Review

Structural Plasticity Guides Functional Versatility of USP7 in Human Diseases: Mechanistic Insights and Therapeutic Targeting

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Abstract: Ubiquitin-specific protease 7 (USP7) is a crucial member of the deubiquitinase family. USP7 exhibits unique structural characteristics, consisting of an N-terminal TRAF domain, a catalytic domain, and C-terminal ubiquitin-like (UBL) domains. Notably, the dynamic switch between inactive and active conformations in the catalytic domain confers precise control of its enzymatic activity. USP7 plays pivotal roles in cell cycle progression, DNA damage repair, and key signaling pathways through deubiquitinating critical regulatory factors. Dysregulation of USP7 triggers various diseases, including cancers, metabolic disorders, neurodegenerative diseases, and Hao-Fountain syndrome. This review systematically summarizes structural features and physiological functions of USP7, and elucidates its regulatory mechanisms in disease pathogenesis. Additionally, currently reported USP7 targeted modulators, including inhibitors, agonists, and degraders, are also summarized. These insights provide theoretical foundations for developing novel regulators and potential therapeutic strategies for related diseases.

Keywords: USP7; deubiquitination; small molecular modulators

1. Introduction

Ubiquitination is a key post-translational modification of proteins that performs a variety of important physiological functions in cells. This modification not only catalyzes protein degradation, but also mediates cell cycle progression, signal transduction, and DNA damage repair [1]. The process of ubiquitination is carried out synergistically by ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligase enzymes (E3). These three enzymes cascade the formation of an isopeptide bond between the C-terminus of ubiquitin (Ub) and lysine residues of the substrate protein (Figure 1A) [2]. In contrast, deubiquitinating enzymes (DUBs) prevent target proteins from being degraded by preventing the extension of the ubiquitin chain and hydrolyzing the ubiquitin chain attached to the target protein [3].

The human genome encodes approximately 100 DUBs. They can be classified into seven subfamilies based on differences in their structural domains (Figure 1B) [4]. The USP family, the largest component of the DUB system, has more than 50 identified members [5]. Specifically, USP7 is a key member of the USP subfamily.

In 1997, Everett et al. identified a novel protease with a molecular weight of 135 kDa that interacts strongly and specifically with immediate-early protein ICP0 of herpes simplex virus 1 (HSV-1) to modulate the stability of HSV-1 infection. This protease is known as herpes virus-associated protease (HAUSP), as well as ubiquitin-specific protease 7 (USP7) [6]. As an important member of USPs, USP7 has a unique TRAF domain, a catalytic domain, and ubiquitin-like domains (UBLs), with its enzymatic activity being precisely modulated by the conserved catalytic triad (Cys223/His464/Asp481) [7]. Accumulating evidence demonstrates that USP7 plays critical regulatory roles in diverse cellular processes, including the cell cycle, DNA damage repair, and cancer-related signaling pathways such as mouse double minute 2 homolog (MDM2)-p53, Wnt/β-catenin, and NF-κB [8–12]. Therefore, dysregulation of USP7 will lead to cancer, metabolic diseases, neurodegenerative diseases, and Hao-Fountain syndrome (HAFOUS) [13–16].

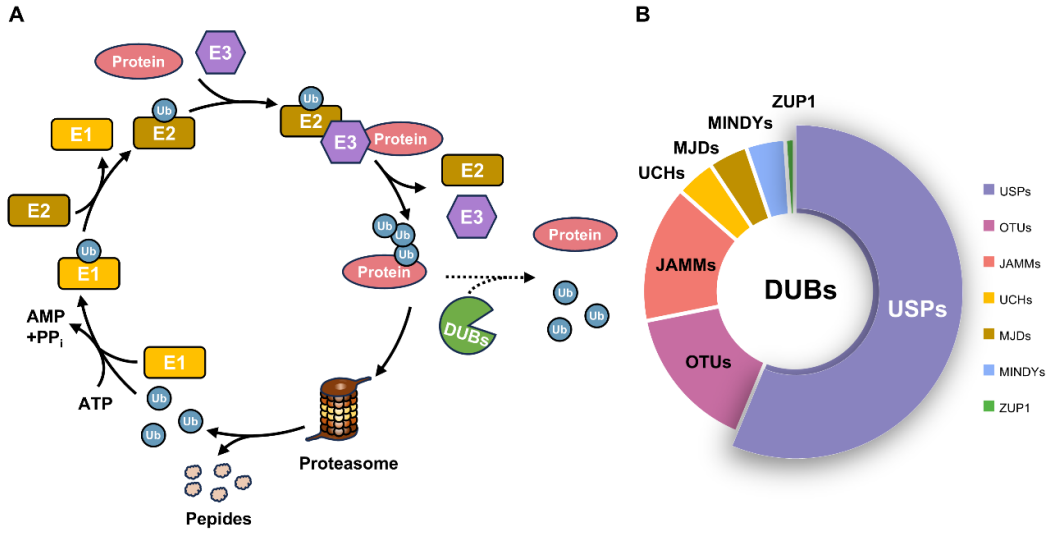


Figure 1. (**A**) Ubiquitination and deubiquitination pathways. E1 activates and binds Ub in an ATP-dependent manner. Then, the activated Ub is transferred to E2, where it is coupled to the target protein in the presence of E3. Target proteins tagged with Ub can be degraded by the proteasome, and Ub is recycled. Deubiquitinating enzymes remove Ub from target proteins, rescuing them from degradation. (**B**) DUB can be categorized into seven families: ubiquitin-specific proteases (USPs, purple), ovarian tumor-like proteases (OTUs, rose), Jab1/Pab1/MPN domain containing metalloproteinases (JAMMs, pink), ubiquitin C-terminal hydrolases (UCHs, yellow), Machado-Joseph domain proteases (MJDs, brown), MIU containing novel DUB family (MINDYs, blue), and zinc-binding metalloprotease (ZUP1, green).

HAFOUS, a recently identified neurodevelopmental disorder resulting from pathogenic USP7 gene mutations, is clinically characterized by a triad of core features: speech delay, intellectual disability, and autism spectrum disorder (ASD) [16]. Given the key regulatory role of USP7 in pathological processes, it has emerged as a highly promising target for new drugs.

Significant progress has been made in developing both covalent and non-covalent USP7 inhibitors. Besides, some of them have been utilized as warheads to obtain USP7 targeted chimeras (PROTACs) for inducing USP7 degradation. These compounds decrease USP7 activity through different mechanisms, laying the foundation for precise treatment of related diseases [17]. The need to target rare neurodevelopmental disorders such as HAFOUS has driven the exploration of agonist research. Recent studies have found that the antidepressant Sertraline, the antihistamine drug Astemizole, and compound MS-8 can act as USP7-specific agonists to rescue the functional defects of HAFOUS causing mutants, providing new ideas for the treatment of the disease [18,19].

In this review, we systematically summarize the structural information of USP7, its physiological functions and key roles in the incidence of diseases, and then comprehensively elaborate on the progress in the development of related regulators. With its distinctive domain architecture and catalytic mechanism, USP7 participates in regulating diverse biological processes and plays an indispensable role in maintaining cellular homeostasis. Importantly, this review presents the first systematic synthesis of recent advances in HAFOUS research and reveals the pathogenic mechanism by which USP7 haploinsufficiency or heterozygous mutations cause WASH protein inactivation, ultimately leading to protein cycling disorders. Afterwards, we illustrate the latest research progress of USP7 agonists currently developed for HAFOUS. These findings not only reveal new perspectives for understanding the biological functions of USP7 but also provide potential targets for diseases.

2. USP7 Structure

USP7 contains 1102 amino acids with three distinct domains: a TRAF-like domain (amino acids 62-208) at the N-terminal end, a catalytic domain (amino acids 208-560), and five tandemly linked ubiquitin-like (UBL) domains (amino acids 560-1102) at the C-terminal end (Figure 2A,B) [7]. The TRAF domain recognizes and binds proteins involved ubiquitination pathway through the conserved "(P/A/E)XXS" motifs, such as Epstein-Barr nuclear antigen 1 (EBNA1), tumor suppressor proteins p53, MDM2, and phosphatase and tensin homolog (PTEN), while affecting the nuclear localization of USP7 [7,20].

The catalytic domain (CD), which serves as the structural basis for USP7 activity, binds to the Ub molecule and cleaves the isopeptide bond between Ub and the substrate, thus catalyzing the deubiquitination of the substrate

[21,22]. Crystal structure analysis reveals that it contains 10 α-helices and 14 β-sheet and can be divided into three parts: a finger region, a thumb region, and a palm region, which is akin to an outstretched right hand. The catalytic triad, composed of Cys223, His464 and Asp481 is located in the catalytic cleft between the thumb and palm region, which is mainly responsible for regulating the enzymatic activity of USP7. Besides, the finger region mainly binds to Ub. With the combined action of three components, the ubiquitin chain linked to target proteins can be removed. Both the structure and function of the catalytic domain of USP7 are highly similar to papain enzymes from plant. Normally, the residues of the catalytic triad are spatially distant from each other and exist in an unproductive conformation. Upon binding to Ub, the residues in the active site undergo a rearrangement and shift into an active conformation that can realize the function of deubiquitylation [23,24]. When the carboxyl group at the C-terminal end of Ub conjugates to the amino group of the substrate, the following reactions are stimulated: (1) His464 deprotonates the sulfhydryl group of Cys223 to produce thiol anion; (2) The thiol anion mediates nucleophilic attack on the isopeptide bond between the substrate and Ub, resulting in Ub dissociation from the substrate; (3) The thioester group reacts with a water molecule to release Ub. In this process, Asp481 promotes the catalytic reaction by polarizing His464 and restricting its side chain rotation, rather than directly participating in the attack reaction (Figure 2C) [25].

