Molecular Pathways: Translational Potential of Deubiquitinases as Drug Targets

Pádraig D’Arcy and Stig Linder

Abstract

The ubiquitin proteasome system (UPS) is the main system for controlled protein degradation and a key regulator of fundamental cellular processes. The dependency of cancer cells on a functioning UPS coupled with the clinical success of bortezomib for the treatment of multiple myeloma have made the UPS an obvious target for drug development. Deubiquitinases (DUB) are components of the UPS that encompass a diverse family of ubiquitin isopeptidases that catalyze the removal of ubiquitin moieties from target proteins or from polyubiquitin chains, resulting in altered signaling or changes in protein stability. Increasing evidence has implicated deregulation of DUB activity in the initiation and progression of cancer. The altered pattern of DUB expression observed in many tumors can potentially serve as a clinical marker for predicting disease outcome and therapy response. The finding of DUB overexpression in tumor cells suggests that they may serve as novel targets for the development of anticancer therapies. Several specific and broad-spectrum DUB inhibitors are shown to have antitumor activity in preclinical models with low levels of systemic toxicity. Future studies will hopefully establish the clinical potential for DUB inhibitors as a strategy to treat cancer. Clin Cancer Res; 20(15); 3908–14. ©2014 AACR.

Disclosure of Potential Conflicts of Interest

S. Linder has ownership interest (including patents) in Vivolux AB. No potential conflicts of interest were disclosed by the other author.

CME Staff Planners' Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

Upon completion of this activity, participants should have a better understanding of the diverse role of deubiquitinases (DUB) in cancer as well as the potential of DUBs as novel targets for the development of new cancer therapeutics.

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Background

The ubiquitin proteasome system

Since its initial description more than 30 years ago, the ubiquitin proteasome system (UPS) has emerged as a central node for the regulation of cellular homeostasis. Although, perhaps more renowned for its role in mediating controlled protein degradation, the UPS impinges on numerous aspects of cellular proliferation and survival, including DNA damage repair, macromolecule trafficking, signaling, and immunologic recognition (1, 2). At its most basic level, the UPS consists of a ubiquitin tagging system that covalently modifies target proteins with the 76 amino acid protein ubiquitin and the 26S proteasome, a large multisubunit complex that acts as a molecular shredder by degrading ubiquitin-tagged substrates. The 26S proteasome is composed of two subparticles, the barrel-like 20S core particle (20S CP) composed of four stacks of heptameric subunits and the 19S regulatory particle (19S RP) that regulates the translocation of ubiquitinated substrates to the 20S CP in which proteolysis takes place (3, 4). Specificity is conferred by ubiquitin E1 activating, E2 conjugating, and E3 ligases that mediate the attachment of ubiquitin to lysine residues on target substrates (5). A ubiquitin code exists whereby ubiquitin molecules linked via internal K48 and K11 residues are involved in proteasome degradation, whereas those linked by other linkages are generally involved in nonproteolytic roles such as DNA repair and endocytosis (6). The route to the proteasome is
not a one-way street: A ubiquitin deconjugation system also exists that catalyzes the removal of ubiquitin from conjugated substrates, thus altering their stability and/or function (Fig. 1A). More than 90 deubiquitinases (DUB) have been described and divided into five classes based on the presence of conserved catalytic domains. The ubiquitin-specific proteases (USP), ubiquitin-C terminal hydrolases (UCH), ovarian tumor domain (OTU), and Machado–Joseph domain (MJD) DUBs are all thiol proteases, whereas the Jab1/MPN (JAMM) class are zinc-dependent metalloproteases. The overexpression of several DUBs has been implicated in neoplastic transformation, including USP1 (Fanconi anemia), USP2 (prostate cancer), USP4 (adenocarcinoma), USP7/USP2a/USP10 (via stabilization of p53), USP9x (leukemias and myelomas), and USP15 (glioblastoma), providing potential therapeutic targets for drug development. Perhaps the first realization of this clinical potential
was the observation that specific DUBs negatively regulated the stability of the p53 tumor suppressor. Transfection of cells with siRNA specific for USP7 or USP2a led to increased expression of p53 and downstream targets via disruption of the p53-HDM2 axis; HDM2 is itself an E3 ligase that ubiquitinates both p53 and itself (14–16). Such findings have prompted the development of small-molecule inhibitors of USP7 as a means to increase p53 levels in cancer cells in a manner analogous to the development of the HDI2 inhibitor, Nutlin-3 (17, 18). Additional DUBs have been implicated in p53 regulation. USP22 indirectly inhibits p53 transcriptional activation by deubiquitinating the class III histone deacetylase Sirt-1 as well as other important targets in gene regulation such as H2A and H2B (19, 20). USP42 forms a direct complex with p53 and controls ubiquitination levels during stress responses (21).

