreted by an

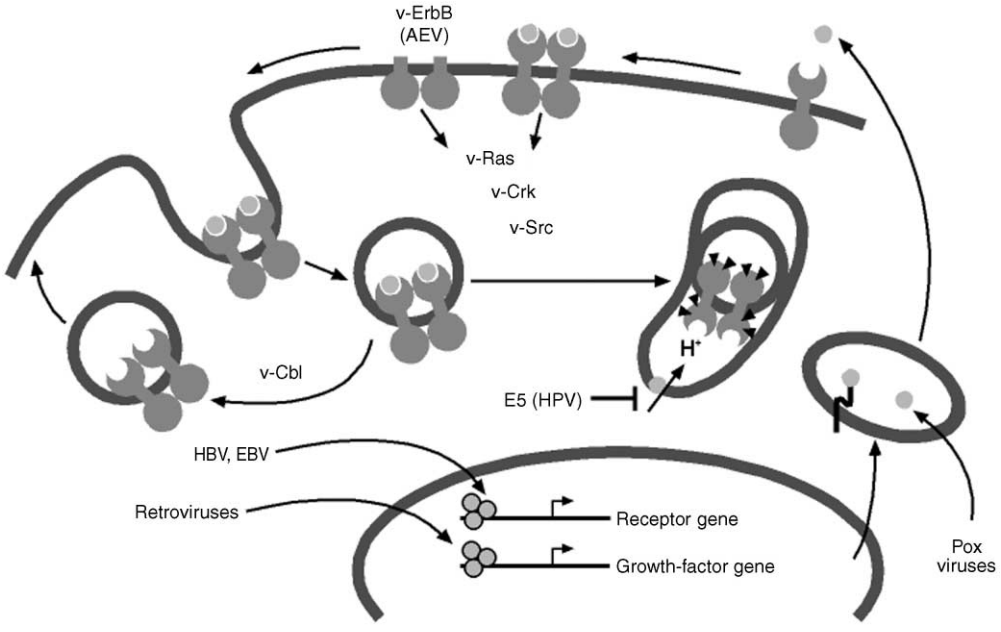

Fig. 4. Oncogenic viruses harness epidermal growth factor receptor (EGFR) signalling. A number of oncogenic viruses transform infected cells by activating the mitogen-activated protein kinase (MAPK) pathway and stimulating the proliferation of infected host cells. Different virally-encoded oncoproteins stimulate receptor activation, inhibit degradation or induce the expression of growth factors or receptors. Abbreviations: AEV = avian erythroblastosis virus; EBV = Epstein–Barr virus; HBV = hepatitis B virus; HPV = human papillomavirus. Reprinted by permission from *Nat Rev Mol Cell Biol*; **2**, 129–132, 2001 Macmillan Magazines Ltd [12].

anchor cell binds to LET-23 receptors on adjacent multipotent vulval precursor cells. The resulting inductive signal stimulates these cells to assume a vulval fate while surrounding vulval precursor cells that do not receive adequate LET-23 activation become part of the epidermis (reviewed in Ref. [9]).

Structural features of LIN-3—a characteristic motif of six cysteine residues forming three disulphide bonds—and the LET-23 receptor were retained in higher organisms while the complexity of the signalling network grew. Four ligands and a single receptor are present in insects such as *Drosophila melanogaster* (reviewed in Ref. [10]), and moving further up the evolutionary ladder, four receptors and at least 10 ligands have been identified in mammals (reviewed in Refs. [9,11]) (Fig. 2). The presence of multiple ligands and receptors in mammals imparts the signalling system with greater specificity and an expanded repertoire of potential responses, as the four receptors can potentially form ten distinct homo- and heterodimers that are activated by different ligands (Fig. 3).

