Abstract

The ubiquitin system of protein modification has emerged as a crucial mechanism involved in the regulation of a wide array of cellular processes. As our knowledge of the pathways in this system has grown, so have the ties between the protein ubiquitin and human disease. The power of the ubiquitin system for therapeutic benefit blossomed with the approval of the proteasome inhibitor Velcade in 2003 by the FDA. Current drug discovery activities in the ubiquitin system seek to (i) expand the development of new proteasome inhibitors with distinct mechanisms of action and improved bioavailability, and (ii) validate new targets. This review summarizes our current understanding of the role of the ubiquitin system in various human diseases ranging from cancer, viral infection and neurodegenerative disorders to muscle wasting, diabetes and inflammation. I provide an introduction to the ubiquitin system, highlight some emerging relationships between the ubiquitin system and disease, and discuss current and future efforts to harness aspects of this potentially powerful system for improving human health.

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Broad overview of family

Overview of the ubiquitin system

The ubiquitin system is a hierarchical enzymatic cascade in which a ubiquitin-activating enzyme (E1) activates the 76 amino acid protein UBIQ (ubiquitin) in an ATP-dependent manner and transfers it to the active site cysteine of ubiquitin-conjugating enzymes (E2s) [1]. Ubiquitin ligases (E3s) have a central role in the process of protein modification with UBIQ (known as ‘ubiquitination’ or ‘ubiquitylation’); they recognize specific substrates and facilitate UBIQ transfer from the E2 onto the substrate. Although the precise number of human E3s is unknown, about 500 or more have been proposed to exist [2-5], supportive of the broad role for the ubiquitin system in regulating diverse cellular processes. Ubiquitin-like proteins (UBLs) have also been identified with varying degrees of identity to UBIQ and are conjugated onto proteins through similar enzymatic cascades as UBIQ.

Numerous deubiquitylating enzymes (DUBs) have roles in processing polyubiquitin precursor proteins and may also have regulatory roles, e.g. counteracting the ubiquitylation of a particular protein by its cognate E3 and/or proofreading synthesized UBIQ chains. There are also emerging roles for DUBs in disease (see [6]). Ubiquitin binding proteins also have diverse functions and may represent viable therapeutic targets (see [7]). In a general sense, they act as ‘effector’ proteins that sense a protein’s modification with UBIQ and facilitate downstream signaling.
Two major classes of E3s have been identified and this classification is largely based on how they facilitate UBIQ transfer from E2 onto substrate. HECT (homologous to E6AP C-terminus) domain E3s form a catalytic UBIQ intermediate on a conserved cysteine residue prior to covalent UBIQ transfer (see [8]). The second class of E3s, which contains RING-type and structurally related ligases, facilitates the direct transfer of UBIQ from E2 onto substrate. In general, E3s facilitate covalent UBIQ transfer by properly positioning the site to be modified (i.e. a lysine residue of its recognized substrate) such that it can perform nucleophilic attack of a thioesterified UBIQ molecule either on the active site of the E2 for RING-type E3s or on the conserved cysteine of HECT domain E3s, resulting in isopeptide bond formation [9].

Lysine residues appear to be major sites of UBIQ attachment on proteins, although N-terminal and cysteine modifications have also been reported [10-17]. The type of UBIQ modification could confer distinct encoded protein fate and we are only beginning to understand how this process occurs and how it is recognized and interpreted. Mono-ubiquitylation (i.e. the attachment of a single UBIQ molecule to a single site on a protein) may be involved in histone regulation, receptor endocytosis and signaling [18-22]. UBIQ chains using a lysine residue of one UBIQ molecule attached via an isopeptide bond to the C-terminus of another UBIQ molecule add further complexity to UBIQ-encoded protein fate. Lys48-linked UBIQ chains can trigger degradation by the 26S proteasome [23-26] and Lys63-linked UBIQ chains may regulate signaling pathways [27-30] when attached to a protein. Other types of linkages (including those containing heterogeneous mixtures of linkages or branched chains) could also exist [31-33]; however their roles and physiological significance are currently unclear.

Target validation

Implication of the ubiquitin system in human disease

The basic functions of the UBIQ (ubiquitin) protein were first described in 1980 [34-36], yet its implication in human disease has only recently started to become appreciated. Below, I describe some relationships between the ubiquitin system and various human diseases.

Cancer is associated with alterations in UBIQ-dependent regulation

The ubiquitin system has a widely appreciated role in regulating cellular proliferation. As expected and described in the examples below, alterations in specific pathways involving UBIQ have been associated with cancer.

The stability of P53 (p53) is regulated by ubiquitin ligases and a deubiquitylating enzyme (DUB)

The transcription factor P53 has a crucial role in cellular anticancer mechanisms and it has been estimated that >50% of tumors contain mutations in the P53 gene [37]. MDM2 is a major regulator of P53 function – it binds directly to P53 and targets P53 for degradation through its RING ubiquitin ligase activity [38-42]. MDM2-P53 binding, MDM2-dependent P53 degradation by the proteasome, and P53 ubiquitylation by MDM2 have been demonstrated in cell-based and in vitro systems by a large number of groups.

P53 regulates the stability of the interaction between MDM2 and the DUB known as UBP7 (also known as USP7, HAUSP, herpesvirus-associated ubiquitin-specific protease [43]) [44-47]. Work from the laboratory of Wei Gu demonstrated that partial reduction of UBP7 by RNAi in human cell lines (NHF-1, IMR90, U2OS and H1299 cells were tested) promotes decreased levels of both MDM2 and P53 [45]. By contrast, however, total reduction of UBP7 decreases MDM2 yet stabilizes P53. This observation suggests that the absence of UBP7 promotes MDM2 downregulation, which in turn eliminates MDM2’s function as the ubiquitin ligase for P53. This mechanism requires MDM2-dependent function, as depletion of UBP7 in cells inactive for MDM2-dependent P53 turnover (i.e. HeLa cells [48]) results in P53 stabilization.

Cullin-RING ubiquitin ligases and the APC/C regulate cellular proliferation

Cullin-RING ubiquitin ligases (CRLs) and the anaphase-promoting complex/cyclosome (APC/C) are multi-subunit RING ubiquitin ligases that have fundamental roles in controlling the eukaryotic cell cycle (see [49]). CRLs contain a cullin protein (CUL1, CUL2, CUL3, CUL4A, CUL5, CUL7) that binds within the cullin homology domain to the RING protein RBX1 [50-52]. The distinct N-terminal regions of the cullins interact with specific classes of substrate receptors that promote the recruitment of a large number of proteins for ubiquitylation [53]. The APC/C consists of at least 11 subunits and has a mass of 1.5 mDa [54,55]. One of its subunits, APC2, contains a domain similar to cullins and associates with the RING protein APC11, suggesting that its enzymatic core is similar to CRLs [56-62].

The CUL1-based ubiquitin ligase known as SCF (SKP1-CUL1-F-box) recognizes its substrates through various receptor proteins containing the F-box motif [63]. The SKP2 F-box protein, which functions with SCF in the ubiquitylation of CDN1B (the cyclin-dependent kinase inhibitor p27) at the G1/S transition of the cell cycle [64-66], has garnered attention as a potential oncology target.
SKP2, in conjunction with the adapter protein CKS1, recognizes phosphorylated CDN1B late in G1, recruiting it for ubiquitylation by SCF [67,68]. An inverse correlation between SKP2 overexpression and low CDN1B levels has been found in a variety of human tumors and transgenic mouse models, and has led to the proposal that SKP2 is a proto-oncogene [69-74].

The protein encoded by the tumor suppressor gene VHL (von Hippel-Lindau) serves as a substrate receptor for a CUL2-based ubiquitin ligase [50,75-80]. Mutations in VHL are associated with lung cancer, sporadic clear cell renal carcinomas and an autosomal dominant familial cancer known as von Hippel-Lindau disease ([81-93] and see [94]). Many of these mutations prevent VHL associating with the other subunits of its ubiquitin ligase, as judged by in vitro binding and co-immunoprecipitation experiments [79,80,95]. A substrate for this ubiquitin ligase is a marker of tumor hypoxia, the transcription factor HIF1A (HIF1α, hypoxia-inducible factor 1α), which stimulates angiogenesis [96]. Numerous biochemical and structural studies have determined that HIF1A binds to VHL when hydroxylated on two proline residues through the activity of prolyl hydroxylases, which results in its ubiquitylation and ultimate degradation [77,78,97-102]. Transgenic mice overexpressing a stabilized form of HIF1A that cannot be recognized by VHL develop hypervascularity without leakage or inflammation [103].

**Cervical cancer is linked to HPV infection and involves downregulation of P53 and RB (Rb)**

HPVs encode two oncogenic proteins known as E6 and E7, and the sexually transmitted types of HPV have a strong association with cervical cancer (see [104]). Whereas E7 may facilitate the degradation of the tumor suppressor RB through an unclear mechanism, the role of E6 in cellular transformation is more established [105]. E6 binds to a cellular protein known as UBE3A (E6AP), which is a HECT domain ubiquitin ligase [106]. This interaction promotes the recruitment of the tumor suppressor P53 to this complex, resulting in its ubiquitylation and subsequent degradation by the 26S proteasome [107,108].

**Colorectal cancers are associated with defects in the regulation of CTNB1 (β-catenin) stability through mutations in adenomatous polyposis coli**

The tumor suppressor gene adenomatous polyposis coli (APC, not to be confused with the ubiquitin ligase APC/C, anaphase-promoting complex/cyclosome, described above) is frequently mutated in colorectal cancers [109-114]. Many of these mutations truncate APC and/or alter its ability to interact with proteins, which may lead to altered regulation of cellular proliferation.

