Unraveling the Mechanism of Action of Ubiquitin-Specific Protease 5 and Its Inhibitors in Tumors

Clinical Medicine Insights: Oncology
Volume 18: 1–11
© The Author(s) 2024
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/11795549241281932

Jie Wang*, Shizhen Fang*, Yang Jiang and Qingquan Hua

Department of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University, Wuhan, China.

ABSTRACT: Ubiquitin-specific protease 5 (USP5), a member of the ubiquitin-specific proteases (USPs) family, functions by specifically removing ubiquitin chains from target proteins for stabilization and degrading unbound polyubiquitin chains to maintain a steady-state monoubiquitin pool. Ubiquitin-specific protease 5 regulates various cellular activities, including DNA double-strand break repair, transmission of neuropathic and inflammatory pain signals, immune response, and tumor cell proliferation. Furthermore, USP5 is involved in the development of multiple tumors such as liver, lung, pancreatic, and breast cancers as well as melanoma. Downstream regulatory mechanisms associated with USP5 are complex and diverse. Ubiquitin-specific protease 5 has been revealed as an emerging target for tumor treatment. This study has introduced some molecules upstream to control the expression of USP5 at the levels of transcription, translation, and post-translation. Furthermore, the study incorporated inhibitors known to be associated with USP5, including partially selective deubiquitinase (DUB) inhibitors such as WP1130, EOAI3402143, vialinin A, and chalcone derivatives. It also included the ubiquitin-activating enzyme E1 inhibitor, PYR-41. These small molecule inhibitors impact the occurrence and development of various tumors. Therefore, this article comprehensively reviews the pivotal role of USP5 in different signaling pathways during tumor progression and resumes the progress made in developing USP5 inhibitors, providing a theoretical foundation for their clinical translation.

KEYWORDS: Ubiquitin-specific protease 5, ubiquitination, deubiquitination, tumor, inhibitor

RECEIVED: March 3, 2024. **ACCEPTED:** August 21, 2024.

TYPE: Emerging Next Generation Biomarkers in Cancer – Review

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Health Commission of Hubei Province (WJ2023M175).

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHORS: Yang Jiang, Department of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University, 238 Jie-Fang Road, Wuhan 430060, Hubei, China. Email: yangj_1977@163.com

Qingquan Hua, Department of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University, 238 Jie-Fang Road, Wuhan 430060, Hubei, China. Email: RM001637@whu.edu.cn

Introduction

Ubiquitination, a crucial post-translational protein modification in tumor cells, regulates various physiological functions and pathological processes such as cell cycle progression, signal transduction, and carcinogenesis.[1,2] Ubiquitination primarily involves three classes of enzymes: ubiquitin-activating enzyme E1, ubiquitin-conjugating enzyme E2, and ubiquitin ligase E3.[3] The assembly of ubiquitin into mixed chains, including seven lysine (Lys) residues and N-terminal methionine (Met1) determines substrate protein fate, including degradation, specific interactions, transport, and activity regulation. Ubiquitination, along with its counterpart process—deubiquitination—establishes a dynamic equilibrium, maintaining cellular homeostasis by governing protein degradation and stability.[4] Approximately 100 deubiquitinating enzymes in the human genome fall into seven homologous families based on differences in amino acid sequence and structure: ubiquitin-specific proteases (USPs), ubiquitin C-terminal hydrolases (UCHs), Otubain proteases (OTUs), Machado-Joseph disease proteases (MJDs), MINDYs protease family, ZUP1 protease family, and AB1/MPN/Mov34 metalloenzymes (JAMMs).[5–9] Except for JAMMs, which are metalloproteases, all other deubiquitinases belong to the cysteine protease family.[10]

The functions of deubiquitinases in the human body can be categorized into three groups: removing monoubiquitin from substrate proteins, eliminating polyubiquitin chains from post-translationally modified protein substrates, resulting in a reversal of ubiquitin signaling, and altering ubiquitin modification by trimming ubiquitin chains.[11] Ubiquitin-specific protease 5 (USP5), also known as isopeptidase T (ISOT), is a cysteine deubiquitinating enzyme in the USP family.[12]

Increasing evidence indicates the involvement of USP5 in various cellular processes such as DNA repair,[13] stress response,[14] and inflammation.[15] Moreover, it is closely associated with tumor occurrence and development,[16] promoting the biological characteristics of multiple tumors, including drug resistance. This review focuses on elucidating the structure and function of USP5 in cancer, exploring its targeted inhibitors, and discussing their therapeutic potential.

Landscape of USP5

Ubiquitin-specific protease 5 was initially identified in 1995[12] and subsequently recognized as a functional homolog of USP14 in 1997.[17] This gene generates three isoforms of USP5: ISOT-S (initially referred to as ISOT, later renamed USP5), ISOT-L, and ISOT-3. The ISOT-S and ISOT-L exhibit a different splicing pattern of the 15th exon. The ISOT-L, unlike ISOT-S, presents a deletion of 23 amino acids in its ubiquitin-binding domain.[18] Ubiquitin-specific protease 5 is highly conserved between humans and mice, with a similarity of 98.7%. In humans, it has a molecular weight of 93.3 kDa.[19] Its

*Jie Wang and Shizhen Fang contributed equally.

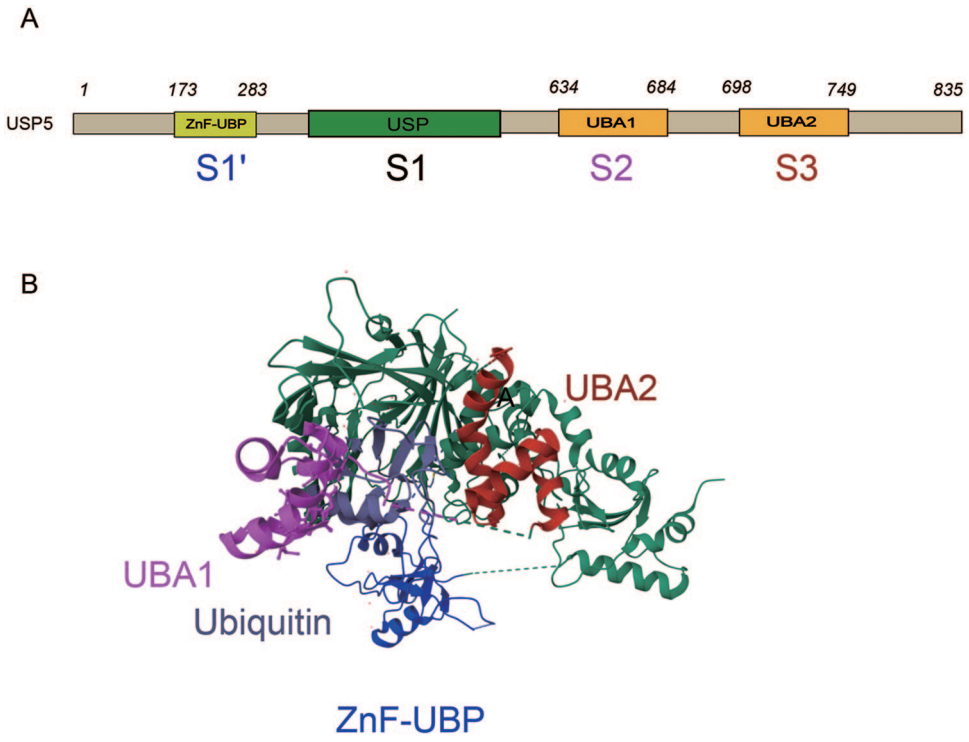

Figure 1. Schematic representation of the USP5 domains.
(A) USP5 domain structure with ubiquitin binding site.[24,25] Adapted from: Reyes-Turcu et al.[24] Licensed under CC-BY 4.0. And Ning et al.[25] With permission from Elsevier. ZnF-UBP domain (residues 173-283) is shown in yellow at S1´ binding site. S1 site is in USP domain colored green. S2 site is in UBA1 domain (residues 634-684) colored orange. S3 site is in UBA2 domain (residues 698-749) colored orange. (B) 3D structure of covalent ubiquitin-USP5 complex (PDB ID:3ihp https://www.rcsb.org/structure/3ihp). Ubiquitin molecules is shown in purple. ZnF-UBP is shown in blue. UBA1 is shown in purplish red. UBA2 is shown in red.

encoding gene is located on chromosome 12p13, near the human *CD4* gene.[20]

Unlike most deubiquitinases that exhibit substrate-dependent enzymatic activity, USP5 uniquely removes ubiquitin molecules directly from free polyubiquitin chains, stabilizing the free ubiquitin pool. Extensive studies confirm the ability of USP5 to cleave five types of linkages: K6, K29, K48, K63, and linear linkages. Notably, USP5 exhibits a higher affinity for K48-linked polyubiquitin chains and a lower affinity for linear ubiquitin chains and those linked to K63.[19,21]

