Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1766 (2006) 120–139
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.elsevier.com/locate/bbacan

Review

The complexity of targeting EGFR signalling in cancer: From expression to turnover

Sinto Sebastian [a], Jeffrey Settleman [b], Stephan J. Reshkin [c], Amalia Azzariti [a], Antonia Bellizzi [a], Angelo Paradiso [a,*]

[a] *Clinical Experimental Oncology Laboratory, National Cancer Institute, Via Amendola, 209, 70126, Bari, Italy*
[b] *MGH Cancer Center and Harvard Medical School, Charlestown, MA 02129, USA*
[c] *Department of General and Environmental Physiology, University of Bari, Bari, Italy*

Received 21 February 2006; received in revised form 8 June 2006; accepted 15 June 2006
Available online 23 June 2006

Abstract

The epidermal growth factor receptor (ErbB1 or EGFR) has been found to be altered in a variety of human cancers. A number of agents targeting these receptors, including specific antibodies directed against the ligand-binding domain of the receptor and small molecules that inhibit kinase activity are either in clinical trials or are already approved for clinical treatment. However, identifying patients that are likely to respond to such treatments has been challenging. As a consequence, it still remains important to identify additional alterations of the tumor cell that contribute to the response to EGFR-targeted agents. While EGFR-mediated signalling pathways have been well established, there is still a rather limited understanding of how intracellular protein–protein interactions, ubiquitination, endocytosis and subsequent degradation of EGFR contribute to the determination of sensitivity to EGFR targeting agents and are emerging areas of investigation. This review primarily focuses on the basic signal transduction pathways mediated through activated membrane bound and/or endosomal EGFR and emphasizes the need to co-target additional proteins that function either upstream or downstream of EGFR to improve cancer therapy.

Keywords: Cancer; EGFR; Cell signalling; Trafficking; Turnover; EGFR inhibitors

Contents

* Corresponding author. Tel./fax: +39 080 5555561.
E-mail address: a.paradiso@oncologico.bari.it (A. Paradiso).

0304-419X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbcan.2006.06.001

1. Introduction

Cancer arises from multiple distinct causes but typically involves a basic mechanism of immortalization with alteration of specific signalling pathways. ErbB family receptors, in particular EGFR, have been described as implicated in such immortalization in several solid tumors including breast, gastric, ovarian, non-small cell lung cancer, head and neck cancers, prostate and others [1].

The Epidermal growth factor receptor (EGFR also known as ErbB1, HER or EGFR) was the first receptor identified of the ErbB family of receptors [2]. Since then, the ErbB family proteins have increased to four, including EGFR-1 itself (HER-1, ErbB1), HER-2 (ErbB2), HER-3 (ErbB3) and human HER-4 (ErbB-4) [3]. EGFR has been associated with a variety of human malignancies [1,3–6].

Over-activated EGFR can convert a normal to a malignant cell by providing sustained signals for cell proliferation, anti-apoptosis, angiogenesis and metastasis, which are the basic properties of cancer [7]. Both theoretically and practically, it is possible to inhibit EGFR activation by several strategies and if successful, this could selectively eradicate cancer cells [8]. However, in cases where a tumor cell is not dependent on EGFR activity, such treatments would not be expected to produce clinical benefit. Therefore, there is a need to predict which patients are likely to benefit from anti-EGFR treatments [9].

From the clinical point of view, several strategies are now available to block EGFR receptor mediated oncogenic function. Two types of agents, monoclonal antibodies against EGFR and small molecule tyrosine kinase inhibitors are in clinical settings or furthest in development [10].

Pre-clinical studies suggest that both types of agents inhibit tumor cell proliferation and angiogenesis, induce apoptosis as monotherapy, and that there may be synergistic or of additive benefit when combined with conventional anticancer therapy [11–14]. With the exception of some EGFR mutation-positive lung cancers, there have been questions raised regarding the clinical benefit from EGFR-targeted treatments. EGFR and its associated heterodimeric partners produce complex signalling outputs, which knowledge is clearly needed for the optimal development of specific targeting therapies. In fact, it is unlikely that EGFR over-expression alone, which is seen in a high proportion of solid tumors, will correlate with drug sensitivity [9].

As there are four ErbB receptors, and each receptor can homo or hetero dimerise with other family members, it is difficult to predict which ErbB receptor combination needs to be targeted for an effective anti-cancer therapy. The total amount of each receptor present in the cell membrane determines, to some degree, its dimerising potential, subsequent signalling output, and endocytosis, highlighting the potential difficulty in targeting any one receptor as an anticancer therapy [15].

This review focuses on the complexity of EGFR signalling, from its regulated expression to receptor activation, endocytosis, and turnover, in the context of developing a better understanding of EGFR biology and the role of EGFR as a target for anticancer therapy.

2. EGFR and solid tumors

In the early 1980s, the discovery that alterations in the EGFR signalling pathway contribute to malignant transformation was initially made in studies of oncogenic viruses that demonstrated that the EGFR is the cellular homolog of the avian erythroblastosis virus v-erbB oncogene. This encodes a truncated EGF receptor (or closely related protein) lacking the external EGF-binding domain but retaining the transmembrane domain and the domain involved in stimulating cell proliferation [16–18]. Subsequently, it was discovered that activation of the EGFR signalling pathway mediates the malignant transformation of virus-infected cells [19]. Additional supportive evidence of the critical role of EGFR in oncogenesis comes from studies in transgenic mice: overexpression of TGF-α and/or neu, the mouse homolog of ErbB2, was associated with morphologic abnormalities in the mammary gland and with the development of spontaneous carcinomas, becoming markedly more frequent when simultaneous over expression of both proteins is present [20,21]. Furthermore, overexpression of members of the ErbB family, EGFR and ErbB2, has been demonstrated to induce malignant transformation in NIH-3T3 cells [22].

Malignant transformation as a consequence of EGFR dysregulation can occur in humans by different mechanisms, including receptor overexpression, activating mutations, alterations in the dimerisation process, activation of autocrine growth factor loops, limited or enhanced endocytosis of activated receptor, deficiency of specific phosphatases deactivating the phosphorylated EGFR tyrosine residues, and limited turnover. EGFR gene overexpression, without gene amplification, and EGFR activation, by TGF-α in an autocrine loop, are two of the main frequent mechanisms implicated in cancer development and progression [23,24].

Moreover, it has been reported that EGFR is mutated in some cancers and this is particularly prevalent in glioblastoma [25]. While multiple types of mutations have been found, one specific mutation is far and away the most common. This mutation results in the expression of a truncated EGFR designated

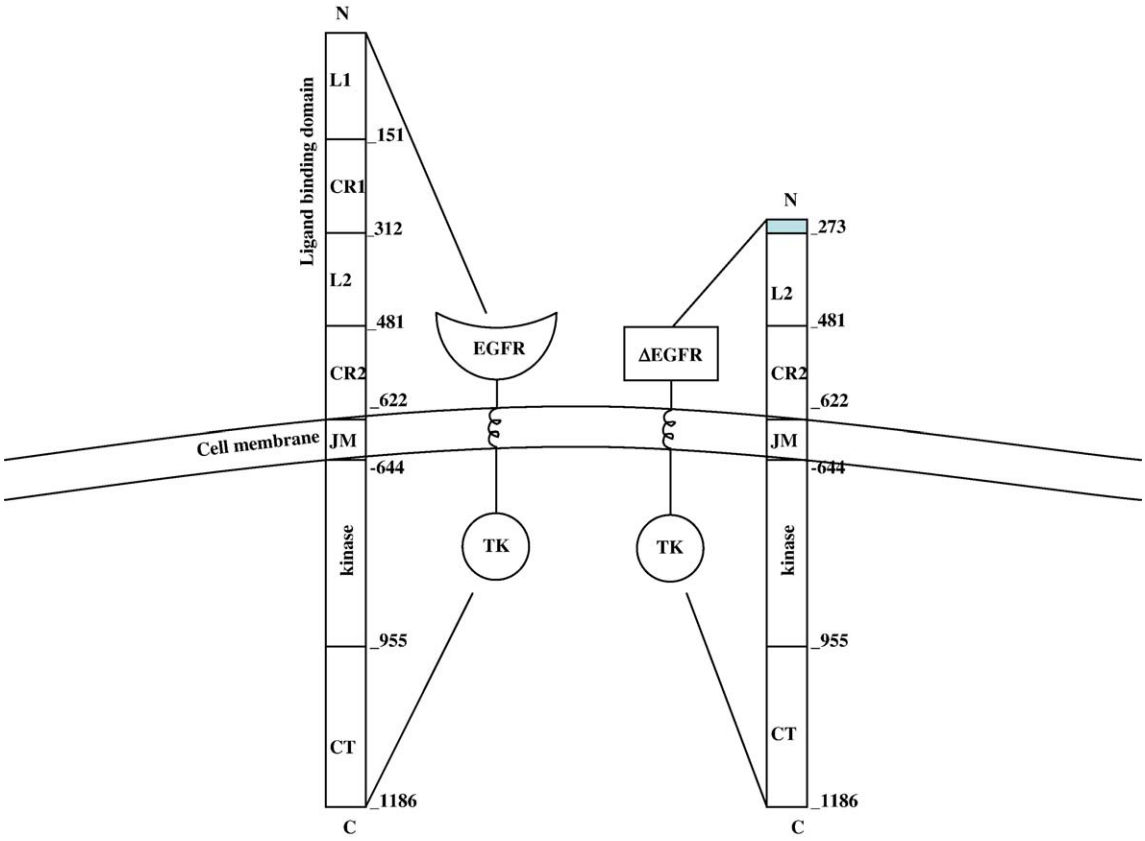

Fig. 1. Schematic representation of EGFR wild type and EGFRvIII. In-frame deletion of the coding exon results in a truncated mutant known as EGFRvIII lacks a portion of the ligand-binding region, is constitutively active and has lost some phosphorylation sites. The transmembrane domain (residues 622–644) is between the CR2 and the juxtamembrane domains. The abbreviations used: L and CR for the ligand binding and the cysteine-rich domains [also known as I(L1), II(CR1), III(L2), and IV(CR2) or S1(CR1) and S2(CR1), where L and S refer to large and small]; JM and CT, juxtamembrane domain and carboxy-terminal terminus.

EGFRvIII (also referred to as del2-7 EGFR or ΔEGFR in the literature) see Fig. 1 [26–28]. In this mutation, the information coded for by exons 2 through 7 of the 26-exon EGFR gene is lost. Transcription of the mutated gene gives rise to an mRNA with an 801 base pair deletion. This deletion is in-frame, and a new glycine codon is formed at the fusion junction. Translation of this mRNA in turn gives rise to an EGFR in which amino acids 6–273 are replaced by a single glycine residue, resulting in a 145-kDa glycoprotein with constitutive, ligand-independent activation of the receptor's tyrosine kinase activity [29,30] (see Fig. 1). Other than this well known truncated EGFR variant, single nucleotide somatic missense mutations, as well as small in-frame deletions and insertions have recently been reported in the EGFR tyrosine kinase domain. These somatic mutations (L858 and del747–752) also appear to be oncogenic in nature, and may provide insights into the potential role of altered EGFR signalling pathways in tumorigenesis [31,32].

Many cell types normally and widely express EGFR, including those of epithelial and mesenchymal lineages [33]. The variability in over-expression or dysregulation of EGFR has been also described for human malignancies [7,34]. Epidemiological evidence accumulated over the last 22 years in human tumors supports the notion that aberrant EGFR expression and signalling contribute to the development of multiple epithelial malignancies in humans (Table 1), including squamous carcinomas of the skin and breast cancer among others [35–39]. In particular, EGFR expression is dysregulated in at least 33–50% of human epithelial tumors [15,40–42]. This variability may be partially attributable to a lack of standardization in quantitation methodology, which typically utilise either simple immunohistochemistry or more sophisticated fluorescence in situ hybridisation techniques. Overall, it does appear that many of the most common human epithelial cancers express relatively high levels of EGFR at advanced stages of

Table 1
Percentage of different tumor types expressing EGFR

Tumor type	Tumors with expressed EGFR (%)	References
Head and Neck	90–95	[237]
Breast	82–90	[238]
Renal carcinoma	76–89	[239]
Cervix/uterus	90	[240]
Esophagael	43–89	[241]
Pancreatic	30–89	[242]
Non-small-cell lung	40–80	[243]
Prostate	40–80	[244]
Colon	25–77	[245]
Ovarian	35–70	[246]
Glioma	40–63	[247–248]
Bladder	31–48	[249]
Gastric	4–33	[250]

malignancy and increased metastatic potential of the disease [43,44].

However, the level of EGFR expression for a particular tumor appears to be less important than the degree of the activation of EGFR with regard to oncogenic potential as well as response to treatment [9]. Factors affecting activation status include receptor mutation, heterodimerisaton and increased expression of ligands [7] (Table 2). However, signalling complexity due to interactions among the four receptors and their binding with nearly ten ligands makes difficult to definitively measure signalling output for each receptor type and within individual human tumors [15].

3. EGFR in tissues development

The ErbB family has long been known to contribute to the development of a number of important organs and tissue systems [45,46]. Although not restricted to mammary tissue or epithelial cells, studies of ErbB isoforms suggest that each receptor isoform has a different function in gland development. In fact, while EGFR promotes ductal growth, signalling through ErbB2 and ErbB4 contributes to lobuloalveolar differentiation and lactation [47,48]. The development of transgenic mice has been invaluable as a source of information related to the physiologic role of these receptors. Interestingly, loss-of-function phenotypes differ dramatically depending on whether the targeted knockout disrupts EGFR-specific ligands or the receptor itself. For example, mice deficient for the gene encoding TGF-α develop wavy coats, curly whiskers and ocular aberrations including corneal opacity, external discharge and micro-ophthalmia [49,50]. The knockout of major three EGF related peptide growth factors (EGF, transforming growth factor (TGF)-α and amphiregulin) results in mice having similar physical abnormalities but a greater penetrance of defects. Remarkably, ligand-knockout mice have similar survival durations as their wild-type counterparts and are still able to reproduce. In contrast, knockout of the EGFR is uniformly lethal and mice show profound abnormalities of nervous, gastrointestinal, dermatological and respiratory systems [51]. Of further interest, it is the grave CNS morbidity described in mice models [52]: in a cohort of EGFR-null mutant mice, hemorrhagic lesions in the forebrain suddenly appear after birth followed, after 2 weeks, by a marked brain decreased size with respect to control mice. Deletion of the EGFR gene is strikingly pernicious as all live-born mutants succumb within 3 weeks after birth.

Table 2
Mutations of EGFR detected in tumor cells

Type	Alteration in sequence	References
EGFR L858R	Missense Mutation Leu858–Arg858	[231]
EGFR delL747–P753	The 18-base pair inframe deletion	[231]
EGFR vI	Translation starts at aa 543	[72]
EGFR vII	Deletion of aa 521–603	[72]
EGFR vIII	Deletion of aa 6–273	[72]
EGFR vIII/Δ12–13	Deletions of aa 6–273 and 409–520	[72]
EGFR vIV	Deletions of aa 959–1030	[72]
EGFR vV	Truncation at residue 958	[72]
EGFR.TDM/2–7	Tandem duplication of 6–273	[72]
EGFR.TDM/18–25	Tandem duplication of 664–1030	[72]
EGFR.TDM/18–26	Tandem duplication of 664–1014	[72]

4. EGFR protein structure

The EGFR gene encodes a transmembrane protein containing 1186 amino acids, of which 621 residues comprise the extracellular region (or ectodomain). The ectodomain is further divided into four sub-domains (see Fig. 1). Early binding studies proposed that the major ligand-binding site was located between amino acids 294 and 543, a fragment restricted primarily to L2 or domain III [53]. A follow-up study revealed that, in addition to domain III, a portion of domain I (or L1) appeared to play a role, albeit a minor one, in growth factor binding [54].

Determination of the crystal structure of the EGFR resulted in the identification of three ligand-binding sites within a C-shaped configuration between domains I and III (L1 and L2) [55]. The CR1 and CR2 (domain II and IV) domains consist of a number of small modules, each appearing to be held together by one or two disulfide bonds. A large loop that protrudes from the back of the CR1 domain makes contact with the CR1 domain of the other receptor in the dimer [55,56]. The first module of the CR1 and CR2 domains contain conserved tryptophan residues (Trp176 and Trp 492) that intercalate between the fourth and fifth helical turns of the β-helical L domain and sit in a hydrophobic environment that includes other conserved tryptophan residues (Trp 140 and Trp 453). An EGFR construct consisting of residues 1–476 lacks this second tryptophan interaction and does not bind ligand with high affinity [57]. Residues 557–617 in the CR2 domain are considered sufficient to target more than half of EGFR to the caveolae/raft component of the cell membrane prior to ligand binding [58].