Most data on EGFR function in mammals have come from studies with transgenic mice in which the expression of both receptors and ligands has been manipulated. These studies have shown that the EGFR signalling module plays an essential role in mammalian development. Mice lacking the EGFR have abnormal eyes and epidermal tissues and die due to defects in the development of epithelial organs [13–15]. Indeed, the mammalian EGFR signalling module is involved in the morphogenesis of many organs. For example, in the mouse intestine, the mesenchyme secretes several EGFR ligands that induce local epithelial cells to differentiate [16]. The EGFR signalling module is also involved in the later stages of mammary gland development that occur during pregnancy when EGF-like ligands secreted by the mesenchyme stimulate growth and branching of the mammary duct [17]. In contrast to the lethal effects of a disrupted *EGFR* gene, mice homozygous for a disrupted gene encoding *TGF-α* are viable and fertile, but display abnormal eye development and hair follicles [18,19]. Thus, the phenotypic effects of disrupting the function of the receptor protein rather than a single ligand are far more dramatic.

3. EGFR signal transduction—a complex network

EGFR ligands and receptors clearly play critical roles in many aspects of morphogenesis. However, the complexity of this signal transduction system in most cases precludes tracing a signalling event from its initiation at the cell surface with a ligand and receptor dimer through particular adapter and effector proteins to culminate in gene activation and a cellular response. Instead, the EGFR signalling module is perhaps best thought of in terms of a richly layered network (Fig. 3). The first level of this network is the input layer composed of ligands and receptors. Hidden layers made up of cascades of adapter and effector proteins are specific at the level of the inductive homo- or heterodimer. This specificity is also reflected at the level of transcriptional control and the eventual output, which may affect apoptosis, cellular migration, differentiation, adhesion or, in most cases, proliferation.

