

Emerging Potential of Therapeutic Targeting of Ubiquitin-Specific Proteases in the Treatment of Cancer

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Abstract

The ubiquitin–proteasome system (UPS) has emerged as a therapeutic focus and target for the treatment of cancer. The most clinically successful UPS-active agents (bortezomib and lenalidomide) are limited in application to hematologic malignancies, with only marginal efficacy in solid tumors. Inhibition of specific ubiquitin E3 ligases has also emerged as a valid therapeutic strategy, and many targets are currently being investigated. Another emerging and promising approach in regulation of the UPS involves targeting deubiquitinases (DUB). The DUBs comprise a relatively small group of proteins, most with cysteine protease activity that target several key proteins involved in regulation of tumorigenesis, apoptosis, senescence, and autophagy. Through their multiple contacts with ubiquitinated protein substrates involved in these pathways, DUBs provide an untapped means of modulating many important regulatory proteins that support oncogenic transformation and progression. Ubiquitin-specific proteases (USP) are one class of DUBs that have drawn special attention as cancer targets, as many are differentially expressed or activated in tumors or their microenvironment, making them ideal candidates for drug development. This review attempts to summarize the USPs implicated in different cancers, the current status of USP inhibitor-mediated pharmacologic intervention, and future prospects for USP inhibitors to treat diverse cancers. *Cancer Res*; 74(18); 4955–66. ©2014 AACR.

Introduction

Most of the current molecular cancer therapies target protein kinases and mediate their antitumor activity through the deactivation of an aberrantly expressed, tumor-dependent, or unregulated target enzyme. Clinically successful kinase-targeting examples include Herceptin (Her2), imatinib (Bcr-Abl and c-Kit), vemurafenib (BRAF), and ibrutinib (BTK; ref. 1). However, kinase inhibition has restricted application and limited long-standing efficacy, as intrinsic resistance reduces benefit to only a fraction of patients and most responders will acquire resistance due to additional mutations or activation of compensating pathways (1). A deeper understanding of the molecular pathways associated with cancer has implicated posttranslational regulation through ubiquitination/deubiquitination of many target proteins as an additional and attractive targeted therapeutic approach.

Ubiquitin Proteasome System-Targeted Anticancer Therapeutics

Covalent attachment of ubiquitin, a 76–amino acid protein, to a target protein is a means of regulating protein half-life, localization, and activity. Because protein homeostasis is essential for the survival of all cells, but more essential to cancer cells, modulation of individual ubiquitin–proteasome system (UPS) components may present an opportunity for therapeutic targeting. As proof-of-principle, many compounds with proteasome inhibitory activity have been developed, including bortezomib (or Velcade; Millenium Pharmaceuticals), which is a synthetic dipeptide boronic acid that reversibly inhibits the chymotryptic-like activity of the 20S enzymatic core of the proteasome and induces apoptosis in several malignancies. Velcade is approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with mantle cell lymphoma and multiple myeloma, even those resistant to doxorubicin, mitoxantrone, melphalan, and dexamethasone, and is commonly used in combination with many of these agents (2). Amplified protein synthesis (immunoglobulin) in many myeloma cells may underlie their clinical sensitivity to bortezomib and other proteasome inhibitors as most solid tumors do not have a similar commitment to elevated protein synthesis and are not clinically responsive to these drugs. This narrow therapeutic application, combined with some toxicity (sensory neuropathy), may be circumvented by novel proteasome inhibitory molecules (2).

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Deubiquitinases as Emerging Targets for Anticancer therapeutics

