Minireview

Ubiquitin-dependent proteolysis: its role in human diseases and the design of therapeutic strategies

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Abstract

Protein degradation is one of the tactics employed by the cell for irreversibly inactivating proteins. In eukaryotes, ATP-dependent protein degradation in the cytoplasm and nucleus is carried out by the 26S proteasome. Most proteins are targeted to the 26S proteasome by covalent attachment of a multi-ubiquitin chain. A key component of the enzyme cascade that results in attachment of the multi-ubiquitin chain to the target or labile protein is the ubiquitin ligase that controls the specificity of the ubiquitination reaction. Defects in ubiquitin-dependent proteolysis have been shown to result in a variety of human diseases, including cancer, neurodegenerative diseases, and metabolic disorders. This review focuses on the role of ubiquitin-dependent degradation in human disease and potential clinical applications that are being developed to exploit the cells natural proteolytic machinery to treat diseases. © 2002 Published by Elsevier Science (USA).

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1. Introduction

Ubiquitin (Ub)-dependent proteolysis of key regulatory proteins impacts various cellular processes such as cell cycle progression, transcription, antigen presentation, receptor endocytosis, fate determination, and signal transduction [1,2]. With so many cellular pathways affected, it is therefore not surprising that derangements in the Ub-proteolytic pathway contribute to the etiology of several diseases. In this review, we will summarize our current understanding of how Ub-dependent degradation takes place in the cell and the various steps at which an impairment of the normal pathway contributes to the diseased state. We will conclude by summarizing the different ways we, as well as others, are utilizing the cellular degradation machinery to develop therapeutic strategies to treat human diseases.

2. Background

2.1. The Ubiquitin system

Ubiquitin is one of the most conserved proteins in eukaryotes. It is a small 76-amino acid protein that is conjugated to other proteins through an energy-dependent enzymatic pathway [1,3]. Conjugation is initiated by the activation of Ub by the Ub-activating enzyme, E1, which forms a high-energy Ub-thiol ester bond in the presence of ATP (Fig. 1). It then transfers the activated Ub to a Ub-conjugating enzyme, E2, forming an E2-thiol ester bond. Finally, ubiquitin is transferred to a target substrate protein through an isopeptide linkage between the conserved C-terminal glycine residue of ubiquitin and the ε-amino group of the lysine residue of the substrate. In many cases, the transfer of ubiquitin from an E2 to a target protein requires the involvement of a ubiquitin ligase, E3, which is discussed in the next section. Sequential conjugation of the internal lysine residue of ubiquitin to a C-terminal glycine residue of a
new ubiquitin molecule results in formation of a polyubiquitin chain, which targets proteins for degradation by the 26S proteasome [4,5].

2.2. Ubiquitin ligases

The E3 ubiquitin ligases cooperate with E2 ubiquitin conjugating enzymes to assemble multi-ubiquitin chains on substrate proteins. Generally, ubiquitin ligases, whether monomeric, or multimeric complexes, contain domains that recognize substrate proteins specifically, as well as domains that interact with E2 enzymes, thereby promoting ubiquitin transfer from the E2 to the substrate. Based on sequence homology and catalytic mechanisms, all known ubiquitin ligases can be divided into two major classes: the catalytic HECT domain E3s and the adaptor RING finger based E3s [4,6]. Variants of the latter class, the PHD domain containing E3s and the U box E3s are only now beginning to be defined [7–9].

The HECT domain proteins are found in all eukaryotes and are defined by a 350 amino acid C-terminal HECT homology domain (Homologous to E6-AP C-Terminus), originally identified in E6-AP [10]. E6-AP is the cellular ubiquitin ligase recruited by the human papilloma virus E6 oncoprotein to induce degradation of the p53 tumor suppressor. Multiple HECT domain proteins have been identified, including RSP5, UFD4, and TOM1 in *Saccharomyces cerevisiae*; Publ in *Schizosaccharomyces pombe*, and NEDD4 in humans. The large N-terminal domains allow the ligases to bind substrates and the C-terminal HECT domains serve to directly transfer ubiquitin to the substrates through an E1–E2–E3 ubiquitin thiol ester cascade. Thus this class of ligases functions catalytically [6,11].

