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Review

The role of USP1 deubiquitinase in the pathogenesis and therapy of cancer

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Ubiquitin-specific protease 1 (USP1) is an important deubiquitinating enzyme (DUB) involved in the maintenance of genome integrity, cell cycle, and cell homeostasis. USP1 overexpression is a characteristic feature of various cancers, correlating with a poor prognosis. The review summarizes the recent knowledge in understanding the role of deubiquitinase USP1 in the stabilization of oncoproteins and tumor suppressors, as a critical event in cancer development and progression. The putative mechanisms of USP1 involvement in some prevalent human cancers are discussed. The numerous data demonstrate that inhibition of USP1 suppresses the proliferation and viability of malignant cells, sensitizes them to radiation and increases their sensitivity to various chemotherapeutic agents, which opens up new opportunities for combined therapy of malignant neoplasms.

Keywords: Deubiquitinating enzyme (DUB), USP1, cancer, targeted therapy

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Abbreviations: AR, androgen receptor; B-ALL, B-cell acute lymphoblastic leukemia; BC, breast cancer; BCAA, branched-chain amino acid; CML, chronic myeloid leukemia; COSMIC, catalog of somatic mutations in cancer; DUB, deubiquitinating enzyme; HCC, hepatocellular carcinoma; ID2, DNA binding 2; MINDYs, motif-interacting with ubiquitin-containing novel DUB family; MJDs, Machado-Joseph disease proteases; NES, nuclear export signal; NLSs, nuclear localization signals; NSCLC, non-small cell lung carcinoma cells; OTUs, ovarian tumor proteases; PDAC, pancreatic ductal adenocarcinoma; RPs, ribosomal proteins; ROS, reactive oxygen species; T-ALL, T-cell acute lymphoblastic leukemia; TBLR1, transducin β-like X-linked receptor 1; UAF1, USP1 cofactor-associated protein 1; USPs, ubiquitin-specific proteases; UCHs, ubiquitin carboxy-terminal hydrolases

INTRODUCTION

Deubiquitinating enzymes (DUB) are proteases that regulate ubiquitin dynamics by selectively cleaving mono- or polyubiquitin from protein substrates (Henning *et al.*, 2021; Caba *et al.*, 2022). The deubiquitination is crucial for maintaining the stability of target proteins, and in the absence of proteolytic load on proteasome it affects the activity of substrates, cellular localization, interactions with other proteins, activation or silencing of gene expression, and the functioning of signaling pathways (Snyder *et al.*, 2021; Tu *et al.* 2022; Estavoyer *et al.*, 2022 Trulsson *et al.*, 2022). More than 500 protease genes have been identified in the human genome, about 100 of which belong to DUBs (Bonacci & Emanuele, 2021). Based on the homology of the active site, DUBs are classified into six families: ubiquitin-specific proteases (USPs), ubiquitin carboxy-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), the Machado–Joseph disease proteases (MJDs), motif-interacting with ubiquitin-containing novel DUB family (MINDYs) and JAB1/PAB1/MPN (JAMMs). The first five families (USPs, UCHs, OTUs, MJDs, MINDYs) are cysteine proteases, while the JAMMs family belongs to metalloproteases (Mullard *et al.*, 2021; Lei *et al.*, 2021a; Caba *et al.*, 2022). The largest family among DUBs is USPs. Members of the USPs family have a conserved structural organization consisting of three subdomains, namely the "thumb" and "palm" with a catalytic site between them and the "fingers" that provide interaction with ubiquitin on substrates (Fraile *et al.*, 2012; Snyder *et al.,* 2021*)*. Sometimes USPs have additional domains, such as ubiquitin-binding zinc finger domain, ubiquitin-interacting motifs and ubiquitin-associated, etc. (Du *et al.*, 2019; Yang *et al.*, 2019).

The physiological role of DUB is to ensure the homeostasis of critical cell functions, including genome stability, gene expression, cell cycle progression, proliferation, stem cell differentiation, chromosome segregation, growth factor signaling, redox regulation, endocytosis, apoptosis, etc. Deregulation of functional activity and expression of DUB correlates with neurodegenerative and immune diseases, development and progression of cancer (Jerabkova *et al.*, 2020; Wang *et al.*, 2022; Tu *et al.*, 2022; Liu *et al.*, 2022). The growing interest in DUBs as markers of oncogenic transformation and new therapeutic targets for cancer treatment seems to be justified.

The most studied DUB to date is ubiquitin-specific protease 1 (USP1). Quite often, DUB expression is altered in many cancers (Poondla *et al.*, 2019; Lai *et al.*, 2020). Hyperexpression of USP1 is observed in glioma, osteosarcoma, leukemia, hepatocellular carcinoma, gastric cancer, breast cancer, ovarian cancer, prostate cancer, colorectal cancer, etc., being associated with low patient survival and malignant neoplasm progression (Williams *et al.*, 2011; Xu *et al.*, 2019; Ma *et al.*, 2019b; Kuang *et al.*, 2021; Liao *et al.*, 2021a; Liang *et al.*, 2022; Li *et al.*, 2021; Chen *et al.*, 2022).

