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THE UBIQUITIN-PROTEASOME SYSTEM IN CANCER: MECHANISMS, TARGETS AND THERAPEUTIC POTENTIAL

The ubiquitin-proteasome system (UPS) is the central mechanism for regulated intracellular protein degradation in eukaryotic cells, controlling essential biological processes including cell cycle progression, DNA repair, apoptosis, and signal transduction. Through a hierarchical enzymatic cascade, ubiquitin is covalently attached to substrate proteins, often as polyubiquitin chains, marking them for selective degradation by the 26S proteasome. Dysregulation of this system is a hallmark of cancer, where altered ubiquitination dynamics can drive malignant transformation by promoting the degradation of tumor suppressors or stabilizing oncogenic proteins. Deubiquitinating enzymes (DUBs), particularly the ubiquitin-specific protease (USPs) family, reverse ubiquitination and help maintain protein homeostasis. Many USPs are aberrantly expressed or genetically altered in tumors, contributing to oncogenic signaling, resistance to apoptosis, and therapy evasion. This review presents a comprehensive overview of the architecture and function of the UPS, focusing on ubiquitination mechanisms, proteasomal activity, and context-dependent roles of DUBs in cancer. Here, we highlight emerging therapeutic strategies that target various UPS components, including FDA-approved proteasome inhibitors, inhibitors of E3 ligase function, PROTAC-based protein degradation, and small-molecule USP inhibitors. While drugging DUBs remains challenging due to issues of specificity and toxicity, advances in structure-based design and ubiquitin code mapping are accelerating progress. Overall, the UPS is a key regulatory hub in cancer biology and a promising target in precision oncology. Therapeutic modulation of this pathway offers new opportunities for destabilizing oncogenic networks and overcoming resistance mechanisms in cancer.

Keywords: ubiquitin-proteasome system, ubiquitination, ubiquitin-specific proteases, deubiquitinating enzymes, cancer, targeted therapy.

Post-translational modification by ubiquitin is a universal mechanism for regulating protein stability, activity, and localization in eukaryotic cells. Through covalent attachment, often in the form of polyubiquitin chains, ubiquitin marks the substrates for degradation by the 26S proteasome or alters their cellular functions [1, 2]. The ubiquitin-proteasome system (UPS) plays a central role in

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protein homeostasis and governs essential cellular processes such as cell cycle control, DNA repair, signal transduction, and apoptosis. Remarkably, the UPS is estimated to mediate the degradation of 80—90% of short-lived intracellular proteins, making it a cornerstone of protein quality control in both cytoplasmic and nuclear compartments [3].

In cancer, where signaling networks are regularly rewired and protein turnover is accelerated, the UPS is commonly hijacked. The aberrant ubiquitination or defective degradation of regulatory proteins can drive tumor progression. For example, hyperactive E3 ligases may eliminate tumor suppressors, while loss of specific ligases or increased deubiquitinase activity can stabilize oncoproteins [4].

The role of the UPS in cancer is evident from the clinical success of proteasome inhibitors and the identification of multiple UPS components as therapeutic targets. Cancer cells often depend on heightened UPS activity, creating a window for selective intervention [5]. Deubiquitinating enzymes (DUBs), particularly ubiquitin-specific proteases (USPs), regulate protein stability by reversing ubiquitination. Many USPs are overexpressed or mutated in tumors, promoting proliferation, metastasis, and therapy resistance [6]. Their substrate specificity makes them promising targets for cancer drug development.

Key structural and catalytic modules of the UPS

The UPS is a tightly regulated and compartmentalized machinery responsible for selective protein degradation in eukaryotic cells. Its core components include ubiquitin, three classes of ubiquitin-modifying enzymes (E1, E2, and E3), DUBs, ubiquitin-binding receptors, and the 26S proteasome complex. Together, these elements orchestrate the recognition, modification, and degradation of intracellular proteins, maintaining proteostasis and regulating essential processes such as cell cycle progression, transcription, signal transduction, and stress responses.

26S proteasome. At the center of the UPS is the 26S proteasome, a 2.5 MDa ATP-dependent protease complex, which catalyzes the degradation of polyubiquitinated proteins. It is composed of a 20S core particle (CP) and one or two 19S regulatory particles (RP), forming either singly or doubly capped proteasomes [3, 7].

The 20S core is a barrel-shaped structure consisting of four stacked heptameric rings arranged in the order α_7–β_7–β_7–α_7. The two inner β-rings contain the proteolytic active sites, with three subunits (β1, β2, and β5) providing caspase-like, trypsin-like, and chymotrypsin-like protease activities [3]. These active sites face inward toward the central catalytic chamber, which ensures that degradation occurs in a spatially confined environment. The outer α-rings form a gated entry portal that restricts access to the chamber, preventing uncontrolled proteolysis in the cytoplasm.