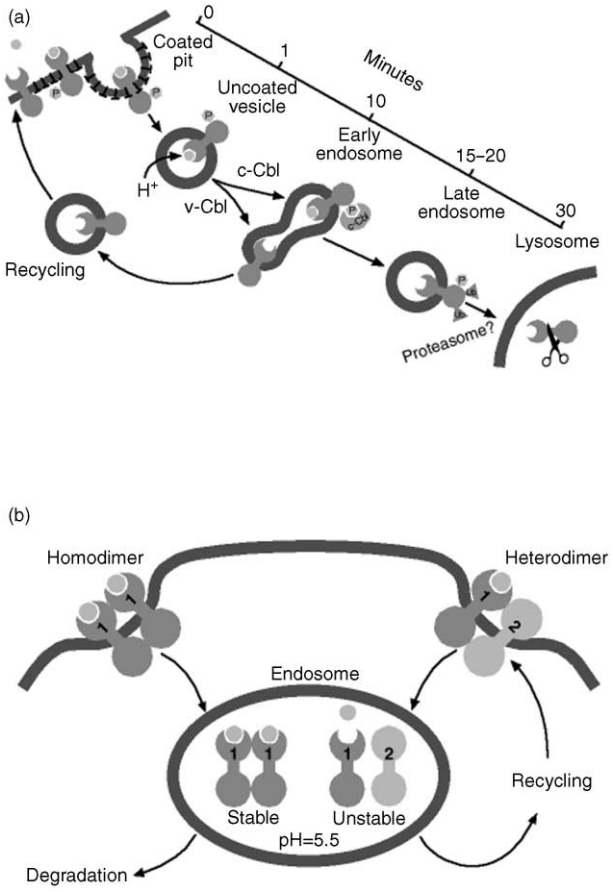

Fig. 5. Two factors determine the fate of internalised receptor complexes. (a) Cbl influences the fate of internalised epidermal growth factor receptor (EGFR). Once an EGFR–ligand complex is internalised in early endosomes, it can either be recycled back to the plasma membrane or targeted for ubiquitination and degradation. The Cbl ubiquitin ligase interacts with a phosphorylated tyrosine residue present on activated EGFR and shunts early endosomes through degradative pathways. In the absence of Cbl recruitment or if v-Cbl, the viral, oncogenic version of the protein, is expressed, the ligand–receptor complex will instead undergo recycling to the plasma membrane. Adapted with permission from *Genes Dev* [22]. (b) The stability of the activated ligand–receptor complex in the endosomal environment also affects receptor fate. Once in the sorting endosome, activated EGFR–HER2 heterodimers are relatively unstable. The ligand–receptor complex dissociates, releasing Cbl, and the receptors are recycled back to the plasma membrane. In contrast, stable EGFR homodimeric complexes remain associated with Cbl in the endosomal environment and are directed through an endocytic pathway that culminates in receptor degradation. Adapted with permission from *Adv Cancer Res* [24].

4. Oncogenic viruses harness EGFR signalling

Another avenue of research that has highlighted the importance of EGFR signalling is the study of viral oncogenes. Viruses exploit this signalling network in many different ways, altering both receptor tyrosine kinase activity and gene expression (Fig. 4). For example, hepatitis B virus and Epstein–Barr virus both activate EGFR expression during the invasion process. In contrast, avian erythoblastosis virus encodes a truncated form of EGFR which is constitutively active, while the human papilloma virus E5 appears to block the degradation of the activated receptor by inhibiting an endosomal ATPase. This returns internalised receptors to the plasma membrane where they may again bind ligand and stimulate proliferative pathways (reviewed in Ref. [20]). Pox viruses are large DNA viruses that encode soluble forms of growth factors that bind to the EGFR family of receptor tyrosine kinases. These growth factors contribute to virulence, but are not essential for viral replication. Although most viral growth factors have a lower affinity for the EGFR and related receptors than do their mammalian homologues, they act as more potent mitogens by evading normal receptor endocytosis, producing sustained EGFR phosphorylation and signalling [21].

5. Internalised ligand–receptor complexes are recycled or degraded

During signal termination, activated EGFR complexes are endocytosed in clatherin-coated pits. Two distinct processes have been identified that determine the fate of the internalised receptors (Fig. 5). The first of these processes involves a ubiquitin ligase known as Cbl. Recruitment of Cbl to ligand–receptor complexes in early endosomes targets receptors for lysosomal degradation by promoting receptor ubiquitination. In the absence of Cbl or when v-Cbl, the viral, oncogenic form of Cbl, is present, receptors are instead recycled to the plasma membrane [22]. A single tyrosine residue (tyrosine 1045) on the EGFR is essential for Cbl-mediated downregulation of EGFR signalling. Consistent with this model, mutation of Y1045 leads to potentiated EGFR signalling which is more mitogenic than that of the wild-type protein [23]. A second determinant of receptor fate, as mentioned previously, is the stability of the activated ligand–receptor complex in the mildly acidic endosomal environment. Activated EGFR homodimers are relatively stable and remain bound to Cbl. These relatively stable interactions result in endocytic sorting to lysosomes and receptor degradation. In contrast, EGFR–HER2 heterodimers are less stable and