Recently, many breakthroughs have been made in elucidating the role of USP1 as an important regulator of basic cellular processes including metabolism, proliferation, and apoptosis. Ensuring the balance between ubiquitination and deubiquitination, USP1 is critical for maintaining the integrity of signaling networks, the proper performance of functions controlled in a spatio-temporal mode, and the homeostasis of the cell as a whole. The impairment of this balance could be important for tumorigenesis and cancer progression (Cui *et al.*, 2020; Meng *et al.*, 2022; Sun *et al.*, 2022). USP 1

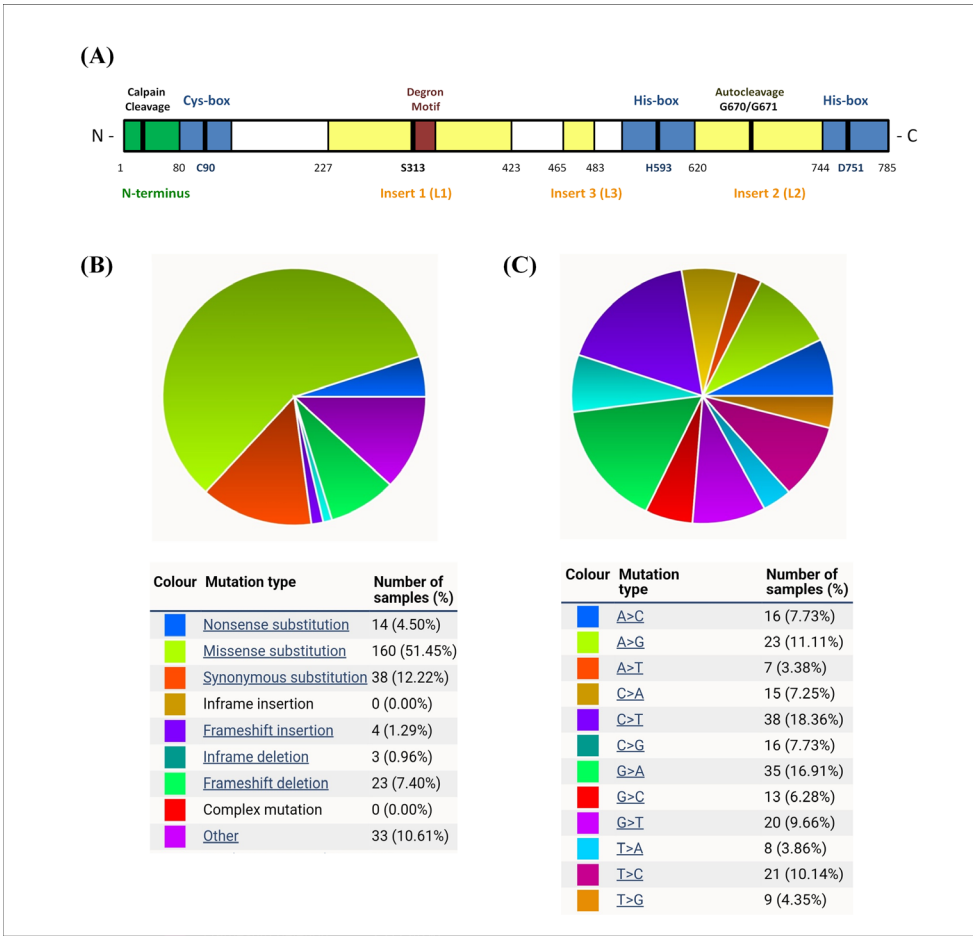
could be a valuable diagnostic marker in various types of cancer. USP1 is directly involved in tumorigenesis by regulating the stability of oncoproteins or tumor suppressors (Sonego *et al.*, 2019; Coleman *et al.*, 2022; Song *et al.*, 2022). Finally, USP1 ability to modulate the level of oncoproteins accumulating in cancer cells makes it an attractive therapeutic target for cancer treatment.

THE STRUCTURE AND FUNCTION OF USP1

USP1 belongs to the most numerous and diverse USPs of the DUB family (Cruz *et al.*, 2021). The protein was first identified in 1998 as part of the Human Genome project by a group of scientists from Japan (Fujiwara *et al.*, 1998). The USP1 gene is localized to the p31.3-p32.1 band of chromosome 1. The USP1 protein consists of 785 amino acid residues with a predicted molecular weight of 88.2 kDa. In the structure of USP1, there is a typical DUB conserved USP domain, which consists of a N-terminal Cys box motif with a C90 catalytic residue and a C-terminal His box motif with H593 and D751 catalytic residues. (Fig. 1A). It is believed that these three amino acids form the so-called catalytic triad, which actually forms the catalytic core of DUB (Yu *et al.*, 2017; Woo *et al.*, 2022). The catalytic domain of the USP1 protein is one of the largest in the USPs family due to additional insertions (Bishop *et al.*, 2016). The first additional insert L1 (within 227-433 amino acid residues) located between box 2 and 3 is able to enhance USP1 activity due to natural affinity to DNA and allosteric activation after binding of UAF1 (Dharadhar *et al.*, 2021). Within L1 there are phosphorylation sites, including the most studied S313, nuclear localization signals (NLSs) and a degradation motif (degron). A second additional L2 insert (between amino acid residues 602-744) is located between boxes 5 and 6 and includes an autocleavage site (G670-G671). The third smallest L3 insert is located between 465-483 amino acid residues. It is believed that L1 and L3 insertions together are able to cause autoinhibition that could be reversed by binding to UAF1 cofactor. Co-deletion of L1 and L3 leads to hyperactivation of USP1, while deletion of the L2 or L3 insert does not affect its enzymatic activity (Dharadhar *et al.*, 2021).

