Front. Biol. 2012, 7(1): 1–13
DOI 10.1007/s11515-011-1181-z

REVIEW

Growth factor receptor trafficking as a potential therapeutic target in pediatric cancer

Peter E. ZAGE (✉)[1,2,3], Andrew J. BEAN[4,5]

[1] *Department of Pediatrics, Section of Hematology-Oncology, 1102 Bates, Suite 1220, Houston, TX 77030, USA*
[2] *Texas Children's Cancer and Hematology Centers, Houston, TX 77030, USA*
[3] *Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX 77030, USA*
[4] *Department of Neurobiology and Anatomy, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA*
[5] *Division of Pediatrics, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA*

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2012

Abstract **Growth factor receptors (GFRs) are often aberrantly expressed in tumor cells, and altered GFR expression and activity contribute to the pathogenesis of many types of cancer. A variety of mechanisms have been identified that result in enhanced GFR expression and activity in cancer cells. Defects in the pathways responsible for GFR internalization and intracellular trafficking are likely to be involved in altered GFR expression in a variety of cancers. The roles of GFR trafficking pathways in the regulation of GFR expression, in the pathogenesis of tumors, and in the response of tumors to treatment have not been fully delineated, but the likely contributions of GFR signaling to the development and progression of various malignancies suggest that therapies that modify GFR trafficking may be effective as anticancer treatments.**

The intracellular trafficking of GFRs is regulated by a number of protein complexes and by protein ubiquitination. Many of the proteins required for this trafficking are products of tumor suppressor genes, and the expression and function of the protein machinery utilized for intracellular trafficking is frequently altered in tumor cells, consistent with the likely role of GFR trafficking in tumorigenesis. Many of the proteins involved in GFR trafficking have been identified as potential targets for anticancer treatment, and novel treatments directed against these targets are currently in preclinical development and in clinical trials. Ubiquitin ligases are critical for GFR trafficking and represent potentially important targets for the development of novel therapies.

The genes for the ubiquitin ligases *c-Cbl* and *UBE4B* are located in chromosome regions commonly altered in a variety of tumors and therefore are likely to be important for tumorigenesis. c-Cbl ubiquitinates a number of GFRs and directs them for degradation. Mutations in c-Cbl have been identified in cases of myeloid leukemia and myelodysplasia, providing a link between GFR ubiquitination and trafficking and leukemogenesis. We have shown that UBE4B plays a crucial role in GFR trafficking and degradation in tumor cells, suggesting a previously uncharacterized link between UBE4B and tumorigenesis. With the critical need for new and effective therapies for pediatric malignancies, the recently identified roles for the GFR trafficking pathway in the pathogenesis of various forms of cancer confirm the importance of the further development of novel therapies targeting this pathway in children with cancer.

Keywords growth factor receptor, tyrosine kinase, trafficking, UBE4B, neuroblastoma

Introduction

Multi-modality treatment for pediatric cancer with chemotherapy, surgery, and radiation therapy has been used for the past half-century with considerable improvement in overall survival for many pediatric malignancies. However, many types of pediatric cancer remain extremely difficult to cure, even with this aggressive therapy, and these treatments are associated with significant morbidity. Therapies targeted against biologically relevant pathways for tumorigenesis or for treatment resistance provide alternative options for these

Received September 1, 2011; accepted October 13, 2011
Correspondence: Peter E. ZAGE
E-mail: zage@bcm.tmc.edu

types of pediatric malignancies and will potentially improve long-term outcomes and reduce long-term treatment-related sequelae.

Growth factors stimulate a variety of cellular behaviors via binding to specific growth factor receptors (GFRs) on the surface of target cells. GFRs are transmembrane proteins that are activated by ligand binding, leading to downstream signaling that modifies cellular behavior. Nontransformed cells require specific growth factors and GFR signaling for growth and survival, and loss of this requirement is a feature of malignant transformation.

Receptor tyrosine kinases (RTKs) are a family of cell-surface GFRs whose signaling induced by ligand binding regulates a variety of normal and pathologic cellular processes, including cellular growth, proliferation, migration, and survival. Ligand binding to their cognate RTKs induces receptor multimerization, resulting in conformational changes within the receptor cytoplasmic domains that allow for kinase activation and transphosphorylation of specific tyrosine residues. These phosphorylated tyrosines serve as binding sites for other signaling proteins, including phospholipase C, phosphatidylinositol-3-kinase (PI3K), and Src family members, and for other adaptor proteins that recruit additional signaling proteins to the RTK cytoplasmic domain. These signaling complexes function to relay and amplify signals that ultimately lead to changes in gene transcription as well as altered cell shape, motility, and adhesion. These signals downstream of receptor activation need precise coordination and integration to maintain cellular homeostasis during embryonic development and continuing throughout childhood and adult life (Pawson, 2007; Abella and Park, 2009).

Regulation of the cell surface expression of GFRs is critical for their normal function. Aberrant growth factor receptor expression and activity has been shown to be important in the pathogenesis of a variety of malignancies, and a variety of growth factors and GFRs are aberrantly expressed in pediatric cancer cells and tumors (Goumnerova 1996; Abella and Park 2009), including the neurotrophin receptors TrkA, TrkB, and TrkC (Thiele et al., 2009); fibroblast growth factor receptors (Janet et al., 1995; Duplan et al., 2002; Goldstein et al., 2007), platelet-derived growth factor receptors (PDGFR) (Matsui et al., 1993; Östman and Heldin, 2007; Thorarinsdottir et al., 2008; Shimada et al., 2008), vascular endothelial growth factor receptors(VEGFR) (Meister et al., 1999; Langer et al., 2000, Fakhari et al., 2002; Nowicki et al., 2007; Slongo et al., 2007), c-kit (Shimada et al., 2008; Blom et al., 2010; Puputti et al., 2010), RET (Hishiki et al., 1998), c-Met (Hecht et al., 2004; Li et al., 2005; Diomedi-Camassei et al., 2008), and the ErbB receptor family (EGFR, her-2, her-3, her-4) (Bredel et al., 1999; Bodey et al., 2005; Vasei et al., 2009; Patereli et al., 2010; Izycka-Swieszewska et al., 2011). However, the mechanisms responsible for the regulation of GFR expression in pediatric cancer cells are not well understood. Moreover, the role of GFR trafficking in the regulation of GFR expression in pediatric cancer cells and the mechanisms by which altered GFR trafficking affects tumorigenesis in children and responses of pediatric tumors to treatment have not been delineated. GFR trafficking pathways therefore represent a novel target for the development of innovative, biologically-directed anticancer therapies.

In this review, we will focus on our current understanding of the mechanisms of GFR trafficking and its potential roles in the tumorigenesis of pediatric cancers. We will summarize the roles of ubiquitination and ubiquitin ligases in GFR trafficking in pediatric cancer cells and present data identifying the GFR trafficking pathway as a novel therapeutic target.

Growth factor receptor trafficking pathways and regulation

After ligand binding, GFRs are internalized and directed through various morphologically defined intracellular compartments in a complex series of events, leading to GFR degradation or recycling back to the plasma membrane for repeated ligand binding and signaling (Fig. 1). Ligand-induced GFR activation initiates downstream signal transduction cascades as well as mechanisms that modulate receptor signaling and downregulate plasma membrane GFR expression levels. These mechanisms include receptor dephosphorylation by specific phosphatases as well as internalization and intracellular sorting for subsequent lysosomal GFR degradation (Östman and Böhmer, 2001; Wiley and Burke, 2001). GFR internalization and intracellular trafficking have been identified as pathways responsible for downregulation of the cell surface expression and subsequent signaling of a variety of growth factor receptors, such as the epidermal growth factor receptor (EGFR) (Wells et al., 1990; Wiley et al., 1991; Futter et al., 1996; Carter and Sorkin, 1998), c-Met (Abella et al., 2010), PDGFR (Mori et al., 1993), c-kit (Masson et al., 2006), VEGFR2 (Ewan et al., 2006), and the nerve growth factor receptors TrkA and TrkB (Geetha et al., 2005; Huang et al., 2009). Regulation of the sorting and trafficking of GFRs can modulate the amplitude and kinetics of signaling of GFR, and downregulation of GFR expression through lysosomal degradation serves to inhibit the unrestrained signaling that is characteristic of transformed cells (Waterman et al., 1999; Schlessinger, 2000; Burke et al., 2001; Ciardiello et al., 2004; El-Rayes and LoRusso, 2004; Marmor and Yarden, 2004).