The original assignment of the transmembrane domain to residues 622–644 was performed by visual analysis of the EGFR sequence [59], but other prediction methods indicate variation in the assignment of the boundaries of the transmembrane [60]. Nuclear magnetic resonance analysis of a peptide corresponding to the EGFR transmembrane domain and the beginning of the cytoplasmic domain indicate that residues 626–647 are α-helical, suggesting that the transmembrane α-helix continues into the juxtamembrane domain [60]. The juxtamembrane region appears to have a number of regulatory functions, including receptor dimerisation and ligand-dependent internalisation events [61], basolateral sorting of the EGFR in polarized cells [62], and association with proteins such as eps8 [63] or the inter-competitive association with calmodulin and protein kinase C (PKC) [64,65]. The carboxy-terminal domain of the EGFR contains tyrosine residues whose phosphorylation modulates EGFR-mediated signal transduction [66]. There are also several serine/threonine residues (and another tyrosine residue) where phosphorylation has been inferred to be important for receptor down-regulation processes and sequences thought to be necessary for endocytosis [67]. Residues 984–996 in the C-terminus have been identified as a binding site for actin [68] and may well be

involved in the formation of higher order receptor oligomers and/or receptor clustering after ligand activation of the kinase domain.

In addition to the structural features described above, post-translational modifications, as well as expression levels, play a role in determining the exact three-dimensional conformation of the EGFR important for ligand binding, dimerisation and signal transduction. Over 20% of the receptor's 170-kDa mass is N-linked glycosylated and this is required for translocation of the EGFR to the cell surface and subsequent acquisition of function [69]. Over-expression of the EGFR or altered glycosylation can reveal peptide epitopes suitable for antibody therapies [70]. The sequence identity of the EGFR family varies from 37% (53% similarity) for EGFR and ErbB3 to 49% (64% similarity) for EGFR and ErbB2 [71]. The amino acid identities can also vary significantly among the various domains, with the tyrosine kinase domains having the highest sequence identities (average 59–81% identity) and the carboxy-terminal domains having the lowest (average 12–30% identity). The three-dimensional folds of corresponding domains of the different EGFR homologues are expected to be similar with the possible exception of the heavily divergent C-terminal domains. The influence of the various mutations on the three-dimensional configuration of the receptor and its dimerisation and signal transduction potentials are difficult to study, but recent results, from well-characterized mutated EGFRs, have revealed that their signalling mechanisms and endocytic regulation is different when compared with wild-type EGFR [72–74].

5. ErbB receptors and its binding ligands

These receptors have an extracellular ligand-binding domain and a single hydrophobic transmembrane domain [75]. The intracellular domain of ErbB receptors consists of a highly conserved tyrosine kinase domain, although the kinase domain of ErbB-3 contains substitutions of critical amino acids and therefore lacks kinase activity [76]. The extracellular domains are less conserved among the four receptors, suggesting that they have different specificity in ligand binding [71,74,77]. Extensive receptor–receptor interactions and the existence of numerous ligands highlight the enormous potential for diversification of biological messages mediated by the ErbB family [77]. Growth factors that bind and activate the ErbB receptors belong to the EGF-family of growth factors [71,77]. This family of growth factors is characterized by the presence of an EGF-like domain composed of three disulfide-bonded intramolecular groups conferring binding specificity, and additional structural motifs such as immunoglobulin-like domains, heparin-binding sites and glycosylation sites. They are produced as transmembrane precursors that are biologically active and able to interact with receptors expressed on adjacent cells; subsequently, they are processed by proteolysis leading to the shedding of soluble growth factors [78].

Based on their affinity for one or more ErbB receptors, *EGF*-related growth factors can be divided into three groups [77,79]. The first group includes EGF, transforming growth factor α (TGF-α) and amphiregulin (AR), which bind to the EGFR; the second group includes growth factors that have dual binding specificity for EGFR and ErbB4, such as betacellulin (BTC), heparin-binding growth factors (HB-EGF) and epiregulin (EPR). The third group includes tomoregulin and the neuregulins (NRGs) or heregulins (HRG) that can be divided in two subgroups based upon their ability to bind ErbB3 and ErbB4 (NRG-1 and NRG-2) or only ErbB4 (NRG-3 and NRG-4) [80–84]. No direct high-affinity ligand for ErbB-2 has been described.

While ErbB receptors are usually located in the basolateral membrane of epithelial cells, most of the ErbB polypeptide ligands, such as EGF, TGF-α, AR, NRGs, HB-EGF, BTC and EPR, are also found in the extracellular matrix, suggesting that ErbBs play an important role in mediating signals between the epithelium and stroma [85–87]. Moreover, the expression and processing of the ligand precursor are highly regulated [87,88]. For example, transformation by active Ras, or exposure to steroid hormones leads to increased expression of several ErbB ligands, while cleavage of ligand precursors by a metalloproteinase can be stimulated by activated G-protein-coupled receptors (GPCR) [88,89].

Although, in general, formation of the ligand-bound complex induces receptor dimerisation and oligomerization, not all RTKs appear to be activated in the same way. Unlike the vascular endothelial growth factor (VEGF)–VEGF receptor 1 and the fibroblast growth factor (FGF)–FGF receptor complexes, in which the ligand is believed to drive dimerisation, a new receptor-mediated paradigm has emerged for monomeric EGFR [55,90]. The TGF-α–EGFR complex induces conformational changes in each receptor, which exposes a previously concealed interface, thereby promoting dimerisation through receptor–receptor interactions [91].

While all ErbB proteins have a similar transmembrane-spanning region, differences exist in terms of ligand specificity of the extracellular ligand-binding portion and in intrinsic tyrosine kinase activity of the intracellular catalytic kinase and regulatory domains [81]. 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, whereas ErbB2 seems to have no direct ligand [76,87], leading to the hypothesis that neither ErbB2 nor ErbB3 can support linear signalling. Most inter-receptor interactions are mediated by ligands, and ErbB2-containing heterodimers are formed preferentially [88,92]. 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 [93].

6. EGFR expression regulation in anticancer therapy

Several reports have revealed that two of the main mechanisms underlying over-expression of EGFR are gene amplification and/or increased transcription of EGFR. Transcription is clearly a requisite step in the over-expression of EGFR in the presence or absence of gene-amplified cases and this fact demands a detailed investigation of its transcriptional

regulation in cancer. Interestingly, the evidence of a more predominant EGFR expression in cells of epithelial origin (it is not expressed in haematopoietic cells), suggests that major transcription factors influencing EGFR over-expression could also be co-expressed with EGFR in various epithelial cancers.

The EGFR promoter region is a GC-rich, TATA-less regulatory region with multiple transcription initiation sites and specificity protein 1 (Sp1) binding sites [94]. Many DNA-binding factors have been identified that interact with the promoter region, including p53, activator protein2 (AP2) and interferon regulated factor-1 (IRF-1) [95]. AP-1 was determined to bind to at least seven sites in the promoter and to mediate phorbol ester induced EGFR expression [96]. Wild type and mutated p53 can differentially transactivate the EGFR promoter by binding to a response element present in the EGFR promoter. However, EGFR promoter sequence requirements for transactivation by wild type p53 and mutated p53 are different [97]. In addition to p53, its closely related homologue p63 has also been shown to regulate the EGFR promoter, through multiple encoded proteins (TAp63, ΔNp63). In specific, the p63 isoform, TAp63gamma, represses EGFR expression by directly interacting with Sp1 and impairing Sp1 binding to the target DNA [98]. Also, promyelocytic leukemia protein (PML) and ligand-activated thyroid hormone receptor (T3R) have been reported to repress EGFR transcription by impairing Sp1 binding to the EGFR promoter [94,99]. In addition, Sp1 mediated transactivation of the EGFR promoter can be enhanced by ligand-bound estrogen receptor alpha (Eralpha) [100]. Finally both Vitamin D and retinoic acid also have been shown to modulate EGFR expression; in particular, both Vitamin D and retinoic acid, after the binding to their respective nuclear receptors, can suppress EGFR expression in various cell contexts by binding a responsive element located within the EGFR promoter [101,102]. In this regard, it has been hypothesized that these agents could be of clinical utilisation to overcome the EGFR overexpression induced by long-term treatment with tyrosine kinase inhibitors [103,104].

7. Influence of EGFR dimerisation in anticancer therapy

The EGFR is known to activate with other ErbB receptors by ligand-induced and/or -independent homodimerisation or heterodimerisation [105,106]. All the various domains of EGFR can potentially influence its dimerisation properties but also some mutations may positively or negatively regulate EGFR dimerisation and activation [107]. A recombinant form of the EGFR, consisting of only the transmembrane and kinase domains, is capable of self-association demonstrating that both these domains have active roles in stabilizing the dimer [108].

The formation of heterodimers of the ErbB family in vitro is less well characterized than the formation of the EGFR homodimer. However, it is known that ErbB2 is the preferred interacting partner for the EGFR [92,109] and this interaction has been reported to reduce the rate of EGFR endocytosis and degradation [110,111]. It has been suggested that so-called heterodimers may actually be heterotetramers, possibly organized around a nucleating ErbB homodimer [112]. The formation of secondary hetero-oligomers can be induced by a ligand for a third ErbB protein. For example, EGF stimulates the formation of ErbB2–ErbB3 hetero-oligomers in cells that also express the EGFR [113,114]. In addition, EGF stimulation can induce EGFR to heterodimerise with ErbB2 and result in a higher phosphorylation of ErbB2 residue Tyr1248 and subsequent activation ErbB2 down-stream pathways [115]. The biological and clinical implications of these phenomena may be seen in tumors that co-express both EGFR and high levels of ErbB2 [116,117]. For example, the highly aggressive behaviour of lung adenocarcinomas may be due to EGF-mediated formation of EGFR and ErbB2 heterodimers compared with squamous cell carcinomas in which high EGFR expression is more prevalent and homodimers expected to be more dominant [92]. The preferential pairing of ErbB2 with EGFR or ErbB3 in breast and ovarian cancers could also be enhanced by a similar mechanism or by high-affinity cross-linking of heregulin with ErbB3 [118].

In addition to EGFR dimerisation with other ErbB receptor family members, EGFR can undergo direct interactions with other cell surface receptors, such as the platelet-derived growth factor (PDGF) receptor and the insulin-like growth factor receptor (IGFR) [119]. Recently, a reported association of EGFR with the Fas receptor has generated some excitement about a potential signalling mechanism by which EGFR can directly influence cell survival and death as a function of cell context [120–122]. How the dynamics of this dimerisation may influence anti-EGFR therapy is certainly of great interest.

In conclusion, the analysis of EGFR dimerisation dynamics could be relevant in the establishment and development of potent anti-EGFR drugs preventing active receptor dimer formation. For example, most anti-EGFR tyrosine kinase inhibitors, such as Gefitinib (AstraZeneca Pharmaceuticals, UK), induces the formation of inactive EGFR/HER2 and EGFR/HER3 heterodimers [123], leading to the hypothesis that anti-EGFR and anti-ErbB2 drugs could be used together [124]; moreover, in a tumor expressing both the death receptor (e.g. Fas) and EGFR, combined therapy with Apo2L/TRAIL ligand and EGFR tyrosine kinase inhibitors might result in a synergistic effect on tumor cell apoptosis. This seems also suggested by the evidence that death receptor-mediated apoptosis is inhibited by activated EGFR [125,126].

8. The EGFR kinase domain; a junction for originate multiple signalling pathways

Epidermal growth factor receptor (EGFR) signalling is initiated by ligand binding, followed by receptor homo- and hetero-dimerisation, and subsequent autophosphorylation by its kinase domain as well as phosphorylation of other cytoplasmic substrates. This process of receptor activation subsequently results in a signalling cascade that drives a wide range of cellular responses, such as cytoskeletal rearrangements, changes in gene expression, increased cell proliferation and anti-apoptosis [71,127].

Various ligands can differentially influence specific tyrosine sites of receptor activation [128]. For example, the EGFR

ligands betacellulin (BTC) and epidermal growth factor (EGF) promote phophorylation of different tyrosine residues within the EGFR c-terminal domain: i.e. BTC mediated pTyr1173 is markedly reduced compared with EGF stimulation reducing BTC stimulation of Shc tyrosine phosphorylation, Ras, and Raf-1 activation [128]. On the other hand, Y1068 phosphorylation is significantly increased after BTC stimulation, compared with EGF stimulation, leading in parallel to a greater extent of Erk phosphorylation. Although BTC and EGF share overlapping signalling properties, the ability of BTC to enhance Erk activation occurs independently from Ras and yields anti-apoptotic activity [128].

Besides these differences in tyrosine residue phosphorylation by different ligands, multiple residues of the EGFR cytoplasmic kinase domain are autophosphorylated upon EGFR dimerisation and, thereafter, recruit a variety of downstream substrates. Substrates for these autophosphorylated tyrosine residues include GRB-2 an adaptor that binds pY1068 and pY1086, the Shc adaptor that binds pY1148 and pY1173, the Dok-R adaptor that binds pY1086 and pY1148, phospholipase (PLC-γ), which is recruited by pY1173 and pY992, phosphatase (PTB-1B) which can interact with pY992 and pY1148, c-Cbl, which binds pY1045, the SHP-1 phosphatase which binds pY1173, and the Abl tyrosine kinase which binds pY1086 (see Fig. 2) [129]. Some of these adapters have multiple tyrosine binding sites within the EGFR c-terminal domain suggesting that their preferential binding site can vary depending on the presence of different dimerisation partners, the availability of particular receptor ligands, the expressional status of the adapter itself, and the presence of competing substrates.

Although the major tyrosine sites of the EGFR c-terminal domain seem to be autophosphorylated after ligand-induced dimerisation, some tyrosine residues are phosphorylated by other intracellular tyrosine kinases. For example, EGFR Tyr845,

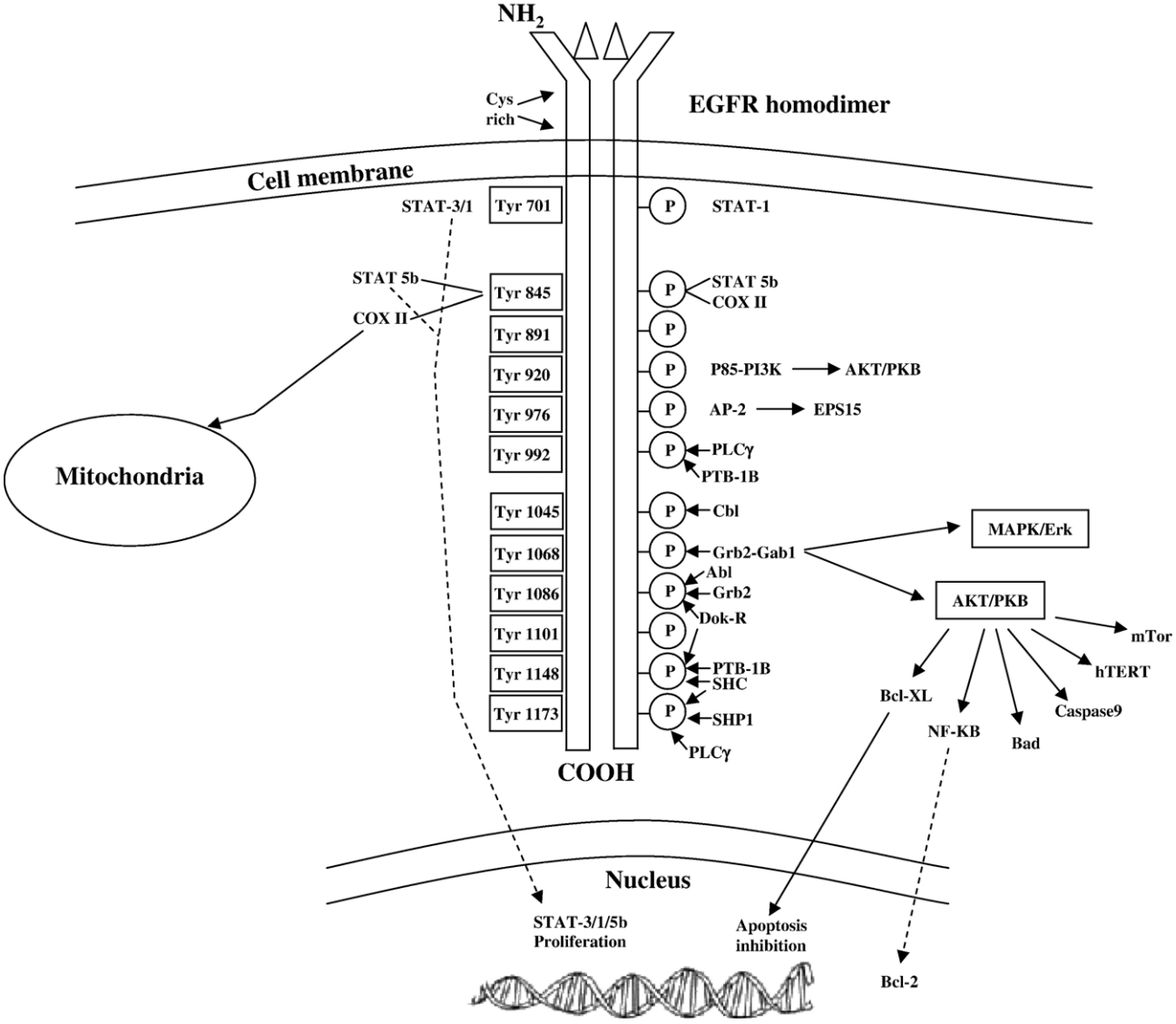

Fig. 2. The EGFR kinase domains. Schematic representation of major tyrosine residues can get phosphorylated at EGFR kinase domain after EGFR stimulation by EGF related ligands or autophosphorylated during homodimerisation. Possible adaptors and signalling proteins can get activated from each phosphorylated tyrosine residues and major signalling pathways can be generated from the junction of EGFR kinase domain.