The database "Catalog of somatic mutations in cancer" (COSMIC) contains information on dozens of mutations in USP1 (Fig. 1B), among which 51% are missense substitutions, 12% are synonymous substitutions, slightly more than 7% are frameshift deletions, and 4% are nonsense substitutions. It is known that mutation of any of the C90, H593 or D751 amino acid residues significantly reduces the catalytic activity of the USP1 protein. While the functional effect of the vast majority of mutations has not been fully elucidated, about forty different mutations have been detected in various types of cancer.

For the first time, as a critical regulator of genome integrity, USP1 was described in Fanconi anemia, where its functions are realized by FANCD2 deubiquitination (Nijman *et al.*, 2005). It is believed that USP1 in complex with the cofactor UAF1 moves along the replication fork



Colour	Mutation type	Number of samples (%)
	Nonsense substitution	14 (4.50%)
	Missense substitution	160 (51.45%)
	Synonymous substitution	38 (12.22%)
	Inframe insertion	0 (0.00%)
	Frameshift insertion	4 (1.29%)
	Inframe deletion	3 (0.96%)
	Frameshift deletion	23 (7.40%)
	Complex mutation	0 (0.00%)
	Other	33 (10.61%)

Colour	Mutation type	Number of samples (%)
	A>C	16 (7.73%)
	A>G	23 (11.11%)
	A>T	7 (3.38%)
	C>A	15 (7.25%)
	C>T	38 (18.36%)
	C>G	16 (7.73%)
	G>A	35 (16.91%)
	G>C	13 (6.28%)
	G>T	20 (9.66%)
	T>A	8 (3.86%)
	T>C	21 (10.14%)
	T>G	9 (4.35%)

Figure 1. USP1: structure and mutations.
A – USP1 domain organization. B – Distribution of different mutation types for USP1. The COSMIC database was used to analyze the mutation distribution for USP1. C – A breakdown of the observed substitution mutations in USP1.

and deubiquitinates its partner proteins (Lim *et al.*, 2018; Dharadhar *et al.*, 2021). DNA-dependent metalloprotease Spartan can cause USP1 molecule to be removed from DNA acting selectively only on catalytically active forms of USP1, while minimally affecting mutants with changes in the autocleavage domain. This defines a new role of USP1 autocleavage in its increased retention on DNA. Spartan does not specifically act on protease sequences, cleaving substrates mainly in unstructured regions near lysine, arginine, and serine residues (Coleman *et al.*, 2022). The preferential association of Spartan with ubiquitin-modified proliferating cell nuclear antigen (PCNA) protects against PCNA deubiquitylation by USP1 and facilitates the access of polymerase responsible for a translesion DNA synthesis to the replication fork (Juhasz *et al.*, 2012). These facts highlight the role of USP1 autocleavage regulation as one of the components maintaining the steady function of DNA replication machinery and genome integrity (Coleman *et al.*, 2022).

It is also known that USP1 participates in the regulation of centrosome duplication, therefore, a violation of its activity causes the formation of an abnormal mitotic spindle, amplification of centrosomes, incorrect segregation of chromosomes, which leads to aneuploidy, instability of the genome, thus creating prerequisites for oncogenic transformation of cells (Jung *et al.*, 2016; Twest *et al.*, 2017). Today, a wide range of USP1 cellular substrates is known, the dynamic balance between the activity of DAB and ubiquitin E3 ligase underlies the modulation of protein degradation and localization, the formation of protein-protein interactions, gene expression, activation and deactivation of signaling pathways, ensures homeostasis and the performance of critical cell functions, such as metabolism, proliferation and cell differentiation, autophagy, apoptosis.

USP1/UAF1 PROTEIN COMPLEX

The catalytic activity of USP1 is dramatically increased in complex with the USP1 cofactor-associated protein 1 (UAF1), also known as protein 48, or WDR48 (Yu *et al.*,