The five tandem UBL (UBL-1, UBL-2, UBL-3, UBL-4, UBL-5) at the C-terminal end are arranged in the form of "2-1-2" and connected by flexible linkers [26].To be specific, UBL-123 is involved in the binding of ICP0, ubiquitin-like and ring finger domain 1 (UHRF1), DNA methyltransferase1 (DNMT1), and MDM2 [27–29]. UBL-45 recognizes and binds p53 and forkhead box family 4 (FOXO4) [30]. Furthermore, the C-terminal region of USP7 is essential for USP7 activation. UBL-45 deletion does not significantly alter the protein levels of p53 or MDM2 in transfected human osteosarcoma cells U2OS. Additionally, Ub-AMC hydrolysis assays are conducted using a chimeric mutant protein in which USP7CD is directly fused to UBL-45 (USP7CD-45). The enzymatic activity profile of USP7CD-45 closely resembles that of the USP7CD-UBL, demonstrating that UBL-45 can effectively activate USP7 catalytic function through its interaction with the "switching loop" in the catalytic domain. Although UBL-123 cannot directly activate the catalytic domain, it binds to guanosine monophosphate synthetase (GMPS) to stabilize the interaction between UBL-45 and USP7CD. This stabilization maintains USP7 in a constitutively active conformation, thereby promoting its activated state. The above studies illustrate that the enzymatic activity function of USP7 can be precisely regulated, providing a theoretical basis for the discovery of targeted USP7 regulators [31].

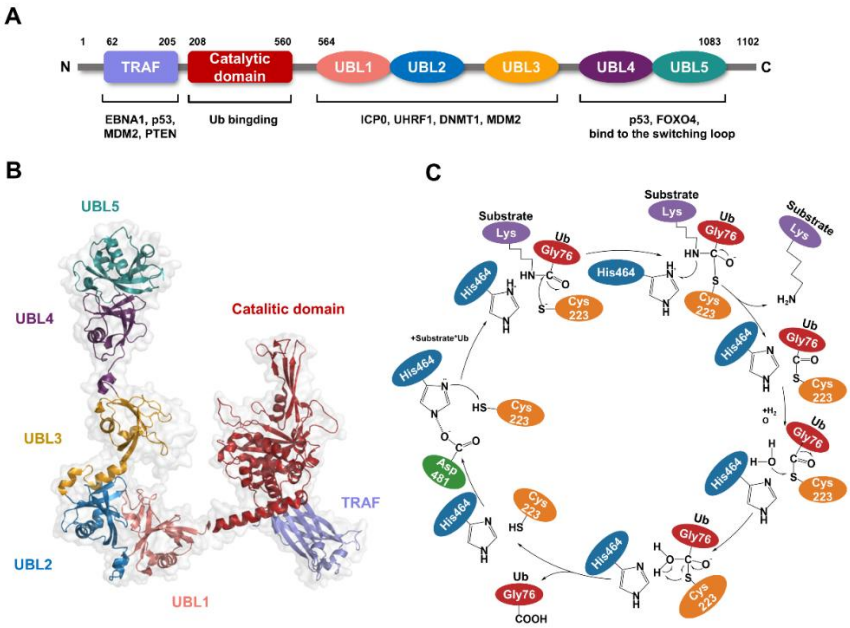


Figure 2. Structural illustration of USP7. (**A**) Structural domains of USP7 and recognized substrates. The domains are mainly classified into the TRAF domain, the catalytic domain and five UBL domains. Numbers indicate residue regions. (**B**) Schematic representation of USP7 protein crystal structure. The full-length USP7 structural model consists of overlapping partial fragments, including the TRAF domain (PDB ID: 2F1W), the catalytic domain (PDB ID: 5FWI) and the UBL1-5 domain (PDB ID: 2YLM). Each structural domain is labeled and color coded: TRAF (light purple), catalytic domain (red), UBL1 (pink), UBL2 (blue), UBL3 (yellow), UBL4 (aubergine) and UBL5 (cyan). (**C**) Mechanism of ubiquitin cleavage by USP7.

3. Physiological Functions of USP7

3.1. Regulation of the Cell Cycle

The mitotic cell cycle (G1, S, G2, M phases) proceeds in an orderly manner under the regulation of DNMT1, its cofactor UHRF1, and cyclin-dependent kinase (CDK) family members. The RING domain of UHRF1 is essential for the E3 ligase activity in vitro and plays a critical role in regulating the growth of tumor cells [32]. The SRA domain of UHRF1 can bind to hemimethylated DNA, recruiting DNMT1 to lysine 9 methylated histone H3 (H3K9Me2/3) on DNA substrates and promoting DNA methylation [33]. The ubiquitination of DNMT1 is tightly regulated by UHRF1 and USP7 (Figure 3A). The overexpression of UHRF1 and USP7 exhibited contrasting effects on the ubiquitination and stability of DNMT1. The former promotes the ubiquitination of DNMT1 and reduces the stability, while the latter inhibits the recruitment of DNMT1 to replication forks by mediating the deubiquitination of DNMT1 and UHRF1, which improves the stability of DNMT1 and also helps avoid excessive DNA methylation of DNMT1, thus ensuring the cell cycle can proceed in a homeostatic manner [34,35].

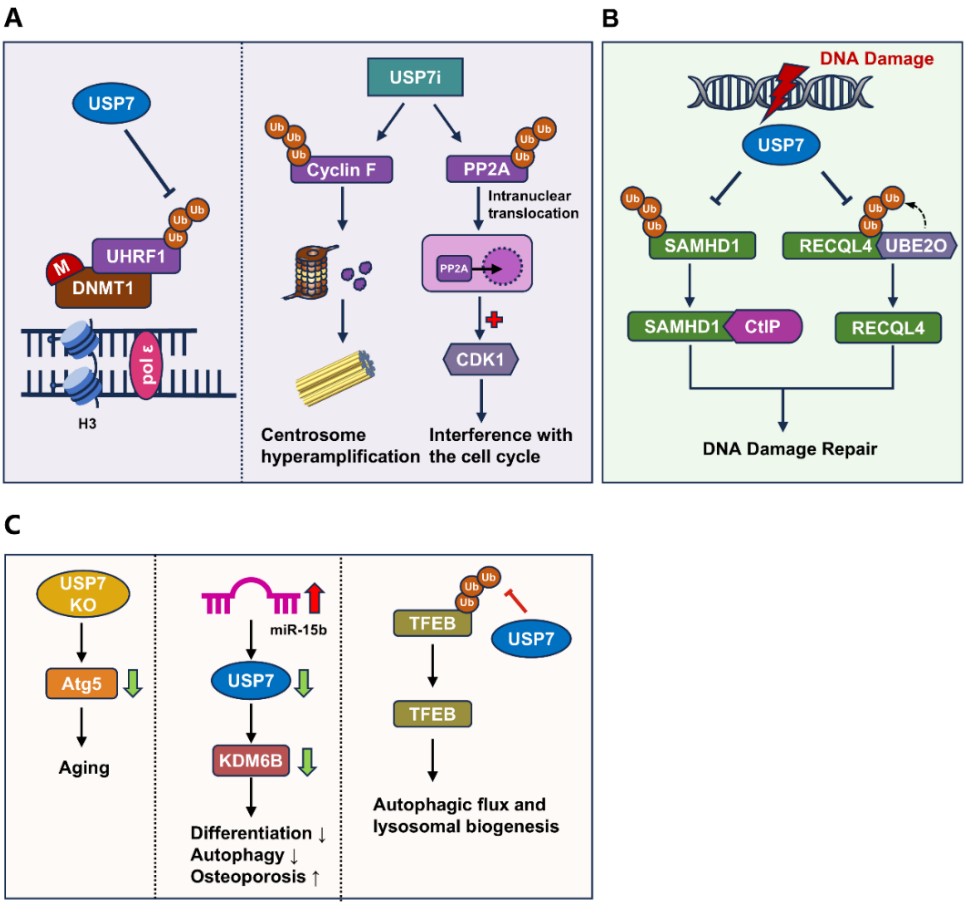


Figure 3. Physiological functions of USP7 (**A**) Cell cycle regulation by USP7. USP7 affects the cell cycle by interacting with UHRF1 and DNMT1 to regulate DNA methylation and promote their recruitment to H3K9Me2/3. In addition, both the cell cycle protein cyclin F and the protein phosphatase PP2A are affected when USP7 is inhibited, which in turn interferes with the homeostatic operation of mitosis. (**B**) Repair of DNA damage by USP7. During DNA damage, USP7 deubiquitinates and stabilizes SAMHD1, a dNTP hydrolase, which binds to the DSB repair promoter CtIP and promotes DNA damage repair. At the same time, USP7 antagonizes UBE2O-mediated ubiquitination of human RecQ DNA helicase RECQL4, thereby rescuing the damage caused by DSB. (**C**) Regulation of autophagy by USP7. Knockdown of USP7 decreases protein and mRNA expression levels of Atg5, which in turn causes shortened lifespan. In osteoporosis, upregulated miR-15b negatively regulates the USP7/KDM6B axis, inhibiting osteoblast differentiation and autophagy. In addition, USP7 deubiquitinates TFEB, and thus adjusts autophagic flux and lysosomal biogenesis.

The oscillating activity of CDKs is crucial for cell cycle progression and is regulated by cyclin F. USP7 interacts with cyclin F and influences its abundance and stability through both deubiquitinase activity-dependent and deubiquitinase activity-independent mechanisms. USP7 inhibition reduces cyclin F levels, causing centrosome

amplification and genomic instability [36]. Additionally, USP7 suppression facilitates nuclear translocation and inactivation of PP2A, contributing to CDK1 activation and increased phosphorylated histone H3 (H3S10P) levels, a mitotic marker, which confirms disrupted cell cycle progression [37].