Tyr891, Tyr920, and Tyr1101 have been proposed to be phosphorylated by the Src kinase following EGF stimulation [66,130] indicating that interactions between EGFR and the non-receptor tyrosine kinase c-Src may contribute to an aggressive phenotype in multiple human tumors [130,131]. Reports also provide strong evidence of association between c-Src and EGFR as an EGF–dependent heterocomplex in vivo; this interaction is mediated by Src's SH2 domain, which can directly bind to pY891, pY920 and pY1101 sites [131]. Biscardi et al. also reported that c-Src-mediated phosphorylation of EGFR Tyr845 is involved in regulation of receptor function, including its role in tumor progression [131]. One of the proposed binding targets for EGFR pTyr845 is Stat5b (a transcription factor known to be overexpressed in several tumors), potentially providing a direct link between EGFR at the cell surface and a transcriptional response [132]. Another pathway that appears to be regulated through Tyr845 following EGF stimulation is that mediated by the cytochrome-c oxidase subunit II (CoxII)-related trafficking of EGFR to mitochondria and contributing to regulate cell survival [133]. However, the exact molecular mechanism underlying this trafficking is unknown.

9. Major signalling pathways are integrated at the EGFR c-terminal domain

Depending upon the type of ligand and the EGFR dimerisation partner, several different signal transduction pathways can be engaged. The best studied pathways include the Ras/Raf/MEK/ERK and PI3K/PDK1/Akt pathways; but, the PLC-γ and JAK/STAT pathways can also be engaged by activated EGFR through overlapping or independent mechanisms [129]. However, it still remains a challenge to determine which are the principal pathways in cancer initiation and progression.

The most thoroughly studied EGFR downstream pathway is that involving Ras and Raf/MEK/ERK. The key player in EGF-dependent Ras activation is the adaptor protein Grb2, through its ability to associate with the receptor either directly via Y1068 and Y1086 [134,135] or indirectly, by binding to EGFR-associated, tyrosine phosphorylated Shc. It has been suggested that association of Shc with EGFR via its PTB domain, which leads to its tyrosine phosphorylation and to the recruitment of Grb2, is the critical step in EGF-dependent induction of the Ras/MAPK pathway [136]. Translocation of the Grb2/Sos complex to the receptor in the plasma membrane facilitates the interaction of membrane-associated Ras with Sos, resulting in the activation of Ras proteins (H-Ras, K-Ras and N-Ras). This stimulation induces, consecutively, the activation of Raf family kinases (A-Raf, B-Raf and Raf-1) [137,138], of the mitogen activated extracellular signal regulated kinases (MEKs) and consequently of Erk1 and Erk2. Finally, Erk1/2 kinases positively regulate cell proliferation by activating the major transcription factors associated with cell proliferation; e.g., c-Myc, and ribosomal subunit kinase (RSK) family isoforms [139,140].

In the modulation of balance between cell proliferation, apoptosis and senescence, the EGFR/PI3K/PDK1/Akt pathway is crucial in promoting sustained proliferation mediated by EGFR/Ras/Raf/MEK/ERK pathway (Fig. 2). The EGFR c-terminal domain contains Y920, which can provide a docking site for the p85 subunit of PI3K either directly or indirectly through binding to Grb2 [130]. PI3K generates phosphatidylinositol-3, 4, 5-tris-phosphate (PIP3), which recruits the protein kinase Akt and phosphoinositide-dependent kinase (PDK)-1 via their PH domains, which bind to PIP3. Phosphorylated Akt appears to be able to prevent programmed cell death through targeted inhibition (phosphorylation) of Bad (a pro-apoptotic member of the Bcl-2 family) and caspase-9 (an enzyme in the Fas-mediated death pathway) [141,142].

In addition to the Akt survival pathway, EGFR can regulate STAT pathways through two different mechanisms: a Janus kinase (JAK)-dependent and a JAK-independent mechanism [132,143]. Stimulation of EGFR induces Tyr701 phosphorylation of STAT1 and initiates formation of complexes STAT1 and STAT3 with JAK1 and JAK2. Thereafter, the STATs translocate to the nucleus within 15 min [143,144] and can potentially contribute to cancer cell survival via effects on gene expression. A JAK-independent mechanism of activation has also been proposed for STAT5b, which has a direct docking site at EGFR (Tyr845) [132].

EGFR-mediated signalling pathways have also been shown to contribute to the regulation of both angiogenesis and metastasis. One of the most interesting roles in tumor progression is the EGFR-mediated angiogenesis via up-regulation of vascular endothelial growth factor (VEGF) and metalloproteinase's (MMPs) [145]. Moreover, a PLCγ dependent pathway can directly influence metastasis when the EGFR cytoplasmic kinase domain (Tyr992) is phosphorylated. EGFR Tyr992 is the direct docking site of PLC-γ and, thus, EGF receptor-mediated activation of PLC-γ is believed to be critical for the reorganization of the actin cytoskeleton and for the initiation of an asymmetric motile phenotype [146].

EGFR-mediated signalling pathways are likely to be involved in multiple properties of tumor cells and, as a consequence, a better understanding of how the various EGFR-mediated signalling pathways contribute to tumorigenesis will certainly be of value in future efforts to target appropriate EGFR-mediated signals as a rational cancer therapy.

10. EGFR endocytosis and its importance in anticancer therapy

The functionality of all proteins is stringently regulated through various post-translational modifications, including ubiquitination and subsequent degradation [147]. Indeed, receptor activation not only engages the multiple positively acting pathways described above, but also sets in motion mechanisms that will ultimately terminate signalling [148]. Upon ligand activation of many tyrosine kinase receptors, such as the EGFR, there is a rapid decrease in the cell surface number of the receptor and an eventual decrease in the cellular content of activated receptors — a process known as down-regulation [149]. After activation of the EGFR by ligand, Cbl proteins are

recruited rapidly to the EGFR and mediate its ubiquitination [61,150].

Cbl proteins are a family of proteins that are conserved throughout metazoans, have E3 ubiquitin ligase activity and contain an amino-terminal phosphotyrosine binding (PTB) domain and a C3HC4 RING finger [67]. Cbl proteins can bind to the EGFR via two types of interactions: they can interact directly via their PTB domain with a phosphorylated tyrosine in the EGFR cytoplasmic tail (Y1045) or they can interact indirectly via binding to Grb2 [151,152]. Upon EGFR activation, Cbl proteins are tyrosine phosphorylated by Src kinases [153]. In addition, phosphorylation of Cbl proteins enhances their interaction with another adaptor protein, the 85 kDa Cbl interacting protein (CIN85) that is constitutively associated with endophilins implicated in the control of clathrin-mediated endocytosis [154,155]. After phosphorylation of clathrin by EGFR, the redistribution of clathrin to the cell periphery has also been described [156].

The monoubiquitylation of both EGFR-pathway substrates EPS15 and epsin is also regulated by EGFR [157]. As both EPS15 and epsin are required for EGFR internalisation, they probably function as a complex during EGFR internalisation [158]. However, a further twist to the story is that mono-ubiquitylated epsin-1 loses its capacity to bind to PtdIns (4, 5)

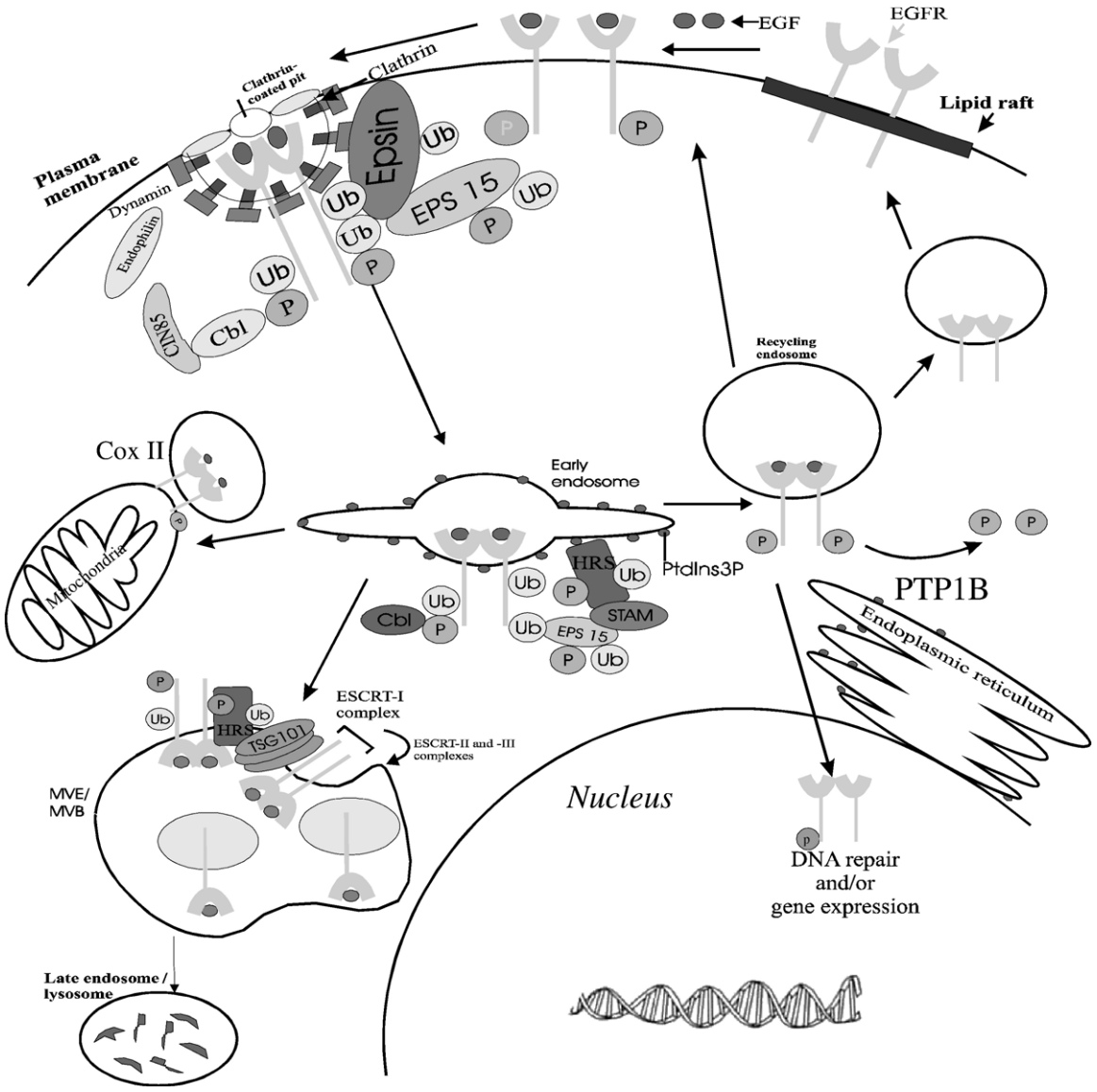

Fig. 3. Epidermal growth factor-receptors endocytosis, trafficking and turnover. From the cell surface, epidermal growth factor (EGF)-bound EGF receptors (EGFRs) might move out of lipid rafts. They are then internalised into clathrin-coated pits. Phosphorylated EGFRs recruit the E3 ubiquitin ligase Cbl, which multiubiquitylates EGFR and associates with Cbl-interacting protein of 85 kDa (CIN85) and endophilins. Later EGFR phosphorylates and induces the monoubiquitylation of EGFR-pathway substrate-15 (EPS15) and epsin, the latter of which interacts with adaptor protein-2 (AP2), and clathrin form an early endosome. From the early endosomes, EGFRs are recycled back to the cell surface after deactivation (dephosphorylation) and/or not at endoplasmic reticulum where PTP1 was located. Moreover, this endocytosised EGFR can move to mitochondrion in association with CoxII or move to nucleus depends upon different activation mode and cell contexts. In absence of recycling they are sorted towards the multi vesicular endosome/multivesicular body (MVE/MVB), and subsequently to late endosomes/lysosomes where they are degraded. P, phosphate; Ub, ubiquitin.

P2 and to interact with adaptor protein-2 (AP2) and clathrin, thus indicating that ubiquitylation might also promote the release of internalised EGFR from clathrin adaptors [159]. After internalisation into the early endosomes, EGFR can be sorted to the recycling endosome from which it travels back to the cell surface. Alternatively, the receptors can form a ternary complex together with EPS15, STAM (signal transduction adaptor molecule) and HRS (hepatocyte-growth-factor-regulated tyrosine-kinase substrate; also known as HGS) [160]. The latter directs the receptors to TSG101 (tumor susceptibility gene-101) and the ESCRT (endosomal sorting complex required for transport-I) complexes and then into the intralumenal vesicles of multivesicular endosomes, which results in EGFR degradation and signal termination [159,161] (Fig. 3).

An additional process for terminating EGFR-mediated cell signalling is the de-activation (de-phosphorylation) of endocytosed EGFR at the endoplasmic reticulum, where the protein tyrosine phosphatase-1B (PTP1B) resides [162]. However, at present, it is still not clear how endocytosis and trafficking of EGFR determine de-activation (dephosphorylation) and recycling back to the cell membrane and/or degradation in late endosomes (see the schematic representation, Fig. 3).

In addition to the above-described mechanisms positively influencing EGFR endocytosis, some adaptor proteins are reported to negatively regulate EGFR endocytosis, such as the Na+/H+ exchanger regulatory factor1 (NHERF1) and Sprouty2. NHERF1 stabilizes EGFR at the cell surface and retards receptor down-regulation [163]. The NH2-terminal PDZ domain (PDZ1) of NHERF1 specifically binds to an internal peptide motif within the 164-aa COOH-terminal regulatory domain of EGFR [163]. Stabilization of EGFR at the cell membrane may have some biological role, since membrane-bound EGFR is an important activator of the PLCγ pathway following serum stimulation, which may influence the metastatization process [164]. Moreover, competing interactions may exclude Grb2 from EGFR proximity as a means of elevating the activity of PLCγ [165]. Through this pathway, NHERF1 may stabilize EGFR at the cell membrane in a serum rich microenvironment and thereby promote increased metastasis thorough the PLCγ pathway, whereas in a serum-poor microenvironment, it may confer increased motility and invasion of breast cancer cells by activating the Na+/H+ exchanger isoform 1 (NHE1) pathway [166,167]. However, the precise nature of the interplay between these two pathways in low/high serum environments has yet to be explored.

Another well-studied inhibitor of Cbl-mediated endocytosis of EGFR is Sprouty2, an inducible regulator that becomes phosphorylated on a conserved tyrosine residue (Y55) after EGFR activation. This phosphorylated tyrosine functions as a docking site for the SH2 domain of Cbl, and competes with pY1045 within the activated EGFR [168–170]. Thus, Sprouty2 removes Cbl from activated EGFRs and blocks Cbl-mediated EGFR ubiquitination, endocytosis and degradation, which lead to sustained receptor signalling [169,170]. Whether or not NHERF1 and Sprouty2 negatively regulate EGFR endocytosis in a coordinated process or as independent mechanisms is also not known. It is worth noting that sorting of internalised ligand bound EGFR is not an all-or-nothing process. It involves multiple rounds of internalisation, sorting, recycling and re-internalisation.

In addition to the ligand-mediated EGFR endocytosis mechanisms described above, EGFR undergoes spontaneous metabolic turnover with a half-life of approximately 10–14 h in both fibroblasts and epithelial cells [156,171] and of 20–48 h in transformed cells [171,172]. Thus, on average, a receptor will cycle through the endocytic pathway dozens of times during its life span, with a low probability of being degraded. This disparity between the relatively high constitutive flux of EGFR through the endocytic pathway and the low probability of lysosomal degradation has significant consequences. It means that a small increase in the fraction of receptors shunted to the lysosomal pathway can have a large effect on receptor degradation rates [173]. Much less is known regarding the constitutive turnover of the other members of the ErbB family, but their behaviour appears similar to that described for the EGFR indicating that, in the absence of ligands, receptors are still internalised as a consequence of normal membrane flow through the cell [172,174].