One major target subjected to regulation through APC is CTNB1, a crucial component of Wnt signaling and cell adhesion [115,116]. The phosphorylation of CTNB1 through APC-associated kinase activity promotes its recognition by the F-box protein β-TRCP [117,118]. Numerous cell-based and in vitro experiments have demonstrated that the F-box motif of β-TRCP interacts with SKP1 and assembles into an SCF complex with CUL1 [63,117-119]. Overexpression of β-TRCP containing an F-box deletion results in an increased stability of CTNB1, as demonstrated in pulse chase experiments [117,119]. Other studies have identified the RING protein SIAH1 (a *Drosophila* seven in absentia homolog) as a P53-inducible and APC-associated ubiquitin ligase that can also regulate the stability of CTNB1 [120,121].

**Mutations in the BRCA1 ubiquitin ligase complex correlate with breast and ovarian cancer**

Germline mutations in the gene encoding the RING protein BRCA1 are associated with the inherited predisposition for breast and ovarian cancer [122-124]. BRCA1 forms a heterodimer with another RING protein known as BARD1 and this complex has E3 activity in vitro [125-128]. These studies, utilizing bacterially expressed proteins and overexpression in mammalian cells, demonstrated that the RING motif of BRCA1 serves as the binding site for the E2 enzyme UB2D3 (UbcH5c) and that BRCA1/BARD1 together have an increased ability to conjugate UBIQ with UB2D3 in vitro than BRCA1 alone. Rachel Klevit’s group determined that several of the cancer-predisposing mutations in BRCA1 result in defective E3 activity in vitro by disrupting BRCA1/BARD1 heterodimer formation or by altering the RING domain structure of BRCA1 [129-131]. Cell-based overexpression experiments demonstrated that the abundance of each protein is dependent upon the presence of its binding partner.

Phosphorylated RBBP8 (CtIP) is a reported substrate for ubiquitylation by BRCA1 [132]. Originally identified in a yeast two-hybrid screen for proteins that bind to the BRCA1 C-terminus (BRCT) domain of BRCA1 and confirmed through in vitro binding experiments, RBBP8 interacts with BRCA1 during G2 of the cell cycle [133-135]. Cell-based and in vitro ubiquitylation assays have demonstrated that BRCA1 ubiquitylates phosphorylated RBBP8 [132]. Rather than promoting RBBP8 degradation by the proteasome, UBIQ modification may cause RBBP8 to associate with chromatin following DNA damage to regulate the G2/M transition of the cell cycle, as determined by cellular localization studies in response to DNA damage [132,136]. BRCA1 could also be associated with the DNA repair activities of the Fanconi anemia (FA) pathway (see next section), as both physical and functional
interactions between BRCA1 and FA complex proteins in response to DNA damage have been described [137].

The Fanconi anemia pathway involves a ubiquitin ligase complex and is associated with increased cancer susceptibility

As described in the [138], studies on the rare autosomal recessive genetic disorder known as Fanconi anemia (FA) have identified a pathway crucial for the cellular response to DNA damage [139-151]. Alterations in this pathway promote increased susceptibility to cancer and have been associated with a wide variety of tumor types, even in non-FA patients [152-166]. Upon DNA damage, two proteins in this pathway are mono-ubiquitylated; FANCD2 (FANCD2) and FANCI [137,141], and recruited to chromatin within nuclear foci. These nuclear foci contain other DNA repair proteins, suggesting that they are sites of DNA damage [137,147,167-169].

The role and exact molecular mechanisms underlying the regulation of FANCD2 and FANCI mono-ubiquitylation are unclear; however, a protein complex (the FA core complex) contains a subunit known as FANCL that contains a RING motif and likely confers their modification [140]. Work from the D'Andrea and Dutta laboratories identified a ubiquitin-conjugating enzyme, UBE2T, that binds directly to FANCL in a yeast two-hybrid screen and through in vitro pull-down experiments with bacterially expressed proteins [170]. siRNA depletion of UBE2T in U2OS cells diminished the mono-ubiquitylation of FANCD2 in response to DNA damage and promoted the formation of abnormal chromosomes [170]. An siRNA screen for DUBs important for removing ubiquitin from FANCD2 implicated UBP1 (USP1) as an enzyme that could attenuate the role of FANCD2 in DNA damage repair [171].

Viruses exploit the ubiquitin system

Viruses utilize clever mechanisms to exploit their host to facilitate their own propagation. Modification of proteins with ubiquitin during infection promotes viral replication and immune response evasion, suggesting potential antiviral strategies.

HIV encodes proteins that hijack cellular cullin-RING ubiquitin ligases

The rapid evolution of viral subtypes resistant to available treatments suggests that there is still significant need for new anti-HIV therapeutics. Two HIV-encoded proteins, VIF and VPU, interact with distinct cullin-RING ubiquitin ligases to hijack their activity and promote the ubiquitylation of cellular proteins.

VIF interacts directly with a cellular cytidine deaminase, ABC3G (APOBEC3G), and facilitates its proteasome-dependent degradation [172,173]. In the absence of VIF, ABC3G is packaged into progeny virion particles, which renders them defective in replication [172,174,175]. Immunoprecipitation of hemagglutinin (HA)-tagged VIF from H9 cells (human T-cell line) infected with engineered HIV, followed by mass spectrometry, demonstrated that VIF associates with CUL5, ELOB (elongin B) and ELOC (elongin C). Western blotting was used to confirm the presence of all proteins including RBX1 [176]. In vitro ubiquitylation of ABC3G purified from transfected cells has been demonstrated using a reconstituted complex of these proteins from baculovirus-infected insect cells [177,178].

The CD4 cell surface receptor found on a subclass of T-cells is downregulated through the activity of VPU [179,180]. As CD4 is a co-receptor for HIV entry into cells, this downregulation could optimize viral replication by blocking further infection, allow for progeny viral particles to be efficiently released, and promote immune response evasion [179]. Co-expression of VPU and CD4 in HeLa cells results in the degradation of CD4, which can be blocked by the proteasome inhibitor MG132 [181]. Work by Margottin et al. originally identified the F-box protein β-TRCP from a yeast two-hybrid screen for VPU-interacting proteins [182]. After demonstrating the formation of the ternary CD4/VPU/β-TRCP complex by overexpressing these proteins in HeLa cells, the authors showed that the F-box motif of β-TRCP is necessary for CD4 degradation in these cells. It was later reported that VPU may block the ubiquitylation of IkBα (IkBα) and CTNB1, which are phosphorylation-dependent cellular substrates of SCFβ-TRCP, and that VPU itself may also be ubiquitylated by this complex [183-186].

Herpesviruses encode ubiquitin ligases and modulate cellular ubiquitin ligases

Herpesviruses often employ strategies to utilize the host cell’s ubiquitin system for their own benefit. The gammaherpesvirus Kaposi’s sarcoma herpesvirus (KSHV, alternatively human herpesvirus types 8, HHV8), which is associated with AIDS-related cancer (see [187]), encodes two ubiquitin ligases; K3 (MIR1) and K5 (MIR2), which downregulate a wide range of immunoreceptors (MHC class I, ICAM1, CD86, CD1D (CD1d)) from the surface of infected cells [188-193]. This mechanism could promote immune system evasion by blocking detection by cytotoxic T-lymphocytes.

The molecular details of how K3 and K5 promote immunoreceptor downregulation through the ubiquitin system are beginning to emerge. Laurent Coscoy’s group recently reported the unexpected observation that transient expression of K3 in human BJAB cells stably expressing the MHC class I allele HLA.B7 can lead to downregulation of this receptor in the absence of cytoplasmic lysine residues if a cysteine residue is present [17]. Paul Lehner’s laboratory used siRNA experiments to identify UBC13 and UB2D2/
UB2D3 (UbchH5b/c) as the E2 enzymes important for K3-dependent downregulation of MHC class I [194]. This study suggests that K3-mediated modification of MHC class I occurs through a sequential mechanism in which Lys63-linked UBIQ chains synthesized by UBC13 are added after initial mono-ubiquitylation by UB2D2/UB2D3, thereby promoting receptor endocytosis and lysosomal targeting.

Herpesviruses also encode proteins that modulate cellular ubiquitin ligase activity, such as LMP1 (latent membrane protein 1) – encoded by EBV. LMP1 is required for EBV latency in B-cells and is sufficient to induce transformation [195,196]. Recent work from Joseph Pagano’s laboratory has uncovered differential effects of LMP1 on the SIAH1 ubiquitin ligase, dependent upon cell type [197-199]. EBV-positive B-cells expressing LMP1 or cells transiently transfected to express LMP1 manifest an upregulation of CTNB1, a component of the Wnt signaling pathway whose increased stability has been associated with cancer (see section on Colorectal cancers are associated with defects in the regulation of CTNB1 (β-catenin) stability through mutations in adenomatous polyposis coli) [197]. This observation was attributed to LMP1-mediated downregulation of SIAH1, a component of a RING ubiquitin ligase complex that regulates CTNB1 stability, at the transcriptional level [120,121]. By contrast, human epithelial cells expressing LMP1 manifest increased SIAH1 protein levels and a resulting decrease in the SIAH1 substrates prolyl hydroxylases 1 and 3 (PHD1, PHD3 [also known as EGLN2 and EGLN3]) [198,200]. These decreases promote the stability of the transcription factor HIF1A as it cannot be hydroxylated, an event required for its association with the VHL-containing ubiquitin ligase (see section on Cullin-RING ubiquitin ligases and the APC/C regulate cellular proliferation).

