

Signalling networks in focus

Epidermal growth factor receptor degradation: An alternative view of oncogenic pathways[☆]

Andreas Kirisits, Dietmar Pils, Michael Krainer^{*}

Clinical Division of Oncology, Department of Medicine I, Medical University Vienna, Austria

Received 26 April 2007; received in revised form 16 July 2007; accepted 25 July 2007

Available online 1 August 2007

Abstract

Positive regulation of epidermal growth factor receptor signalling is related to many human malignancies. Besides overexpression and gain of function mutations, the escape from negative regulation through an increase in epidermal growth factor receptor stability has evolved as yet another key factor contributing to enhanced receptor activity. Intensive research over the past years has provided considerable evidence concerning the molecular mechanisms which provide epidermal growth factor receptor degradation. c-Cbl mediated ubiquitination, endocytosis via clathrin-coated pits, endosomal sorting and lysosomal degradation have become well-investigated cornerstones. Recent findings on the interdependency of the endosomal sorting complexes required for transport in multivesicular body sorting, stress the topicality of receptor tyrosine kinase downregulation. Here, we review the degradation pathway of the epidermal growth factor receptor, following the receptor from ligand binding to the lysosome and illustrating different modes of oncogenic deregulation.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: EGFR; Receptor degradation; Ubiquitination; MVB; ESCRT; Cancer

Facts on epidermal growth factor receptor degradation:

- The pathway of receptor degradation shares main events and molecules with the mitogenic signalling cascade of the epidermal growth factor receptor.
- Negative regulation of certain components of this pathway bears oncogenic potential.

- Epidermal growth factor receptor degradation requires preceding ligand binding and c-Cbl mediated monoubiquitination.
- Internalisation of the epidermal growth factor receptor via clathrin coated pits is followed by its uptake into a specialised endosomal organelle, called multivesicular body.
- Epidermal growth factor receptor routing within the multivesicular body is determined by three endosomal sorting complexes required for transport.

[☆] This work was supported by FWF (Fonds zur Förderung der wissenschaftlichen Forschung) Grant 13473.

^{*} Corresponding author at: Clinical Division of Oncology, Department of Medicine I, University Hospital, Währinger Gürtel 18-20, A-1090 Vienna, Austria. Tel.: +43 1 40400 4449; fax: +43 1 40400 4451.

E-mail address: michael.krainer@meduniwien.ac.at (M. Krainer).

1. Introduction

The ErbB family of receptor tyrosine kinases (RTK) represents a highly conserved signal transduction network that plays a pivotal role in physiological cell proliferation and behaviour. Following on from that, dys-

regulation of epidermal growth factor receptor (EGFR) signalling by constitutive overexpression, overstimulation or mutational activation has turned out to be a driving force behind the pathogenesis of various tumour entities (Yarden & Sliwkowski, 2001). The mechanisms underlying EGFR degradation are probably the best understood among all RTKs and there is evidence that besides the dysregulating thus tumourigenic effects mentioned above, an impaired or ineffective EGFR-degradation pathway plays a major role in increased ErbB-signalling and accordingly in cancer development.

This review will therefore focus on ligand-induced downregulation of EGFR, addressing the post-translational modification of the receptor and the intracellular machinery regulating the sequence of EGFR internalisation, endosomal and multivesicular body (MVB) sorting and lysosomal degradation. Taking into consideration the complex interdependency of proteins in this anti-proliferative cascade (Table 1), we will furthermore illustrate different modes of dysregulation as well as their impact on malignant cell transformation.

2. The EGFR degradation cascade—key events and molecules

2.1. Dimerisation and phosphorylation

Ligand binding to the initially inactive monomeric EGF receptor leads to either homodimerisation or heterodimerisation with any of the other ErbB family members (Fig. 1). Dimerisation in turn triggers mutual receptor transphosphorylation on intracellular tyrosine residues, which not only initiates the signalling cascade but also provides the docking sites necessary for the recruitment of the cytoplasmic phosphotyrosine-binding proteins that are responsible for the proper degradation of ligand-activated EGFR (Sweeney & Carraway, 2004).

Although the formation of all 10 possible ErbB dimers has been observed, there was a striking preference of Her2 as a heterodimerisation partner for EGFR (Sweeney & Carraway, 2004). This aspect is of remarkable significance, as participation of Her2 in dimer formation increases signalling potency, and has even been found to activate EGFR independent of preceding ligand binding. Furthermore, Her2 overexpression is associated with a slow rate of receptor internalisation and an increased fraction of internalised EGFR being recycled back to the cell surface, thus diminishing EGFR down-regulation (Worthylake, Opresko, & Wiley, 1999).

2.2. c-Cbl and ubiquitination: modifying EGFR

One of the major players involved in EGFR degradation is the E3 ligase c-Cbl, which is recruited to the plasma membrane upon ligand stimulation. It is subject to tyrosine phosphorylation by EGFR and mediates ubiquitination as well as intracellular receptor handling (Fig. 1). The Cbl family plays a key role in negative regulation not only of EGFR but of a vast range of RTKs, including platelet derived growth factor receptor (PDGFR), colony stimulating factor (CSF-1) receptor and Her2 (Thien & Langdon, 2001). c-Cbl occupies the phosphorylated tyrosine residue 1045 (pTyr 1045) of EGFR and attaches ubiquitin to the bound receptor (Katzmann, Odorizzi, & Emr, 2002). The fact that c-Cbl binds phosphorylated Tyr 1045 underlines the importance of tyrosine kinase activity for consecutive receptor degradation. In addition to that, growth factor receptor binding protein 2 (Grb2) was shown to facilitate an indirect, supplementary interaction between EGFR and c-Cbl, apparently concerned in receptor endocytosis (Huang & Sorkin, 2005).

