Published OnlineFirst April 22, 2014; DOI: 10.1158/1078-0432.CCR-13-3175

Clinical Cancer Research

Molecular Pathways: Turning Proteasomal Protein Degradation into a Unique Treatment Approach

Sebastian Stintzing and Heinz-Josef Lenz

Abstract

Cancer treatment regimens have evolved from single cytotoxic substances affecting all proliferative tissues toward antibodies and kinase inhibitors targeting tumor-specific pathways. Treatment efficacy and cancer survival have improved overall, and side effects have become less frequent. The ubiquitin-proteasome system–mediated proteasomal protein degradation is the most critical pathway to regulate the quantity of signal proteins involved in carcinogenesis and tumor progression. These processes are, as well as protein recycling, highly regulated and offer targets for biomarker and drug development. Unspecific proteasome inhibitors such as bortezomib and carfilzomib have shown clinical efficacy and are approved for clinical use. Inhibitors of more substrate-specific enzymes of degradation processes are being developed and are now in early clinical trials. The novel compounds focus on the degradation of key regulatory proteins such as p53, $p27^{Kip1}$, and β-catenin, and inhibitors specific for growth factor receptor kinase turnover are in preclinical testing. *Clin Cancer Res; 20(12); 3064–70. ©2014 AACR.*

Background

Ubiquitin-proteasome system

The closely regulated ubiquitin-proteasome system (UPS) clears the cell plasma from damaged, misfolded, and aged proteins. More than 80% of intracellular proteins are processed by the UPS (1), and the remaining proteins are handled by the lysosome system. UPS is also involved in the inactivation of regulatory proteins by initiating the post-translational addition of multiple ubiquitin motifs, which sorts intracellular proteins for degradation.

Ubiquitin is a small and highly conserved protein of 76 amino acids. Polyubiquitination is facilitated by isopeptide bonds between the last amino acid of ubiquitin (glycine) and one lysine (K) of another ubiquitin that functions as the substrate. Ubiquitin has seven lysine positions (K6, K11, K27, K29, K33, K48, and K63) with K48 and K63 being the most common positions where polyubiquitination occurs. The position of polyubiquitination determines whether a protein will be degraded (K48-linked) or will be activated (K63-linked; ref. 2). Little is known about ubiquitination at the other lysine positions.

Ubiquitin-like proteins

More than 20 ubiquitin-like proteins, such as NEDD8 (neural precursor cell expressed, developmentally down-regulated 8), SUMO (small ubiquitin-related modifier), and ISG15 (IFN-induced 17-kDa protein), have been described that play important roles in posttranslational protein modification (3).

NEDD8 most importantly is modifying the ubiquitin-dependent degradation process by interacting with cullin-like E3 ligases (4). It activates cullin E3 ligases, leading to a higher rate of polyubiquitination, and therefore drives the degradation of proteins that are turned over by cullin E3 ligases (5).

SUMO, like ubiquitin, facilitates lysine amino acids within the substrate to bind to other proteins. SUMOylation therefore competes with ubiquitylation and can inhibit ubiquitin-dependent proteolysis (6). It has been described in neurodegenerative disorders such as Alzheimer and Parkinson disease (7). SUMO modification of multiple substrates supports their physical interaction (SUMO glue) and thereby stimulates complex formation. This complex formation plays an important role in DNA repair mechanisms, ribosomal biogenesis, and genome maintenance (8) and links SUMOylation to multiple diseases such as melanoma and renal cell carcinoma and to cell stemness, making it an interesting field for drug development (9).

ISG15 also modifies proteins by a lysine–glycin isopeptide bond and is involved in the inflammatory response to IFN-1. Its role in alternating lethality to virus infection has been investigated to a wide extent (10). As virus replication is for the most part not affected and function differs between different viruses and host species, many questions remain unaddressed. It is known that ISG15 targets newly translated virus and host proteins under the influence of IFN-1 (11) and therefore that it is involved in the modulation of immune response to viral infections.

Authors' Affiliation: USC/Norris Comprehensive Cancer Center, Keck School of Medicine, Sharon Carpenter Laboratory, Los Angeles, California

Corresponding Author: Heinz-Josef Lenz, Norris Comprehensive Cancer Center, University of Southern California, 1441 Eastlake Avenue, Los Angeles, CA 90033. Phone: 323-865-3967; Fax: 323-865-0061; E-mail: lenz@usc.edu

doi: 10.1158/1078-0432.CCR-13-3175

Published OnlineFirst April 22, 2014; DOI: 10.1158/1078-0432.CCR-13-3175

Ubiquitin activation (E1)

The UPS can be separated into four different processes: (i) ubiquitin activation by E1 enzymes, (ii) ubiquitin conjugation by E2 enzymes, (iii) ubiquitin ligation by E3 enzymes, and (iv) the proteolysis of the substrate in a 26S-proteasome (12). Deubiquitinases (DUB) can reverse this process by dissociating ubiquitin from the substrate and enable protein recycling.

Ubiquitin activation is ATP dependent and is achieved by one of the two known E1 human enzymes. UBE1, the principal ubiquitin activating protein in eukaryotes, and the recently described UBE1L2, add an energy-rich thioester bond to the C-terminal end of ubiquitin. Inhibitors of E1 enzymes are designed to interfere with this thioester bond.