Ubiquitin-specific protease 5 is a multi-domain enzyme with 835 residues.[22] Ubiquitin-specific protease 5 contains at least four ubiquitin binding sites[4,22,23] (S1´, S1, S2, and S3). The S1´ site binds to the zinc finger ubiquitin-specific protease (ZnF-UBP) domain (residues 173-283), the S1 site binds to ubiquitin—specific processing protease (USP), the S2 and S3 sites bind to two ubiquitin-associated (UBA) domains (residues 634-684 and 698-749)[24] (see Figure 1). The ZnF-UBP domain is involved in substrate affinity and specificity.[22] The ZnF-UBP domain recognizes ubiquitin, binding to its di-glycine motifs and facilitating proximal cleavage. The USP domain does not directly bind to ubiquitin but enhances the catalytic activity of USP5 through conformational effects. The UBA domains interact with distal ubiquitin via the S2 and S3 sites. These domains, connected through flexible peptide loops, enable USP5 to cleave various types of polyubiquitin chains.[24,26,27]

The cBioPortal online platform ((https://www.cbioportal.org/), utilizing The Cancer Genome Atlas (TCGA) data, demonstrated the genetic mutations of USP5 across various types of cancer. The top five most frequent alterations in the *USP5* gene were found in uterine corpus endometrial carcinoma (UCEC), uterine carcinosarcoma (UCS), ovarian serous cystadenocarcinoma (OV), skin cutaneous melanoma (SKCM), and testicular germ cell tumors (TGCT). The most common forms of genetic change detected in the *USP5* gene were mutation, amplification, and deep deletion. In UCEC, USP5 had the highest frequency of *USP5* gene mutation (6.05%), and in UCS, USP5 had the highest frequency of gene amplification (7.02%). The specific mutations can be shown in Figure 2.

Multiple molecules located upstream directly or indirectly control the expression of USP5. The E2F1 acts as a transcription factor that positively regulates the expression of USP5 in mesenchymal glioma stem cells (MES GSCs) at the transcriptional level, as determined by dual luciferase reporter gene analysis.[28] Ubiquitin-specific protease 5 is targeted and negatively regulated by miR-1256.[29] MiR-23a-3p specifically acts on USP5, leading to decreased expression and perhaps enhancing the relief of inflammatory pain *via* modulating the HDAC2/NRF2 pathway.[30] At the translational level, METTL5 promotes USP5 translation in an m6A-dependent manner.[31] In addition, post-translational modifications, including ubiquitination and SUMOylation, also have an impact on USP5. Smurf1, a HECT-type E3 ligase, facilitates the ubiquitination of USP5 and suppresses the production of Tumor Necrosis Factor-alpha (TNF-α).[32] The interaction

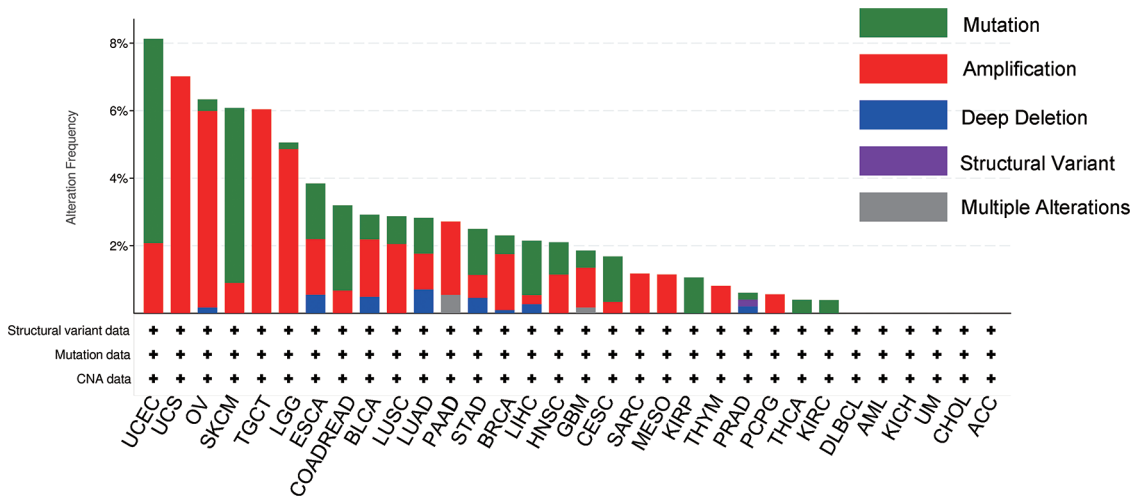

Figure 2. *USP5* genetic alteration in TCGA PanCancer Atlas (https://www.cbioportal.org/).

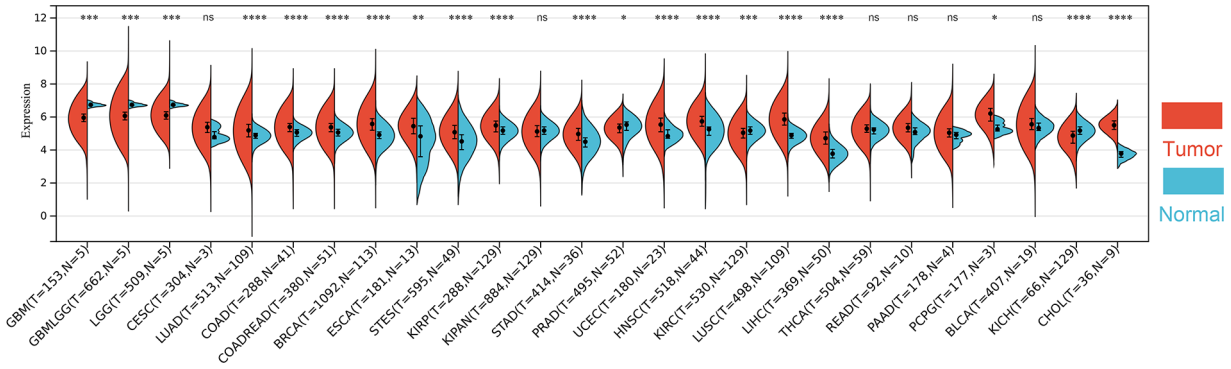

Figure 3. USP5 is aberrantly expressed in different tumors.
The data were analyzed using the SangerBox online analysis platform (http://sangerbox.com/home.html) based on TCGA Pan-Cancer from the UCSC database (https://xenabrowser.net/). USP5 is upregulated in 14 types of tumors (LUAD, COAD, COADREAD, BRCA, ESCA, STES, KIRP, TAD, UCEC, HNSC, LUSC, LIHC, PCPG, CHOL). USP5 is downregulated in six types of tumors (GBM, GBMLGG, LGG, PRAD, KIRC, KICH). Non-paired Wilcoxon Rank Sum and Signed Rank Tests were employed for significance analysis. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$; ns, not significant. BRCA indicates breast invasive carcinoma; BLCA, bladder urothelial carcinoma; CESC, cervical squamous cell Carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; COADREAD, colorectal adenocarcinoma; ESCA, esophageal adenocarcinoma; GBM, glioblastoma multiforme; GBMLGG, glioblastoma and low-grade glioma; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIPAN, pan-kidney cohort; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocacinoma; STAD, stomach adenocarcinoma; STES, stomach and esophageal carcinoma; UCEC, uterine corpus endometrial carcinoma; THCA, thyroid carcinoma.

between USP5 and Cav3.2 calcium channels can be influenced by the SUMOylation state of USP5. When the SUMOylation of USP5 is decreased, there is an increase in the interactions between USP5 and Cav3.2 calcium channels.[33] The KRas indirectly regulates USP5 activity by upregulating cellular ROS levels, leading to the formation of USP5 homodimers, enhanced USP5 enzymatic activity, and the accumulation of USP5 protein.[34] Long non-coding RNAs (LncRNAs) interact with specific proteins to regulate downstream effectors. Ubiquitin-specific protease 5 is the specific RNA-binding protein (RBP) for LOC85009. It inhibits USF1 ubiquitination, while LOC85009 sequesters USP5, causing the USF1 protein to destabilize.[35] The LNC01468 had a high affinity for USP5, attracting it to deubiquitylate PAI1 and inhibit its degradation.[36] The LncRNA MAFG-AS1 functions to stabilize Hu antigen R by recruiting USP5, thereby preventing the degradation process.[37]

Underlying Mechanism Associated to USP5 in Human Cancer

Based on the TCGA Pan-Cancer data from the UCSC database (https://xenabrowser.net/), aberrant mRNA expression was observed in 20 tumors, with 14 demonstrating upregulation and 6 showing downregulation compared with normal tissues (see Figure 3). The role of USP5 in tumors is underscored by a study by Meyers et al.[38] In a whole-genome CRISPR-Cas9 knockout screening of 804 tumor cell lines across 28 cancer types, *USP36* and *USP5* emerged as the most crucial tumor-related genes within the USPs family. Ubiquitin-specific protease 5 regulates the stability of downstream proteins such as p53,[21] FoxM1,[39] β-catenin,[40] HDAC2,[30] and TUFM[41] (see Figure 4), promoting or inhibiting effects on cancer development across various tumors. Research indicates that USP5 promotes the occurrence and progression of liver, lung, breast, ovarian, and colorectal cancers[41-48] (see Figure 3), while