**Neurodegenerative diseases often have associated impairment of the ubiquitin system**

The formation of protein aggregates containing UBIQ has long been associated with neurodegenerative diseases such as Parkinson’s, Alzheimer’s, Huntington’s, and others. For example, polyglutamine repeat expansion in proteins associated with Huntington’s disease and the spinocerebellar ataxias could promote the formation of protein aggregates that are resistant to degradation by the proteasome and also impair proteasome function (see [201]). Similarly in Alzheimer’s disease, the formation of neurofibrillary tangles and plaques associated with amyloid-β protein aggregation and/or ubiquitylated TAU (tau) accumulation could impair proteasome function (see [202]).

Another example is found with autosomal-recessive juvenile Parkinson’s disease, in which mutations in the ubiquitin ligase PRKN2 (parkin) manifest as defects in its ligase activity in vitro [203-205], suggesting that accumulation of its substrates could contribute to disease development.

**Metabolic diseases such as diabetes could have associated defects in aspects of the ubiquitin system**

The exact relationships between the ubiquitin system and metabolic processes are only beginning to be understood (see [206]). Insulin resistance, associated with diabetes and obesity, manifests as defects in sensing and signaling mechanisms. The ubiquitin system has been associated with insulin signaling through regulating the stability of insulin receptor substrate (IRS) proteins.

IRS proteins serve as adapter molecules, functioning between receptor tyrosine kinases and downstream signaling molecules. IRS2 in particular has a crucial function in controlling the growth and survival of pancreatic β-cells – the body’s source of insulin. IRS2 knockout mice are diabetic and exhibit dramatic reduction in β-cell mass, and decreasing IRS2 expression via siRNA in β-cells promotes apoptosis and decreased cell survival [207]. Thus, signaling through IRS2 has a crucial function in regulating the body’s response to changes in glucose.

IRS2 function is regulated by phosphorylation and UBIQ-mediated degradation by the proteasome [208,209]. In pancreatic β-cells, activation of the kinase FRAP (mTOR), as demonstrated by adenoviral delivery of constitutively active FRAP to rat INS-1 cells, promotes IRS2 phosphorylation and degradation by the proteasome [210]. IRS2 interacts with suppressors of cytokine signaling (SOCS) proteins SOCS1 and SOCS3 in human HEK293 cells, mouse 3T3-L1 adipocytes, and mouse hepatocytes [210]. These proteins contain a ‘SOCS box’ motif, which promotes their interaction with ELOC, a component of a cullin-based ubiquitin ligase, which in turn recruits IRS2 for ubiquitylation and targets it for degradation by the proteasome [211]. Future studies aimed at understanding how IRS2 abundance relates to mechanisms of glucose sensing may lead to novel approaches for combating the growing epidemic of diabetes.

**Muscle wasting disorders have increases in ubiquitin system function**

Decreases in skeletal muscle mass associated with aging, cancer, disuse and other physiological circumstances occur through proteolytic mechanisms involving calpain proteases and UBIQ-dependent protein degradation (see [212]).

Experimental evidence suggests that numerous genes of the ubiquitin system are upregulated during muscle atrophy, including those encoding a muscle-specific ubiquitin
inflammation and innate immunity

NFκB activation that could allow for the modulation of this complexity provides distinct levels of regulation of NFκB pathways [28,30]. Also, these signaling mechanisms can non-proteolytic functions in regulating specific signaling of NFκB [118,218-220] and the proteasome-dependent processing inhibitor IKBA for degradation by the proteasome [221,222], are mixed with increased stability of an epithelial Na+ channel. Alterations of CYLD increases the ability of transfected TRAF6 or TRAF7 to activate an NFκB-dependent reporter gene [226].

UBIQ-dependent ion channel stability has implications in cardiovascular diseases

Alterations in ion channel stability have been associated with cystic fibrosis and Liddle’s syndrome. Cystic fibrosis, one of the most common genetic diseases, is characterized by a wide array of recessive mutations in CFTR (cystic fibrosis transmembrane conductance regulator), a Cl- ion channel protein (see [229]). These mutations promote CFTR misfolding and subsequent clearance through protein quality control pathways of the ubiquitin system. Exactly how CFTR downregulation promotes lung disease is unclear. By contrast, Liddle’s syndrome is associated with increased stability of an epithelial Na+ channel (ENaC, see [230]). This autosomal dominant disorder is characterized by mutations in ENaC that block its recognition by the HECT ligase NEDD4 (see [8]), which promotes ENaC accumulation at the cell surface. Alterations of proper ionic balance in the kidney through increases in ENaC may increase blood volume and blood pressure, promoting cardiovascular disease [231,232].

UBIQ is involved in NFκB activation to regulate inflammation and innate immunity

Numerous distinct pathways can promote the activation of NFκB in response to distinct stimuli (such inflammatory cytokines, DNA damaging agents and microbes) and UBIQ has diverse and complex roles in this process. Conventional roles for UBIQ, such as targeting the NFκB inhibitor IKBA for degradation by the proteasome [118,218-220] and the proteasome-dependent processing of NFκB precursor proteins [221,222], are mixed with non-proteolytic functions in regulating specific signaling pathways [28,30]. Also, these signaling mechanisms can be attenuated by specifically associated DUBs [223]. This complexity provides distinct levels of regulation of NFκB activation that could allow for the modulation of this process associated with a wide range of diseases. NFκB activation through TLR2 (receptor activator of NFκB, RANK), for example, has an important role in bone homeostasis and is associated with diseases such as osteoporosis, rheumatoid arthritis and Paget’s disease of bone (see [224]).

The importance of regulating NFκB signaling through distinct pathways is highlighted by the tumors associated with the genetic disorder cylindromatosis [225]. Afflicted individuals contain mutations in a DUB known as CYLD. This enzyme functions downstream of the tumor necrosis factor α receptor and is involved in attenuating NFκB activation by deubiquitylating the signaling molecules TRAF2, TRAF6 and TRAF7 [226-228]. The regulation of TRAF2 signaling by CYLD was identified by screening an RNAi library targeting DUBs for ones that attenuate NFκB activation [227] and through a two-hybrid screen for proteins that interact with the regulatory subunit of the IkB kinase complex that could also bind to TRAF2 [228]. Later work studying signaling through the toll-like receptor 2 (TLR2) performed cell-based experiments to demonstrate that CYLD binds to TRAF6 and TRAF7 and that depletion of CYLD increases the ability of transfected TRAF6 or TRAF7 to activate an NFκB-dependent reporter gene [226].

Lead discovery

Current drug discovery activities focused on the ubiquitin system

The 26S proteasome is the only validated therapeutic target of the ubiquitin system, with a single commercially available drug known as Velcade. Current drug discovery activities related to the ubiquitin system focus on three major areas: (i) expanding indications of proteasome inhibition in therapy, (ii) developing proteasome inhibitors targeting different activities of the proteasome and with improved bio-availability, and (iii) validating the potential of other targets of the ubiquitin system.

Proteasome inhibition is a treatment for cancer

Changes in proteasome function have been implicated in the development of various diseases (see [233]). In 2003, a small molecule proteasome inhibitor known as Velcade
that has been shown to regulate the interaction between the p53 pathway at a range of 1–3 μM, stimulating cell cycle arrest and apoptosis in tumor cells. By contrast, treatment of untransformed cells results in cell cycle arrest, but no apoptosis [239].

Efforts are underway by several other companies to develop inhibitors that target distinct activities of the proteasome. One such company is Proteolix, which has developed PR-171, a synthetic analog of epoxomycin that irreversibly inhibits the chymotryptic site of the proteasome. Treatment of xenograft models over the course of two days demonstrated that PR-171 has a stronger antitumor effect than Velcade [236]. Phase I trials are currently underway for PR-171 to evaluate its role in treating multiple myelomas and non-Hodgkin’s lymphoma. Another proteasome-targeting drug that is currently in Phase I clinical trials is salinoporamide A (NPI-0052), developed by Nereus Pharmaceuticals. Pre-clinical studies have demonstrated that NPI-0052 achieves a higher and more sustained level of proteasome inhibition when compared with Velcade [237]. It is also well tolerated and improves the response of multi-drug treatment of a colon cancer xenograft model [237]. These two drugs highlight the therapeutic potential of proteasome inhibition when treating various cancerous states. Indeed, Cytomics Systems, Eisai, Novartis AG, Bristol-Myers Squibb, Cell Therapeutics, Cephalon and Ergon Pharmaceuticals are all developing proteasome inhibitors for this purpose.

Small molecules can promote P53 (p53) stability

As described in the section Cancer is associated with alterations in UBIQ-dependent regulation, MDM2 is a RING ubiquitin ligase that has a crucial role in regulating P53 stability. The development of inhibitors that disrupt this interaction will play a major role in the regulation of cell cycle progression and potentially the treatment of cancer. One group of small molecules currently in development is the nutlins, by Roche. These represent the first small molecules that can interfere with the ability of MDM2 to mediate P53 ubiquitylation [238]. Nutlins bind to the pocket domain of P53, and inhibit xenograft tumor growth in vivo with no obvious toxicity. They activate the P53 pathway at a range of 1–3 μM, stimulating cell cycle arrest and apoptosis in tumor cells. By contrast, treatment of untransformed cells results in cell cycle arrest, but no apoptosis [239].