Following internalization, GFRs are localized in early endosomes. After the transition from early to late endosomes, GFRs destined for lysosomal degradation are internalized into the lumen of the late endosome via a process of membrane invagination and vesicle fission (Futter et al., 1996; Lemmon and Traub, 2000; Gruenberg 2001). This fission reaction results in the formation of an organelle with a limiting membrane and intralumenal vesicles, called a multivesicular

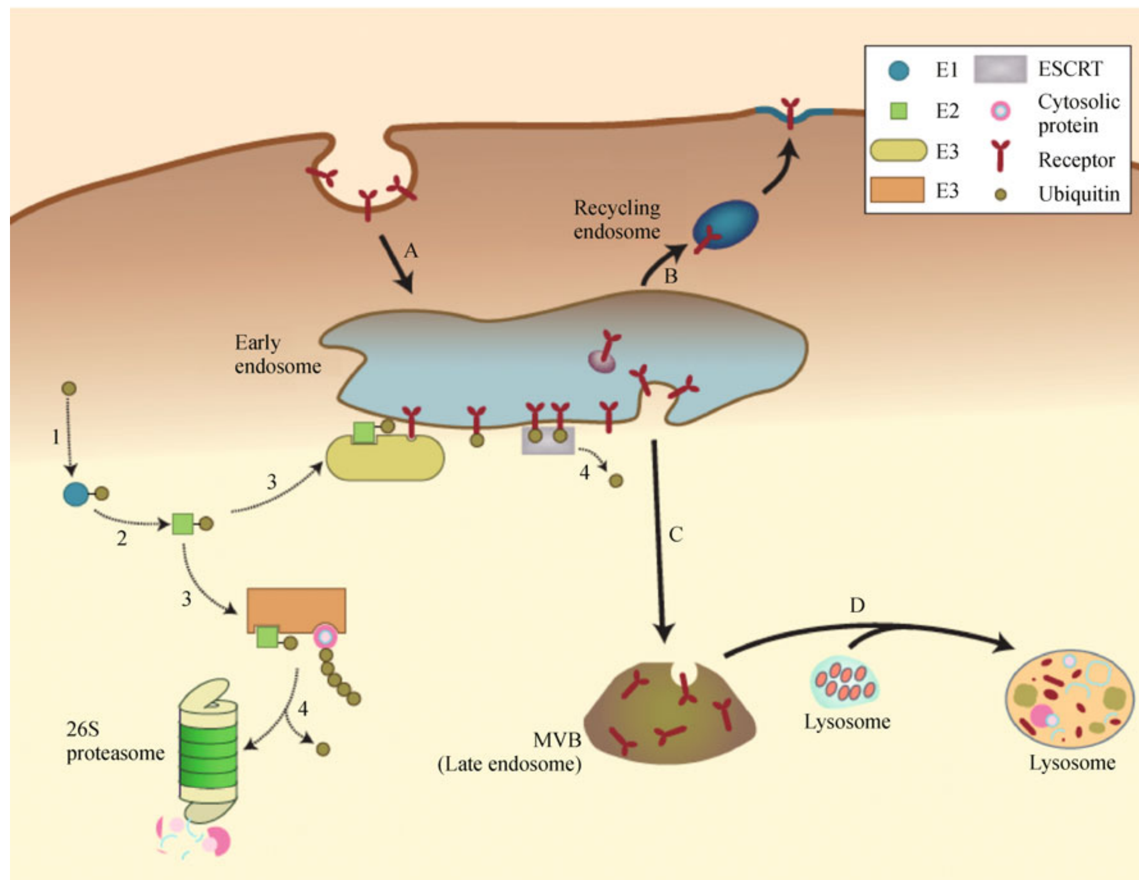

Figure 1 Trafficking Pathways for Sorting, Recycling, and Degradation of Growth Factor Receptors (GFRs). Following internalization, GFR-containing vesicles fuse with the early endosome (A). From the early endosome, GFRs can be recycled back to the cell surface via recycling endosomes (B) and/or remain in the endosome as it matures into a multi-vesicular body (MVB) (C). GFRs may also be recycled back to the plasma membrane from the MVB (not shown). Remaining GFRs are then sorted into internal vesicles of the MVB in an ESCRT-mediated, ubiquitin-dependent pathway. MVB fusion with lysosomes results in the degradation of MVB vesicles and their contents (D).

Protein Ubiquitination Pathway. The E1 activating enzyme activates ubiquitin using one molecule of ATP (1). Activated ubiquitin is then transferred to the E2 ubiquitin conjugating enzyme (2). E3 ubiquitin protein ligases transfer the ubiquitin molecules from the E2 enzyme to substrates at the early endosome for lysosomal targeting or in the cytosol for proteasomal degradation, after additional polyubiquitination by E4 ligases (3). Deubiquitination of endosomal substrates occurs after ESCRT-mediated sorting but before MVB lumenal vesicle formation, while deubiquitination of cytosolic substrates occurs during proteasomal degradation (4), allowing for the maintenance of a constant pool of cytoplasmic ubiquitin.

body (MVB) (Fig. 1) (Futter et al., 1996; Lemmon and Traub, 2000; Gruenberg, 2001). The manner in which proteins are sorted on the limiting membrane of the MVB and marked for internalization into lumenal vesicles involves the attachment of ubiquitin sorting signals (see below and Fig. 1). MVB sorting and the subsequent lysosomal degradation of activated cell surface receptors is a critical mechanism for regulating agonist-induced signaling (Schlessinger, 2000; Marmor and Yarden, 2004).

Four Endosomal Sorting Complexes Required for Transport (ESCRT 0, I, II, and III) have been proposed to be necessary for endosomal sorting (Katzmann et al., 2001). The ESCRT-0 complex contains two peripheral membrane proteins, Hrs (Hepatocyte growth factor-regulated tyrosine kinase substrate) and STAM (Signal-Transducing Adaptor Molecule), that are thought to function in the initial recruitment of proteins to be internalized into lumenal MVB vesicles. Hrs binds to and recruits a variety of proteins to the endosome membrane that are required for the sorting of ubiquitinated cargo (Katzmann et al., 2001; Bilodeau et al., 2002; Bache et al., 2003), endosome fusion (Sun et al., 2003), and endosome motility (Yan et al., 2005). Hrs physically interacts with a number of membrane trafficking proteins, including eps15 (Bean et al., 2000), SNX-1 (Chin et al., 2001), TSG101/Vps23 (Pornillos et al., 2003) and SNAP-25 (Bean et al., 1997), and appears to be a key regulator of endosomal trafficking. Hrs recruits the ESCRT I complex to endosomes through binding with TSG101, a member of

ESCRT I (Babst et al., 2000; Katzmann et al., 2001). TSG101, like Hrs and a number of proteins in the ESCRT complexes, contains a low affinity ubiquitin binding site and may aid Hrs in binding ubiquitinated proteins (Hierro et al., 2004; Sundquist et al., 2004; Slagsvold et al., 2005). Following ESCRT I binding to endosomes, ESCRT II and III bind (Katzmann et al., 2001; Babst et al., 2002a; Babst et al., 2002b). Although the function of ESCRT II is not clear, the ESCRT III complex is thought to perform the fission reaction, allowing the liberation of protein-laden vesicles from the limiting MVB membrane into the MVB lumen. Once protein cargo has been sorted into vesicles, the ESCRT complexes are dissociated by the action of Vps4, an AAA ATPase (Babst et al., 1998), a process that allows for their repeated use in sorting.

Ubiquitin-mediated growth factor receptor degradation

The protein machinery required for MVB sorting, including the ESCRT complexes delineated above, is responsible for recognizing and sorting transmembrane proteins into lumenal MVB vesicles. The sorting process of membrane proteins is regulated through the tagging of proteins with ubiquitin, a ubiquitously expressed 76 amino acid cytoplasmic polypeptide (Fig. 1). Ubiquitin tagging provides a generalized signal that is used for intracellular protein targeting (Thrower et al., 2000; Passmore and Barford, 2004). Ubiquitin tagging of membrane proteins allows for timed internalization from the cell surface and provides sorting labels to enable transport to different intracellular locations. Proteins are internalized from membranes or directed to the 26S proteasome for their destruction by virtue of the covalent addition of one or more ubiquitin moieties. Ubiquitin-mediated proteolysis can provide a mechanism to regulate protein quality and cellular processes, such as cell cycle progression, that require rapid alterations in protein levels and that are essential for cell survival (Ciechanover et al., 2000; Thrower et al., 2000; Passmore and Barford, 2004). While differences have been noted between proteasomal and lysosomal ubiquitin targeting (e.g. poly- vs. monoubiquitination, respectively; Fig. 1) (Thrower et al., 2000; Haglund et al., 2003; Hicke and Dunn 2003; Mosesson et al., 2003), the precise role of ubiquitin and the molecular mechanisms that regulate the tagging of membrane proteins for internalization and sorting at the MVB are unclear.

Covalent attachment of ubiquitin to protein substrates occurs through the sequential action of multiple enzymes resulting in isopeptide bond formation between ubiquitin and a residue in the target protein (Aravind and Koonin, 2000; Thrower et al., 2000; Guardavaccaro and Pagano, 2004; Passmore and Barford, 2004) (Fig. 1). An activating enzyme (E1) performs the initial ATP-dependent activation of ubiquitin, forming a high-energy thiol-ester bond between the C terminus of ubiquitin and a cysteine in the E1 enzyme. Subsequently, activated ubiquitin is transferred to a cysteine residue of a ubiquitin-conjugating enzyme (E2). Transfer of ubiquitin to substrate proteins generally requires a third class of enzymes, ubiquitin protein ligases (E3), which enable substrate recognition and the formation of stable isopeptide linkages (Aravind and Koonin, 2000; Thrower et al., 2000; Guardavaccaro and Pagano, 2004; Passmore and Barford 2004). E3 ligases bind directly to their target proteins and provide substrate specificity; they are generally classified into three families based on structural composition and mechanism of action: the HECT (Homologous to the E6-AP Carboxyl Terminus) family, the RING (Really Interesting New Gene) finger family, and the U-box family (Thrower et al., 2000; Guardavaccaro and Pagano, 2004; Passmore and Barford 2004). An additional class of ubiquitin-modifying enzymes, termed E4 ligases, drives multiubiquitin chain assembly, yielding long ubiquitin chains on substrate proteins. E4 ligases were initially shown to be necessary for degradation of some proteins via the ubiquitin fusion degradation pathway in yeast (Koegl et al., 1999).