The 19S RP, also known as PA700, functions in substrate recognition, deubiquitination, unfolding, and translocation. It consists of two functional modules: a base and a lid. The base contains six ATPases of the AAA^+ family (Rpt1 through Rpt6), which form a hexameric ring driving substrate unfolding and translocation into the 20S core [3, 8]. Non-ATPase subunits such as Rpn1 and Rpn2 provide structural scaffolding and docking sites for ubiquitinated proteins. The lid includes several non-ATPase subunits (Rpn3, Rpn5 through Rpn9, Rpn11, and Rpn12) involved in substrate processing. Among these, Rpn10 and Rpn13 serve as ubiquitin receptors, while Rpn11 is a JAMM-family metalloprotease that removes ubiquitin chains from substrates at the proteasome entry site, allowing ubiquitin to be recycled before degradation begins [2,9].

Beyond the standard 26S proteasome, cells express alternative proteasome complexes. The immunoproteasome, predominantly found in immune cells, substitutes the β1, β2, and β5 subunits with inducible isoforms (β1i, β2i, and β5i), enhancing the generation of peptides suitable for MHC class I presentation. The 20S core can also associate with the 11S regulator PA28, which promotes ATP-independent degradation of short peptides and unstructured proteins, particularly during oxidative stress or immune activation. Hybrid proteasomes, containing both 19S and 11S caps, further highlight the structural and functional flexibility of this system [7, 8].

Key enzymes and accessory components of the UPS. The ubiquitination process depends on a cascade of three enzyme types: E1 activating enzymes, E2 conjugating enzymes, and E3 ligases. In humans, two canonical E1 enzymes (UBA1 and UBA6) activate ubiquitin in an ATP-dependent

manner. The activated ubiquitin is then transferred to one of approximately 30 E2 enzymes, which cooperate with over 500 E3 ligases to achieve substrate-specific ubiquitin conjugation [2]. E3 ligases confer substrate selectivity and are categorized into RING, HECT, and RBR families based on their catalytic mechanism.

DUBs remove or modify ubiquitin chains, regulating the stability, localization, and activity of target proteins. DUBs act at multiple points in the UPS, either upstream to reverse ubiquitination or downstream to recycle ubiquitin during proteasomal degradation. Among them, ubiquitin-specific proteases (USPs) represent the largest subfamily and have gained increasing attention for their roles in oncogenesis and drug resistance [6].

Ubiquitin-binding proteins, or shuttle receptors, guide polyubiquitinated substrates to the proteasome. These include intrinsic proteasomal receptors such as Rpn10 and Rpn13, as well as adaptor proteins like Rad23 and Dsk2, which contain both ubiquitin-binding domains and proteasome-interacting motifs [3].

The coordinated activity of these components enables the UPS to operate with high specificity and temporal precision. Disruption of this system, whether due to genetic mutations, aberrant expression, or loss of regulatory control, can destabilize proteome integrity and contribute to disease. In cancer, where protein turnover is tightly coupled to proliferation and survival, several UPS components have emerged as promising therapeutic targets. These include the proteasome itself, select E3 ligases, and cancer-associated DUBs, all of which are currently being explored for targeted intervention strategies [4].

From ubiquitination to degradation: How the UPS functions

The UPS orchestrates selective protein degradation through a tightly regulated sequence of enzymatic steps, which determine protein fate, from initial ubiquitin tagging to final proteolytic breakdown. This cascade plays a central role in maintaining cellular homeostasis and regulating key biological processes such as cell cycle progression, signal transduction, DNA repair, and apoptosis [1, 2]. Its dysregulation is frequently implicated in cancer pathogenesis, where altered protein turnover can support uncontrolled proliferation, drug resistance, and immune evasion [4].

The process begins with ubiquitination, a post-translational modification in which the 76-amino-acid protein ubiquitin is covalently attached to lysine residues on substrate proteins. Ubiquitin conjugation occurs through an ATP-dependent three-step cascade involving E1 activating enzymes, E2 conjugating enzymes, and E3 ubiquitin ligases. The E1 enzyme forms a high-energy thioester bond with the C-terminal glycine of ubiquitin, then transfers ubiquitin to the active-site cysteine of an E2 enzyme [2]. The E2, in collaboration with a substrate-specific E3 ligase, facilitates the final attachment of ubiquitin to the target protein. E3 ligases are responsible for substrate recognition and determine the specificity of the reaction. In humans, over 500 E3 ligases have been identified, reflecting the diversity of ubiquitin signaling and its integration with cellular regulatory networks [5].

Substrates can be modified by mono-ubiquitination, multi-mono-ubiquitination, or polyubiquitination. Polyubiquitin chains are formed through linkages between the C-terminal glycine of one ubiquitin and any of seven lysine residues or the N-terminal methionine of another. The type of chain linkage determines the fate of a modified protein. K48- and K11-linked chains serve as canonical degradation signals, targeting proteins for recognition by the 26S proteasome. In contrast, K63-linked chains are associated with non-proteolytic functions such as DNA repair, endocytosis, and signaling cascades, including NF-κB activation [2]. Branched and mixed-linkage chains further increase the signaling capacity of ubiquitin modifications, forming what is often referred to as the ubiquitin code.