The identification of RING finger domains in SCF (Skp1–Cullin–F box) and other non-HECT domain ubiquitin ligases suggested that these domains function to ubiquitinate proteins. The RING domain is a zinc binding motif that is defined by an octet of cysteine and histidine residues in a consensus sequence CX2CX [9–39] CX [1–3] HX [2,3] C/HX2CX [4–48] CX2C [4,6,12]. RING finger E3s play prominent roles in diverse cellular processes, including cell cycle progression, signaling, transcription, apoptosis, proliferation, and DNA repair.

The SCF subfamily of E3s was originally discovered and studied in budding yeast *S. cerevisiae*. The SCF complex consists of at least four subunits: CUL1/CDC53, HRT1/RBX1/ROC1, SKP1, and an F-box protein [4,13,14]. The known SCF subunits are con-
served throughout evolution. The Cullin and the RING finger protein HRT1 form a catalytic core of E3s that binds to and activates E2 CDC34 or UBCH5 [15,16]. HRT1 is a small 121 amino acid RING finger protein that binds directly to Cullins and CDC34, likely tethering E2 to the CH domain, which is confirmed by the crystal structure [17]. SKP1 binds to the CDC53/HRT1 core and mediates recruitment of various F-box adapter proteins. These F-box proteins contain a 45 amino acid motif called an F-box that binds substrates through protein–protein interaction domains, thus conferring substrate specificity to this family of E3s. Multiple F-box proteins have been shown to bind SKP1. There are over 400 F-box proteins currently in the database, with 20 F-box proteins in *S. cerevisiae*, over 100 in *Caenorhabditis elegans*, and over 50 described so far in mammals [18].

Many proteins have been demonstrated to be substrates of SCF ubiquitin ligase [4]. These proteins participate in a variety of cellular functions, including regulation of cyclin-dependent kinase activity, activation of transcription, signal transduction, and DNA replication. In all cases of SCF Ub-ligases working in transcription, signal transduction, and DNA regulation of cyclin-dependent kinase activity, activating a variety of cellular functions, including protein–protein interaction and binding to DNA. Recently, it was shown that the MEKK1 PHD domain has E3 ubiquitin ligase activity, suggesting at least in some cases, these proteins may behave as E3s [7]. Naturally occurring mutations have been identified in several diseases such as ING1 in head and neck squamous cell carcinomas [39]. AIRE in autoimmune polygladular syndrome, type I [40], and MLL and CBP in myeloid leukemias [41]. Furthermore, genes encoding PHD proteins have been identified in deleted regions of several contiguous gene deletion syndromes such as Williams syndrome [42]. The occurrence of mutations in PHD domains suggests that the PHD domain E3 activity could play an important role in human disease [43].

Recently, a sequence-profile analysis demonstrated that the U box is a derived version of the RING-finger domain that lacks the metal-chelating residues but is likely to function similarly to the RING-finger in mediating ubiquitin-conjugation of protein substrates [9]. Interestingly, the signature cysteines of the RING finger are not conserved in the U box. Multiple alignment of the U box with selection of RING fingers shows that, except for the loss of the hallmark cysteines and a histidine, the U box retains the same pattern of amino acid residue conservation. Thus, the U box, like the RING finger are likely to activate ubiquitination and multi-ubiquitination by facilitating the interaction between E2 proteins and their substrates [9].

3. Protein degradation in human disease

3.1. Diseases arising due to impairment of ubiquitin ligases

3.1.1. Cervical cancer

Among the best-characterized associations between cancer and the ubiquitin ligase pathway is that of cancer of the uterine cervix. This is the third most common cancer diagnosed in women. While the precise cause of cervical cancer remains uncertain, the disease is strongly associated with infections by the oncogenic forms of HPV, types 16 and 18. The E6 and E7 proteins of these
high risk strains are often detected in cervical cancers [44]. In these same carcinomas, the levels of tumor suppressor p53 are very low, suggesting a link between E6 and p53. In fact, the p53 protein forms a ternary complex with high risk HPV E6 oncoprotein E6-16 or -18 and the ubiquitin ligase E6-AP [10]. E6-AP can interact with E6 in the absence of p53, but can only interact with p53 in the presence of E6. The E6/E6-AP complex promotes ubiquitination of p53, resulting in degradation by the 26S proteasome. The strong correlation between different polymorphisms of p53 to E6-mediated destruction and the prevalence of cervical carcinoma in women further supports the link between p53 degradation by the ubiquitin pathway and malignant transformation. The tumor suppressive effects of p53 are most likely exerted through its apoptotic function or checkpoint activity such that accelerated degradation promotes malignant transformation.