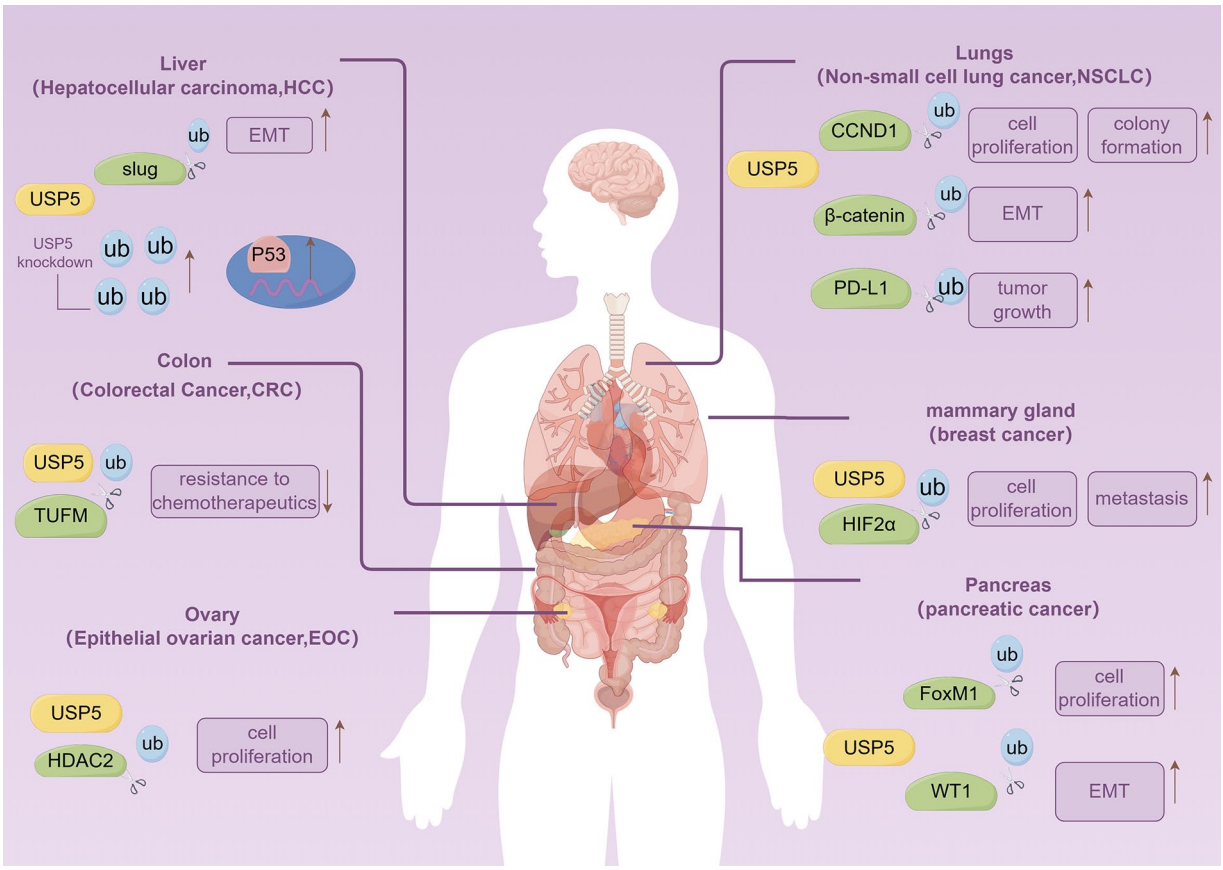

Figure 4. The role of USP5 in tumors (by Figdraw2.0).
The downstream proteins regulated by USP5 are depicted in green, including slug, TUFM, HDAC2, CCND1, β-catenin, PD-L1, HIF2α, FoxM1, and WT1. Ubiquitin (Ub) is indicated in blue. Tumor phenotypes are showed in purple. The dark blue region indicates an increase in p53 transcriptional activity after the knockdown of USP5.

simultaneously exhibiting inhibitory effects on melanoma tumor growth.[14,49]

The role of USP5 in the proliferation of cancer

Lung cancer cells exhibit rapid growth and high proliferation rates, which are closely related to short cell cycle intervals.[50] D-type cyclins, including cyclin D1 (CCND1), cyclin D2 (CCND2), and cyclin D3 (CCND3), crucially interact with cyclin-dependent kinases 4/6 (CDK4/6) in lung cancer, activating them.[51-53] In humans, these cyclins are encoded by the cyclin D geneIn lung cancer, elevated levels of CCND1 promote carcinogenesis, cell cycle progression, metastasis, and drug resistance.[54] The USP5 binds to CCND1, reducing its polyubiquitination, stabilizing it, and prolonging its half-life. This promotes non-small cell lung cancer (NSCLC) cell proliferation and colony formation.[55] Thus, inhibiting USP5 expression can lead to CCND1 degradation in NSCLC cells and cell cycle arrest. In lung cancer cells, epithelial–mesenchymal transition (EMT) causes loss of intercellular adhesion and cellular polarity, increases the expression of mesenchymal markers and migration ability, induces resistance to apoptosis, and promotes the production of extracellular matrix components. Ubiquitin-specific protease 5 induces EMT through the glycogen synthase kinase 3-mediated activation of the Wingless/integrated/β-catenin pathway.[40] Hepatocellular carcinoma (HCC) is the most prevalent malignant liver tumor. p53 is a classic regulator of cell proliferation and has been associated with carcinogenesis,[56] modulating cell cycle arrest and apoptosis. Both p53 and its inhibitor Mdm2 undergo ubiquitination and proteasomal degradation.[57] In general, deubiquitinases regulate the stability of p53 by removing ubiquitin from substrate proteins or stabilizing E3 ligases. Ubiquitin-specific protease 5 removes single ubiquitin chains from unanchored substrates with polyubiquitin chains at their proximal ends. Inhibiting USP5 expression allows polyubiquitin chains to competitively bind to proteasomes, preventing p53 degradation.[21] In addition, inhibition of USP5 activates the p14 ARF/p53 signaling pathway in HCC cells, indicating USP5 as a potential therapeutic target for HCC treatment.[45]

Pancreatic ductal adenocarcinoma (PDAC) is the most aggressive human malignant tumor, with a 5-year overall survival rate of only 6%.[58] FoxM1 belongs to the Forkhead transcription factor superfamily, promoting pancreatic cancer cell proliferation and progression.[39] In addition, USP5 expression is significantly upregulated within pancreatic cancer cell lines, enhancing FoxM1 stability and extending its half-life.[48] Treatment with MG-132, a proteasome inhibitor, restores FoxM1 expression levels. Furthermore, inhibiting USP5 expression accelerates the FoxM1 degradation rate, ultimately

slowing cellular proliferation in pancreatic cancer cell lines and growth in xenograft tumor mouse models.

Hypoxia-inducible factor 2α (HIF2α) is pivotal in breast tumor growth and metastasis.[59] Ubiquitin-specific protease 5 interacts with HIF2α within breast tumors, stabilizing and preventing its ubiquitin-proteasome degradation. Consequently, this stabilization promotes the transcription of HIF2α target genes such as *SLC2A1*, *PLOD2*, *P4HA1*, and *VEGFA*.[46] Suppressing USP5 expression attenuates breast cancer cell proliferation, colony formation. Targeting USP5, by enhancing the stability of HIF2α may serve as a promising therapeutic strategy against breast cancer.

Epithelial ovarian cancer (EOC) is a prevalent gynecological malignancy with a typically unfavorable prognosis.[43] The USP5 expression is upregulated in ovarian cancer, exhibiting a negative correlation with the prognosis of patients with serous ovarian carcinoma. An isoform of histone deacetylase 2 (HDAC2), serves as a cellular adaptive factor governing cell cycle progression *in vitro* and metastasis *in vivo*.[60] Suppressing USP5 expression reduces post-translational HDAC2 levels, enhancing the transcriptional activity of p27,[61] a crucial cell cycle inhibitor. This induces G0/G1 phase arrest in ovarian cancer cells and inhibits xenograft growth in nude mice. These findings suggest that USP5 promotes ovarian cancer and may be involved in its regulatory mechanisms.

The role of USP5 in the migration of cancer cells

Hepatocellular carcinoma is closely linked to EMT.[62,63] Epithelial–mesenchymal transition enables tumor cells to acquire an invasive mesenchymal phenotype, enhancing their migratory capacity. Consequently, inhibiting EMT represents a crucial strategy for suppressing tumor metastasis. In HCC cells, high USP5 expression levels are significantly associated with tumor malignancy and pathological grading.[47] The zinc finger transcription factor SLUG inhibits E-cadherin transcription by binding to E-box elements in the proximal promoter region during the EMT.[64-67] Aberrant SLUG expression, observed in various cancers, regulates tumor cell invasion and metastasis.[68] In liver cancer cells, the overexpression of SLUG indicates the presence of a stabilizing factor.[47] Through pull-down and mass spectrometry analysis, an interaction between USP5 and SLUG has been identified in liver cancer cells, inhibiting SLUG ubiquitination and stabilizing it. Knocking down USP5 increases the degradation of SLUG in HCC, suppressing EMT and liver cancer cell metastasis. Furthermore, inhibiting USP5 expression causes cell cycle arrest at the G1 phase, inhibiting tumor growth in liver cancer cells.[45]

As an oncogene, WT1 plays a pivotal role in regulating downstream proteins including cyclin D1, B-cell lymphoma 2 (Bcl-2), and E-cadherin, enhancing pancreatic cancer cell proliferation, inhibiting cell apoptosis, and promoting cancer cell metastasis.[69-71] Overexpression of USP5 was shown to deubiquitinate WT1 protein and stabilize its expression *in vivo*. This stabilization reduces E-cadherin expression while promoting pancreatic cancer metastasis.[72] Moreover, USP5 also activates the STAT3 signaling pathway, facilitating the occurrence and progression of pancreatic cancer.[42] Thus, targeting USP5 emerges as a potential therapeutic strategy for treating pancreatic cancer.