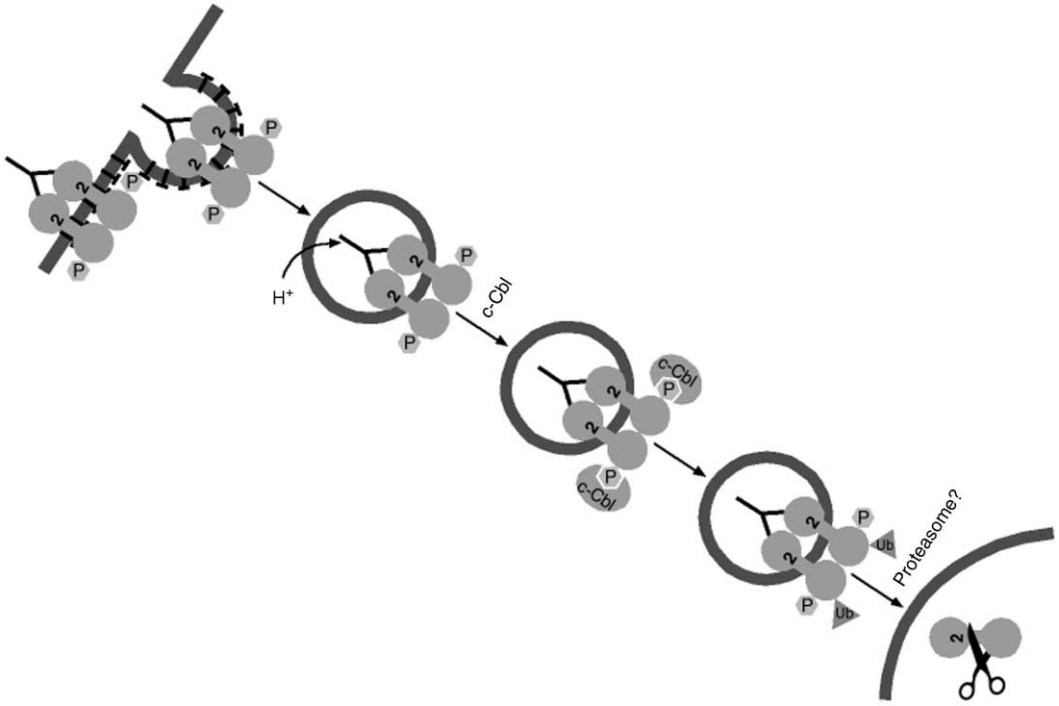

Fig. 6. Model of antibody-mediated receptor degradation. Anti-HER2 antibodies bind to receptors at the cell surface and induce HER2 internalisation. The receptors are then ubiquitinated and degraded through a process that requires both Cbl and HER2 tyrosine 1112. Antibody-induced receptor degradation may contribute to the efficacy of anti-HER2 monoclonal antibodies (MAbs) and is potentially a mechanism of action common to MAbs directed against other members of the EGFR family. Adapted with permission from *Genes and Development* [22].

uncouple in early endosomes, causing Cbl to dissociate from the receptor complex. The receptors then travel through the default recycling pathway which returns them to the cell surface [5].

A recent study by Klapper and colleagues [25] indicates that endocytic sorting, and Cbl in particular, play an essential role in the antitumour effects of antibodies directed against EGFR family members. In this study, treatment with L26, an anti-HER2 antibody with antitumour properties, was shown to promote the degradation of HER2 receptors in N87 human gastric cancer cells. The antibody-induced degradation of HER2 was accompanied by increased receptor ubiquitination and was dependent upon both Cbl expression and a putative Cbl docking site on the receptor at tyrosine 1112. This residue is homologous to EGFR Y1045. Similarly, EGF induced HER2 ubiquitination and degradation in a Cbl-dependent manner. This suggests that the mechanism of action of HER2-targeted immunotherapy, such as trastuzumab (Herceptin®; Roche) involves Cbl-mediated receptor degradation [25] (Fig. 6). It remains to be seen if antibodies specific to EGFR act in a similar fashion and direct EGFR to a degradative pathway by recruiting Cbl and elevating receptor ubiquitination.

6. Conclusion

The EGFR family of growth-factor receptors form part of a complex signal transduction network which is at the centre of many important cellular responses. This evolutionarily conserved signalling module plays a crucial role in the morphogenesis of many different organisms and also mediates a variety of cellular processes, including cell proliferation, migration, survival and adhesion. Decades of research have revealed multiple mechanisms that modulate the strength and duration of EGFR signals and shown that aberrant activation of this potent signalling module may promote the growth and development of cancer. Analysis of the mechanics of the EGFR signalling module has provided opportunities for the development of many novel cancer therapeutics designed to specifically inhibit EGFR signalling activity, such as anti-EGFR monoclonal antibodies (MAbs), tyrosine kinase inhibitors and molecules that bind to EGFR chaperones and induce EGFR and HER2 degradation.