Ubiquitination was originally considered to be the signal responsible for proteasomal delivery of its substrates. However, as EGFR is degraded in lysosomal vesicles, this alternative pathway requires a slightly modified mechanism of ubiquitination. Two distinct means of ubiquitin-attachment were shown to officiate as sorting signals: proteasomal recognition of a protein requires a chain of ubiquitin molecules appended during a process termed polyubiquitination. EGFR, by contrast, is monoubiquitinated at multiple sites to enter its endosomal trafficking route (Haglund et al., 2003).

Oncogenic variants of the c-Cbl protein, dysfunctional in transferring ubiquitin while retaining their ability to bind EGFR are thought to compete with wild type c-Cbl in occupying pTyr 1045. This is of certain interest, as they have been identified in murine tumours and are hence held responsible for RTK-dependent cell transformation (Thien & Langdon, 2001). Besides that, the cytoplasmic tyrosine kinase c-Src, degrading c-Cbl, and deregulated in multiple human cancers, was shown to stabilise EGFR at the cell surface if aberrantly activated (Bao, Gur, & Yarden, 2003). Therefore, negative regulation of c-Cbl can be regarded as an indirect way to enhance EGFR's stability and EGF-induced mitogenesis.

2.3. Receptor endocytosis

Ligand-induced internalisation of EGFR is performed via clathrin coated pits (CCPs) and involves a vast

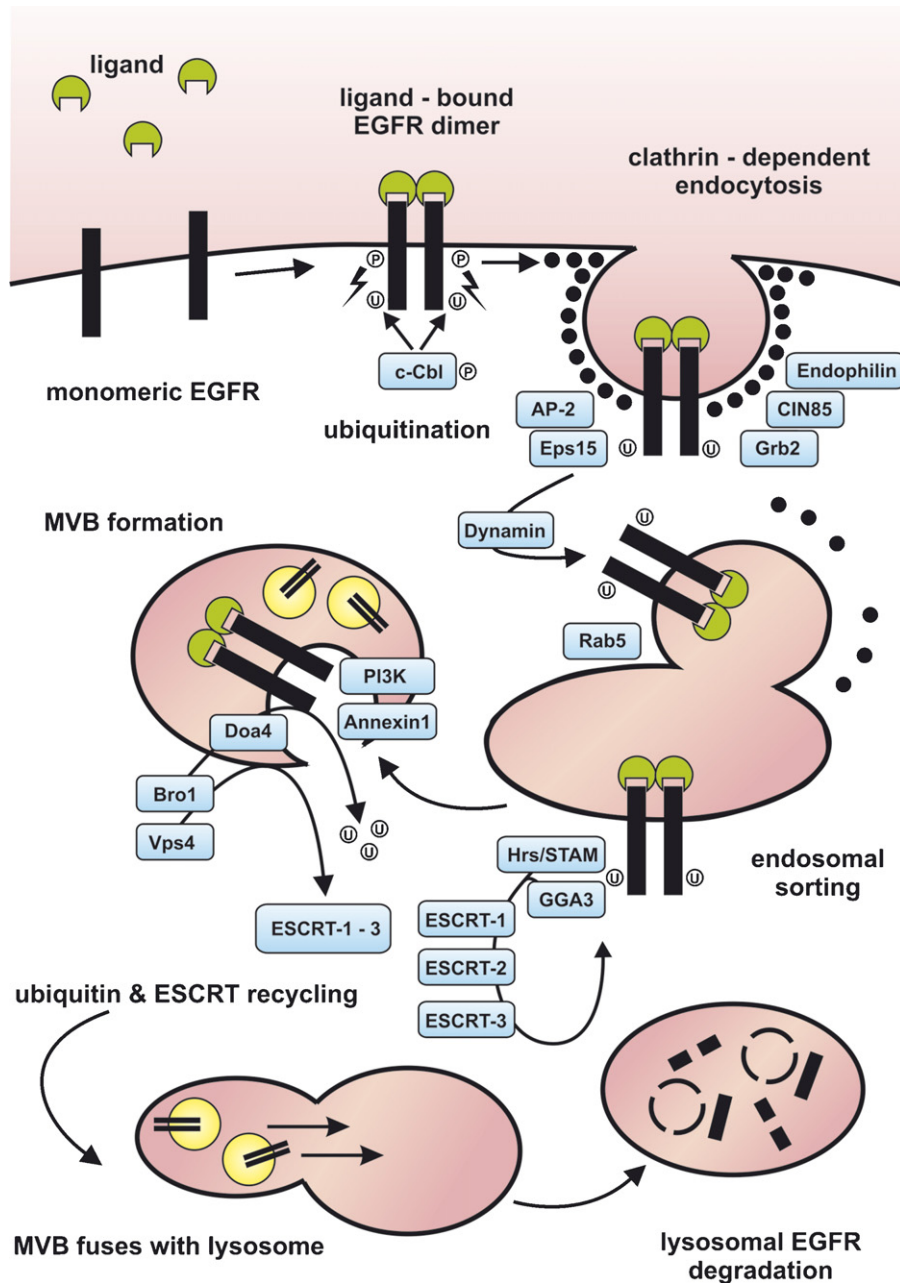


Fig. 1. EGFR degradation pathway. Ligand binding to the monomeric EGFR leads to dimerisation and autophosphorylation. c-Cbl is then phosphorylated and recruited to the receptor where it induces monoubiquitination. Ubiquitinated EGFRs aggregate in clathrin coated pits and are internalised, both upon mediation of the multiprotein endocytic apparatus. Clathrin is shed and the internal vesicles fuse with early endosomes. Hrs/STAM and GGA3 provide the link between ESCRT components and EGFR at the limiting endosomal membrane where they meet. The endosomal sorting cascade, consisting of ESCRT-I, ESCRT-II and ESCRT-III, in turn triggers MVB formation (for a detailed depiction of MVB sorting please see Fig. 2). Before the internal vesicles are formed, ESCRT complexes and ubiquitin are recycled to the cytoplasmic pool. The MVB fuses with the lysosome and delivers the EGFR-containing inner vesicles to the proteolytic lysosomal interior where they are degraded.