Ubiquitin-conjugating enzymes (E2)

Ubiquitin-conjugating enzymes (E2) are capable of transferring the activated ubiquitin onto a ubiquitin ligase (E3) enzyme–substrate complex. About 50 E2 enzymes have been identified. The central functional motif is a ubiquitin-conjugating catalytic (UBC) fold. The UBC exhibits a catalytic cysteine residue that, together with the thioester bond of the activated ubiquitin, forms a high-energetic conjugate. E2 enzymes define the position of ubiquitination (e.g., K48 vs. K63) and consequently determine the further destiny of the protein substrate (12). Characterized by the extensions to the UBC, four different classes of E2 enzymes have been defined: class I, no extension; class II, N-terminal extension; class III, C-terminal extension; and class IV, extension on both ends (13).

Ubiquitin ligases (E3)

E3 ligase enzymes are highly substrate specific with more than a thousand enzymes estimated (14). The principal function of E3 ligases is to recruit specific proteins (substrates) and to interact with E2 enzymes to catalyze the covalent binding of ubiquitin. Three major classes of E3 enzymes have been defined according to the structure of the catalytic domain (15): (i) HECT (homologous to the E6AP carboxyl terminus), (ii) U-Box, and (iii) RING (really interesting new gene) E3s. An important subgroup of RING E3s is cullin RING ligases (CRL), which ubiquitinate proteins with key roles in cell-cycle progression and signal transduction. The functional motif of many CRL is the SCF (Skp-Cullin-F-box containing) complex, making all three components interesting targets for drug development. F-box is a structural motif of about 50 amino acids that mediates protein–protein interactions (16). Skp (seventeen-kDa protein) is a trimeric periplasmic chaperone that assists outer membrane proteins in their folding and insertion into membranes. Cullins are a family of proteins scaffolding the E3 ligase activity that are regulated by neddylation (4). The addition of NEDD8 to CRLs drives the turnover of multiple regulatory proteins toward degradation such as growth factor receptor proteins (5).

26S proteasome

Degradation within the UPS is processed by the 26S proteasome, which consists of a 20S core particle and two regulatory 19S regulatory caps (17). Polyubiquitinated proteins are broken down by proteolysis. In cancer cell lines, augmented proteasome activity is a common phenomenon, including degradation of proteins involved in tumor progression, apoptosis, and cell-cycle regulation.

Clinical–Translational Advances

Novel inhibitors of the UPS have been developed targeting key proteins of the major circuits of carcinogenesis, as defined by Hanahan and Weinberg (ref. 18; Fig. 1). Most of the compounds (Table 1) are still in early clinical development (phase I) and therefore are under examination for toxicity and tolerability. As single substance efficacy is not anticipated, studies testing drugs in further development (phase Ib/II) use combinations of standard-of-care chemotherapeutic substances such as antimetabolites (e.g., cytarabine, 5-fluorouracil) or mitotic inhibitors (e.g., paclitaxel) depending on the underlying disease.

Cell-cycle regulation

Accelerated degradation of regulatory cell-cycle proteins causing lower intracellular expression of tumor suppressors such as p53 has been demonstrated in a variety of neoplasias. E3 ligases reducing intracellular p53 levels are associated with carcinogenesis and prognosis. Examples are E6AP ubiquitin-protein ligase (E6-AP), which is activated by human papillomavirus (HPV; ref. 13) and leads to HPV-associated carcinomas, and RING finger and CHY zinc finger domain-containing protein 1 (aka Pirh2), which is overexpressed in hepatocellular carcinoma (HCC), head and neck cancers, lung cancer, and prostate cancer and correlates with poor overall survival in HCC (19).

Given the central role of p53 in DNA damage repair and cell-cycle regulation, the turnover of p53 has been extensively studied and several potential regulatory proteins of p53 degradation have been identified. Higher levels of Hdm2, which is the most important E3 ligase for p53, were identified in leukemias, lymphomas, and solid tumors (12), making it a valuable target for drug development. Substances interacting with the Hdm2-p53–binding site have been shown to increase p53 levels in p53 nonmutant cell lines and consequently led to cell-cycle arrest and apoptosis (20). Among those, nutlins were the first class discovered and two members (RO5045337 and RO5503781) made it to phase I trials, but results have not been reported. Recently, clinical data on serdemetan (JNJ-26854165), a tryptamine compound belonging to the second class of Hdm2 antagonists, have been reported (21). Serdemetan has been shown to increase p53 levels radio-sensitizing tumors in xenograft models (22). It was well tolerated in a phase I trial. Spiro-oxindoles are the third class of small inhibiting molecules interacting with Hdm2 (23), with MI-773 (SAR405838) currently being tested in two clinical trials (NCT01636479 and NCT01985191). Other compounds interacting with Hdm2 are being developed and are in early