3.2. Repair of DNA Damage

The human body maintains genomic homeostasis through DNA-damage response (DDR) pathways that repair endogenous and exogenous DNA lesions [38]. However, in cancer cells, activated DDR counteracts DNA damage from oxidative stress and cytotoxic agents, thus promoting chemotherapy drug resistance [39]. Both USP7 and SAMHD1, a dNTP hydrolase, are upregulated in cancer cells (Figure 3B). It has been found that USP7 specifically recognizes and binds to the HD domain of SAMHD1, which in turn removes the K48-linked polyubiquitin chain at the K421 site. Then the stabilized SAMHD1 interacts with the DNA double-strand break (DSB) repair initiator CtIP to trigger damage repair, overcome oncogenic stress, and promote cell survival [40,41].

The human RecQ DNA helicase RECQL4 has been shown to stabilize the genome through the DDR process and is engaged in the regulation of several cancers and aging [42]. The E2/E3 hybrid ubiquitin-conjugating enzyme UBE2O binds the Sld2-like domain of RECQL4 to cause proteasomal degradation and inhibits homologous recombination (HR) mediated repair of DSB. In contrast, USP7 antagonizes UBE2O-mediated ubiquitination and RECQL4 degradation, rescuing the damage [43].

3.3. Regulation of Autophagy

Autophagy is a stress-responsive catabolic process that maintains cellular and tissue homeostasis through lysosomal degradation and recycling of intracellular components (Figure 3C) [44,45]. The study of USP7 in the aging pathway is still relatively limited. Using *Drosophila* as a study subject, it has been found that knockdown of USP7 significantly decreases the protein and mRNA expression levels of autophagy-associated 5 (Atg5). In addition, autolysosomes are reduced in midgut epithelial cells. The use of DMC, a derivative of celecoxib, can rescue USP7 knockdown-induced shortened lifespan, reduced climbing ability, decreased resilience, and loss of intestinal barrier integrity, independent of increased antioxidant capacity [46]. In osteoporosis, miR-15b expression is upregulated and negatively affects the expression of USP7/KDM6B axis. The above processes inhibit osteoblast differentiation and autophagy, further aggravating osteoporosis [47]. As a master regulator of lysosomal biogenesis and autophagy, transcription factor EB (TFEB) is predominantly post-translationally modified by USP7. By stabilizing TFEB through deubiquitination, USP7 maintains TFEB-mediated transcriptional responses to nutrient deprivation, as well as regulates autophagic flux and lysosomal biogenesis. These findings set the stage for targeting the USP7-TFEB axis to treat conditions of TFEB dysregulation and metabolic abnormalities, especially in certain cancers [48].

3.4. Regulation of Classical Signaling Pathways

3.4.1. MDM2-p53 Signaling Pathway

USP7 has emerged as one of the most extensively studied deubiquitinases in the USP family, primarily due to its important role in regulating the MDM2-p53 signaling pathway. By regulating the cell cycle, mediating programmed cell death, and activating DNA damage repair, p53 serves as a regulator of genomic stability and cellular homeostasis. Dysregulation of these p53-mediated processes is mechanistically linked to the pathogenesis of diverse diseases, including malignancies, neurodegenerative disorders, and ischemic conditions [49]. MDM2, the oncogenic RING E3 ligase of p53, accelerates p53 degradation through ubiquitination and silences transcription of downstream target genes [50].

As a negative regulator of p53, USP7 can directly deubiquitinate p53 and MDM2 (Figure 4) [51,52]. The mechanism is as follows: (1) Under physiological conditions, USP7 preferentially binds and stabilizes MDM2 by preventing its autoubiquitination, which indirectly triggers the degradation of p53; (2) Under cellular stress, GMPS competitively displaces MDM2 from the USP7-MDM2-p53 complex, allosterically activating USP7 to deubiquitinate and stabilize p53 [53–55]. Therefore, pharmacological inhibition of USP7 promotes MDM2 degradation, relieving its negative regulation on p53 and restoring tumor suppression, which provides a novel therapeutic strategy for targeted cancer therapy [24].

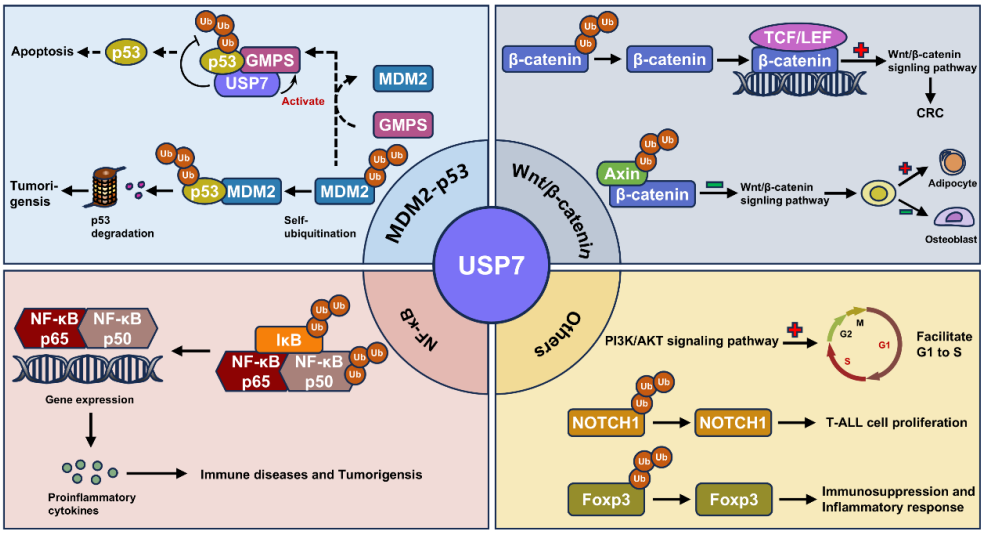


Figure 4. Regulation of classical signaling pathways by USP7. MDM2-p53: Under normal conditions, USP7 preferentially deubiquitinates MDM2, an E3 ubiquitin ligase, which promotes the degradation of the oncogenic factor p53 and induces tumorigenesis. Under cellular stress, GMPS displaces MDM2 in the complex and activates USP7, which stabilizes p53 through the process of deubiquitination and activates apoptosis. The solid line is the normal condition and the dashed line is the cellular stress state. Wnt/β-catenin: USP7 deubiquitinates β-catenin and the scaffolding protein Axin in colon cancer cells and osteoblasts/adipocytes, respectively, the former activating the Wnt/β-catenin signaling pathway to induce CRC, and the latter inhibiting the Wnt/β-catenin signaling pathway, thereby inhibiting osteoblast differentiation and promoting adipocyte differentiation. NF-κB signaling pathway: USP7 directly deubiquitinates NF-κB or upstream factor IκB, activating NF-κB signaling and thus increasing the expression of cellular inflammatory factors. Other pathways: USP7 activates the PI3K/AKT signaling pathway while deubiquitinating NOTCH1 and Foxp3, affecting cell cycle and inflammatory responses.

3.4.2. Wnt/β-Catenin Signaling Pathway

Colorectal cancer (CRC) pathogenesis is strongly associated with aberrant activation of Wnt/β-catenin signaling pathway. In the absence of Wnt, the homeostatic regulation of β-catenin is mainly mediated by the destruction complex, which contains the scaffolding protein Axin, the tumor suppressor adenomatous polyposis coli (APC), glycogen synthase kinase 3 (GSK3) and casein kinase 1α (CK1α) [56]. In the nucleus, β-catenin binds to transcription factors TCF/LEF to form a complex that activates the Wnt signaling pathway, and subsequently induces tumors [57].

In 2017, Novellasdemunt et al. proposed that USP7 can directly interact with the N-terminus of β-catenin to prevent its proteasomal degradation. In CRC with APC truncating mutations, reduced recruitment of the E3 ligase β-TrCP to the destruction complex switches β-catenin stabilization from proteasomal degradation to USP7-mediated deubiquitination. The process ultimately results in β-catenin accumulation and Wnt pathway activation. Therefore, the inhibition of USP7 restores β-catenin ubiquitination and degradation, effectively suppressing oncogenic Wnt/β-catenin signaling to inhibit tumor proliferation and stimulate terminal differentiation [58]. Subsequent work by Li et al. revealed that USP7 preferentially interacted with Axin through the TRAF domain, stabilizing it by blocking proteasomal degradation and consequently exerting negative regulation on Wnt/β-catenin signaling, without direct β-catenin binding. Surprisingly, the next-generation USP7 inhibitors unexpectedly potentiated Wnt/β-catenin signaling, which contrasted with prior findings and may stem from the off-target effects of early-generation inhibitors [11]. These findings demonstrated the potential of USP7 as a Wnt inhibitor for APC-mutant CRC. Novellasdemunt's team systematically evaluated the function of USP7 in CRC models, proving that USP7 inhibitors significantly suppressed growth in both patient-derived organoids (PDOs) and xenografts carrying APC truncations. This evidence established USP7 as a promising tumor-specific therapeutic target in APC-mutant CRC through Wnt pathway inhibition. Notably, all analyzed PDOs harbored p53 mutations regardless of treatment response, confirming USP7-mediated Wnt regulation occurs independently of p53. The apparent functional discrepancies between studies may reflect tissue-specific effects, with Novellasdemunt et al. focusing on the intestinal system while Li et al. primarily examined osteoblast/adipocyte differentiation [59].

3.4.3. Nuclear Factor (NF)-kB Signaling Pathway

The transcription factor NF-κB is closely associated with the development of inflammatory responses and may contribute to immune diseases and hematopoietic malignancies when NF-κB signaling is over-activated [60]. NF-κB is induced to translocate to the nucleus by Toll-like receptor (TLR) ligand or TNFα. USP7 can directly deubiquitinate upstream factor lκB as well as p65 and interacts with the promoter proximal end of NF-κB in a DNA-dependent manner. By counteracting proteasomal degradation, it enhances the transcriptional stability of NF-κB, which in turn promotes the release of proinflammatory cytokines [61,62]. Clinically, the overexpression of USP7 correlates with poor prognosis in various cancers. Targeting USP7 to disrupt NF-κB signaling consequently suppresses inflammatory cytokine production and overcomes drug resistance in multiple myeloma (MM) [63].