Another important aspect in this regard, is how ErbB family receptor heterodimerisation influences ubiquitination and endocytosis. Activation of the EGFR causes c-Cbl association within the endosome compartment, but studies have failed to reveal a significant association of c-Cbl with ErbB2 [175]. Other studies indicate that EGFR/ErbB2 heterodimerisation inhibits c-Cbl binding [176]. This is consistent with the observed inhibition of EGFR degradation following ErbB2 overexpression [177]. It should be noted, however, that c-Cbl was found to stimulate ErbB2 degradation following a massive overexpression of this receptor [178]. Recent work has shown that the receptor (ErbB2) down-regulation induced by trastuzumab (Herceptin, Genentech, South San Francisco, CA, USA) involves recruitment of Cbl proteins to and subsequent ubiquitination of ErbB2 [178–180]. It is likely that the newly approved EGFR targeting antibody (Cetuximab) also enhances endocytosis-meditated receptor degradation and may exhibit similar clinical activity [181]. It may also be possible to develop strategies to inhibit the function of proteins such as Sprouty2 and NHERF1 [182], which can negatively regulate Cbl function and endocytosis of EGFR. An understanding of the mechanisms that negatively regulate growth factor signalling and endocytosis and characterization of the function of such negative regulators in both normal and transformed cells could lead to the identification of additional therapeutic targets for the treatment of human cancers.

11. EGFR signalling from the endosome: is there a link to cancer development?

Endocytosis of EGFR is initiated upon ligand binding but, as described above, multiple molecular factors are involved in the process. Endosome-associated EGFR is able to generate most, if not all, of its signal transduction output during its endocytosis [183–185]. Following EGF stimulation activated EGFR remains at the plasma membrane only briefly (5 to 10 min)

but remains associated with the endosome for a considerably longer time (1 h) [185,186].

Interestingly, signalling pathways activated by endosomal EGFR differ from those engaged by EGFR at the cell membrane [187,188]. EGFR endocytosis can direct membrane-bound EGFR to various cellular compartments, such as mitochondria, endoplasmic reticulum and, possibly, the nucleus [133,162]. Nuclear localisation of EGFR has now been confirmed by several groups [189,190], although a role for ligand-binding in this localization or a role in cancer has yet to be established. Notably, one study has reported that cancer cells exhibit relatively increased nuclear localization of EGFR in comparison with normal cells [190]. Recently, ionising radiation, but not stimulation with EGF, was reported to trigger EGFR import into the nucleus in a karyopherin-alpha-dependent manner. An increase in nuclear EGFR has also been observed after treatment with H_2O_2, heat or cisplatin, and EGFR nuclear import has been linked to the activation of DNA-dependent protein kinase during the DNA repair process [191].

Studies of EGFR signalling in the context of endocytosis have revealed that endosomally localized EGFR associates with many, if not all, of its downstream effectors, including SHC (SH2-domain-containing transforming protein) and Grb2 [159,192]. Furthermore, this leads to the recruitment of the Ras activator Son-of-Sevenless and to the endosomally localized activation of Ras, Raf, MEK1 and the mitogen-activated protein kinases (MAPK) cascade [192–194]. In addition, by blocking either cell-surface EGFR activation or its recycling, it has been established that internalised activated receptors are able to propagate signal transduction from the endosome to cell survival pathways [195]. Earlier studies proposed that PLCγ and PI3K activities are restricted to the cell surface and depend on the availability of PI (4, 5) P2 [196]. Accordingly, there is no evidence that enzymes such as Akt that engage lipid products of type 1 PI3Ks, are activated at the endosomal membrane. However, more recent signalling studies of endosomal EGFR have revealed that, similar to plasma membrane activation of EGFR, activation of endosome-associated EGFR supports cell survival by stimulating the PI3K-Akt pathway [195]. Ras can be activated in endosomes as well as at the plasma membrane and there are a number of corroborative reports linking internalised EGFR to the activation of Raf, MEK, and Erk. However, this relationship is complicated by Ras-independent mechanisms that contribute to the activation of this cascade [197]. Thus, Erk activation was strongly inhibited in HeLa cells expressing dominant-negative dynamin, which blocks EGFR endocytosis, as compared to cells over expressing wild-type dynamin [198].

Recent studies suggested that EGFR endocytosis is also important for functional STAT signalling. Upon stimulation of cell surface EGFR, Stat3 protein is recruited to activated receptors through an interaction between the Stat3 SH2 domain and phosphotyrosine docking sites on the EGFR [199]. Subsequent tyrosine phosphorylation of Stat3 occurs directly via the receptor kinase itself or indirectly by activation of intermediary kinases, including members of the Janus kinase (JAK) family, which are able to induce Stat3 dimerisation [199,200]. Another well-known Stat family transcription factor activity potentially activated through a direct interaction with the Y845 EGFR docking site is Stat5 [132]. Even though Stat3 and Stat5 transcription factors are activated by cell surface bound EGF receptor, it is unclear through which route these factors that regulate transcription of major anti-apoptotic and cell proliferative genes, takes to the nucleus. Recent studies support a model in which Stat3 is transported through the cytoplasm by EGFR-mediated endocytosis, and inhibition of receptor-mediated endocytosis by Amph A1, Epsin 2a or PAO leads to the abrogation of Stat3 transport to the perinuclear region, exclusion of functional Stat3 DNA-binding activity from the nucleus, and suppression of Stat3-mediated transcriptional events [201]. Altogether, such findings demonstrate the importance of EGFR endocytosis in Stat3 signalling, but the role of direct association with Stat5b in EGFR endocytosis still remains unclear.

In summary, endosomal EGFR signalling is important for many, if not all, aspects of EGFR-dependent signal transduction and EGFR internalisation can play a major role in determining specific signal generation, as well as the strength and duration of EGFR signalling. However, the relevance of endocystosis of EGFR to the excessive EGFR signalling seen in many human tumors still remains unclear. Moreover, the precise role of EGFR over-expression or mutations in endocytosis and ligand-independent EGFR signalling in cancer remains an important area for future research [183].

12. Targeting EGFR for anticancer treatment

Targeting EGFR as an option in the management of cancer was initially proposed in the 1980s [2]. Subsequently, advances in our understanding of the biology and regulation of EGFR and its family members has led to the development of several anti-EGFR strategies for cancer treatment and this is now a crucial area of clinical study for the treatment of various solid tumors. There are several potential strategies targeting the EGFR, including monoclonal antibodies (mAbs) directed towards the extracellular domain of EGFR, such as cetuximab (Erbitux) [202] see Table 3, and small molecule tyrosine kinase inhibitors that interfere with receptor signalling (TKIs) by targeting the catalytic kinase domain of EGFR [8] such as gefitinib (Iressa) and erlotinib (Tarceva) see Table 4. In addition to the above-mentioned clinically approved strategies, approaches using antisense oligonucleotides and ribozymes that block receptor translation [203,204] or disruption of receptor trafficking to the

Table 3
Major Anti-EGFR antibodies in clinical trials

Agent	Type	Trade name	Institution/Company
Cetuximab	Chimeric IgG1	Erbitux	ImClone Systems
ABX-EGF	Human IgG2	Panitumumab	Abgenix
EMD 72000	Humanized IgG1	Matuzumab	EMD Pharmaceuticals
MDX-447	Bispecific antibody	–	Merck KgaA
TheraCIM hR-3	Humanized	TheraCIM	YM Biosciences/CIM
Mab 806	Anti-EGFR vIII	–	Ludwig Institute

Table 4
Major small molecule Anti-ErbB2 inhibitors in clinical trials

Agent	Target	Trade name	Company/Institution
ZD1839	EGFR (ErbB1)	Gefitinib/Iressa	AstraZeneca
OSI-774	EGFR (ErbB1)	Erlotinib/Tarceva	OSI/Genentech/Roche
CI-1033	pan ErbB	Canertinib	Pfizer
EKB-569	EGFR/ErbB2		Wyeth Ayerst
GW2016	EGFR/ErbB2	Lapatinib	GlaxoSmithKline
PKI-166	EGFR/ErbB2		Novartis
HKI-272	EGFR/ErbB2		Wyeth Ayerst
AG-1478	ErbB1		Merck KgaA

cell surface with intracellular single chain Fv antibody fragments [205] are currently in development. In addition to discussing the various strategies being developed to antagonise EGFR activation, we will also discuss below additional issues associated with the clinically approved strategies.

12.1. Anti-EGFR antibodies

Cetuximab (Erbitux; ImClone Systems; formerly known as C225) is the most extensively studied and clinically approved chimeric mAb designed to specifically inhibit EGFR [202]. Cetuximab exhibits a 2-log higher affinity for EGFR than EGF itself, thereby inhibiting EGF binding and receptor autophosphorylation and inducing its internalisation and degradation [206–208]. In addition, cetuximab blocks the production of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), IL-8 and beta-fibroblast growth factor [209]. In preclinical models, cetuximab inhibits the proliferation of a range of human tumor cell lines in a dose-dependent manner [210,211]. This inhibition of cell proliferation reflects cell-cycle arrest in the G1 phase and/or an increase in apoptosis [212]. The increase of p27KIPI and an endogenous cyclin-dependent kinase inhibitor provides the basis for interpretations of the molecular mechanism of inhibition of cell growth in G1 phase following blockade of the EGFR [213]. The growth inhibitory impact of cetuximab in tumor xenograft models is often more pronounced than that observed in cell culture, suggesting that additional anticancer mechanisms are involved. One such mechanism involves anti-angiogenesis [42]. In tumor xenografts, Cetuximab inhibits vascular endothelial growth factor (VEGF) production in epidermoid carcinoma cells, resulting in a reduction in the number of tumor blood vessels and consequent inhibition of tumor growth. Furthermore, down regulation of VEGF, interleukin (IL)-8 and basic fibroblast growth factor (bFGF) expression by Cetuximab were also shown [214].

Cetuximab has been evaluated in clinical trials both as a single agent and in combination with conventional chemotherapy or radiation therapy. Because EGFR inhibition and conventional anticancer therapy act potentially via different cytotoxic mechanisms, theoretically, combination therapy offers the potential advantage of additive or synergistic activity without overlapping toxicity profiles [215]. A Phase I study performed to determine the tumor EGFR saturation dose of cetuximab [216], administrated i.v. at doses of 200–400 mg/m2 with a mean half-life of 114 h (range 75–188 h) which argues for weekly administration. Using tyrosine kinase activity as a surrogate marker, the investigators also observed a significant reduction in receptor phosphorylation following the third weekly dose (250 mg/m2). Adverse effects were minimal with a maximum tolerable dose (MTD) not reached. Antibodies against cetuximab were detected in only one patient out of total 52 subjects treated [216]. Importantly, toxicity was relatively mild, with acne form rash, fatigue and hypersensitivity reactions being the most frequent drug-related toxicities. Recent studies on colorectal cancer patients have revealed symptomatic hypomagnesaemia in cetuximab treated patients suggesting the need of serum electrolyte chemistry analysis during the administration of cetuximab [217].

In a large Phase III trial of cetuximab alone or in combination with irinotecan in patients with metastatic colorectal cancer who had progressed on irinotecan-containing regimen, the combination led to a response rate of 10.8% [218]. This led to the approval of cetuximab for the treatment of metastatic colorectal cancer. In another phase III trial in patients with squamous cell carcinoma of the head and neck, patients with loco-regionally advanced disease were randomized to receive either radiation alone, or radiation and weekly cetuximab. The addition of cetuximab to high-dose radiation resulted in a significant prolongation in overall survival without any increase in the overall toxicity profile [219,220]. There are a number of ongoing trials evaluating the efficacy of cetuximab alone and in combination with other targeted agents such as bevacizumab and cytotoxic chemotherapy in NSCLC, metastatic/recurrent squamous cell carcinoma of the head and neck, metastatic colorectal cancer and advanced gynaecological malignancies (clinicaltrials.gov). These combinations seem to be well tolerated [216,221] but efficacy data for these trials is only now becoming available.

In an effort to predict the response of individual patients to cetuximab, Vallbohmer et al. tried to correlate the response to cetuximab with mRNA expression levels of various members of the EGFR pathway in patients with metastatic colorectal cancer [222]. They observed that higher VEGF gene expression levels were associated with resistance to cetuximab and, furthermore, that the combination of low gene expression level of cyclooxygenase-2, EGFR and IL-8 was associated with a significantly better overall survival in cetuximab treated patients.

In addition to cetuximab, several monoclonal antibodies have already entered in Phase I clinical trials; among them Panitumumab (Abgenix Inc./Amgen, Inc), Matuzumab (EMD 72000) and h-R3 are closest to clinical development [223]. Further clinical studies will be required to identify the most appropriate antibody-based treatments for particular tumor types as well as for particular patients.

12.2. Small-molecule TK inhibitors

A second approach to inhibit the EGFR signalling network that has been widely investigated in clinical trials utilises agents blocking the activation of the EGFR tyrosine kinase (TK) domain. The basic mechanism of action of these agents is their competitive inhibition of the binding of ATP through interaction

with the TK domain of the receptor resulting in selective inhibition of EGFR autophosphorylation [11,224]. The tyrosine kinase inhibitors (TKIs) are synthetic, mainly quinazoline-derived and of low molecular weight, and they interact with the intracellular tyrosine kinase domain of several receptors including EGFR, where they inhibit ligand-induced receptor phosphorylation by competitive binding with the intracellular Mg-ATP-binding site [11]. Novel compounds with improved pharmacologic properties were discovered using a molecular modelling approach and were tested in pre-clinical studies. Several such small molecule tyrosine kinase inhibitors are currently being tested in clinical settings and a few have now been approved for clinical use.

12.3. Gefitinib (Iressa or ZD1839)

Gefitinib, the first small molecule EGFR tyrosine kinase inhibitor was approved for non-small lung cancer treatment by the US Food and Drug Administration (FDA) in May 2003 and was subsequently approved in many other countries including Japan, Australia, and countries in Europe. Gefitinib (4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline) is an orally active, low molecular weight (447 kDa), synthetic anilinoquinazoline that inhibits several receptors tyrosine kinases, particularly EGFR [11,224]. Gefitinib inhibits the kinase activity of isolated EGFR with an IC_{50} in the nanomolar range. However, higher concentrations may be required to block EGFR activity in vivo, due to the high intracellular concentration of ATP [224]. At concentrations more than 100-fold greater than those required for inhibiting EGFR, gefitinib inhibits other tyrosine kinase receptors, including ErbB2 [225]. Interestingly, gefitinib has been reported to inhibit autophosphorylation of ErbB2 with an IC_{50} of 1 μM in breast cancer cells in which ErbB2 is preferentially activated by heterodimerisation with EGFR [225,226]. Gefitinib demonstrates antiproliferative activity in tumor cell cultures and in human tumor xenografts, both as a single agent and in combination with cytotoxic chemotherapy. When utilised in combination with other drugs gefitinib shows additive or even synergistic effect [12–14,227]. The additive or synergistic effects of gefitinib achieved in combination therapy did not necessarily require high levels of EGFR expression in the tumor models tested.

Studies indicate that gefitinib affects many of the same intracellular signalling pathways inhibited by anti-EGFR mAB therapy. Gefitinib inhibits the growth of a range of human cancer cells in vitro and in vivo, and there is evidence that the inhibitor acts by inducing cell cycle arrest and/or apoptosis [12,228,229]. As with the EGFR-specific mAbs, Gefitinib appears to indirectly inhibit angiogenesis since treatment is associated with growth inhibition of human colon, breast, ovarian, and gastric cancer cells in vitro, accompanied by a in vivo reduction in vascular endothelial growth factor (VEGF) and tumor growth factor alpha (TGF-α) production [11]. Although gefitinib treatment was associated with the rapid regression of established tumors, the tumors re-grew when the drug was discontinued, suggesting that long-term drug administration will be required to maintain a tumor response in patients. Based on the early clinical activity observed in the phase I studies, anti-EGFR TK inhibitors (TKIs) were preferentially studied in patients with advanced NSCLC.

Two large Phase III trials evaluated the role of gefitinib in the treatment of chemotherapy-naïve patients with advanced stage NSCLC. In the first study, Giaccone et al. randomized 1093 patients to a combination of gemicintabine, paclitaxel and either gefitinib or placebo but no difference in median survival, median time to progression and response rates were observed [230]. A recent randomized placebo-controlled trial with gefitinib in patients with advanced NSCLC unable to tolerate chemotherapy showed no survival advantage with gefitinib either in the general population or in patients with adenocarcinoma with an overall response rate in patients who received gefitinib of 8.2%. As gefitinib did not show activity in a majority of patients, but did show a rapid and dramatic activity in particular sub-sets of patients, Lynch et al. tried to investigate the molecular mechanisms responsible for the sensitivity of gefitinib [231]. They examined the primary tumors from patients with NSCLC for mutations in the EGFR gene and found that eight of nine patients with gefitinib-responsive lung cancer had somatic mutations in the tyrosine kinase domain of the EGFR gene [232].