Targeted inhibition of ubiquitin-conjugating enzymes and ligases could provide another therapeutic avenue. Inhibition of NEDD8-associated E1 enzyme by MLN4924, E2 enzyme hCdc34 by CC0651, and E3 ligase MDM2 by RITA (NSC652287) and MI-219 reflects this ongoing effort (3). Deubiquitinases (DUB) are another class of emerging anticancer target that regulate specific substrate proteins by reversing their ubiquitination through the hydrolysis of isopeptide or α -peptide bonds linking ubiquitin to the target protein (4). If the target protein is an oncogene, the DUB activity of the associated DUB may stabilize its cellular expression and supports identification of DUB inhibitors that could reestablish normal protein turnover, location, or activity (4). This approach may also avoid the deleterious side effects associated with direct targeting of the proteasome. With around 55 members, ubiquitin-specific proteases (USP) comprise the largest and most diverse family of DUBs. Genetic and/or functional analysis have placed USPs in the category of cancer-associated proteases, and their unique biochemical structures have made them desirable targets for anticancer therapies. More than 30 USPs have been associated with cancer directly or indirectly. These numbers are not surprising, considering the various critical cellular functions regulated by different USPs and the diversity of substrates used and regulated by them. A comprehensive list of USPs altered in different cancers is provided in Table 1.

Role of Ubiquitin Specific Proteases in Cancer

USP-associated mutations and gene fusions in hematologic malignancies

Recurrent mutations of USPs are rare in cancer with the exception of *CYLD*. Germline mutations of the tumor-suppressor gene *CYLD* are prevalent in familial cylindromatosis, a genetic condition that leads to predisposition for developing multiple skin tumors (5). The only known chromosomal translocation involving a USP is the fusion of the promoter of *CDH11* to the full-length *USP6* gene that leads to upregulated transcript levels of *USP6* (6). USP gene fusions are reported for *USP42* and *USP16* that are fusion gene partners with *RUNX*, which has high-frequency gene alterations in hematologic diseases such as chronic myelomonocytic leukemia and acute myeloid leukemia (AML; refs. 7, 8). *USP18* transcript levels are upregulated in mice expressing the AML fusion protein *AML1-ETO* (9). *USP33/VDU1* mRNA levels are overexpressed in B-cell acute lymphoblastic leukemias (10). Increased mRNA levels of *USP50* have also been reported in AML (11). Upregulated transcript levels of *USP9x* have been correlated with poor prognosis in multiple myeloma and are associated with *Mcl-1* accumulation in patients with follicular lymphoma (12). Thus, USPs are repeatedly implicated in many hematologic malignancies.

USPs as important regulators of p53 signaling and DNA damage response

An important aspect of hematopoietic tumors is that p53 mutations are rare; however, p53 levels are tightly regulated by ubiquitination/deubiquitination (13). Thus, modulation of p53

levels in these tumors can provide a therapeutic advantage by inducing p53-dependent apoptosis and cell-cycle arrest. A key molecule involved in p53 ubiquitination is the E3 ligase MDM2, which is a negative regulator of p53 stability (13). Although *USP7* and *USP2a* have been reported to deubiquitinate MDM2, leading to degradation of p53 and antiapoptotic activity, *USP7* and *USP10* can directly deubiquitinate p53 under specific conditions (14, 15). Under normal conditions, *USP7* stabilizes MDM2 and leads to degradation of p53, whereas *USP10* deubiquitinates and stabilizes p53. In response to DNA damage and following ATM kinase activation, the substrate preference of *USP7* switches from MDM2 to p53, leading to its stabilization (14, 16). Similarly, in response to DNA damage, *USP10* is stabilized in an ATM kinase-dependent manner and a portion of it translocates to the nucleus where it joins *USP7* in deubiquitinating and stabilizing p53. Other USPs known to regulate p53 levels in the cell include *USP4* and *USP5* (17, 18). *USP4* reduces p53 levels in the cell through direct binding and deubiquitination of the E3 ligase *HUWE1*, also called *MULE* or *ARF-BP1* (17). *USP5* depletion in the cell leads to accumulation of unanchored polyubiquitin chains, competing with p53 for proteasomal destruction, which results in nuclear accumulation of p53 and transcriptional activation of p53 target genes (18). Very recently, *USP15* was shown to target MDM2 with effects on the stability of both p53 and the T-cell transcription factor, *NFATc*. Thus, *USP15* inhibition could result in direct tumor cell apoptosis and increased T-cell responsiveness (19).