array of adaptors and enzymes (Fig. 1). Receptor guidance to CCPs is driven by concomitant interaction of epidermal growth factor pathway substrate 15 (Eps15) with the clathrin adaptor complex 2 (AP-2) and either

the ubiquitinated receptor or EGFR-associated c-Cbl (de Melker, van der Horst, & Borst, 2004). Moreover, another protein called Cbl-interacting protein of 85 kDa (CIN85) was also shown to link EGFR-c-Cbl com-

plexes to CCPs through interaction with Endophilin, a CCP-regulatory component (Soubeyran, Kowanetz, Szymkiewicz, Langdon, & Dikic, 2002). Together with the aforementioned Grb2 which contributes to receptor aggregation in CCPs, the concomitance of different cellular strategies in internalising EGFR becomes evident.

A GTPase called dynamin carries out the vesicle fission from the plasma membrane (Fig. 1) (McNiven, Cao, Pitts, & Yoon, 2000). After that, clathrin is shed from the EGFR-laden vesicle which hereupon fuses with cytoplasmic vesicular structures, a process mediated by another GTPase named Ras-associated protein 5 (Rab5), to form the early endosome (Fig. 1) (Zerial & McBride, 2001).

The topic of ubiquitination in endocytosis is a controversial one. The primal idea, that pTyr 1045-coupled ubiquitination of EGFR was necessary for the ensuing internalisation step, has come under discussion. Two hypotheses have emerged: one asserts that Grb2-mediated EGFR ubiquitination might serve as a prerequisite for receptor endocytosis (Jiang, Huang, Marusyk, & Sorkin, 2003). According to the other, ubiquitination of the proteins representing the endocytic apparatus, rather than of EGFR itself, might be substantial for clathrin-dependent endocytosis (CDE) (Waterman et al., 2002). The finding that c-Cbl overexpression does not augment the rate of EGFR internalisation, while at the same time an increase in degradation of already endocytosed receptors can be observed, has raised additional questions (Duan et al., 2003). Contradictory at first sight, it points out that c-Cbl's principal task should be seen in facilitating proper intracellular trafficking of EGFR, not its endocytosis.

2.4. Endosomal sorting

2.4.1. The multivesicular body (MVB)

Effective degradation of EGFR requires its uptake into a highly specialised organelle termed multivesicular body (MVB) (Gruenberg & Stenmark, 2004). The formation of this late endosomal compartment through invagination of the limiting endosomal membrane towards the compartmental interior, thus creating the eponymous multiplicity of vesicles, is necessary to solve the topological problem of lumenally degrading a transmembrane protein like EGFR. This event entails the eventual cessation of EGFR signalling by segregating the still active receptor domain from its cytoplasmic transducers, as EGFR was observed to maintain signalling potency even after being internalised and conveyed to

the early endosome (Miaczynska, Pelkmans, & Zerial, 2004).

MVB formation and cargo recognition have been extensively studied in *Saccharomyces cerevisiae*, and the proteins involved have been classified as class E vacuolar protein sorting (Vps) proteins, all of them having their mammalian homologues identified (Babst, 2005). Loss of function of E Vps proteins is phenotypically reflected by altered trafficking of transmembrane proteins which, in consequence, accumulate in the yeast-specific class E compartment now unable to form an MVB. Analogous observations regarding the mammalian E Vps counterparts suggest that their task in protein sorting is an evolutionarily conserved feature in eukaryotic cells (Babst, 2005).

2.4.2. Endosomal sorting complexes: ESCRT-I–III

Certain E Vps proteins catenate to three so-called endosomal sorting complexes required for transport (ESCRT-I–III), acting consecutively in MVB sorting (Fig. 1). ESCRT-I consists of three proteins, namely tumour susceptibility gene 101 (TSG101), human Vps28 (hVps28) and hepatocellular carcinoma related protein 1 (HCRP1) (Vps23, Vps28 and Vps37 in yeast, respectively) (Fig. 2) (Katzmann, Babst, & Emr, 2001; Bache et al., 2004). TSG101 has a ubiquitin-conjugating-like (UBC) domain to bind ubiquitinated EGFR directly (Pornillos et al., 2002). TSG101 is therefore considered responsible for cargo recognition in the ESCRT-I complex. Experiments inhibiting the subunits of ESCRT-I separately, either using antibodies or siRNA, resulted in retardation of EGFR degradation. This indicates the essential importance of all three constituents in MVB sorting, even though the scope of functions for hVps28 and HCRP1 has so far not been specified (Bishop, Horman, & Woodman, 2002).