3.4.4. Other Signaling Pathways

USP7 also plays important roles in other oncogenic pathways. In Hepatoblastoma (HB), the expression of USP7 is upregulated, which activates the PI3K/AKT signaling pathway and promotes the transition of tumor cells from G1 phase to S phase. When USP7 is inhibited, HB cell proliferation, migration, and invasion are decreased, which is expected to be a new target for HB treatment [64]. Similarly, in the majority of T-cell acute lymphoblastic leukemia (T-ALL) cases, USP7 expression is significantly upregulated while the NOTCH1 signaling pathway is aberrantly activated. USP7 specifically binds to the NOTCH1 protein through its MATH and UBL domains, thereby deubiquitinating and stabilizing NOTCH1. Consequently, inhibiting USP7 promotes NOTCH1 degradation, suppresses the transcriptional activity, and simultaneously induces apoptosis in T-ALL cells, providing a novel molecular basis for targeted therapy of T-ALL [65–67]. Beyond direct tumorigenic effects, USP7 modulates immune responses by stabilizing Foxp3 in regulatory T cells. USP7 inhibition diminishes Foxp3 stability, thereby alleviating immunosuppression and enhancing antitumor immunity. This mechanism holds promise for cancer immunotherapy [68,69].

4. USP7 and Diseases

4.1. Cancer

USP7 is a vital therapeutic target in oncology due to its multifaceted regulation of tumor-associated factors and signaling pathways. Clinically, USP7 is overexpressed in multiple malignancies, including lung cancer, prostate cancer, breast cancer, and colorectal cancer (CRC) (Figure 5). Inhibition or knockdown of USP7 induces cell death and restores the sensitivity to anticancer drugs.

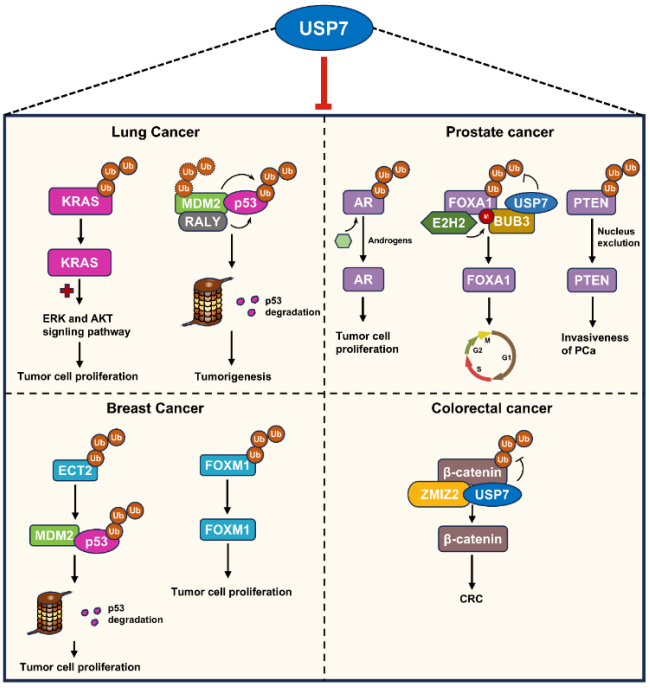


Figure 5. Regulatory mechanisms of USP7 in different cancer types. In lung cancer, prostate cancer, breast cancer, and colorectal cancer, USP7 triggers tumorigenesis by deubiquitinating multiple substrate proteins.

4.1.1. Lung Cancer

Lung cancer, one of the most prevalent malignancies, is primarily classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with NSCLC accounting for approximately 80%. KRAS, frequently mutated in NSCLC, drives aberrant signaling [70,71]. USP7 recognizes and binds KRAS through the TRAF domain and removes K48-linked polyubiquitin chains at the K147 site, thereby activating downstream pathways and promoting NSCLC proliferation [72]. Additionally, USP7 inhibition reprograms tumor-associated macrophages (TAMs) in the tumor microenvironment, restoring anti-tumor immunity and synergizing with PD-1 antibodies to suppress immune evasion [73]. The deubiquitinase activity of USP7 toward MDM2 is amplified by the RNA-binding protein RALY, leading to enhanced stability of MDM2. This activity subsequently inhibits p53 and induces lung tumorigenesis [74].

4.1.2. Prostate Cancer

Prostate cancer (PCa), one of the most common malignancies in men, is closely related to the androgen receptor (AR). As a cofactor of AR, USP7 binds to AR in an androgen-dependent manner and induces its deubiquitination. The USP7-AR synergy upregulates the expression of proliferation-related genes in prostate cancer cells [75]. Importantly, USP7 inhibition not only counteracts androgen-induced AR activation but also reduces the tumor suppressor protein CCDC6, thus enhancing cancer cell sensitivity to PARP inhibitors [76].

Forkhead box protein A1 (FOXA1), a key transcriptional cofactor of AR, primarily drives androgen-dependent cell growth while partially suppressing dedifferentiation pathways in prostate cells [77,78]. BUB3 protein containing WD40 repeats recognizes FOXA1 methylated by Polycomb group (PcG) protein enhancer of zeste homolog 2 (EZH2) and subsequently recruits USP7 to deubiquitinate FOXA1. BUB3 and USP7 jointly regulate cell cycle progression, which is closely associated with the development of aggressive PCa [79,80]. Consequently, FOXA1-driven PCa may be effectively targeted by combined inhibition of EZH2 and USP7.

Moreover, USP7 modulates the tumor suppressor PTEN by altering its subcellular localization. Knockdown of USP7 retains PTEN in the nucleus and maintains its tumor-suppressive activity. However, USP7 overexpression promotes PTEN deubiquitination and cytoplasmic translocation, impairing its tumor-suppressive role and contributing to PCa progression [81].

4.1.3. Breast Cancer

Breast cancer is the second leading cause of cancer-related deaths in women. The epithelial cell transforming factor ECT2 promotes breast cancer cell growth through both guanine nucleotide exchange factor (GEFs)-dependent and GEFs-independent mechanisms. ECT2 and USP7 stabilize each other through a positive feedback loop, thus enhancing MDM2 expression. The underlying mechanisms include: (1) ECT2 antagonizes USP7 polyubiquitination in a GEF-independent manner, thereby stabilizing USP7; (2) USP7 directly deubiquitinates and stabilizes ECT2 [82].

Triple-negative breast cancer (TNBC) is the most aggressive and metastatic subtype of breast cancer [83]. It has been revealed that the oncogenic transcription factor FOXM1 is highly expressed in TNBC cells, with USP7 identified as its interacting partner. The USP7 PROTAC PU-7 effectively retards FOXM1 transcription, significantly inhibiting cancer cell proliferation and exerting tumor-suppressive effects, which proposes a novel approach to the treatment of this malignant tumor [84].

4.1.4. Colorectal Cancer

Aberrant activation of the β-catenin signaling pathway is closely associated with CRC development. The nuclear protein ZMIZ2 recruits USP7 to β-catenin and mediates its deubiquitination, significantly enhancing β-catenin stability. This stabilization activates downstream oncogenic signaling pathways and drives tumor progression [85]. In CRC with truncated APC mutations, USP7 inhibitors effectively block p53-independent Wnt/β-catenin signaling, suppressing the growth of patient-derived organoids and xenografts [59].

4.2. Metabolic Diseases

The role of USP7 in metabolic diseases is also becoming increasingly apparent (Figure 6). As a negative regulator of the glycolytic deacetylase Sirtuin 7 (SIRT7), USP7 removes Lys63-linked polyubiquitin chains to suppress SIRT7 enzymatic activity rather than regulate its stability. Then, the H3K18 acetylation on the promoter of the gluconeogenic gene glucose-6-phosphatase catalytic subunit (G6PC) activates the expression and consequently modulates glucose metabolism [86]. In diabetic cardiomyopathy (DCM), USP7 exacerbates myocardial injury and

mitochondrial dysfunction through the activation of the PGC1-β/PPARα signaling pathway [87]. In addition, USP7 induces pathological hepatic de novo lipogenesis (DNL). On the one hand, USP7 deubiquitinates and stabilizes zinc finger protein 638 (ZNF638) in hepatocytes. On the other hand, sterol regulatory element binding protein (SREBP1C) is cleaved through the USP7/ZNF638 axis. These coordinated actions facilitate the formation of a USP7/ZNF638/SREBP1C nuclear complex that activates lipogenic enzymes, eliciting fatty liver disease and other hepatic disorders [88]. Beyond lipid metabolism, inhibition of USP7 also slows the progression of atherosclerosis (AS) by targeting EZH2 [89].