Because ubiquitination/deubiquitination is also an important mechanism essential for regulation of the DNA-damage repair (DDR), a large number of USPs are implicated in regulating or coordinating DDR. *USP3*, *USP7*, *USP10*, *USP11*, *USP16*, *USP21*, and *USP22* are reported to regulate DDR sensor proteins. *USP1*, *USP2*, *USP4*, *USP7*, *USP10*, *USP11*, *USP24*, *USP29*, and *USP47* are directly implicated in the regulation of DDR repair proteins (20). Single-strand DNA breaks are repaired by nucleotide excision repair (NER) and base excision repair (BER) pathways that deal with various DNA helix-distorting lesions and single-strand breaks; mismatch repair pathways that repair base mismatches and insertions/deletions; the nonhomologous end-joining pathway (NHEJ) and/or homologous recombination (HR) pathways, and the Fanconi anemia (FA) pathway, which in conjunction with certain HR factors act to recognize and repair inter-strand cross-links (ICL) DNA lesions. *USP1* and *USP7* act in multiple DDR pathways. *USP1* removes monoubiquitin from *FANCD2* and *PCNA*, and thus regulate ICL and trans-lesional DNA synthesis (20). *USP1* levels are well regulated during the cell cycle and their depletion leads to genomic instability. *USP1* knockouts show defects in FA and HR repair (21). Like *USP1*, *USP7* also acts in multiple DDR pathways. In response to DSB, the DSB sensor kinase ATM is activated, which leads to downregulation of *USP7S*, a *USP7* isoform, resulting in the activation of a p53 response and regulation of the G₂-M checkpoint (20). *USP7* is also a major regulator of the oxidative stress response. Through its deubiquitinating activity, *USP7* can modulate *FOXO4* transcriptional activity in response to oxidative stress. *USP7* can also modulate BER of oxidative lesions through chromatin remodeling directly by deubiquitination of histones, or

Table 1. A comprehensive list of the USPs implicated in human cancers

USP	Cancer-associated activity	Implicated signaling pathway	Tissue	Pharmacologic inhibitor	Crystal structure (Ref.)	Structure comments	Disease reference
CYLD	Tumor suppressor	NF- κ B, JNK	Cylindromatosis of the scalp, trichoepithelioma of hair follicles, colitis, hepatocellular carcinoma		2VHF.pdb—catalytic domain (res. 583–928), 2.8 Å (41). 1WHL, 1WHM, 1IXD.pdb—C-term interaction domains [res. 125–206, 228–304, 460–550], solution NMR (to be published [tbp]). Not available.	Catalytic domain includes a Zn-binding B box domain suggested to play a protein interaction role in subcellular localization.	(42–44)
USP1	Tumor promoter	FA pathway	Overexpressed in melanoma, myeloma, gastric, cervical, brain, liver, lung and colorectal cancers	Pimozide GW7647 ML323			(21, 32, 45)
USP2a	Tumor promoter	Fas/p53, NF- κ B, Myc	Prostate cancer, glioma		3NHE.pdb—catalytic domain (res. 277–600) complex with Ub, 1.3 Å (tbp). 2HD5.pdb—catalytic domain (res. 259–605) in complex with Ub (46). 2IBI.pdb—covalent complex catalytic domain (res. 263–605) with Ub (tbp). 3V6C.pdb, 3Y6E.pdb—catalytic domain (res. 263–599) complex with high-affinity Ub mutant (47). Not available.	High Ub:DUB affinity. Ub complex suggests trapped partial acyl-enzyme covalently bound to catalytic Cys. High-affinity Ub-mutant complex is first of a series with multiple DUBs generated via phage display/directed evolution.	(15, 48–50)
USP3	Cancer associated	DDR	Increased mRNA levels reported in bladder, brain and prostate cancers; reduced levels reported in leukemia and colon cancers				(51–53)
USP4	Oncogene	TGF β , NF- κ B, Wnt, p53	Can transform NIH3T3 cells, can induce tumorigenesis in athymic nude mice. Increased expression in human small cell and adenocarcinoma lung tumors and metastatic breast carcinomas		2Y6E.pdb—minimal catalytic domain of two linked pieces (res. 312–490, 765–930) 2.4 Å (54). 3JYU.pdb—N-terminus (res. 7–226), DUSP and Ub-like (Ubi) domains (tbp).	Illustrates how flexible loops can block Ub access to the DUB active site. Although not present in the structure, Ubi domain also auto-down regulates.	(55–58)

