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Nuclear deubiquitination in the spotlight: the multifaceted nature of USP7 biology in disease

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Abstract

Ubiquitination is a versatile and tightly regulated post-translational protein modification with many distinct outcomes affecting protein stability, localization, interactions, and activity. Ubiquitin chain linkages anchored on substrates can be further modified by additional post-translational modifications, including phosphorylation and SUMOylation. Deubiquitinases (DUBs) reverse these ubiquitin marks with matched levels of precision. Over hundred known DUBs regulate a wide variety of cellular events. In this review, we focus on ubiquitin-specific protease 7 (USP7, also known as herpesvirus-associated ubiquitin-specific protease, or HAUSP) as one of the best studied, disease-associated DUBs. By highlighting the functions of USP7, particularly in the nucleus, and the emergence of the newest generation of USP7 inhibitors, we illustrate the importance of individual DUBs in the nucleus, and the therapeutic prospects of DUB targeting in human disease.

Review

Ubiquitination, the addition of a small, 76-amino acid protein called ubiquitin to other proteins ('substrates'), regulates numerous biological processes, from protein degradation, interaction, and localization, to cell signaling, division, and proliferation. A cascade between the relatively few E1 ligases, ~100 E2 conjugating enzymes, and ~600 E3 ubiquitin ligases confers specificity to the addition of ubiquitin to protein substrates [1–4]. Ubiquitination patterns add complexity, as chains form between one ubiquitin's c-terminus and any of another ubiquitin's internal lysine residues. These branching patterns, along with

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modifications like phosphorylation or SUMOylation [4–6], create a landscape of ubiquitin patterns that regulate most major processes in the cell [7].

Deubiquitinases, or DUBs, are enzymes that disassemble these complex ubiquitin patterns. DUBs consist of six families of different cysteine and metallo-peptidases [7–18], of which the Ubiquitin-Specific Peptidases (USP) are the largest, with >50 members. USP7 is one of the best-studied disease-associated DUBs, as the discovery of USP7's regulation of known tumor suppressors spurred extensive research into its effects on proteins - whether by altering their stability, localization, or activity - and processes ranging from apoptotic cascades to transcriptional activation. USP7 regulates numerous substrates directly implicated in human disease, yet many of these substrates are either 'undruggable' or without established direct targeting strategies, such as p53 [19]. To circumvent these limitations, targeting USP7 with small molecule inhibitors has provided an alternative approach to targeting key factors in human disease (i.e. p53 in cancer) [20**]. The first generation of USP7 inhibitors were promising in *in vitro* and *in vivo* models, and the recent development of the more potent, selective, and mechanistically diverse second generation of USP7 inhibitors provides new opportunities to understand deubiquitination as a mechanistic driver of disease, through the targets, regulation, and effects of USP7 activity. Several groups have reviewed USP7's well-known interactions, in numerous cellular compartments and with varied functions [21,22]. Here, we contextualize recent findings of USP7's nuclear roles and the regulation of USP7 itself while providing a comprehensive review of the new generation of USP7 inhibitors.

USP7 in the nucleus: pervasive regulation p53 and beyond: USP7 as a context-specific modulator in apoptosis and cancer

Although numerous substrates have been suggested for USP7 [7], the best characterized role of USP7 is in the regulation of p53 levels (Figure 1a). Under normal conditions, USP7 stabilizes MDM2, resulting in p53 turnover [11,23]; however, upon cell stress, USP7 'switches' from stabilizing MDM2 to stabilizing p53 [12]. Since the discovery of the MDM2-USP7-p53 complex, similar 'switch' interactions have been well-reviewed for USP [22] and also discovered for other E3 ligases and DUBs (APC^{CDC20}-USP44, and KPC1-USP19 [24–26]). Still, the USP7-MDM2-p53 axis remains the paradigm of USP7 interactions in the nucleus, and new research continues to show how USP7 promotes p53-dependent apoptosis in disease. For example, in esophageal cancer, USP7 inhibition upregulates Noxa, which in turn mediates p53-dependent apoptosis [27,28].

Beyond the USP7-MDM2-p53 axis, however, modulation of p53 levels and activity may not always be the end product of USP7 activity. Genetic knockout of *Usp7* in mice is embryonic lethal and cannot be fully rescued by p53, implying USP7 effects extend beyond p53 regulation [29]. USP7 knockdown in colorectal cancer cells with varied TP53 status inhibits proliferation, further demonstrating that USP7's effects can be independent of p53 [30]. Studies in hematological malignancies illuminate aspects of both USP7's p53-dependent and independent effects in disease. In chronic myelogenous leukemia (CML), BCR-ABL enhances USP7-induced PTEN deubiquitination resulting in PTEN's exclusion

from the nucleus [31] and subsequent increase in PI3K/AKT/mTOR signaling. DAXX leads to decreased USP7-mediated deubiquitination of PTEN [10].

In chronic lymphocytic leukemia (CLL), USP7 inhibition arrests cell growth and induces p53-independent apoptosis by restoring PTEN in the nucleus [32]. CK2's stabilization of USP7 further contributes to MDM2 stabilization and downregulation of p53, and after ionizing radiation, the ATM-dependent protein phosphatase PPM1G downregulates USP7, reversing its effects on MDM2 and p53 [33]. These dynamics demonstrate how, even in the absence of intact p53, USP7 remains a key regulator of apoptotic signaling pathways [20[•], 34^{*}].