Other DUBs have been shown to regulate the apoptotic response. USP9x has been shown to stabilize the prosurvival BCL2 family member MCL1 and correlated to elevated MCL1 expression in human lymphomas (12). USP9x has also been shown to be an important factor for prodrug resistance TGFβ signaling by deubiquitinating SMAD4 (22). Such findings suggest that small-molecule USP9x inhibitors may present a therapeutic strategy.

Other DUBs that could serve as potential drug targets include USP8, which regulates the cell-surface expression of EGFR (23), USP19, which regulates the levels of the anti-apoptosis regulators c-IAP (24), and USP28, which stabilizes the MYC oncoprotein in colon and breast carcinoma cells (25).

The three proteasome-associated DUBs, USP14, UCHL5, and POH1 are in a unique position in that they regulate a critical node in the pathway of protein waste disposal. Proposed roles of the proteasome DUBs are to facilitate degradation of proteins by removing polyubiquitin chains, editing function and control of gate opening (26, 27). As such, proteasome DUBs may represent an Achilles’ heel in cancer cells and provide a target for drug development. Growing evidence supports a specific role for each DUB in regulating multiple aspects of cancer biology. The cysteine DUB USP14 has been implicated in the regulation of stability of IκB (28) and β-catenin (29) as well as proliferation in certain cancer types (30). Whereas a degree of redundancy exists with the two cysteine DUBs, the expression of POH1 is essential for viability; POH1 knockdown induced growth arrest and senescence (31). POH1 expression has also been shown to regulate the expression of the receptor tyrosine kinase (RTK) HER2, an oncogenic RTK overexpressed in a subset of breast cancers. The expression of POH1 enhances the stability of HER2 presumably via receptor deubiquitination, rescue from proteasomal degradation, and recycling to the cell surface (32). A nuclear role for POH1 has also been described in the regulation of BRCA1-mediated DNA repair (33, 34). Finally, POH1 has been shown to be a key mediator of the early response by promoting c-Jun stability (35), a component of the heterodimeric transcription factor AP-1 that activates the expression of early-response genes following stimulation with growth factors, cytokines, or various cellular stresses.

Clinical–Translational Advances

The approval of bortezomib for the treatment of relapsed multiple myeloma has verified UPS as a rational target for drug design and accelerated development, with several next-generation proteasome inhibitors already in advanced stages of clinical testing (36, 37). However, despite this success, problems such as dose-limiting side toxicities have raised the possibility of targeting alternative components of the UPS. The advantages of such a strategy may be a reduction of bortezomib-associated side effects as well as providing an alternative target for drug-resistant tumors (38).

Recently, several small-molecule inhibitors targeting the ubiquitin conjugation machinery of the UPS have been described. Compounds that block the activity of the E1-activating enzymes, or E3 ligases regulating cancer-associated pathways have been reviewed elsewhere (39). The sheer number of UPS components regulating cancer cell survival has provided multiple intervention points for the development of anticancer drugs. Although still in its infancy, the development of DUB inhibitors has the potential to equal the breakthrough of proteasome inhibitors for the treatment of cancer. Because of space restrictions, we can only provide an overview of the area here, with the focus on translational aspects. For a more comprehensive review of the subject list, readers should refer to refs. (9, 40).

Achieving specificity is likely to be a problem in targeting DUBs—both at the level of identifying inhibitors to specific DUBs (due to homologies between related enzymes) and the level of promiscuity of DUBs toward ubiquitinated targets. The promiscuity of DUBs such as USP7 and USP2a toward multiple targets may mean that pharmacologic intervention will be complex; for example, USP7 also deubiquitinates p53 as well as HDM2, leading to highly dynamic fluctuations in both protein levels (41). Similarly, several DUBs have been shown to have dual oncogenic and tumor-suppressive functions. For example, although USP9x has an antiapoptotic role in lymphomas (12), it has been shown to function as a tumor-suppressor gene in pancreatic cancer with loss of the USP9x gene enhancing transformation and protecting cells from anoikis (42). These opposing findings are not unexpected, considering that any specific DUB is expected to have multiple targets. The findings do, however, point to the necessity of careful evaluation of the effects of inhibition of different targets.