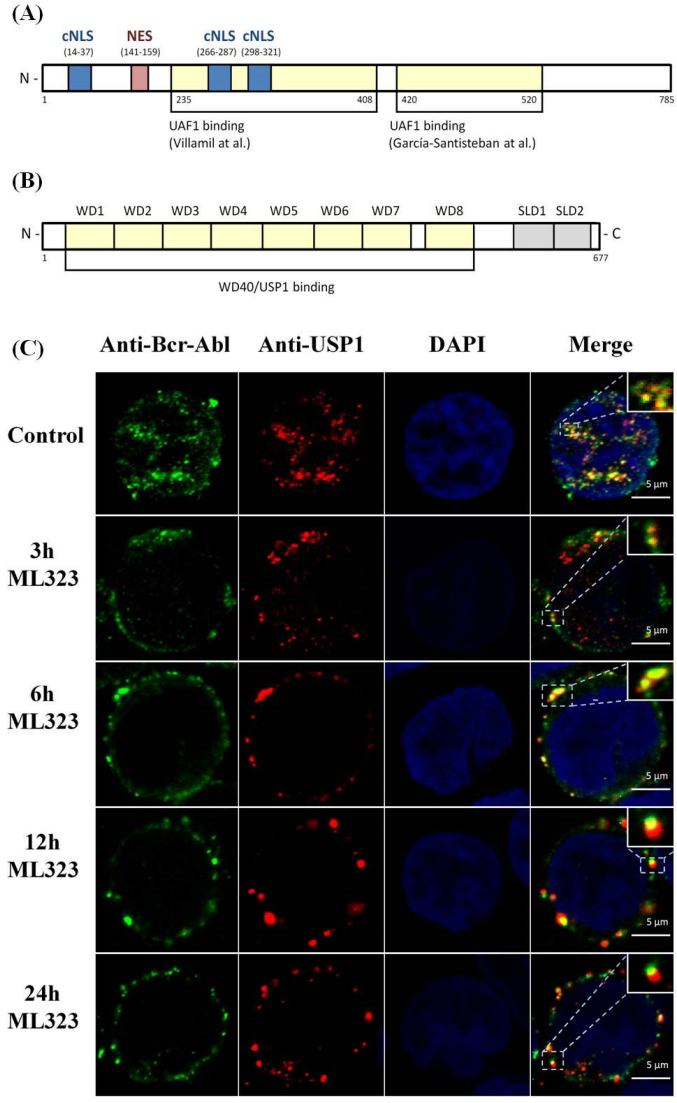


Figure 2. Localization of USP1 and the mechanism of its interaction with the UAF1 cofactor.
A – schematic representation of the location of motifs in the USP1 protein, which determine its localization and interaction with the UAF1 cofactor, B – domain organization of the UAF1 cofactor, a representation of the motifs responsible for the interaction with USP1, C – USP1 is a nuclear protein that colocalizes with the Bcr-Abl oncoprotein in CML cells. In cells treated with ML323 inhibitor, which acts on the protein complex USP1/UAF1, USP1 is localized exclusively in the cytoplasm, which indicates that only its activated form enters the nucleus in a complex with a protein cofactor.

2017; Arkinson *et al.*, 2018). The UAF1 protein contains eight WD40 repeats, seven of which have a β-propeller structure, the eighth repeat belongs to the so-called auxiliary domain. WD40 repeats are localized to the N-terminal domain of UAF1, which binds to USP1 and other substrate proteins, including USP12 and USP46. Deletion of 2–4 repeats of WD40 disrupts the formation of the USP1/UAF1 protein complex, and deletion of the 8th repeat and the adjoining region causes impairment of function of the UAF1 cofactor itself, which is believed to be a consequence of disruption of its tertiary structure. At the C-terminus of UAF1, a SUMO-like domain is located with two subdomains. Deletions in the proximal part of the C-domain do not affect the formation of the USP1/UAF1 protein complex (Yang *et al.*, 2011; Goncalves *et al.*, 2017; Rennie *et al.*, 2022). Villamil et al. suggested that USP1 is activated by the UAF1 cofactor via modulation of the conformation of the active site of DAB, while changes due to remodeling of the ubiquitin-binding site are unlikely (Villamil *et al.*, 2012). The altered UAF1 expression has critical consequences; in particular, UAF1 deficiency causes the death of mice in the embryonic period (Arkinson *et al.*, 2018). The formation of a protein complex involves joint subcellular localization of partner proteins, however, USP1 belongs to nuclear proteins, while UAF1 is localized mainly in the cytoplasm. It is assumed that the USP1/UAF1 complex is formed in the cytoplasm and subsequently imported into the nucleus due to the NLS region of the USP1 protein (Yu *et al.*, 2017). There are contradictory data regarding the mechanisms of formation of the USP1/UAF1 protein complex. Villamil and others (Villamil *et al.*, 2012) showed that the site between 235-408 amino acid residues on the USP1 protein is responsible for cofactor binding. On the other hand, García-Santisteban and others indicated that USP1 binds to its cofactor due to the site within 420–520 amino acid residues (Fig. 2A, B) (García-Santisteban *et al.*, 2013; Yu *et al.*, 2017). Currently, it is not completely clear, which of two USP1 sites is responsible for binding to UAF1 or whether both sites are involved under different physiological conditions.

PHOSPHORYLATION OF USP1

Phosphorylation is an important post-translational modification that can stabilize proteins, increase or decrease their abundance in the cell, influence their activity, localization, interaction with other protein substrates (Wang *et al.*, 2021). Extensive phosphoproteomic studies found that the activity of the vast majority of human USPs is regulated by phosphorylation (Villamil *et al.*, 2012). Phosphorylation at the Ser313 site may be one of the ways of regulating the level of USP1 during the cell cycle, preventing its degradation during mitosis. (Cotto-Rios *et al.*, 2011). Villamil and others (Villamil *et al.*, 2012) showed that phosphorylation of USP1 at the Ser313 site is one of the conditions for the formation of the USP1/UAF1 protein complex, while protein phosphatase treatment can lead to its inactivation. Nevertheless, Olazabal-Herrero and others (Olazabal-Herrero *et al.*, 2015) found that the mutant USP1, which is not phosphorylated at the Ser313 site, is also able to interact with UAF1. In addition, the formation of the USP1/UAF1 protein complex does not depend on the two other known sites, Ser42 and Ser67 (Villamil *et al.*, 2012). Phosphorylation of USP1 at S42 and S331 is a prerequisite for Snail interaction and deubiquitination, which promotes platinum resistance and metastatic progression in ovarian cancer. In turn, under the influence of phosphatases, the level of interaction between Snail/USP1 proteins decreases (Sonego *et al.*, 2019). Phosphorylated forms of USP1 at tyrosine sites were detected in Bcr-Abl-positive chronic myeloid leukemia cells. USP1 is believed to be phosphorylated by interaction with the oncoprotein Bcr-Abl, which has uncontrolled tyrosine kinase activity, leading to deregulation of downstream signaling pathways and disease progression (Antonenko & Telegeev, 2020).