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Table 1. A comprehensive list of the USPs implicated in human cancers (Cont'd)

USP	Cancer-associated activity	Implicated signaling pathway	Tissue	Pharmacologic inhibitor	Crystal structure (Ref.)	Structure comments	Disease reference
USP5	Cancer associated	p53, DDR	Melanoma, glioblastoma	WP1130	2G45.pdb—Ubp ZnF domain alone and in complex with Ub, 2 Å (59).3IHP.pdb—catalytic domain (res. 1–857) covalent complex with Ub-aldehyde, 2.8 Å (60).2DAK, 2DAG.pdb—Uba domains, solution NMR (tbp). Not available	First example of UBP ZnF module recognizing free C-terminus of Ub.Full-length structure includes two UBP and two UBA domains, with some linkers disordered.	(18, 39, 61, 62)
USP6	Oncogene	NF-κB	Aneurymal bone cysts		1NBF.pdb—catalytic domain (res. 208–560). Single crystal has both monomer and covalent complex with Ub-aldehyde (Uba), 2.3 Å (66).1NB8.pdb—Catalytic domain (res. 208–560), 2.3 Å (66).2F1Z.pdb—TRAF-like and catalytic domain (res. 63–553) in complex with p53 and MDM2 peptides, 3.2 Å (67).2F1W, 2F1X, 2F1Y, 2FOJ, 2FOO, 2FOP.pdb—TRAF-like domains (res. 63–200) with p53/MDM2 peptides 1.7–2.3 Å (67, 68).5M5W.pdb, 5M5X.pdb—catalytic domain, 2.2 Å (69).2YLM.pdb—5 Ubi domains from the c-term (res 560–1,084; ref. 67).1YY6, 1YZE.pdb—TRAF-like domains (res 63–204) with peptides, 1.7–2 Å (70).	The first USP catalytic domain structure and complex with Ub-al. Complex of the TRAF-like domain with p53/MDM2 peptides illustrates how these substrates are recognized by a common surface of the TRAF-like domain.The 5 Ubi domains are arranged as dimer-monomer-dimer (2-1-2), and the last Ub-dimer can activate the catalytic domain.	(63–65) (33, 34, 71, 72)
USP7	Tumor promoter	p53, PI3-K, PTEN, FOXO4	Myeloma, prostate cancer, neuroblastoma, gliomas	HBX 41,108 P5091, HBX 19,818	Others:2KVR.pdb—Ubi (res 537–664) NMR.2XXN.pdb—TRAF-like domain.4KG9, 4JJQ.pdb—N-term domain.4PYZ.pdb—2 Ubi domains.		

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Table 1. A comprehensive list of the USPs implicated in human cancers (Cont'd)

USP	Cancer-associated activity	Implicated signaling pathway	Tissue	Pharmacologic inhibitor	Crystal structure (Ref.)	Structure comments	Disease reference
USP8	Tumor promoter	Wnt, hedgehog cytokine receptor signaling	Non-small cell lung cancer	HBX 41,108	2GFO.pdb—catalytic domain (res. 734–1,110), 2.0 Å (73),3N3K.pdb—catalytic domain complex with high-affinity Ub mut, 2.6 Å (47),2A9U.pdb—N-terminal domain (res. 1–142) homodimer, 2.1 Å (73),2GWF.pdb—Rhodanese domain (res. 181–319) complex with RNDP1 USP8-interaction domain (res. 193–317), 2.6 Å (73),1WHB.pdb—Rhodanese domain (res. 174–317), solution NMR (tbp).4FIP, 4FJC, 4FK5, 3MHH, 3MHS, 3M99.pdb—Ubp8/Sgf11/Sus1/Sgf73 SAGA DUB module (res. 1–471) 2.0–2.8 Å (74, 75).	Multiple structures exist for independent domains. The two structures of the multi-subunit SAGA DUB module illustrate allosteric regulation by non-substrate partners.	(76–79)
USP9x	Tumor promoter	TGFB, Mcl-1, ERG, AGS-3	Human lymphoma, myeloma, ductal, colon, prostate and small-cell lung adenocarcinomas, glioblastoma, medulloblastoma	WP1130	Not available.		(12, 39, 40, 80–83)
USP10	Tumor suppressor	ITCH	Mouse pancreatic adenocarcinoma model		Not available.		(16, 84–86)
USP11	Tumor promoter	cMyc, p53 DDR, NF-κB	Renal cell carcinoma Higher recurrence rates and poor survival outcome in breast cancer	Mitoxantrone	4MEL, 4MEM.pdb—N-terminal (res. 75–287) composed of DUBL-Ubl domains (87).	N-terminal domains not regulatory. Speculated to be important in protein interactions or trafficking.	(26, 88–91)