Following from structure studies, there has been speculation about the existence of additional ESCRT-I subunits. Investigators have now found a poorly conserved yeast protein called Mvb12 to transiently associate with ESCRT-I (Curtiss, Jones, & Babst, 2007). While Mvb12 did not seem to be essential for endocytic cargo sorting, lack of Mvb12 resulted in an impaired dissociation of ESCRT-I from the MVB. Further investigation is needed to show whether this can be explained by a role of Mvb12 in ESCRT/ESCRT or rather ESCRT/cargo interaction. Another study of interest identified the breakpoint cluster region (Bcr) protein, well known from the aberrant Bcr-Abl fusion protein expressed by patients with chronic myelogenous leukaemia, as a binding partner for TSG101 in mam-

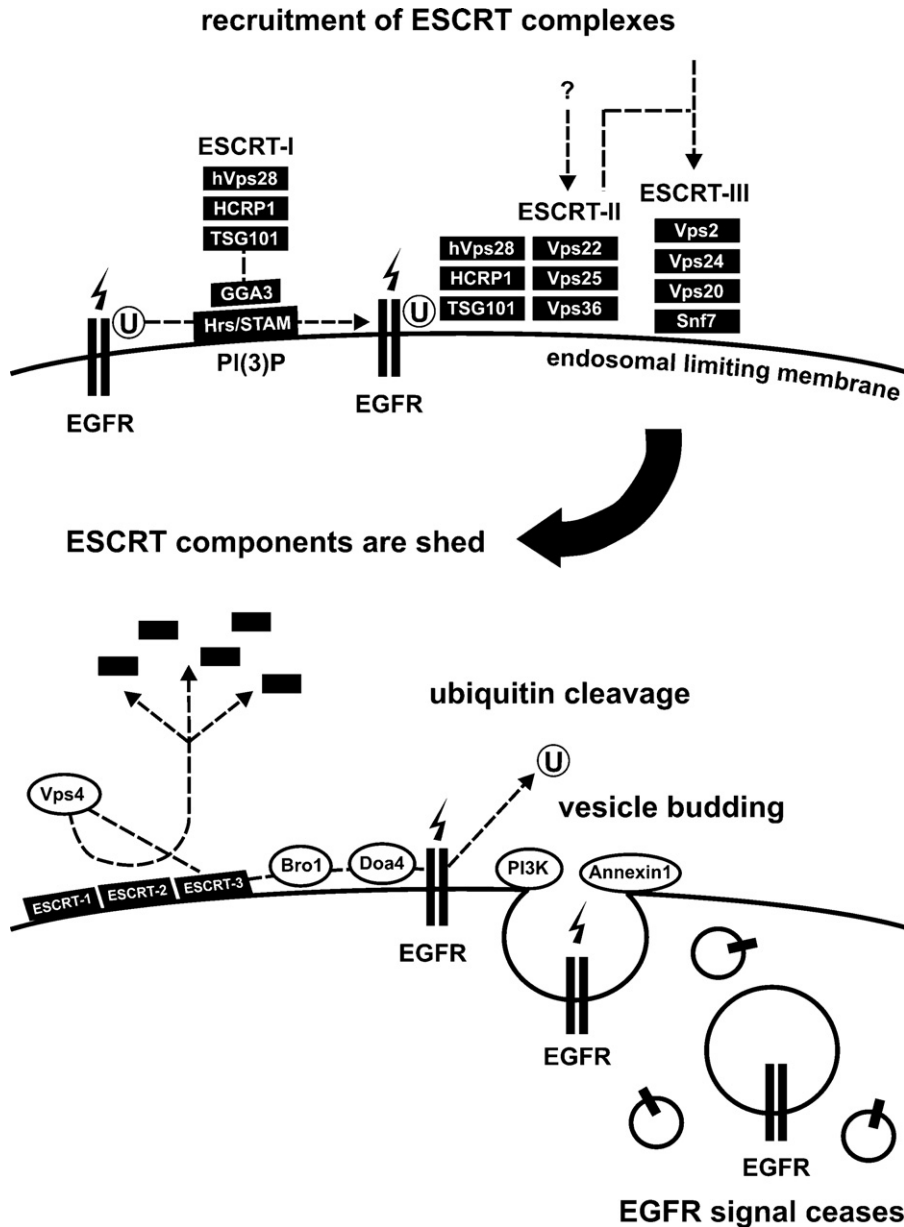


Fig. 2. Multivesicular body sorting. After endocytosis, the still active EGFR reaches the endosome. The ubiquitin moieties attached to the receptor are recognised by the Hrs/STAM complex and GGA3, which in turn guide ESCRT-I to the site of action. ESCRT-II is hereupon recruited from the cytoplasmic pool, however it is unclear how ESCRT-I triggers this step. ESCRT-II then initiates ESCRT-III assembly at the endosomal limiting membrane. At this moment, all three ESCRT complexes are associated with EGFR. Ubiquitin and the ESCRT components are recycled and internal vesicle formation sequesters EGFR away from its cytoplasmic signal transducers.

malian cells (Olabisi et al., 2006). Upon suppression of Bcr, an accumulation of EGFR was observed, suggesting Bcr to be either a regulator or a novel component of ESCRT-I.