The ubiquitin protein contains seven lysine residues that can serve as sites for linkage to target proteins, leading to ubiquitin chains of different configurations and lengths (Ikeda and Dikic, 2008). After a single ubiquitin moeity is conjugated to a protein substrate (monoubiquitination), further ubiquitin molecules can be added to the first to yield a polyubiquitin chain (polyubiquitination) or additional ubiquitin molecules can be added to alternate substrate residues (multimonoubiquitination). Chains conjugated to the lysine residue at position 48 of ubiquitin generally lead to proteasomal degradation, while conjugation to lysine residue 63 is often associated with intracellular trafficking (Bennett and Harper, 2008). Furthermore, whereas polyubiquitination of target proteins with ubiquitin chains is often a signal for proteasomal degradation, mono- or multi-monoubiquitination of proteins serves as a signal for a variety of intracellular protein sorting events, including sorting for lysosomal degradation (Miranda and Sorkin, 2007).

Many proteins and complexes implicated in MVB sorting contain domains that recognize and bind to ubiquitin (Katzmann et al., 2001). Ubiquitin conjugation allows for reversible binding to proteins that contain ubiquitin binding domains such as ubiquitin-interaction motifs (UIMs), ubiquitin-associated domains (UBAs), and ubiquitin-conjugating-like domains (UBC-like) (Peschard and Park, 2003; Woelk et al., 2007). UIM-containing proteins in the ESCRT complexes are thought to transiently interact with cargo proteins (e.g. GFRs) for recruitment to the sorting machinery (Williams and Urbé, 2007), and these interactions with the ESCRT complexes determine whether the cargo is internalized into the MVB lumen. Ubiquitination of a normally non-ubiquitinated protein results in redirection of that protein into MVB vesicles, providing evidence that ubiquitin tagging is sufficient for MVB sorting (Katzmann et al., 2001).

Deubiquitination of a cargo protein occurs just prior to its inclusion in lumenal vesicles of the MVB and after the ESCRT complexes have performed their sorting functions (Fig. 1). Since free ubiquitin molecules are essential for protein sorting, deubiquitination at the MVB is a mechanism that allows for the maintenance of a constant pool of cytoplasmic ubiquitin (Luhtala and Odorizzi, 2004). Disruption of GFR ubiquitination or of ESCRT-mediated sorting has the potential to alter GFR trafficking and to increase cell surface receptor expression, leading to protracted GFR signaling and cellular transformation.

Growth factor receptor trafficking defects in cancer

Pediatric tumor cells exhibit a series of characteristics considered to be hallmarks of cancer, including cellular proliferation independent of growth factor signals, evasion of apoptosis, enhanced angiogenesis, resistance to inhibitory signals, cellular immortality, and enhanced capability for tissue invasion (Hanahan and Weinberg, 2011). These features are primarily driven by cellular adaptations of GFR signaling pathways, including constitutive, ligand-independent activation of oncogenic signaling pathways and inhibition of tumor suppressors whose signals normally serve to limit cellular growth and spread.

Oncogenic activation of intracellular signaling and inhibition of tumor suppressor signaling can occur through a variety of mechanisms. For example, chromosomal translocations or gene mutations can result in modification of GFR expression and kinase activation (Lamorte and Park, 2001). In fact, mutations that increase RTK activity and/or expression levels have been identified in a variety of pediatric cancers, such as rhabdomyosarcoma (Taylor et al., 2009), acute lymphoid and myeloid leukemias (Braoudaki et al., 2009; Meshinchi et al., 2001), osteosarcoma and other sarcomas (Entz-Werlé et al., 2005; Tsuda et al., 2007), and thyroid cancer (Takahashi, 1995). Defects in the GFR trafficking pathway resulting in inhibition of receptor degradation can also result in increased levels of cell-surface GFRs, leading to constitutive kinase activation and malignant progression (Abella and Park, 2009).

There is growing evidence to support an important role for aberrant intracellular trafficking in the process of malignant transformation. Disruption, mutation, or loss of any of the components of the trafficking pathway could effectively delay the internalization, sorting, and degradation of GFRs, leading to the continued recycling of activated GFRs back to the cell surface for unregulated signaling (Levkowitz et al., 1998; Waterman et al., 1999; Yokouchi et al., 1999). The recent identification of novel mutations in the genes coding for proteins involved in the internalization and intracellular sorting of membrane proteins, resulting in delayed GFR degradation and enhanced GFR-mediated oncogenic signaling, underscores the importance of this mechanism of GFR regulation in the process of oncogenesis (Abella and Park, 2009; Mosesson et al., 2003; Peschard and Park 2003).

Recent studies have demonstrated that overexpression of the endocytic adaptor Huntington interacting protein 1 (HIP1) *in vitro* is associated with increased cell surface RTK levels. HIP1 has been shown to be overexpressed in many cancer types, including breast, prostate, and colon cancers, and overexpression is associated with poor clinical outcome (Rao et al., 2002; Rao et al., 2003; Hyun et al., 2004), providing an example of the association of a mutation affecting the GFR trafficking pathway with oncogenesis. Expression of another endocytic adaptor protein, Numb, results in inhibition of EGFR endocytosis (Salcini et al., 1997; Santolini et al., 2000), and Numb expression is significantly reduced in approximately half of human breast cancers (Pece et al., 2004), suggesting a linkage between Numb expression, EGFR trafficking, and the pathogenesis of breast cancer.

Several studies in *Drosophila* have suggested tumor suppressor roles for components of the ESCRT machinery. Mutations in TSG101 (ESCRT I), vsp25 (ESCRT II) or dVps4 (ESCRT III) result in increased levels of actively signaling ubiquitinated GFRs on endosomes (Thompson et al., 2005; Vaccari and Bilder 2005; Rodahl et al., 2009). Aberrant receptor signaling leads to ectopic secretion of cytokine-like molecules that induce proliferation of surrounding wild-type cells. When additional mutations disrupt the ability of these mutant cells to undergo apoptosis, overproliferation ensues, analogous to the multiple sequential mutations that occur during carcinogenesis in humans (Thompson et al., 2005; Vaccari and Bilder 2005; Rodahl et al., 2009).

E3 ubiquitin ligases have also been linked to the process of tumorigenesis, due to their role in the proteasomal targeting of oncogenes and/or tumor suppressors for degradation (Hoeller et al., 2006; Nakayama and Nakayama, 2006; Bernassola et al., 2008). Depending upon their substrates, E3 ligases can induce or suppress cell transformation, and may function as tumor suppressors in some cancers while acting as tumor promoters in others. Mdm2 (Mouse double minute 2) is a RING-type E3 ligase that promotes ubiquitin-mediated degradation of p53, a known tumor suppressor inactivated in over 50% of human cancers (Haupt et al., 1997; Bond et al., 2005; Vousden and Prives, 2005). The E3 ligase E6-AP interacts with the human papillomavirus protein E6, and the subsequent dimer binds to p53 and also targets it for destruction, leading to tumor development. Another E3 ligase, Nedd4-1, targets the tumor suppressor PTEN for degradation and is overexpressed in bladder and prostate cancers (Wang et al., 2007). Many of the other members of the Nedd4-like family of E3 ligases have been shown to regulate the endocytosis and degradation of GFRs and have been implicated in tumorigenesis (Chen and Matesic, 2007).

Growth factor receptor trafficking as a target for cancer treatment

The role of intracellular GFR trafficking in the regulation of GFR expression and activity and in the pathogenesis of many cancers has led to efforts to develop new therapies targeting the GFR trafficking pathway. Some currently available drugs target components of the GFR trafficking and degradation pathways. For example, bortezomib (Velcade, Millennium Pharmaceuticals) is a selective inhibitor of proteasomal chymotrypsin-like activity and has been shown to be effective against a wide range of malignancies (Voorhees and Orlowski 2006). Additionally, nutlins are a class of small molecule inhibitors blocking the interaction between the E3 ligase Mdm2 and the p53 tumor suppressor, leading to stabilized p53 and induced cancer cell senescence. However, despite the early successes of these and other agents targeting GFR trafficking and degradation, many membrane proteins and viruses are also sorted through these pathways, suggesting that nonspecific alteration of GFR trafficking pathways may result in undesirable side effects. Thus, potential targets involved in GFR sorting that offer substrate specificity, such as ubiquitin ligases, might be more appropriate for development of novel therapies. However, the development of small molecule ubiquitin ligase inhibitors has proven difficult.

Ubiquitin ligases as targets of pediatric anticancer therapy

c-Cbl

The c-Cbl protein was initially identified as the full-length clone of the oncogene *v-Cbl*, isolated from an oncogenic retrovirus that causes myeloid leukemia and lymphomas in mice, and was subsequently shown to function as a RING family E3 ubiquitin ligase. c-Cbl is a member of the CBL family of proteins, all of which contain a conserved N-terminal tyrosine kinase binding (TKB) domain and RING finger domains. c-Cbl is a ubiquitously expressed cytoplasmic protein in mammalian cells, with the highest levels of expression in lymphocytes and in the testis. Results of experiments from mice with absent c-Cbl expression suggest that it plays a key role in lymphocyte development and function (Kales et al., 2010).

c-Cbl serves as a ubiquitin ligase for a variety of GFRs, including EGFR, PDGFR, CSF-1R, and c-Met (Thien and Langdon, 2001; Ogawa et al., 2010; Peschard and Park, 2003), and has been hypothesized to be required for the internalization of ligand-bound GFRs from the cell surface for lysosomal degradation. c-Cbl can also act as an adaptor protein, linking GFRs to a variety of other intracellular signaling proteins. The c-Cbl TKB domain contains a series of subdomains, including a 4-helix bundle, an EF-hand, and a variant Src-homology 2 (SH2) domain, that serve to bind RTK cytoplasmic domains. c-Cbl can also be phosphorylated on tyrosine residues and has several proline-rich segments that provide docking sites for SH2 and SH3 domain-containing proteins, respectively. c-Cbl therefore negatively regulates tyrosine kinase signaling and appears to have tumor suppressor functions, the loss of which promotes tumorigenesis.