Published OnlineFirst April 22, 2014; DOI: 10.1158/1078-0432.CCR-13-3175

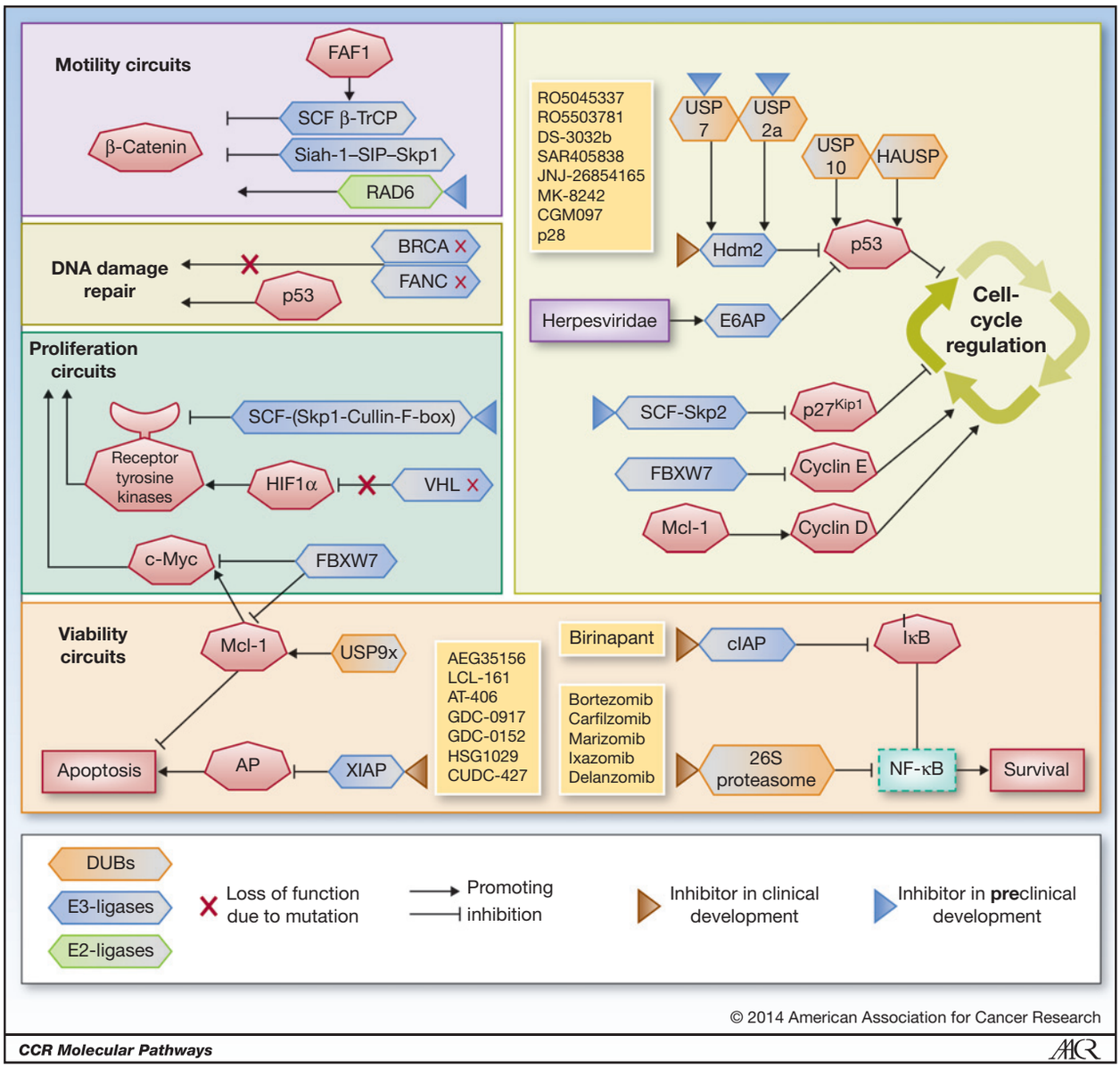

Figure 1. Inhibitors of the UPS-targeting proteins involved in the intracellular circuits of carcinogenesis. AP, apoptosis protein; BRCA1, breast cancer 1, early onset; c-Myc, Myc proto-oncogene protein; FANC, Fanconi anemia (FA) pathway; FBXW7, F-box and WD repeat domain containing 7; Hdm2, human double minute 2 homolog; HIF1α, hypoxia-inducible factor-1α; Mcl-1, induced myeloid leukemia cell differentiation protein; $p27_{Kip1}$, cyclin-dependent kinase inhibitor 1B; p53, tumor suppressor p53; XIAP, X-linked inhibitor of apoptosis protein.

clinical testing (see Table 1). Their specific mechanisms of action have not been reported.

Targeting DUBs play an important role in the regulation of p53 levels. Both the DUB HAUSP (herpesvirus-associated ubiquitin-specific protease) and USP10 (ubiquitin-specific protease 10), targeting polyubiquitinated p53, have been shown to restore p53 levels even when Hdm2 is overexpressed (24, 25).

It has also been shown that inhibition USP7 and USP2a, which are deubiquitinases of Hdm2, increases Hdm2 proteolysis and stabilizes p53 levels in multiple myeloma cells (26, 27), enabling the initiation of apoptosis. The inhibition of USP7 with HBX 41,108, a DUB to Hdm2, has been shown to induce p53-dependent apoptosis with an IC_{50} in submicromolar concentrations. The same principle was demonstrated with other compounds such as P5091 (26) and P22077 (28) in preclinical models; however, no deubiquitinase inhibitor has entered clinical development.