ESCRT-II consisting of Vps22, Vps25 and Vps36 in yeast, is supposed to exert its activity downstream of ESCRT-I (Babst, Katzmann, Snyder, Wendland, & Emr,

2002). ESCRT-II is formed independently of ESCRT-I and was found to be transiently recruited to the endosomal limiting membrane from a soluble cytoplasmic pool to initiate oligomerisation of Vps2, Vps20, Vps24 and sucrose non fermentor 7 (Snf7) through interaction with Vps20, which results in the formation of ESCRT-III (Fig. 2) (Babst, Katzmann, Estepa-Sabal,

Meerloo, & Emr, 2002). The latter complex guides recycling proteins to the site of action, namely Bro1, responsible for the recruitment of Doa4, a deubiquitinating enzyme, and Vps4, an AAA-type ATPase responsible for ESCRT disassembly (Figs. 1 and 2) (Babst, 2005). Ubiquitin-specific protease Y (UBPY) and associated molecule with the SH3 domain of STAM (AMSH) are up for discussion as deubiquitinating counterparts of Vps4 in human cells. While both of them are functionally and topologically linked to the mammalian MVB pathway, the results obtained from knockdown experiments investigating their part in EGFR degradation were inconsistent (Bowers et al., 2006; Ma et al., 2007). The interesting finding that TSG101-associated ligase (Tal) regulates TSG101 activity by means of ubiquitination provides a possible explanation for the methodological difficulties experienced when testing the direct effects of depleting either UBPY or AMSH on EGFR sorting (Amit et al., 2004). Accordingly, as ubiquitination has turned out to be involved in regulating ESCRT function, it will be necessary to answer the question whether the two deubiquitinating enzymes are in fact targeting EGFR or rather components of the MVB machinery.

2.4.3. Consolidating ub-EGFR and ESCRT at the endosome

Hepatocyte growth factor regulated tyrosine kinase substrate (Hrs), the mammalian homologue of Vps27, in combination with signal transducing adaptor molecules 1 and 2 (STAM1, STAM2) and the Golgi-localised, γ -ear-containing, Arf-binding protein 3 (GGA3) are supposed to bind the ubiquitinated cargo upstream of ESCRT-I. Both Hrs-STAM and GGA3 capture and guide ESCRT-I, TSG101 in the strict sense, to the late endosome, and form the prerequisite for the proper localisation and connection of EGFR and the ESCRT apparatus at the endosome (Figs. 1 and 2) (Katzmann, Stefan, Babst, & Emr, 2003; Puertollano & Bonifacino, 2004). Consistent with this, mutations perturbing Hrs/TSG101 interaction resulted in EGFR being insufficiently transferred from early to late endosomes (Lu, Hope, Brasch, Reinhard, & Cohen, 2003). In addition, EGFR degradation was partially inhibited in both Hrs(–/–) and STAM1(–/–)/STAM2(–/–) mouse embryonic fibroblasts (Kanazawa et al., 2003), and similar results could be obtained for cells treated with si-RNA targeting GGA3 (Puertollano & Bonifacino, 2004). In short, both Hrs-STAM and GGA3 can be considered central elements in cargo recognition and cargo feeding into the MVB.

2.4.4. MVB internal vesicle formation and lysosomal fusion

Once the ESCRT machinery has completed cargo reception and concentration, the invagination and formation of EGFR-loaded MVB vesicles from the limiting endosomal membrane need to be conducted (Fig. 1). There is not much evidence on this topic, both ubiquitin and the ESCRT complexes are shed from endosomal EGFR prior to MVB vesicle moulding and are therefore unlikely to be involved directly. There has been speculation about whether the late endosomal phospholipid lysobisphosphatic acid (LBPA) participates in vesicle fission, as LBPA occurs in particularly high concentration in luminal MVB substructures (Babst, 2005; Kobayashi et al., 1998). Human antiphospholipid antibodies that specifically bind LBPA have been shown to restrict MVB sorting, which strengthens this assumption (Kobayashi et al., 1998). Besides LBPA, phosphatidylinositol-3-phosphat (PI3P), a lipid extensively enriched in late endosomal membranes, might play a role in vesicle invagination by recruiting phosphatidylinositol-3-kinase (PI3K) (Vps34 in yeast) to the MVB membrane (Fig. 2). Inhibition of PI3K activity using either wortmannin or anti-human Vps34 antibodies disabled vesicle formation in human cells (Futter, Collinson, Backer, & Hopkins, 2001).

Searching for substrates of the EGFR tyrosine kinase, participating in receptor degradation, the phospholipid-binding protein Annexin 1 has been found to be phosphorylated at the MVB membrane upon stimulation with EGF (Futter, Felder, Schlessinger, Ullrich, & Hopkins, 1993). Later results have shown EGF-mediated receptor activation to stimulate MVB biogenesis as well as internal vesicle formation (Fig. 2). In this connection, Annexin 1 has been found to promote the latter process in an EGF-dependent manner exclusively in MVBs containing EGFR (White, Bailey, Aghakhani, Moss, & Futter, 2006). It is not clear whether phosphorylation of Annexin 1 regulates its localisation or functionality. In a recent review on Annexins in membrane trafficking, the authors are discussing phosphorylation as a possible prerequisite for the final fission of internal MVB vesicles (Futter & White, 2007). Interestingly, the depletion of Annexin 1 had no significant effect on receptor turnover, causing just a minor delay in EGFR degradation (White et al., 2006). Following from that, it is likely that Annexin 1 is not essential for EGFR degradation but catalyses the final sorting steps preceding lysosomal fusion. Ending the intracellular EGFR trafficking process, the MVB eventually fuses with the lysosome, delivering the EGFR-laden intracompartmen-

tal vesicles to the hydrolytic lysosomal interior where they are degraded (Fig. 1).