Under pressure: USP7 as a pro-survival and proliferative factor

USP7 helps regulate the balance of DNA damage tolerance and mutagenesis. USP7-depleted cells fail to elongate nascent daughter strands after UV irradiation, showing decreased DNA damage tolerance [35,36]. Under stress, Rad18 monoubiquitinates PCNA, leading to the recruitment of specialized DNA polymerases like Pol η to promote translesion synthesis, a DNA damage response that repairs or bypasses lesions including thymine dimers or abasic sites that are generated by ultraviolet irradiation [37–39]. The Rad18–PCNA response can contribute to tolerance of DNA damage and lead to mutagenesis [40]. Rad18 itself is deubiquitinated by USP7 [36,41], leading to its nuclear exclusion and inactivity. Pol η is also deubiquitinated by USP7, leading to the recruitment of PCNA to stalled replication forks and PCNA's ubiquitination by Rad 6 and Rad18 [36]. These seemingly contradictory roles of USP7 are reminiscent of its activity in the MDM2–p53 complex, where upon cell stress, USP7's main substrate changes. USP7 may regulate the balance of DNA damage tolerance and mutagenesis in a similar manner, lending sway in response to the cellular environment. Additional novel USP7 roles in regulating the DNA damage response include interactions with CDC25A in BRCA $\frac{1}{2}$ -mediated tumorigenesis [42], suggesting further involvement in the stress response.

USP7's role in cell proliferation is similarly multifaceted. Under normal conditions, USP7 stabilizes the histone demethylase PHF8, and this stabilization increases upon DNA damage, driving aberrantly increased transcription of CCNA2 and other cell cycle associated signals [43]. USP7 may also deubiquitinate SUMOylated proteins at the replication fork, contributing to the SUMO-rich environment of the replisome, the importance of which is not yet known [44].

Top to bottom: USP7 as a regulator at the epigenetic and transcriptional levels

USP7's role as a widespread regulator of post-translational protein stability is well-known and well-accepted, and while many of USP7's roles in epigenetic control are similarly well-established, new advances suggest interesting nuances in USP7's multi-level regulation of the nucleus. While the MDM2–USP7 interaction is best known in the context of p53 stability, it may also regulate p53 targets epigenetically through the SUV39H1 H3K9 methyltransferase [45]. USP7 is also accepted as both an H2B deubiquitinase and a member

of the non-canonical Polycomb Repressive Complex (PRC) 1, which controls H2A ubiquitination levels [46,47], and might play an activating role with regards to gene expression. Both USP7 and USP11 interact with PRC1 [48], and these USPs might form a complex [49], suggesting possible inter-USP regulation, the implications of which would be interesting for USP biology. The physical interaction of USP7 and PRC1 occurs via bridging by SCML2, which connects USP7 to BMI1 and MEL18 [8]. More recent work revealed that the Elongin BC And Polycomb Repressive Complex 2-Associated Protein (EPOP), a modulator of PRC2, controls the levels of H2B ubiquitination via recruitment of USP7 and may regulate mechanisms of cancer proliferation [50]. These studies provide greater clarity into the structural basis of USP7's regulation of histone marks, while raising new insights that hint at possible redundancies or novel types of interactions.

USP7's role in maintenance methylation is an area of investigation. UHRF1 is an E3 ligase and essential component of DNA methylation machinery that binds hemimethylated CpG islands and recruits the maintenance methyltransferase DNMT1 to ensure propagation of DNA methylation patterns through replication [51,52]. UHRF1 is stabilized and conformationally opened by USP7, allowing for efficient H3K9me3 binding [9,53]. USP7 directly regulates DNMT1's DNA-binding ability [15,54], but there is disagreement over whether USP7 directly binds and stabilizes DNMT1 [55,56]. This may suggest that that UHRF1, USP7, and DNMT1 operate as an E3 ligase-USP7-target complex that forms at promoters of known tumor suppressors and represses them by increased DNA methylation and histone modifications [57]. Recently, it was also shown that M-phase CDK1-CCNB phosphorylates UHRF1's USP7 interacting domain, thus decreasing its interaction with USP7 and its stability in M phase [58], suggesting cell cycle control of methylation through USP7.

At the transcriptional level, USP7's pro-oncogenic role extends to its control of the activity of oncogenic transcription factors via levels and localization (Figure 1b). By reversing polyubiquitination, USP7 stabilizes transcription factors, leading to increased expression of their targets. For example, USP7 stabilizes NOTCH1 (a main oncogene in T cell leukemia) [59*,60], and leads to increased expression of NOTCH1 target genes. Upon USP7 inhibition, NOTCH1 polyubiquitination increases, leading to its degradation [59*]. Similar patterns are seen with the stabilization of N-myc [61**], c-Myc [62], FoxP3 [63], β -catenin [64], and both NF- κ B [65] and NEK2, a modulator of NF- κ B [66]. Not only does USP7 regulate transcription factors by altering their stability, but also by altering localization. By reversing monoubiquitination, USP7 regulates nuclear exclusion. As mentioned above, monoubiquitinated PTEN is deubiquitinated by USP7, causing PTEN's exclusion from the nucleus [31] and subsequent increase in PI3K/AKT/mTOR signaling [67]. Similarly, USP7 deubiquitination of FOXO4's monoubiquitinated, transcriptionally active form excludes it from the nucleus, eliminating its activity [14]. USP7's ability to reverse monoubiquitin and poly-ubiquitin marks confers versatility in regulating transcription factors, and the differences, if any, between altering the stability of a transcription factor by reversing polyubiquitination and changing its compartmentalization by reversing monoubiquitylation, are a subject for greater study.

Emerging aspects of USP7 biology in disease: USP7 regulation

USP7's ubiquitous and divergent functions have made it a fascinating subject of clinical research. Patient data and xenograft models reveal clear associations between USP7 expression and the progression or grade of several cancers, although the nature of USP7 as an oncogene or tumor suppressor appears dependent on cancer type [10,69–71] (Figure 2a). The factors that regulate USP7 activity are thus of inherent interest. DUB regulation in general has been reviewed in the past, focusing on protein interactors, subcellular localization, and post-translational modifications [72,73]. New developments with implications for disease involve transcriptional regulation and mutation status (Figure 2b). Key transcription factors that regulate USP7 include NOTCH1, FOXO6, and STAT3. The USP7 gene locus is bound by NOTCH1, which significantly upregulates USP7 levels and activity for USP7 in a subset of T-cell Acute Lymphoblastic Leukemia (T-ALL) cases [59*]. As USP7 controls NOTCH1 stability [59*], there may be a positive feedback loop between USP7 and NOTCH1 in T-ALL as suggested by Jin *et al.* [59*]. USP7 expression is also enhanced by FOXO6, which inhibits proliferation of lung carcinoma [74]. In colon cancer, STAT3 negatively regulates USP7 expression and protein levels [75]. These transcriptional changes regulate the levels of USP7. At the genetic level, USP7 nonsense mutations and chromosomal microdeletions suggest USP7 haploinsufficiency as a mechanism of pathogenesis for neurodevelopmental disorders in humans [76]. USP7 is also mutated in a significant portion of pediatric cancers (Figure 2b) [77,78], and it has just been shown that a group of fatal, relapsed leukemias with defects in DNA damage response can be defined by mutations in three genes, including USP7 [79]. Among the disease-associated mutations identified in a study of pediatric cancer genomes was USP7 D305G [77]. Recent work by Kategaya *et al.* demonstrated the role of this USP7 residue in distal K48 ubiquitin (exo-) cleavage [80**], suggesting that the mutation may alter USP7's ability to cleave K48 chains.