RITA [2,5-bis(5-hydroxymethyl-2-thienyl)furan (NSC652287)], isolated from a screen by the National Cancer Institute (NCI) [240,241], is another compound that has been shown to regulate the interaction between MDM2 and P53. RITA also induces apoptosis in human tumor cells, but has little effect on normal cells. It has been proposed to bind the N-terminus of P53; thereby causing a conformational change that prevents MDM2 binding. However, the sensitivity of human fibroblasts to RITA is varied and depends on the expression level of oncogenes such as MYC (C-MYC) [242]. RITA, as with nutlins, has a significant antitumor effect on mice carrying human tumor xenografts, without apparent toxicity [243], and both are in pre-clinical development. The therapeutic potential of these and other molecules targeting this important regulatory mechanism are currently being explored. Nevertheless, they represent an important class of molecules, which block substrate recognition by a ubiquitin ligase through interfering with a protein-protein interaction interface.

Inhibition of E1 may be a viable therapeutic target

If inhibiting the proteasome has therapeutic utility, then perhaps targeting UBIQ (ubiquitin) activation by E1 might also be beneficial. Both Millennium Pharmaceuticals and Rigel Pharmaceuticals have filed patents disclosing their discovery of E1 inhibitors (Millennium patent: WO2006084281 [244], Rigel patent: WO2005037845 [245], see [246]). These small molecule inhibitors of UBIQ activation may also have potential as cancer therapeutics.

Ubiquitin ligases and deubiquitylating enzymes are emerging therapeutic targets

Proteasome inhibition or E1 inhibition appear to be global approaches to controlling protein activities regulated by UBIQ. As a result, efforts are ongoing to selectively target enzymes involved in specific ubiquitylation pathways. Ubiquitin ligases and deubiquitylating enzymes (DUBs) have gained the most attention due to their direct roles in regulating their recognized protein’s stability. However, the complexity of protein ubiquitylation coupled with the absence of catalytic pockets for small molecule binding has made targeting ubiquitin ligases challenging. Nevertheless, Rigel Pharmaceuticals is currently characterizing ubiquitin ligases as potential therapeutic targets. The company has a broad, on-going program to explore the role of specific ubiquitin ligases in oncology, inflammation, virology and metabolism – with Merck and Daiichi as collaborators. Other companies such as Celgene, Amgen and Genentech also have programs exploring ubiquitin ligases for clinical indications. Thus, the flurry of activity surrounding ubiquitin ligases supports their potential therapeutic importance for developing new treatments for a wide variety of conditions.

In contrast to ubiquitin ligases, DUBs have a more simple mechanism of action and a catalytic pocket for targeted binding of small molecules. Hybrigenics recently dis-
closed the discovery of several small molecules that can inhibit UBP7 (USP7) (HBX 41108) and UBP8 (USP8) (HBX 90397, HBX 90659), which may have oncology applications.

New frontiers in drug discovery for the ubiquitin system
Our understanding of the complexities of the ubiquitin system and its involvement in aspects of physiology is still developing and (perhaps justifiably) so are efforts to discover drugs targeting its enzymes. Here, I summarize some of the current challenges and opportunities that could allow for the potential of the enzymes of the ubiquitin system to be realized as therapeutic targets.

How can ubiquitin ligase activity be inhibited?
Ubiquitin ligases may represent the best target class of the ubiquitin system due to their intrinsic specificity for particular protein substrates. The vast majority of ubiquitin ligases are RING-type. These appear to function primarily as scaffolds to position the substrate to be modified in close juxtaposition with the ubiquitin-conjugating enzyme to promote covalent UBIQ (ubiquitin) transfer [247]. Thus, means to modulate ubiquitin ligase activity may necessarily focus on targeting protein-protein interfaces. Whereas the MDM2-P53 (p53) interface can be disrupted by small molecules, is this possible for other E3-substrate interactions? Do different RING motifs (the binding site for the ubiquitin-conjugating enzyme) look different enough to be considered as targets? If so, how can they be targeted? HECT (homologous to E6AP C-terminus) domain ligases, by contrast, have a catalytic function and may undergo conformational changes [248], suggesting that, at least superficially, they could be more amenable.

Can we harness ubiquitin ligases to artificially target proteins for ubiquitylation?
Increased protein stability correlates with several disease phenotypes. For some of these cases, defects in upstream signaling results in impaired substrate targeting to ubiquitin ligases. In other cases, the ubiquitin ligase itself has functional defects. These observations suggest that artificially recruiting a protein to a ubiquitin ligase could be an approach to restoring proper protein homeostasis. Indeed, there are several reports supporting the potential of this approach [249-251]. One such strategy has involved the development of a chimeric protein or 'ProTaC' (proteolysis targeting chimeric molecule), which targets the protein to the SCFβ-TRCP ubiquitin ligase. SCFβ-TRCP then targets the protein for ubiquitylation, followed by proteasomal degradation. ProTaC consists of a SCFβ-TRCP, binding IKBA (IkBa) phosphopeptide linked to a domain that binds the targeted protein [252]. Further development of this technology could have the potential to realize a powerful and specific tool for the treatment of diseases such as cancer.

Are ubiquitin-conjugating enzymes good therapeutic targets?
Whereas there are considerably fewer ubiquitin-conjugating enzymes than ubiquitin ligases, they may function in specific ubiquitylation pathways. As they represent an essential part of the ubiquitylation process, conferred through their enzymatic activity, they may be reasonable targets. However, these enzymes have a very highly conserved enzymatic core and do not possess defined catalytic pockets [253].

What other approaches should be considered?
Recent work identified small molecules known as ubiquitin-conjugating enzymes that bind to Lys48-linked UBIQ chains in vitro and block UBIQ-dependent protein degradation by the proteasome [254]. Randy King’s group at Harvard identified ubistatins in a chemical genetic screen for small molecules that stabilize Cyclin B in Xenopus extracts, and they were subsequently shown to inhibit the degradation of the yeast cyclin-dependent kinase inhibitor SIC1 (Sic1) in a reconstituted in vitro system. NMR and in vitro binding studies determined that these molecules bind specifically to Lys48-linked UBIQ chains. Whilst these molecules are not cell permeable and have unclear therapeutic potential, they do represent a new approach for blocking protein degradation.

Another direction could be to target mechanisms that regulate ubiquitin ligase assembly. The SCF ubiquitin ligase, for example, is comprised of multiple subunits in which substrate recognition is conferred by a variable receptor subunit (F-box proteins) [53]. The assembly of specific SCF complexes appears to be regulated at multiple levels ranging from receptor abundance to post-translational modification of its enzymatic core. As our knowledge of the intricacies of the ubiquitin system continues to grow, it is likely that we will uncover new regulatory mechanisms associated with protein ubiquitylation.

List of abbreviations
APC: adenomatous polyposis coli; APC/C: anaphase promoting complex/cyclosome; BRCT: BRCA1 C-terminus; CFTR: cystic fibrosis transmembrane conductance regulator; CRLs: Cullin-RING ligases; DUB: deubiquitylating enzyme; E1: ubiquitin-activating enzyme; E2: ubiquitin-conjugating enzyme; E3: ubiquitin ligase; ENaC: epithelial Na+ channel; FA: Fanconi anemia; HECT: homologous to E6AP C-terminus; HHV8: human herpesvirus type 8; HIF1α: hypoxia-inducible factor 1α; IRS: insulin receptor substrate; KSHV: Kaposi’s sarcoma herpesvirus; ProTaC: proteolysis targeting chimeric molecule; RANK: receptor
activator of NFκB; SCF: SKP1-CUL1-F-box ubiquitin ligase; UBLs: ubiquitin-like proteins.

Competing interests

The author was employed by a pharmaceutical company with an interest in the ubiquitin system during the writing of this review.