As *c-Cbl* was initially identified through isolation of the oncogenic *v-Cbl* gene, many studies have also attempted to identify the mechanisms responsible for c-Cbl oncogenic activity. The v-Cbl protein consists only of the TKB domain, and other deletion studies have demonstrated that the oncogenic forms of c-Cbl act in a dominant negative fashion to block endogenous, wild-type c-Cbl interaction with tyrosine kinases, leading to enhanced GFR signaling. c-Cbl can also contribute to activated oncogenic signaling through its binding to signaling molecules such as PI3K and MAPK family kinases (Thien and Langdon, 2001). These dual functions of c-Cbl confirm its role as a regulator of GFR trafficking and as an important potential contributor to tumor development.

c-Cbl in pediatric cancer

Recent publications have identified mutations in c-Cbl in a variety of pediatric myeloid neoplasms, including acute myeloid leukemia (Caligiuri et al., 2007), chronic myelogenous leukemia (Grand et al., 2009), and juvenile myelomonocytic leukemia (Loh et al., 2009). Interestingly, these leukemogenic mutations are often homozygous, with uniparental disomy as a result of duplication of the mutant copy of the *c-Cbl* gene on chromosome 11q23 (Ogawa et al., 2010). Mutations in *c-Cbl* predominantly affect the ubiquitin ligase activity, with reduced or absent ligase activity leading to deregulated GFR trafficking, enhanced GFR signaling, and induction of myeloid leukemogenesis. Interestingly, the *c-Cbl* gene is located within the 11q23.3 region commonly deleted in neuroblastoma (Ando et al., 2008), a solid tumor of childhood, and this deletion is associated with a poor prognosis, suggesting that c-Cbl may also have a tumor suppressor function in these tumors.

c-Cbl as a therapeutic target in pediatric cancer

These well-described functions of c-Cbl also provide insight into potential therapeutic approaches to myeloid disorders harboring Cbl mutations. Therapies targeting mutant Cbl proteins or of downstream signaling pathways could have a role in treatment of myeloid neoplasms with Cbl mutations. The broad spectrum of c-Cbl regulated tyrosine kinases may preclude the efficacy of RTK inhibitors for therapy of c-Cbl mutant malignancies, and suggests that agents acting directly on c-Cbl may be more effective. Piceatannol, a phenol stilbenenoid, is a naturally occurring oilseed product that targets c-Cbl and induces the downregulation of a variety of GFRs (Ogawa et al., 2010), suggesting that the development of c-Cbl targeted agents for anticancer therapy is feasible.

Other novel agents targeted against c-Cbl are currently being developed and may provide more effective therapies for a variety of pediatric malignancies.

UBE4B

UFD2 is a U-box ubiquitin ligase involved in the ubiquitin fusion degradation (UFD) pathway (Johnson et al., 1995). UFD2 was the first E4 ubiquitination factor identified, and the enzymatic activity of UFD2 is required for ubiquitin chain assembly and for ubiquitin-fusion protein degradation (Johnson et al., 1995; Koegl et al., 1999; Hatakeyama et al., 2004). UFD2 associates with cdc48 and has been implicated in cell survival (Koegl et al., 1999).

The mammalian homolog of UFD2, UBE4B, likely functions as both an E3 and an E4 ligase (Hatakeyama et al., 2001). UBE4B is expressed in a variety of tissue types, but is most abundantly expressed in neural tissues (Hatakeyama et al., 2001; Kaneko et al., 2003) and in neural tumors, including astrocytoma, medulloblastoma, and ependymoma (Wu et al., 2011). UBE4B is a cytoplasmic protein in neurons, cortical pyramidal cells, and Purkinje cells in the cerebellum (Kaneko et al., 2003), and the UBE4B protein is also found in skeletal muscle and to a lesser extent in the heart, lung, liver, pancreas, spleen, ovary, and testis (Kaneko et al., 2003).

While the enzymatic activity of UBE4B is well described, its cellular function is not. Deletion of UBE4B in a mouse knockout model results in early embryonic lethality (Kaneko-Oshikawa et al., 2005), although the mechanism for this lethal effect remains unknown. Hemizygous UBE4B deletion in mice results in neurodegenerative disorders with axonal dystrophy and Purkinje cell degeneration (Kaneko-Oshikawa et al., 2005). UBE4B interacts with a limited number of proteins, including the AAA + ATPase valosin-containing protein (VCP), Hsp90 (Hatakeyama et al., 2004; Wang et al., 2004) and FEZ1, a protein implicated in neurite extension (Okumura et al., 2004). UBE4B serves as a ubiquitin ligase for ataxin-3 (Matsumoto et al., 2004), FEZ1 (Okumura et al., 2004), and p73 (Hosoda et al., 2005), although its ligase activity does not necessarily result in degradation of the substrate proteins. UBE4B has also been shown to induce degradation of p53 in medulloblastoma tumor cells through direct interaction with Hdm2 and p53 polyubiquitination (Wu et al., 2011).

UBE4B in growth factor receptor trafficking

We have identified a direct interaction between UBE4B and the ESCRT protein Hrs (Sirisaengtaksin et al., submitted). Characterization of the UBE4B-Hrs interaction revealed that recruitment of UBE4B to endosomal membranes is dependent upon binding with Hrs and that this interaction is important for GFR sorting and degradation. Furthermore depletion of UBE4B in tumor cells decreases GFR degradation rates (Fig. 2; Sirisaengtaksin et al., submitted). These data suggest that the Hrs-UBE4B interaction is required for sorting of growth factor receptors into MVB vesicles that ultimately will result in their lysosomal degradation. These data also suggest that the interaction between UBE4B and Hrs plays a positive role in GFR degradation, implying that inhibition or reduced expression of UBE4B would decrease

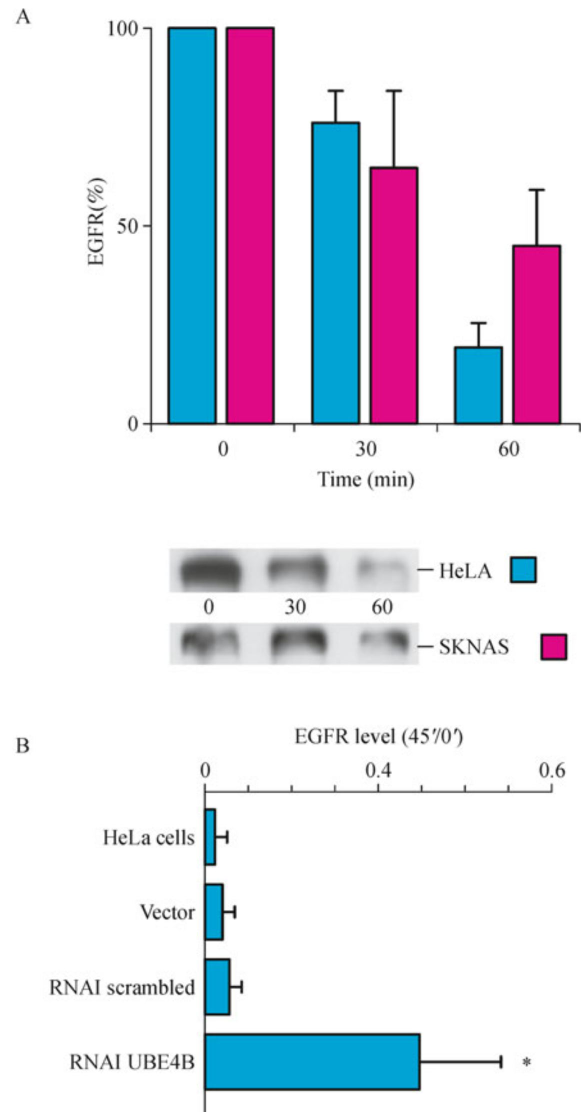

Figure 2 UBE4B mediates EGFR degradation. (A) HeLa and SKNAS neuroblastoma cells were starved, then stimulated with EGF for 0, 30, or 60 minutes. Cell lysates were then examined by immunoblotting for EGFR content. SKNAS cells (red bars) have greater amounts of remaining EGFR, suggesting slower degradation rates, than HeLa cells (blue bars). (B) Cell lysates of untransfected HeLa cells or HeLa cells transfected with shRNA (RNAI) to UBE4B were probed with antibodies to EGFR to show decreased rates of EGFR degradation with UBE4B depletion. Control HeLa cells were transfected with empty vector or with a scrambled shRNA sequence. The results are presented as the amount of remaining EGFR at 45 minutes vs. the amount of EGFR present at 0 minutes (*denotes $p < 0.05$).

GFR degradation and stimulate tumorigenesis.

In preliminary studies exploring the linkage of UBE4B with EGFR trafficking in pediatric preclinical cancer models, we observed a correlation between low UBE4B protein levels and reduced EGFR degradation rates (Fig. 2 and data not shown). In neuroblastoma patient tumor samples, UBE4B protein expression levels were found to be inversely correlated with EGFR levels (data not shown). These data further suggest a correlation between UBE4B levels and GFR trafficking in pediatric tumors and tumor cells.