The role of USP5 in patients' prognosis

The rate of recurrence and clinical staging determines the prognosis of cancer. Elevated levels of USP5 expression have been identified as a negative prognostic indicator for pancreatic cancer, leading to a reduced overall survival time in patients.[48] The study revealed that USP5 interacts with c-Maf, protecting it from degradation by decreasing ubiquitination. This interaction eventually results in the extended life of multiple myeloma cells and accelerates the course of the disease.[73] Moreover, it has been demonstrated that USP5 promotes bladder urothelial carcinoma growth, which is associated with a poor prognosis for patients.[37] Ubiquitin-specific protease 5 also exhibits high expression in NSCLC and is correlated with overall survival and metastasis.[74] The LncRNA LINC01468 plays a role in the progression of lung adenocarcinoma (LUAD). It interacts with USP5 to inhibit ubiquitination-mediated degradation of the plasminogen activator inhibitor-1 (PAI1) protein, thereby stabilizing its expression in lung cancer. This suggests that USP5 may provide new insights into the mechanisms driving the progression of lung cancer.[36]

The role of USP5 in immunity of cancer

Programmed cell death ligand 1 (PD-L1; CD274) is a widely recognized immune inhibitory molecule that enables cancer cells to evade immune surveillance and serves as a key target for cancer immunotherapy.[75,76] T-cell immunity selectively recognizes and eliminates pathogens and abnormal cells, including cancer cells.[75] The interaction between PD-L1 and PD-1 leads to the dephosphorylation of the T-cell receptor.[77] PD-L1 is a crucial immune checkpoint protein that hinders the ability of T-cells to eliminate cancer cells by diminishing their proliferation and activity. Elevated levels of PD-L1 protein expression have been detected in many cancer types, resulting in the facilitation of immune evasion in cancer cells.[78] Antibodies that specifically target PD-L1 have significantly transformed the approach to treating advanced-stage malignancies, including melanoma, lung cancer, breast cancer, kidney cancer, and other types of cancer.[79] Ubiquitin-specific protease 5 directly interacts with PD-L1 and removes its polyubiquitin chains, increasing its stability, preventing its degradation, and increasing its protein level.[44] In NSCLC tissues, the expression of USP5 is elevated and positively correlates with the level of PD-L1, a correlation closely associated with poor prognosis. Knockdown of USP5 delayed tumor growth in a mouse model of lung cancer. Notably, two isoforms of USP5 have been identified in

Table 1. Small molecule inhibitors of USP5 and related diseases.

COMPOUNDS	REPORTED TARGETS	DISEASES
WP1130 (Degrasyn)	USP5, UCH37, UCH-L1, USP9X, USP14	Renal cancer, lung cancer, hepatocellular cancer, chronic myelogenous leukemia,[91] pancreatic ductal adenocarcinoma,[69] melanoma[92]
EOAI3402143 (G9)	USP9X, USP24, USP5	Melanoma,[93] non-small cell lung cancer[55]
PYR-41	USP5, UBE1	Mantle cell lymphoma cancer[94]
Vialinin A	USP5	Colonic cancer, hepatic carcinoma[95]
Chalcone derivatives (AM146, RA-9, RA-14)	UCH-L1, UCH-L3, USP2, USP5, USP8	Breast cancer, ovarian cancer and cervical cancer[90]

UBE, ubiquitin-activating enzyme; UCH, ubiquitin C-terminal hydrolase; USP, ubiquitin-specific protease.

NSCLC, with the shorter form playing a more significant role in regulating the stability of PD-L1, promoting tumor immune evasion, and exerting tumor regulatory effects.

The PD-1 functions as a receptor in colorectal cancer, suppressing T-cells that contribute to the promotion of immune evasion in cancer. Ubiquitin-specific protease 5 removes ubiquitin molecules from PD-1, leading to its stabilization. The ERK phosphorylates PD-1, which enhances the interaction between USP5 and PD-1. The elimination of USP5 in T-cells under specific conditions boosts the production of effector cytokines and hinders the formation of colorectal tumors.[80]

The role of USP5 in the resistance of cancer to therapy

Doxorubicin is a common treatment for colorectal tumors,[81,82] inhibiting DNA replication and damaging DNA to exert an anti-tumor effect. It significantly inhibits the luciferase activity driven by the USP5 promoter and reduces the transcription level of USP5 in colorectal tumor cells.[41] Tu translation elongation factor, mitochondrial (TUFM), a widely expressed mitochondrial protein,[83] plays a crucial role in promoting colorectal adenoma progression to cancerous tissue.[84] Mass spectrometry reveals that USP5 deubiquitinates TUFM at the K48 site, maintaining its stability. This process is regulated by its upstream transcription factor early B-cell factor-1 (EBF1). Furthermore, EBF1 overexpression leads to elevated levels of both USP5 and TUFM while reducing the sensitivity of colorectal cancer cells to doxorubicin. Henceforth, targeting the EBF1/USP5/TUFM axis could be an effective strategy for mitigating resistance in colorectal cancer. In addition, USP5 functions as the deubiquitinase for PD-1. The combined administration of the USP5 inhibitor EOAI3402143 with either anti-CTLA-4 or trametinib has a synergistic effect in inhibiting the growth of mice colorectal cancers.[80]

Small Molecule Inhibitors of USP5

As a deubiquitinase, USP5 cleaves ubiquitin from substrate proteins and unanchored polyubiquitin chains at specific sites, including Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63. Due to its abnormal activation or expression in various malignant tumors or tumor microenvironments and its role in regulating the stability of downstream oncogenic proteins, targeting USP5 inhibition emerges as a promising strategy for cancer therapy.[85] An α, β-unsaturated ketone with a sterically accessible β-carbon is a crucial molecular factor in conferring partial selectivity as a DUB inhibitor and determining its inhibitory activity.[86] When the thiol group of cysteine in DUB attacks the α, β-unsaturated carbonyl group of the DUB inhibitor by a nucleophilic addition reaction, it forms a covalent complex. This complex leads to an increase in the accumulation of polyubiquitin chains and a reduction in the degradation rate. The monomeric ubiquitin pool decreases, and individual DUB activity overall decreases, thereby affecting the cellular level and activity of cancer proteins regulated by DUB.[87] Ubiquitin-specific protease 5 inhibitors with a similar structure include WP1130,[87] EOAI3402143,[88] PYR-41,[89] and chalcone-based derivatives,[90] which are discussed below and summarized in Table 1. The information related to USP5 inhibitors, including chemical structures, PubChem CID, molecular formula and IC50 values, was presented in Figure 5. Consequently, USP5 represents an ideal candidate for anticancer treatment. Developing inhibitors specifically targeting USP5 could represent a novel effective approach to tumor management.

WP1130 (degrasyn)

WP1130, a second-generation tyrphostin derivative (degrasyn), was discovered through screening for AG490-like molecules that inhibit the activation of Stat molecules by interleukin 6 (IL-6) and IL-3.[87] WP1130 has superior effectiveness in inducing apoptosis in both myeloid and lymphoid tumor cells, with an IC50 range of around 0.5 to 2.5 μM.[91] Mantle cell lymphoma Z138 cells displayed the most apoptotic sensitivity to WP1130, with an IC50 of approximately 1 μM.[96] Unlike MG132, which directly inhibits the proteasome, WP1130 rapidly induces the accumulation of polyubiquitinated target proteins, leading to apoptosis in tumor cells.[97] It selectively inhibits deubiquitinases responsible for