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References

1. Pickart CM, Eddins MJ: Ubiquitin: structures, functions, mechanisms. Biochim Biophys Acta 2004, 1695:55-72.
2. Lorick KL, Tsai Y-C, Yang Y, Weissman AM: RING fingers and relatives: Determination of protein fate. Weinheim, Germany: VCH Verlag; 2005.
3. Semple CA: The comparative proteomics of ubiquitination in mouse. Genome Res 2003, 13:1389-1394.
4. von Arnim AG: A hitchhiker’s guide to the proteasome. Sci STKE 2001, 2001:PE2.
5. Wong BR, Parlati F, Qu K, Demo S, Pray T, Huang J, Payan DG, Bennett MK: Drug discovery in the ubiquitin regulatory pathway. Drug Discov Today 2003, 8:746-754.
6. Sindжал S, Taylor MC, Baker RT: Deubiquitylating enzymes and disease. BMC Biochemistry 2008, 9(Suppl 1):S3.
7. Madsen L, Shulze A, Seeger M, Hartmann-Petersen R: Ubiquitin domain proteins in disease. BMC Biochemistry 2007, 8(Suppl 1):S3.
8. Scheffner M, Staub O: HECT E3s and human disease. BMC Biochemistry 2007, 8(Suppl 1):S6.
9. Hershko A, Heller H, Elias S, Ciechanover A: Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. J Biol Chem 1983, 258:8206-8214.
10. Aviel S, Winberg G, Massucci M, Ciechanover A: Degradation of the Epstein-Barr virus latent membrane protein 1 (LMP1) by the ubiquitin-proteasome pathway. Targeting via ubiquitination of the N-terminal residue. J Biol Chem 2000, 275:23491-23499.
11. Ben-Saadon R, Fajerman I, Ziv T, Hellman U, Schwartz AL, Ciechanover A: The tumor suppressor protein p16(INK4a) and the human papillomavirus oncoprotein-S8 E7 are naturally occurring lysine-less proteins that are degraded by the ubiquitin system. Direct evidence for ubiquitination at the N-terminal residue. J Biol Chem 2004, 279:41414-41421.
12. Breitschopf K, Bengal E, Ziv T, Admon A, Ciechanover A: A novel site for ubiquitination: the N-terminal residue, and non internal lysines of MyoD, is essential for conjugation and degradation of the protein. Embo J 1998, 17:5964-5973.
13. Coulombe P, Rodier G, Bonneil E, Thibault P, Meloche S: N-Terminal ubiquitination of extracellular signal-regulated kinase 3 and p21 directs their degradation by the proteasome. Mol Cell Biol 2004, 24:6140-6150.
14. Reinstein E, Scheffner M, Oren M, Ciechanover A, Schwartz A: Degradation of the E7 human papillomavirus oncoprotein by the ubiquitin-proteasome system: targeting via ubiquitination of the N-terminal residue. Oncogene 2000, 19:5944-5950.
15. Seng T, Sivaprasad U, Zhu W, Park JH, Arias EE, Walter JC, Dutta A: PCNA is a cofactor for Cdt1 degradation by CUL4/DDB1-mediated N-terminal ubiquitination. J Biol Chem 2006, 281:6246-6252.
16. Bloom J, Amador V, Bartolini F, DeMartino G, Pagano M: Proteasome-mediated degradation of p21 via N-terminal ubiquitinylation. Cell 2003, 115:71-82.
17. Cadwell K, Coscosay L: Ubiquitination on nonlysine residues by a viral E3 ubiquitin ligase. Science 2005, 309:127-130.
18. Lucero P, Penalver E, Vela L, Lagunas R: Monoubiquitination is sufficient to signal internalization of the maltose transporter in Saccharomyces cerevisiae. J Bacteriol 2000, 182:241-243.
19. Nakatsu F, Sakuma M, Masu S, Aro H, Yamazaki S, Nakamura N, Saito T, Ohno H: A Di-leucine signal in the ubiquitin moiety. Possible involvement in ubiquitination-mediated endocytosis. J Biol Chem 2000, 275:26213-26219.
20. Robzyk K, Recht J, O’Shea HA: Rad6-dependent ubiquitination of histone H2B in yeast. Science 2003, 297:504-505.
21. Roth AF, Davis NG: Ubiquitination of the PEST-like endocytosis signal of the yeast a-factor receptor. J Biol Chem 2000, 275:8143-8153.
22. Terrell J, Shih S, Dunn R, Hicke L: A function for monoubiquitination in the internalization of a G protein-coupled receptor. Mol Cell 1998, 1:193-202.
23. Chau V, Tobias JW, Bachmair A, Marriott D, Ecker DJ, Gonda DK, Varshavsky A: A mult ubiquitin chain is confined to specific lysine in a targeted short-lived protein. Science 1989, 243:1576-1583.
24. Finley D, Sadis S, Monia BP, Boucher P, Ecker DJ, Crooke ST, Chau V: Inhibition of proteolysis and cell cycle progression in a mult ubiquitin-deficient yeast mutant. Mol Cell Biol 1994, 14:5501-5509.
25. Petskow MD, Deshaies RJ: Context of mult ubiquitin chain attachment influences the rate of Sic1 degradation. Mol Cell 2003, 11:1435-1444.
26. Thrower JS, Hoffman L, Rechsteiner M, Pickart CM: Recognition of the poly ubiquitin proteolytic signal. EMBO J 2000, 19:94-102.
27. Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S: Rad6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 2002, 419:135-141.
28. Kanayama A, Seth RB, Sun L, Es CK, Hong M, Shaito A, Chiu YH, Delagrange L, Chen ZJ: TAB2 and TAB3 activate the NF-kappaB pathway through binding to polyubiquitin chains. Mol Cell 2004, 15:535-548.
29. Spence J, Sadis S, Haas AL, Finley D: A ubiquitin mutant with specific defects in DNA repair and mult ubiquitination. Mol Cell Biol 1995, 15:1265-1277.
30. Deng L, Wang C, Spencer E, Yang L, Braun A, You J, Stough R, Pickarts C, Chen ZJ: Activation of the IkappaB kinase complex by TRAF6 requires a multi ubiquitin conjugating enzyme complex and a unique polyubiquitin chain. Cell 2000, 103:351-361.
31. Ben-Saadon R, Zaroor D, Ziv T, Ciechanover A: The polycybomb protein Ring1B generates stably mixed ubiquitin chains required for its in vitro histone H2A ligase activity. Mol Cell 2006, 24:701-711.
32. Kim HT, Kim KP, Liedias F, Ksiliea SF, Scaglione KM, Skowrya D, Gygyp SP, Goldberg AL: Certain Pairs of Ubiquitin-conjugating Enzymes (E2s) and Ubiquitin-Protein Ligases (E3s) Synthesize Nondegradable Forked Ubiquitin Chains Containing All Possible Isopeptide Linkages. J Biol Chem 2007, 282:17375-17386.
33. Kirkpatrick DS, Hathaway NA, Hanna J, Elssasser S, Rush J, Finley D, King RW, Gygi SP: Quantitative analysis of in vitro ubiquitinated cyclin B1 reveals complex chain topology. Nat Cell Biol 2006, 8:700-710.
34. Ciechanover A, Elias S, Heller H, Ferber S, Herschko A: Characterization of the heat-stable poly peptide of the ATP-dependent proteolytic system from reticulocytes. J Biol Chem 1980, 255:7525-7528.
35. Ciechanover A, Heller H, Elias S, Haas AL, Herschko A: ATP-dependent conjugation of reticulocyte proteins with the poly peptide required for protein degradation. Proc Natl Acad Sci USA 1980, 77:1365-1368.
36. Herschko A, Ciechanover A, Heller H, Haas AL, Rose IA: Proposed role of ATP in protein breakdown: conjugation of protein
with multiple chains of the polypeptide of ATP-dependent proteolysis. Proc Natl Acad Sci USA 1980, 77:1783-1786.

37. Leventhal NP, Chambers KA, Pabo CO. The DNA-binding domain of F-box protein Skp2 defines the minimal ubiquitin ligase module of the anaphase-promoting complex. Mol Biol Cell 1999, 12:3839-3851.

38. Hengstermann A, Linares LK, Ciechanover A, Whitaker NJ, Scheffner M. The APC11 RING-H2 finger mediator of E2-dependent ubiquitination and SCF ubiquitin ligase. Science 2000, 288:185-197.

39. Wang Z, Li B, Bharadwaj R, Zhu H, Ozkan E, Hakala K, Deisenhofer J, Shih Y, Hershko A, Pagano M, Kaelin WG Jr. Human breast cancer. Nature 2001, 411:651-656.

40. Sauter T, Staunton J, Aasland S, Grimmer S, Betensky R, Brinkley B, Chippindale P, Ganshirtzer E, Golub T, Keshaviah A, et al. Malignancies induced by conditional expression of Mdm2 in adult transgenic mice. Science 2000, 288:185-197.

41. Elledge SJ, Pietenpol ET, Chau V, Kaelin WG Jr. The von Hippel-Lindau tumor-suppressor gene product functions as a transcriptional corepressor of c-myc. Nature 1995, 378:185-197.

42. Lehenkari PD, Lees M, Pollard J, Bhattacharya S, Pagano M, Hershko A. The cyclin-selective ubiquitin ligase activity, targets cyclins for ubiquitin-mediated degradation. EMBO J 1997, 16:1519-1530.

43. Li M, Brooks CL, Kon N, Gu W. The cell-cycle regulatory protein Cks1 is required for tumor suppressor p53. Mol Cell 2001, 7:639-650.

44. Duan DR, Pause A, Burgess WH, Aso T, Chen DY, Garrett KP, Conaway RC, Cenci G, Pavletich NP, Pellicer A, Inghirami L, Klausner RD. Inverse relation between levels of p27(Kip1) and of its ubiquitin ligase subunit Skp2 in colorectal carcinoma. Cancer 2001, 97:1745-1751.

45.脫 Motegi H, Inoue T, Ohya S, Sakaguchi K, Sakaguchi K, et al. The cyclin-dependent kinase 5 inhibits the ubiquitin ligase activity of the anaphase-promoting complex. Mol Cell 2001, 8:33-44.

46. 脫 Motegi H, Inoue T, Ohya S, Sakaguchi K, Sakaguchi K, et al. The cyclin-dependent kinase 5 inhibits the ubiquitin ligase activity of the anaphase-promoting complex. Mol Cell 2001, 8:33-44.
ber of the Cdc53 family of proteins. Proc Natl Acad Sci USA 1997, 94:2156-2161.

80. Lonergan KM, Illopoulos O, Ohh M, Kamura T, Conaway RC, Conaway JW, Kaelin WG Jr: Regulation of hypoxia-inducible mRNAs by the von Hippel-Lindau tumor suppressor protein requires binding to complexes containing elongins B/C and Cul2. Mol Cell Biol 1998, 18:732-741.

81. Chen F, Kishida T, Husada T, Glavac D, Dean M, Gnarra JR, Orcutt ML, Duh FM, Glenn G, et al.: Germline mutations in the von Hippel-Lindau disease tumor suppressor gene: correlations with phenotype. Hum Mutat 1995, 9:196-75.

82. Crossev PA, Foster K, Richards FM, Phips ME, Latif F, Cory K, Jones MA, Bentley BR, Kumar R, Lerman LM, et al.: Molecular genetic investigations of the mechanism of tumourigenesis in von Hippel-Lindau disease: analysis of allele loss in VHL tumours. Hum Genet 1994, 93:53-58.