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Table 1. A comprehensive list of the USPs implicated in human cancers (Cont'd)

USP	Cancer-associated activity	Implicated signaling pathway	Tissue	Pharmacologic inhibitor	Crystal structure (Ref.)	Structure comments	Disease reference
USP14	Tumor promoter	Wnt	Colorectal cancer, non-small cell lung cancer	IU1	2AYN.pdb—catalytic domain, 3.2 Å (92).2AYO—catalytic domain covalently bound to Ub-ai, 3.5 Å (92).	Structures of the apo and Ub-ai complex illustrate DUB active-site flexibility. Showing conformational changes in regulatory loops that are required to allow access to the active-site.	(35, 93, 94)
USP15	Tumor promoter	NF-κB Wnt	<i>USP15</i> gene is found amplified in human breast and ovarian tumors, and in glioblastoma		4A3O, 4A3P, 3PV1, 3T9L, 4A3O, 3PPA.pdb—DUSP-Ubi domains (res 6–222; refs. 95, 96).3LMN, 1W6V.pdb—DUSP domain (res. 1–133; ref. 97).	Inter-domain orientation suggested to be important for protein recognition.	(98, 99)
USP16	Oncogene	RUNX fusion	AML		2I50.pdb—BUZ UBP domain (res 22–143), solution NMR (100). Not available.	ZnF domain that recognizes the C-terminal tail of Ub.	(7, 47, 101)
USP17	Tumor promoter	GTPase subcellular localization and cell motility, G ₁ -S cell-cycle checkpoint	Breast cancer NSCLC distal metastases				(91, 102, 103)
USP18	Cancer associated	NF-κB	AML		Not available.		(9, 104)
USP19	Cancer associated	ERAD pathway	Breast and prostate cancer		1WH0.pdb—CS domain (res. 273–393), solution NMR (tbp). Not available.		(105, 106)
USP20	Oncogene	NF-κB	Von Hippel-Lindau syndrome				(107, 108)
USP21	Cancer associated	NF-κB	Metastatic urothelial carcinoma		2Y5B.pdb—catalytic domain (res. 254–559) covalent complex with di-Ub-aldehyde, 2.7 Å.3MITN.pdb—catalytic domain (res.210–558) complex with high-affinity Ub mut.3I3T.pdb—catalytic domain (res. 211–558) covalent complex with Ub, 2.6 Å (47).	Structure of the DUB with a di-Ub substrate reveals a second substrate recognition surface on the DUB.	(109, 110)

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Table 1. A comprehensive list of the USPs implicated in human cancers (Cont'd)