Taking into account the role of phosphorylation in USP1 activation, one may suggest that the inhibition of protein kinases that phosphorylate USP1 may impair its functional activity. In fact, we have observed that in CML cells treated with imatinib, the level of colocalization of USP1 and Bcr-Abl is significantly reduced, while part of the USP1 protein remains in the cytoplasm, which may indicate a lack of deubiquitinase activity (Antonenko S., unpublished data). It has been also shown that tyrosine kinase inhibitors reduce the expression of USP47 in CML cells, which can be potentially relevant to other USPs, including USP1 (Lei *et al.*, 2021b).

SUBCELLULAR LOCALIZATION OF USP1

Subcellular localization is a key for the realization of enzymatic activity by any protein, as it ensures the execution of a whole complex of intricately connected pathways and processes within and between different compartments (Garapati *et al.*, 2020). A change in subcellular localization can affect the catalytic activity of a protein, disrupt its physiological functions, protein-protein interactions, downstream signaling pathways, which creates prerequisites for the development of inflammatory and neurodegenerative diseases, malignant neoplasms (Wang & Wang, 2021; Garapati *et al.*, 2021). USP1 belongs to nuclear proteins. Three NLS sites in USP1 are responsible for its nuclear transport. The first sequence, which is between 14–37 amino acid residues, has a weak NLS and ensures uniform distribution of USP1 in the nucleus and cytoplasm. The second region of the NLS, located within 266–287 amino acid residues, ensures almost complete movement to the nucleus, the third region of the NLS within the range of 298–321 amino acid residues, provides a clear, but less marked relocation. In the physiological context, the two NLS signals (266–287), (298–321) are the main motifs of USP1 nuclear-cytoplasmic localization, while the third motif does not have a critical role. It is believed that USP1 and UAF1 form a complex in the cytoplasm, which subsequently translocates to the nucleus thanks to defined NLS (266–287), (298–321) motifs (Garcia-Santisteban, *et al.*, 2012). The nuclear export signal (NES), located within amino acid residues 141-159 of USP1, may mediate the interaction with the export receptor CRM1, although the ultimate physiological significance of this sequence has not been established yet (Fig. 2A) (García-Santisteban *et al.*, 2013). We have demonstrated that in CML cells treated with ML323 inhibitor USP1 remains in cytoplasm suggesting that only the activated form of DUB in a complex with the UAF1 cofactor can enter the nucleus (Fig. 2C) (Antonenko & Telegeev, 2020).

DEGRADATION OF USP1

USP1 level in cells is regulated by its proteasomal degradation. The degradation motif (degron) ensures APC/CCdh1-dependent degradation of USP1 in the G1 phase

of the cell cycle (García-Santisteban *et al.*, 2013). Calpain (CAPNS1) stabilizes USP1 by activating Cdk5, which in turn inhibits cdh1 and consequent USP1 proteolysis. It is believed that the region of interaction with CAPNS1 is located at the N-terminus of the USP1 protein; mutations in this region, in particular the Leu-to-Gly substitution in amino acid 12, dramatically destabilize the DUB (Cataldo *et al.*, 2013). During mitosis, proteolytic degradation of the USP1 protein can be regulated by phosphorylation of the Ser313 site, which leads to the masking of the degradation motif and impairment of proteolysis. The USP1 level is significantly reduced in cells exposed to genotoxic agents (in particular, such as UV) due to autocleavage at the Gly670-Gly671 internal motif (Cotto-Rios *et al.*, 2011). The autocleavage of USP1 leads to the formation of two protein fragments, namely the amino-terminal USP1NT or N-terminal fragment (residues 1–671) and the shorter carboxyl-terminal USP1CT or C-terminal fragment (residues 672–785), which are subsequently subjected to proteasomal degradation (Piatkov *et al.*, 2012). It is believed that USP1 fragments may retain their enzymatic activity because they continue to form complexes with the UAF1 cofactor until their complete destruction. Destruction of the N-terminal fragment occurs at the expense of the C-terminal degron, which is recognized and eliminated by DesCEND pathway with recognition of unusual C-termini by the ubiquitin ligase CRL2. The smaller C-terminal fragment is destroyed by the Arg/N-terminal rule pathway through deamidation of its destabilizing N-terminal Gln24 residue (Piatkov *et al.*, 2012; Coleman *et al.*, 2022).

Four missense mutations G667A, L669P, K673T, A676T are known within the USP1 autocleavage site. Only the L669P mutation reduces protein cleavage, due to conformational changes of the protein impairing access to the cleavage site. USP1 autocleavage shows that the balance of USP1 autocleavage can be disrupted by a cancer-associated mutation (Olazabal-Herrero *et al.*, 2015). USP1 activity is reversibly inactivated in response to stress and the accumulation of reactive oxygen species (ROS) in the cell due to the oxidation of the catalytic cysteine (Cotto-Rios *et al.*, 2012). The mechanism of DUB inactivation involves disruption of isopeptide cleavage but without affecting affinity to ubiquitin (Lee *et al.*, 2013). Disruption of USP1 enzymatic activity as a result of ROS bursts causes accumulation of Ub-PCNA during S-phase, while the effect is negligible during G0 or G1. The accumulation of Ub-PCNA is also observed under the influence of UV irradiation, which leads to the destruction of USP1 (Cotto-Rios *et al.*, 2012; Coleman *et al.*, 2022).