3.1.2. SCF complex and cancer

The invariant subunits of SCF are the E2-interacting subunits Hrt1/ROC1, Cdc53/Cul1, and the F-box interacting protein Skp1. The subunit that varies is the F-box containing protein that confers substrate specificity. Thus the specific form of the SCF ligase is denoted by a superscript. Besides SCF<sup>Cdc4</sup>, SCF<sup>Skp2</sup> is another form of the ligase whose substrate is the cyclin-dependent kinase (cdk) inhibitor, p27Kip1. Recently, a cdk subunit known as Cks1 was found to direct ubiquitin-mediated proteolysis of the CDK-bound p27Kip1 by SCF<sup>Skp2</sup> [45]. The p27 protein plays a major role in maintaining differentiated mammalian cells in a quiescent state by negatively regulating the activities of cyclin-dependent kinases required for initiation of DNA synthesis and inhibiting G1 to S transition [46]. The p27 protein is unstable and expressed at low levels in certain types of cancer [47]. In many cases, decreased p27 expression is associated with a worse prognosis [48,49]. In vivo, mouse embryonic stem cells lacking the Skp2 gene express elevated levels of p27 [50].

Regulation of G1 to S transition in the mammalian cell cycle occurs through the activation of cyclin-dependent kinases (Cdk)s 2, 4, and 6. The cyclin that regulates Cdk2, Cyclin E, is targeted for ubiquitin-mediated proteolysis by an SCF ubiquitin ligase that contains the human homologue of yeast Cdc4, which is an F-box protein containing WD40 repeats. Recently, the F box protein Cdc4 was found to be mutated in breast cancer cells that express high levels of cyclin E [51]. A tandem duplication consisting of a direct repeat of exons 8 and 9 separated by 11 base pairs of intronic sequence was predicted to result in chain termination, eliminating the last four WD40 repeats and rendering the truncated Cdc4 non-functional. Interestingly, aberrant hCdc4 mRNA levels, loss of hCdc4 protein, and loss of heterozygosity in breast cancer cell lines were also observed [51].

Targets for SCF<sup>β-TRCP</sup> ubiquitin ligase include the transcription factor β-catenin and IκB, the inhibitor of the transcription factor NF-κB [25,26,52]. SCF<sup>β-TRCP</sup> complexes recruit phosphorylated β-catenin and IκB for ubiquitination by the E2 enzymes Cdc34 and ubch5. β-Catenin functions as a transcription factor in the Wnt/wingless signaling pathway [53]. The Wnt/wingless family of secreted proteins act as inducers of axis formation and organogenesis in embryonic patterning pathways of many developing tissues. In the absence of a Wnt/wingless signal, β-catenin is rapidly phosphorylated by the glycogen synthase 3-β (GSK-3β) kinase in a reaction that requires axin and the adenomatous polyposis coli (APC) tumor suppressor protein. Phosphorylated β-catenin is targeted for ubiquitination by the SCF<sup>β-TRCP</sup> complex and degraded by the proteasome [54]. In the presence of a Wnt/wingless signal, β-catenin is not phosphorylated and ubiquitinated. The levels of transcriptionally active β-catenin rise, which results in activation of β-catenin-regulated gene expression. Mutations of β-catenin phosphorylation sites, as well as APC mutations also block ubiquitination and degradation of β-catenin and often result in inappropriate expression of β-catenin-regulated genes and in cancer [53,55]. Recent work has suggested that a complex of casein kinase I and axin induces β-catenin phosphorylation at serine 45 that initiates the degradation cascade and serves as a molecular switch for the Wnt pathway [56].