The 26S proteasome is a multisubunit protease complex composed of a 20S catalytic CP and one or two 19S RPs. The 20S CP contains three proteolytic β subunits (β1, β2, and β5) with distinct cleavage specificities [3]. These active sites face the central chamber of the barrel-shaped complex, where proteolysis occurs in a compartmentalized and controlled manner. The 19S RP is responsible for recognizing the polyubiquitinated substrates, removing the ubiquitin chains, unfolding the target protein, and translocating it into the 20S CP for degradation [3]. Recognition is mediated by the ubiquitin receptors such as Rpn10 and Rpn13,

while deubiquitinating enzymes like Rpn11 cleave ubiquitin chains to recycle free ubiquitin before the substrate entry [1].

Unfolding and translocation require ATP hydrolysis performed by the hexameric ring of AAA^+ ATPases in the base of the 19S RP [3]. Once inside the catalytic chamber, the unfolded substrate is cleaved into oligopeptides of 3 to 25 amino acids, which are then released for further processing or antigen presentation [2]. The entire process is highly selective and energy-dependent, ensuring that only proteins with appropriate ubiquitin tags and accessible unstructured regions are committed to degradation.

Throughout this cascade, the specificity is reinforced at multiple levels. Substrate selection by E3 ligases, ubiquitin chain editing by deubiquitinases, and gated access to the proteasome collectively ensure an accurate control of protein stability. The reversibility of ubiquitination, primarily governed by DUBs, adds an additional layer of dynamic regulation [6].

The disruption of this finely tuned system can be involved in cell transformation. An aberrant ubiquitination can lead to the degradation of tumor suppressors or the stabilization of oncoproteins. For instance, the overexpression of the E3 ligase MDM2 promotes proteasomal degradation of p53, while loss of E3 ligases such as FBXW7 can stabilize cyclin E and c-MYC, driving cell cycle progression and transformation [4]. Conversely, impaired deubiquitination may prevent timely protein recycling or interfere with the stress response.

Understanding the molecular mechanisms of the UPS, from ubiquitin tagging to proteasomal degradation, is essential for identifying the possible targets for cancer treatment. The pharmacological strategies that inhibit proteasome activity, modulate E3 ligase function, or target deubiquitinating enzymes are currently being explored to exploit the vulnerabilities of tumors dependent on the aberrant UPS activity [4, 6].

DUBs and USPs

DUBs are a diverse class of proteases that regulate the ubiquitin-proteasome system by removing ubiquitin from modified proteins. They function by cleaving either the isopeptide bond between ubiquitin and its substrate or the internal linkages between ubiquitin molecules within polyubiquitin chains [2]. Through these activities, DUBs perform such essential cellular roles as rescuing proteins from degradation, generating free ubiquitin from precursor forms, and recycling ubiquitin from proteasome-bound substrates [8, 9].

The human genome encodes approximately 100 DUBs, classified into distinct families based on their catalytic mechanisms and structural domains. Among these, the USPs represent the largest group, with over 50 members [6]. USPs are cysteine proteases defined by a conserved catalytic domain that adopts a hand-like configuration composed of "Palm," "Thumb," and "Fingers" subdomains. This unique fold enables the precise recognition and cleavage of ubiquitin from substrates. Many USPs exhibit regulated activity through conformational dynamics: in their inactive state, the catalytic cleft is misaligned and only adopts the catalytically competent conformation upon binding to ubiquitin [1]. Additional regulatory mechanisms include interaction with cofactors and the presence of auxiliary domains that govern subcellular localization, substrate specificity, or enzymatic activation. The structural elucidation of USP7 (also known as HAUSP) provided foundational insights into this mode of regulation and remains a key model for understanding USP function [10, 11].

Other DUB families perform specialized roles in different cellular contexts. The ubiquitin C-terminal hydrolases (UCHs), a small family with four members, primarily process ubiquitin precursors and short ubiquitinated peptides [12]. The ovarian tumor (OTU) family comprises approximately 16 cysteine proteases involved in immune signaling. Notable examples include OTULIN, which cleaves linear M1-linked ubiquitin chains, and CYLD, which hydrolyzes K63-linked chains to negatively regulate the NF-κB pathway [13]. The Machado—Joseph disease (MJD) family, or Josephin domain-containing DUBs, includes ATXN3 and has been implicated in neurodegeneration and transcriptional regulation [13, 14]. In contrast to these cysteine protease families, the JAMM/MPN+ family consists of zinc-dependent metalloproteases such as Rpn11, a core component of the proteasome, as well as AMSH and BRCC3, which function in pathways like DNA damage repair [2, 7].

DUBs are active across nearly all cellular pathways involving ubiquitin. Some of them function

broadly; for example, USP14 and UCHL5 are associated with the 19S regulatory particle of the proteasome, where they trim ubiquitin chains from substrates en route to degradation [3]. Others act with a high substrate specificity. USP4 regulates TGF-β signaling by deubiquitinating its receptor [10]. USP1 is essential for the Fanconi anemia DNA repair pathway, where it reverses the monoubiquitination of FANCD2 and PCNA [6]. In the cell cycle, USP28 stabilizes key checkpoint proteins such as Claspin and also influences c-MYC turnover [15]. In transcription, USP22 functions within the SAGA complex to remove ubiquitin from histones H2A and H2B, thereby modulating chromatin accessibility and gene expression [2].