USP1 IN THE DEVELOPMENT AND PROGRESSION OF CANCER

Since ubiquitination and deubiquitination are suggested to be essential regulators of basic cell functions, the impairment of the coordination in this network is inevitably associated with the characteristic features of cancer cells. In fact, a vast number of data demonstrate the altered expression of one or another DUBs in cancer. Currently, such data are available for more than 40 USPs including USP1, USP2, USP4, USP7, USP9X, USP10, USP11, USP12, USP13, USP14, USP15, USP19, USP22, USP26, USP29, USP33, USP39, USP42, USP44, USP46 and USP51. For example, USP2, USP3, USP4, USP7, USP10, USP29, and USP42 regulate the tumor suppressor protein p53. While USP7, USP10, USP29 and USP42 stabilize the level of p53, USP2 and USP4, on the contrary, contribute to its proteosomal degradation due to the stabilization of E3 ligase. A number of DUBs (USP4, USP11, USP13, USP15, USP28, USP51) contribute to metastasis. For USP4, USP11, USP15, this is possible by altering the level of ubiquitination of the TGFβ receptor, which promotes TGFβ (transforming growth factor β) signaling and invasiveness of malignant cells. The functioning of DUBs is an essential component of cell cycle progression (USP1, USP2, USP3, USP7, USP17, USP22, USP39), DNA damage repair (USP1, USP3, USP4, USP7, USP9x, USP11, USP20, USP21, USP34, USP51), chromatin remodeling (USP7, USP11, USP16, USP21) (Pal *et al.*, 2014; Gorrepati *et al.*, 2018; Poondla *et al.*, 2019; Cruz *et al.*, 2021; Guo *et al.*, 2022). The list of the DUBs whose expression is deregulated in cancer is continuously expanding.

USP1 is a prime example of DUBs modulating the expression level of oncoprotein. Such an aspect is of particular interest from the point of cancer pathogenesis and antitumor therapy.

An expanding number of findings suggest the important role of USP1 in pathogenesis of various cancers via diverse mechanisms. The dynamic balance between USP1 and ubiquitin E3 ligase underlying the modulation of the activity, degradation and localization of proteins contributes to maintaining homeostasis and controlling the critical functions in cell. Overexpression of USP1 facilitates the development of metastatic phenotype as well as chemo- and radioresistance of cancer cells. Besides the expression upregulation, the fact of mutations in DUB genes demonstrated in several human cancers provides further evidence in the support of the putative involvement of these enzymes in the development and progression of various malignancies.

The USP1 activity is important for controlling expression of several proteins related to oncogenesis and cancer progression. Among them are SIK2, MMP-2, GSK-3β, Bcl-2, Stat3, cyclin E1, Notch1, Wnt-1, and cyclin A1. Moreover, deubiquitination stabilizes the level of both oncoproteins and tumor growth suppressors such as EZH2, CHEK, TAZ, PHLPP1, Bcr-Abl, Aurora B, BCAT2, TBLR1, RPS16, c-Kit, KPNA2, KDM4A, ERα, SIX1, Snail, and ID1/ID2/ID3 impairing their intracellular balance (Table 1).

USP1 overexpression is characteristic of various types of cancer. According to GEPIA data from The Cancer Genome Atlas and the Genotype Tissue Expression databases, the highest level of USP1 expression is observed in cervical squamous cell carcinoma and endocervical adenocarcinoma, and it also significantly exceeds expression compared to normal tissue samples in breast invasive carcinoma, sarcoma, cholangiocarcinoma, colon adenocarcinoma, diffuse large B-cell lymphoma, esophageal carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, brain lower grade glioma, rectum adenocarcinoma, thyroid carcinoma, etc. (Fig. 3). Changes in USP gene expression in cancer cells can be caused by various mechanisms, such as DNA repair defects, activation of signaling pathways, post-translational modifications, etc. A characteristic feature of increased USP1 expression for various types of cancer is a violation of cell homeostasis, proliferation, and apoptosis, which can be realized due to the stabilization of ID1, ID2, and ID3 proteins or FANCD2, as a key regulator of genome stability. siRNA knockdown or pharmacological inhibition of USP1 reduces proliferative activity and cell migration, promotes the onset of apoptosis and res-

Table 1. The putative role of USP1 in different forms of cancer.