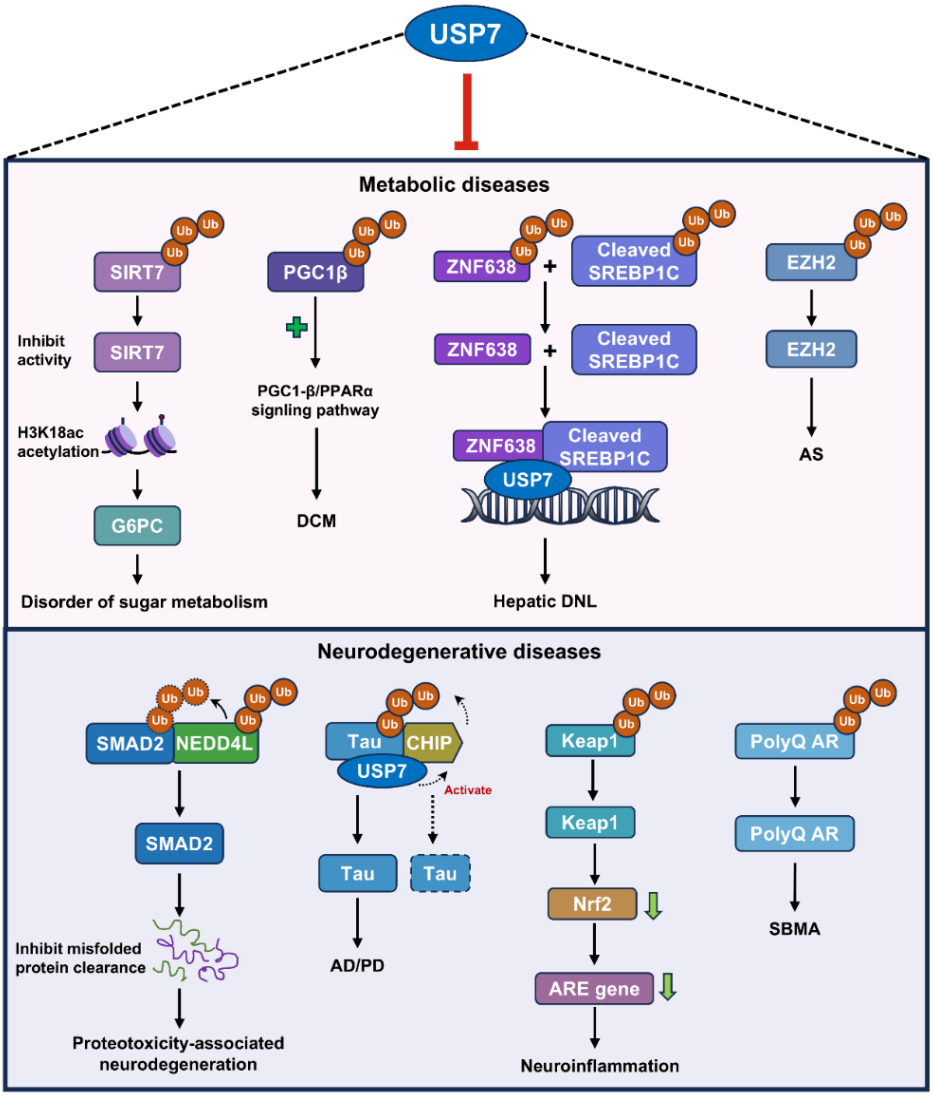


Figure 6. Regulatory mechanisms of USP7 in metabolic and neurodegenerative diseases.

4.3. Neurodegenerative Diseases

Neurodegenerative diseases have recently become a major focus for novel drug target discovery. Current evidence indicates that disorders such as Creutzfeldt-Jakob disease, Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) primarily arise from misfolding or aggregation of pathologic proteins, imbalance of protein homeostasis, and inflammation [90,91].

USP7 functions as a transcriptional switch regulating protein quality control genes. It counteracts the ubiquitination of SMAD2, an ALS-associated protein, by inhibiting the E3 ubiquitin ligase NEDD4L. This action prevents SMAD2 degradation and subsequently enhances clearance of misfolded proteins, which implies the critical role of USP7 in the pathogenesis of proteotoxicity-related neurodegeneration [92]. The development of AD and PD is strongly associated with elevated endogenous Tau levels. Similar to the dual regulatory mechanism of MDM2-p53, USP7 both enhances CHIP activity to destabilize Tau and inhibits CHIP-mediated Tau ubiquitination to stabilize Tau, with the latter effect predominating. Knockdown of USP7 can effectively alleviate memory decline in PS19 mice [93]. Furthermore, inhibition of USP7 contributes to the ubiquitination degradation

of Kelch-like ECH-associated protein 1 (Keap1) and promotes the expression of antioxidant response element (ARE)- dependent genes. The transcription factor Nrf2 mediates this enhancement, engendering the suppression of microglia activity. This study first reveals that targeting USP7 ameliorates microglia-mediated neuroinflammation through the Keap1/Nrf2 signaling axis, casting light on the treatment of neurodegenerative diseases [15].

The role of USP7 in the regulation of AR function has been revealed in PCa [75]. Recently, it has also been pointed out that polyglutamine (polyQ) amplification within the AR will trigger progressive neuromuscular toxicity in spinal and bulbar muscular atrophy (SBMA). USP7, a preferred interactor of polyQ-amplified AR, mediates 5α-dihydrotestosterone (DHT)-dependent mutant AR aggregation and cytotoxicity. Thus, inhibition of USP7 attenuates these effects and ameliorates the disease phenotype in the SBMA mouse model as well as in the SBMA and SCA3 Drosophila models [46].

4.4. Hao-Fountain Syndrome

The MAGE-L2-TRIM27 ubiquitin ligase enhances endosomal protein recycling by mediating K63-linked polyubiquitination of WASH, an actin nucleating protein essential for this process. USP7 has a dual regulatory role in this system: (1) It promotes WASH ubiquitination by counteracting TRIM27 auto-ubiquitination; (2) It prevents WASH overactivation through direct deubiquitination (Figure 7A). This seemingly paradoxical regulation enables precise tuning of WASH activity, maintaining optimal endosomal F-actin levels and ensuring proper protein recycling. Heterozygous loss or mutation of USP7 disrupts WASH-mediated protein recycling, leading to neurodevelopmental disorders. In the first reported seven cases of de novo heterozygous loss-of-function mutations of USP7, Hao et al. have observed that these patients shared characteristic clinical features with MAGE-L2-deficient Schaaf-Yang syndrome (SYS), including developmental delay/intellectual disability (DD/ID), autism spectrum disorder (ASD), hypogonadism, and hypotonia (Figure 7B). Compared to USP7 haploinsufficiency cases, these patients exhibit more pronounced neurological phenotypes, particularly a higher incidence of epilepsy and more prominent aggressive behaviors [16,94].

In 2019, the team further reported 16 additional individuals with de novo pathogenic USP7 variants, expanding the phenotypic spectrum to include feeding difficulties, gastroesophageal reflux disease (GERD), eye anomalies, and abnormal brain magnetic resonance image findings. The analysis confirms relatively high prevalence (>50%) of DD/ID, ASD, and hypotonia, with relatively high frequency (<50%) of seizures and hypogonadism. This neurodevelopmental disorder, characterized by speech delay, intellectual disability, and ASD connected with heterozygous USP7 mutations or deletions, is designated Hao-Fountain syndrome [95]. Subsequent reports identified rare clinical manifestations including isolated tube torsion [96], congenital heart defects, pregnancy complications [97], and abnormal pain thresholds [98].

A recent study has shown that USP7 participates in the process of histone ubiquitination modification by stabilizing the non-classical multi-comb repressive complex (ncPRC1), which in turn regulates the expression of several key neurodevelopmental factors, including AUTS2.

Functionally, these target genes constitute indispensable factors during the neurodevelopmental processes and the pathogenesis of ASD, which supply important clues for a deeper understanding of the molecular mechanisms of HAFOUS [98]. A sensitive and specific DNA methylation (DNAm) epigenetic signature shows promise as a robust diagnostic biomarker for HAFOUS, enabling reclassification of variants with uncertain significance (VUS) in USP7. Wimmer et al. have developed an HAFOUS severity scoring system revealing that patients with missense mutations in the USP7 catalytic domain exhibit significantly higher pathogenicity scores compared to those with mutations in the TRAF or UBL domains. This phenomenon proves that missense variants within the catalytic domain correlate with more severe clinical phenotypes [99].

The forebrain glutamatergic neuron-specific USP7 knockout mice (USP7 cKO) recapitulate HAFOUS-like phenotypes, establishing a valid disease model. USP7 deficiency triggers p53-dependent neuronal apoptosis, though the glutamatergic neuron-associated phenotypes remain p53-independent. Crucially, this work identifies the RNA splicing factor Ppil4 as a direct USP7 target in HAFOUS pathogenesis. Disruption of the USP7-Ppil4 pathway perturbs the synaptic proteome and dendritic spine formation, which is prone to be instrumental in the disease development [100].

Given the established pathogenesis and phenotypic spectrum of HAFOUS, the enhancement of USP7 enzymatic activity through targeted agonists has been regarded as a promising therapeutic strategy. Recent breakthrough findings identify Sertraline and Astemizole as first-reported USP7 agonists. These compounds can bind to the switch-loop region of USP7, effectively restoring deubiquitinase activity in pathogenic USP7 mutants

[18]. Then, MS-8 has also been found to mimic the allosteric autoactivation of the USP7 C-terminal tail, paving the way for a new activation-driven USP7 pharmacology [19].