The identification of somatic activating mutations associated with gefitinib hypersensitivity has established a new paradigm for mutated EGFR signalling in cancer and Gefitinib sensitivity [73,232]. It was initially hypothesized that the mutated kinase simply presented greater access to the inhibitory action of Gefitinib; however, further studies addressing the signalling properties of these EGFR mutants revealed that they exhibit qualitative changes in their signalling output resulting in a selective activation of anti-apoptotic survival pathways. Indeed, inhibition of mutated EGFR expression by allele-specific SiRNA “knockdown” revealed that tumor cells expressing mutant EGFR exhibit a strict dependency on the mutant EGFR for survival [73]. As described above, the EGFR c-terminal domain contains several tyrosine phosphorylation sites that are differentially activated (phosphorylated) as a function of autocrine and/or paracrine ligand binding, interaction with the various dimerisation partners, and effects of intracellular Src kinase. Notably, Gefitinib does not cause the dephosphorylation of pre-existing activated receptor, but instead, it inhibits phosphorylation on these residues by competing with ATP after TKI inhibitor (Gefitinib or Irlotinib) treatment. As such, we suggest the possibility that increased EGFR expression and endocytosis (leading to de-phosphorylation or turnover) should be a reliable marker for predicting Gefitinib sensitivity. While such a relationship remains largely unexplored, recent reports have indirectly provided support for this hypothesis. For example, in transient transfection studies, phosphorylation of the wild-type EGFR is inhibited of 50% by 0.1 μM gefitinib and of 100% by 2.0 μM; conversely, the respective values for mutant EGFR (L858R and del747–752) were approximately 0.015 and 0.2 μM [231]. Furthermore, EGFR gene amplification has also been recently reported in NSCLC and it has been shown to correlate with Gefitinib sensitivity [74]. More interestingly, in all mutated EGFRs exhibiting hypersensitivity

to Gefitinib, several reports have revealed increased phosphorylation at EGFR Tyr1068 compared with wild type. Grb2 adaptor is more interesting in these aspects due to its specific interaction at Tyr1068 of EGFR c-terminal domain and to its interacting properties with several proteins substrates including c-Cbl, Gab and the p80 subunit of PI3K. Moreover, mutant Grb2 or mutation at Tyr1068 also abolishes c-Cbl mediated endocytosis of EGFR [233]. In conclusion, increased phosphorylation of pY1068, Grb2, c-Cbl mediated ubiquitination and subsequent down regulation of EGFR may reliably predict Gefitinib sensitivity. In addition to the well-studied tyrosine kinase inhibitor, Gefitinib, several other TK inhibitors listed in Table 4 are also being explored in clinical settings [234].

13. Concluding notes

Many years of experimental study from a large number of investigators has produced a vast knowledge of ErbB receptors and their major downstream pathways in the context of human cancer. However, only recently has it begun to become clear that selectively targeting these receptors can have therapeutic value and further studies are certainly required to maximize the clinical benefit of EGFR inhibition strategies. Even though EGFR overexpression is increased in most epithelial cancers, EGFR over-expression alone has been suggested to be implicated in the development of resistance to a number of targeted therapeutic agents such as Tamoxifen and Herceptin [235,236] but not as a validated surrogate marker for predicting the success of anti-EGFR therapy. The ErbB signalling network is very complex, and the involvement of numerous ligands and multiple dimerisation partners creates a substantial challenge for establishing the crucial signalling pathways that become activated in the context of tumorigenesis. As we develop a more detailed understanding of regulated EGFR expression, receptor trafficking and turnover, as well as signalling output, we probably should begin to identify additional targets for anti-EGFR therapy. One such important aspect of EGFR inhibition may be at the level of expression but also identification of the major dimerisation partners of ErbB family receptors with EGFR will help to yield pharmacological effects mediated through the various ErbB family receptors. For example, it is possible that in the presence of relatively high EGF and/or TGFα in blood plasma, the use of cetuximab to compete with the above ligands might further enhance the efficacy of Gefitinib by blocking phosphorylation of EGFR during endocytosis promoted by cetuximab. Similarly, gefitinib could be administrated with the ErbB2 specific monoclonal antibody (Herceptin; Trastuzumab) when moderate EGFR and high ErbB2 are present. However, the molecular characterization should not be limited to the screening of these factors alone but it could be increased to include the evaluation of receptors and their main down-stream effectors. Other important aspects in EGFR biology that should be evaluated are EGFR endocytosis and trafficking, important for generation of specific signalling pathways and receptor turnover. A deeper understanding of these various mechanisms of cell signalling from EGFR is certainly expected to enhance the effective clinical utility of anti-EGFR drugs in multiple cancer settings.

Acknowledgments

This review was supported by grants from the Italian Association for Cancer Research (AIRC-2005) and from the Italian Ministry of Health, Region of Sicilia project (2003).

References

[1] D.S. Salomon, R. Brandt, F. Ciardiello, N. Normanno, Epidermal growth factor-related peptides and their receptors in human malignancies, Crit. Rev. Oncol./Hematol. 19 (1995) 183–232.
[2] J.D. Sato, T. Kawamoto, A.D. Le, J. Mendelsohn, J. Polikoff, G.H. Sato, Biological effects in vitro of monoclonal antibodies to human epidermal growth factor receptors, Mol. Biol. Med. 1 (1983) 511–529.
[3] G. Carpenter, Receptors for epidermal growth factor and other polypeptide mitogens, Annu. Rev. Biochem. 56 (1987) 881–914.
[4] A.L. Harris, S. Nicholson, R. Sainsbury, C. Wright, J. Farndon, Epidermal growth factor receptor and other oncogenes as prognostic markers, J. Natl. Cancer Inst. Monogr. 11 (1992) 181–187.
[5] A.J. Wong, S.H. Bigner, D.D. Bigner, K.W. Kinzler, S.R. Hamilton, B. Vogelstein, Increased expression of the epidermal growth factor receptor gene in malignant gliomas is invariably associated with gene amplification, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 6899–6903.
[6] Y. Yamanaka, H. Friess, MS. Kobrin, M. Buchler, J. Kunz, H.G. Beger, M. Korc, Overexpression of HER2/neu oncogene in human pancreatic carcinoma, Hum. Pathol. 24 (1993) 1127–1134.
[7] C.L. Arteaga, Epidermal growth factor receptor dependence in human tumors: more than just expression? Oncologist 4 (2002) 31–39.
[8] C.L. Arteaga, Overview of epidermal growth factor receptor biology and its role as a therapeutic target in human neoplasia, Semin. Oncol. 5 (Suppl. 14) (2002) 3–9.
[9] J.E. Dancey, B. Freidlin, Targeting epidermal growth factor receptor—are we missing the mark? Lancet 362 (2003) 62–64.
[10] C.L. Arteaga, J. Baselga, Clinical trial design and end points for epidermal growth factor receptor-targeted therapies: implications for drug development and practice, Clin. Cancer Res. 9 (2003) 1579–1589.
[11] F. Ciardiello, Epidermal growth factor receptor tyrosine kinase inhibitors as anticancer agents, Drugs 60 (Suppl. 1) (2000) 25–32 (discussion 41–2).
[12] J.M. Xu, A. Azzariti, G. Colucci, A. Paradiso, The effect of gefitinib (Iressa, ZD1839) in combination with oxaliplatin is schedule-dependent in colon cancer cell lines, Cancer Chemother. Pharmacol. 52 (2003) 442–448.
[13] J.M. Xu, A. Azzariti, M. Severino, B. Lu, G. Colucci, A. Paradiso, Characterization of sequence-dependent synergy between ZD1839 (“Iressa”) and oxaliplatin, Biochem. Pharmacol. 66 (2003) 551–563.
[14] A. Azzariti, J.M. Xu, L. Porcelli, A. Paradiso, The schedule-dependent enhanced cytotoxic activity of 7-ethyl-10-hydroxy-camptothecin (SN-38) in combination with Gefitinib (Iressa, ZD1839), Biochem. Pharmacol. 68 (2004) 135–144.
[15] H.S. Earp, B.F. Calvo, C.I. Sartor, The EGF receptor family—multiple roles in proliferation, differentiation, and neoplasia with an emphasis on HER4, Trans. Am. Clin. Climatol. Assoc. 114 (2003) 315–333.
[16] J.E. De Larco, R. Reynolds, K. Carlberg, C. Engle, G.J. Todaro, Sarcoma growth factor from mouse sarcoma virus-transformed cells, purification by binding and elution from epidermal growth factor receptor-rich cells, J. Biol. Chem. 255 (1980) 3685–3690.
[17] J. Downward, Y. Yarden, E. Mayes, G. Scrace, N. Totty, P. Stockwell, A. Ullrich, J. Schlessinger, M.D. Waterfield, Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences, Nature 307 (1984) 521–527.
[18] R. Roskoski, The ErbB/HER receptor protein-tyrosine kinases and cancer, Biochem. Biophys. Res. Commun. 319 (2004) 1–11.

[19] W.E. Miller, H.S. Earp, N. Raab-Traub, The Epstein–Barr virus latent membrane protein 1 induces expression of the epidermal growth factor receptor, J. Virol. 69 (1995) 4390–4398.
[20] Y. Matsui, S.A. Halter, J.T. Holt, B.L. Hogan, R.J. Coffey, Development of mammary hyperplasia and neoplasia in MMTV-TGF alpha transgenic mice, Cell 61 (1990) 1147–1155.
[21] L. Bouchard, L. Lamarre, P.J. Tremblay, P. Jolicoeur, Stochastic appearance of mammary tumors in transgenic mice carrying the MMTV/c-neu oncogene, Cell 57 (1989) 931–936.
[22] P.P. Di Fiore, J.H. Pierce, T.P. Fleming, R. Hazan, A. Ullrich, C.R. King, J. Schlessinger, S.A. Aaronson, Overexpression of the human EGF receptor confers an EGF-dependent transformed phenotype to NIH 3T3 cells, Cell 51 (1987) 1063–1070.
[23] C.A. Athale, T.S. Deisboeck, The effects of EGF-receptor density on multiscale tumor growth patterns, J. Theor. Biol. 238 (2006) 771–779.
[24] A. El-Obeid, G. Hesselager, B. Westermark, M. Nister, TGF-alpha-driven tumor growth is inhibited by an EGF receptor tyrosine kinase inhibitor, Biochem. Biophys. Res. Commun. 290 (2002) 349–358.
[25] V.P. Collins, Epidermal growth factor receptor gene and its transcripts in glioblastomas, Recent Results Cancer Res. 135 (1994) 17–24.
[26] P.A. Humphrey, A.J. Wong, B. Vogelstein, M.R. Zalutsky, G.N. Fuller, G. E. Archer, H.S. Friedman, M.M. Kwatra, S.H. Bigner, D.D. Bigner, Anti-synthetic peptide antibody reacting at the fusion junction of deletion-mutant epidermal growth factor receptors in human glioblastoma, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 4207–4211.
[27] H. Yamazaki, Y. Ohba, N. Tamaoki, M. Shibuya, A deletion mutation within the ligand binding domain is responsible for activation of epidermal growth factor receptor gene in human brain tumors, Jpn. J. Cancer Res. 81 (1990) 773–779.
[28] A.J. Wong, J.M. Ruppert, S.H. Bigner, C.H. Grzeschik, P.A. Humphrey, D.S. Bigner, B. Vogelstein, Structural alterations of the epidermal growth factor receptor gene in human gliomas, Proc. Natl. Acad. Sci. 89 (1992) 2965–2969.
[29] C.T. Chu, K.D. Everiss, C.J. Wikstrand, S.K. Batra, H.J. Kung, D.D. Bigner, Receptor dimerization is not a factor in the signalling activity of a transforming variant epidermal growth factor receptor (EGFRvIII), Biochem. J. 324 (1997) 855–861.
[30] D.K. Moscatello, M. Holgado-Madruga, D.R. Emlet, R.B. Montgomery, A.J. Wong, Constitutive activation of phosphatidylinositol 3-kinase by a naturally occurring mutant epidermal growth factor receptor, J. Biol. Chem. 273 (1998) 200–206.
[31] J. Jiang, H. Greulich, P.A. Janne, W.R. Sellers, M. Meyerson, J.D. Griffin, Epidermal growth factor-independent transformation of Ba/F3 cells with cancer-derived epidermal growth factor receptor mutants induces gefitinib-sensitive cell cycle progression, Cancer Res. 65 (2005) 8968–8974.
[32] H. Greulich, T.H. Chen, W. Feng, P.A. Janne, J.V. Alvarez, M. Zappaterra, S.E. Bulmer, D.A. Frank, W.C. Hahn, S.R. Sellers, M. Meyerson, Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants, PLoS Med. 2 (2005) e313.
[33] A. Wells, EGF receptor, Int. J. Biochem. Cell. Biol. 31 (1999) 637–643.
[34] R.I. Nicholson, J.M. Gee, M.E. Harper, EGFR and cancer prognosis, Eur. J. Cancer 37 (Suppl. 4) (2001) 9–15.
[35] G.T. Merlino, Y.H. Xu, N. Richert, A.J. Clark, S. Ishii, S. Banks-Schlegel, I. Pastan, Elevated epidermal growth factor receptor gene copy number and expression in a squamous carcinoma cell line, J. Clin. Invest. 75 (1985) 1077–1079.
[36] R. Derynck, D.V. Goeddel, A. Ullrich, J.U. Gutterman, R.D. Williams, T.S. Bringman, W.H. Berger, Synthesis of messenger RNAs for transforming growth factors alpha and beta and the epidermal growth factor receptor by human tumors, Cancer Res. 47 (1987) 707–712.
[37] S. Costa, H. Stamm, A. Almendral, H. Ludwig, R. Wyss, D. Fabbro, A. Ernst, A. Takahashi, U. Eppenberger, Predictive value of EGF receptor in breast cancer, Lancet 2 (1988) 1258.
[38] J.R. Sainsbury, S. Nicholson, B. Angus, J.R. Farndon, A.J. Malcolm, A. L. Harris, Epidermal growth factor receptor status of histological subtypes of breast cancer, Br. J. Cancer 58 (1988) 458–460.
[39] S.O. Ibrahim, E.N. Vasstrand, P.G. Liavaag, A.C. Johannessen, J.R. Lillehaug, Expression of c-erbB proto-oncogene family members in squamous cell carcinoma of the head and neck, Anticancer Res. 17 (1997) 4539–4546.
[40] S.A. Aaronson, Growth factors and cancer, Science 254 (1991) 1146–1153.
[41] V. Grunwald, M. Hidalgo, The epidermal growth factor receptor: a new target for anticancer therapy, Curr. Probl. Cancer 26 (2002) 109–164.
[42] J. Mendelsohn, The epidermal growth factor receptor as a target for cancer therapy, Endocr. Relat. Cancer 8 (2001) 3–9.
[43] J.R. Sainsbury, S. Nicholson, B. Angus, J.R. Farndon, A.J. Malcolm, A. L. Harris, Epidermal growth factor receptor status of histological subtypes of breast cancer, Br. J. Cancer 58 (1988) 458–460.
[44] S. Ozawa, M. Ueda, N. Ando, N. Shimizu, O. Abe, Prognostic significance of epidermal growth factor receptor in esophageal squamous cell carcinomas, Cancer 63 (1989) 2169–2173.
[45] P.J. Miettinen, J.E. Berger, J. Meneses, Y. Phung, R.A. Pedersen, Z. Werb, R. Derynck, Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor, Nature 376 (1995) 337–341.
[46] K.F. Lee, H. Simon, H. Chen, B. Bates, M.C. Hung, C. Hauser, Requirement for neuregulin receptor erbB2 in neural and cardiac development, Nature 378 (1995) 394–398.
[47] F.E. Jones, D.F. Stern, Expression of dominant-negative ErbB2 in the mammary gland of transgenic mice reveals a role in lobuloalveolar development and lactation, Oncogene 18 (1999) 3481–3490.
[48] F.E. Jones, T. Welte, X.Y. Fu, D.F. Stern, ErbB4 signaling in the mammary gland is required for lobuloalveolar development and Stat5 activation during lactation, J. Cell Biol. 147 (1999) 77–88.
[49] N.C. Luetteke, T.H. Qiu, R.L. Peiffer, P. Oliver, O. Smithies, D.C. Lee, TGF alpha deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice, Cell 73 (1993) 263–278.
[50] G.B. Mann, K.J. Fowler, A. Gabriel, E.C. Nice, R.L. Williams, A.R. Dunn, 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 73 (1993) 249–261.
[51] P.J. Miettinen, J.E. Berger, J. Meneses, Y. Phung, R.A. Pedersen, Z. Werb, R. Derynck, Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor, Nature 376 (1995) 337–341.
[52] M. Sibilia, J.P. Steinbach, L. Stingl, A. Aguzzi, E.F. Wagner, A strain-independent postnatal neurodegeneration in mice lacking the EGF receptor, EMBO J. 17 (1998) 719–731.
[53] I. Lax, W.H. Burgess, F. Bellot, A. Ullrich, J. Schlessinger, D. Givol, Localization of a major receptor-binding domain for epidermal growth factor by affinity labelling, Mol. Cell. Biol. 8 (1988) 1831–1834.
[54] I. Lax, F. Bellot, R. Howk, A. Ullrich, D. Givol, J. Schlessinger, Functional analysis of the ligand binding site of EGF-receptor utilizing chimeric chicken/human receptor molecules, EMBO J. 8 (1989) 421–427.
[55] H. Ogiso, R. Ishitani, O. Nureki, S. Fukai, M. Yamanaka, J.H. Kim, K. Saito, A. Sakamoto, M. Inoue, M. Shirouzu, S. Yokoyama, Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains, Cell 110 (2002) 775–787.
[56] T.P. Garrett, N.M. McKern, M. Lou, T.C. Elleman, T.E. Adams, G.O. Lovrecz, H.J. Zhu, F. Walker, M.J. Frenkel, P.A. Hoyne, R.N. Jorissen, E. C. Nice, A.W. Burgess, C.W. Ward, Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha, Cell 110 (2002) 763–773.
[57] T.C. Elleman, T. Domagala, N.M. McKern, M. Nerrie, B. Lonnqvist, T.E. Adams, J. Lewis, G.O. Lovrecz, P.A. Hoyne, K.H. Richards, G.J. Howlett, J. Rothacker, R.N. Jorissen, M. Lou, T.P. Garrett, A.W. Burgess, E.C. Nice, C.W. Ward, Identification of a determinant of epidermal growth factor receptor ligand-binding specificity using a truncated, high-affinity form of the ectodomain, Biochemistry 40 (2001) 8930–8939.
[58] M. Yamabhai, R.G. Anderson, Second cysteine-rich region of epidermal growth factor receptor contains targeting information for caveolae/rafts, J. Biol. Chem. 277 (2002) 24843–24846.
[59] A. Ullrich, L. Coussens, J.S. Hayflick, T.J. Dull, A. Gray, A.W. Tam, J. Lee, Y. Yarden, T.A. Libermann, J. Schlessinger, Human epidermal growth factor receptor cDNA sequence and aberrant expression of the