Other potential targets in cell-cycle regulation, such as cyclin E, C-Myc, and $p27^{Kip1}$, are known to be degraded by F-box containing SCF E3 ligases.

Mutations leading to a loss of function in the F-box FBXW7 have been identified in solid and hematologic malignancies (29), leading to increased proliferation. The

Published OnlineFirst April 22, 2014; DOI: 10.1158/1078-0432.CCR-13-3175

Table 1. Modulators of the UPS in clinical development for cancer treatment

Target structure name	Clinical stage	Remarks
Proteasome inhibitors		
Bortezomib	Approved for MM and MCL	Multiple clinical trials testing bortezomib in combination with chemotherapeutic substances in MM and MCL.
Carfilzomib	Approved for MM after two prior therapies	Tested in a phase I study of advanced malignancies (including mCRC) with hepatic impairment (NCT01949545) and in combination with irinotecan in a phase I/II study of irinotecan-sensitive advanced malignancies (including mCRC; NCT01941316)
Oprozomib ONX 0912	Phase I	Oral proteasome inhibitor under evaluation for the treatment of MM and lymphomas and in a phase I study of advanced malignancies (including mCRC; NCT01129349)
Marizomib NPI-0052	Phase I/II	Tested in a phase I study for advanced solid malignancies (including mCRC; NCT00629473)
Delanzomib CEP-18770	Phase I	Tested in a phase I study for advanced solid malignancies (including mCRC) or non-Hodgkin lymphoma (NCT00572637)
Ixazomib MLN9708	Phase I/II	Tested in a phase I study for advanced solid malignancies (including mCRC; NCT00830869)
Nedd8 activating enzyme		
NAE MLN4924	Phase I	Currently tested in AML (NCT01814826), large B-cell lymphoma (NCT01415765) and advanced malignancies (NCT00677170) in combination with chemotherapeutic drugs
E3 ligases interacting Hdm2–p53		
RO5045337	Phase I	Nutlin derivate tested in multiple phase I trials (NCT00559533), results not reported yet
RO5503781	Phase I	Nutlin derivate currently being tested in AML (NCT01773408), advanced malignancies except leukemia (NCT01462175)
DS-3032b	Phase I	Tested in a phase I study for advanced solid malignancies or lymphomas (NCT01877382)
SAR405838	Phase I	Spiro-oxindole tested in phase I studies for advanced malignancies (NCT01636479) and in combination with pimasertib (NCT01985191)
JNJ-26854165	Phase I	Successful phase I study with good tolerability and modest efficacy (21)
MK-8242	Phase I	Tested in phase I studies for advanced solid malignancies (NCT01463696) and in combination with cytarabin in AML (NCT01451437)
CGM097	Phase I	Tested in a phase I study for advanced solid malignancies (NCT01760525)
p28	Phase I	Tested in pediatric patients with recurrent or progressive central nervous system tumors (NCT01975116)
E3 ligases interacting with apoptotic proteins		
Birinapant TL32711	Phase II	Testing the efficacy for ovarian, primary peritoneal or fallopian tube cancer (NCT01681368)
AEG35156	Phase I/II	Preclinical studies showed activity but data of clinical phase I/II studies were disappointing
LCL161	Phase I/II	Tested in solid tumors (NCT01098838), in combination with paclitaxel in solid tumors (NCT01240655), and in breast cancer (NCT01617668)
AT-406 (Debio 1143)	Phase I	Encouraging data in mouse xenotransplant models of human ovarian cancer (56), tested in phase I design (NCT01078649)
GDC-0917	Phase I	(NCT01226277)
GDC-0152	Phase I	Tested in solid tumors (NCT00977067)
HSG1029	Phase I	Tested in solid tumors (NCT00708006) and lymphoid malignancies (NCT01013818)
CUDC-427	Phase I	Tested in solid tumors and lymphomas (NCT01908413)

NOTE: The authors make no claim for completeness of clinical trials listed in this table.

Abbreviations: mCRC, metastatic colorectal cancer; MM, multiple myeloma; NCT, National clinical trial number.

Published OnlineFirst April 22, 2014; DOI: 10.1158/1078-0432.CCR-13-3175

reported frequency of FBXW7 loss of function mutations in T-cell acute lymphoblastic leukemia (T-ALL) is approximately 20% (30), but these mutations are also frequently found in cholangiocarcinoma (~35%), colorectal (~10%), ovarian, and endometrial cell lines (29, 30). Modulating FBXW7 degradation is promising for novel drug development in a variety of tumors (15).

Degradation of $p27^{Kip1}$ is mediated by a specific SCF Skp2, which plays a role in cellular senescence, cancer progression, and metastasis (31). No specific Skp2 inhibitor has been reported in clinical development.

Viability pathways

The downregulation of apoptosis and upregulation of survival pathways are characteristic for cancer development and progression. Inhibitors of apoptosis proteins (IAP) are E3 ligases regulating caspase activity that is required for apoptosis. In a number of hematologic diseases, including acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, and lymphomas, IAPs are overexpressed (32), making them a promising target for drug development.