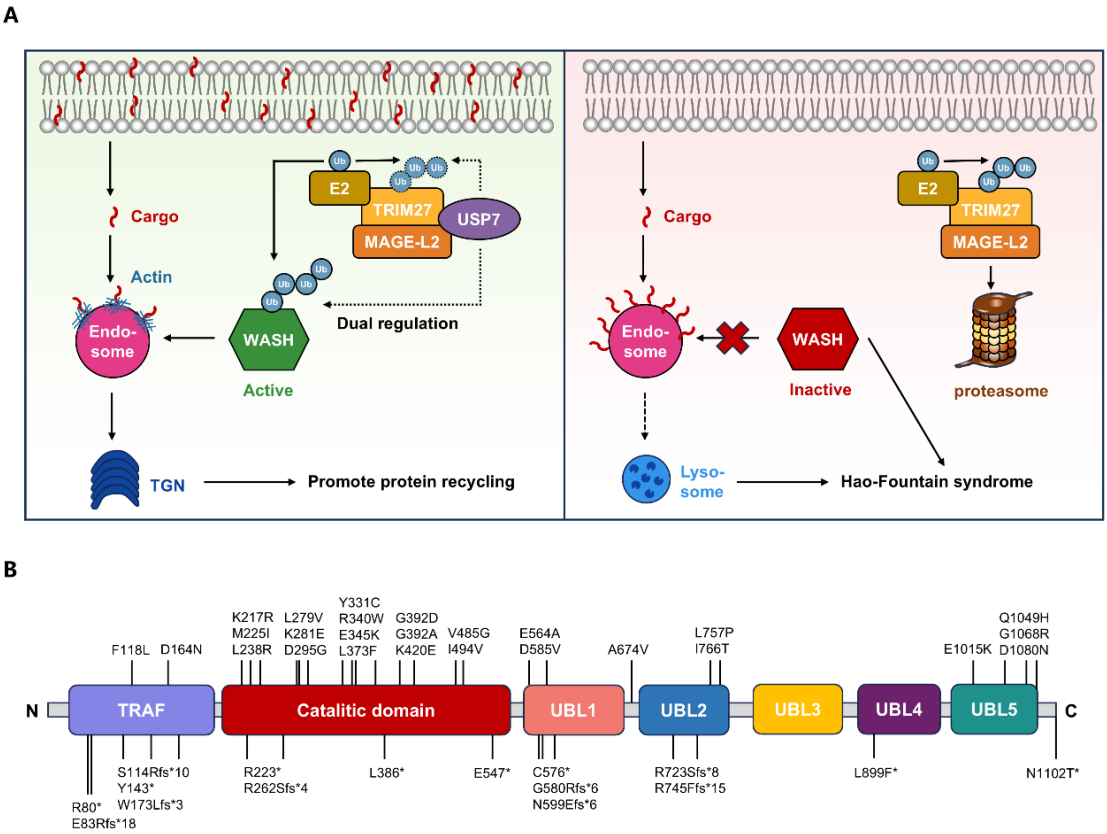


Figure 7. Link between USP7 and Hao-Foutain syndrome. (**A**) USP7 haploinsufficiency/heterozygous mutations cause Hao-Fountain syndrome. Under normal conditions, USP7 interacts with TRIM27 and WASH to exert dual regulatory functions. The activated WASH drives actin assembly on endosomal surfaces, forming an endosomal actin network that facilitates cargo trafficking from endosomes to the trans-Golgi network (TGN), ensuring proper protein recycling. When USP7 is haploinsufficient or carries heterozygous mutations, the TRIM27-MAGE-L2 complex undergoes degradation. Consequently, the WASH complex fails to activate, leading to reduced endosomal actin networks, impaired cargo sorting, and suppression of protein recycling pathways. This causes misrouting of cargo to lysosomes for degradation or accumulation in endosomes, ultimately resulting in neurodevelopmental disorders. (**B**) Distribution of missense and nonsense variants of USP7 in Hao-Fountain syndrome. Known domains of USP7 are labeled, with missense mutations shown above and nonsense mutations below the protein schematic diagram. The variant type represented by "*" is nonsense mutations or frameshifting indels.

5. USP7 Modulators

5.1. Chemical Synthesis Derived Non-Covalent Inhibitors Targeting the Catalytic Domain

As the first identified small-molecule non-covalent inhibitor of USP7, HBX41108 preferentially targets the enzyme-substrate complex rather than competing directly with substrate binding (Table 1). This compound stabilizes and activates p53 in a non-genotoxic manner, inducing p53-dependent apoptosis and suppressing HCT116 colon cancer cell growth [101,102]. This groundbreaking discovery has established the foundation for developing allosteric USP7 inhibitors. In the subsequent analysis of co-crystal structures, it has been found that the non-covalent binding of inhibitors to USP7 is mainly located at two sites: (1) the allosteric pocket away from the catalytic domain, (2) the narrow and long catalytic groove occupied by the C-terminal tail of ubiquitin (Figure 8). Remarkably, most inhibitors interact with the catalytic site of the latter [103].

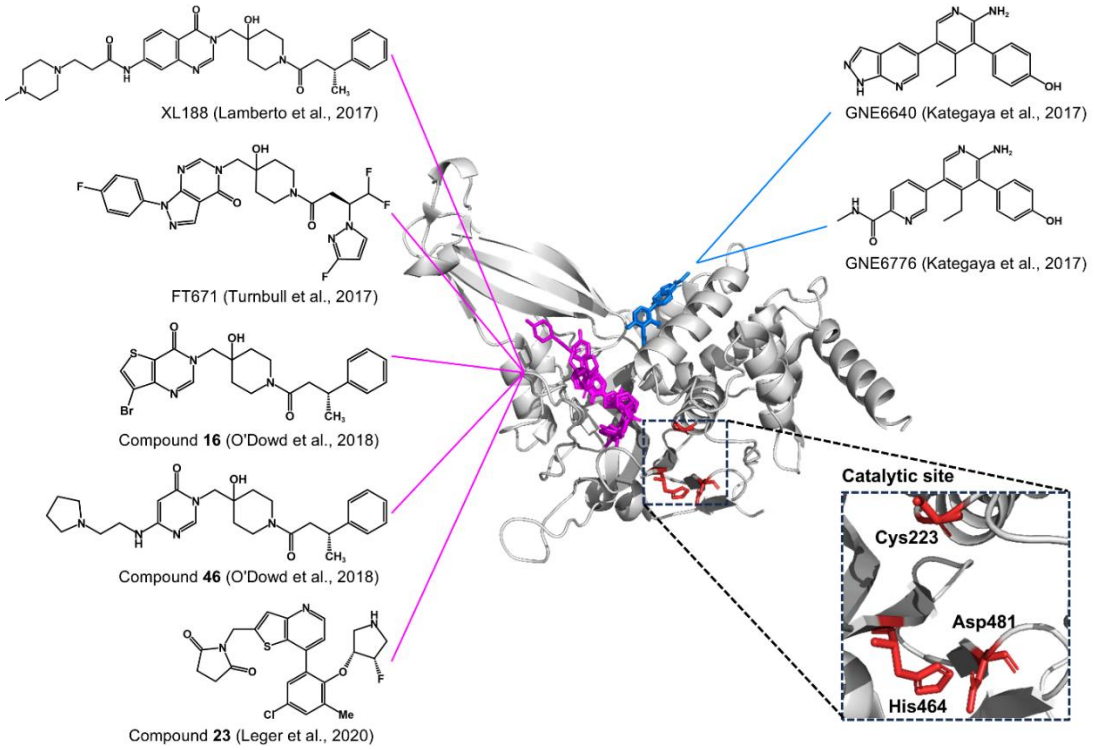


Figure 8. The two binding sites of non-covalent inhibitors on the USP7 catalytic domain (PDB ID: 1NB8). Compounds bound to the allosteric binding site and inhibitors targeting the catalytic cleft site are colored in marine and magenta, respectively. GNE6640 (PDB ID: 5UQV), GNE6776 (PDB ID: 5UQX) [104], XL188 (PDB ID: 5VS6) [105], FT671 (PDB ID: 5NGE) [106], Compound **23** (PDB ID: 6VN3) [107], Compound **16** (PDB ID: 6F5H), Compound **46** (PDB ID: 5N9R) [108]. The catalytic triad (Cys223, His464, Asp481) is represented by red sticks.

5.1.1. Allosteric Binding Site

In 2017, there was a spurt in the development of USP7 inhibitors. By using nuclear magnetic resonance (NMR) screening and co-crystal structure analysis, Kategaya et al. first revealed the mechanism of far-catalytic metastable regulation of USP7. GNE-6640 and GNE-6776 are identified as bound to a metastable site at the interface of the finger and thumb regions 12 Å away from the catalytic cysteine. These inhibitors suppress USP7 activity by blocking α5 helix conformational changes and disrupting ubiquitin Lys48-acidic residue interactions, exhibiting potential efficacy for therapeutics on AML and breast cancer [55,104,109]. Nevertheless, inhibitor development targeting this site remains relatively limited.

5.1.2. Catalytic Cleft Site

The catalytic cleft site has emerged as a prominent research target, with numerous structurally analogous compounds having been reported in recent years. The quinazoline derivative XL188, designed in 2017, inhibits USP7 activity by occupying the S4 and S5 subsites, about 5 Å from the catalytic triad. The quinazolin-4-one and 4-hydroxypiperidine rings form a key hydrogen bonding network with the catalytic domain, which plays a decisive role in the stability of the complex [105,110]. In the same year, *Nature* reported FT671, a high-affinity and high-specificity non-covalent inhibitor of USP7. The study first resolved the USP7-FT671 co-crystal structure, corroborating its allosteric mechanism: FT671 competitively binds to the dynamic pocket near the catalytic triad, sterically hindering ubiquitin binding to inhibit USP7 enzymatic activity. Functionally, FT671 demonstrates potent suppression of tumor progression in breast cancer, MM, and CRC [106]. Subsequently, Gavory et al. employed surface plasmon resonance (SPR) screening and structure-based drug design to identify compound **4**, a highly potent and selective non-covalent reversible inhibitor of USP7. This inhibitor binds to a previously unreported allosteric pocket located 5.5 Å from the catalytic cysteine, sterically hindering the C-terminal end of ubiquitin binding to the pocket. This mechanism ultimately induces MDM2 degradation and stabilizes p53 [111]. Using the covalent inhibitor L55 as a lead compound, the easily metabolizable methyl ester moiety is replaced with a cyclic functional group to obtain compound X36, whose oral pharmacokinetic (PK) properties are significantly enhanced. This advanced inhibitor modulates the tumor immune microenvironment by enhancing CD8+ T cell, natural killer

(NK) cell, and natural killer T (NKT) cell infiltration while suppressing regulatory Tregs and myeloid-derived suppressor cells (MDSCs), ultimately suppressing tumor growth and paving the way for novel cancer immunotherapies [112].