USP7 mutations also raise questions about the p53–MDM2 axis. The complex nature of USP7–MDM2–p53 axis, cancer-associated differences in p53 expression, H2Aub-associated gene expression, the presence of p53 mutations, and the oncogenic role of USP7 in some cancers add complexity that must be explored. Although it has been suggested that USP7 mutations may affect the polyubiquitination, and consequent degradation, of p53, research is required to clarify how USP7 mutations, or mutation-driven inactivation, may affect the p53–MDM2 axis. Potential context-specific roles for USP7 depending on p53 status are also possible, as we are beginning to see in Ewing's sarcoma [20**] and colorectal cancer [34*].

USP7 inhibition: the new frontier

USP7's disease relevance has prompted the development of DUB inhibitors that take advantage of the structural properties of deubiquitinases. The structural characteristics of DUBs and their catalytic cycle have been previously reviewed in detail [65]. The cysteine protease family of DUBs, which includes the USPs, relies on three crucial amino acid residues within a catalytic pocket [86]. When bound to the ubiquitin C-terminus, the USPs share sufficient homology that their catalytic regions can be superimposed [87]. However, in the absence of ubiquitin, different USPs display various conformations. For example, USP7

[88*] and USP14 [89] rest in catalytically inactive states, known as apo configurations, and only become active after binding to ubiquitin. For USP7, activation involves a conformational change that enables the interaction between histidine (H) 464, aspartate (D) 481, and the catalytic cysteine (C) 223 [83]. The presence or absence of apo configurations, as well as the protein sequences and structures around the catalytic pockets of the DUBs have facilitated the development of increasingly specific inhibitors.

HBX 41108, the first reversible, uncompetitive DUB inhibitor with selectivity for USP7, was able to stabilize p53 and p21 and inhibit HCT116 colon cancer cell growth [90]. Additional inhibitors with increased USP7 specificity (HBX 19818, 28258) were reported thereafter [91], along with several dual USP7/USP47 inhibitors [92]. P5091 suppressed *in vivo* tumor growth [30], caused apoptosis of Multiple Myeloma (MM) cells, and prolonged survival in xenograft models [93]. Optimization of P5091 led to P22077 [94] and P50429 [92]. P22077 downregulated USP7 targets MDM2, claspin, and Chk1 [95] and inhibited neuroblastoma growth [94], and P50429 inhibited HCT116 proliferation [92,96].

These first-generation compounds, with IC50s in the micromolar range and moderate selectivity, have formed the basis of tools for inhibiting USP7. These inhibitors, and particularly P5091 and P22077, have been shown to induce degradation of many USP7 substrates and phenocopy USP7 knockdown. In many cancers, expression of USP7's targets is associated with poor prognosis, and first-generation inhibitors are able to decrease tumor growth and even overcome drug resistance by inhibiting USP7. In Multiple Myeloma (MM), where patients with activated NFkB signaling though NEK2 have poorer outcomes, USP7 inhibitors effectively inhibit myeloma cell growth and overcome NEK2-induced and NEK2-acquired drug resistance in xenograft models [66]. Similarly, in CLL, where USP7 is upregulated and contributes to aberrant homologous recombination repair, USP7 inhibition induces tumor cell death due to the buildup of DNA damage and additionally sensitizes p53-defective, chemotherapy-resistant CLL to clinically achievable doses of chemotherapeutic agents in mouse xenograft models [97]. Targeting USP7 with first-generation inhibitors may have therapeutic potential even in tumors with defective p53 or ibrutinib resistance [97]. While effective, these nitrothiophene-based compounds have posed solubility challenges, prompting the optimization of P5091 to generate P217564 [98], another USP7/47 dual inhibitor that was recently used in studies on Foxp3 T-regulatory cells [98] and also in preclinical models of T-ALL, where it extended xenograft mouse survival and decreased tumor growth by reducing oncogenic NOTCH1 levels [59*].

The promise of these compounds and the desire to better understand the effects of USP7 inhibition have sparked research into the structural characteristics of USP7 and its inhibitors. Using nuclear magnetic resonance (NMR), Pozhidaeva *et al.* showed USP7's irreversible conformational change upon use of the first generation inhibitors P22077 and P50429 [88*]. Greater understanding of the structure has enabled researchers to develop new lead compounds with a variety of modes of action (Table 1).

Gavory *et al.* employed fragment-based methods and surface plasmon resonance (SPR) to identify noncompetitive inhibitors for USP7 that bind ~5.5 Å from the catalytic cysteine, with activity at nanomolar concentrations [99*]. Kategaya *et al.* used NMR screening and

structure-based design to develop GNE6640 and GNE6776, compounds that interfere with ubiquitin binding by non-covalently targeting USP7 12 Å away from the catalytic cysteine [80**]. This seminal study further showed USP7's binding preference for ubiquitin's free Lysine (Lys) 48 side chains over other configurations and the importance of USP7's Aspartate (D) 305 residue in ubiquitin cleavage. Lamberto *et al.* generated XL188 by improving the potency of a previous compound from Hybrigenics [100–102] with a structure guided approach and solving X-ray crystal structures of USP7-small molecule complexes [103*]. Turnbull *et al.* used a ubiquitin-rhodamine screening assay and SPR to evaluate the inhibitory potential of 500 000 compounds from FORMA Therapeutics, identifying a PyrZPPip-based covalent (FT671) and non-covalent inhibitor (FT827) that target a dynamic pocket near the catalytic residue [104**]. Each inhibitor has been validated *in vitro* and, in some cases, *in vivo*.