A detailed review of IAP inhibitors in cancer has been recently published (33) and multiple IAP inhibitors are currently being tested in phase I and II trials (Table 1). AEG 35156 is the only substance with clinical outcome data. Unfortunately, due to a lack of efficacy shown with this agent in two phase II trials (34, 35), further development was terminated.

NF-κβ is a transcriptional factor involved in inflammatory processes, cell proliferation, and cell survival. Active NF-κβ is released after degradation of inhibitor of κβ (Iκβ). Canonical release through TNF-α signaling and noncanonical activation through NF-κβ inducing kinase are both dependent on cellular IAPs (cIAP; ref. 33). Birinapant is an antagonist inhibiting cIAP1 and cIAP2 and was shown to restore TNF-α–dependent apoptosis in breast and melanoma cancer cells (36, 37). Birinapant is currently being tested in phase II trials in ovarian, fallopian tube, and primary peritoneal cancer.

Another target for novel drug development interacting with viability circuits is USP9x, which deubiquitinases the induced myeloid leukemia cell differentiation protein Mcl-1. USP9x is overexpressed in lymphomas, chronic myelogenous leukemia, and multiple myeloma, where higher Mcl-1 levels cause a block in apoptosis (38). In preclinical models, the USP9x inhibitor WP1130 has been shown to increase proapoptotic proteins and to decrease antiapoptotic proteins (39) and therefore increase tumor cell sensitivity to multiple chemotherapeutic agents (40). Further clinical testing will depend on whether promising data are generated in preclinical models.

Regulation of cell motility

A key regulatory protein that is commonly associated with a large number of cancers and hematologic tumors is β-catenin. Depending on its intracellular localization, this protein has distinct functions in cell proliferation and cell motility. Usually bound to cytoskeleton proteins, cell plasma levels are critical for the function as a transcription factor. The F-Box E3 ligase SCF β-TrCP (41) and Siah-1–SIP–Skp1 (42) degrade cell plasma β-catenin, reducing wnt-β-catenin signaling. FAS-associated factor 1 (FAF1) has been shown to increase β-catenin degradation by activating the β-TrCP F-Box complex (43). K63 ubiquitination of β-catenin with the help of the E2 ligase RAD6 (ubiquitin-conjugating enzyme E2 B) increases cell plasma β-catenin. K63 polyubiquitinated β-catenin is not available for K48 ubiquitination (44) but is functionally active. RAD6 has been shown to be overexpressed in breast cancer (45), and inhibitors are in preclinical development (46).

Growth factor–dependent pathways

Growth factor pathway–dependent carcinogenesis, proliferation, and metastasis are activated through extracellular ligands, transmembrane receptors, or by activating mutations within the intracellular part of the pathways. The amount of receptors and ligands expressed on the cell surface is dependent on recycling and degradation processes.

Solid tumors are in need of growth factors to migrate, proliferate, and create their own vessel system through angiogenesis. These signaling pathways are often regulated through growth factor receptor kinases such as ErbB family members or members of the VEGF receptor family. After ligand binding, the growth factor receptor is internalized, the tyrosine activity is shut down, and the protein is degraded with the help of c-Cbl, an SCF (Skp1-Cullin-F-box) ligase that is activated by neddylation (47, 48). Only inhibitors of neddylation (ML4232) are in clinical development (4).

The von Hippel–Lindau (VHL) syndrome is caused by a mutation in the E3-ligease (VHL), reducing degradation of hypoxia-inducible factor-1α (HIF1α) and leading to increased signaling of proangiogenic cytokines (49), which further supports the key role of degradation in the regulation of growth factor signaling.

DNA repair mechanisms

There are no inhibitors of the UPS-associated DNA repair mechanisms available; however, alterations in the BRCA and FANC E3 ligases are a good example for the clinical significance of degradation for diagnosis, prognosis, prevention, and treatment strategies. Reduced DNA repair mechanisms lead to accumulation of DNA damage, promoting carcinogenesis. One of the best known hereditary cancer syndromes is caused by mutations within the *BRCA1* gene, which is an E3 ligase involved in DNA repair, emphasizing the importance of alterations in the UPS in carcinogenesis and tumor progression. Loss-of-function mutations in FANC E3 ligases that are involved in the ubiquitination of the FANCI-FANCD2, which is also associated with DNA repair, cause Fanconi anemia (50) and are associated with childhood T-ALL and testicular seminoma (51).

Published OnlineFirst April 22, 2014; DOI: 10.1158/1078-0432.CCR-13-3175

Inhibitors of neddylation and the 26S proteasome

Inhibition of the 26S proteasome. Inhibition of the 26S proteasome causes an accumulation of intracellular proteins that leads to an inhibition of NF-κβ activity and angiogenesis, alters degradation of cell cycle and apoptotic proteins, and effects endoplasmatic reticulum stress (1, 52). The reversible inhibitor, bortezomib (53), and the irreversible inhibitor, carfilzomib (54), have been approved for treatment of multiple myeloma. Bortezomib is also approved for the clinical use of mantle cell lymphoma (MCL) after disease progression after one prior therapy and is currently being tested in clinical trials in combination with chemotherapeutic agents in AML and ALL. More potent and less toxic proteasome inhibitors (Table 1) are under evaluation in clinical trials for patients with multiple myeloma and advanced solid tumors.