However, as discussed by Cohen and Tcherkapov (43), lack of specificity does not necessarily constitute a disadvantage. The lessons from the development of protein kinase inhibitors have taught us that antineoplastic effects may rely on the inhibition of multiple targets, and such inhibitors may be beneficial in avoiding the development of drug resistance. Instead of targeting specific components of the UPS, an alternative strategy may be the development of broad-spectrum drugs that interfere with essential nodes in cancer cell signaling, an analogous spanner in the
works as opposed to the silver bullet approach for drug development. Interference with the UPS-mediated regulation of vital systems controlling cellular homeostasis, such as endoplasmic reticulum–associated protein degradation (ERAD), mitochondrial fusion/fission cycles, DNA synthesis and repair, and proteasome degradation, may be a more viable option than targeting specific E3 ligases or DUBs.

Several DUB inhibitors have emerged from preclinical testing, but as of now, no results from clinical trials are available. The data, so far, look promising with robust antitumor activity and low toxicity-associated side effects reported across a broad spectrum of cancer models and in drug-resistant cell lines. Here, we describe some of the DUB inhibitors for which preclinical data are available (Table 1).

### Preclinical activity of DUB inhibitors

WP1130 (degrasyn) was initially identified from a functional screen for compounds that suppressed interleukin-mediated STAT activation. Studies showed that WP1130 induced growth arrest and apoptosis in melanoma and chronic myelogenous leukemia (CML) cells in a manner dependent on the proteasomal degradation of the c-Myc transcription factor and Bcr/Abl oncoprotein, respectively (44, 45). In vivo studies also showed antitumor activity on melanoma and CML xenograft models at doses that were well tolerated. Subsequent studies on the mechanism of action of WP1130 showed it to be a potent inhibitor of cellular DUB activity at apoptosis-inducing concentrations (46). In the case of CML, it seems that the apoptotic effect is due to the dual inhibition of the deubiquitination of the Bcr/Abl fusion protein resulting in sequestering in aggresomal structures and inhibition of USP9x and its antiapoptotic activity (47). Interestingly, imatinib-resistant CML cells harboring a mutation in the drug-binding domain of the Bcr/Abl fusion protein retained sensitivity to WP1130, suggesting a potential therapeutic strategy for relapsed CML (47).

Betulinic acid (BA) is a plant-derived small molecule with proapoptotic activity in cancer cells (48). Results from a recent study by Reiner and colleagues has suggested BA to be a pan-DUB inhibitor that selectively kills prostate cancer but not normal cells. BA was shown to significantly inhibit tumor growth and promote apoptosis in a transgenic model of prostate cancer (49). Although no specific DUBs were identified, the authors propose a mechanism whereby BA inhibition of DUB activity leads to the enhanced proteasomal degradation of progrowth mediators such as the androgen receptor and cyclin D1 as well as antiapoptotic regulators. BA was developed as part of the NCI’s Rapid Access to Intervention Development (RAID) program and is currently undergoing a phase I–II clinical trial as a treatment for dysplastic nevi (50).

P5091 is a USP7-specific inhibitor shown to induce apoptosis in multiple myeloma cells (Fig. 1B; ref. 18). In vivo studies on multiple myeloma xenografts showed that P5091 inhibited tumor growth, blocked angiogenesis, and prolonged survival at efficacies similar to those achieved with bortezomib. Furthermore, P5091 synergized with lenalidomide, dexamethasone (both common therapies for multiple myeloma), and the histone deacetylase inhibitor vorinostat. Importantly, P5091 was equally effective on multiple myeloma tumors that had acquired resistance to bortezomib, suggesting the potential for the development of treatments for bortezomib-relapsed multiple myeloma (18).

b-AP15 was identified in a cell-based screen for compounds that induce lysosomal-dependent apoptosis, and induced a cellular response characteristic of proteasome inhibitors (51). b-AP15 inhibits the activity of proteasomal cysteine DUBs USP14/UCHL5, resulting in a buildup of ubiquitinated substrates, while not altering the proteolytic activity of the 20S CP (Fig. 1C; ref. 52). In vivo b-AP15 was shown to have antitumor activity on a variety of tumors of diverse origin. Importantly b-AP15 was also shown to have antitumor activity on multiple myeloma cells that had acquired resistance to bortezomib as well as prolonging the life of myeloma-bearing xenografts, raising the possibility of a treatment option for bortezomib-resistant relapsed myeloma (53).

AC17 is a 4-arylidene curcumin analogue that has recently been shown to have DUB-inhibiting properties (54). Unlike its parent molecule curcumin, which inhibits the proteolytic activities of the 20S CP, the AC17 derivative irreversibly inhibits the DUB activity of the 19S RP without altering the activities of the 20S CP (Fig. 1C; refs. 54, 55). In vivo AC17 significantly inhibited tumor growth in a lung carcinoma xenograft model with no observable toxicity.