83. Crossev PA, Maher ER, Jones MA, Richards FM, Latif F, Phips ME, MacLellan MJ, Zbar B, Tarsa NA, Ferguson-Smith MA, et al.: Identification of intragenic mutations in the von Hippel-Lindau disease tumour suppressor gene and correlation with disease phenotype. Hum Mol Genet 1994, 3:1303-1308.

84. Eng C, Crossev PA, Mulligan LM, Healey CS, Houghton C, Prowse A, Chew SL, Dahl RJ, O'riordan J, Toledo SP, et al.: Mutations in the RET proto-oncogene and the von Hippel-Lindau disease tumour suppressor gene in sporadic and syndromic phaeochromocytomas. J Med Genet 1995, 32:943-947.

85. Foster K, Crossev PA, Cairns P, Hetherington JW, Richards FM, Jones MA, Bentley BR, Paffa NA, Ferguson-Smith MA, Maher ER. Molecular genetic investigation of sporadic renal cell carcinoma: analysis of allele loss on chromosomes 3p, 11p, 17 and 22. Br J Cancer 1994, 69:230-234.

86. Foster K, Prowse A, Berg A van den, Flemming S, Hultsbeek MM, Crossev PA, Richards FM, Paffa NA, Ferguson-Smith MA, et al.: Somatic mutations of the von Hippel-Lindau disease tumour suppressor gene in non-familial clear cell renal carcinoma. Hum Mol Genet 1994, 3:2169-2173.

87. Gallou C, Joly D, Mejean A, Staroz F, Martin N, Tarle G, Orfanelli M, Bouvier-Fabre S, Staroz D, Chretien Y, et al.: Mutations of the VHL gene in sporadic renal cell carcinoma: of a risk factor for VHL patients to develop an RCC. Hum Mutat 1999, 13:464-475.

88. Maher ER, Webster AR, Richards FM, Green JS, Crossev PA, Payne SJ, Moore AT: Phenotypic expression in von Hippel-Lindau disease: correlations with germline VHL gene mutations. J Med Genet 1996, 33:328-332.

89. Sekido Y, Bader S, Latif F, Gnarra JR, Gazdar AF, Linehan WM, Zbar B, Lerman ML, Minna JD: Molecular analysis of the von Hippel-Lindau disease tumor suppressor gene in human lung cancer cell lines. Oncogene 1994, 9:1599-1604.

90. Stolle C, Glenn G, Zbar B, Humphrey JS, Choyke P, Walther M, Pack S, Hurley K, Andrey C, Klausner R, Linehan WM: Improved detection of germline mutations in the von Hippel-Lindau disease tumor suppressor gene. Hum Mutat 1996, 12:417-423.

91. Zbar B, Kishida T, Chen F, Kaelin WG Jr, Illopoulos O: The von Hippel-Lindau tumor suppressor protein is required for proper assembly of an extracellular fibronectin matrix. Mol Cell 1998, 1:959-968.

92. Wang GL, Jiang BH, Rue EA, Semenza GL: Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 1995, 92:1510-5514.

93. Bruck RK, McKnight SL: A conserved family of prolyl-4-hydroxylases that modify HIF. Science 2001, 294:1337-1340.

94. Hum WC, Wilson MI, Hynes N, Eden T, Pugh CW, Maxwell PH, Ratcliffe PJ, Stuart DJ, Jones YE: Structural basis for the recognition of hydroxyproline in HIF-1 alpha by PYP. Nature 2002, 417:975-978.

95. Ivan M, Kondo K, Yang H, Kim W, Valiani J, Ohh M, Salic A, Asara JM, Jun WS, Kaelin WG Jr: O2-Regulated destruction by proline hydroxylation: implications for O2 sensing. Science 2001, 292:464-468.

96. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegesheim A, Hebestreit HF, Mukherji M, Schofield CJ, et al.: Targeting of trans-α-Val to the von Hippel-Lindau ubiquitlom complex by O2-regulated proly hydroxylation. Science 2001, 292:468-472.

97. Masson N, William C, Maxwell PH, Pugh CW, Ratcliffe PJ: Independent function of two destruction domains in hypoxia-inducible factor achain alpha activated by prolyl hydroxylation. Embo J 2001, 20:5197-5206.

98. Yu F, White SB, Zhao Q, Lee FS: HIF-1α-binding to VHL is regulated by stimulus-sensitive proline hydroxylation. Proc Natl Acad Sci USA 2001, 98:6360-6365.

99. Eison DA, Thurston G, Huang LE, Ginzinger DG, McDonald DM, Johnson RS, Arbein JM: Induction of hypervascularlity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible-factor-1αpha. Genes Dev 2001, 15:2520-2532.

100. Beaudenon S, Hulbregse JM: HPV E6, E6-AP and cervical cancer. BMJ 2008, 3:215-224.

101. Wang J, Sampath A, Hulbregse JM: Genetic linkage analysis of allele loss on chromosomes 3p, 5q, 11p, 17 and 22. Br J Cancer 1994, 70:230-234.

102. Foster K, Prowse A, Berg A van den, Flemming S, Hultsbeek MM, Crossev PA, Richards FM, Paffa NA, Ferguson-Smith MA, et al.: Somatic mutations of the von Hippel-Lindau disease tumour suppressor gene in non-familial clear cell renal carcinoma. Hum Mol Genet 1994, 3:2169-2173.

103. Zbar B, Lerman AO, Louis DN, Gavin BJ, Kley N, Kaelin WG Jr, Illopoulos O: The von Hippel-Lindau tumor suppressor protein is required for proper assembly of an extracellular fibronectin matrix. Mol Cell 1998, 1:959-968.

104. Wang GL, Jiang BH, Rue EA, Semenza GL: Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 1995, 92:1510-5514.
118. Winston JT, Strack P, Beer-Romero P, Chu CY, Elledge SJ, Harper JW: The SCFbeta-TRCP ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IkappaBalpha and beta-catenin and stimulates IkappaBalpha ubiquitination in vitro. Genes Dev 1999, 13:270-283.

119. Latres E, Chiaour DS, Pagano M: The human F box protein beta-TRCP associates with the Cull/Sipc1 complex and regulates the stability of beta-catenin. Oncogene 1999, 18:849-854.

120. Liu J, Stevens J, Rote CA, Yost HJ, Hu Y, Neufeld KL, White RL, Matsunari N: Siah-1 mediates a novel beta-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein. Mol Cell 2001, 7:927-936.

121. Matsuura SI, Redd JC: Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin degradation linked to p53 responses. Mol Cell 2001, 7:915-926.

122. Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC: Linkage of early-onset familial breast cancer to chromosome 17q21. Science 1990, 250:1684-1689.

123. Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigan S, Bennett LM, Haugan-Strano A, Swensen J, Miki Y, et al.: BRCA1 mutations in primary breast and ovarian carcinomas. Science 1994, 266:120-122.

124. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigan S, Liu Q, Cochran C, Bennett LM, Ding W, et al.: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994, 266:66-71.

125. Brzovic PS, Keeffe JR, Nishikawa H, Miyamoto K, Fox D 3rd, Fukuda M, Ohta T, Lai M: Binding and recognition in the assembly of an active BRCA1/BARD1 ubiquitin-ligase complex. Proc Natl Acad Sci USA 2003, 100:5646-5651.

126. Chen A, Kleiman FE, Manley JL, Ouchi T, Pan ZQ: Auto ubiquitination of the BRCA1/BARD1 ubiquitin ligase. J Biol Chem 2002, 277:22001-22007.

127. Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y, Hashizume M, Oka T, Klevit R: The RING heterodimer BRCA1-BARD1 is a BRCA1 ubiquitin ligase inactivated by a breast cancer-derived mutation. Nat Struct Biol 2000, 7:1227-1233.

128. Xia Y, Pao GM, Chen HW, Verma IM, Hunter T: BRCA1 E3 ubiquitin ligase activity through direct interaction of the BRCT domains of BRCA1 with CtIP, a proline-rich DNA-binding protein. J Biol Chem 2000, 275:26636-26640.

129. Meetei AR, Levellie F, van Berkel CG, Roosens MA, Weel L van De, Aleem J, D'Andrea AD, Alon N, Brzovic PS, Meetei AR, et al.: Isolation of a cDNA representing the Fanconi anemia complementation group E gene. Am J Hum Genet 2000, 67:1306-1308.

130. de Winter JP, Leveille F, van Berkel CG, Roosens MA, Weel L van De, Aleem J, Schillinger E, D'Andrea AD, Alon N, Brzovic PS, Meetei AR, et al.: The Fanconi anemia gene FANC E encodes a novel protein with homology to ROM. Nat Genet 2000, 24:15-16.

131. Kleiman FE, Manley JL: BRCA1: a tumor suppressor gene that regulates cell cycle checkpoint control by the BRCA1-CtIP complex. Cancer Res 1999, 59:4139-4146.

132. Brzovic PS, Rajagopal P, Hoyt DW, King MC, Kleit RE: Structure and effects of protein-protein interactions. J Biol Chem 2001, 276:41399-41406.

133. Brzovic PS, Rajagopal P, Hoyt DW, King MC, Kleit RE: Structure and effects of protein-protein interactions. J Biol Chem 2001, 276:41399-41406.

134. Chen A, Kleiman FE, Manley JL, Ouchi T, Pan ZQ: Auto ubiquitination of the BRCA1/BARD1 ubiquitin ligase. J Biol Chem 2002, 277:22001-22007.

135. Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y, Ohtsu T, Ohta T, Klevit R: The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. J Biol Chem 2001, 276:14537-14540.

136. Xia Y, Pao GM, Chen HW, Verma IM, Hunter T: Enhancement of BRCA1 E3 ubiquitin ligase activity through direct interaction with the BARD1 protein. J Biol Chem 2003, 278:5255-5263.