Inhibiting ubiquitin and NEDD8-activating enzymes. Inhibitors of E1 enzymes have been identified using high-throughput screening for substances targeting p53 and p27. PYR-41 was the first ubiquitin-activating enzyme (UAE) inhibitor to be tested in preclinical models and was able to inhibit p53 degradation and downregulate cytokine-induced NF-κβ signaling (12). In addition to p53 levels, the structurally related substance PYZD-4409 was able to stabilize cyclin D3 levels and to induce cell death by induction of endoplasmatic reticulum stress (55). The UAE inhibitors have shown activity in preclinical models, but no clinical trials are under way, so far. Many E3 ligases are cullin-RING-ubiquitin ligases, which are activated by neddylation. Neddylation accelerates K48-linked polyubiquitination of multiple regulatory proteins such as p53, p21, $p27^{Kip1}$, growth factor receptor tyrosine kinases, and apoptosis proteins. One inhibitor of NEDD8 activation (MLN4924) is in early clinical development.

Conclusions

Degradation plays a key regulatory role in all major cell circuits representing the hallmarks of cancer. The challenge to translate these novel compounds successfully into the clinic is to identify the tumor tissue-specific degradation processes to personalize therapy with specific inhibitors.

Targeting the UPS for cancer treatment is a unique approach that has been proved to be effective, as the proteasome inhibitors bortezomib and carfilzomib are established in the treatment of multiple myeloma and MCL. Inhibitors of specific UPS enzymes of key regulatory proteins of carcinogenesis and tumor progression such as apoptosis proteins and p53 are currently being tested in phase I and phase II trials in a variety of cancers, including leukemia and solid tumors.

Increased molecular understanding of the regulation of protein degradation in cancer and liquid tumors will be essential for development of more specific and more effective and less toxic compounds. Identification of tumor-specific UPS enzymes in cancers and liquid tumors will be critical for selection of patients who will benefit the most from specific UPS inhibitors.

Disclosure of Potential Conflicts of Interest

S. Stintzing reports receiving speakers bureau honoraria from Amgen, Merck KgaA, Roche/Genentech, and Sanofi and is a consultant/advisory board member for Bristol-Myers Squibb, Merck KgaA, and Roche/Genentech. H.-J. Lenz reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Bristol-Myers Squibb and Merck.

Authors' Contributions

Conception and design: S. Stintzing, H.-J. Lenz
Development of methodology: S. Stintzing, H.-J. Lenz
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.-J. Lenz
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Stintzing, H.-J. Lenz
Writing, review, and/or revision of the manuscript: S. Stintzing, H.-J. Lenz
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.-J. Lenz
Study supervision: H.-J. Lenz

Grant Support

H.-J. Lenz is supported by the NIH (P30CA014089) and the Nancy Bernstein Research Fund. S. Stintzing is supported by a postdoctoral fellowship from the German Cancer Aid (Mildred-Scheel Foundation).

Received January 9, 2014; revised March 24, 2014; accepted April 2, 2014; published OnlineFirst April 22, 2014.

References

1. Crawford LJ, Irvine AE. Targeting the ubiquitin proteasome system in haematological malignancies. Blood Rev 2013;27:297–304.
2. Nathan JA, Kim HT, Ting L, Gygi SP, Goldberg AL. Why do cellular proteins linked to K63-polyubiquitin chains not associate with proteasomes? EMBO J 2013;32:552–65.
3. Hochstrasser M. Origin and function of ubiquitin-like proteins. Nature 2009;458:422–9.
4. Micel LN, Tentler JJ, Smith PG, Eckhardt GS. Role of ubiquitin ligases and the proteasome in oncogenesis: novel targets for anticancer therapies. J Clin Oncol 2013;31:1231–8.
5. Kirisits A, Pils D, Krainer M. Epidermal growth factor receptor degradation: an alternative view of oncogenic pathways. Int J Biochem Cell Biol 2007;39:2173–82.
6. Desterro JM, Rodriguez MS, Hay RT. SUMO-1 modification of IkappaBalpha inhibits NF-kappaB activation. Mol Cell 1998;2:233–9.
7. Krumova P, Weishaupt JH. Sumoylation in neurodegenerative diseases. Cell Mol Life Sci 2013;70:2123–38.
8. Jentsch S, Psakhye I. Control of nuclear activities by substrate-selective and protein-group SUMOylation. Annu Rev Genet 2013;47:167–86.
9. Yang XJ, Chiang CM. Sumoylation in gene regulation, human disease, and therapeutic action. F1000Prime Rep 2013;5:45.
10. Morales DJ, Lenschow DJ. The antiviral activities of ISG15. J Mol Biol 2013;425:4995–5008.
11. Durfee LA, Lyon N, Seo K, Huibregtse JM. The ISG15 conjugation system broadly targets newly synthesized proteins: implications for the antiviral function of ISG15. Mol Cell 2010;38:722–32.
12. Shen M, Schmitt S, Buac D, Dou QP. Targeting the ubiquitin-proteasome system for cancer therapy. Expert Opin Ther Targets 2013;17:1091–108.