3. Therapeutic implications and future prospects

Its involvement in the formation of different tumours has led to the development of two therapeutic approaches targeting the EGFR pathway: ATP-competitive inhibition of the intracellular receptor tyrosine kinase by means of small-molecule tyrosine kinase inhibitors (TKI) and monoclonal antibodies (mAB) directed against the extracellular receptor domain. Amongst the TKIs, Erlotinib has been approved for the last-line treatment of non-small cell lung cancer (NSCLC) and first-line treatment of pancreatic cancer, whereas Gefitinib has so far only been approved for the last-line treatment of NSCLC. Cetuximab, a chimeric mAB, has received approval for the treatment of colorectal carcinoma, and quite recently for head and neck squamous-cell cancer. Phase I–III trials, testing other substances and their efficacy in a wide range of cancer types, are underway. Furthermore, the genetic profile of EGFR and the cellular response to EGFR-targeted antitumour therapy have evolved as important fields of research. This is due to the fact that even though the preclinical results obtained with TKIs and mABs were promising, the actual clinical benefit lags behind (Scaltriti & Baselga, 2006).

Remembering the close connection between positive EGFR signalling and its degradation, the question is whether or not the application of either mABs or TKIs stabilises EGFR at the cell surface. It is conceivable that the number of EGFR at the cell surface increases during treatment using any of the two. A recent study investigating the effect of Gemcitabine on EGFR phosphorylation and degradation follows similar lines (Feng et al., 2007). Future research should therefore deal with the question of whether this supposable augmentation of receptor molecules is substantial and whether it affects tumour growth, in case the therapy with EGFR inhibitors needs to be paused or discontinued.

More than a decade ago, the detection of intact EGFR dimers along with other RTKs in the cell nucleus started a lively discussion about the mechanisms behind nuclear translocalisation and the possible functional relevance of this entirely different way of EGFR signalling. Significant levels of nuclear EGFR have thereupon been detected in different tumour types and were moreover found to correlate with poor clinical outcome in breast cancer and oropharyngeal squamous cell carcinoma (Lo et al., 2005; Psyrri et al., 2005). Furthermore, Cyclin D1, B-Myb and most recently, inducible nitric oxide synthase (iNOS), all of which involved in normal and

tumour cell proliferation, have been identified as transcriptional targets for nuclear EGFR, while the mode of interaction between the receptor molecule and its target genes remains unclear (Lo & Hung, 2006; Massie & Mills, 2006). Of special interest in the context of receptor degradation is the fact that the alternative intracellular route of nuclear translocalisation has been found to be dependent on preceding ligand-induced internalisation (Lo and Hung, 2006); therefore, both pathways seem to share certain trafficking passages. But what are the triggers for EGFR being redirected from the degradation machinery? To answer the evident question of how to transfer a transmembrane protein like EGFR from the lipid bilayer into the cell nucleus will be another major challenge. First results concerning this matter indicate an involvement of the endoplasmatic reticulum and its Sec61 translocon, normally acting in the turnover of misfolded proteins, which might just define another cell organelle to be participating in EGFR processing (Liao & Carpenter, 2007).

Turning back to the degradation cascade itself, we pointed out that the EGFR signal does not cease until the receptor is safely embedded within the MVB. Interestingly enough, TSG101, the first identified human homologue in the MVB machinery, is a well-known player in cancer genetics. Several years ago, a functional screen for novel tumour suppressor genes showed that the knockout of a gene consequently called tumour susceptibility gene 101 (TSG101) induces malignant transformation of NIH 3T3 cells (Li & Cohen, 1996), a fact that, together with the new insights gained in ESCRT functionality, renders the role of TSG101 in tumour development or progression an interesting topic for future investigation. Besides TSG101, HCRP1, another promising human homologue of an E Vps gene participating in the same ESCRT complex was recently identified and was found to influence hepatocellular carcinogenesis (Xu, Liang, Wang, Li, & Zhao, 2003).

While the field has just accepted the importance of receptor ubiquitination pertaining to the dysregulation of growth receptors in cancer, the next challenge, that is to determine the subsequent sorting steps until the mitogenic signal ceases, is right around the corner. The knowledge we have about the role of the MVB offers intriguing hints regarding a presumable involvement of these events in the pathogenesis of cancer. Moreover, it might be of interest to explore the potential the degradation pathway of EGFR holds for specific therapeutical approaches, as this aspect has so far mostly been disregarded. A better understanding of EGFR downregulation will not only enhance our diagnostic and prognostic

Table 1

Components of the EGFR degradation cascade

Mammalian	Yeast	Complex	Function
Grb2	Ede1		Recruitment of c-Cbl to EGFR Regulation of endocytosis
Eps15			EGFR aggravation in CCPs
CIN85			Guidance of EGFR into CCPs in interaction with Endophilin
Endophilin			CCP regulatory component
Hrs STAM GGA3	Vps27 Hse1	Hrs/STAM	Consolidation of ub-EGFR and ESCRT-I at the endosome Consolidation of ub-EGFR and ESCRT-I at the endosome
TSG101 hVps28 HCRP1 Bcr	Vps23 Vps28 Vps37 Mvb12	ESCRT-I ESCRT-I ESCRT-I	Cargo recognition; Activation of ESCRT-II Potentially involved in dissociation of ESCRT-I Potential regulator or additional component of ESCRT-I
Eap30 Eap25 Eap45	Vps22 Vps25 Vps36	ESCRT-II ESCRT-II ESCRT-II	Cargo routing; Recruitment of ESCRT-III
Chmp2 Chmp6 Chmp3 Chmp4/hSnf7	Vps2 Vps20 Vps24 Snf7	ESCRT-III ESCRT-III ESCRT-III ESCRT-III	Cargo concentration; Recruitment of recycling proteins
UBPY AMSH	Doa4		Deubiquitination; Ub-recycling Deubiquitination Deubiquitination
	Vps4		Recycling of the ESCRT components
Annexin 1			EGFR-specific MVB internal vesicle invagination
PI3K	Vps34		MVB vesicle invagination

armamentarium, but should also be considered a promising arena of therapeutic intervention.