As with all inhibitors, toxicity is a concern when evaluating therapeutic potential. Genetic deletion of *Usp7* in mice results in early embryonic death between embryonic days E6.5 and E7.5 [13], suggesting that USP7 is essential for development at the embryonic stage. However, the breadth of *in vitro* studies on USP7 suggest that inhibition may be safe in non-embryonic tissues and cell lines. *In vivo* inhibitor studies also indicate that USP7 inhibition is tolerable at doses that are effective in tumor suppression, causing no significant weight loss and cachexia. Overcoming potential off-target effects or achieving synergistic treatment may also be possible with combined use of inhibitors at different concentrations. Each study has shown significant specificity of the compounds against a panel of deubiquitinases and other enzymes, but the specificity of the new compounds against related enzymes, including desumoylases and deneddylases, as well as improvements in the solubility and bioavailability of these compounds remain to be seen.

Concluding remarks

New aspects of USP7 biology have been recently elucidated, demonstrating a context-specific role for this enzyme as well as the potential of targeting the protein in certain types of cancer. USP7 interactions, as demonstrated here and by others [22], span the breadth of nuclear regulation. While USP7 is considered a predominantly nuclear DUB [22], some additional roles have been identified in other cellular compartments, for example in the endosome, where USP7 participates in another E3 ligase-target complex to regulate endosomal trafficking [76]. There is undoubtedly a need for continued mechanistic studies of USP7's ever-expanding list of functions. Different USP7 complexes, in the context of each environment, should be used to test the new inhibitors, which provide exciting prospects for understanding both nuclear and non-nuclear functions of USP7, as well as for inhibiting deubiquitination in a therapeutic context. Beyond the effects summarized above, USP7 inhibitors enhance chemotherapeutic efficacy and the cytotoxicity of targeted compounds, including PIM kinase inhibitors [80**], PARP inhibitors [106,107], and GSKJ4 inhibitor against the NOTCH1 partner JMJD3 [59*], further demonstrating the therapeutic prospects of USP7 inhibition.

Similarly, other DUBs should be studied for their potential roles in cell regulation and disease. USP7 is uniquely well-studied, both because it is widely expressed and because its

main substrates include classical tumor suppressors like p53 and PTEN. Other DUBs may also have notable roles in cancer and disease, and ongoing work on the activities of the USPs and DUBs in general [7] may pave the way for new discoveries in the biology of disease.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Komander D, Rape M: The ubiquitin code. *Annu Rev Biochem* 2012, 81:203–229. [PubMed: 22524316]
2. Schulman BA, Harper JW: Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. *Nat Rev Mol Cell Biol* 2009, 10:319–331. [PubMed: 19352404]
3. Ye Y, Rape M: Building ubiquitin chains: E2 enzymes at work. *Nat Rev Mol Cell Biol* 2009, 10:755–764. [PubMed: 19851334]
4. Yau R, Rape M: The increasing complexity of the ubiquitin code. *Nat Cell Biol* 2016, 18:579–586. [PubMed: 27230526]
5. Mevissen TET, Komander D: Mechanisms of deubiquitinase specificity and regulation. *Annu Rev Biochem* 2017, 86:159–192. [PubMed: 28498721]
6. Swatek KN, Komander D: Ubiquitin modifications. *Cell Res* 2016, 26:399–422. [PubMed: 27012465]
7. Sowa ME et al.: Defining the human deubiquitinating enzyme interaction landscape. *Cell* 2009, 138:389–403. [PubMed: 19615732]
8. Lecona E, Narendra V, Reinberg D: USP7 cooperates with SCML2 to regulate the activity of PRC1. *Mol Cell Biol* 2015, 35:1157–1168. [PubMed: 25605328]
9. Zhang ZM et al.: An allosteric interaction links USP7 to deubiquitination and chromatin targeting of UHRF1. *Cell Rep* 2015, 12:1400–1406. [PubMed: 26299963]
10. Song MS et al.: The deubiquitylation and localization of PTEN are regulated by a HAUSP-PML network. *Nature* 2008, 455:813–817. [PubMed: 18716620]
11. Li M et al.: A dynamic role of HAUSP in the p53-Mdm2 pathway. *Mol Cell* 2004, 13:879–886. [PubMed: 15053880]
12. Li M et al.: Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. *Nature* 2002, 416:648–653. [PubMed: 11923872]
13. Kon N et al.: Inactivation of HAUSP in vivo modulates p53 function. *Oncogene* 2010, 29:1270–1279. [PubMed: 19946331]
14. van der Horst A et al.: FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. *Nat Cell Biol* 2006, 8:1064–1073. [PubMed: 16964248]
15. Du Z et al.: DNMT1 stability is regulated by proteins coordinating deubiquitination and acetylation-driven ubiquitination. *Sci Signal* 2010, 3:ra80.