3.1.3. SCF<sup>β-TRCP</sup> complex and immune modulation

The SCF<sup>β-TRCP</sup> complex also participates in activation of transcription factor NF-κB by targeting the inhibitor IκB for ubiquitination and degradation by the proteasome, as well as being implicated in processing of the p105 precursor. Extracellular signals mediated by cytokines such as TNFα activate phosphorylation of IκB by the IκB kinases (IKK) [57]. Phosphorylated IκBα is targeted for ubiquitination by the SCF<sup>β-TRCP</sup> complex and degraded by the proteasome, resulting in nuclear translocation of NF-κB and activation of expression of NF-κB-regulated genes. More recent work has shown that the signal transducer in the NF-κB pathway, TRAF6, a ring domain protein, functions together with a ubiquitin conjugating enzyme Ubc13/Uev1A to catalyze the synthesis of unique polyubiquitin chains linked through lysine 63 (K63) of ubiquitin. Inhibition of this polyubiquitin chain synthesis prevents the activation of IKK by TRAF6, suggesting a new role for ubiquitination in immune regulation [58].

3.1.4. von Hippel-Lindau (VHL) disease

The VHL syndrome is an autosomal dominant familial cancer syndrome that predisposes affected individuals to tumors, including cerebellar hemangioblastomas, hemangiomas, renal cell carcinomas, retinal angiomas, and pheochromocytomas [59]. The VHL tu-
mor suppressor gene is located on chromosome 3p25.5 and is mutated in the majority of sporadic clear cell renal carcinomas in addition to tumors associated with the VHL syndrome. The VHL protein resides in the cytoplasm but can translocate to the nucleus in a process that is Ran and energy-dependent. VHL forms stable, multimeric complexes that contains Elongin B, Elongin C, CUL2, and RBX1 [60]. The knowledge that Elongin C resembles SKP1 adaptor protein of SCF and VHL associates with the cullin family member CUL2, suggested that the VHL/Elongin/CUL2 complex might function similarly to SCF in yeast. The crystal structure of VHL bound to Elongin B and C showed that the region of VHL that binds to Elongin C loosely resembles an F-box and that Elongin C resembles SKP1 [29]. Mutations found in VHL disease are clustered in two regions that appear to be critical for complex formation and substrate recognition. Anti-VHL immunoprecipitates contain ubiquitin ligase activity, indicating that VHL is associated with ubiquitination. It is now known that VHL ubiquitinates the HIF transcription factor, which regulates hypoxia-inducible genes and is degraded in the presence of oxygen [60]. Furthermore, VHL binds to a short HIF-derived peptide on a conserved proline residue only when the peptide is hydroxylated. Since this modification most likely plays a critical role in oxygen sensing in mammalian cells [61].

Clear cell renal carcinoma cells lack a functional VHL protein and exhibit a diverse array of phenotypes, including cell cycle defects [62], overexpression of TGFβ [63], defects in endoplasmic reticulum associated degradation of misfolded proteins [64], and defects in assembly of extracellular matrix [65]. One interesting feature of VHL tumors is their high vascularity, which is thought to result from deregulation by VHL of hypoxia-inducible genes including the angiogenesis-promoting factor vascular endothelial growth factor (VEGF) [66,67]. The expression of VEGF and other hypoxia-regulated genes are repressed under normoxic growth conditions in cells, but are strongly induced under hypoxic conditions and constitutively expressed in cells lacking functional VHL.

3.1.5. Cbl ubiquitin ligase and cancer

Cbl is an adapter protein that contains both SH2 and RING-HC finger domains. Cbl functions as a monomeric ubiquitin ligase that recognizes phosphorylated tyrosine on receptor tyrosine kinases (RPTKs) through its SH2 domain and negatively regulates signaling by promoting active receptor ubiquitination and degradation [68–72]. Oncogenic versions of Cbl containing deletions or point mutations in the C-terminal tyrosine kinase binding domain result in transformation. Another oncogenic form of c-Cbl, termed 70Z-Cbl, exhibits a 17-amino acid deletion that removes C381, the first cysteine residue of the RING finger, and most of the linker domain between TKB and the RING finger. These mutants lack E3 activity, suggesting that they act as dominant-negative proteins competing with wild-type c-Cbl for tyrosine-phosphorylated tyrosine kinases abrogating the negative-regulatory effect of c-Cbl in cell signaling [70].