Cancer	Known pathways affected	The role USP1 in the pathogenesis of the cancer	References
Glioma	PDGF-E2F-USP1-ID2 β-catenin-USP1-EZH2 USP1-CHEK1 USP1-ID1	Support for cell survival of the proneural subtype of glioblastoma. Promotion of tumorigenesis. Survival support. GSCs and resistance to treatment. Reduction of NgR1 promoting myelin-related infiltration	Rahme *et al.*, 2016; Ma *et al.*, 2019a; Lee *et al.*, 2016; Lee *et al.*, 2016
Osteosarcoma	USP1-ID1/ID2/ID3 USP1-TAZ	Suppression of osteoblast differentiation, increased proliferation, invasiveness, metastasis. Violation of the Hippo signaling pathway, increased proliferation and migration of malignant cells.	Williams *et al.*, 2011 Yuan *et al.*, 2022
Non-small cell lung carcinoma	USP1-PHLPP1-Akt	Disease progression. Unregulated proliferation of cancer cells.	Zhiqiang *et al.*, 2012
B-cell acute lymphoblastic leukemia	USP1-ID1- AKT	Support for cell growth and viability. Disease progression. Suppression of cell apoptosis.	Kuang *et al.*, 2021
T-cell acute lymphoblastic leukemia	USP1-Aurora B	Promotion of chemoresistance of T-ALL cells. Promotion of cell invasion. Inhibition of glucocorticoid receptor expression and apoptosis.	Gong *et al.*, 2021
Multiple myeloma	USP1-ID-Notch-Sox2	Promotion of cell viability and resistance to bortezomib treatment. Enhances cell growth and inhibits apoptosis through the activation of caspase-3, caspase-8, and caspase-9.	Das *et al.*, 2017
Chronic myeloid leukemia	USP1-Bcr-Abl	Stabilization of the Bcr-Abl oncoprotein level in cells as one of the conditions for disease progression.	Antonenko & Telegeev, 2020
Esophageal squamous cell carcinoma	D1/CDK4/CDK6	Maintenance of esophageal cancer cell viability and colony formation. Promotion of genetic stability of cells.	Sun *et al.*, 2022
Stomach cancer	USP1-ID2	Promotes proliferation, metastasis, epithelial-mesenchymal transition of gastric cancer cells.	Li *et al.*, 2021
Pancreatic ductal adenocarcinoma	USP1-BCAT2	Promotes cell proliferation, the formation of ductal clones in pancreatic adenocarcinoma cells.	Li *et al.*, 2022b
Hepatocellular carcinoma	USP1-TBLR1 USP1-RPS16-Twist1/Snail USP1-c-Kit	Promotes the survival of circulating tumor cells, promotes metastasis. Supports cell proliferation and metastasis. Decreased overall patient survival. Resistance to treatment with lenvatinib.	Li *et al.*, 2020 Liao *et al.*, 2021b Chen *et al.*, 2022
Breast cancer	USP1-KPNA2 USP1-ERα USP1-TAZ	Correlates with a poor prognosis for patients, promotes metastatic progression of breast cancer cells. Supports breast cancer cell proliferation and invasion *via* estrogen signaling. Promotes the proliferation and migration of malignant cells in triple-negative breast cancer.	Ma *et al.*, 2019a Niu *et al.*, 2020 Mussell *et al.*, 2020
Prostate cancer	USP1-KDM4A-AR-c-Myc GRP75-USP1-SIX1	Promotes the proliferation and survival of prostate cancer cells. Supports the growth and proliferation of prostate cancer cells. Associated with a poor prognosis for patients. Promotes cell resistance to AR-targeted therapy.	Cui *et al.*, 2020 Liao *et al.*, 2021a, Liao *et al.*, 2022
Colorectal cancer	Upregulation of Bcl-2, Mcl1, A1, D1, E1 cyclins and DNA-repair related substrates FANCD2 and ID1	Associated with short overall patient survival. Promotes the growth and survival of colorectal cancer cells, resistance to radio- and chemotherapy.	Xu *et al.*, 2019
Ovarian cancer	ATM/ATR-USP1-Snail	Facilitates resistance to platinum treatment and promotes metastasis.	Sonego *et al.*, 2019

toration of sensitivity to antitumor therapy (Das *et al.*, 2015; Gong *et al.*, 2021; Rennie *et al.*, 2022).

USP1-mediated stabilization of inhibitors of DNA binding and cell differentiation (ID proteins family) has been reported in many cancers (glioma, osteosarcoma, B-cell acute lymphoblastic leukemia, multiple myeloma, esophageal squamous cell carcinoma, gastric cancer, etc.). The disrupted functional activity of ID proteins is associated with deregulation of cell proliferation, metastasis, and apoptosis impairment. Moreover, the inhibition of USP1 deubiquitination activity diminishes proliferation and colony-forming activity of cancer cells, decreases their metastatic ability and restores the sensitivity to anticancer drugs. The findings suggesting the putative role of USP1 targeting in future design of therapeutic modal-