16. Espinosa JM: Histone H2B ubiquitination: the cancer connection. *Genes Dev* 2008, 22:2743–2749. [PubMed: 18923072]
17. Chen ST et al.: The deubiquitinating enzyme USP7 regulates androgen receptor activity by modulating its binding to chromatin. *J Biol Chem* 2015, 290:21713–21723.
18. Hermanns T et al. : A family of unconventional deubiquitinases with modular chain specificity determinants. *Nat Commun* 2018, 9:799. [PubMed: 29476094]
19. Lazo JS, Sharlow ER: Drugging undruggable molecular cancer targets. *Annu Rev Pharmacol Toxicol* 2016, 56:23–40. [PubMed: 26527069]
20. Stolte B et al. : Genome-scale CRISPR-Cas9 screen identifies druggable dependencies in TP53 wild-type Ewing sarcoma. *J Exp Med* 2018, 215:2137–2155. [PubMed: 30045945] ••In this study, the authors identified USP7 as a dependency in TP53 wildtype tumors. They demonstrated that inhibition of USP7 as a single therapy or in combination with MDM2 or another NFkB modulator (PPM1D) can be a valid therapeutic strategy in a subset of cancers.
21. Bhattacharya S et al. : Emerging insights into HAUSP (USP7) in physiology, cancer and other diseases. *Signal Transduct Target Ther* 2018, 3:17. [PubMed: 29967688]
22. Kim RQ, Sixma TK: Regulation of USP7: a high incidence of E3 complexes. *J Mol Biol* 2017, 429:3395–3408. [PubMed: 28591556]
23. Cummins JM et al. : Tumour suppression: disruption of HAUSP gene stabilizes p53. *Nature* 2004, 428 1 p following 486.
24. Lu Y et al.: USP19 deubiquitinating enzyme supports cell proliferation by stabilizing KPC1, a ubiquitin ligase for p27Kip1. *Mol Cell Biol* 2009, 29:547–558. [PubMed: 19015242]
25. Stegmeier F et al. : Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. *Nature* 2007, 446:876–881. [PubMed: 17443180]
26. Ventii KH, Wilkinson KD: Protein partners of deubiquitinating enzymes. *Biochem J* 2008, 414:161–175. [PubMed: 18687060]
27. Oda E et al.: Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science* 2000, 288:1053–1058. [PubMed: 10807576]
28. Hu T et al. : Targeting the overexpressed USP7 inhibits esophageal squamous cell carcinoma cell growth by inducing NOXA-mediated apoptosis. *Mol Carcinog* 2018, 58:42–54. [PubMed: 30182448]
29. Kon N et al. : Roles of HAUSP-mediated p53 regulation in central nervous system development. *Cell Death Differ* 2011, 18:1366–1375. [PubMed: 21350561]
30. An T et al.: USP7 inhibitor P5091 inhibits Wnt signaling and colorectal tumor growth. *Biochem Pharmacol* 2017, 131:29–39. [PubMed: 28216017]
31. Morotti A et al.: BCR-ABL disrupts PTEN nuclear-cytoplasmic shuttling through phosphorylation-dependent activation of HAUSP. *Leukemia* 2014, 28:1326–1333. [PubMed: 24317448]
32. Carra G et al.: Therapeutic inhibition of USP7-PTEN network in chronic lymphocytic leukemia: a strategy to overcome TP53 mutated/deleted clones. *Oncotarget* 2017, 8:35508–35522.
33. Khoronenkova SV et al. : ATM-dependent downregulation of USP7/HAUSP by PPM1G activates p53 response to DNA damage. *Mol Cell* 2012, 45:801–813. [PubMed: 22361354]
34. >Tavana O, Sun H, Gu W: Targeting HAUSP in both p53 wildtype and p53-mutant tumors. *Cell Cycle* 2018, 17:823–828. [PubMed: 29616860] • This work and the work by Stolte et al. collectively show that inhibitors of USP7 and MDM2 can synergistically activate p53 and induce p53-dependent apoptosis in human cancer cells. This work also shows the main role of USP7 in cancer in relation to the USP7- MDM2-p53 axis.
35. Qian J et al.: USP7 modulates UV-induced PCNA monoubiquitination by regulating DNA polymerase eta stability. *Oncogene* 2015, 34:4791–4796. [PubMed: 25435364]
36. Zlatanou A et al.: USP7 is essential for maintaining Rad18 stability and DNA damage tolerance. *Oncogene* 2016, 35:965–976. [PubMed: 25961918]
37. Andersen PL, Xu F, Xiao W: Eukaryotic DNA damage tolerance and translesion synthesis through covalent modifications of PCNA. *Cell Res* 2008, 18:162–173. [PubMed: 18157158]
38. Huang TT et al.: Regulation of monoubiquitinated PCNA by DUB autocleavage. *Nat Cell Biol* 2006, 8:339–347. [PubMed: 16531995]