137. Brzovic PS, Meza J, King MC, Kleit RE: The cancer-predisposing mismatch repair gene MSH6 disrupts homologous recombination in the NHR-terminal BRCA1 ring finger domain. J Biol Chem 1999, 273:7797-7799.

138. Brzovic PS, Meza JE, King MC, Kleit RE: BRCA1 RING domain cancer-predisposition mutations. Structural consequences and effects of protein-protein interactions. J Biol Chem 2001, 276:41399-41406.

139. Brzovic PS, Rajagopal P, Hoyt DW, King MC, Kleit RE: Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. Nat Struct Biol 2001, 8:833-837.

140. Yen Y, Fu S, Lai M, Baer R, Chen J: BRCA1 ubiquitinates its phosphorylation-dependent binding partner CtIP. Genes Dev 2002, 16:1721-1726.

141. Wong AK, Ormonde PA, Perez R, Chen Y, Lian L, Salada G, Berry S, Lawrence Q, Dayananth P, Ha P, et al.: Characterization of a carcinoma-specific terminal BRCA1 interacting protein. Oncogene 1998, 17:2279-2285.

142. Yu X, Wu LC, Bowcock AM, Aronheim A, Baer R: The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtIP/Bpatr pathway of transcriptional repression. J Biol Chem 2001, 276:25388-25392.

143. Yu X, Chen J: DNA damage-induced cell cycle checkpoint control requires CtIP, a phosphorylation-dependent binding partner of BRCA1 C-terminal domains. Mol Cell Biol 2004, 24:9167-9177.

144. Bara AK, Brown RS, Birrane G, Ladas IA: Structural basis for cell cycle checkpoint control by the BRCA1-CtIP complex. Biochemistry 2005, 44:10941-10946.

145. Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Heijna J, Grompe M, D'Andrea AD: Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. Mol Cell 2001, 7:249-262.

146. Matsuura SI, Reed JC: Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin degradation linked to p53 responses. Mol Cell 2001, 7:915-926.

147. Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC: Linkage of early-onset familial breast cancer to chromosome 17q21. Science 1990, 250:1684-1689.
Anemia-BRCA pathway in cervical cancer. Cancer Res 2004, 64:2994-2997.

158. Wood AD, Wei M: FANCF methylation contributes to chemoresistance in ovarian cancer. Cancer Cell 2003, 3:171-200.

159. Rogers CD, Heijden MS van der, Brune K, Yeoh CJ, Hruban RH, Kern SE, Goggins M: The genetics of FANCC and FANCG in familial pancreatic cancer. Cancer Biother 2004, 3:167-169.

160. Taniguchi T, Tischkowitz M, Ameziane N, Hodgson SV, Mathew CG, Joenje H, Mok SC, D’Andrea AD: Disruption of the Fanconi anemia-BRCA pathway in cisplatin-sensitive ovarian tumors. Nat Med 2003, 9:568-574.

161. Koscheiwitzi M, Ameziane N, Waissfisz Q, de Winter JP, Harris R, Taniguchi T, Tischkowitz M, Ameziane N, Hodgson SV, Mathew CG, Joenje H: Bilallelic silencing of the Fanconi anemia gene FANCF in sporadic myeloid leukaemia. Br J Haematol 2003, 123:469-471.

162. Tischkowitz M, Ameziane N, Waissfisz Q, de Winter JP, Harris R, Taniguchi T, Tischkowitz M, Ameziane N, Hodgson SV, Mathew CG, Joenje H: Bilallelic silencing of the Fanconi anemia gene FANCA in sporadic myeloid leukemia. Leukemia 2004, 18:420-423.

163. Heijden MS van der, Brody JR, Gallmeier E, Cunningham SC, Dezentje DA, Shen D, Hruban RH, Kern SE: Functional defects in the fand sl gene pathway in pancreatic cancer cells. Am J Pathol 2004, 165:651-657.

164. Heijden MS Van Der, Brody JR, Kern SE: Functional screen of the fanconi anemia pathway in cancer cells by Fancd2 immunoblot. Cancer Biother 2004, 3:534-537.

165. Taniguchi T, van der Heijden MS, Shen D, Hruban RH, Kern SE: Fanconi anemia gene mutations in young-onset pancreatic cancer. Cancer Res 2003, 63:2585-2588.

166. Wang Z, Li M, Lu S, Zhang Y, Yang H: Promoter hypermethylation of FANCF plays an important role in the occurrence of ovarian cancer through disruption of Fanconi anemia-BRCA pathway. Cancer Biother 2006, 5:256-260.

167. Hussain S, Wilson JB, Medhurst AL, Hejna J, Witt E, Ananth S, Davies A, Masson JF, Moses R, West SC, et al.: Direct interaction of FANCDO with BRCA2 in DNA damage response pathways. Hum Mol Genet 2004, 13:1241-1248.

168. Nakashiki K, Taniguchi T, Ranganathan V, New HV, Moreau LA, Storksky M, Mathew CG, Kastan MB, Weaver DT, D’Andrea AD: Interaction of FANCDO and NBS1 in the DNA damage response pathway. Nat Cell Biol 2002, 4:913-920.

169. Wang X, Andreaens PR, D’Andrea AD: Functional interaction of monoubiquitinated FANCDO and BRCA2/FANCDO in chromatin. Mol Cell Biol 2004, 24:5850-5862.

170. Machida YJ, Machida Y, Chen Y, Gurtan AM, Kupfer GM, D’Andrea AD: UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. Mol Cell Biol 2006, 23:589-596.

171. Nijman SM, Huang TT, Dirac AM, Brummelkamp TR, Kerkhoven RM, D’Andrea AD, Bernards R: The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. Mol Cell 2005, 17:331-339.

172. Sheehy AM, Gaddis NC, Malim MH: The antiretroviral enzyme Elongin B-Elongin C complex is essential for Vif function. J Biol Chem 2005, 280:18573-18578.

173. Shirakawa K, Takao-Kondo A, Kobayashi M, Tomonaga M, Izumi T, Fukunaga K, Sasada A, Abudu A, Miyasuchi Y, Akira H, et al.: Ubiquitination of APOBEC3 proteins by the Vif-Cullin5-ElonginB-ElonginC complex. Virology 2006, 344:263-266.

174. Willey RL, Maldarelli F, Martin MA, Strebkel: Human immunodeficiency virus type 1 Vpu protein induces rapid degradation of CD4. J Virol 1992, 66:7193-7200.

175. Willey RL, Maldarelli F, Martin MA, Strebkel: Human immunodeficiency virus type 1 Vpu protein regulates the formation of intracellular gp160-CD4 complexes. J Virol 1992, 66:222-234.

176. Schubert U, Anton LC, Back C, Cox JH, Bour S, Bennink JR, Orfowski M, Strebkel K, Yewdell JW: CD4 glycoprotein degradation induced by human immunodeficiency virus type 1 Vpu protein requires the function of proteasomes and the ubiquitination-conjugating pathway. J Virol 1998, 72:2280-2288.

177. Margetts F, Bour SP, Durand H, Selig L, Benichou S, Richard V, Thomas D, Strebkel K, Benarous R: A novel human WD protein, beta-TrCP, that interacts with HIV-1 Vpu connects CD4 to the ER degradation pathway through an F-box motif. Mol Cell 2004, 15:653-574.

178. Akari H, Bour S, Kao S, Adachi A, Strebkel K: The human immunodeficiency virus type 1 accessory protein Vpu induces apoptosis by suppressing the nuclear factor kappaB-dependent expression of antiapoptotic factors. J Exp Med 2001, 193:299-311.

179. Belaidouni N, Marchal C, Benarous R, Bernard-Guerin C: Involvement of the betaTrCP in the ubiquitination and stability of the HIV-1 Vpu protein. Biochem Biophys Res Commun 2007, 357:688-693.

180. Bernard-Guerin C, Belaidouni N, Lassot I, Segere J, Jobart A, Marchal C, Benarous R: HIV-1 Vpu sequesters beta-transducin repeat-containing protein (betaTrCP) in the cytoplasm and provokes the accumulation of beta-catenin and other SCFbetaTrCP substrates. J Biol Chem 2004, 279:788-793.

181. Boon S, Perrin C, Akari H, Strebel K: The human immunodeficiency virus type 1 Vpu protein inhibits NF-kappaB activation by interfering with betaTrCP-mediated degradation of IkappaB. J Biol Chem 2001, 276:15920-15928.

182. Shackelford J, Pagano JS: Role of the ubiquitin system and tumor viruses in AIDS-related cancer. BMC Biochemistry 2007, 8(Suppl 1):S8.

183. Coscos L, Ganem D: Kaposi's sarcoma-associated herpesvirus encodes two proteins that block cell surface display of MHC class I chains by enhancing their endocytosis. Proc Natl Acad Sci USA 2000, 97:8051-8056.

184. Coscos L, Sanchez DJ, Ganem D: A novel class of herpesvirus-encoded membrane-bound 3 ubiquitin ligases regulates endocytosis of proteins involved in immune recognition. J Virol 2002, 76:6126-6137.

185. Hewitz EW, Duncan L, Mufo D, Baker J, Stevenson PG, Lehrer PJ: Ubiquitilation of MHC class I by the K3 viral protein signals internalization and TSG101-dependent degradation. J Virol 2002, 76:6126-6137.

186. Ishido S, Choi JK, Lee BS, Wang C, DeMaria M, Johnson RP, Cohen GB, Jung JU: Inhibition of natural killer cell-mediated cytotoxicity by Kaposi's sarcoma-associated herpesvirus K5 protein. Immunity 2000, 13:365-374.