Researchers developed compound **41** (USP7-797), through structural simplification of benzofuranamide to an ortho-methyl ether compound and replacement of the ether linkage with a carbon bond connecting piperazine and morpholine. This optimized inhibitor demonstrates both remarkably potent and highly selective USP7 inhibition along with excellent oral bioavailability. Similar to FT671, USP7-797 binds to the analogous allosteric pocket and manifests dual p53-dependent and independent antitumor activity in MM and SCLC [107,113]. However, the subsequent study has found that the V517F mutation in USP7 changes the conformation of the variant pocket, leading to drug resistance [114].

Structural optimization of inhibitors based on this site has continued over the past two years. To further enhance the inhibitory effect of FT671, researchers use a backbone jumping strategy to screen a novel inhibitor, YCH2823, which not only induces cell cycle arrest, apoptosis, and expression of the proto-oncogene B-cell lymphoma 6 (BCL6), but also exhibits significant anti-proliferative activity against tumor cells. In addition, YCH2823 has a synergistic effect with the mammalian target protein of rapamycin(mTOR) inhibitor, which pioneers an unprecedented strategy for cancer combination therapy [115]. Besides, compound **61** with low nanomolar affinity is prepared using a modular approach based on the identified quinazoline derivatives related USP7 inhibitors. This optimized compound demonstrates favorable pharmacokinetic properties in vitro, effectively suppressing tumor growth and reducing platelet counts [107,110].

5.2. Chemical Synthesis Derived Covalent Inhibitors Targeting the Catalytic Domain

A series of important advances have been made in the development of USP7 covalent inhibitors. The representative compounds, HBX19818 and HBX28258, form a covalent binding with the Cys223 located in the active site of USP7 through nucleophilic attack. In particular, the basic amino groups simultaneously generate strong electrostatic interactions with two acidic residues (Asp295 and Glu298) located at the entrance of the ubiquitin binding pocket. This unique mechanism can effectively inhibit the proliferation of HCT116 cells, induce apoptosis, and block the cell cycle [116].

The dichlorophenylthio/nitro/acetyl-substituted compound P5091 obtained by structural optimization has a broader spectrum of inhibitory effects. It not only degrades a variety of USP7 substrates and inhibits tumor angiogenesis, but also synergizes with lenalidomide, the histone deacetylase (HDAC) inhibitor SAHA, or dexamethasone against MM [117]. Significantly, P5091 induced apoptosis even in a p53-deficient chronic lymphocytic leukemia (CLL) model, confirming the existence of a p53-independent pathway for USP7 inhibitors [118]. Moreover, the development of P22077 and its optimized product P50429 based on the Ub-CHOP reporter gene platform has further expanded the application of covalent inhibitors. These compounds competitively target Cys223 to exhibit potent anti-proliferative influence on HCT116 cells [119–121]. In neuroblastoma (NB) treatment, P22077 restores chemosensitivity in drug-resistant LA-N-6 cells by stabilizing p53 through MDM2 degradation [122,123]. However, poor solubility and dose-limiting toxicity necessitate further structural optimization of this series. A new generation of thiazole derivatives, C7 and C19, has emerged based on the retention of the thiophene electron-withdrawing moiety. These compounds induce cell death by competitive block of ubiquitin binding in a dual p53-dependent and p53-independent mechanism, which displays moderate inhibitory activity against both USP7 enzymes and cancer cell lines [124].

In contrast to the non-covalent inhibitor FT671, its derivative FT827 achieves irreversible inhibition through the covalent bond formation between the ethylenesulfonamide structure and the Cys223 residue of USP7 [106]. Structural optimization of FT671 yielded the N-benzyl piperidinol derivative L55 with higher selectivity and inhibitory potency. The co-crystal analysis reveals L55 occupies the ubiquitin C-terminal cleft between the palm and thumb domains of the USP7 catalytic domain. Mechanically, a large upshift of Phe409 allows the pyrazole ring of L55 to form a stable π-π interaction. This may be caused by the lack of hydrogen bonding interactions with Tyr465, leading to the outward stretching of the benzyl group. In addition, the 2-chloro and 4-methoxycarbonyl groups on the benzyl group are close to the binding pocket boundary, limiting the substitution of larger groups [125]. The newly developed XL177A, as the first subnanomolar inhibitor, achieves precise inhibition by specifically labeling Cys223 and enhancing conformational changes in the α2-α4 region, marking a breakthrough in covalent inhibitor development [126]. These systematic advances have laid a solid foundation for the development of safer and more effective USP7-targeted drugs.

5.3. Naturally Derived USP7 Inhibitors

Research on naturally derived USP7 inhibitors has uncovered multiple lead compounds with distinct mechanisms of action. Spongiacidin C represents the first identified natural USP7 inhibitor, utilizing the hydantoin ring for enzymatic inhibition. However, its high cytotoxicity and limited cellular activity constrain its therapeutic potential [127]. Then, more exploration of alternative natural products, with pentacyclic triterpenoids offering new possibilities. Among these compounds, ursolic acid demonstrates the strongest inhibitory activity, dose-dependently suppressing myeloma cell proliferation. Molecular docking reveals its dual binding mechanism within the ubiquitin binding pocket: the core scaffold forms hydrophobic interactions with Met328, Tyr367, Ala369 and Val393, while the 17-carboxyl group and 3-hydroxyl group establish critical hydrogen bonds with Glu371 and Gln351, respectively [128].

As the first covalent inhibitor targeting the UBL domains, the natural compound eupalinolide B (EB) represents a landmark achievement. EB forms covalent bonds with the allosteric site Cys576 by anchoring the negatively charged cavity between UBL1 and UBL2. This unique mode of action not only leads to the degradation of Keap1, but also activates Nrf2-dependent transcription of anti-neuroinflammatory genes in microglial cells, which offers an innovative framework for the treatment of neurodegenerative diseases [15].

5.4. USP7 PROTACs

Proteolysis targeting chimeras (PROTACs) represent an emerging chemical modality capable of reversibly targeting traditionally undruggable proteins with high selectivity [129]. These bifunctional molecules consist of an E3 ligase ligand linked to a protein of interest (POI) binder through a chemical linker, enabling polyubiquitination and subsequent proteasomal degradation of the target protein [130]. In recent years, PROTACs incorporating USP7 inhibitors as warheads have provided innovative approaches for inducing USP7 degradation.

U7D-1, the first highly potent and selective PROTAC degrader of USP7, is designed using Compound **4** as the target protein ligand. This molecule demonstrates robust USP7 degradation across multiple cell lines. Significantly, U7D-1 exhibits particularly pronounced anti-proliferative activity in p53-mutant cancer cells [131]. Subsequently, Murgai et al. reported a PROTAC incorporating XL188 as the USP7-targeting moiety. It not only maintains high potency and selectivity for USP7 but also effectively suppresses cancer cell viability [132]. Furthermore, PU7-1 exerts tumor suppressor function by targeting USP7 in TNBC and antagonizing the FOXM1 network. This novel degradative agent with XL177A as a ligand provides new insights and strategies for the treatment of TNBC [84].

5.5. USP7 Agonists

Although current USP7 small molecule research is mainly focused on inhibitor development, a deeper understanding of the pathogenesis of HAFOUS has driven the exploration of USP7 agonists. A recent study has revealed that the clinical drugs Sertraline and Astemizole specifically bind to the switch loop region of USP7 to enhance the enzymatic activity. The co-crystal analysis demonstrates that both compounds occupy an activation cleft formed by the switch loop (F283-D295) and adjacent α5 helix (V296-M311). Additionally, Ub-AMC hydrolysis assays confirm their ability to upregulate deubiquitinase activity in HAFOUS-associated USP7 mutants, with cellular studies further validating the activating effect of Astemizole on full-length USP7 mutants [18]. MS-8, a newly discovered small molecule agonist of USP7, activates USP7 by occupying the allosteric C-terminal peptide binding (CPB) pocket of USP7. It activates mutant USP7 in the cellular environment, which affects the downstream MDM2-p53 pathway [19].

Table 1. Inhibitors and agonists partially targeting USP7.

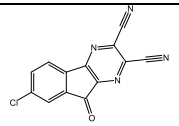
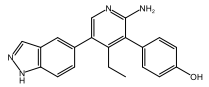
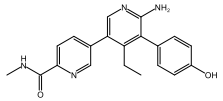
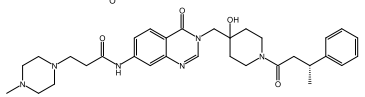
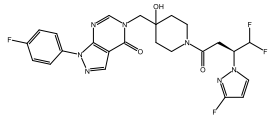
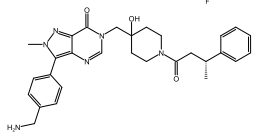
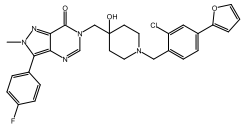
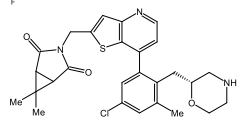
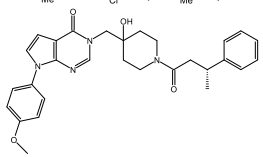
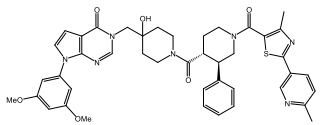
Class	Compound	Chemical Structure	PDB ID	Binding Mode	IC50 (μM)/ EC50 (μM)	Targeted Diseases	Year	Reference
Non-covalent inhibitors	HBX41108		N/A	Allosteric	0.424	Induces P53-dependent apoptosis，CRC	2009	[101,102]
	GNE-6640		5UQV	Allosteric	0.75	AML, Breast Cancer, Osteosarcoma, CRC	2017	[55,104,109]
	GNE-6776		5UQX	Allosteric	1.34	AML, Breast Cancer, Osteosarcoma, CRC	2017	[55,104,109]
	XL188		5VS6	Catalytic	0.193	Breast Cancer, MM	2017	[105]
	FT671		5NGE	Catalytic	0.052	Breast Cancer, MM，CRC	2017	[106]
	Compound **4**		N/A	Catalytic	0.0015	Breast Cancer, CRC	2018	[111]
	X36		N/A	Catalytic	0.0645	CRC	2022	[112]
	USP7-797/ Compound **41**		N/A	Catalytic	0.00044	MM, SCLC	2022	[107,113,114]
	YCH2823		N/A	Catalytic	0.0496	MM	2024	[115]
	Compound **61**		N/A	Catalytic	low nanomolar	MM	2024	[107,110]