UBE4B in pediatric cancer

Alterations of the chromosome 1p36 region are found in a wide range of pediatric neural tumors, including neuroblastoma, gliomas, and Ewing's sarcoma, and are often associated with poor outcomes. Deletion of the chromosome 1p36 region is found in approximately one-third of neuroblastoma tumors and is associated with high-risk tumor features and a poor prognosis (Caron et al., 1996; Maris et al., 2001; Attiyeh et al., 2005). The *UBE4B* gene is located in this consensus deleted 1p36 region (Carén et al., 2006). Previously published studies have identified a mutation in the *UBE4B* gene in the tumor DNA of a patient with high-risk neuroblastoma (Krona et al., 2003) and have shown that UBE4B expression is reduced in a cohort of high-stage/poor-outcome neuroblastoma tumors compared to a group of low-stage/favorable-outcome tumors (Ohira et al., 2000; Krona et al., 2003; Carén et al., 2005). Analysis of publicly available neuroblastoma tumor gene expression databases revealed a significant association between low *UBE4B* gene expression and poor patient outcomes (Fig. 3), suggesting that UBE4B may function as a tumor suppressor in neuroblastoma tumors.

Conversely, UBE4B expression is elevated in some pediatric neural tumor cell lines and primary tumor samples, and this overexpression is associated with *UBE4B* gene amplification (Wu et al., 2011). In medulloblastoma model systems, UBE4B expression levels are inversely correlated with p53 levels, presumably due to UBE4B-mediated p53 ubiquitination and degradation. Furthermore, UBE4B cooperates with activated *Hras* to transform primary fibroblasts, and expression of UBE4B results in increased tumorigenesis of NIH3T3 cells *in vivo* (Wu et al., 2011). These results further emphasize the importance of cellular context in studies of the roles of UBE4B and the GFR trafficking pathway in oncogenesis.

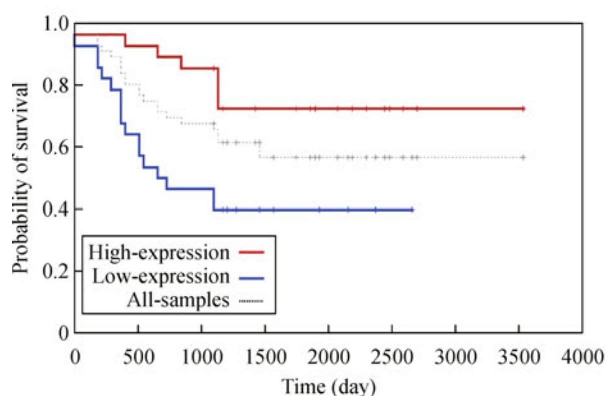

Figure 3 Outcomes of neuroblastoma patients based on *UBE4B* expression. The NCI Oncogenomics databases were evaluated for outcomes of neuroblastoma patients (http://pob.abcc.ncifcrf.gov/cgi-bin/JK). The resulting Kaplan-Meier curve shows overall survival of patients with low (blue) and high (red) *UBE4B* gene expression ($p < 0.001$).

The potential role of UBE4B in resistance to treatment

Previous studies have demonstrated that EGFR expression is associated with chemotherapy resistance in neuroblastoma tumor cells and that chemotherapy is synergistic with EGFR inhibitors (Meyers et al., 1988; Michaelis et al., 2008). The etiology of this EGFR-associated chemotherapy resistance in neuroblastoma tumor cells and the mechanisms responsible for this association of EGFR with resistance to chemotherapy are unknown. Additional studies have identified EGFR ubiquitination and degradation as a mechanism for colon cancer cell resistance to cetuximab, an FDA-approved monoclonal EGFR-blocking antibody (Lu et al., 2007). The observed roles of UBE4B in GFR trafficking above implicates UBE4B and the GFR trafficking pathway in the sensitivity of neuroblastoma tumor cells to treatment and suggests that UBE4B-mediated GFR trafficking, through regulation of EGFR expression, may be responsible for the association of EGFR expression and resistance to chemotherapy. However, the mechanisms by which UBE4B and GFR trafficking might affect neuroblastoma tumor cell responses to therapy are unknown, and increased understanding of these mechanisms will likely lead to improved efficacy of current therapy and allow for identification of patient populations that might be more or less sensitive to GFR inhibitor treatment.

Conclusions and perspectives

GFRs are often aberrantly expressed in tumor cells, and altered GFR expression and activity contribute to the pathogenesis of many types of cancer. Dysregulation of GFR trafficking is clearly emerging as an important mechanism for oncogenesis in a variety of human cancers. A large and growing body of evidence clearly demonstrates that disruption of the GFR trafficking pathways can alter cell expression and activity of GFRs, leading to the development and progression of cancer. The roles of GFR trafficking in various malignancies suggest that therapies that modify GFR trafficking may be effective as anticancer treatments. The ubiquitin ligases c-Cbl and UBE4B have been recently shown to play roles in the pathogenesis of pediatric cancers and provide potential targets for novel treatment strategies. Therefore, developing a better understanding of the GFR trafficking pathway is critical in the understanding of the

process of tumorigenesis and in the development and use of novel therapies.

Abbreviations

GFR- growth factor receptor; RTK- receptor tyrosine kinase; PI3K- phosphatidylinositol-3-kinase; EGFR- epidermal growth factor receptor; MVB- multivesicular body; ESCRT- endosomal sorting complex required for transport; Hrs- hepatocyte growth factor-regulated tyrosine kinase substrate; STAM- signal-transducing adaptor molecule; HIP1- huntingtin interacting protein 1; UIM- ubiquitin-interacting motifs; VCP- valosin-containing protein; TKB- tyrosine kinase binding; RING- really interesting new gene; UFD- ubiquitin fusion degradation

Acknowledgements

Natalie Sirisaengtaksin for figure preparation and proofreading of the manuscript

References

Abella J V, Parachoniak C A, Sangwan V, Park M (2010). Dorsal ruffle microdomains potentiate Met receptor tyrosine kinase signaling and down-regulation. J Biol Chem, 285(32): 24956–24967

Abella J V, Park M (2009). Breakdown of endocytosis in the oncogenic activation of receptor tyrosine kinases. Am J Physiol Endocrinol Metab, 296(5): E973–E984

Ando K, Ohira M, Ozaki T, Nakagawa A, Akazawa K, Suenaga Y, Nakamura Y, Koda T, Kamijo T, Murakami Y, Nakagawara A (2008). Expression of TSLC1, a candidate tumor suppressor gene mapped to chromosome 11q23, is downregulated in unfavorable neuroblastoma without promoter hypermethylation. Int J Cancer, 123 (9): 2087–2094

Aravind L, Koonin E V (2000). The U box is a modified RING finger — a common domain in ubiquitination. Curr Biol, 10(4): R132–R134

Attiyeh EF, London W B, Mossé Y P, Wang Q, Winter C, Khazi D, McGrady P W, Seeger R C, Look A T, Shimada H, Brodeur G M, Cohn S L, Matthay K K, Maris J M, Children's Oncology Group (2005). Chromosome 1p and 11q deletions and outcome in neuroblastoma. N Engl J Med, 353(21): 2243–2253

Babst M, Katzmann D J, Estepa-Sabal E J, Meerloo T, Emr S D (2002a). Escrt-III: an endosome-associated heterooligomeric protein complex required for mvb sorting. Dev Cell, 3(2): 271–282

Babst M, Katzmann D J, Snyder W B, Wendland B, Emr S D (2002b). Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body. Dev Cell, 3(2): 283–289

Babst M, Odorizzi G, Estepa E J, Emr S D (2000). Mammalian tumor susceptibility gene 101 (TSG101) and the yeast homologue, Vps23p, both function in late endosomal trafficking. Traffic, 1(3): 248–258

Babst M, Wendland B, Estepa E J, Emr S D (1998). The Vps4p AAA ATPase regulates membrane association of a Vps protein complex required for normal endosome function. EMBO J, 17(11): 2982–2993

Bache K G, Brech A, Mehlum A, Stenmark H (2003). Hrs regulates multivesicular body formation via ESCRT recruitment to endosomes. J Cell Biol, 162(3): 435–442

Bean A J, Davanger S, Chou M F, Gerhardt B, Tsujimoto S, Chang Y (2000). Hrs-2 regulates receptor-mediated endocytosis via interactions with Eps15. J Biol Chem, 275(20): 15271–15278

Bean A J, Seifert R, Chen Y A, Sacks R, Scheller R H (1997). Hrs-2 is an ATPase implicated in calcium-regulated secretion. Nature, 385 (6619): 826–829

Bennett E J, Harper J W (2008). DNA damage: ubiquitin marks the spot. Nat Struct Mol Biol, 15(1): 20–22

Bernassola F, Karin M, Ciechanover A, Melino G (2008). The HECT family of E3 ubiquitin ligases: multiple players in cancer development. Cancer Cell, 14(1): 10–21

Bilodeau P S, Urbanowski J L, Winistorfer S C, Piper R C (2002). The Vps27p Hse1p complex binds ubiquitin and mediates endosomal protein sorting. Nat Cell Biol, 4(7): 534–539

Blom T, Roselli A, Häyry V, Tynninen O, Wartiovaara K, Korja M, Nordfors K, Haapasalo H, Nupponen N N (2010). Amplification and overexpression of KIT, PDGFRA, and VEGFR2 in medulloblastomas and primitive neuroectodermal tumors. J Neurooncol, 97(2): 217–224

Bodey B, Kaiser H E, Siegel S E (2005). Epidermal growth factor receptor (EGFR) expression in childhood brain tumors. In Vivo, 19 (5): 931–941