amplified gene in A431 epidermoid carcinoma cells, Nature 309 (1984) 418–425.
[60] A.C. Rigby, C.W. Grant, G.S. Shaw, Solution and solid state conformation of the human EGF receptor transmembrane region, Biochim. Biophys. Acta 1371 (1998) 241–253.
[61] S.J. Kil, C. Carlin, EGF receptor residues leu(679), leu(680) mediate selective sorting of ligand-receptor complexes in early endosomal compartments, J. Cell. Physiol. 185 (2000) 47–60.
[62] C. He, M. Hobert, L. Friend, C. Carlin, The epidermal growth factor receptor juxtamembrane domain has multiple basolateral plasma membrane localization determinants, including a dominant signal with a polyproline core, J. Biol. Chem. 277 (2002) 38284–38293.
[63] P. Castagnino, Z. Biesova, W.T. Wong, F. Fazioli, G.N. Gill, P.P. Di Fiore, Direct binding of eps8 to the juxtamembrane domain of EGFR is phosphotyrosine- and SH2-independent, Oncogene 10 (1995) 723–729.
[64] J. Martin-Nieto, A. Villalobo, The human epidermal growth factor receptor contains a juxtamembrane calmodulin-binding site, Biochemistry 37 (1998) 227–236.
[65] H. Li, A. Villalobo, Evidence for the direct interaction between calmodulin and the human epidermal growth factor receptor, Biochem. J. 362 (2002) 499–505.
[66] C.R. Lombardo, T.G. Consler, D.B. Kassel, In vitro phosphorylation of the epidermal growth factor receptor autophosphorylation domain by c-src: identification of phosphorylation sites and c-src SH2 domain binding sites, Biochemistry 34 (1995) 16456–16466.
[67] C.B. Thien, W.Y. Langdon, Cbl: many adaptations to regulate protein tyrosine kinases, Nat. Rev. Mol. Cell. Biol. 2 (2001) 294–307.
[68] J.C. Den Hartigh, P.M. van Bergen en Henegouwen, A.J. Verkleij, J. Boonstra, The EGF receptor is an actin-binding protein, J. Cell Biol. 119 (1992) 349–355.
[69] L.J. Slieker, T.M. Martensen, M.D. Lane, Synthesis of epidermal growth factor receptor in human A431 cells. Glycosylation-dependent acquisition of ligand binding activity occurs post-translationally in the endoplasmic reticulum, J. Biol. Chem. 261 (1986) 15233–15241.
[70] T.G. Johns, E. Stockert, G. Ritter, A.A. Jungbluth, H.J. Huang, W.K. Cavenee, F.E. Smyth, C.M. Hall, N. Watson, E.C. Nice, W.J. Gullick, L.J. Old, A.W. Burgess, A.M. Scott, Novel monoclonal antibody specific for the de2–7 epidermal growth factor receptor (EGFR) that also recognizes the EGFR expressed in cells containing amplification of the EGFR gene, Int. J. Cancer 98 (2002) 398–408.
[71] Y. Yarden, The EGFR family and its ligands in human cancer. Signalling mechanisms and therapeutic opportunities, Eur. J. Cancer 37 (Suppl. 4) (2001) 3–8.
[72] C.T. Kuan, C.J. Wikstrand, D.D. Bigner, EGF mutant receptor vIII as a molecular target in cancer therapy, Endocr. Relat. Cancer 8 (2001) 83–96.
[73] R. Sordella, D.W. Bell, D.A. Haber, J. Settleman, Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways, Science 305 (2004) 1163–1167.
[74] F.R. Hirsch, M. Varella-Garcia, P.A. Bunn, M.V. Di Maria, R. Veve, R.M. Bremmes, A.Z. Baron, C. Zeng, W.A. Franklin, Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis, J. Clin. Oncol. 21 (2003) 3798–3807.
[75] M.A. Olayioye, R.M. Neve, H.A. Lane, N.E. Hynes, The ErbB signaling network: receptor heterodimerization in development and cancer, EMBO J. 19 (2000) 3159–3167.
[76] P.M. Guy, J.V. Platko, L.C. Cantley, R.A. Cerione, K.L. Carraway, Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 8132–8136.
[77] Y. Yarden, M.X. Sliwkowski, Untangling the ErbB signalling network, Nat. Rev. Mol. Cell. Biol. 2 (2001) 127–137.
[78] J. Massague, A. Pandiella, Membrane-anchored growth factors, Annu. Rev. Biochem. 62 (1993) 515–541.
[79] N. Normanno, C. Bianco, A. De Luca, M.R. Maiello, D.S. Salomon, Target-based agents against ErbB receptors and their ligands: a novel approach to cancer treatment, Endocr. Relat. Cancer 10 (2003) 1–21.
[80] N. Normanno, C. Bianco, A. De Luca, D.S. Salomon, The role of EGF-related peptides in tumor growth, Front. Biosci. 6 (2001) 685–707.
[81] R.R. Beerli, N.E. Hynes, Epidermal growth factor-related peptides activate distinct subsets of ErbB receptors and differ in their biological activities, J. Biol. Chem. 271 (1996) 6071–6076.
[82] H. Toyoda, T. Komurasaki, D. Uchida, S. Morimoto, Distribution of mRNA for human epiregulin, a differentially expressed member of the epidermal growth factor family, Biochem. J. 326 (1997) 69–75.
[83] L.M. Wang, A. Kuo, M. Alimandi, M.C. Veri, C.C. Lee, V. Kapoor, N. Ellmore, X.H. Chen, J.H. Pierce, ErbB2 expression increases the spectrum and potency of ligand-mediated signal transduction through ErbB4, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 6809–6814.
[84] D.J. Riese, E.D. Kim, K. Elenius, S. Buckley, M. Klagsbrun, D.D. Plowman, D.F. Stern, The epidermal growth factor receptor couples transforming growth factor-alpha, heparin-binding epidermal growth factor-like factor, and amphiregulin to Neu, ErbB-3, and ErbB-4, J. Biol. Chem. 271 (1996) 20047–20052.
[85] K.L. Troyer, D.C. Lee, Regulation of mouse mammary gland development and tumorigenesis by the ERBB signaling network, J. Mammary Gland. Biol. Neoplasia 6 (2001) 7–21.
[86] J.P. Borg, S. Marchetto, A. Le Bivic, V. Ollendorff, F. Jaulin-Bastard, H. Saito, E. Fournier, J. Adelaide, B. Margolis, D. Birnbaum, ERBIN: a basolateral PDZ protein that interacts with the mammalian ERBB2/HER2 receptor, Nat. Cell Biol. 2 (2000) 407–414.
[87] L.N. Klapper, S. Glathe, N. Vaisman, N.E. Hynes, G.C. Andrews, M. Sela, Y. Yarden, The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4995–5000.
[88] E. Tzahar, H. Waterman, X. Chen, G. Levkowitz, D. Karunagaran, S. Lavi, B.J. Ratzkin, Y. Yarden, A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor, Mol. Cell. Biol. 16 (1996) 5276–5287.
[89] O.M. Fischer, S. Hart, A. Gschwind, A. Ullrich, EGFR signal transactivation in cancer cells, Biochem. Soc. Trans. 31 (2003) 1203–1208.
[90] P.M. Guy, J.V. Platko, L.C. Cantley, R.A. Cerione, K.L. Carraway, Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 8132–8136.
[91] M.A. Lemmon, Z. Bu, J.E. Ladbury, M. Zhou, D. Pinchasi, I. Lax, D.M. Engelman, J. Schlessinger, Two EGF molecules contribute additively to stabilization of the EGFR dimer, EMBO J. 16 (1997) 281–294.
[92] D. Graus-Porta, R.R. Beerli, J.M. Daly, N.E. Hynes, ErbB-2, the preferred eterodimerization partner of all ErbB receptors, is a mediator of lateral signalling, EMBO J. 16 (1997) 1647–1655.
[93] B.S. Hendriks, L.K. Opresko, H.S. Wiley, D. Lauffenburger, Quantitative analysis of HER2-mediated effects on HER2 and epidermal growth factor receptor endocytosis: distribution of homo- and heterodimers depends on relative HER2 levels, J. Biol. Chem. 278 (2003) 23343–23351.
[94] S. Vallian, K.V. Chin, K.S. Chang, The promyelocytic leukemia protein interacts with Sp1 and inhibits its transactivation of the epidermal growth factor receptor promoter, Mol. Cell. Biol. 18 (1998) 7147–7156.
[95] Y.R. Rubinstein, K.N. Proctor, M. Bergel, B. Murphy, A.C. Johnson, Interferon regulatory factor-1 is a major regulator of epidermal growth factor receptor gene expression, FEBS Lett. 431 (1998) 268–272.
[96] A.C. Johnson, B.A. Murphy, C.M. Matelis, Y. Rubinstein, E.C. Piebenga, L.M. Akers, G. Neta, C. Vinson, M. Birrer, Activator protein-1 mediates induced but not basal epidermal growth factor receptor gene expression, Mol. Med. 6 (2000) 17–27.
[97] J.H. Ludes-Meyers, M.A. Subler, C.V. Shivakumar, R.M. Munoz, P. Jiang, J.E. Bigger, D.R. Brown, S.P. Deb, S. Deb, Transcriptional activation of the human epidermal growth factor receptor promoter by human p53, Mol. Cell. Biol. 16 (1996) 6009–6019.
[98] H. Nishi, M. Senoo, K.H. Nishi, B. Murphy, T. Rikiyama, Y. Matsumura, S. Habu, A.C. Johnson, p53 Homologue p63 represses epidermal growth factor receptor expression, J. Biol. Chem. 276 (2001) 41717–41724.
[99] J. Xu, K.L. Thompson, L.B. Shephard, L.G. Hudson, G.N. Gill, T3 receptor suppression of Sp1-dependent transcription from the epidermal growth factor receptor promoter via overlapping DNA-binding sites, J. Biol. Chem. 268 (1993) 16065–16073.

[100] L. Salvatori, L. Ravenna, M.P. Felli, M.R. Cardillo, M.A. Russo, L. Frati, A. Gulino, E. Petrangeli, Identification of an estrogen-mediated deoxyribonucleic acid-binding independent transactivation pathway on the epidermal growth factor receptor gene promoter, Endocrinology 141 (2000) 2266–2274.
[101] K.R. McGaffin, L.E. Acktinson, S.A. Chrysogelos, Growth and EGFR regulation in breast cancer cells by vitamin D and retinoid compounds, Breast. Cancer Res. Treat. 86 (2004) 55–73.
[102] J.A. Fernandez-Pol, Modulation of EGF receptor protooncogene expression by growth factors and hormones in human breast carcinoma cells, Crit. Rev. Oncog. 2 (1991) 173–185.
[103] A. Jimeno, B. Rubio-Viqueira, M.L. Amador, D. Oppenheimer, N. Bouraoud, P. Kulesza, V. Sebastiani, A. Maitra, M. Hidalgo, Epidermal growth factor receptor dynamics influences response to epidermal growth factor receptor targeted agents, Cancer Res. 65 (2005) 3003–3010.
[104] F. Recchia, G. Saggio, A. Cesta, E. Alesse, R. Gallo, S. Necozione, S. Rea, Phase II randomized study of interleukin-2 with or without 13-cis retinoic acid as maintenance therapy in patients with advanced cancer responsive to chemotherapy, Anticancer Res. 25 (2005) 3149–3157.
[105] T. Moriki, H. Maruyama, I.N. Maruyama, Activation of preformed EGF receptor dimers by ligand-induced rotation of the transmembrane domain, J. Mol. Biol. 311 (2000) 1011–1026.
[106] X. Yu, K.D. Sharma, T. Takahashi, R. Iwamoto, E. Mekada, Ligand-independent dimer formation of epidermal growth factor receptor (EGFR) is a step separable from ligand-induced EGFR signaling, Mol. Biol. Cell 13 (2002) 2547–2557.
[107] A. Sorokin, Activation of the EGF receptor by insertional mutations in its juxtamembrane regions, Oncogene 11 (1995) 1531–1540.
[108] A. Chantry, The kinase domain and membrane localization determine intracellular interactions between epidermal growth factor receptors. growth factor receptors, J. Biol. Chem. 270 (1995) 3068–3073.
[109] D. Karunagaran, E. Tzahar, R.R. Beerli, X. Chen, D. Graus-Porta, B.J. Ratzkin, R. Seger, N.E. Hynes, Y. Yarden, ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer, EMBO J. 15 (1996) 254–264.
[110] C. Haslekas, K. Breen, K.W. Pedersen, L. Johannessen, E. Stang, I.H. Madshus, The inhibitory effect of ErbB2 on EGF-induced formation of clathrin-coated pits correlates with retention of EGFR–ErbB2 oligomeric complexes at the plasma membrane, Mol. Biol. Cell 16 (2005) 5832–5842.
[111] A.E. Lenferink, A.D. De Roos, M.J. Van Vugt, M.L. Van de Poll, E.J. Van Zoelen, The linear C-terminal regions of epidermal growth factor (EGF) and transforming growth factor-alpha bind to different epitopes on the human EGF receptor, Biochem. J. 336 (1998) 147–151.
[112] K.M. Ferguson, J.M. Kavran, V.G. Sankaran, E. Fournier, S.J. Isakoff, E. Y. Skolnik, M.A. Lemmon, Structural basis for discrimination of 3-phosphoinositides by pleckstrin homology domains, Mol. Cell 6 (2000) 373–384.
[113] G.C. Huang, X. Ouyang, R.J. Epstein, Proxy activation of protein ErbB2 by heterologous ligands implies a heterotetrameric mode of receptor tyrosine kinase interaction, Biochem. J. 331 (1998) 113–119.
[114] D.C. Gamett, G. Pearson, R.A. Cerione, I. Friedberg, Secondary dimerization between members of the epidermal growth factor receptor family, J. Biol. Chem. 272 (1997) 12052–12056.
[115] L.E. Johannessen, K.E. Haugen, A.C. Ostvold, E. Stang, I.H. Madshus, Heterodimerization of the epidermal-growth-factor (EGF) receptor and ErbB2 and the affinity of EGF binding are regulated by different mechanisms, Biochem. J. 356 (2001) 87–96.
[116] F.R. Hirsch, B. Helfrich, W.A. Franklin, M. Varella-Garcia, D.C. Chan, P. A. Bunn, Preclinical studies of gemcitabine and trastuzumab in breast and lung cancer cell lines, Clin. Breast. Cancer 3 (Suppl. 1) (2002) 12–16.
[117] D.P. Hughes, D.G. Thomas, T.J. Giordano, L.H. Baker, K.T. McDonagh, Cell surface expression of epidermal growth factor receptor and Her-2 with nuclear expression of Her-4 in primary osteosarcoma, Cancer Res. 64 (2004) 2047–2053.
[118] M.X. Sliwkowski, G. Schaefer, R.W. Akita, J.A. Lofgren, V.D. Fitzpatrick, A. Nuijens, B.M. Fendly, R.A. Cerione, R.L. Vandlen, K. L. Carraway III, Coexpression of erbB2 and erbB3 proteins reconstitutes a high affinity receptor for heregulin, J. Biol. Chem. 269 (1994) 14661–14665.
[119] Y. Saito, J. Haendeler, Y. Hojo, K. Yamamoto, B.C. Berk, Receptor heterodimerization: essential mechanism for platelet-derived growth factor-induced epidermal growth factor receptor transactivation, Mol. Cell. Biol. 21 (2001) 6387–6394.
[120] S. Gibson, S. Tu, R. Oyer, S.M. Anderson, G.L. Johnson, Epidermal growth factor protects epithelial cells against Fas-induced apoptosis. Requirement for Akt activation, J. Biol. Chem. 274 (1999) 17612–17618.
[121] R. Reinehr, F. Schliess, D. Haussinger, Hyperosmolarity and CD95L trigger CD95/EGF receptor association and tyrosine phosphorylation of CD95 as prerequisites for CD95 membrane trafficking and DISC formation, FASEB J. 17 (2003) 731–733.
[122] D. Peng, Z. Fan, Y. Lu, T. DeBlasio, H. Scher, J. Mendelsohn, Anti-epidermal growth factor receptor monoclonal antibody 225 up-regulates p27KIP1 and induces G1 arrest in prostatic cancer cell line DU145, Cancer Res. 56 (1996) 3666–3669.
[123] J. Anido, P. Matar, J. Albanell, M. Guzman, F. Rojo, J. Arribas, S. Averbuch, J. Baselga, ZD1839, a specific epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, induces the formation of inactive EGFR/HER2 and EGFR/HER3 heterodimers and prevents heregulin signaling in HER2-overexpressing breast cancer cells, Clin. Cancer Res. 9 (2003) 1274–1283.
[124] H. Nakamura, S. Takamori, T. Fujii, M. Ono, H. Yamana, M. Kuwano, K. Shirouzu, Cooperative cell-growth inhibition by combination treatment with ZD1839 (Iressa) and trastuzumab (Herceptin) in non-small-cell lung cancer, Cancer Lett. 230 (2005) 33–46.
[125] J.P. Steinbach, C. Eisenmann, A. Klumpp, M. Weller, Co-inhibition of epidermal growth factor receptor and type 1 insulin-like growth factor receptor synergistically sensitizes human malignant glioma cells to CD95L-induced apoptosis, Biochem. Biophys. Res. Commun. 321 (2004) 524–530.
[126] T.J. Sayers, W.J. Murphy, Combining proteasome inhibition with TNF-related apoptosis-inducing ligand (Apo2L/TRAIL) for cancer therapy, Cancer Immunol. Immunother. 55 (2006) 76–84.
[127] A. Ullrich, J. Schlessinger, Signal transduction by receptors with tyrosine kinase activity, Cell 61 (1990) 203–212.
[128] T. Saito, S. Okada, K. Ohshima, E. Yamada, M. Sato, Y. Uehara, H. Shimizu, J.E. Pessin, M. Mori, Differential activation of epidermal growth factor (EGF) receptor downstream signaling pathways by betacellulin and EGF, Endocrinology 145 (2004) 4232–4243.
[129] R.N. Jorissen, F. Walker, N. Pouliot, T.P. Garrett, C.W. Ward, A.W. Burgess, Epidermal growth factor receptor: mechanisms of activation and signalling, Exp. Cell. Res. 284 (2003) 31–53.
[130] D.R. Stover, M. Becker, J. Liebetanz, N.B. Lydon, Src phosphorylation of the epidermal growth factor receptor at novel sites mediates receptor interaction with Src and P85 alpha, J. Biol. Chem. 270 (1995) 15591–15597.
[131] J.S. Biscardi, M.C. Maa, D.A. Tice, M.E. Cox, T.H. Leu, S.J. Parsons, c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and receptor on Tyr845 and Tyr1101 is associated with modulation of receptor function, J. Biol. Chem. 274 (1999) 8335–8343.
[132] M.T. Kloth, K.K. Laughlin, J.S. Biscardi, J.L. Boerner, S.J. Parsons, C.M. Silva, STAT5b, a mediator of synergism between c-Src and the epidermal growth factor receptor, J. Biol. Chem. 278 (2003) 1671–1679.
[133] J.L. Boerner, M.l. Demory, C. Silva, S.J. Parsons, Phosphorylation of Y845 on the epidermal growth factor receptor mediates binding to the mitochondrial protein cytochrome c oxidase subunit II, Mol. Cell. Biol. 24 (2004) 7059–7071.
[134] A.G. Batzer, D. Rotin, J.M. Urena, E.Y. Skolnik, J. Schlessinger, Hierarchy of binding sites for Grb2 and Shc on the epidermal growth factor receptor, Mol. Cell. Biol. 14 (1994) 5192–5201.
[135] E.J. Lowenstein, R.J. Daly, A.G. Batzer, W. Li, B. Margolis, R. Lammers, A. Ullrich, E.Y. Skolnik, D. Bar-Sagi, J. Schlessinger, The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signalling, Cell 70 (1992) 431–442.
[136] S.B. Waters, D. Chen, A.W. Kao, S. Okada, K.H. Holt, J.E. Pessin, Insulin and epidermal growth factor receptors regulate distinct pools of