3.1.6. Angelman syndrome

Angelman syndrome is a rare neurological disorders characterized by mental retardation, seizures, absence of speech, excessive laughter, and abnormal gait. The affected gene is the E3 ubiquitin ligase E6-AP. It is thought that the syndrome results from aberrant accumulation of substrates of E6-AP [73]. Although the targets are unknown, studies have suggested a role for E6-AP in brain development. Studies also show that mutation of the E6-AP ubiquitin ligase reduces nuclear inclusions and accelerate polyglutamine-induced neurodegeneration [74].

3.1.7. Parkinson disease

One of the most common forms of familial Parkinson disease is the autosomal recessive juvenile Parkinsonism (AR-JP). AR-JP is characterized by selective dopaminergic neural cell death and the absence of the Lewy body, a cytoplasmic inclusion body consisting of abnormally accumulated proteins [75].

AR-JP was first described by Yamamura and colleagues [76]. The gene was mapped to chromosome 6q25.2-q27 and contains 12 exons with a molecular mass of 52kDa. This gene is highly conserved throughout evolution, including Drosophila, C. elegans, and mammals. The N-terminus of Parkin demonstrates a ubiquitin-like domain, while the C-terminal half region of Parkin has two RING-finger motifs. Parkin is thought to act as a ubiquitin ligase through association with the ubiquitin-conjugating enzyme UbcH7. Mutations in Parkin in AR-JP patients demonstrate decreased ubiquitin ligase activity [77]. Recent findings suggest that accumulation of proteins that are substrates of Parkin causes selective neuronal cell death without formation of Lewy bodies.

Further link between Parkinson disease and the ubiquitin system has been suggested in the studies of two gene products, UCH-L1 (ubiquitin carboxyl-terminal hydrolase) and α-synuclein, whose mutations cause autosomal dominant familial Parkinson disease [78,79]. UCH-L1 is thought to regenerate ubiquitin by both cleaving polyubiquitin chains and releasing ubiquitin from small adducts such as glutathione and cellular amines [80]. α-Synuclein, one of the major components of Lewy bodies [81] is degraded by the 26S Proteasome [82], suggesting that it is modified by ubiquitin, and mutations in Parkin may extend the half-life of the protein.

Other neurological disorders including Huntington disease and spinocerebellar ataxias have been thought
also to result from mutant proteins that aggregate in intranuclear accumulation bodies that fail to be degraded due to expanded CAG/polyglutamine repeats [83,84]. In prion diseases, pathology results from accumulation of a conformationally altered prion protein, which forms aggregates [85]. Thus, it is postulated that prionogenic and amyloidogenic proteins escape correct protease and chaperone responses of the cellular quality control program.

### 3.2. Diseases arising due to impairment of substrates of ubiquitin ligases

#### 3.2.1. Alzheimer disease

In many neurodegenerative disorders, such as Alzheimer disease, inclusions containing ubiquitinated proteins have been identified in the brain, suggesting a role for ubiquitin-dependent proteolysis of neuronal proteins. Prevention of ubiquitination inhibited the neurotoxic effects of β-amyloid. In the central nervous system, proteasome-mediated protein degradation plays a major role in the breakdown of proteins damaged by oxidative stress or other insults, including glucose and oxygen deprivation. Inclusions containing ubiquitinated proteins are commonly found in many neurogenetic disorders [86]. Since β-amyloid itself could cause protein ubiquitination and inhibiting protein ubiquitination or proteasome activity can block β-amyloid toxicity, new prophylactic and therapeutic avenues for treatment of neurodegenerative disease are being developed. Pharmacological inhibition of ubiquitination or proteasome-mediated degradation of ubiquitinated proteins may prevent, alleviate, or inhibit the progression of chronic neurodegenerative diseases such as Alzheimer disease. One caution, however, is that protein aggregation has been recently shown to impair directly the function of the ubiquitin-proteasome system [87].