References

- Amit, I., Yakir, L., Katz, M., Zwang, Y., Marmor, M. D., Citri, A., et al. (2004). Tal, a Tsg101-specific E3 ubiquitin ligase, regulates receptor endocytosis and retrovirus budding. *Genes Dev.*, 18, 1737–1752.
- Babst, M. (2005). A protein's final ESCRT. *Traffic*, 6, 2–9.
- Babst, M., Katzmann, D. J., Estepa-Sabal, E. J., Meerloo, T., & Emr, S. D. (2002). Escrt-III: An endosome-associated heterooligomeric protein complex required for MVB sorting. *Dev. Cell*, 3, 271–282.
- Babst, M., Katzmann, D. J., Snyder, W. B., Wendland, B., & Emr, S. D. (2002). Endosome-associated complex, ESCRT-II, recruits

- transport machinery for protein sorting at the multivesicular body. *Dev. Cell*, 3, 283–289.
- Bache, K. G., Slagsvold, T., Cabezas, A., Rosendal, K. R., Raiborg, C., & Stenmark, H. (2004). The growth-regulatory protein HCRP1/hVps37A is a subunit of mammalian ESCRT-I and mediates receptor down-regulation. *Mol. Biol. Cell*, 15, 4337–4346.
- Bao, J., Gur, G., & Yarden, Y. (2003). Src promotes destruction of c-Cbl: Implications for oncogenic synergy between Src and growth factor receptors. *Proc. Natl. Acad. Sci. U.S.A.*, 100, 2438–2443.
- Bishop, N., Horman, A., & Woodman, P. (2002). Mammalian class E Vps proteins recognize ubiquitin and act in the removal of endosomal protein-ubiquitin conjugates. *J. Cell Biol.*, 157, 91–101.
- Bowers, K., Piper, S. C., Edeling, M. A., Gray, S. R., Owen, D. J., Lehner, P. J., et al. (2006). Degradation of endocytosed epidermal growth factor and virally ubiquitinated major histocompatibility complex class I is independent of mammalian ESCRTIII. *J. Biol. Chem.*, 281, 5094–5105.
- Curtiss, M., Jones, C., & Babst, M. (2007). Efficient cargo sorting by ESCRT-I and the subsequent release of ESCRT-I from multivesicular bodies requires the subunit Mvb12. *Mol. Biol. Cell*, 18, 636–645.
- de Melker, A. A., van der Horst, G., & Borst, J. (2004). Ubiquitin ligase activity of c-Cbl guides the epidermal growth factor receptor into clathrin-coated pits by two distinct modes of Eps15 recruitment. *J. Biol. Chem.*, 279, 55465–55473.
- Duan, L., Miura, Y., Dimri, M., Majumder, B., Dodge, I. L., Reddi, A. L., et al. (2003). Cbl-mediated ubiquitinylation is required for lysosomal sorting of epidermal growth factor receptor but is dispensable for endocytosis. *J. Biol. Chem.*, 278, 28950–28960.
- Feng, F. Y., Varambally, S., Tomlins, S. A., Chun, P. Y., Lopez, C. A., Li, X., et al. (2007). Role of epidermal growth factor receptor degradation in gemcitabine-mediated cytotoxicity. *Oncogene*, 26(23), 3431–3439.
- Futter, C. E., Collinson, L. M., Backer, J. M., & Hopkins, C. R. (2001). Human VPS34 is required for internal vesicle formation within multivesicular endosomes. *J. Cell Biol.*, 155, 1251–1264.
- Futter, C. E., Felder, S., Schlessinger, J., Ullrich, A., & Hopkins, C. R. (1993). Annexin I is phosphorylated in the multivesicular body during the processing of the epidermal growth factor receptor. *J. Cell Biol.*, 120, 77–83.
- Futter, C. E., & White, I. J. (2007). Annexins and endocytosis. *Traffic*. [Epub ahead of print].
- Gruenberg, J., & Stenmark, H. (2004). The biogenesis of multivesicular endosomes. *Nat. Rev. Mol. Cell Biol.*, 5, 317–323.
- Haglund, K., Sigismund, S., Polo, S., Szymkiewicz, I., Di Fiore, P. P., & Dikic, I. (2003). Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. *Nat. Cell Biol.*, 5, 461–466.
- Huang, F., & Sorkin, A. (2005). Growth factor receptor binding protein 2-mediated recruitment of the RING domain of Cbl to the epidermal growth factor receptor is essential and sufficient to support receptor endocytosis. *Mol. Biol. Cell*, 16, 1268–1281.
- Jiang, X., Huang, F., Marusyk, A., & Sorkin, A. (2003). Grb2 regulates internalization of EGF receptors through clathrin-coated pits. *Mol. Biol. Cell*, 14, 858–870.
- Kanazawa, C., Morita, E., Yamada, M., Ishii, N., Miura, S., Asao, H., et al. (2003). Effects of deficiencies of STAMs and Hrs, mammalian class E Vps proteins, on receptor downregulation. *Biochem. Biophys. Res. Commun.*, 309, 848–856.
- Katzmann, D. J., Babst, M., & Emr, S. D. (2001). Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell*, 106, 145–155.
- Katzmann, D. J., Odorizzi, G., & Emr, S. D. (2002). Receptor downregulation and multivesicular-body sorting. *Nat. Rev. Mol. Cell Biol.*, 3, 893–905.
- Katzmann, D. J., Stefan, C. J., Babst, M., & Emr, S. D. (2003). Vps27 recruits ESCRT machinery to endosomes during MVB sorting. *J. Cell Biol.*, 162, 413–423.
- Kobayashi, T., Stang, E., Fang, K. S., de Moerloose, P., Parton, R. G., & Gruenberg, J. (1998). A lipid associated with the antiphospholipid syndrome regulates endosome structure and function. *Nature*, 392, 193–197.
- Li, L., & Cohen, S. N. (1996). Tsg101: A novel tumor susceptibility gene isolated by controlled homozygous functional knockout of allelic loci in mammalian cells. *Cell*, 85, 319–329.
- Liao, H. J., & Carpenter, G. (2007). Role of the Sec61 translocon in EGF receptor trafficking to the nucleus and gene expression. *Mol. Biol. Cell*, 18, 1064–1072.
- Lo, H. W., & Hung, M. C. (2006). Nuclear EGFR signalling network in cancers: Linking EGFR pathway to cell cycle progression, nitric oxide pathway and patient survival. *Br. J. Cancer*, 94, 184–188.
- Lo, H. W., Xia, W., Wei, Y., li-Seyed, M., Huang, S. F., & Hung, M. C. (2005). Novel prognostic value of nuclear epidermal growth factor receptor in breast cancer. *Cancer Res.*, 65, 338–348.
- Lu, Q., Hope, L. W., Brasch, M., Reinhard, C., & Cohen, S. N. (2003). TSG101 interaction with HRS mediates endosomal trafficking and receptor down-regulation. *Proc. Natl. Acad. Sci. U.S.A.*, 100, 7626–7631.
- Ma, Y. M., Boucrot, E., Villen, J., Affar, el B., Gygi, S. P., Gottlinger, H. G., et al. (2007). Targeting of AMSH to endosomes is required for epidermal growth factor receptor degradation. *J. Biol. Chem.*, 282, 9805–9812.
- Massie, C., & Mills, I. G. (2006). The developing role of receptors and adaptors. *Nat. Rev. Cancer*, 6, 403–409.
- McNiven, M. A., Cao, H., Pitts, K. R., & Yoon, Y. (2000). The dynamin family of mechanoenzymes: Pinching in new places. *Trends Biochem. Sci.*, 25, 115–120.
- Miaczynska, M., Pelkmans, L., & Zerial, M. (2004). Not just a sink: Endosomes in control of signal transduction. *Curr. Opin. Cell Biol.*, 16, 400–406.
- Olabisi, O. O., Mahon, G. M., Kostenko, E. V., Liu, Z., Ozer, H. L., & Whitehead, I. P. (2006). Bcr interacts with components of the endosomal sorting complex required for transport-I and is required for epidermal growth factor receptor turnover. *Cancer Res.*, 66, 6250–6257.
- Pornillos, O., Alam, S. L., Rich, R. L., Myska, D. G., Davis, D. R., & Sundquist, W. I. (2002). Structure and functional interactions of the Tsg101 UEV domain. *EMBO J.*, 21, 2397–2406.
- Psyrr, A., Yu, Z., Weinberger, P. M., Sasaki, C., Haffty, B., Camp, R., et al. (2005). Quantitative determination of nuclear and cytoplasmic epidermal growth factor receptor expression in oropharyngeal squamous cell cancer by using automated quantitative analysis. *Clin. Cancer Res.*, 11, 5856–5862.
- Puertollano, R., & Bonifacio, J. S. (2004). Interactions of GGA3 with the ubiquitin sorting machinery. *Nat. Cell Biol.*, 6, 244–251.
- Scaltriti, M., & Baselga, J. (2006). The epidermal growth factor receptor pathway: A model for targeted therapy. *Clin. Cancer Res.*, 12, 5268–5272.
- Soubeyran, P., Kowanetz, K., Szymkiewicz, I., Langdon, W. Y., & Dikic, I. (2002). Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. *Nature*, 416, 183–187.