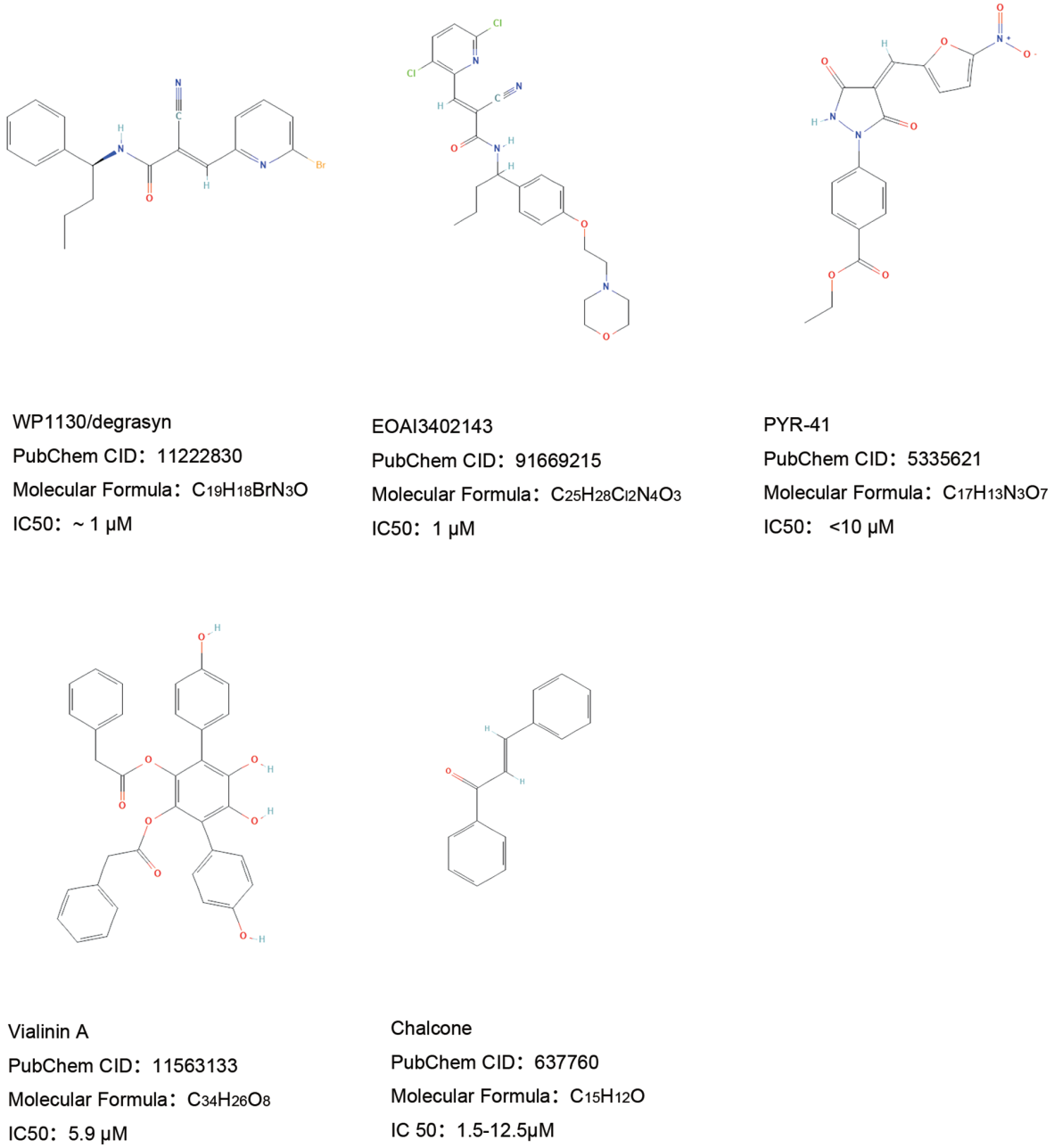

Figure 5. Chemical structures of USP5 inhibitors.
The chemical structures of USP5 inhibitors with PubChem CID, molecular formula and IC50 values.

the removal of K48- and K63-specific ubiquitin chains, including USP5, UCH37, UCH-L1, USP9X,[98] and USP14.[87] The molecular structure of WP1130 contains a cross-conjugated α, β-unsaturated dienone with two sterically accessible electrophilic β-carbons.[86] The α, β-unsaturated carbonyl group in WP1130 can create covalent adducts with the cystine in the active site of USP5, which can hinder its deubiquitinating activity.[87] Consequently, inhibition of USP5 leads to an accumulation of unanchored polyubiquitin chains within cells and a reduction in monomeric ubiquitin, increasing p53 levels in a dose-dependent manner and suppressing tumor formation.[21]

WP1130 effectively inhibits tumor development in mouse models with xenograft melanoma and chronic myeloid leukemia tumor cells.[91] Furthermore, derivatives of WP1130 with reduced activity, such as WP1066 and WP1034, exhibit antiproliferative and pro-apoptotic effects in diseases such as chronic myelogenous leukemia,[91] glioblastoma multiforme,[99] and myeloproliferative disorders.[100] It hinders the spread of PDAC by blocking the USP5-induced production of WT1 and E-cadherin, which are responsible for metastasis. Moreover, degrasyn hampers the occurrence and advancement of melanoma and leukemia by degrading c-Myc and BCR-ABL via ubiquitin-mediated pathways.[92,101]

EOAI3402143(G9)

EOAI3402143(G9) was selected as a small molecule inhibitor from more than 220 compounds that retained the inhibitory activity of WP1130 against Usp9x and improved its solubility. G9 was better than WP1130 in USP9x catalytic activity, water-soluble activity, and cell USP9x inhibitory activity.[88] G9 inhibits USP9x, USP24, and USP5. The IC50 of G9 is reported to be 1 μM.[93] G9 inhibits the function of Usp9x and Usp24 by creating a covalent, gradually reversible connection with cysteine residues.[88] However, the mechanism by which G9 inhibits USP5 has yet to be identified. Moreover, clinical trials for G9 have not yet been conducted.

G9 suppresses USP9X, leading to aggresomal translocation, ultimately triggering apoptosis in FLT3-ITD-positive acute myeloid leukemia (AML) cells[102] and Ruxolitinib-resistant JAK2-V617F-positive leukemic cells.[103] G9 exhibited a dose-dependent inhibition of Usp9x and Usp24 activity. It also induced apoptosis in tumor cells and led to either full regression or suppression of myeloma tumors.[88] When G9 is utilized, similar effects are observed as when USP5 is knocked down, leading to the induction of FAS and sensitization to apoptosis. This ultimately results in significant inhibition of melanoma tumor growth.[93] G9 inhibits the function of USP5, resulting in reduced production of the CCND1 protein and eventually suppressing the proliferation of NSCLC cells *in vitro* and limiting the formation of tumors *in vivo*.[55]

PYR-41

Ubiquitin-activating enzyme 1 (UBE1) plays a pivotal role in the regulation of the ubiquitination cycle. Its inhibitor PYR-41 is an effective blocker of UBE1-mediated ubiquitination reactions, exhibiting significant inhibitory effects on USP5.[94] The IC50 of PYR-41 is reported to be less than 10 μM.[89] PYR-41 possesses an α, β-unsaturated enone functional group that has a readily available β-carbon. The α, β-unsaturated enones exhibit a rapid reaction with sulfhydryl groups via a Michael addition reaction. The suppression of both UBE1 and USP5 was achieved by PYR-41-induced covalent protein cross-linking, which was accompanied by the inhibition of the enzymatic activity of the target proteins.[94] Elevated levels of PYR-41 in lymphoma tumor cells downregulate the expression of USP5 by inducing protein crosslinking into W structures instead of degradation pathways. This selective protein crosslinking may impact signal transduction and ubiquitin cycling, modulating protein expression. PYR-41-related compounds have demonstrated promising anti-tumor activity in animal models of prostate[104] and breast cancers,[105] among others, providing potential alternative strategies for cancer treatment.

Vialinin A

Vialinin A, a small molecule compound extracted and isolated from the Chinese fungus *Ganoderma l*ucidum, possesses antioxidant properties.[106] Vialinin A displayed competitive binding with Ub at the S1 and/or S1′ sites of USP5, with an IC50 value of 5.9 μM. It exhibited the highest efficacy in the competition. However, further research is needed to investigate the mechanism and binding characteristics of vialinin A with USP5.[107] Vialinin A effectively regulates the production of TNF-α and prevents the progression of rat eosinophilic leukemia.[108] Furthermore, vialinin A dose-dependently inhibits vascular endothelial growth factor expression, antagonizing human umbilical vein endothelial cell-mediated neovascularization.[109] Ultimately, it exerts anticancer effects through its antioxidant and anti-angiogenic actions. Toshio Norikura et al[95] were the first to discover that vialinin A reduces the proliferative activity of human colon tumor and liver cancer cells.

Chalcones derivatives

Chalcones serve as precursors for flavonoids and isoflavonoids, which are common chemical structures found in many naturally occurring substances.[110] A multitude of chalcone derivatives were synthesized due to their simple synthesis procedure. The chalcone-based derivatives, such as RA-14, AM146, and RA-9, from the "RA" and "AM" series of compounds exhibit partially selective inhibitory activity against DUB. These small molecules possess a reactive α, β-unsaturated carbonyl group that may undergo a Michael addition reaction with the sulfhydryl of cysteines at the active sites in DUB, resulting in the formation of covalent compounds. This leads to the rapid accumulation of polyubiquitinated proteins and a reduction in the available free ubiquitin. These chalcone derivatives effectively inhibit the activity of DUB, such as USP5, UCH-L3, USP2, UCH-L1, and USP8, leading to irreversible cell cycle arrest in breast, ovarian, and cervical cancer cells (IC50 = 1.5-12.5 μM), as well as inhibiting their proliferation and initiating apoptosis.[90]

Discussion

With the advancement of research, the understanding of USP5 has been progressively elucidated. Ubiquitin-specific protease 5 plays a crucial role in the onset and progression of malignancies and has become a prospective target for therapy. However, there are inherent difficulties that must be resolved in the development of USP5 inhibitors. First, these inhibitors do not specifically inhibit the expression of USP5, complicating their effects on different types of tumors.[21,111-113] Second, since USP5 is involved in various *in vivo* physiological activities such as DNA repair[114] and normal immune responses,[115] its inhibitors may cause certain side effects during tumor treatment. Therefore, it is crucial to comprehensively understand the role of USP5 in different tumors, explore specific regulatory mechanisms, and assess the effects of USP5 inhibitors through animal experiments.