Published OnlineFirst April 22, 2014; DOI: 10.1158/1078-0432.CCR-13-3175

13. van Wijk SJ, Timmers HT. The family of ubiquitin-conjugating enzymes (E2s): deciding between life and death of proteins. FASEB J 2010; 24:981–93.
14. Li W, Bengtson MH, Ulbrich A, Matsuda A, Reddy VA, Orth A, et al. Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. PLoS ONE 2008;3:e1487.
15. Lau AW, Fukushima H, Wei W. The Fbw7 and betaTRCP E3 ubiquitin ligases and their roles in tumorigenesis. Front Biosci (Landmark Ed) 2012;17:2197–212.
16. Bai C, Sen P, Hofmann K, Ma L, Goebl M, Harper JW, et al. SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. Cell 1996;86:263–74.
17. Gallastegui N, Groll M. The 26S proteasome: assembly and function of a destructive machine. Trends Biochem Sci 2010;35:634–42.
18. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
19. Halaby MJ, Hakem R, Hakem A. Pirh2: an E3 ligase with central roles in the regulation of cell cycle, DNA damage response, and differentiation. Cell Cycle 2013;12:2733–7.
20. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 2004;303:844–8.
21. Tabernero J, Dirix L, Schoffski P, Cervantes A, Lopez-Martin JA, Capdevila J, et al. A phase I first-in-human pharmacokinetic and pharmacodynamic study of serdemetan in patients with advanced solid tumors. Clin Cancer Res 2011;17:6313–21.
22. Chargari C, Leteur C, Angevin E, Bashir T, Schoentjes B, Arts J, et al. Preclinical assessment of JNJ-26854165 (Serdemetan), a novel tryptamine compound with radiosensitizing activity in vitro and in tumor xenografts. Cancer Lett 2011;312:209–18.
23. Mohammad RM, Wu J, Azmi AS, Aboukameel A, Sosin A, Wu S, et al. An MDM2 antagonist (MI-319) restores p53 functions and increases the life span of orally treated follicular lymphoma bearing animals. Mol Cancer 2009;8:115.
24. Li M, Chen D, Shiloh A, Luo J, Nikolaev AY, Qin J, et al. Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. Nature 2002;416:648–53.
25. Yuan J, Luo K, Zhang L, Cheville JC, Lou Z. USP10 regulates p53 localization and stability by deubiquitinating p53. Cell 2010;140: 384–96.
26. Chauhan D, Tian Z, Nicholson B, Kumar KG, Zhou B, Carrasco R, et al. A small molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance. Cancer Cell 2012;22:345–58.
27. Stevenson LF, Sparks A, Allende-Vega N, Xirodimas DP, Lane DP, Saville MK. The deubiquitinating enzyme USP2a regulates the p53 pathway by targeting Mdm2. EMBO J 2007;26:976–86.
28. Dar A, Shibata E, Dutta A. Deubiquitination of Tip60 by USP7 determines the activity of the p53-dependent apoptotic pathway. Mol Cell Biol 2013;33:3309–20.
29. Song JH, Schnittke N, Zaat A, Walsh CS, Miller CW. FBXW7 mutation in adult T-cell and B-cell acute lymphocytic leukemias. Leuk Res 2008;32:1751–5.
30. Akhoondi S, Sun D, von der Lehr N, Apostolidou S, Klotz K, Maljukova A, et al. FBXW7/hCDC4 is a general tumor suppressor in human cancer. Cancer Res 2007;67:9006–12.
31. Wang G, Chan CH, Gao Y, Lin HK. Novel roles of Skp2 E3 ligase in cellular senescence, cancer progression, and metastasis. Chin J Cancer 2012;31:169–77.
32. Fulda S. Inhibitor of apoptosis proteins in hematological malignancies. Leukemia 2009;23:467–76.
33. Fulda S. Molecular pathways: targeting inhibitor of apoptosis proteins in cancer–from molecular mechanism to therapeutic application. Clin Cancer Res 2014;20:289–95.
34. Schimmer AD, Herr W, Hanel M, Borthakur G, Frankel A, Horst HA, et al. Addition of AEG35156 XIAP antisense oligonucleotide in reinduction chemotherapy does not improve remission rates in patients with primary refractory acute myeloid leukemia in a randomized phase II study. Clin Lymphoma Myeloma Leuk 2011;11:433–8.
35. Mahadevan D, Chalasani P, Rensvold D, Kurtin S, Pretzinger C, Jolivet J, et al. Phase I trial of AEG35156 an antisense oligonucleotide to XIAP plus gemcitabine in patients with metastatic pancreatic ductal adenocarcinoma. Am J Clin Oncol 2013;36:239–43.
36. Allensworth JL, Sauer SJ, Lyerly HK, Morse MA, Devi GR. Smac mimetic Birinapant induces apoptosis and enhances TRAIL potency in inflammatory breast cancer cells in an IAP-dependent and TNF-alpha-independent mechanism. Breast Cancer Res Treat 2013;137:359–71.