39. Soria G et al. : DNA damage induced Pol eta recruitment takes place independently of the cell cycle phase. *Cell Cycle* 2009, 8:3340–3348. [PubMed: 19806028]
40. Kashiwaba S et al.: USP7 is a suppressor of PCNA ubiquitination and oxidative-stress-induced mutagenesis in human cells. *Cell Rep* 2015, 13:2072–2080. [PubMed: 26673319]
41. Zlatanou A, Stewart GS: Damaged replication forks tolerate USP7 to maintain genome stability. *Mol Cell Oncol* 2016, 3: e1063571.
42. Biswas K et al. : BRE/BRCC45 regulates CDC25A stability by recruiting USP7 in response to DNA damage. *Nat Commun* 2018, 9:537. [PubMed: 29416040]
43. Wang Q et al. : Stabilization of histone demethylase PHF8 by USP7 promotes breast carcinogenesis. *J Clin Invest* 2016, 126:2205–2220. [PubMed: 27183383]
44. Lecona E et al.: USP7 is a SUMO deubiquitinase essential for DNA replication. *Nat Struct Mol Biol* 2016, 23:270–277. [PubMed: 26950370]
45. Mungamuri SK et al.: USP7 enforces heterochromatinization of p53 target promoters by protecting SUV39H1 from MDM2- mediated degradation. *Cell Rep* 2016, 14:2528–2537. [PubMed: 26971997]
46. Vidal M, Starowicz K: Polycomb complexes PRC1 and their function in hematopoiesis. *Exp Hematol* 2017, 48:12–31. [PubMed: 28087428]
47. Hu B et al.: HSCARG, a novel regulator of H2A ubiquitination by downregulating PRC1 ubiquitin E3 ligase activity, is essential for cell proliferation. *Nucleic Acids Res* 2014, 42:5582–5593. [PubMed: 24711370]
48. Maertens GN et al. : Ubiquitin-specific proteases 7 and 11 modulate Polycomb regulation of the INK4a tumour suppressor. *EMBO J* 2010, 29:2553–2565. [PubMed: 20601937]
49. Georges A et al. : Identification and characterization of USP7 targets in cancer cells. *Sci Rep* 2018, 8:15833.
50. Liefke R, Karwacki-Neisius V, Shi Y: EPOP interacts with elongin BC and USP7 to modulate the chromatin landscape. *Mol Cell* 2015, 64:659–672.
51. Bostick M et al. : UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* 2007, 317:1760–1764. [PubMed: 17673620]
52. Sharif J et al. : The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 2007, 450:908–912.
53. Gao L et al.: An intramolecular interaction of UHRF1 reveals dual control for its histone association. *Structure* 2018, 26:304–311.e3.
54. Yamaguchi L et al. : Usp7-dependent histone H3 deubiquitylation regulates maintenance of DNA methylation. *Sci Rep* 2017, 7:55. [PubMed: 28246399]
55. Cheng J et al. : Molecular mechanism for USP7-mediated DNMT1 stabilization by acetylation. *Nat Commun* 2015, 6:7023. [PubMed: 25960197]
56. Yarychivska O et al.: Independent functions of DNMT1 and USP7 at replication foci. *Epigenetics Chromatin* 2018, 11:9. [PubMed: 29482658]
57. Beck A et al.: Overexpression of UHRF1 promotes silencing of tumor suppressor genes and predicts outcome in hepatoblastoma. *Clin Epigenetics* 2018, 10:27. [PubMed: 29507645]
58. Ma H et al. : M phase phosphorylation of the epigenetic regulator UHRF1 regulates its physical association with the deubiquitylase USP7 and stability. *Proc Natl Acad Sci USA* 2012, 109:4828–4833. [PubMed: 22411829]
59. Jin Q et al.: USP7 cooperates with NOTCH1 to drive the oncogenic transcriptional program in T cell leukemia. *Clin Cancer Res* 2018, 25:222–239. [PubMed: 30224337] • The authors identified USP7 as both a NOTCH1 target and regulator. They showed that USP7 can regulate NOTCH at both the transcriptional and post-translational levels, showing the multitude of ways that USP7 can regulate transcription factors, and in turn, transcriptional programs in disease. The authors also identified USP7 as a therapeutic target in T cell leukemia.
60. Shan H et al.: USP7 deubiquitinates and stabilizes NOTCH1 in T-cell acute lymphoblastic leukemia. *Signal Transduct Target Ther* 2018, 3:29. [PubMed: 30370059]
61. Tavana O et al.: HAUSP deubiquitinates and stabilizes N-Myc in neuroblastoma. *Nat Med* 2016, 22:1180–1186. [PubMed: 27618649] ••This work demonstrates a new, critical target for USP7 in

neuroblastoma, the oncogenic transcription factor N-Myc. These studies may also explain some of the phenotypic characteristics of Usp7 knockout animals, or the neurodegenerative phenotypes seen in Usp7 haploinsufficient patients.

62. Nicklas S et al. : A complex of the ubiquitin ligase TRIM32 and the deubiquitinase USP7 balances the level of c-Myc ubiquitination and thereby determines neural stem cell fate specification. *Cell Death Differ* 2018, 26:728–740. [PubMed: 29899379]
63. van Loosdregt J et al.: Stabilization of the transcription factor Foxp3 by the deubiquitinase USP7 increases Treg-cell- suppressive capacity. *Immunity* 2013, 39:259–271. [PubMed: 23973222]
64. Novellasdemunt L et al.: USP7 is a tumor-specific WNT activator for APC-mutated colorectal cancer by mediating beta-catenin deubiquitination. *Cell Rep* 2017, 21:612–627. [PubMed: 29045831]
65. Colleran A et al.: Deubiquitination of NF-kappaB by ubiquitin- specific protease-7 promotes transcription. *Proc Natl Acad Sci USA* 2013, 110:618–623. [PubMed: 23267096]
66. Franqui-Machin R et al.: Destabilizing NEK2 overcomes resistance to proteasome inhibition in multiple myeloma. *J Clin Invest* 2018, 128:2877–2893. [PubMed: 29863498]
67. Barata JT: The impact of PTEN regulation by CK2 on PI3K- dependent signaling and leukemia cell survival. *Adv Enzyme Regul* 2011, 51:37–49. [PubMed: 21035501]
68. Reddy BA et al.: Nucleotide biosynthetic enzyme GMP synthase is aTRIM21-controlled relay of p53 stabilization. *Mol Cell* 2014, 53:458–470. [PubMed: 24462112]
69. Bhattacharya S, Ghosh MK: HAUSP, a novel deubiquitinase for Rb - MDM2 the critical regulator. *FEBS J* 2014, 281:3061–3078. [PubMed: 24823443]
70. Cheng C et al.: Expression of HAUSP in gliomas correlates with disease progression and survival of patients. *Oncol Rep* 2013, 29:1730–1736. [PubMed: 23483195]
71. Masuya D et al.: The HAUSP gene plays an important role in non-small cell lung carcinogenesis through p53-dependent pathways. *J Pathol* 2006, 208:724–732. [PubMed: 16450335]
72. Nijman SM et al.: A genomic and functional inventory of deubiquitinating enzymes. *Cell* 2005, 123:773–786. [PubMed: 16325574]
73. Komander D, Clague MJ, Urbe S: Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol* 2009, 10:550–563. [PubMed: 19626045]
74. Hu HJ et al.: FoxO6 inhibits cell proliferation in lung carcinoma through up-regulation of USP7. *Mol Med Rep* 2015, 12:575–580. [PubMed: 25695151]
75. Yang Z et al.: STAT3 repressed USP7 expression is crucial for colon cancer development. *FEBS Lett* 2012, 586:3013–3017. [PubMed: 22750444]
76. Hao YH et al. : USP7 acts as a molecular rheostat to promote WASH-dependent endosomal protein recycling and is mutated in a human neurodevelopmental disorder. *Mol Cell* 2015, 59:956–969. [PubMed: 26365382]
77. Huether R et al.: The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. *Nat Commun* 2014, 5:3630. [PubMed: 24710217]
78. Liu Y et al. : The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. *Nat Genet* 2017, 49:1211–1218. [PubMed: 28671688]
79. Richter-Pechanska P et al. : Identification of a genetically defined ultra-high-risk group in relapsed pediatric T- lymphoblastic leukemia. *Blood Cancer J* 2017, 7:e523. [PubMed: 28157215]
80. Kategaya L et al.: USP7 small-molecule inhibitors interfere with ** ubiquitin binding. *Nature* 2017, 550:534–538. [PubMed: 29045385] ••In this seminal study, the authors used a crystal structure-based method to identify USP7 inhibitors. The inhibitors act non-covalently 12 Å away from the catalytic cysteine, and antagonize ubiquitin binding. These studies also show that USP7 has a preference for removing ubiquitin with free lysine 48 side chains, illuminating a potential role of D305, an amino acid mutated in leukemia, in the regulation of USP7's K48-related activity.
81. Sheng Y et al. : Molecular recognition of p53 and MDM2 by USP7/HAUSP. *Nat Struct Mol Biol* 2006, 13:285–291. [PubMed: 16474402]
82. Felle M et al.: The USP7/Dnmt1 complex stimulates the DNA methylation activity of Dnmt1 and regulates the stability of UHRF1. *Nucleic Acids Res* 2011, 39:8355–8365. [PubMed: 21745816]