Bond G L, Hu W, Levine A J (2005). MDM2 is a central node in the p53 pathway: 12 years and counting. Curr Cancer Drug Targets, 5(1): 3–8

Braoudaki M, Karpusas M, Katsibardi K, Papathanassiou Ch, Karamolegou K, Tzortzatou-Stathopoulou F (2009). Frequency of FLT3 mutations in childhood acute lymphoblastic leukemia. Med Oncol, 26(4): 460–462

Bredel M, Pollack I F, Hamilton R L, James C D (1999). Epidermal growth factor receptor expression and gene amplification in high-grade non-brainstem gliomas of childhood. Clin Cancer Res, 5(7): 1786–1792

Burke P, Schooler K, Wiley H S (2001). Regulation of epidermal growth factor receptor signaling by endocytosis and intracellular trafficking. Mol Biol Cell, 12(6): 1897–1910

Caligiuri M A, Briesewitz R, Yu J, Wang L, Wei M, Arnoczky K J, Marburger T B, Wen J, Perrotti D, Bloomfield C D, Whitman S P (2007). Novel c-CBL and CBL-b ubiquitin ligase mutations in human acute myeloid leukemia. Blood, 110(3): 1022–1024

Carén H, Ejeskär K, Fransson S, Hesson L, Latif F, Sjöberg R M, Krona C, Martinsson T (2005). A cluster of genes located in 1p36 are down-regulated in neuroblastomas with poor prognosis, but not due to CpG island methylation. Mol Cancer, 4(1): 10

Carén H, Holmstrand A, Sjöberg R M, Martinsson T (2006). The two human homologues of yeast UFD2 ubiquitination factor, UBE4A and UBE4B, are located in common neuroblastoma deletion regions and are subject to mutations in tumours. Eur J Cancer, 42(3): 381–387

Caron H, van Sluis P, de Kraker J, Bökkerink J, Egeler M, Laureys G, Slater R, Westerveld A, Voûte P A, Versteeg R (1996). Allelic loss of chromosome 1p as a predictor of unfavorable outcome in patients with neuroblastoma. N Engl J Med, 334(4): 225–230

Carter R E, Sorkin A (1998). Endocytosis of functional epidermal growth factor receptor-green fluorescent protein chimera. J Biol Chem, 273(52): 35000–35007

Chen C, Matesic L E (2007). The Nedd4-like family of E3 ubiquitin ligases and cancer. Cancer Metastasis Rev, 26(3–4): 587–604

Chin L S, Raynor M C, Wei X, Chen H Q, Li L (2001). Hrs interacts with sorting nexin 1 and regulates degradation of epidermal growth factor receptor. J Biol Chem, 276(10): 7069–7078

Ciardiello F, De Vita F, Orditura M, Tortora G (2004). The role of EGFR inhibitors in nonsmall cell lung cancer. Curr Opin Oncol, 16(2): 130–135

Ciechanover A, Orian A, Schwartz A L (2000). The ubiquitin-mediated proteolytic pathway: mode of action and clinical implications. J Cell Biochem Suppl, 77(S34): 40–51

Diomedi-Camassei F, McDowell H P, De Ioris M A, Uccini S, Altavista P, Raschellà G, Vitali R, Mannarino O, De Sio L, Cozzi D A, Donfrancesco A, Inserra A, Callea F, Dominici C (2008). Clinical significance of CXC chemokine receptor-4 and c-Met in childhood rhabdomyosarcoma. Clin Cancer Res, 14(13): 4119–4127

Duplan S M, Théorêt Y, Kenigsberg R L (2002). Antitumor activity of fibroblast growth factors (FGFs) for medulloblastoma may correlate with FGF receptor expression and tumor variant. Clin Cancer Res, 8 (1): 246–257

El-Rayes B F, LoRusso P M (2004). Targeting the epidermal growth factor receptor. Br J Cancer, 91(3): 418–424

Entz-Werlé N, Marcellin L, Gaub M P, Guerin E, Schneider A, Berard-Marec P, Kalifa C, Brugiere L, Pacquement H, Schmitt C, Tabone M D, Jeanne-Pasquier C, Terrier P, Dijoud F, Oudet P, Lutz P, Babin-Boilletot A (2005). Prognostic significance of allelic imbalance at the c-kit gene locus and c-kit overexpression by immunohistochemistry in pediatric osteosarcomas. J Clin Oncol, 23(10): 2248–2255

Ewan L C, Jopling H M, Jia H, Mittar S, Bagherzadeh A, Howell G J, Walker J H, Zachary I C, Ponnambalam S (2006). Intrinsic tyrosine kinase activity is required for vascular endothelial growth factor receptor 2 ubiquitination, sorting and degradation in endothelial cells. Traffic, 7(9): 1270–1282

Fakhari M, Pullirsch D, Paya K, Abraham D, Hofbauer R, Aharinejad S (2002). Upregulation of vascular endothelial growth factor receptors is associated with advanced neuroblastoma. J Pediatr Surg, 37(4): 582–587

Futter C E, Pearse A, Hewlett L J, Hopkins C R (1996). Multivesicular endosomes containing internalized EGF-EGF receptor complexes mature and then fuse directly with lysosomes. J Cell Biol, 132(6): 1011–1023

Geetha T, Jiang J, Wooten M W (2005). Lysine 63 polyubiquitination of the nerve growth factor receptor TrkA directs internalization and signaling. Mol Cell, 20(2): 301–312

Goldstein M, Meller I, Orr-Urtreger A (2007). FGFR1 over-expression in primary rhabdomyosarcoma tumors is associated with hypomethylation of a 5′ CpG island and abnormal expression of the AKT1, NOG, and BMP4 genes. Genes Chromosomes Cancer, 46 (11): 1028–1038

Goumnerova L C (1996). Growth factor receptors and medulloblastoma. J Neurooncol, 29(1): 85–89

Grand F H, Hidalgo-Curtis C E, Ernst T, Zoi K, Zoi C, McGuire C, Kreil S, Jones A, Score J, Metzgeroth G, Oscier D, Hall A, Brandts C, Serve H, Reiter A, Chase A J, Cross N C (2009). Frequent CBL mutations associated with 11q acquired uniparental disomy in myeloproliferative neoplasms. Blood, 113(24): 6182–6192

Gruenberg J (2001). The endocytic pathway: a mosaic of domains. Nat Rev Mol Cell Biol, 2(10): 721–730

Guardavaccaro D, Pagano M (2004). Oncogenic aberrations of cullin-dependent ubiquitin ligases. Oncogene, 23(11): 2037–2049

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(5): 461–466

Hanahan D, Weinberg R A (2011). Hallmarks of cancer: the next generation. Cell, 144(5): 646–674

Hatakeyama S, Matsumoto M, Yada M, Nakayama K I (2004). Interaction of U-box-type ubiquitin-protein ligases (E3s) with molecular chaperones. Genes Cells, 9(6): 533–548

Hatakeyama S, Yada M, Matsumoto M, Ishida N, Nakayama K I (2001). U box proteins as a new family of ubiquitin-protein ligases. J Biol Chem, 276(35): 33111–33120

Haupt Y, Maya R, Kazaz A, Oren M (1997). Mdm2 promotes the rapid degradation of p53. Nature, 387(6630): 296–299

Hecht M, Papoutsi M, Tran H D, Wilting J, Schweigerer L (2004). Hepatocyte growth factor/c-Met signaling promotes the progression of experimental human neuroblastomas. Cancer Res, 64(17): 6109–6118

Hicke L, Dunn R (2003). Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. Annu Rev Cell Dev Biol, 19(1): 141–172

Hierro A, Sun J, Rusnak A S, Kim J, Prag G, Emr S D, Hurley J H (2004). Structure of the ESCRT-II endosomal trafficking complex. Nature, 431(7005): 221–225

Hishiki T, Nimura Y, Isogai E, Kondo K, Ichimiya S, Nakamura Y, Ozaki T, Sakiyama S, Hirose M, Seki N, Takahashi H, Ohnuma N, Tanabe M, Nakagawara A (1998). Glial cell line-derived neurotrophic factor/neurturin-induced differentiation and its enhancement by retinoic acid in primary human neuroblastomas expressing c-Ret, GFR alpha-1, and GFR alpha-2. Cancer Res, 58(10): 2158–2165

Hoeller D, Hecker C M, Dikic I (2006). Ubiquitin and ubiquitin-like proteins in cancer pathogenesis. Nat Rev Cancer, 6(10): 776–788

Hosoda M, Ozaki T, Miyazaki K, Hayashi S, Furuya K, Watanabe K, Nakagawa T, Hanamoto T, Todo S, Nakagawara A (2005). UFD2a mediates the proteasomal turnover of p73 without promoting p73 ubiquitination. Oncogene, 24(48): 7156–7169

Huang S H, Zhao L, Sun Z P, Li X Z, Geng Z, Zhang K D, Chao M V, Chen Z Y (2009). Essential role of Hrs in endocytic recycling of full-length TrkB receptor but not its isoform TrkB.T1. J Biol Chem, 284 (22): 15126–15136

Hyun T S, Rao D S, Saint-Dic D, Michael L E, Kumar P D, Bradley S V, Mizukami I F, Oravecz-Wilson K I, Ross T S (2004). HIP1 and HIP1r stabilize receptor tyrosine kinases and bind 3-phosphoinositides via epsin N-terminal homology domains. J Biol Chem, 279(14): 14294–14306

Ikeda F, Dikic I (2008). Atypical ubiquitin chains: new molecular signals. 'Protein Modifications: Beyond the Usual Suspects' review series. EMBO Rep, 9(6): 536–542