USP	Cancer-associated activity	Implicated signaling pathway	Tissue	Pharmacologic inhibitor	Crystal structure (Ref.)	Structure comments	Disease reference
USP22	Tumor promoter	C-Myc	Increased expression in salivary duct carcinoma, papillary thyroid carcinoma, non-small cell lung carcinoma, oral squamous cell carcinoma and colorectal cancer and poor prognosis in glioblastoma. Novel prognostic marker for improving treatment efficiency for patients with glioblastoma.		Not available.		(111–116)
USP25	Cancer associated	ERAD pathway	Overexpressed in human breast cancer		1VDL.pdb—UBA domain (res 1–67) solution NMR (tbp). Not available. Not available.		(84, 117, 118) (119, 120) (121, 122)
USP29	Cancer associated	p53	Overexpressed in breast cancer				
USP32	Tumor promoting		Von Hippel-Lindau syndrome, B-cell acute lymphoblastic leukemia				
USP33	Oncogene		AML		2UZG.pdb—UBP domain (res 36–130) solution NMR (123).	UBP domain does not appear to bind Ub. 3 Zn ions.	(10, 124)
USP42	Oncogene	p53, RUNX fusion gene	AML		Not available.		(56, 125)
USP50	Cancer associated	G ₂ -M checkpoint	AML		Not available.		(126)
DUB3	Cancer associated	G ₂ -M checkpoint	Breast cancer		Not available.		(127, 128)

indirectly by regulating the cellular levels of E3 ubiquitin ligases involved in histone ubiquitination. USP29 also mediates the oxidative stress response by stabilizing p53 in a FBP/JTV1-dependent manner. USP7 is also implicated in transcription-coupled NER (TCNER), a subpathway of NER that efficiently removes highly toxic RNA polymerase II blocking lesions on DNA causing Cockayne syndrome and UV-sensitive syndrome (UVSS). Apart from USP7, USP47 is the only other DUB that is reported to regulate BER through the stabilization of newly synthesized polymerase β (Pol- β). USP47-silenced cells show aberrant DDR growth inhibition and chemosensitization. USP3 and USP16 deubiquitinate histone H2A and USP16 also opposes the RNF8–RNF18 pathway-mediated DDR to double-strand breaks (DSB), leading to accumulation of replication stress in the cell. USP24 stabilizes DDB2, also called p48, which is an important protein in the recognition and repair of UV-induced lesions in the NER pathway (22). In response to DNA damage, USP28 binds with 53BP1 and its loss induces ionizing radiation-induced apoptosis. USP44 has been identified as a DUB critical for spindle assembly checkpoint activity and in centrosome regulation. USP44-deficient mice are susceptible to spontaneous tumors of the lung, supporting a role for USP44 as a tumor-suppressor gene (reviewed in ref. 20). These findings are especially noteworthy because current radiation and chemotherapy regimens used for the treatment of various cancers rely heavily on inducing DNA damage to the cancer cell. Developing specific or even partially specific inhibitors that can target several USPs, resulting in cell-cycle arrest or apoptosis, in the cancer cell may be advantageous.

USPs as regulators of cancer-associated pathways

USPs are also implicated as important regulators of several cancer-associated pathways. The NF- κ B pathway is constitutively activated in a vast range of cancers and is implicated in tumorigenesis and metastasis. Several USPs are reported as negative regulators of NF- κ B signaling. CYLD binds to the NEMO component of the I κ B kinase complex and regulates its activity through deubiquitination of TRAF2. Inhibition of CYLD increases resistance to apoptosis, an effect that can be relieved by inhibiting NF- κ B activity using aspirin derivatives (23). USP31 has also been identified as a TRAF2-interacting protein that regulates NF- κ B activation by members of the TNF receptor superfamily (24). USP4 directly interacts and exerts deubiquitinating activity on multiple NF- κ B pathway-associated molecules, such as TRAF2, TRAF6, and TAK1 kinase, and negatively regulates TNF α and IL1 β -induced cancer cell migration. USP4 negatively regulates receptor-interacting protein 1 (RIP1)-mediated NF- κ B activation and promotes TNF α -induced apoptosis in head and neck squamous cell carcinomas through direct interaction with RIP1 and deubiquitination of K63-linked ubiquitin from RIP1 (25). USP11 mediates downregulation of TNF α -mediated NF- κ B activation through modulation of I κ B α stability by its deubiquitination (26). USP14 removes the ubiquitin chain of I- κ B, thereby inducing I- κ B degradation and increasing cytokine release in lung epithelial cells (27). COP9 signalosome (CSN) regulates assembly and activity of cullin-RING ubiquitin ligases (CRL), which are involved in the ubiquitination of I κ - α . USP15

is identified as a CSN-associated DUB that reverses the ubiquitinating activity of CSN on I κ B α , thus negatively regulating NF- κ B signaling (28).