83. Hu M et al.: Crystal structure of a UBP-family deubiquitinating enzyme in isolation and in complex with ubiquitin aldehyde. *Cell* 2002, 111:1041–1054. [PubMed: 12507430]
84. Ding X et al.: Mixed lineage leukemia 5 (MLL5) protein stability is cooperatively regulated by O-GlcNAc transferase (OGT) and ubiquitin specific protease 7 (USP7). *PLoS One* 2015, 10: e0145023.
85. Zhao Y et al. : Noncanonical regulation of alkylation damage resistance by the OTUD4 deubiquitinase. *EMBO J* 2015, 34:1687–1703. [PubMed: 25944111]
86. Storer AC, Menard R: Catalytic mechanism in papain family of cysteine peptidases. *Methods Enzymol* 1994, 244:486–500. [PubMed: 7845227]
87. Komander D, Barford D: Structure of the A20 OTU domain and mechanistic insights into deubiquitination. *Biochem J* 2008, 409:77–85. [PubMed: 17961127]
88. Pozhidaeva A et al.: USP7-specific inhibitors target and modify the enzyme's active site via distinct chemical mechanisms. *Cell Chem Biol* 2017, 24:1501–1512.e5. • Here, the authors demonstrated that ubiquitinated substrates are required to change USP7's conformation from the inactive (apo) to the catalytically active conformation, a shift that was previously thought to rely solely upon the presence of ubiquitin. They also showed the mechanism of P22077 and P50429 inhibition of USP7, providing a structural basis for the actions of first-generation inhibitors.
89. Hu M et al.: Structure and mechanisms of the proteasome- associated deubiquitinating enzyme USP14. *EMBO J* 2005, 24:3747–3756. [PubMed: 16211010]
90. Colland F et al.: Small-molecule inhibitor of USP7/HAUSP ubiquitin protease stabilizes and activates p53 in cells. *Mol Cancer Ther* 2009, 8:2286–2295. [PubMed: 19671755]
91. Reverdy C et al.: Discovery of specific inhibitors of human USP7/HAUSP deubiquitinating enzyme. *Chem Biol* 2012, 19:467–477. [PubMed: 22520753]
92. Weinstock J et al.: Selective dual inhibitors of the cancer- related deubiquitylating proteases USP7 and USP47. *ACS Med Chem Lett* 2012, 3:789–792. [PubMed: 24900381]
93. Chauhan D et al.: A small molecule inhibitor of ubiquitin- specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance. *Cancer Cell* 2012, 22:345–358. [PubMed: 22975377]
94. Fan YH et al.: USP7 inhibitor P22077 inhibits neuroblastoma growth via inducing p53-mediated apoptosis. *Cell Death Dis* 2013, 4:e867. [PubMed: 24136231]
95. Altun M et al.: Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes. *Chem Biol* 2011, 18:1401–1412. [PubMed: 22118674]
96. Cao P, et al. Patent: Anti-neoplastic compounds, compositions and methods. 2009, Progenra (Malvern, PA, US): United States.
97. Agathangelou A et al.: USP7 inhibition alters homologous recombination repair and targets CLL cells independently of ATM/p53 functional status. *Blood* 2017, 130:156–166. [PubMed: 28495793]
98. Wang F et al.: Active site-targeted covalent irreversible inhibitors of USP7 impair the functions of Foxp3+ T-regulatory cells by promoting ubiquitination of Tip60. *PLoS One* 2017, 12: e0189744.
99. Gavory G et al.: Discovery and characterization of highly potent and selective allosteric USP7 inhibitors. *Nat Chem Biol* 2018, 14:118–125. [PubMed: 29200206] •The authors used the UbiPlex platform and structural studies to discover new compounds that are active at the nanomolar range of concentrations, have efficacies comparable to MDM2 inhibitors, and bind to a previously unidentified exosite ~5.5Å from the catalytic triad.
100. Ritorto MS et al.: Screening of DUB activity and specificity by MALDI-TOF mass spectrometry. *Nat Commun* 2014, 5:4763. [PubMed: 25159004]
101. Kessler BM: Selective and reversible inhibitors of ubiquitin- specific protease 7: a patent evaluation (WO2013030218). *Expert Opin Ther Pat* 2014, 24:597–602. [PubMed: 24456106]
102. Colland F, Gourdel M-E, Patent: Selective and reversible inhibitors of ubiquitin specific protease 7, W.I.P. Organization: Hybrigenics SA; 2011.
103. Lamberto I et al. : Structure-guided development of a potent and selective non-covalent active-site inhibitor of USP7. *Cell Chem Biol* 2017, 24:1490–1500.e11. • The authors used a structure-guided approach with NMR and mass- spectrometry to optimize previous inhibitors. They identified XL188, a non-covalent, allosteric second-generation USP7 inhibitor.