Izycka-Swieszewska E, Wozniak A, Drozynska E, Kot J, Grajkowska W, Klepacka T, Perek D, Koltan S, Bien E, Limon J (2011). Expression and significance of HER family receptors in neuroblastic tumors. Clin Exp Metastasis, 28(3): 271–282

Janet T, Lüdecke G, Otten U, Unsicker K (1995). Heterogeneity of human neuroblastoma cell lines in their proliferative responses to basic FGF, NGF, and EGF: correlation with expression of growth

factors and growth factor receptors. J Neurosci Res, 40(6): 707–715

Johnson E S, Ma P C, Ota I M, Varshavsky A (1995). A proteolytic pathway that recognizes ubiquitin as a degradation signal. J Biol Chem, 270(29): 17442–17456

Kales S C, Ryan P E, Nau M M, Lipkowitz S (2010). Cbl and human myeloid neoplasms: the Cbl oncogene comes of age. Cancer Res, 70 (12): 4789–4794

Kaneko C, Hatakeyama S, Matsumoto M, Yada M, Nakayama K, Nakayama K I (2003). Characterization of the mouse gene for the U-box-type ubiquitin ligase UFD2a. Biochem Biophys Res Commun, 300(2): 297–304

Kaneko-Oshikawa C, Nakagawa T, Yamada M, Yoshikawa H, Matsumoto M, Yada M, Hatakeyama S, Nakayama K, Nakayama K I (2005). Mammalian E4 is required for cardiac development and maintenance of the nervous system. Mol Cell Biol, 25(24): 10953–10964

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 (2): 145–155

Koegl M, Hoppe T, Schlenker S, Ulrich H D, Mayer T U, Jentsch S (1999). A novel ubiquitination factor, E4, is involved in multi-ubiquitin chain assembly. Cell, 96(5): 635–644

Krona C, Ejeskär K, Abel F, Kogner P, Bjelke J, Björk E, Sjöberg R M, Martinsson T (2003). Screening for gene mutations in a 500 kb neuroblastoma tumor suppressor candidate region in chromosome 1p; mutation and stage-specific expression in UBE4B/UFD2. Oncogene, 22(15): 2343–2351

Lamorte L, Park M (2001). The receptor tyrosine kinases: role in cancer progression. Surg Oncol Clin N Am, 10(2): 271–288, viii

Langer I, Vertongen P, Perret J, Fontaine J, Atassi G, Robberecht P (2000). Expression of vascular endothelial growth factor (VEGF) and VEGF receptors in human neuroblastomas. Med Pediatr Oncol, 34 (6): 386–393

Lemmon S K, Traub L M (2000). Sorting in the endosomal system in yeast and animal cells. Curr Opin Cell Biol, 12(4): 457–466

Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon W Y, Beguinot L, Geiger B, Yarden Y (1998). c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. Genes Dev, 12(23): 3663–3674

Li Y, Lal B, Kwon S, Fan X, Saldanha U, Reznik T E, Kuchner E B, Eberhart C, Laterra J, Abounader R (2005). The scatter factor/hepatocyte growth factor: c-met pathway in human embryonal central nervous system tumor malignancy. Cancer Res, 65(20): 9355–9362

Loh M L, Sakai D S, Flotho C, Kang M, Fliegauf M, Archambeault S, Mullighan C G, Chen L, Bergstraesser E, Bueso-Ramos C E, Emanuel P D, Hasle H, Issa J P, van den Heuvel-Eibrink M M, Locatelli F, Stary J, Trebo M, Wlodarski M, Zecca M, Shannon K M, Niemeyer C M (2009). Mutations in CBL occur frequently in juvenile myelomonocytic leukemia. Blood, 114(9): 1859–1863

Lu Y, Li X, Liang K, Luwor R, Siddik Z H, Mills G B, Mendelsohn J, Fan Z (2007). Epidermal growth factor receptor (EGFR) ubiquitination as a mechanism of acquired resistance escaping treatment by the anti-EGFR monoclonal antibody cetuximab. Cancer Res, 67(17): 8240–8247

Luhtala N, Odorizzi G (2004). Bro1 coordinates deubiquitination in the multivesicular body pathway by recruiting Doa4 to endosomes. J Cell Biol, 166(5): 717–729

Maris J M, Guo C, Blake D, White P S, Hogarty M D, Thompson P M, Rajalingam V, Gerbing R, Stram D O, Matthay K K, Seeger R C, Brodeur G M (2001). Comprehensive analysis of chromosome 1p deletions in neuroblastoma. Med Pediatr Oncol, 36(1): 32–36

Marmor M D, Yarden Y (2004). Role of protein ubiquitylation in regulating endocytosis of receptor tyrosine kinases. Oncogene, 23 (11): 2057–2070

Masson K, Heiss E, Band H, Rönnstrand L (2006). Direct binding of Cbl to Tyr568 and Tyr936 of the stem cell factor receptor/c-Kit is required for ligand-induced ubiquitination, internalization and degradation. Biochem J, 399(1): 59–67

Matsui T, Sano K, Tsukamoto T, Ito M, Takaishi T, Nakata H, Nakamura H, Chihara K (1993). Human neuroblastoma cells express alpha and beta platelet-derived growth factor receptors coupling with neurotrophic and chemotactic signaling. J Clin Invest, 92(3): 1153–1160

Matsumoto M, Yada M, Hatakeyama S, Ishimoto H, Tanimura T, Tsuji S, Kakizuka A, Kitagawa M, Nakayama K I (2004). Molecular clearance of ataxin-3 is regulated by a mammalian E4. EMBO J, 23 (3): 659–669

Meister B, Grünebach F, Bautz F, Brugger W, Fink F M, Kanz L, Möhle R (1999). Expression of vascular endothelial growth factor (VEGF) and its receptors in human neuroblastoma. Eur J Cancer, 35(3): 445–449

Meshinchi S, Woods W G, Stirewalt D L, Sweetser D A, Buckley J D, Tjoa T K, Bernstein I D, Radich J P (2001). Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. Blood, 97(1): 89–94

Meyers M B, Shen W P, Spengler B A, Ciccarone V, O'Brien J P, Donner D B, Furth M E, Biedler J L (1988). Increased epidermal growth factor receptor in multidrug-resistant human neuroblastoma cells. J Cell Biochem, 38(2): 87–97

Michaelis M, Bliss J, Arnold S C, Hinsch N, Rothweiler F, Deubzer H E, Witt O, Langer K, Doerr H W, Wels W S, Cinatl J Jr (2008). Cisplatin-resistant neuroblastoma cells express enhanced levels of epidermal growth factor receptor (EGFR) and are sensitive to treatment with EGFR-specific toxins. Clin Cancer Res, 14(20): 6531–6537

Miranda M, Sorkin A (2007). Regulation of receptors and transporters by ubiquitination: new insights into surprisingly similar mechanisms. Mol Interv, 7(3): 157–167

Mori S, Heldin C H, Claesson-Welsh L (1993). Ligand-induced ubiquitination of the platelet-derived growth factor beta-receptor plays a negative regulatory role in its mitogenic signaling. J Biol Chem, 268(1): 577–583

Mosesson Y, Shtiegman K, Katz M, Zwang Y, Vereb G, Szollosi J, Yarden Y (2003). Endocytosis of receptor tyrosine kinases is driven by monoubiquitylation, not polyubiquitylation. J Biol Chem, 278 (24): 21323–21326

Nakayama K I, Nakayama K (2006). Ubiquitin ligases: cell-cycle control and cancer. Nat Rev Cancer, 6(5): 369–381

Nowicki M, Ostalska-Nowicka D, Kaczmarek M, Miskowiak B, Witt M (2007). The significance of VEGF-C/VEGFR-2 interaction in the neovascularization and prognosis of nephroblastoma (Wilms' tumour). Histopathology, 50(3): 358–364

Ogawa S, Shih L Y, Suzuki T, Otsu M, Nakauchi H, Koeffler H P, Sanada M (2010). Deregulated intracellular signaling by mutated c-CBL in myeloid neoplasms. Clin Cancer Res, 16(15): 3825–3831

Ohira M, Kageyama H, Mihara M, Furuta S, Machida T, Shishikura T, Takayasu H, Islam A, Nakamura Y, Takahashi M, Tomioka N, Sakiyama S, Kaneko Y, Toyoda A, Hattori M, Sakaki Y, Ohki M, Horii A, Soeda E, Inazawa J, Seki N, Kuma H, Nozawa I, Nakagawara A (2000). Identification and characterization of a 500-kb homozygously deleted region at 1p36.2-p36.3 in a neuroblastoma cell line. Oncogene, 19(37): 4302–4307

Okumura F, Hatakeyama S, Matsumoto M, Kamura T, Nakayama K I (2004). Functional regulation of FEZ1 by the U-box-type ubiquitin ligase E4B contributes to neuritogenesis. J Biol Chem, 279(51): 53533–53543

Östman A, Böhmer F D (2001). Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatases. Trends Cell Biol, 11(6): 258–266

Östman A, Heldin C H (2007). PDGF receptors as targets in tumor treatment. Adv Cancer Res, 97: 247–274

Passmore L A, Barford D (2004). Getting into position: the catalytic mechanisms of protein ubiquitylation. Biochem J, 379(3): 513–525

Patereli A, Alexiou GA, Stefanaki K, Moschovi M, Doussis-Anagnostopoulou I, Prodromou N, Karentzuo O (2010). Expression of Epidermal Growth Factor Receptor and Her-2 in Pediatric Embryonal Brain Tumors.