Several USPs are implicated in the regulation of TGF β signaling at various levels of the pathway. USP4, USP11, and USP15 interact with the TGF β receptor I, deubiquitinate, and stabilize this receptor to engage sustained Smad activation, resulting in enhancement of TGF β signaling. Inhibiting USP4 and USP11 inhibits TGF β -mediated epithelial–mesenchymal transition (EMT) and invasion in breast cancer while USP15 inhibition blocks EMT and invasion in glioblastomas (29). USP4 has also been shown to drive cross-talk between TGF β and AKT signaling in breast cancer. Inhibiting USP4 suppresses AKT-mediated breast cancer cell migration. These findings underscore the relevance of USP4 in the pathway as AKT activation has been associated with poor prognosis in breast cancer (29). USP9X reverses the mono-ubiquitination of Smad4 at Lys 519, a modification that prevents its binding to phosphorylated Smad2 and inhibition of TGF β signaling. Thus, Smad4 deubiquitination by USP9X reinstates TGF β signaling (12). USP9X can also deubiquitinate E3 ubiquitin ligase SMURF1, whose substrates include TGF β receptor and coreceptor Smads (30). Recent findings in our laboratory show an oncogenic potential of USP9X in established breast cancer cells and inhibiting USP9X inhibits cell growth in tumorigenic cells and induces apoptosis in triple-negative breast cancers (Unpublished Data). Thus, there is accumulating evidence in favor of therapeutic targeting of USPs like USP4, USP15, and USP9X in breast cancer.

Inhibitor of differentiation (ID) proteins (ID1-4) regulate differentiation and maintain stem cell fate through inhibition of basic–helix–loop–helix transcription factors. ID protein levels are deregulated during cancer and have been associated with an aggressive clinical phenotype and poor patient outcomes. The expression levels of ID proteins are tightly regulated through ubiquitination by the APC/Cdh1 E3 ligase complex, which leads to proteasomal degradation and shorter half-life of ID proteins in the cell (31). Recently, USP1 was shown to stabilize ID1, ID2, and ID3 protein expression levels in normal and malignant stem cell populations through its deubiquitinating activity. USP1 was found to be overexpressed in a subset of osteosarcomas where it stabilized ID1, ID2, and ID3, leading to repression of p21 levels and aberrant osteogenic differentiation (32). USP1 abundance correlated with ID2 protein abundance levels in human osteosarcoma tumors. Thus, small molecule-mediated targeting of USP1 may provide an additional modality for treatment of cancer through modulation of ID proteins. Despite the growing interest in USPs and in acquiring USP inhibitors, their development has been hampered by some of the biochemical properties of the USPs themselves and mostly unavailable three-dimensional (3D) structures. To date, only seven USPs have defined 3D structures (Table 1). USPs can range in size from 350 to 3,500 amino acids. They comprise a central catalytic domain, which could be 295 to 850 amino acids long and shows an average 22% identity among USPs and many different additional domains like the ubiquitin-like domain in USP7 and USP14. Such versatility in this small family of DUBs could be a boon in the development

of partially selective or specific inhibitors and will benefit from unraveling the structural information of more USPs.