Table 1. *Cont.*

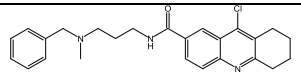
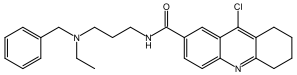
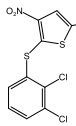
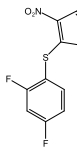
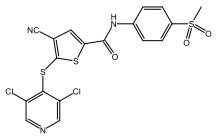
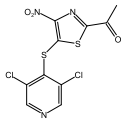
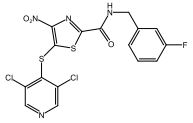
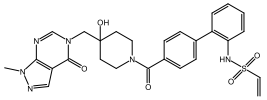
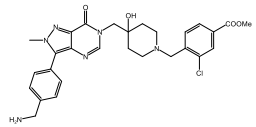
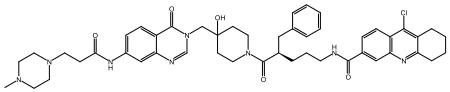
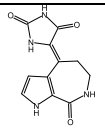
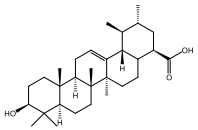
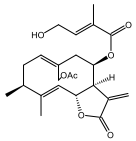
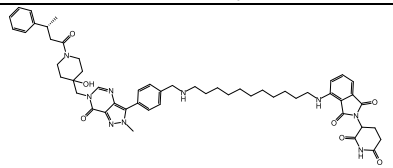
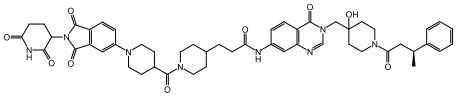
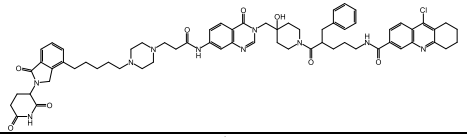
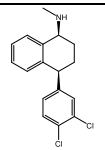
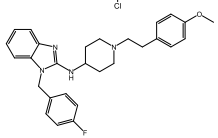
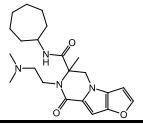
Class	Compound	Chemical Structure	PDB ID	Binding Mode	IC50 (μM)/ EC50 (μM)	Targeted Diseases	Year	Reference
Covalent inhibitors	HBX19818		N/A	Covalent	28.1	CRC	2012	[116]
	HBX28258		N/A	Covalent	22.6	CRC, Influence on cell activity, Induce apoptosis, Disrupts the cell cycle	2012	[116]
	P5091		N/A	Covalent	4.2	Prostate cancer, CRC, MM, CLL	2012	[117,118]
	P22077		N/A	Covalent	8	CRC, Neuroblastoma (NB)	2011	[121]
	P50429		N/A	Covalent	0.42	CRC	2012	[122]
	C7		N/A	Covalent	0.67	CRC	2017	[124]
	C19		N/A	Covalent	1.35	CRC	2017	[124]
	FT827		5NGF	Covalent	0.065	Breast Cancer, MM and CRC	2017	[106]
	L55		6M1K	Covalent	0.0408	Lymphoblastic leukemia, Prostate cancer	2020	[125]
	XL177A		N/A	Covalent	0.00034	Breast Cancer	2020	[126]

Table 1. *Cont.*

Class	Compound	Chemical Structure	PDB ID	Binding Mode	IC50 (μM)/ EC50 (μM)	Targeted Diseases	Year	Reference
Naturally derived inhibitors	Spongiacidin C		N/A	Unknown	3.8	Non-cellular activity and cytotoxicity	2013	[127]
	Ursolic acid		N/A	Allosteric	7	MM	2017	[128]
	Eupalinolide B		7XPY	Covalent	N/A	Neurodegenerative diseases	2022	[15]
USP7 PROTACs	U7D-1		N/A	Catalytic	0.2633	Non-Hodgkin lymphoma, Pre-B lymphoblastic leukemia, Acute T cell leukemia, Mantle cell lymphoma, Myeloma	2022	[131]
	PROTAC 17		N/A	Catalytic	N/A	MM, SCLC, Lung cancer, and PCa	2022	[132]
	PU7-1		N/A	Covalent	N/A	TNBC	2023	[84]
Agonists	Sertraline		9IJU	Allosteric	61.02	HAFOUS	2025	[18]
	Astemizole		9IML	Allosteric	32.24	HAFOUS	2025	[18]
	MS-8		N/A	Allosteric	N/A	HAFOUS	2025	[19]

6. Conclusions and Future Perspectives

As one of the most widely studied members of the USPs, USP7 extensively participates in protein degradation, cell cycle regulation, DNA damage repair, and regulation of classical signaling pathways. Dysregulation of USP7 contributes to various pathologies, including cancer, metabolic diseases, and neurological diseases. In the native state, the catalytic triad of the USP7 catalytic domain exhibits a misarranged conformation, resulting in a low basal enzymatic activity. Studies have shown that the binding of ubiquitin substrates or their terminal peptide can significantly activate the catalytic activity of USP7, revealing the precisely regulated enzymatic mechanism [26]. These characteristics render USP7 an attractive target for developing activity modulators as novel therapeutic interventions for related diseases.

Currently, the process of developing regulators targeting USP7 still faces multiple challenges. Although the catalytic domain and some protein complex structures of USP7 have been resolved, the complete conformation of the full length, the regulatory mechanisms of post-translational modifications, and the protein-interaction network in physiological environments are still not fully elucidated. In the future, with the help of proteomics studies and computational simulations, it will precisely characterize the mechanism of USP7 with related substrates or compounds, and provide prerequisites for the discovery of USP7 targeted regulators [24].

Clinical translation of USP7 inhibitors still needs to break through several bottlenecks. The difficulty in obtaining co-crystal structures of USP7 inhibitor complexes has limited full elucidation of the molecular mechanisms, constraining drug optimization strategies based on rational structural design. Another critical obstacle is the insufficient selectivity and specificity of compounds, a major barrier to clinical translation. Thiophene inhibitors, such as P5091, P22077 and P50429, despite exhibiting high inhibitory activity against USP7, demonstrate comparable activity against USP47, leading to potential off-target effects and cellular toxicity [122,123,133]. Meanwhile, the cellular activity and pharmacokinetic studies of USP7 should not be neglected. Although non-covalent inhibitors CP4 and L55 significantly improve affinity and selectivity and showed potent inhibition in vitro, they also suffer from poor membrane permeability, unstable metabolism or low oral bioavailability, which limited the in vivo evaluation [108,125]. These challenges underscore the need for further exploration to develop highly efficient and specific USP7 inhibitors.

Research on HAFOUS has presented the therapeutic potential of USP7 agonists, though the development remains in early stages with only Sertraline, Astemizole and MS-8 reported as active compounds to date [18,19]. Meanwhile, the development of agonists encounters several problems. Firstly, there are no published crystal structures of USP7 agonist complexes to guide allosteric site optimization. Secondly, neurological delivery requires breaching the blood-brain barrier (BBB). Notably, Sertraline can cross the BBB despite its lack of intracellular activation potency against USP7 mutants [18]. Thirdly, overactivation of USP7 may also disrupt the balance of important signaling pathways, such as MDM2-p53, leading to the complication of other diseases. These obstacles highlight the need to address selectivity, delivery, and activity control for successful USP7 agonist development through integrated medicinal chemistry and a novel delivery system approach. Prodrug design and nanoparticle delivery systems (e.g., liposomes) can effectively solve the problem of poor membrane permeability of compounds, which in turn improves brain delivery efficiency [134].

Simultaneously, AI-based structural prediction tools, such as AlphaFold, have revolutionized drug discovery by providing detailed insights into protein structures, thus enabling the structure-guided design of USP7 agonists. AlphaFold-derived models allow researchers to simulate the conformational landscape of USP7 and identify cryptic binding pockets that may transiently form during its active state. Furthermore, molecular dynamics simulations based on these predicted structures help refine binding hypotheses and assess agonist-induced conformational changes in USP7 [135,136]. Although no potent USP7 agonists have yet entered clinical development, the integration of AI-assisted structural modeling with biophysical validation holds significant promise for accelerating the discovery of functionally selective USP7 agonists.

Recently, USP7 inhibitors have been found to act as a starting point for the synthesis of PROTACs, which can induce degradation of USP7. Similarly, deubiquitinase-targeting chimeras (DUBTACs), a heterobifunctional stabilizer, deubiquitinates and stabilizes target proteins with protective functions in disease by recruiting USP7 [137]. The team led by Wei reported the first USP7-based DUBTACs, MS6869 and MS8118, which could stabilize cystic fibrosis transmembrane conductance regulator (CFTR) and AMPKβ1, respectively [138]. Consequently, USP7 agonists such as Sertraline, Astemizole, and MS-8 are expected to be recruitment motifs for DUBTACs, which is important for the development of new pharmacological modalities for targeted protein stabilization (TPS) [19].

In summary, USP7, as a key post-translational modifier, critically drives the development of many diseases. It is of great scientific significance and clinical value to deeply investigate its biological mechanism of action and develop targeted active modulators.

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