Pawson T (2007). Dynamic control of signaling by modular adaptor proteins. Curr Opin Cell Biol, 19(2): 112–116

Pece S, Serresi M, Santolini E, Capra M, Hulleman E, Galimberti V, Zurrida S, Maisonneuve P, Viale G, Di Fiore P P (2004). Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. J Cell Biol, 167(2): 215–221

Peschard P, Park M (2003). Escape from Cbl-mediated downregulation: a recurrent theme for oncogenic deregulation of receptor tyrosine kinases. Cancer Cell, 3(6): 519–523

Pornillos O, Higginson D S, Stray K M, Fisher R D, Garrus J E, Payne M, He G P, Wang H E, Morham S G, Sundquist W I (2003). HIV Gag mimics the Tsg101-recruiting activity of the human Hrs protein. J Cell Biol, 162(3): 425–434

Puputti M, Tynninen O, Pernilä P, Salmi M, Jalkanen S, Paetau A, Sihto H, Joensuu H (2010). Expression of KIT receptor tyrosine kinase in endothelial cells of juvenile brain tumors. Brain Pathol, 20(4): 763–770

Rao D S, Bradley S V, Kumar P D, Hyun T S, Saint-Dic D, Oravecz-Wilson K, Kleer C G, Ross T S (2003). Altered receptor trafficking in Huntingtin Interacting Protein 1-transformed cells. Cancer Cell, 3(5): 471–482

Rao D S, Hyun T S, Kumar P D, Mizukami I F, Rubin M A, Lucas P C, Sanda M G, Ross T S (2002). Huntingtin-interacting protein 1 is overexpressed in prostate and colon cancer and is critical for cellular survival. J Clin Invest, 110(3): 351–360

Rodahl L M, Haglund K, Sem-Jacobsen C, Wendler F, Vincent J P, Lindmo K, Rusten T E, Stenmark H (2009). Disruption of Vps4 and JNK function in Drosophila causes tumour growth. PLoS ONE, 4(2): e4354

Salcini A E, Confalonieri S, Doria M, Santolini E, Tassi E, Minenkova O, Cesareni G, Pelicci P G, Di Fiore P P (1997). Binding specificity and in vivo targets of the EH domain, a novel protein-protein interaction module. Genes Dev, 11(17): 2239–2249

Santolini E, Puri C, Salcini A E, Gagliani M C, Pelicci P G, Tacchetti C, Di Fiore P P (2000). Numb is an endocytic protein. J Cell Biol, 151 (6): 1345–1352

Schlessinger J (2000). Cell signaling by receptor tyrosine kinases. Cell, 103(2): 211–225

Shimada A, Hirato J, Kuroiwa M, Kikuchi A, Hanada R, Wakai K, Hayashi Y (2008). Expression of KIT and PDGFR is associated with a good prognosis in neuroblastoma. Pediatr Blood Cancer, 50(2): 213–217

Sirisaengtaksin N, Sun W, Yan Q, Zage P E, Bean A J. The Ubiquitin Ligase, UBE4B, Promotes Multivesicular Body Sorting of EGF Receptors. (Manuscript submitted)

Slagsvold T, Aasland R, Hirano S, Bache K G, Raiborg C, Trambaiolo D, Wakatsuki S, Stenmark H (2005). Eap45 in mammalian ESCRT-II binds ubiquitin via a phosphoinositide-interacting GLUE domain. J Biol Chem, 280(20): 19600–19606

Slongo M L, Molena B, Brunati A M, Frasson M, Gardiman M, Carli M, Perilongo G, Rosolen A, Onisto M (2007). Functional VEGF and VEGF receptors are expressed in human medulloblastomas. Neuro-oncol, 9(4): 384–392

Sun W, Yan Q, Vida T A, Bean A J (2003). Hrs regulates early endosome fusion by inhibiting formation of an endosomal SNARE complex. J Cell Biol, 162(1): 125–137

Sundquist W I, Schubert H L, Kelly B N, Hill G C, Holton J M, Hill C P (2004). Ubiquitin recognition by the human TSG101 protein. Mol Cell, 13(6): 783–789

Takahashi M (1995). Oncogenic activation of the ret protooncogene in thyroid cancer. Crit Rev Oncog, 6(1): 35–46

Taylor J G 6th, Cheuk A T, Tsang P S, Chung J Y, Song Y K, Desai K, Yu Y, Chen Q R, Shah K, Youngblood V, Fang J, Kim S Y, Yeung C, Helman L J, Mendoza A, Ngo V, Staudt L M, Wei J S, Khanna C, Catchpoole D, Qualman S J, Hewitt S M, Merlino G, Chanock S J, Khan J (2009). Identification of FGFR4-activating mutations in human rhabdomyosarcomas that promote metastasis in xenotransplanted models. J Clin Invest, 119(11): 3395–3407

Thiele C J, Li Z, McKee A E (2009). On Trk—the TrkB signal transduction pathway is an increasingly important target in cancer biology. Clin Cancer Res, 15(19): 5962–5967

Thien C B F, Langdon W Y (2001). Cbl: many adaptations to regulate protein tyrosine kinases. Nat Rev Mol Cell Biol, 2(4): 294–307

Thompson B J, Mathieu J, Sung H H, Loeser E, Rørth P, Cohen S M (2005). Tumor suppressor properties of the ESCRT-II complex component Vps25 in Drosophila. Dev Cell, 9(5): 711–720

Thorarinsdottir H K, Santi M, McCarter R, Rushing E J, Cornelison R, Jales A, MacDonald T J (2008). Protein expression of platelet-derived growth factor receptor correlates with malignant histology and PTEN with survival in childhood gliomas. Clin Cancer Res, 14 (11): 3386–3394

Thrower J S, Hoffman L, Rechsteiner M, Pickart C M (2000). Recognition of the polyubiquitin proteolytic signal. EMBO J, 19 (1): 94–102

Tsuda M, Davis I J, Argani P, Shukla N, McGill G G, Nagai M, Saito T, Laé M, Fisher D E, Ladanyi M (2007). TFE3 fusions activate MET signaling by transcriptional up-regulation, defining another class of tumors as candidates for therapeutic MET inhibition. Cancer Res, 67 (3): 919–929

Vaccari T, Bilder D (2005). The Drosophila tumor suppressor vps25 prevents nonautonomous overproliferation by regulating notch trafficking. Dev Cell, 9(5): 687–698

Vasei M, Modjtahedi H, Ale-Booyeh O, Mosallaei A, Kajbafzadeh A M, Shahriari M, Ghaderi A A, Soleymanpour H, Kosari F, Moch H, Sauter G (2009). Amplification and expression of EGFR and ERBB2 in Wilms tumor. Cancer Genet Cytogenet, 194(2): 88–95

Voorhees P M, Orlowski R Z (2006). The proteasome and proteasome inhibitors in cancer therapy. Annu Rev Pharmacol Toxicol, 46(1): 189–213

Vousden K H, Prives C (2005). P53 and prognosis: new insights and further complexity. Cell, 120(1): 7–10

Wang Q, Song C, Li C C (2004). Molecular perspectives on p97-VCP: progress in understanding its structure and diverse biological functions. J Struct Biol, 146(1–2): 44–57

Wang X, Trotman L C, Koppie T, Alimonti A, Chen Z, Gao Z, Wang J, Erdjument-Bromage H, Tempst P, Cordon-Cardo C, Pandolfi P P, Jiang X (2007). NEDD4-1 is a proto-oncogenic ubiquitin ligase for PTEN. Cell, 128(1): 129–139

Waterman H, Levkowitz G, Alroy I, Yarden Y (1999). The RING finger of c-Cbl mediates desensitization of the epidermal growth factor receptor. J Biol Chem, 274(32): 22151–22154

Wells A, Welsh J B, Lazar C S, Wiley H S, Gill G N, Rosenfeld M G (1990). Ligand-induced transformation by a noninternalizing epidermal growth factor receptor. Science, 247(4945): 962–964

Wiley H S, Burke P M (2001). Regulation of receptor tyrosine kinase signaling by endocytic trafficking. Traffic, 2(1): 12–18

Wiley H S, Herbst J J, Walsh B J, Lauffenburger D A, Rosenfeld M G, Gill G N (1991). The role of tyrosine kinase activity in endocytosis, compartmentation, and down-regulation of the epidermal growth factor receptor. J Biol Chem, 266(17): 11083–11094

Williams R L, Urbé S (2007). The emerging shape of the ESCRT machinery. Nat Rev Mol Cell Biol, 8(5): 355–368

Woelk T, Sigismund S, Penengo L, Polo S (2007). The ubiquitination code: a signalling problem. Cell Div, 2(1): 11

Wu H, Pomeroy S L, Ferreira M, Teider N, Mariani J, Nakayama K I, Hatakeyama S, Tron V A, Saltibus L F, Spyracopoulos L, Leng R P (2011). UBE4B promotes Hdm2-mediated degradation of the tumor suppressor p53. Nat Med, 17(3): 347–355

Yan Q, Sun W, Kujala P, Lotfi Y, Vida T A, Bean A J (2005). CART: an Hrs/actinin-4/BERP/myosin V protein complex required for efficient receptor recycling. Mol Biol Cell, 16(5): 2470–2482

Yokouchi M, Kondo T, Houghton A, Bartkiewicz M, Horne W C, Zhang H, Yoshimura A, Baron R (1999). Ligand-induced ubiquitination of the epidermal growth factor receptor involves the interaction of the c-Cbl RING finger and UbcH7. J Biol Chem, 274(44): 31707–31712