A defining feature of DUBs is their ability to counterbalance the activity of E3 ubiquitin ligases. This antagonistic relationship allows DUBs to act as molecular switches or brakes in the signaling pathways. In the p53 regulatory axis, for example, the E3 ligase MDM2 promotes p53 degradation by polyubiquitination, while USP7 can remove ubiquitin from both p53 and MDM2. This dual role allows USP7 to either stabilize p53 or enhance MDM2 activity, depending on the cellular context, creating a tightly regulated feedback loop [1, 11]. Such interplay illustrates how DUBs fine-tune protein stability thresholds and integrates ubiquitination with the broader regulatory networks.

Taken together, DUBs play critical roles in maintaining protein homeostasis, modulating signal transduction, and controlling gene expression. Their dysregulation is increasingly recognized as a driver of pathological conditions, especially in cancer, where they can influence cell proliferation, DNA repair capacity, and therapeutic resistance [16—18]. Given their specificity and central regulatory functions, DUBs have emerged as attractive targets for drug development and are under active investigation as modulators of oncogenic pathways [5, 6].

USPs in cancer

USPs are the central regulators of protein stability, signaling, and chromatin structure, which are frequently dysregulated in cancer. As the largest subfamily of DUBs, USPs are strategically positioned within the UPS to balance protein degradation and stabilization. Their substrate specificity and dynamic regulation make them both the key contributors to cancer biology and attractive targets for therapeutic intervention [19, 20].

In cancer cells, USPs are often aberrantly expressed, mutated, or co-opted by oncogenic pathways. An overexpression of certain USPs can stabilize oncoproteins, promote proliferation, and inhibit apoptosis. Conversely, a loss of tumor-suppressive USPs may lead to the degradation of proteins, which normally restrain tumor growth. This context-dependent duality reflects the nuanced roles of USPs across different cancer types [11, 12].

USP1 as a prominent example of DUBs, playing a significant role in the pathogenesis of various cancers, is considered a promising therapeutic target. An increasing body of evidence highlights the involvement of USP1 in malignant transformation through the stabilization of oncoproteins and apoptosis inhibitors (particularly ID family proteins), thereby promoting cell proliferation, metastasis, and drug resistance. USP1 stabilizes FANCD2 and PCNA and has been implicated in maintaining BCR-ABL in chronic myeloid leukemia. USP1 is overexpressed in numerous malignant tumors, including cervical, breast, gastric, and esophageal cancers, as well as brain gliomas. The alterations in its activity are associated with disrupted cellular homeostasis and genomic instability [21—25].

USP7 (also known as HAUSP) is among the most extensively studied USPs in the context of cancer. It regulates the stability of both p53 and its E3 ligase MDM2. In many tumors, USP7 is overexpressed, tipping the balance toward MDM2 stabilization and p53 degradation, thereby promoting tumor progression. An inhibition of USP7 has been shown to restore p53 function and induce apoptosis in p53-wildtype cancers, making it a compelling therapeutic target [11].

USP28 plays a role in stabilizing oncogenic transcription factors such as c-MYC and in regulating DNA damage checkpoints. Its overexpression is associated with genomic instability and poor prognosis in several cancers [15]. USP22, a component of the SAGA chromatin-modifying complex, removes ubiquitin from histones H2A and H2B and is implicated in transcriptional reprogramming, metastasis, and immune evasion. An elevated USP22 level has been proposed as a marker of the cancer stem cell phenotype correlating with a poor clinical outcome [6].

Several USPs intersect with major oncogenic pathways, including Wnt/β-catenin, NF-κB, TGF-

Examples of USPs involved in cancer, their key substrates ("target genes"), and the mechanism by which they influence tumorigenesis. References indicate primary literature or reviews supporting these roles