Targeted gene therapy is a promising way of cancer therapy. A galactose-decorated lipopolyplex (Gal-SLP) is developed to co-delivery sorafenib and USP22 shRNA for

synergetic hepatocellular carcinoma (HCC) therapy.[116] DeUBiquitinase-TArgeting Chimeras (DUBTACs) has been revealed as a promising approach against cancer. It facilitates the recruitment of a deubiquitinase to prevent the degradation of the target protein by the proteasome, thereby stabilizing the target protein.[117] Deubiquitinating enzymes that are specifically targeted can be found in two different kinds of binding: covalently bound and non-covalently bound. The ligand EN523 forms a covalent bond with OTUB1 by selectively interacting with the non-catalytic allosteric cysteine C23 in OTUB1. This interaction synergistically combines with lumacaftor to stabilize the ΔF508-CFTR protein and increase chloride channel conductance in human cystic fibrosis bronchial epithelial cells.[118] USP7 inhibitors interact with USP7 as the ligand in a non-covalent manner, resulting in the formation of DUBTAC. DUBTAC, a compound that binds to the CFTR ligand Lumacaftor, effectively stabilizes the ΔF508-CFTR mutant protein in human cystic fibrosis bronchial epithelial cells (CFBE41o-4.7 ΔF508-CFTR). Its efficiency is equivalent to that of OTUB1-based CFTR DUBTAC NJH-2-057.[119] USP5 plays a significant role in the development of the majority of malignancies, indicating that it might be a reliable target for treating tumors. Thus, further investigation of USP5 in DUBTAC is needed.

Conclusions

In conclusion, this review provides a comprehensive examination of USP5, shedding light on its functional domains and diverse roles in various cancers. Ubiquitin-specific protease 5 influences processes like proliferation, apoptosis, autophagy, and migration of tumor cells. In the past decade, research on deubiquitinases has advanced, presenting them as promising targets for tumor treatment. The current investigation of small-molecule inhibitors, such as WP1130, PYR-41, and vialinin A, has revealed the possibility of targeting USP5 and shown interesting opportunities for treating tumors.

Acknowledgements

We thank Department of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University and the Health Commission of Hubei Province (WJ2023M175) for support and encouragement. And we also thank the drawing tools provided by Figdraw (https://www.home-for-researchers.com).

Author Contributions

QH and YJ conceived and designed this topic, JW and SF drafted the article. All authors read and approved the final article.

Availability of Data and Materials

Main data are shown in this article, and additional data about this study could be obtained from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Not applicable.

ORCID iDs

Shizhen Fang https://orcid.org/0009-0001-6466-1719
Qingquan Hua https://orcid.org/0000-0002-6499-0280

REFERENCES

1. Komander D, Rape M. The ubiquitin code. *Annu Rev Biochem*. 2012;81:203-229.
2. Deng L, Meng T, Chen L, Wei W, Wang P. The role of ubiquitination in tumorigenesis and targeted drug discovery. *Signal Transduct Target Ther*. 2020;5:11.
3. Neutzner M, Neutzner A. Enzymes of ubiquitination and deubiquitination. *Essays Biochem*. 2012;52:37-50.
4. Mevissen TET, Komander D. Mechanisms of deubiquitinase specificity and regulation. *Annu Rev Biochem*. 2017;86:159-192.
5. Nijman SMB, Luna-Vargas MPA, Velds A, et al. A genomic and functional inventory of deubiquitinating enzymes. *Cell*. 2005;123:773-786.
6. Clague MJ, Urbé S, Komander D. Breaking the chains: deubiquitylating enzyme specificity begets function. *Nat Rev Mol Cell Biol*. 2019;20:338-352.
7. Abdul Rehman SA, Kristariyanto YA, Choi SY, et al. MINDY-1 is a member of an evolutionarily conserved and structurally distinct new family of deubiquitinating enzymes. *Mol Cell*. 2016;63:146-155.
8. Haahr P, Borgermann N, Guo X, et al. ZUFSP deubiquitylates K63-linked polyubiquitin chains to promote genome stability. *Mol Cell*. 2018;70:165-174e6.
9. Hermanns T, Pichlo C, Woiwode I, et al. A family of unconventional deubiquitinases with modular chain specificity determinants. *Nat Commun*. 2018;9:799.
10. Reyes-Turcu FE, Ventii KH, Wilkinson KD. Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu Rev Biochem*. 2009;78:363-397.
11. Komander D, Clague MJ, Urbé S. Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol*. 2009;10:550-563.
12. Wilkinson KD, Tashayev VL, O'Connor LB, Larsen CN, Kasperek E, Pickart CM. Metabolism of the polyubiquitin degradation signal: structure, mechanism, and role of isopeptidase T. *Biochemistry*. 1995;34:14535-14546.
13. Mattiroli F, Vissers JHA, van Dijk WJ, et al. RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell*. 2012;150:1182-1195.
14. Izaguirre DI, Zhu W, Hai T, Cheung HC, Krahe R, Cote GJ. PTBP1-dependent regulation of USP5 alternative RNA splicing plays a role in glioblastoma tumorigenesis. *Mol Carcinog*. 2012;51:895-906.
15. García-Caballero A, Gadotti VM, Stemkowski P, et al. The deubiquitinating enzyme USP5 modulates neuropathic and inflammatory pain by enhancing Cav3.2 channel activity. *Neuron*. 2014;83:1144-1158.
16. Colwill K, Renewable Protein Binder Working Group,Gräslund S.A roadmap to generate renewable protein binders to the human proteome. *Nat Methods*. 2011;8:551-558.
17. Amerik AY, Swaminathan S, Krantz BA, Wilkinson KD, Hochstrasser M. In vivo disassembly of free polyubiquitin chains by yeast Ubp14 modulates rates of protein degradation by the proteasome. *EMBO J*. 1997;16:4826-4838.
18. Gabriel JM, Lacombe T, Carobbio S, et al. Zinc is required for the catalytic activity of the human deubiquitinating isopeptidase T. *Biochemistry*. 2002;41:13755-13766.
19. Reyes-Turcu FE, Shanks JR, Komander D, Wilkinson KD. Recognition of polyubiquitin isoforms by the multiple ubiquitin binding modules of isopeptidase T. *J Biol Chem*. 2008;283:19581-19592.
20. Ansari-Lari MA, Muzny DM, Lu J, et al. A gene-rich cluster between the CD4 and triosephosphate isomerase genes at human chromosome 12p13. *Genome Res*. 1996;6:314-326.
21. Dayal S, Sparks A, Jacob J, Allende-Vega N, Lane DP, Saville MK. Suppression of the deubiquitinating enzyme USP5 causes the accumulation of unanchored polyubiquitin and the activation of p53. *J Biol Chem*. 2009;284:5030-5041.
22. Avvakumov GV, Walker JR, Xue S, et al. Two ZnF-UBP domains in isopeptidase T (USP5). *Biochemistry*. 2012;51:1188-1198.
23. Young MJ, Hsu KC, Lin TE, Chang WC, Hung JJ. The role of ubiquitin-specific peptidases in cancer progression. *J Biomed Sci*. 2019;26:42.
24. Reyes-Turcu FE, Horton JR, Mullally JE, Heroux A, Cheng X, Wilkinson KD. The ubiquitin binding domain ZnF UBP recognizes the C-terminal diglycine motif of unanchored ubiquitin. *Cell*. 2006;124:1197-1208.
25. Ning F, Xin H, Liu J, et al. Structure and function of USP5: insight into physiological and pathophysiological roles. *Pharmacol Res*. 2020;157:104557.
26. McGouran JF, Gaertner SR, Altun M, Kramer HB, Kessler BM. Deubiquitinating enzyme specificity for ubiquitin chain topology profiled by di-ubiquitin activity probes. *Chem Biol*. 2013;20:1447-1455.