#### 3.2.2. Cystic fibrosis

Cystic fibrosis (CF) is the most common autosomal recessive genetic disorder among those of northern European descent, and is characterized by severe bronchopulmonary infections and pancreatic insufficiency. The gene associated with CF encodes a transmembrane protein (CFTR) which is a chloride ion channel normally localized to the plasma membrane of epithelial cells. Because it is a large protein, most of the wild-type protein is degraded from the ER by the ubiquitin system and only a small fraction reaches the cell surface. In CF, mutations in the protein, the most common of which is ΔF508, interfere with the folding of the protein. Although it is functional, the ΔF508 protein is completely retained in the ER, from which it is polyubiquitinated and degraded by the proteasome [88,89]. The efficient degradation of the mutant protein leads to a lack of expression of ΔF508 CFTR at the cell surface, resulting in CF [90]. In this disease, a number of therapeutic strategies are possible, including transient transfection of airway epithelial cells with intact CFTR; modification of the post-transcriptional quality control mechanisms: Activation of residual membrane-associated CFTR; activation of second messenger pathways; activation of alternative CF channels in the luminal membrane; and inhibition of ENaC by amiloride [90].

#### 3.2.3. Wilson disease

Wilson disease is an autosomal recessive inherited disorder of copper metabolism that leads to neuronal degeneration and hepatic cirrhosis. The Wilson protein is a copper-transporting P-type ATPase that when mutated, becomes trapped in the ER and is presumed to be the molecular basis of disease [91]. Like the CFTR ΔF508 mutant protein, the mutated Wilson protein is rapidly degraded.

#### 3.2.4. Liddle syndrome

In contrast to CFTR and the Wilson protein, stabilization of a normally degraded protein is the basis of Liddle syndrome. This disease is an autosomal dominant form of hypertension characterized by early onset hypertension and hypokalemia, and suppression of plasma renin activity and aldosterone. The Liddle syndrome results from a deletion of the proline rich region in the C-terminal β and γ subchannel (ENaC), which leads to constitutive activation of the protein. ENaC normally has a short half-life because the α and γ chains are rapidly ubiquitinated. NEDD4 is a HECT domain protein that binds to the proline rich motif of ENaC through a highly conserved WW domain. Mutations in the β or γ subunits result in stabilization [92], leading to reabsorption of sodium and water and development of hypertension.

#### 3.2.5. Diabetes mellitus

A hereditary form of diabetes mellitus has been shown to be caused by missense mutations in the insulin receptor (IR). Various mutations in the IR gene have been reported in individuals with severe insulin resistance [93]. Several of these mutations result in decreased numbers of IRs at the cell surface, either as a consequence of impaired processing of the mutant pror receptors and accumulation in the ER or increased degradation of the mutant pror receptors. Recent studies have demonstrated that the degradation of mutant IR is preceded by a cleavage of the misfolded pror receptor, resulting in the accumulation of two proteins, 120 and 80 kDa, which associate with the IR [94]. The precise role of these interacting proteins has not been determined.

#### 3.2.6. α1-Antitrypsin Z

α1-Antitrypsin (α1-AT) is the most common genetic cause of infantile liver disease and also causes adult-type
emphysema. Most α1-AT-deficient individuals are protected from liver damage by rapid degradation of the mutant α1-AT in the ER; however, in some of these patients who develop severe liver disease, there is decreased degradation of α1-AT resulting in ER accumulation and hepatotoxicity [95]. The protein appears first to translocate in a retrograde manner into the cytosol, since components of the ubiquitin proteasome pathway have not been shown to exist in the ER. As in the cases of α1-antitrypsin, CFTR, and the Wilson protein, the ubiquitin ligases that attach the ubiquitin onto these proteins have not been identified.

3.2.7. Cytolytic T cell response

The ubiquitin-proteasome system plays an important role in cellular immunity. Peptides from foreign antigens are presented as MHC class I molecules to elicit a cytolytic T cell response [96]. In cells infected with viruses, viral antigens are targeted for degradation by the ubiquitin-proteasome pathway. The display of viral peptides on MHC class I molecules is a critical component of host defense. Therefore, the ability to avoid the CTL response to viral infections is an effective way for a virus to escape immune surveillance in the host [97]. An example is the Epstein–Barr virus (EBV) in

![Diagram of PTCM](image)

**Fig. 2.** Proteolysis targeting chimeric molecule (PTCM). A chimeric molecule PTCM, recruits the substrate, which is in this case MetAP-2, to the E3 SCF\(\beta\)-TrCP for ubiquitination and degradation. The PTCM consists of an \(\alpha\)Xβ\(\alpha\) phosphopeptide moiety at one end and the protein that binds the target at the other end. \(\beta\)-TrCP is the F-box protein, which recognizes the substrate or PTCM, in this case. The adaptor protein Skp1 links together the F-box protein \(\beta\)-TrCP and the ligase CUL1. HRT1 is a ring domain protein that facilitates ubiquitination of the substrate. Ubc is the E2 or ubiquitin conjugating enzyme.