Grb2-SOS in the control of Ras activation, J. Biol. Chem. 271 (1996) 18224–18230.
[137] R. Marais, C.J. Marshall, Control of the ERK MAP kinase cascade by Ras and Raf, Cancer Surv. 27 (1996) 101–125.
[138] J.K. Voice, R.L. Klemke, A. Le, J.H. Jackson, Four human ras homologs differ in their abilities to activate Raf-1, induce transformation, and stimulate cell motility, J. Biol. Chem. 274 (1999) 17164–17170.
[139] L.O. Murphy, S. Smith, R.H. Chen, D.C. Fingar, J. Blenis, Molecular interpretation of ERK signal duration by immediate early gene products, Nat. Cell Biol. 4 (2002) 556–564.
[140] A.C. Gavin, A.R. Nebreda, A MAP kinase docking site is required for phosphorylation and activation of p90(rsk)/MAPKAP kinase-1, Curr. Biol. 9 (1999) 281–284.
[141] S.R. Datta, H. Dudek, X. Tao, S. Masters, H. Fu, Y. Gotoh, M.E. Greenberg, Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery, Cell 91 (1997) 231–241.
[142] T.F. Franke, D.R. Kaplan, L.C. Cantley, PI3K: downstream AKTion blocks apoptosis, Cell 88 (1997) 435–437.
[143] C.D. Andl, T. Mizushima, K. Oyama, M. Bowser, H. Nakagawa, A.K. Rustgi, EGFR-induced cell migration is mediated predominantly by the JAK-STAT pathway in primary esophageal keratinocytes, Am. J. Physiol.: Gastrointest. Liver Physiol. 287 (2004) 1227–1237.
[144] D.W. Leaman, S. Pisharody, T.W. Flickinger, M.A. Commane, J. Schlessinger, I.M. Kerr, D.E. Levy, G.R. Stark, Roles of JAKs in activation of STATs and stimulation of c-fos gene expression by epidermal growth factor, Mol. Cell. Biol. 16 (1996) 369–375.
[145] N. Ferrara, Molecular and biological properties of vascular endothelial growth factor, J. Mol. Med. 77 (1999) 527–543.
[146] A. Wells, M.F. Ware, F.D. Allen, D.A. Lauffenburger, Shaping up for shipping out: PLCgamma signaling of morphology changes in EGF-stimulated fibroblast migration, Cell Motil. Cytoskelet. 44 (1999) 227–233.
[147] A. Mani, E.P. Gelmann, The ubiquitin-proteasome pathway and its role in cancer, J. Clin. Oncol. 23 (2005) 4776–4789.
[148] I. Dikic, Mechanisms controlling EGF receptor endocytosis and degradation, Biochem. Soc. Trans. 31 (2003) 1178–1181.
[149] H. Waterman, Y. Yarden, Molecular mechanisms underlying endocytosis and sorting of ErbB receptor tyrosine kinases, FEBS Lett. 490 (2001) 142–152.
[150] C.B. Thien, F. Walker, W.Y. Langdon, RING finger mutations that abolish c-Cbl-directed polyubiquitination and downregulation of the EGF receptor are insufficient for cell transformation, Mol. Cell 7 (2001) 355–365.
[151] H. Waterman, M. Katz, C. Rubin, K. Shtiegman, S. Lavi, A. Elson, T. Jovin, Y. Yarden, A mutant EGF-receptor defective in ubiquitylation and endocytosis unveils a role for Grb2 in negative signaling, EMBO J. 21 (2002) 303–313.
[152] G. Levkowitz, H. Waterman, S.A. Ettenberg, M. Katz, A.Y. Tsygankov, I. Alroy, S. Lavi, K. Iwai, Y. Reiss, A. Ciechanover, S. Lipkowitz, Y. Yarden, Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1, Mol. Cell 4 (1999) 1029–1040.
[153] E.A. Feshchenko, W.Y. Langdon, A.Y. Tsygankov, Fyn, Yes, and Syk phosphorylation sites in c-Cbl map to the same tyrosine residues that become phosphorylated in activated T cells, J. Biol. Chem. 273 (1998) 8323–8331.
[154] P. Soubeyran, K. Kowanetz, I. Szymkiewicz, W.Y. Langdon, I. Dikic, Cbl–CIN85–endophilin complex mediates ligand-induced downregulation of EGF receptors, Nature 416 (2002) 183–187.
[155] A. Petrelli, G.F. Gilestro, S. Lanzardo, P.M. Comoglio, N. Migone, S. Giordano, The endophilin–CIN85–Cbl complex mediates ligand-dependent downregulation of c-Met, Nature 416 (2002) 187–190.
[156] P.M. Burke, H.S. Wiley, Human mammary epithelial cells rapidly exchange empty EGFR between surface and intracellular pools, J. Cell. Physiol. 180 (1999) 448–460.
[157] S. Polo, S. Sigismund, M. Faretta, M. Guidi, M.R. Capua, G. Bossi, H. Chen, P. De Camilli, P.P. Di Fiore, A single motif responsible for ubiquitin recognition and monoubiquitination in endocytic proteins, Nature 416 (2002) 451–455.
[158] A.E. Salcini, H. Chen, G. Iannolo, P. De Camilli, P.P. Di Fiore, Epidermal growth factor pathway substrate 15, Eps15, Int. J. Biochem. Cell. Biol. 31 (1999) 805–809.
[159] M.D. Marmor, Y. Yarden, Role of protein ubiquitylation in regulating endocytosis of receptor tyrosine kinases, Oncogene 23 (2004) 2057–2070.
[160] K.G. Bache, C. Raiborg, A. Mehlum, H. Stenmark, STAM and Hrs are subunits of a multivalent ubiquitin-binding complex on early endosomes, J. Biol. Chem. 278 (2003) 12513–12521.
[161] D.J. Katzmann, G. Odorizzi, S.D. Emr, Receptor downregulation and multivesicular-body sorting, Nat. Rev. Mol. Cell. Biol. 3 (2002) 893–905.
[162] F.G. Haj, P.J. Verveer, A. Squire, B.G. Neel, P.I. Bastiaens, Imaging sites of receptor dephosphorylation by PTP1B on the surface of the endoplasmic reticulum, Science 295 (2002) 1708–1711.
[163] C.S. Lazar, C.M. Cresson, D.A. Lauffenburger, G.N. Gill, The Na+/H+ exchanger regulatory factor stabilizes epidermal growth factor receptors at the cell surface, Mol. Biol. Cell 15 (2004) 5470–5480.
[164] H. Xie, M.A. Pallero, K. Gupta, P. Chang, M.F. Ware, W. Witke, D.J. Kwiatkowski, D.A. Lauffenburger, J.E. Murphy-Ullrich, A. Wells, EGF receptor regulation of cell motility: EGF induces disassembly of focal adhesions independently of the motility-associated PLCgamma signaling pathway, J. Cell Sci. 111 (1998) 615–624.
[165] J.H. Choi, W.P. Hong, S. Yun, H.S. Kim, J.R. Lee, J.B. Park, Y.S. Bae, S. H. Ryu, P.G. Suh, Grb2 negatively regulates epidermal growth factor-induced phospholipase C-gamma1 activity through the direct interaction with tyrosine-phosphorylated phospholipase C-gamma1, Cell. Signal. 17 (2005) 1289–1299.
[166] R.A. Cardone, A. Bagorda, A. Bellizzi, G. Busco, L. Guerra, A. Paradiso, V. Casavola, M. Zaccolo, S.J. Reshkin, Protein kinase A gating of a pseudopodial-located RhoA/ROCK/p38/NHE1 signal module regulates invasion in breast cancer cell lines, Mol. Biol. Cell 16 (2005) 3117–3127.
[167] R.A. Cardone, V. Casavola, S.J. Reshkin, The role of disturbed pH dynamics and the Na+/H+ exchanger in metastasis, Nat. Rev. Cancer 5 (2005) 786–795.
[168] J.E. Egan, A.B. Hall, B.A. Yatsula, D. Bar-Sagi, The bimodal regulation of epidermal growth factor signaling by human Sprouty proteins, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 6041–6046.
[169] A.B. Hall, N. Jura, J. DaSilva, Y.J. Jang, D. Gong, D. Bar-Sagi, hSpry2 is targeted to the ubiquitin-dependent proteasome pathway by c-Cbl, Curr. Biol. 13 (2003) 308–314.
[170] C. Rubin, V. Litvak, H. Medvedovsky, Y. Zwang, S. Lev, Y. Yarden, Sprouty fine-tunes EGF signaling through interlinked positive and negative feedback loops, Curr. Biol. 13 (2003) 297–307.
[171] C.M. Stoscheck, G. Carpenter, Down regulation of epidermal growth factor receptors: direct demonstration of receptor degradation in human fibroblasts, J. Cell Biol. 98 (1984) 1048–1053.
[172] H.S. Wiley, J.J. Herbst, B.J. Walsh, D.A. Lauffenburger, M.G. Rosenfeld, G.N. Gill, The role of tyrosine kinase activity in endocytosis, compartmentation, and down-regulation of the epidermal growth factor receptor, J. Biol. Chem. 266 (1991) 11083–11094.
[173] A.R. French, G.P. Sudlow, H.S. Wiley, D.A. Lauffenburger, Postendocytic trafficking of epidermal growth factor–receptor complexes is mediated through saturable and specific endosomal interactions, J. Biol. Chem. 269 (1994) 15749–15755.
[174] J. Baulida, M.H. Kraus, M. Alimandi, P.P. Di Fiore, G. Carpenter, All ErbB receptors other than the epidermal growth factor receptor are endocytosis impaired, J. Biol. Chem. 271 (1996) 5251–5257.
[175] G. Levkowitz, L.N. Klapper, E. Tzahar, A. Freywald, M. Sela, Y. Yarden, Coupling of the c-Cbl protooncogene product to ErbB-1/EGF-receptor but not to other ErbB proteins, Oncogene 12 (1996) 1117–1125.
[176] S.K. Muthuswamy, M. Gilman, J.S. Brugge, Controlled dimerization of ErbB receptors provides evidence for differential signaling by homo- and heterodimers, Mol. Cell. Biol. 19 (1999) 6845–6857.
[177] R. Worthylake, L.K. Opresko, H.S. Wiley, ErbB-2 amplification inhibits down-regulation and induces constitutive activation of both ErbB-2 and epidermal growth factor receptors, J. Biol. Chem. 274 (1999) 8865–8874.