27. Raasi S, Varadan R, Fushman D, Pickart CM. Diverse polyubiquitin interaction properties of ubiquitin-associated domains. *Nat Struct Mol Biol*. 2005;12:708-714.
28. Jiang X, You H, Niu Y, et al. E2F1-regulated USP5 contributes to the tumorigenic capacity of glioma stem cells through the maintenance of OCT4 stability. *Cancer Lett*. 2024;593:216875.
29. Cai J, Chen Z, Wang J, et al. circHECTD1 facilitates glutaminolysis to promote gastric cancer progression by targeting miR-1256 and activating β-catenin/c-Myc signaling. *Cell Death Dis*. 2019;10:576.
30. Qu Y, Xu Y, Jiang Y, Yu D, Jiang X, Zhao L. Macrophage-derived extracellular vesicles regulates USP5-mediated HDAC2/NRF2 axis to ameliorate inflammatory pain. *FASEB J*. 2021;35:e21332.
31. Xia P, Zhang H, Lu H, et al. METTL5 stabilizes c-Myc by facilitating USP5 translation to reprogram glucose metabolism and promote hepatocellular carcinoma progression. *Cancer Commun (Lond)*. 2023;43:338-364.
32. Qian G, Ren Y, Zuo Y, et al. Smurf1 represses TNF-α production through ubiquitination and destabilization of USP5. *Biochem Biophys Res Commun*. 2016;474:491-496.
33. Garcia-Caballero A, Zhang FX, Chen L, M'Dahoma S, Huang J, Zamponi GW. SUMOylation regulates USP5-Cav3.2 calcium channel interactions. *Mol Brain*. 2019;12:73.
34. Li J, Wang Y, Luo Y, et al. USP5-Beclin 1 axis overrides p53-dependent senescence and drives Kras-induced tumorigenicity. *Nat Commun*. 2022;13:7799.
35. Yu Z, Tang H, Chen S, et al. Exosomal LOC85009 inhibits docetaxel resistance in lung adenocarcinoma through regulating ATG5-induced autophagy. *Drug Resist Updat*. 2023;67:100915.
36. Yuan Y, Zhou D, Chen F, Yang Z, Gu W, Zhang K. SIX5-activated LINC01468 promotes lung adenocarcinoma progression by recruiting SERBP1 to regulate SERPINE1 mRNA stability and recruiting USP5 to facilitate PAI1 protein deubiquitylation. *Cell Death Dis*. 2022;13:312.
37. Xiao M, Liu J, Xiang L, et al. MAFG-AS1 promotes tumor progression via regulation of the HuR/PTBP1 axis in bladder urothelial carcinoma. *Clin Transl Med*. 2020;10:e241.
38. Meyers RM, Bryan JG, McFarland JM, et al. Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells. *Nat Genet*. 2017;49:1779-1784.
39. Cui J, Xia T, Xie D, et al. HGF/Met and FOXM1 form a positive feedback loop and render pancreatic cancer cells resistance to Met inhibition and aggressive phenotypes. *Oncogene*. 2016;35:4708-4718.
40. Xue S, Wu W, Wang Z, et al. Corrigendum: USP5 promotes metastasis in non-small cell lung cancer by inducing epithelial-mesenchymal transition via Wnt/β-catenin pathway. *Front Pharmacol*. 2020;11:948.
41. Xu X, Huang A, Cui X, et al. Ubiquitin specific peptidase 5 regulates colorectal cancer cell growth by stabilizing Tu translation elongation factor. *Theranostics*. 2019;9:4208-4220.
42. Lian J, Liu C, Guan X, et al. Ubiquitin specific peptidase 5 enhances STAT3 signaling and promotes migration and invasion in pancreatic cancer. *J Cancer*. 2020;11:6802-6811.
43. Du Y, Lin J, Zhang R, et al. Ubiquitin specific peptidase 5 promotes ovarian cancer cell proliferation through deubiquitinating HDAC2. *Aging*. 2019;11:9778-9793.
44. Pan J, Qiao Y, Chen C, et al. USP5 facilitates non-small cell lung cancer progression through stabilization of PD-L1. *Cell Death Dis*. 2021;12:1051.
45. Liu Y, Wang WM, Lu YF, et al. Usp5 functions as an oncogene for stimulating tumorigenesis in hepatocellular carcinoma. *Oncotarget*. 2017;8:50655-50664.
46. Huang W, Liu X, Zhang Y, et al. USP5 promotes breast cancer cell proliferation and metastasis by stabilizing HIF2α. *J Cell Physiol*. 2022;237:2211-2219.
47. Meng J, Ai X, Lei Y, et al. USP5 promotes epithelial-mesenchymal transition by stabilizing SLUG in hepatocellular carcinoma. *Theranostics*. 2019;9:573-587.
48. Li XY, Wu HY, Mao XF, Jiang LX, Wang YX. USP5 promotes tumorigenesis and progression of pancreatic cancer by stabilizing FoxM1 protein. *Biochem Biophys Res Commun*. 2017;492:48-54.
49. Xue Y, Zhou Y, Wu T, et al. Genome-wide analysis of PTB-RNA interactions reveals a strategy used by the general splicing repressor to modulate exon inclusion or skipping. *Mol Cell*. 2009;36:996-1006.
50. Ramalingam SS, Owonikoko TK, Khuri FR. Lung cancer: new biological insights and recent therapeutic advances. *CA Cancer J Clin*. 2011;61:91-112.
51. Massagué J. G1 cell-cycle control and cancer. *Nature*. 2004;432:298-306.
52. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature*. 2004;432:316-323.
53. Gautschi O, Ratschiller D, Gugger M, Betticher DC, Heighway J. Cyclin D1 in non-small cell lung cancer: a key driver of malignant transformation. *Lung Cancer*. 2007;55:1-14.
54. Wikman H, Kettunen E. Regulation of the G1/S phase of the cell cycle and alterations in the RB pathway in human lung cancer. *Expert Rev Anticancer Ther*. 2006;6:515-530.
55. Zhang Z, Cui Z, Xie Z, et al. Deubiquitinase USP5 promotes non-small cell lung cancer cell proliferation by stabilizing cyclin D1. *Transl Lung Cancer Res*. 2021;10:3995-4011.
56. Liu J, Zhang C, Hu W, Feng Z. Tumor suppressor p53 and its mutants in cancer metabolism. *Cancer Lett*. 2015;356:197-203.
57. Kwon SK, Saindane M, Baek KH. p53 stability is regulated by diverse deubiquitinating enzymes. *Biochim Biophys Acta Rev Cancer*. 2017;1868:404-411.
58. Gupta R, Amanam I, Chung V. Current and future therapies for advanced pancreatic cancer. *J Surg Oncol*. 2017;116:25-34.
59. Gonzalez FJ, Xie C, Jiang C. The role of hypoxia-inducible factors in metabolic diseases. *Nat Rev Endocrinol*. 2018;15:21-32.
60. Krauß L, Urban BC, Hastreiter S, et al. HDAC2 facilitates pancreatic cancer metastasis. *Cancer Res*. 2022;82:695-707.
61. Razavipour SF, Harikumar KB, Slingerland JM. p27 as a transcriptional regulator: new roles in development and cancer. *Cancer Res*. 2020;80:3451-3458.
62. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71:209-249.
63. Lee GA, Hwang KA, Choi KC. Roles of dietary phytoestrogens on the regulation of epithelial-mesenchymal transition in diverse cancer metastasis. *Toxins*. 2016;8:162.
64. Hajra KM, Chen DYS, Fearon ER. The SLUG zinc-finger protein represses E-cadherin in breast cancer. *Cancer Res*. 2002;62:1613-1618.
65. Kajita M, McClinic KN, Wade PA. Aberrant expression of the transcription factors snail and slug alters the response to genotoxic stress. *Mol Cell Biol*. 2004;24:7559-7566.
66. Barrallo-Gimeno A, Nieto MA. The Snail genes as inducers of cell movement and survival: implications in development and cancer. *Development*. 2005;132:3151-3161.
67. Dhasarathy A, Phadke D, Mav D, Shah RR, Wade PA. The transcription factors Snail and Slug activate the transforming growth factor-beta signaling pathway in breast cancer. *PLoS ONE*. 2011;6:e26514.
68. Nieto MA. The snail superfamily of zinc-finger transcription factors. *Nat Rev Mol Cell Biol*. 2002;3:155-166.
69. Li J, Li H, Zhu W, et al. Deubiquitinase inhibitor degrasyn suppresses metastasis by targeting USP5-WT1-E-cadherin signalling pathway in pancreatic ductal adenocarcinoma. *J Cell Mol Med*. 2020;24:1370-1382.
70. Glienke W, Maute L, Wicht J, Bergmann L. Wilms' tumour gene 1 (WT1) as a target in curcumin treatment of pancreatic cancer cells. *Eur J Cancer*. 2009;45:874-880.
71. Oka Y, Kawase I. [Cancer antigen WT1-targeting treatment for the malignancies]. *Nihon Rinsho Meneki Gakkai Kaishi*. 2008;31:375-382.
72. Kaistha BP, Krattenmacher A, Fredebohm J, et al. The deubiquitinating enzyme USP5 promotes pancreatic cancer via modulating cell cycle regulators. *Oncotarget*. 2017;8:66215-66225.
73. Inhibition of the deubiquitinase USP5 leads to c-Maf protein degradation and myeloma cell apoptosis. Accessed June 14, 2024. https://pubmed.ncbi.nlm.nih.gov/28933784/
74. Zhou XJ, Li R, Liu X, Qu YQ. Advances in deubiquitinating enzymes in lung adenocarcinoma. *J Cancer*. 2021;12:5573-5582.
75. Cha JH, Chan LC, Li CW, Hsu JL, Hung MC. Mechanisms controlling PD-L1 expression in cancer. *Mol Cell*. 2019;76:359-370.
76. Daassi D, Mahoney KM, Freeman GJ. The importance of exosomal PDL1 in tumour immune evasion. *Nat Rev Immunol*. 2020;20:209-215.
77. Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. *J Exp Med*. 2012;209:1201-1217.
78. Gou Q, Dong C, Xu H, et al. PD-L1 degradation pathway and immunotherapy for cancer. *Cell Death Dis*. 2020;11:955.
79. Gong J, Chehrazi-Raffle A, Reddi S, Salgia R. Development of PD-1 and PD-L1 inhibitors as a form of cancer immunotherapy: a comprehensive review of registration trials and future considerations. *J Immunother Cancer*. 2018;6:8.
80. Xiao X, Shi J, He C, et al. ERK and USP5 govern PD-1 homeostasis via deubiquitination to modulate tumor immunotherapy. *Nat Commun*. 2023;14:2859.
81. Carvalho C, Santos RX, Cardoso S, et al. Doxorubicin: the good, the bad and the ugly effect. *Curr Med Chem*. 2009;16:3267-3285.
82. Ma K, Xu Q, Wang S, et al. Nuclear accumulation of Yes-Associated Protein (YAP) maintains the survival of doxorubicin-induced senescent cells by promoting survivin expression. *Cancer Lett*. 2016;375:84-91.
83. Shi H, Hayes M, Kirana C, et al. TUFM is a potential new prognostic indicator for colorectal carcinoma. *Pathology*. 2012;44:506-512.
84. Xi HQ, Zhang KC, Li JY, Cui JX, Zhao P, Chen L. Expression and clinicopathologic significance of TUFM and p53 for the normal-adenoma-carcinoma sequence in colorectal epithelia. *World J Surg Oncol*. 2017;15:90.