![Graph of ubiquitination results](image)

**Fig. 3.** PTCM recruits MetAP-2 to the SCF\(\beta\)-TrCP for ubiquitination. MetAP-2-PTCM mixture was added to either SCF\(\beta\)-TrCP (+) or mock control beads (−) supplemented with ATP plus purified E1 (rabbit UBA1, Boston Biochem.), E2 (ubch5a, Boston Biochem.) and ubiquitin (10 mg/ml). Reactions were incubated for 1 h at 30°C and were evaluated by SDS–PAGE followed by Western blot analysis with anti-MetAP-2 antisera. Ubiquitination of MetAP-2 is inhibited by addition of \(\alpha\)Xβ\(\alpha\) phosphopeptide but not ovalicin, demonstrating that the reaction is PTCM dependent. SCF\(\beta\)-TrCP is the E3 or ubiquitin ligase; \(\alpha\)Xβ\(\alpha\) phosphopeptide binds the F-box protein \(\beta\)-TrCP. Ovalicin binds MetAP-2 ([102], Copyright 2001 National Academy of Sciences, USA). Addition of the \(\alpha\)Xβ\(\alpha\) phosphopeptide + ovalicin separately does not result in MetAP-2 ubiquitination.
which the Epstein–Barr nuclear antigen (EBNA) is expressed in latently infected B lymphocytes where it persists in healthy individuals. It is the only viral protein regularly detected in EBV-associated malignancies such as nasopharyngeal carcinomas and lymphomas, suggesting that escape from immune surveillance might promote transformation [98]. The human cytomegalovirus (CMV) avoids immune surveillance by down-regulating MCH class I. Finally, HIV utilizes an ER-associated proteasomal degradation pathway to induce the down-regulation of its CD4 receptor in infected cells [99]. In the case of HIV, unlike EBV and CMV, the E3 that ubiquitinates CD4 is known. Proteasomal degradation of CD4 occurs through a ternary complex between CD4, the viral VPU protein, and the WD protein β-TRCP, which recruits the SKP1 adaptor protein of the SCF complex [100]. VPU is an 81-amino acid membrane phosphoprotein that interacts with CD4. In HIV infected cells, expression of the viral envelope glycoprotein precursor gp160 results in the formation of stable CD4-gp160 complexes that are trapped in the ER. The VPU expressed in the ER then associates with the CD4 cytoplasmic tail and recruits β-TRCP to the ER membrane. β-TRCP, in turn, recruits Skp1, resulting in CD4 ubiquitination by the E3 ligase, SCF.

4. Potential therapeutic strategies

Given the fact that all mammalian cells have ubiquitin ligases and proteasomes, selectively targeting proteins for ubiquitination and degradation is a potential avenue for drug development. Moreover, since there is recent information about the crystal structure of ubiquitin ligases and their substrates, one could imagine using low molecular weight compounds to stabilize or degrade proteins to treat a variety of diseases. Several approaches have been considered, including gene therapy to selectively target proteins for ubiquitin-dependent proteolysis. Another approach has been to use chimeric molecules to recruit disease-promoting proteins to E3s for ubiquitination and subsequent degradation.

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Fig. 4. Ubiquitination of MetAP-2 by Cbl. MetAP-2 bound to Zap70 phosphopeptide-ovalacin was added to purified Cbl, with E1, and E2 (Ubc4), and ubiquitin (Ub). The reaction was incubated for 30 min at room temperature. Western blot analysis was performed with anti-MetAP-2 antisera as previously described [102]. These results suggest that PTCM technology can be applied to different ubiquitin ligases.
Tyrosine kinase inhibitors also promote protein degradation through chaperones. We will discuss these approaches and their potential therapeutic applications.