- Sweeney, C., & Carraway, K. L. (2004). Negative regulation of ErbB family receptor tyrosine kinases. *Br. J. Cancer*, 90, 289–293.
- Thien, C. B., & Langdon, W. Y. (2001). Cbl: Many adaptations to regulate protein tyrosine kinases. *Nat. Rev. Mol. Cell Biol.*, 2, 294–307.
- Waterman, H., Katz, M., Rubin, C., Shtiegman, K., Lavi, S., Elson, A., et al. (2002). A mutant EGF-receptor defective in ubiquitylation and endocytosis unveils a role for Grb2 in negative signaling. *EMBO J.*, 21, 303–313.
- White, I. J., Bailey, L. M., Aghakhani, M. R., Moss, S. E., & Futter, C. E. (2006). EGF stimulates annexin 1-dependent inward vesiculation in a multivesicular endosome subpopulation. *EMBO J.*, 25, 1–12.
- Worthylake, R., Opresko, L. K., & Wiley, H. S. (1999). ErbB-2 amplification inhibits down-regulation and induces constitutive activation of both ErbB-2 and epidermal growth factor receptors. *J. Biol. Chem.*, 274, 8865–8874.
- Xu, Z., Liang, L., Wang, H., Li, T., & Zhao, M. (2003). HCRP1, a novel gene that is downregulated in hepatocellular carcinoma, encodes a growth-inhibitory protein. *Biochem. Biophys. Res. Commun.*, 311, 1057–1066.
- Yarden, Y., & Sliwkowski, M. X. (2001). Untangling the ErbB signalling network. *Nat. Rev. Mol. Cell Biol.*, 2, 127–137.
- Zerial, M., & McBride, H. (2001). Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell Biol.*, 2, 107–117.