Development of Small Molecule Inhibitors against USPs

USP7 inhibitors: HBX 41, 108, and P5091

Among the earliest reported DUB inhibitors are cyclopentanone prostaglandins that induce apoptosis and also increase the cellular content of poly-ubiquitinated proteins, suggesting they are nonselective DUB inhibitors (3). A small molecule discovered in screens for DUB inhibitors, PR-619, is selective for DUBs over other cysteine proteases, but inhibits all DUBs tested with moderate potency. More recently, HBX 41,108, which was originally reported to be a USP7 inhibitor, was confirmed to be a nonselective DUB inhibitor. HBX 41,108 stabilizes p53 in HEK293 cells and induces caspase-3 and PARP cleavage in both p53^{+/+} and p53^{-/-} HCT-116 cells. The specificity of HBX 41,108 is limited as it can also inhibit USP5, USP8, UCH-L3, and caspase-3 with a potency (70–200 nmol/L) greater than its activity against USP7 (530 nmol/L). However, because of the critical regulatory functions played by USP7 in the cell there is tremendous interest in developing USP7-specific inhibitors (33). Progenra has identified the novel P5091 small-molecule USP7-specific inhibitor that can stabilize p53, inhibit cancer cell proliferation, and is an active antitumor agent in various tumor models. P5091 induces apoptosis in multiple myeloma cells resistant to conventional and bortezomib therapies (34). P5091 is well-tolerated in animals, inhibits tumor growth, prolongs survival, and triggers synergistic anti-multiple myeloma activity in combination with other chemotherapeutic agents such as lenalidomide, HDAC inhibitor, or dexamethasone. Hit-to-lead optimization identified additional analogs of P5091 (e.g., aqueous soluble derivative P045204) that increases the steady-state levels of p53 and its transcriptional target p21 in a time-dependent manner in HCT-116 cells.

USP14 inhibitors: b-AP15

b-AP15, also known as VLX1500, is a unique class of proteasome inhibitor that inhibits the activity of the 19S regulatory particle-associated DUBs, UCHL5, a ubiquitin C terminal hydrolase, and USP14. b-AP15 induces apoptosis in tumor cells irrespective of their mutant p53 status and BCL2 overexpression and is effective in inhibiting tumor progression in multiple solid tumor mouse models and dissemination of an *in vivo* AML model. The apoptotic effects of b-AP15 are mediated through the induction of oxidative and endoplasmic reticulum (ER) stress in response to b-AP15-mediated accumulation of poly-ubiquitinated proteins (35).

USP1-UAF1 inhibitors: Pimozide, GW7647, and ML323

Pimozide and GW7647 inhibit USP1-UAF1 complex through noncompetitive binding, which provides for more selectivity and specificity toward USP1-UAF1. These inhibitors act in synergy with cisplatin to inhibit cell proliferation in cisplatin-resistant non-small cell lung cancer cells (36). Pimozide also inhibits leukemic cell growth through degradation of USP1 substrate ID1 (37). ML323 is another highly potent inhibitor of the USP1-UAF1 complex that provides for excellent selectivity against DUBs. In non-small cell lung cancer and osteosarcomas, ML323 potentiates cisplatin-induced cytotoxicity (38). ML323-mediated inhibition of USP1 inhibits deubiquitination of FANCD2 and PCNA and compromises TLS and FA pathways (38). Thus, ML323 may provide a means to sensitize cancer cells to platinum-based therapies.

USP9X inhibitors: WP1130 and its derivatives

In our laboratory, we described and developed the small-molecule inhibitor WP1130, also known as Degrasyn, which was derived from a compound with JAK2 kinase-inhibitory activity. WP1130 rapidly induces accumulation of poly-ubiquitinated proteins, resulting in induction of apoptosis. WP1130 is a partially selective inhibitor that directly inhibits the deubiquitinating activity of USP9X, USP5, and USP14, all of which regulate survival protein stability and proteasome function. In several studies done in the recent past, USP9X inhibition by WP1130 promotes apoptosis by reducing MCL-1 levels and increased tumor cell sensitivity to chemotherapy (39). Recently, USP9X inhibition by WP1130 was shown to inhibit the growth of ERG-positive tumors *in vitro* and in mouse xenograft models of prostate cancer (40). Our laboratory has been actively engaged in identifying derivatives with greater selectivity, activity, and drug-like properties. We recently identified and are developing a compound with improved specificity toward USP9X and antitumor activity in mouse models of myeloma, lymphoma, and melanoma (unpublished data).

Conclusively, there is accumulating experimental evidence that a large number of USPs could be targeted for anticancer therapeutics. Early evidence for antitumor efficacy with the currently available USP inhibitors is more than encouraging and sets the stage for the development of selective, as well as partially selective, small-molecule DUB inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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