USP	Cancer	Target substrate(s)	Mechanisms in cancer origin and progression	References
USP1	B-cell acute lymphoblastic leukemia	ID1 (Inhibitor of Differentiation 1)	Deubiquitinates and stabilizes ID1, maintaining elevated ID1 levels. Sustained ID1 leads to persistent PI3K/AKT signaling, promoting leukemic cell survival and proliferation	[27]
	Chronic myeloid leukemia	USP1- BCR-ABL	Stabilization of BCR-ABL oncoprotein levels contributes to the disease progression	[20]
	Glioma	PDGF–E2F–USP1–ID2	Promotion of cell survival in the proneural subtype of glioblastoma	[28]
		USP1–ID1	Downregulation of NgR1 facilitates myelin-associated infiltration.	[29]
	Pancreatic ductal adenocarcinoma	USP1- BCAT2	Enhances cell proliferation and supports ductal clone formation in pancreatic adenocarcinoma cells	[30]
	Ovarian cancer	ATM /ATR — USP1 — Snail	Contributes to platinum resistance and enhances metastatic potential	[31]
USP5	Bladder cancer	c-JUN (AP-1 transcription factor)	Removes ubiquitin from c-JUN, preventing its proteasomal degradation. The stabilized c-JUN hyperactivates JNK signaling, driving tumor cell proliferation, migration, and malignant progression	[32]
USP7	Non-small cell lung cancer (NSCLC)	KRAS (oncogenic GTPase)	Directly deubiquitinates KRAS, increasing KRAS protein stability. Elevated KRAS levels enhance oncogenic signaling, fueling NSCLC cell proliferation and tumor growth	[33]
		c-ABL kinase	USP7-mediated stabilization of c-ABL may promote survival signaling and contribute to tumor progression under genotoxic stress conditions	[34]
USP9X	Oral squamous cell carcinoma	MCL1 (anti-apoptotic BCL2-family protein)	Cleaves ubiquitin from MCL1, markedly extending its half-life. Accumulation of MCL1 blocks apoptosis and supports tumor cell survival, driving OSCC progression	[35]
USP10	Acute myeloid leukemia	FLT3 (tyrosine kinase oncoprotein)	USP10 deubiquitinates mutant FLT3, preventing its ubiquitin-mediated destruction. Stabilized FLT3 sustains aberrant signaling that boosts proliferation of AML cells	[36]
		SYK (spleen tyrosine kinase)	USP10 deubiquitinates and stabilizes SYK, a non-receptor tyrosine kinase that activates downstream signaling pathways NF-κB and STAT3. This enhances survival and proliferation signals in leukemia cells	[37, 38]

USP11	T-cell acute lymphoblastic leukemia	LCK (lymphocyte-specific protein tyrosine kinase)	In cooperation with USP7, USP11 removes ubiquitin chains from LCK, enhancing LCK stability and activity. Hyperactive LCK signaling promotes leukemic cell expansion and confers resistance to glucocorticoid therapy	[39]
USP15	Gastric cancer	HKDC1 (Hexokinase Domain Containing 1)	USP15 interacts directly with HKDC1 and removes K48-linked polyubiquitin chains, thereby preventing its proteasomal degradation	[40, 41]
		Wnt/β-catenin	Stabilizes nuclear β-catenin, enhancing Wnt/β-catenin signaling and promoting gastric cancer cell proliferation	[42]
USP20	ER-negative breast cancer	SNAI2 (Snail Family Transcriptional Repressor 2)	Deubiquitinates SNAI2, protecting it from proteasomal degradation. Stabilized SNAI2 drives epithelial–mesenchymal transition (EMT), increasing cancer cell migration, invasion and metastasis	[43]
USP22	Melanoma	YAP (Yes-associated protein)	Interacts with and deubiquitinates YAP to prevent its turnover. Accumulation of YAP enhances pro-oncogenic gene expression, promoting melanoma cell proliferation and mediating resistance to BRAF inhibitors	[44]
USP28	Ovarian cancer	SOX9 (SRY-box 9 transcription factor)	Binds to SOX9 and removes FBXW7-mediated ubiquitin, thereby stabilizing SOX9, which upregulates DNA damage repair genes, increasing repair capacity and driving resistance to PARP inhibitor therapy	[45]

β, and PI3K/AKT. For instance, USP9X stabilizes anti-apoptotic protein MCL-1, promoting survival in hematological malignancies. USP10 can act as a tumor suppressor by stabilizing p53 or PTEN, though its function varies depending on the cellular context [13, 15, 26]. Examples of USPs potentially involved in cancer and the mechanisms of their possible involvement are listed in the Table.

The therapeutic appeal of USPs lies in their enzymatic activity, druggability, and frequently cancer-specific expression profiles. Several small-molecule inhibitors, especially those targeting USP7 and USP1, have advanced into preclinical and early clinical development [5]. These compounds aim to disrupt USP-substrate interactions, modulate oncogenic signaling, or rewire protein degradation pathways. Achieving selective inhibition remains a major challenge due to the conserved catalytic domains across USPs, but advances in the covalent targeting and structure-based design are making selectivity increasingly attainable [6, 10, 23].

UPS in cancer treatment: Molecular targets and therapeutic approaches

Given the pervasive involvement of the UPS in cancer cell biology, it offers multiple points of intervention for therapy. Indeed, the success of proteasome inhibitors in oncology provided proof-of-concept that the UPS can be targeted for cancer treatment [4, 16]. Targeting the UPS can induce cancer cell death by causing accumulation of pro-apoptotic factors, misfolded proteins, and cell cycle inhibitors, or by destabilizing oncoproteins critical for tumor survival [5, 8].

Proteasome inhibitors: Validating the UPS as a target. The most established example of targeting the UPS in cancer therapy is bortezomib, a boronic acid dipeptide, which reversibly inhibits the proteasome's β5 chymotrypsin-like subunit. Approved by the FDA in 2003 for relapsed multiple myeloma, bortezomib remains a cornerstone of treatment. By blocking proteasomal degradation, it causes the accumulation of pro-apoptotic proteins, inhibits NF-κB signaling via IκBα stabilization, and triggers apoptosis in plasma cells. The high protein turnover in myeloma makes these cells particularly susceptible, validating the UPS as a therapeutic target [2].