[178] L.N. Klapper, H. Waterman, M. Sela, Y. Yarden, Tumor-inhibitory antibodies to HER-2/ErbB-2 may act by recruiting c-Cbl and enhancing ubiquitination of HER-2, Cancer Res. 60 (2000) 3384–3388.
[179] D.J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga, L. Norton, Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2, N. Engl. J. Med. 344 (2001) 783–792.
[180] M. Cuello, S.A. Ettenberg, A.S. Clark, M.M. Keane, R.H. Posner, M.M. Nau, P.A. Dennis, S. Lipkowitz, Down-regulation of the erbB-2 receptor by trastuzumab (herceptin) enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in breast and ovarian cancer cell lines that overexpress erbB-2, Cancer Res. 61 (2001) 4892–4900.
[181] J. Harding, B. Burtness, Cetuximab: an epidermal growth factor receptor chimeric human-murine monoclonal antibody, Drugs Today (Barc) 41 (2005) 107–127.
[182] S. Harguindey, G. Orive, J. Luis Pedraz, A. Paradiso, S.J. Reshkin, The role of pH dynamics and the Na+/H+ antiporter in the etiopathogenesis and treatment of cancer, Two faces of the same coin–one single nature, Biochim. Biophys. Acta 1756 (2005) 1–24.
[183] Y. Wang, S. Pennock, X. Chen, Z. Wang, Endosomal signaling of epidermal growth factor receptor stimulates signal transduction pathways leading to cell survival, Mol. Cell. Biol. 22 (2002) 7279–7290.
[184] S. Pennock, Z. Wang, Stimulation of cell proliferation by endosomal epidermal growth factor receptor as revealed through two distinct phases of signalling, Mol. Cell. Biol. 23 (2003) 5803–5815.
[185] G. Carpenter, Receptors for epidermal growth factor and other polypeptide mitogens, Annu. Rev. Biochem. 56 (1987) 881–914.
[186] A. Sorkin, A. Eriksson, C.H. Heldin, B. Westermark, L. Claesson-Welsh, Pool of ligand-bound platelet-derived growth factor beta-receptors remain activated and tyrosine phosphorylated after internalisation, J. Cell. Physiol. 156 (1993) 373–382.
[187] H.S. Huang, M. Nagane, C.K. Klingbeil, H. Lin, R. Nishikawa, X.D. Ji, C.M. Huang, G.N. Gill, H.S. Wiley, W.K. Cavenee, The enhanced tumorigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signalling, J. Biol. Chem. 272 (1997) 2927–2935.
[188] M.H. Schmidt, F.B. Furnari, W.K. Cavenee, O. Bogler, Epidermal growth factor receptor signaling intensity determines intracellular protein interactions, ubiquitination, and internalisation, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 6505–6510.
[189] G. Carpenter, Nuclear localization and possible functions of receptor tyrosine kinases, Curr. Opin. Cell Biol. 15 (2003) 143–148.
[190] H.W. Lo, W. Xia, Y. Wei, M. Ali-Seyed, S.F. Huang, M.C. Hung, Novel prognostic value of nuclear epidermal growth factor receptor in breast cancer, Cancer Res. 65 (2005) 338–348.
[191] K. Dittmann, C. Mayer, B. Fehrenbacher, M. Schaller, U. Raju, L. Milas, D.J. Chen, R. Kehlbach, H.P. Rodemann, Radiation-induced EGFR nuclear transport is linked to activation of DNA-dependent protein kinase, J. Biol. Chem. 280 (2005) 31182–31189.
[192] G.M. Di Guglielmo, P.C. Baass, W.J. Ou, B.I. Posner, J.J. Bergeron, Compartmentalization of SHC, GRB2 and mSOS, and hyperphosphorylation of Raf-1 by EGF but not insulin in liver parenchyma, EMBO J. 13 (1994) 4269–4277.
[193] J.M. Haugh, A.C. Huang, H.S. Wiley, A. Wells, D.A. Lauffenburger, Internalized epidermal growth factor receptors participate in the activation of p21(ras) in fibroblasts, J. Biol. Chem. 274 (1999) 34350–34360.
[194] O. Kranenburg, I. Verlaan, W.H. Moolenaar, Dynamin is required for the activation of mitogen-activated protein (MAP) kinase by MAP kinase kinase, J. Biol. Chem. 274 (1999) 35301–35304.
[195] Y. Wang, S. Pennock, X. Chen, Z. Wang, Internalization of inactive EGF receptor into endosomes and the subsequent activation of endosome-associated EGF receptors. Epidermal growth factor, Sci. STKE 2002 (2002) PL17.
[196] J.M. Haugh, T. Meyer, Active EGF receptors have limited access to PtdIns(4,5)P(2) in endosomes: implications for phospholipase C and PI 3-kinase signalling, J. Cell Sci. 115 (2002) 303–310.
[197] M.A. Rizzo, K. Shome, S.C. Watkins, G. Romero, The recruitment of Raf-1 to membranes is mediated by direct interaction with phosphatidic acid and is independent of association with Ras, J. Biol. Chem. 275 (2000) 23911–23918.
[198] A.V. Vieira, C. Lamaze, S.L. Schmid, Control of EGF receptor signaling by clathrin-mediated endocytosis, Science 274 (1996) 2086–2089.
[199] J.E. Darnell Jr., Studies of IFN-induced transcriptional activation uncover the Jak-Stat pathway, J. Interferon Cytokine Res. 18 (1998) 549–554.
[200] T. Bowman, R. Garcia, J. Turkson, R. Jove, STATs in oncogenesis, Oncogene 19 (2000) 2474–2488.
[201] A.H. Bild, J. Turkson, R. Jove, Cytoplasmic transport of Stat3 by receptor-mediated endocytosis, EMBO J. 21 (2002) 3255–3263.
[202] M. Azemar, M. Schmidt, F. Arlt, P. Kennel, B. Brandt, A. Papadimitriou, B. Groner, W. Wels, Recombinant antibody toxins specific for ErbB2 and EGF receptor inhibit the in vitro growth of human head and neck cancer cells and cause rapid tumor regression in vivo, Int. J. Cancer 86 (2000) 269–275.
[203] F. Ciardiello, R. Caputo, T. Troiani, G. Borriello, E.R. Kandimalla, S. Agrawal, J. Mendelsohn, A.R. Bianco, G. Tortora, Antisense oligonucleotides targeting the epidermal growth factor receptor inhibit proliferation, induce apoptosis, and cooperate with cytotoxic drugs in human cancer cell lines, Int. J. Cancer 93 (2001) 172–178.
[204] H. Yamazaki, H. Kijima, Y. Ohnishi, Y. Abe, Y. Oshika, T. Tsuchida, T. Tokunaga, A. Tsugu, Y. Ueyama, N. Tamaoki, M. Nakamura, Inhibition of tumor growth by ribozyme-mediated suppression of aberrant epidermal growth factor receptor gene expression, J. Natl. Cancer Inst. 90 (1998) 581–587.
[205] C.B. Jannot, R.R. Beerli, S. Mason, W.J. Gullick, N.E. Hynes, Intracellular expression of a single-chain antibody directed to the EGFR leads to growth inhibition of tumor cells, Oncogene 13 (1996) 275–282.
[206] V. Grunwald, M. Hidalgo, Developing inhibitors of the epidermal growth factor receptor for cancer treatment, J. Natl. Cancer Inst. 95 (2003) 851–867.
[207] B.W. Ennis, M.W. Lippman, R.B. Dickson, The EGF receptor system as a target for antitumor therapy, Cancer Invest. 9 (1991) 553–562.
[208] J.S. de Bono, E.K. Rowinsky, Therapeutics targeting signal transduction for patients with colorectal carcinoma, Br. Med. Bull. 64 (2002) 227–254.
[209] A.M. Petit, J. Rak, M.C. Hung, P. Rockwell, N. Goldstein, B. Fendly, R. S. Kerbel, Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells in vitro and in vivo: angiogenic implications for signal transduction therapy of solid tumors, Am. J. Pathol. 151 (1997) 1523–1530.
[210] F. Ciardiello, R. Bianco, V. Damiano, S. De Lorenzo, S. Pepe, S. De Placido, Z. Fan, J. Mendelsohn, A.R. Bianco, G. Tortora, Antitumor activity of sequential treatment with topotecan and anti-epidermal growth factor receptor monoclonal antibody C225, Clin. Cancer Res. 5 (1999) 909–916.
[211] M.C. Prewett, A.T. Hooper, R. Bassi, L.M. Ellis, H.W. Waksal, D.J. Hicklin, Enhanced antitumor activity of anti-epidermal growth factor receptor monoclonal antibody IMC-C225 in combination with irinotecan (CPT-11) against human colorectal tumor xenografts, Clin. Cancer Res. 8 (2002) 994–1003.
[212] M. Ng, D. Cunningham, Cetuximab (Erbitux)—an emerging targeted therapy for epidermal growth factor receptor-expressing tumours, Int. J. Clin. Pract. 58 (2004) 970–976.
[213] D. Peng, Z. Fan, Y. Lu, T. Deblasio, H. Scher, J. Mendelsohn, Anti-epidermal growth factor receptor monoclonal antibody 225 up-regulates p27KIP1 and induces G1 arrest in prostatic cancer cell line DU145, Cancer Res. 56 (1996) 3666–3669.
[214] P. Perrotte, T. Matsumoto, K. Inoue, H. Kuniyasu, B.Y. Eve, D.J. Hicklin, R. Radinsky, C.P. Dinney, Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice, Clin. Cancer Res. 5 (1999) 257–265.

[215] E.S. Kim, F.R. Khuri, R.S. Herbst, Epidermal growth factor receptor biology (IMC-C225), Curr. Opin. Oncol. 13 (2001) 506–513.
[216] D.M. Shin, N.J. Donato, R. Perez-Soler, H.J. Shin, J.Y. Wu, P. Zhang, K. Lawhorn, F.R. Khuri, B.S. Glisson, J. Myers, G. Clayman, D. Pfister, J. Falcey, H. Waksal, J. Mendelsohn, W.K. Hong, Epidermal growth factor receptor-targeted therapy with C225 and cisplatin in patients with head and neck cancer, Clin. Cancer Res. 7 (2001) 1204–1213.
[217] D. Schrag, K.Y. Chung, C. Flombaum, L. Saltz, Cetuximab therapy and symptomatic hypomagnesemia, J. Natl. Cancer Inst. 97 (2005) 1221–1224.
[218] D. Cunningham, Y. Humblet, S. Siena, D. Khayat, H. Bleiberg, A. Santoro, D. Bets, M. Mueser, A. Harstrick, C. Verslype, I. Chau, E. Van Cutsem, Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer, N. Engl. J. Med. 351 (2004) 337–345.
[219] J.A. Bonner, D.J. Buchsbaum, S.M. Russo, J.B. Fiveash, H.Q. Trummell, D.T. Curiel, K.P. Raisch, Anti-EGFR-mediated radiosensitization as a result of augmented EGFR expression, Int. J. Radiat. Oncol. Biol. Phys. 59 (Suppl. 2) (2004) 2–10.
[220] J.A. Bonner, P.M. Harari, J. Giralt, N. Azarnia, D.M. Shin, R.B. Cohen, C.U. Jones, R. Sur, D. Raben, J. Jassem, R. Ove, M.S. Kies, J. Baselga, H. Youssoufian, N. Amellal, E.K. Rowinsky, K.K. Ang, Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck, N. Engl. J. Med. 354 (2006) 567–578.
[221] L.B. Saltz, N.J. Meropol, P.J. Loehrer Sr., M.N. Needle, J. Kopit, R.J. Mayer, Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor, J. Clin. Oncol. 22 (2004) 1201–1208.
[222] D. Vallbohmer, W. Zhang, M. Gordon, D.Y. Yang, J. Yun, O.A. Press, K. E. Rhodes, A.E. Sherrod, S. Iqbal, K.D. Danenberg, S. Groshen, H.J. Lenz, Molecular determinants of cetuximab efficacy, J. Clin. Oncol. 23 (2005) 3536–3544.
[223] J. Buter, G. Giaccone, EGFR inhibitors in lung cancer, Oncology (Williston Park) 19 (2005) 1707–1711.
[224] C.L. Arteaga, D.H. Johnson, Tyrosine kinase inhibitors-ZD1839 (Iressa), Curr. Opin. Oncol. 13 (2001) 491–498.
[225] S.L. Moulder, F.M. Yakes, S.K. Muthuswamy, R. Bianco, J.F. Simpson, C.L. Arteaga, Epidermal growth factor receptor (HER1) tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER2/neu (erbB2)-overexpressing breast cancer cells in vitro and in vivo, Cancer Res. 61 (2001) 8887–8895.
[226] N.G. Anderson, T. Ahmad, K. Chan, R. Dobson, N.J. Bundred, ZD1839 (Iressa), a novel epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, potently inhibits the growth of EGFR-positive cancer cell lines with or without erbB2 overexpression, Int. J. Cancer 94 (2001) 774–782.
[227] F.M. Sirotnak, M.F. Zakowski, V.A. Miller, H.I. Scher, M.G. Kris, Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase, Clin. Cancer Res. 6 (2000) 4885–4892.
[228] R.B. Lichtner, A. Menrad, A. Sommer, U. Klar, M.R. Schneider, Signaling-inactive epidermal growth factor receptor/ligand complexes in intact carcinoma cells by quinazoline tyrosine kinase inhibitors, Cancer Res. 61 (2001) 5790–5795.
[229] E. Di Gennaro, M. Barbarino, F. Bruzzese, S. De Lorenzo, M. Caraglia, A. Abbruzzese, A. Avallone, P. Comella, F. Caponigro, S. Pepe, A. Budillon, Critical role of both p27KIP1 and p21CIP1/WAF1 in the antiproliferative effect of ZD1839 ('Iressa'), an epidermal growth factor receptor tyrosine kinase inhibitor, in head and neck squamous carcinoma cells, J. Cell. Physiol. 195 (2003) 139–150.
[230] G. Giaccone, The role of gefitinib in lung cancer treatment, Clin. Cancer Res. 10 (2004) 4233s–4237s.
[231] T.J. Lynch, D.W. Bell, R. Sordella, S. Gurubhagavatula, R.A. Okimoto, B.W. Brannigan, P.L. Harris, S.M. Haserlat, J.G. Supko, F.G. Haluska, D. N. Louis, D.C. Christiani, J. Settleman, D.A. Haber, Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib, N. Engl. J. Med. 350 (2004) 2129–2139.
[232] J. Settleman, Inhibition of mutant EGF receptors by gefitinib: targeting an achilles' heel of lung cancer, Cell Cycle 3 (2004) 1496–1497.
[233] X. Jiang, F. Huang, A. Marusyk, A. Sorkin, Grb2 regulates internalization of EGF receptors through clathrin-coated pits, Mol. Biol. Cell 14 (2003) 858–870.
[234] D.A. Haber, J. Settleman, Overcoming acquired resistance to Iressa/ Tarceva with inhibitors of a different class, Cell Cycle 4 (2005) 1057–1059.
[235] C.K. Osborne, R. Schiff, Growth factor receptor cross-talk with estrogen receptor as a mechanism for tamoxifen resistance in breast cancer, Breast 12 (2003) 362–367.
[236] C.L. Arteaga, Trastuzumab, an appropriate first-line single-agent therapy for HER2-overexpressing metastatic breast cancer, Breast Cancer Res. 5 (2003) 96–100.
[237] S.J. Rogers, K.J. Harrington, P. Rhys-Evans, P. O-Charoenrat, S.A. Eccles, Biological significance of c-erbB family oncogenes in head and neck cancer, Cancer Metastasis Rev. 24 (2005) 47–69.
[238] Z. Suo, J.M. Nesland, Type 1 protein tyrosine kinases in breast carcinoma: a review, Ultrastruct. Pathol. 26 (2002) 125–135.
[239] H. Moch, G. Sauter, N. Buchholz, T.C. Gasser, L. Bubendorf, F.M. Waldman, M.J. Mihatsch, Epidermal growth factor receptor expression is associated with rapid tumor cell proliferation in renal cell carcinoma, Hum. Pathol. 28 (1997) 1255–1259.
[240] R.J. Hale, C.H. Buckley, W.J. Gullick, H. Fox, J. Williams, F.L. Wilcox, Prognostic value of epidermal growth factor receptor expression in cervical carcinoma, J. Clin. Pathol. 46 (1999) 149–153.
[241] H. Kuwano, H. Kato, T. Miyazaki, M. Fukuchi, N. Masuda, M. Nakajima, Y. Fukai, M. Sohda, H. Kimura, A. Faried, Genetic alterations in esophageal cancer, Surg. Today 35 (2005) 7–18.
[242] H.Q. Xiong, J.L. Abbruzzese, Epidermal growth factor receptor-targeted therapy for pancreatic cancer, Semin. Oncol. 29 (5 Suppl. 14) (2002) 31–37.
[243] W.A. Franklin, R. Veve, F.R. Hirsch, B.A. Helfrich, P.A. Bunn Jr., Epidermal growth factor receptor family in lung cancer and premalignancy, Semin. Oncol. 29 (1 Suppl. 4) (2002) 3–14.
[244] H.G. Kim, J. Kassis, J.C. Souto, T. Turner, A. Wells, EGF receptor signaling in prostate morphogenesis and tumorigenesis, Histol. Histopathol. 14 (1999) 1175–1182.
[245] M.L. Janmaat, G. Giaccone, The epidermal growth factor receptor pathway and its inhibition as anticancer therapy, Drugs Today (Barc) 39 (Suppl. C) (2003) 61–80.
[246] S.C. Juneja, J.M. Lafky, H. Lee, J.L. Reiter, K.C. Podratz, EGF/ErbB receptor family in ovarian cancer, Cancer Treat. Res. 107 (2002) 247–258.
[247] N. Nathoo, S. Goldlust, M.A. Vogelbaum, Epidermal growth factor receptor antagonists: novel therapy for the treatment of high-grade gliomas, Neurosurgery 54 (2004) 1480–1489.
[248] N. Shinojima, K. Tada, S. Shiraishi, T. Kamiryo, M. Kochi, H. Nakamura, K. Makino, H. Saya, H. Hirano, J. Kuratsu, K. Oka, Y. Ishimaru, Y. Ushio, Prognostic value of epidermal growth factor receptor in patients with glioblastoma multiforme, Cancer Res. 63 (2003) 6962–6970.
[249] A.J. Colquhoun, J.K. Mellon, Epidermal growth factor receptor and bladder cancer, Postgrad. Med. J. 78 (2002) 584–589.
[250] R. Kopp, E. Rothbauer, M. Ruge, H. Arnholdt, J. Spranger, M. Muders, D.G. Pfeiffer, F.W. Schildberg, A. Pfeiffer, Clinical implications of the EGF receptor/ligand system for tumor progression and survival in gastrointestinal carcinomas: evidence for new therapeutic options, Recent. Results Cancer Res. 162 (2003) 115–132.