85. Yuan T, Yan F, Ying M, et al. Inhibition of ubiquitin-specific proteases as a novel anticancer therapeutic strategy. *Front Pharmacol*. 2018;9:1080.
86. Mullally JE, Fitzpatrick FA. Pharmacophore model for novel inhibitors of ubiquitin isopeptidases that induce p53-independent cell death. *Mol Pharmacol*. 2002;62:351-358.
87. Kapuria V, Peterson LF, Fang D, Bornmann WG, Talpaz M, Donato NJ. Deubiquitinase inhibition by small-molecule WP1130 triggers aggresome formation and tumor cell apoptosis. *Cancer Res*. 2010;70:9265-9276.
88. Peterson LF, Sun H, Liu Y, et al. Targeting deubiquitinase activity with a novel small-molecule inhibitor as therapy for B-cell malignancies. *Blood*. 2015;125:3588-3597.
89. Yang Y, Kitagaki J, Dai RM, et al. Inhibitors of ubiquitin-activating enzyme (E1), a new class of potential cancer therapeutics. *Cancer Res*. 2007;67:9472-9481.
90. Issaenko OA, Amerik AY. Chalcone-based small-molecule inhibitors attenuate malignant phenotype via targeting deubiquitinating enzymes. *Cell Cycle*. 2012;11:1804-1817.
91. Bartholomeusz GA, Talpaz M, Kapuria V, et al. Activation of a novel Bcr/Abl destruction pathway by WP1130 induces apoptosis of chronic myelogenous leukemia cells. *Blood*. 2007;109:3470-3478.
92. Bartholomeusz G, Talpaz M, Bornmann W, Kong LY, Donato NJ. Degrasyn activates proteasomal-dependent degradation of c-Myc. *Cancer Res*. 2007;67:3912-3918.
93. Potu H, Peterson LF, Pal A, et al. Usp5 links suppression of p53 and FAS levels in melanoma to the BRAF pathway. *Oncotarget*. 2014;5:5559-5569.
94. Kapuria V, Peterson LF, Showalter HDH, Kirchhoff PD, Talpaz M, Donato NJ. Protein cross-linking as a novel mechanism of action of a ubiquitin-activating enzyme inhibitor with anti-tumor activity. *Biochem Pharmacol*. 2011;82:341-349.
95. Norikura T, Fujiwara K, Narita T, et al. Anticancer activities of thelephantin O and vialinin A isolated from *Thelephora aurantiotincta*. *J Agric Food Chem*. 2011;59:6974-6979.
96. Kapuria V, Levitzki A, Bornmann WG, et al. A novel small molecule deubiquitinase inhibitor blocks Jak2 signaling through Jak2 ubiquitination. *Cell Signal*. 2011;23:2076-2085.
97. D'Arcy P, Brnjic S, Olofsson MH, et al. Inhibition of proteasome deubiquitinating activity as a new cancer therapy. *Nat Med*. 2011;17:1636-1640.
98. Kim S, Woo SM, Min KJ, et al. WP1130 enhances TRAIL-induced apoptosis through USP9X-dependent miR-708-mediated downregulation of c-FLIP. *Cancers*. 2019;11:344.
99. Iwamaru A, Szymanski S, Iwado E, et al. A novel inhibitor of the STAT3 pathway induces apoptosis in malignant glioma cells both in vitro and in vivo. *Oncogene*. 2007;26:2435-2444.
100. Verstovsek S, Manshouri T, Quintás-Cardama A, et al. WP1066, a novel JAK2 inhibitor, suppresses proliferation and induces apoptosis in erythroid human cells carrying the JAK2 V617F mutation. *Clin Cancer Res*. 2008;14:788-796.
101. Sun H, Kapuria V, Peterson LF, et al. Bcr-Abl ubiquitination and Usp9x inhibition block kinase signaling and promote CML cell apoptosis. *Blood*. 2011;117:3151-3162.
102. Akiyama H, Umezawa Y, Ishida S, Okada K, Nogami A, Miura O. Inhibition of USP9X induces apoptosis in FLT3-ITD-positive AML cells cooperatively by inhibiting the mutant kinase through aggresomal translocation and inducing oxidative stress. *Cancer Lett*. 2019;453:84-94.
103. Akiyama H, Umezawa Y, Watanabe D, et al. Inhibition of USP9X downregulates JAK2-V617F and induces apoptosis synergistically with BH3 mimetics preferentially in ruxolitinib-persistent JAK2-V617F-positive leukemic cells. *Cancers*. 2020;12:406.
104. Itoh A, Nonaka Y, Ogawa T, Nakamura T, Nishi N. Galectin-9 induces atypical ubiquitination leading to cell death in PC-3 prostate cancer cells. *Glycobiology*. 2019;29:22-35.
105. Pesiri V, Totta P, Marino M, Acconcia F. Ubiquitin-activating enzyme is necessary for 17β-estradiol-induced breast cancer cell proliferation and migration. *IUBMB Life*. 2014;66:578-585.
106. Xie C, Koshino H, Esumi Y, Takahashi S, Yoshikawa K, Abe N. Vialinin A, a novel 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenger from an edible mushroom in China. *Biosci Biotechnol Biochem*. 2005;69:2326-2332.
107. Okada K, Ye YQ, Taniguchi K, et al. Vialinin A is a ubiquitin-specific peptidase inhibitor. *Bioorg Med Chem Lett*. 2013;23:4328-4331.
108. Onose J, Yoshioka Y, Ye YQ, et al. Inhibitory effects of vialinin A and its analog on tumor necrosis factor-α release and production from RBL-2H3 cells. *Cell Immunol*. 2012;279:140-144.
109. Sonowal H, Shukla K, Kota S, Saxena A, Ramana KV. Vialinin A, an edible mushroom-derived p-terphenyl antioxidant, prevents VEGF-induced neovascularization in vitro and in vivo. *Oxid Med Cell Longev*. 2018;2018:1052102.
110. Ouyang Y, Li J, Chen X, Fu X, Sun S, Wu Q. Chalcone derivatives: role in anticancer therapy. *Biomolecules*. 2021;11:894.
111. Schwickart M, Huang X, Lill JR, et al. Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. *Nature*. 2010;463:103-107.
112. Shan J, Zhao W, Gu W. Suppression of cancer cell growth by promoting cyclin D1 degradation. *Mol Cell*. 2009;36:469-476.
113. Hussain S, Zhang Y, Galardy PJ. DUBs and cancer: the role of deubiquitinating enzymes as oncogenes, non-oncogenes and tumor suppressors. *Cell Cycle Georget Tex*. 2009;8:1688-1697.
114. Nakajima S, Lan L, Wei L, et al. Ubiquitin-specific protease 5 is required for the efficient repair of DNA double-strand breaks. *PLoS ONE*. 2014;9:e84899.
115. Kummari E, Alugubelly N, Hsu CY, Dong B, Nanduri B, Edelmann MJ. Activity-based proteomic profiling of deubiquitinating enzymes in salmonella-infected macrophages leads to identification of putative function of UCH-L5 in inflammasome regulation. *PLoS ONE*. 2015;10:e0135531.
116. Xu S, Ling S, Shan Q, et al. Self-activated cascade-responsive sorafenib and USP22 shRNA co-delivery system for synergetic hepatocellular carcinoma therapy. *Adv Sci (Weinh)*. 2021;8:2003042.
117. Noblejas-López MDM, Tébar-García D, López-Rosa R, et al. TACkling Cancer by Targeting Selective Protein Degradation. *Pharmaceutics*. 2023;15:2442.
118. Henning NJ, Boike L, Spradlin JN, et al. Deubiquitinase-targeting chimeras for targeted protein stabilization. *Nat Chem Biol*. 2022;18:412-421.
119. Liu J, Hu X, Luo K, et al. USP7-based deubiquitinase-targeting chimeras stabilize AMPK. *J Am Chem Soc*. Published online April 10, 2024. doi:10.1021/jacs.4c02373