References

1. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *Cell* 1990, **61**, 203–212.
2. Wells A. Molecules in focus: EGF receptor. *Int J Biochem Cell Biol* 1999, **31**, 637–643.
3. Yarden Y, Kelman Z. Transmembrane signalling receptors for cytokines and growth factors. *Curr Opin Struct Biol* 1991, **1**, 582–589.
4. Tzahar E, Waterman H, Chen X, *et al*. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol Cell Biol* 1996, **16**, 5276–5287.
5. Lenferink AE, Pinkas-Kramarski R, van de Poll ML, *et al*. Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers. *Embo J* 1998, **17**, 3385–3397.
6. Karunagaran D, Tzahar E, Beerli RR, *et al*. ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer. *Embo J* 1996, **15**, 254–264.
7. Aroian RV, Koga M, Mendel JE, Ohshima Y, Sternberg PW. The let-23 gene necessary for Caenorhabditis elegans vulval induction encodes a tyrosine kinase of the EGF receptor subfamily. *Nature* 1990, **348**, 693–699.
8. Hill RJ, Sternberg PW. The gene lin-3 encodes an inductive signal for vulval development in C. elegans. *Nature* 1992, **358**, 470–476.
9. Chang C, Sternberg PW. C. elegans vulval development as a model system to study the cancer biology of EGFR signaling. *Cancer Metastasis Rev* 1999, **18**, 203–213.
10. Schweitzer R, Shilo BZ. A thousand and one roles for the Drosophila EGF receptor. *Trends Genet* 1997, **13**, 191–196.
11. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER2/neu oncogene. *Science* 1987, **235**, 177–182.
12. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2001, **2**, 129–132.
13. Threadgill DW, Dlugosz AA, Hansen LA, *et al*. Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science* 1995, **269**, 230–234.
14. Sibilia M, Wagner EF. Strain-dependent epithelial defects in mice lacking the EGF receptor. *Science* 1995, **269**, 234–238.
15. Miettinen PJ, Berger JE, Meneses J, *et al*. Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* 1995, **376**, 337–341.
16. Birchmeier C, Birchmeier W. Molecular aspects of mesenchymal-epithelial interactions. *Annu Rev Cell Biol* 1993, **9**, 511–540.
17. Wiesen JF, Young P, Werb Z, Cunha GR. Signaling through the stromal epidermal growth factor receptor is necessary for mammary ductal development. *Development* 1999, **126**, 335–344.
18. Mann GB, Fowler KJ, Gabriel A, Nice EC, Williams RL, Dunn AR. Mice with a null mutation of the TGF alpha gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. *Cell* 1993, **73**, 249–261.
19. Luetteke NC, Qiu TH, Peiffer RL, Oliver P, Smithies O, Lee DC. TGF alpha deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. *Cell* 1993, **73**, 263–278.
20. Miller WE, Raab-Traub N. The EGFR as a target for viral oncoproteins. *Trends Microbiol* 1999, **7**, 453–458.
21. Tzahar E, Moyer JD, Waterman H, *et al*. Pathogenic poxviruses reveal viral strategies to exploit the ErbB signaling network. *Embo J* 1998, **17**, 5948–5963.
22. Levkowitz G, Waterman H, Zamir E, *et al*. c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev* 1998, **12**, 3663–3674.
23. Levkowitz G, Waterman H, Ettenberg SA, *et al*. Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol Cell* 1999, **4**, 1029–1040.
24. Klapper LN, Kirschbaum MH, Sela M, Yarden Y. Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors. *Adv Cancer Res* 2000, **77**, 25–79.
25. Klapper LN, Waterman H, Sela M, Yarden Y. Tumor-inhibitory antibodies to HER-2/ErbB-2 may act by recruiting c-Cbl and enhancing ubiquitination of HER-2. *Cancer Res* 2000, **60**, 3384–3388.