37. Krepler C, Chunduru SK, Halloran MB, He X, Xiao M, Vultur A, et al. The novel SMAC mimetic birinapant exhibits potent activity against human melanoma cells. Clin Cancer Res 2013;19:1784–94.
38. Schwickart M, Huang X, Lill JR, Liu J, Ferrando R, French DM, et al. Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. Nature 2010;463:103–7.
39. Kapuria V, Peterson LF, Fang D, Bornmann WG, Talpaz M, Donato NJ. Deubiquitinase inhibition by small-molecule WP1130 triggers aggresome formation and tumor cell apoptosis. Cancer Res 2010;70: 9265–76.
40. Peddaboina C, Jupiter D, Fletcher S, Yap JL, Rai A, Tobin RP, et al. The downregulation of Mcl-1 via USP9X inhibition sensitizes solid tumors to Bcl-xl inhibition. BMC Cancer 2012;12:541.
41. Liu C, Kato Y, Zhang Z, Do VM, Yankner BA, He X. beta-Trcp couples beta-catenin phosphorylation-degradation and regulates Xenopus axis formation. Proc Natl Acad Sci U S A 1999;96:6273–8.
42. Liu J, Stevens J, Rote CA, Yost HJ, Hu Y, Neufeld KL, et al. Siah-1 mediates a novel beta-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein. Mol Cell 2001;7:927–36.
43. Zhang L, Zhou F, Li Y, Drabsch Y, Zhang J, van Dam H, et al. Fas-associated factor 1 is a scaffold protein that promotes beta-transducin repeat-containing protein (beta-TrCP)-mediated beta-catenin ubiquitination and degradation. J Biol Chem 2012;287:30701–10.
44. Gerard B, Sanders MA, Visscher DW, Tait L, Shekhar MP. Lysine 394 is a novel Rad6B-induced ubiquitination site on beta-catenin. Biochim Biophys Acta 2012;1823:1686–96.
45. Shekhar MP, Tait L, Gerard B. Essential role of T-cell factor/beta-catenin in regulation of Rad6B: a potential mechanism for Rad6B overexpression in breast cancer cells. Mol Cancer Res 2006;4:729–45.
46. Sanders MA, Brahemi G, Nangia-Makker P, Balan V, Morelli M, Kothayer H, et al. Novel inhibitors of Rad6 ubiquitin conjugating enzyme: design, synthesis, identification, and functional characterization. Mol Cancer Ther 2013;12:373–83.
47. Chitalia V, Shivanna S, Martorell J, Meyer R, Edelman E, Rahimi N. c-Cbl, a ubiquitin E3 ligase that targets active beta-catenin: a novel layer of Wnt signaling regulation. J Biol Chem 2013;288:23505–17.
48. Lui TT, Lacroix C, Ahmed SM, Goldenberg SJ, Leach CA, Daulat AM, et al. The ubiquitin-specific protease USP34 regulates axin stability and Wnt/beta-catenin signaling. Mol Cell Biol 2011;31:2053–65.
49. Lonser RR, Glenn GM, Walther M, Chew EY, Libutti SK, Linehan WM, et al. von Hippel-Lindau disease. Lancet 2003;361:2059–67.
50. Garner E, Smogorzewska A. Ubiquitylation and the Fanconi anemia pathway. FEBS Lett 2011;585:2853–60.
51. Smetsers S, Muter J, Bristow C, Patel L, Chandler K, Bonney D, et al. Heterozygote FANCD2 mutations associated with childhood T Cell ALL and testicular seminoma. Fam Cancer 2012;11:661–5.
52. Paramore A, Frantz S. Bortezomib. Nat Rev Drug Discov 2003;2:611–2.
53. Bonvini P, Zorzi E, Basso G, Rosolen A. Bortezomib-mediated 26S proteasome inhibition causes cell-cycle arrest and induces apoptosis in CD-30+ anaplastic large cell lymphoma. Leukemia 2007; 21:838–42.
54. Hajek R, Bryce R, Ro S, Klencke B, Ludwig H. Design and rationale of FOCUS (PX-171-011): a randomized, open-label, phase 3 study of carfilzomib versus best supportive care regimen in patients with relapsed and refractory multiple myeloma (R/R MM). BMC Cancer 2012;12:415.
55. Xu GW, Ali M, Wood TE, Wong D, Maclean N, Wang X, et al. The ubiquitin-activating enzyme E1 as a therapeutic target for the treatment of leukemia and multiple myeloma. Blood 2010;115:2251–9.
56. Brunckhorst MK, Lerner D, Wang S, Yu Q. AT-406, an orally active antagonist of multiple inhibitor of apoptosis proteins, inhibits progression of human ovarian cancer. Cancer Biol Ther 2012;13:804–11.

Published OnlineFirst April 22, 2014; DOI: 10.1158/1078-0432.CCR-13-3175

AACR American Association for Cancer Research

Clinical Cancer Research

Molecular Pathways: Turning Proteasomal Protein Degradation into a Unique Treatment Approach

Sebastian Stintzing and Heinz-Josef Lenz

Clin Cancer Res 2014;20:3064-3070. Published OnlineFirst April 22, 2014.