Bortezomib's success led to second-generation inhibitors with improved pharmacologic profiles. Carfilzomib, an irreversible epoxyketone, selectively targets the β5 site and is approved for relapsed/refractory myeloma. It has a reduced neurotoxicity compared to bortezomib but carries cardiovascular risks. Ixazomib, the first orally available proteasome inhibitor, offers more convenient dosing and is effective in combination with immunomodulatory agents like lenalidomide [4].

Marizomib (salinosporamide A) irreversibly inhibits multiple proteasome subunits and crosses the blood–brain barrier, making it a candidate for solid tumors and bortezomib-resistant myeloma. Broader clinical trials have tested proteasome inhibitors in solid tumors, including lung cancer, though success has been limited due to a lower proteasome dependence and drug delivery challenges [16, 46].

Toxicity remains a key limitation. Bortezomib is linked to peripheral neuropathy, likely from the impaired proteostasis in neurons. Carfilzomib reduces this but introduces cardiovascular concerns. These challenges have driven efforts to develop next-generation inhibitors with improved selectivity, reduced toxicity, and expanded utility across tumor types [7, 10].

Targeting ubiquitin ligases and substrate-specific degradation. An alternative to global proteasome inhibition is targeting specific E3 ligases or their interactions with the substrates, offering a greater specificity and potentially fewer side effects. One leading strategy involves blocking the p53–MDM2 interaction. In tumors with wild-type p53, MDM2 is often overactive, leading to p53 degradation. Small molecules like nutlin-3 and idasanutlin (RG7388) disrupt this interaction, stabilizing p53 and reactivating apoptosis. Several MDM2 inhibitors have entered clinical trials for cancers such as leukemia and liposarcoma [11]. Similar efforts target MDM4 and other ligases, though the p53–MDM2 axis remains the most advanced.

Another approach targets neddylation, a modification essential for activating cullin-RING ligases (CRLs). Pevonedistat (MLN4924), an inhibitor of the NEDD8-activating enzyme, blocks CRL function, leading to the accumulation of substrates like CDT1 and inducing DNA re-replication stress and apoptosis. It turned out promising in clinical trials for AML and MDS and is being tested in solid tumors, often in combination regimens [47, 48].

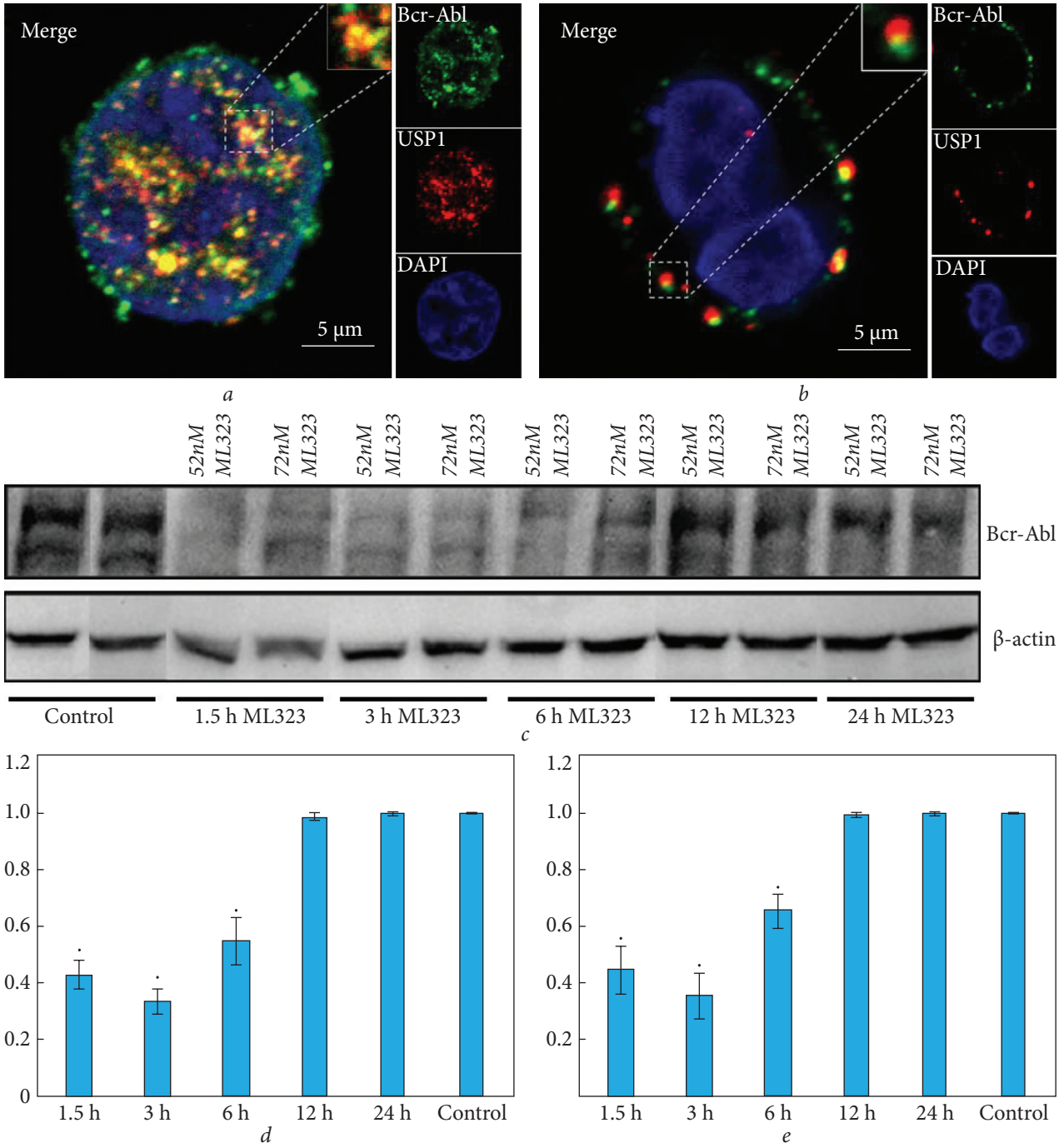
A major innovation is PROTACs, bifunctional molecules that link an E3 ligase to a target protein to induce ubiquitination and proteasomal degradation. Unlike inhibitors, PROTACs eliminate disease-driving proteins, including those considered "undruggable". Clinical examples include ARV-110 (androgen receptor), ARV-471 (estrogen receptor), and ARV-825 (BRD4) with the use of ligases like CRBN or VHL to direct target degradation. PROTACs exemplify how engineered UPS recruitment can achieve a precise protein elimination, expanding therapeutic possibilities and redefining targeted cancer therapy [4, 5].