### Table 1. Summary of DUB inhibitors and targets displaying preclinical activity in in vivo tumor models

<table>
<thead>
<tr>
<th>Compound</th>
<th>DUB targets</th>
<th>Cancer type</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP1130 (degrasyn)</td>
<td>USP9x, USP5, USP14, UCHL5</td>
<td>CML</td>
<td>Preclinical</td>
</tr>
<tr>
<td>BA</td>
<td>Multiple</td>
<td>Pancreatic</td>
<td>Phase I–II for dysplastic nevi</td>
</tr>
<tr>
<td>P5091</td>
<td>USP7</td>
<td>Multiple myeloma</td>
<td>Preclinical</td>
</tr>
<tr>
<td>b-AP15</td>
<td>USP14, UCHL5</td>
<td>Lung adenocarcinoma; colon, breast, head and neck; multiple myeloma</td>
<td>Preclinical</td>
</tr>
<tr>
<td>AC17</td>
<td>USP14, UCHL5</td>
<td>Lung adenocarcinoma</td>
<td>Preclinical</td>
</tr>
<tr>
<td>USP8i</td>
<td>USP8</td>
<td>NSCLC</td>
<td>Preclinical</td>
</tr>
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Abbreviation: NSCLC, non–small cell lung cancer.
9-ethyloxyimino-9H-indeno[1,2-b]pyrazine-2,3-dicarbonitrile (USP8i) was shown to inhibit the activity of USP8. This inhibitor has been shown to display preferential activity on non–small cell lung carcinoma (NSCLC) cells overexpressing USP8 but not on normal epithelial cells. Inhibition of USP8 activity induced the downregulation and activity of RTKs such as EGFR, ERBB2, ERBB3, and Met, a group of oncogenic RTKs whose activity is enhanced in a variety of cancers (Fig. 1D). Of particular importance, cells that had developed resistance to the RTK inhibitor gefitinib still remained sensitive to USP8 inhibition in vivo, suggesting a potential treatment option for RTK inhibitor–resistant NSCLC (56).

DUBs as clinical markers

The observation that several DUBs display altered activity and/or expression levels in diverse cancer types also raises the possibility of using DUBs as markers for predicting disease response to chemotherapeutic drugs.

Using an in situ hybridization (ISH) approach, Luise and colleagues (57) analyzed the expression of 89 DUBs on multituire microarrays representing a variety of normal and tumor tissues. Of the DUBs detected by ISH, 67% showed deregulated expression when compared with normal tissue. NSCLC showed overexpression of JOSD1, COP5, UCHL1, and USP9x, with UCHL1 and USP9x expression correlating to Ki67, indicative of rapidly proliferating tumors. Comparisons of malignant melanoma showed overexpression of USP10, USP11, and USP22 that was significantly correlated to metastatic grade. Interestingly, gastric cancer showed a loss of USP1 expression, a DUB implicated in the DNA damage response, suggesting a tumor-suppressive role for this protein in preventing gastric cancer development.

In the study by Rolén and colleagues (58), the authors profiled DUB activity in a panel of paired biopsies of normal and malignant tissue from HPV+ cervical carcinoma using active site-directed probes. Upregulation of DUB activity was observed in the majority of tumor samples compared with healthy tissue, with the activities of UCHL3 and UCHL5 displaying the greatest increase in activity. Only UCHL1 showed decreased activities in the tumor tissues, potentially indicating a tumor-suppressive role for this protein. Other studies have also indicated loss of UCHL1 expression as a common occurrence; silencing methylation of the UCHL1 promoter was observed in biopsies of pancreatic cancer (42 of 42) and prostate cancer (18 of 22; refs. 59, 60).

The expression of the proteasomal DUB, POH1, has been shown to mediate resistance to a variety of anticancer drugs and UV radiation, possibly due to its role in DNA repair (33, 34, 61). The expression of several RTK receptors on the cell surface is also regulated by specific DUBs with USP8/EGFR and POH1/ERB2 being the most characterized (23, 32). Although to our knowledge no studies have been performed, it would be interesting to determine whether these DUBs have clinical relevance in predicting patient response to treatments with RTK inhibitors or HER2-targeting antibodies. Analysis of DUB expression and activity following chemotherapy regimens would also be interesting in not only elucidating potential roles for DUBs but also as predictors of treatment response.

Authors’ Contributions

Writing, review, and/or revision of the manuscript: P. D’Arcy, S. Linder

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