104. Turnbull AP et al. : Molecular basis of USP7 inhibition by selective small-molecule inhibitors. *Nature* 2017, 550:481–486. [PubMed: 29045389] •• Turnbull et al. ran a comprehensive structure-based screen of 500 000 compounds from FORMA Therapeutics, using a ubiquitin-rhodamine screening assay and SPR to identify inhibitors that bind a dynamic pocket characteristic of the inactive (apo) configuration of USP7.
105. Chen C et al.: Synthesis and biological evaluation of thiazole derivatives as novel USP7 inhibitors. *Bioorg Med Chem Lett* 2016, 27:845–849.
106. Malapelle U et al. : USP7 inhibitors, downregulating CCDC6, sensitize lung neuroendocrine cancer cells to PARP-inhibitor drugs. *Lung Cancer* 2017, 107:41–49. [PubMed: 27372520]
107. Morra F et al. : The combined effect of USP7 inhibitors and PARP inhibitors in hormone-sensitive and castration-resistant prostate cancer cells. *Oncotarget* 2017, 8:31815–31829.

Figure 1.

Landscape of USP7 targets and modes of action.

(a) The USP7–MDM2–p53 axis. Under unstressed conditions, USP7 stabilizes MDM2, resulting in p53 turnover [11,23]. Upon cell stress, guanosine monophosphate synthetase (GMPS) and TRIM21 dissociate in the cytoplasm, resulting in GMPS translocation to the nucleus [68], where it disrupts the interaction between USP7 and MDM2. GMPS displaces MDM2 in the USP7–MDM2–P53 complex and allosterically activates USP7. This activation both upregulates USP7's deubiquitinase activity and prompts USP7 to 'switch' from deubiquitinating MDM2 to deubiquitinating, and thus stabilizing, p53 [12].

(b) USP7 regulates transcription factors. When reversing polyubiquitination, USP7 confers stability. USP7 binds to the androgen receptor (AR), and upon stimulation with androgens, allows AR to bind DNA in prostate cancer [17]. USP7 also deubiquitinates NOTCH1, stabilizing it and activating the NOTCH pathway. Similarly, USP7 stabilizes many other transcription factors including N-myc in neuroblastoma [61**], c-Myc in neural stem cell fate specification [62], FoxP3 to maintain T-regulatory cell function [63], β -catenin for Wnt activation in colorectal cancer [64], and NF- κ B [65] and its modulator NEK2 [66]. Separately, USP7's reversal of monoubiquitination controls the activity of transcription factors through nuclear exclusion, notably for FOXO4 [14] and PTEN [31].

Figure 2.
Regulation of USP7 in disease.

(a) In disease. USP7 levels are associated with disease. While elevated levels of USP7 are observed in prostate cancer and glioma [10,69,70], reduced expression is seen in non-small cell lung cancers and some colon cancers [71]. Further research is needed to examine how USP7's disease-specific roles and regulation can explain these differences and their impact on disease. Note: these survival curves are illustrative of trends and not numerical data.

(b) Transcriptional regulation. At the USP7 promoter, NOTCH1 [59^{*}] and FOXO6 [74] activate USP7 transcription, and STAT3 [75] represses USP7 transcription.

(c) Genetic variation. Select USP7 mutations and their effects are shown. D164A, W165A [81]; C223A [10–12,14,58,82,83]; C223S [10–12,14,58,82–85]; D305G [77,80^{**}]; H464A [83].

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Table 1

New lead compounds for USP7 inhibition

Inhibitor	Mechanism	Validation	IC50 (range)	Citations
Thiazole derivatives C1–21	C7 and C19 are the most potent of the set. C7 may bind the ubiquitin-binding pocket of USP7's catalytic domain and competitively inhibit USP7-ubiquitin binding.	- HCT116 cell line: decreases proliferation. - Some are more potent than P5091 at destabilizing N-myc [34*]	Low micromolar	Progenra Inc. [105,34*]
1–4	Reversible, non-competitive inhibitors that bind an exosite 5.5 Å away from Cys223, and may partially occlude the channel occupied by ubiquitin's C-terminal	- HCT116, MCF7 cell lines: increases p53, decreases MDM2 levels. - RS4; 11, LNCaP cell lines: stabilizes p53, p21. - Cell-line dependent effects on apoptosis (Jurkat EC50: 2 nM, LNCaP not significant)	Nanomolar	Almac discovery [99*]
GNE-6640, GNE-6776	Non-covalent inhibitors that bind 12 Å from USP7's catalytic cysteine, prevent USP7 activation by preventing a conformation change of the α5 helix and by sterically inhibiting ubiquitin binding, and interact with acidic residues that mediate USP7's interactions with ubiquitin Lysine (Lys) 48 side chains.	- GNE6640: decreased viability of 108 out of 441 cell lines screened. GNE 6776 decreased viability of 6 out of 181. - <i>In vivo</i> , 6640 is orally bioavailable, and promotes on-target pathway modulation, and xenograft growth delay. - Combining 6640 with doxorubicin and cisplatin increases inhibitor efficacy.	Low micromolar	Genentech [80**]
XL188	Selective, non-covalent active-site allosteric inhibitor accompanied by its inactive enantiomer, paired for control studies. Occupies a subsite approximately 5 Å from the catalytic triad.	- MCF7, MM.1S cell lines: XL188 increases p53 and p21 levels, decreases MDM2 levels.	Nanomolar	Dana Farber [103**]
FT671, FT827	Competitive inhibitors that bind the inactive (apo) form of USP7 at the ubiquitin-binding site.	FT671: - HCT116, U20S, MM.1S cell lines: stabilizes p53, increases expression of p53 target genes, induces p21, promotes growth arrest and apoptosis. - FT671 in IMR-32 (neuroblastoma cell line); promotes n-MYC degradation, p53 upregulation, degradation of UHRF1 and DNMT1. - MM.1S xenograft mouse: significant dose-dependent inhibition of tumor growth, well tolerated with no significant weight loss or cachexia.	Nanomolar	Forma therapeutics [104*]