DUB inhibitors: Targeting "undegraders". Given the oncogenic role of many DUBs, especially USPs, in stabilizing cancer-promoting proteins, pharmacological DUB inhibition represents a compelling therapeutic strategy. By preventing the removal of ubiquitin chains, DUB inhibitors shift the balance toward substrate degradation, forcing the destruction of proteins, which are otherwise protected in cancer cells [49, 50].

USP7 inhibitors have emerged among the most advanced DUB-targeting compounds. Small molecules such as P5091, P22077, and GNE-6776 have shown efficacy in restoring p53 function or suppressing oncogenic pathways. P5091, for example, induced apoptosis in multiple myeloma cells resistant to bortezomib, while P22077 showed activity in leukemia models. FT671, a more selective and potent USP7 inhibitor, stabilizes p53 and reduces the MDM2 levels in solid tumor models. These effects mimic MDM2 antagonists but act upstream by blocking ubiquitin removal rather than preventing E3-substrate interaction [11, 51].

The inhibition of USP1 represents another promising strategy for cancer therapy. Earlier, we have shown that ML323, a low-molecular-weight USP1 inhibitor, demonstrated high selectivity and disrupted the USP1–UAF1 complex, leading to BCR-ABL degradation in chronic myeloid leukemia K562 cells (Figure) [23]. However, the effect was reversible, and in 12 h, the BCR-ABL level returned to the control values (Figure, *d, e*).

Other compounds capable of inhibiting USP1 activity are also known, including pimozide, GW7647, SJB2-043, C527, KSQ-4279, flupentixol,



Effects of ML323 — low-molecular-weight USP1 inhibitor — on USP1–BCR-ABL interaction and BCR-ABL level in chronic myeloid leukemia K562 cells. Colocalization of USP1 and the BCR-ABL oncoprotein in control K562 cells (*a*) and after 24-h treatment with 52 nM ML323 (*b*). Immunofluorescence confocal microscopy images show endogenous USP1 (red fluorescence) and BCR-ABL (green fluorescence) localization. Yellow areas indicate colocalization of USP1 and BCR-ABL proteins within the nucleus (blue fluorescence, DAPI). The reduction in colocalization indicates the disruption of the USP1–BCR-ABL interaction. The Western blot analysis showing BCR-ABL protein levels after 24-h incubation with ML323 (52 nM and 76 nM) (*c*) and normalized values of band areas in Western blot after treatment with 52 nM (*d*) or 76 nM (e) ML323. * $p < 0.01$ as compared to control

trifluoperazine, and rottlerin. Inhibition of USP1 deubiquitinating activity results in decreased proliferation, metastasis, and survival of malignant cells across various cancer types [23, 24].

USP9X, a DUB known to stabilize MCL-1, has been targeted by WP1130 (Degrasyn), a broad-spectrum DUB inhibitor, which also inhibits USP5, USP14, and UCHL5. WP1130 reduces the

MCL-1 levels and restores chemosensitivity in leukemia and solid tumor models [4]. However, its off-target effects, including potential kinase inhibition, highlight the need for more selective USP9X inhibitors.