4.1. Selective degradation of non-SCF targets by chimeric F box proteins

Recently, it was shown that one could successfully engineer a substrate receptor of an F box protein to direct the degradation of otherwise stable cellular proteins in yeast and in mammalian cells. The yeast F box-containing Cdc4 protein normally targets the phosphorylated cyclin-dependent kinase inhibitor Sic1 for degradation. To target the tumor suppressor retinoblastoma (Rb) protein to the SCF machinery in yeast, derivatives of Cdc4 were fused to the E7 protein encoded by the human papillomavirus type 16. Using the N-terminal 35 residues of E7 (E7N), which contains a conserved LXCXE Rb binding motif, hybrid F box proteins were produced containing the F box/WD-40 repeat domains of Cdc4 sufficient to bind Skp1 and the HPV-16 E7N fused in frame [101]. In yeast, Rb was shown to be degraded in cells expressing the fusion protein. Similar hybrid proteins were engineered using the mammalian F Box-containing β-TRCP fused to HPV-16 E7N. This fusion protein targeted Rb for degradation and inhibited RB-induced growth arrest in human osteosarcoma SAOS-2 cells. These results suggest that the mammalian and yeast SCF machinery could be harnessed to degrade targets that are not normally substrates of SCF. Such an approach is a potentially useful tool to evaluate whether a protein is a target for drug intervention or to knock out cellular proteins to study their functions. As a therapeutic modality, there are limitations as with other gene therapy approaches such as efficiency of transduction, duration of expression, and targeting to the right cells.

4.2. Chimeric molecules to target proteins for ubiquitination and degradation

To circumvent the problem of transducing cells at high efficiency, we sought to target deliberately a protein to the SCF complex by developing a chimeric compound, known as a proteolysis targeting chimeric molecule (PTCM) (Fig. 2, [102], Copyright 2001 National Academy of Sciences, USA). We first tested whether the PTCM could recruit methionine aminopeptidase-2 (MetAP-2) to the SCFβ-TRCP complex resulting in ubiquitination. Addition of PTCM also resulted in degradation of MetAP-2 in Xenopus extracts [102].

To determine whether PTCM could be generalized to other ubiquitin ligases, we performed ubiquitination assays with Cbl. Cbl is a monomeric ubiquitin ligase that attaches ubiquitin to signaling molecules and receptor tyrosine kinases resulting in proteolysis. We generated a PTCM that consisted of ovalicin and the Zap70 phosphopeptide, which binds Cbl [103]. Ubiquitination reactions were performed with purified Cbl, various E1s, ubch4 (E2), ubiquitin, ATP, Met-AP-2, and the Zap70-ovalicin PTCM. We demonstrated that PTCM promotes ubiquitination of MetAP-2 by Cbl in vitro Fig. 4. These results suggest that PTCM can be generalized to other ubiquitin ligases (Fig. 5, [102]).
4.3. Ubiquitination with tyrosine kinase inhibitors

Recently, tyrosine kinase inhibitors (TKIs) have been found to not only inhibit tyrosine phosphorylation, but enhance ubiquitination and degradation of the receptor tyrosine kinase, ErbB-2. ErbB-2/HER-2 is associated with aggressive tumors, including breast cancer. Therapy to target the oncoprotein is currently being used to treat cancer patients. A potent, irreversible TKI, CI-1033 was found to alkylate a cysteine residue specific to ErbB receptors. The pathway stimulated by TKIs appears to be chaperone mediated, and is common to the heat shock protein 90 (Hsp90) antagonist geldanamycin through a stress-induced mechanism [104]. More recently, geldanamycin dimers and hybrid compounds containing geldanamycin linked to estradiol or testosterone have been shown to induce selective degradation of the estrogen receptor or androgen receptor, respectively, in cancer cells [105–107]. These results suggest that selectively targeting chaperoned oncosignaling products for destruction is an alternative strategy to treat human diseases such as cancer.

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References


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Future work will focus on testing other targets that promote tumorigenesis, e.g., androgen receptor in prostate cancer cells. If cell permeable PTCMs prove to increase turnover and degrade proteins in cells, this would lead to potential therapeutic applications in patients with cancer and other diseases.


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