187. Ishido S, Wang C, Lee BS, Cohen GB, Jung JU: Downregulation of major histocompatibility complex class I molecules by Kaposi's sarcoma-associated herpesvirus K3 and K5 proteins. J Virol 2000, 74:5300-5309.

188. Sanchez DJ, Coscos L, Ganem D: Functional organization of MIR2, a novel viral regulator of selective endocytosis. J Biol Chem 2002, 277:12646-12671.

189. Duncan LM, Piper S, Dodd RB, Saville MK, Sanderson CM, Lazio JP, Lehrer PJ: Lysine-63-linked ubiquitination is required for endolysosomal degradation of class I molecules. J Biol Chem 2006, 281:1635-1645.

190. Baichwal VR, Sugden AH: Ubiquitination of proteins by the K3 viral protein. Nature 1988, 335:819-822.

191. Kung AW, Kung AW, Kung AW, Kung AW: Functional organization of MIR2, a novel viral regulator of selective endocytosis. J Biol Chem 2002, 277:12646-12671.

192. Baichwal VR, Sugden AH: Ubiquitination of proteins by the K3 viral protein. Nature 1988, 335:819-822.

193. Baichwal VR, Sugden AH: Ubiquitination of proteins by the K3 viral protein. Nature 1988, 335:819-822.

194. Baichwal VR, Sugden AH: Ubiquitination of proteins by the K3 viral protein. Nature 1988, 335:819-822.

195. Baichwal VR, Sugden AH: Ubiquitination of proteins by the K3 viral protein. Nature 1988, 335:819-822.
198. Kondo S, Seo SY, Yoshizaki T, Wakasaka N, Furukawa M, Joab I, Jang KL, Pagano JS: EBV latent membrane protein 1 up-regulates hypoxia-inducible factor 1 alpha through Siah1-mediated down-regulation of prolyl-hydroxylases 1 and 2 in nasopharyngeal epithelial cells. *Cancer Res* 2006, 66:9870-9877.

199. Shackelford J, Maier C, Pagano JS: Epstein-Barr virus activates beta-catemin in type III latently infected B lymphocyte lines: association with desubiquitinating enzymes. *Proc Natl Acad Sci USA* 2003, 100:15572-15576.

200. Nakayama K, Frew IJ, Hagensen M, Skals M, Babelh H, Bhoomik A, Kadoya T, Erdjument-Bromage H, Tempst P, Frappell PB, et al.: Siah1 regulates stability of prolyl-hydroxylases, controls HIF1 alpha abundance, and modulates physiological responses to hypoxia. *Cell* 2004, 117:941-952.

201. Davies JE, Sarkar S, Rubinstein DC: The ubiquitin proteasome system in Huntington's disease and the spinocerebellar ataxias. *BMC Biochemistry* 2007, 8(Suppl 1):S2.

202. Panteleyev AA, Grzeschik KH, Kadowitz PJ, Vollmar AM, Krieglstein K: Muscle-specific ring finger proteins as potential regulators of some degradation pathway. *Proc Natl Acad Sci USA* 2005, 102:20312-20317.

203. Imai Y, Soda M, Takahashi R: Centner T, Yano J, Kimura E, McElhinny AS, Pelin K, Witt CC, Bang SH, Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ, Pavletich NP: Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science* 1996, 274:948-953.

204. Alkalay I, Yaron A, Hatzubai A, Orian A, Ciechanover A, Ben-Neriah Y: Stimulation-dependent I kappa B alpha phosphorylation marks the NF-kappa B inhibitor for degradation via the ubiquitin-proteasome pathway. *Proc Natl Acad Sci USA* 1992, 99:10599-10603.

205. Spencer E, Jiang J, Chen ZJ: Signal-induced ubiquitination of IkappaBalpha by the F-box protein Slimb/beta-TRCP. *Genes Dev* 1999, 13:284-294.

206. Yaron A, Hatzubai A, Davis M, Lavon I, Amit S, Manning AM, Andersen JS, Mann M, Mercurio F, Ben-Neriah Y: Identification of the receptor component of the IkappaBalpha-ubiquitin ligase. *Nature* 1998, 396:590-594.

207. Seftonbiek U, Cao Y, Xiao G, Greten FR, Krahn G, Bonizzi G, Chen Y, Hu Y, Fong A, Sun SC, Karin M: Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. *Science* 2001, 293:1495-1499.

208. Xiao G, Harhay EW, Sun SC: NF-kappaB-inducing kinase regulation in the processing of NF-kappaB2 p100. *Mol Cell* 2001, 7:401-409.

209. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, Wu P, Wiesmann C, Baker R, Boone DL, et al.: De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signaling. *Nature* 2000, 406:1141-1146.

210. Layfield R, Shaw B: Ubiquitin-mediated signaling and Paget's disease of bone. *BMC Biochemistry* 2007, 8(Suppl 1):S5.

211. Bignell GR, Warren W, Seal S, Takahashi M, Rasley E, Barfoot R, Green H, Brown C, Biggs P, Lakhani SR, et al.: Identification of the familial cylindromatosis tumour-suppressor gene. *Nat Genet* 2000, 25:160-165.

212. Yoshida H, Jono H, Kai H, Li JD: The tumor suppressor cylindromatosis (CYLD) acts as a negative regulator for toll-like receptor 2 signaling via negative cross-talk with TRAF6 and TNF-alpha. *J Biol Chem* 2005, 280:66566-66566.

213. Nary N, Dao JT, Nishizuka Y, Yamada K, Yoneda Y, Tsuchida N: Another possible target of PR-171, a novel irreversible inhibitor of the proteasome. *Antitumor activity in multiple myeloma*.

214. Brummelkamp TR, Nijman SM, Dirac AM, Bernards R: Identification of beta-catemin in type III latently infected B lymphocyte lines: some degradation pathway. *Proc Natl Acad Sci USA* 2005, 102:20312-20317.

215. Panteleyev AA, Grzeschik KH, Kadowitz PJ, Vollmar AM, Krieglstein K: Muscle-specific ring finger proteins as potential regulators of some degradation pathway. *Proc Natl Acad Sci USA* 2005, 102:20312-20317.

216. Yamada K, Yoneda Y, Tsuchida N: Identification of beta-catemin in type III latently infected B lymphocyte lines: some degradation pathway. *Proc Natl Acad Sci USA* 2005, 102:20312-20317.

217. Yamada K, Yoneda Y, Tsuchida N: Identification of beta-catemin in type III latently infected B lymphocyte lines: some degradation pathway. *Proc Natl Acad Sci USA* 2005, 102:20312-20317.
240. Lane DP, Crawford LV: T antigen is bound to a host protein in SV40-transformed cells. Nature 1979, 278:261-263.

241. Gata SA, Wismuller L: p53 in recombination and repair. Cell Death Differ 2006, 13:1003-1016.

242. Issaeva N, Bozko P, Enge M, Protopopova M, Verhoeof LG, Masucci M, Pramanik A, Selivanova G: Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. Nat Med 2004, 10:1321-1328.

243. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N, Liu EA: In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 2004, 303:844-848.

244. Critchley S, Gant TG, Langston SP, Olhava EJ, Peluso S, [Millennium Pharmaceuticals Inc.]: Inhibitors of E1 activating enzymes. WO2006084281.

245. Parlati F, Ramesh UV, Singh R, Payan DG, Lowe R, Look GC, [Rigel Pharmaceuticals Inc.]: Benzothiazole and Thiazole 5,5-B!Pyridine compositions and their use as Ubiquitin ligase Inhibitors. WO2005037845.

246. Gudeat P, Colland F: Patented small molecule inhibitors in the ubiquitin proteasome system. BMC Biochemistry 2007, 8(Suppl 1):S14.

247. Passmore LA, Barford D: Getting into position: the catalytic mechanisms of protein ubiquitylation. Biochem J 2004, 379:S13-S25.

248. Verdecia MA, Joaizeiro CA, Wells NJ, Ferrer JL, Bowman ME, Hunter T, Noel JP: Conformational flexibility underlies ubiquitin ligation mediated by the WWF1 HECT domain E3 ligase. Mol Cell 2003, 11:249-259.

249. Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies Rj: Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. Proc Natl Acad Sci USA 2001, 98:8354-8359.

250. Sakamoto KM, Kim KB, Verma R, Ransick A, Stein B, Crews CM, Deshaies Rj: Development of Protacs to target cancer-promoting proteins for ubiquitination and degradation. Mol Cell Proteomics 2003, 2:1350-1358.

251. Schneekloth J Jr, Fonseca PN, Koldobskiy M, Mandal A, Deshaies R, Sakamoto K, Crews CM: Chemical genetic control of protein levels: selective in vivo targeted degradation. J Am Chem Soc 2004, 126:3748-3754.

252. Sakamoto KM, Kim KB, Verma R, Ransick A, Stein B, Crews CM, Deshaies Rj: Development of Protacs to target cancer-promoting proteins for ubiquitination and degradation. Mol Cell Proteomics 2003, 2:1350-8.

253. Winn PJ, Religa TL, Battey JN, Banerjee A, Wade RC: Determinants of functionality in the ubiquitin conjugating enzyme family. Structure 2004, 12:1563-1574.

254. Verma R, Peters NR, D’Onofrio M, Tochtop G, Sakamoto KM, Varadan R, Zhang M, Coffino P, Fushman D, Deshaies Rj, King RW: Ubistatins inhibit proteasome-dependent degradation by binding the ubiquitin chain. Science 2004, 306:117-120.

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