Other USPs under investigation include USP10, USP11, USP13, USP30, and USP33, with early-stage inhibitors being explored in both academic and pharmaceutical settings [17, 26, 38]. USP30 inhibitors, for example, are being evaluated in the contexts of mitochondrial quality control and cancer metastasis, whereas USP13 inhibition may sensitize ovarian tumors to DNA-damaging agents by destabilizing DNA repair proteins [33].

Fragment-based and structure-guided approaches have facilitated progress in this field, particularly through covalent targeting of the catalytic cysteine residue in cysteine protease DUBs [2]. High-throughput screening assays using fluorogenic substrates, such as ubiquitin-rhodamine or ubiquitin-AMC, have enabled the identification of novel inhibitory scaffolds [9].

Although DUB inhibitors have not yet reached routine clinical application, multiple studies have confirmed their druggability and therapeutic relevance. The inhibition of oncogenic USPs has been shown to suppress tumor growth, restore apoptosis, and overcome resistance to other treatments. Importantly, many USPs are non-essential in normal cells but become critical in cancer, creating a potential therapeutic window driven by oncogene addiction [6, 10].

Conclusion and future perspectives

The UPS is essential for maintaining protein homeostasis and regulating critical cellular processes, including cell cycle progression, DNA repair, and apoptosis. In cancer, this tightly controlled system is frequently dysregulated to support malignant growth, immune evasion, and resistance to therapy. Among the UPS components, USPs have emerged as key regulators of oncogenic signaling, often through selective stabilization of cancer-promoting proteins.

Therapeutically, the UPS has proven to be a viable target. Proteasome inhibitors remain a cornerstone of treatment for hematologic malignancies, and newer approaches such as E3 ligase modulation, DUB inhibition, and targeted protein degradation via PROTACs offer the potential for a greater specificity and broader applicability. USPs, in particular, represent a promising class of drug targets because of their enzymatic activity and context-dependent oncogenic roles.

Looking ahead, challenges remain in achieving a selective inhibition of UPS components, minimizing toxicity, and identifying biomarkers that predict responses. Structural and mechanistic insights into UPS enzymes enable rational drug design, whereas combinatorial strategies integrating UPS inhibitors with DNA-damaging agents, immunotherapies, or targeted kinase inhibitors may enhance therapeutic efficacy.

As our understanding of the ubiquitin landscape deepens, particularly in the context of tumor-specific vulnerabilities, UPS-targeted therapies are poised to expand their role in precision oncology. Continued efforts in mapping USP–substrate networks and refining drug development will be critical for unlocking the full therapeutic potential of this essential cellular machinery.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DN wrote and finalized the draft. SV conceived the presented idea. DN, SV, and DG contributed to the conceptualization of this comment and writing the manuscript. GT led the project. All authors critically revised the manuscript, offering substantial edits and comments to the original draft, and approved the final version of the manuscript.

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УБІКВІТИН-ПРОТЕАСОМНА СИСТЕМА ПРИ РАКУ: МЕХАНІЗМИ, МІШЕНІ ТА ТЕРАПЕВТИЧНИЙ ПОТЕНЦІАЛ

Убіквітин-протеасомна система (UPS) є центральним механізмом контрольованої внутрішньоклітинної деградації білків у еукаріотів. Вона регулює ключові біологічні процеси, зокрема перебіг клітинного циклу, відновлення ДНК, апоптоз і сигнальну трансдукцію. За допомогою ієрархічного каскаду ферментів убіквітин ковалентно приєднується до білків-мішеней, часто у вигляді поліубіквітинових ланцюгів, позначаючи їх для селективного розщеплення 26S протеасомою. Порушення роботи UPS є характерною ознакою раку: змінена динаміка убіквітинування може сприяти злоякісній трансформації, стимулюючи деградацію пухлинних супресорів або стабілізуючи онкогенні білки. Деубіквітинуючі ферменти (DUB), зокрема родина убіквітин-специфічних протеаз (USP), здійснюють зворотне відщеплення убіквітину та підтримують білковий гомеостаз. Багато USP виявляють аномальну експресію або генетичні зміни в пухлинах, що посилює онкогенну сигналізацію, сприяє резистентності до апоптозу та уникненню терапії. У цьому огляді подано узагальнений аналіз будови та функцій UPS з акцентом на механізми убіквітинування, активність протеасоми та роль DUB у канцерогенезі. Розглянуто новітні терапевтичні стратегії, спрямовані на різні компоненти UPS: схвалені FDA інгібітори протеасоми, блокатори функції E3-лігаз, технології деградації білків на основі PROTAC та низькомолекулярні інгібітори USP. Попри складність "націлювання" на DUB через проблеми специфічності та токсичності, досягнення у структурному дизайні та картуванні "убіквітинового коду" пришвидшують прогрес. Загалом, UPS виступає ключовим регуляторним центром у біології раку та є перспективною мішенню для прецизійної онкології. Терапевтична модуляція цього шляху відкриває нові можливості для дестабілізації онкогенних мереж і подолання механізмів резистентності в злоякісних клітинах.

Ключові слова: убіквітин-протеасомна система, убіквітинування, убіквітин-специфічні протеази, деубіквітинізуючі ферменти, рак, таргетна терапія.