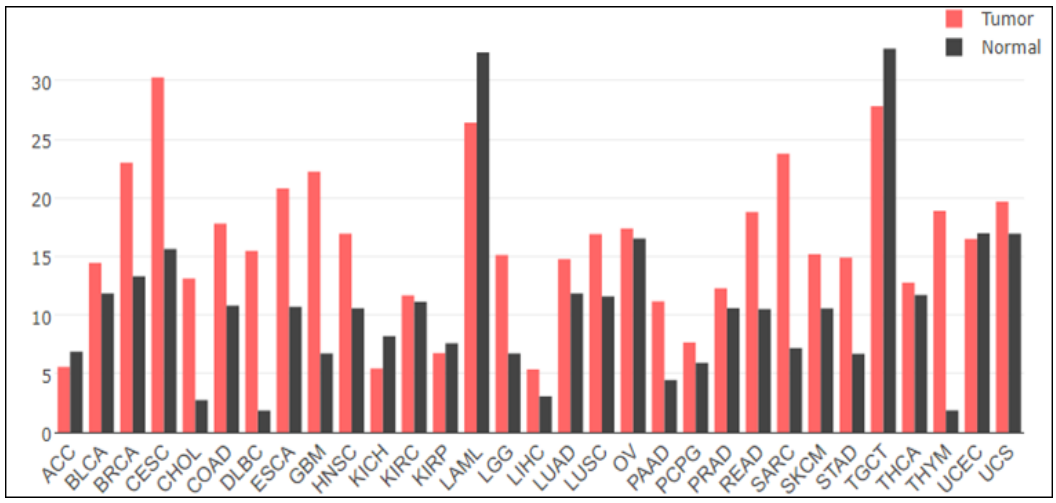


Figure 3. USP1 expression in normal samples (grey box) and tumor samples (red box) from patients with cancer compiled by GEPIA data from The Cancer Genome Atlas and the Genotype Tissue Expression databases.
ACC – adrenocortical carcinoma, BLCA – bladder urothelial carcinoma, BRCA – breast invasive carcinoma, CESC – cervical squamous cell carcinoma and endocervical adenocarcinoma, CHOL – cholangiocarcinoma, COAD – colon adenocarcinoma, DLBC – diffuse large B-cell lymphoma, ESCA – esophageal carcinoma, GBM – glioblastoma multiforme, HNSC – head and neck squamous cell carcinoma, KICH – kidney chromophobe carcinoma, KIRC – renal clear cell carcinoma, KIRP – renal papillary cell carcinoma, LAML – acute myeloid leukemia, LGG – low grade glioma, LIHC – hepatocellular carcinoma, LUAD – lung adenocarcinoma, LUSC – lung squamous cell carcinoma, MESO – mesothelioma, OV – ovarian serous cystadenocarcinoma, PAAD – pancreatic adenocarcinoma, PCPG – pheochromocytoma and paraganglioma, PRAD – prostate adenocarcinoma, READ – rectal adenocarcinoma, SARC – sarcoma, SKCM – cutaneous melanoma, STAD – stomach adenocarcinoma, TGCT – testicular germ cell tumors, THCA – thyroid carcinoma, THYM – thymoma, UCEC – uterine corpus endometrial carcinoma, UCS – uterine carcinosarcoma, UVM – uveal melanoma

ities for cancer treatment are considered below for various forms of malignancies.

USP1 in glioma

The functioning of more than 20 ubiquitin-specific proteases, including USP1, is associated with the progression of glioma. USP1 expression is upregulated in glioma, in particular in glioblastoma stem-initiating cells ($CD133^+$ or $CD15^+$) cells that are associated with clonogenic activity of malignant cells and radioresistance. The involvement of USP1 in glioma progression could be related to stabilization of ID1 and CHEK1 proteins, which promotes cell survival, while genetic or pharmacological inhibition of USP1 reduced tumor growth in experimental models and sensitized tumors to chemotherapeutic agents. The expression level of USP1 significantly increases upon inhibition of IRE-1α, the most evolutionarily conserved resident protein of the endoplasmic reticulum membrane. Thus, USP1 responds to endoplasmic reticulum stress and hypoxia, potentially contributing to the regulation of cell apoptosis and proliferation (Minchenko *et al.*, 2016). A critical factor in glioma progression and recurrence is the high infiltrative capacity of glioma stem cells caused by a decrease in the level of NgR1, negatively regulated through the USP1/ID1 signaling pathway. Pharmacological inhibition of USP1 with pimozide correlates with increased NgR1 expression and decreased infiltration capacity of glioma cells (Lee *et al.*, 2016; Liang *et al.*, 2022). USP1 expression in proneural glioblastoma cells is PDGF-dependent. PDGF upregulates the expression of E2F transcription factors that directly bind to and activate USP1, which in turn stabilizes the inhibitor of DNA binding 2 (ID2). Thus, activation of the PDGF–E2F–USP1–ID2 signaling pathway is crucial for glioma cell survival and may be considered as a valuable therapeutic strategy in proneural glioblastoma (Rahme *et al.*, 2016).

USP1 in osteosarcoma

IDs proteins are also involved in progression of osteosarcoma. In osteosarcoma cells, USP1 stabilizes IDs, particularly ID1, ID2, and ID3, through its deubiquitinating activity, and directly interacts with and deubiquitinates TAZ, leading to disruption of the Hippo signaling pathway. In fact, overexpression of USP1 was found in 26 out of 30 osteosarcoma samples as compared to normal bone tissues. Interestingly, USP1 stabilizes TAZ also in breast cancer, which promotes the proliferation and migration of malignant cells. Suppression of USP1 destabilizes ID proteins, disrupts osteoblast differentiation, negatively affects cell growth, colony formation, and metastasis (Williams *et al.*, 2011; Liu *et al.*, 2016; Mussell *et al.*, 2020; Yuan *et al.*, 2022).

USP1 in non-small cell lung carcinoma

In non-small cell lung carcinoma cells (NSCLC), USP1 forms a protein complex with the negative Akt regulator protein PHLPP1, deubiquitinates and stabilizes it. Knockdown of USP1 leads to rapid accumulation of ubiquitinated PHLPP1 and reduced its half-life, which is a trigger for Akt1 phosphorylation and disease progression. USP1 expression in non-small cell lung carcinoma cells is regulated by PXN and ITGB4. Current data on USP1 expression in lung cancer are somewhat controversial. Zhiqiang and others (Zhiqiang *et al.*, 2012) claim that the level of USP1 protein in non-small cell lung carcinoma is reduced, in particular in A549, H157, H2126, and H1770 cell lines, while its overexpression suppresses the growth of lung cancer cells and promotes cell death. Nevertheless, García-Santisteban and others (García-Santisteban *et al.*, 2012) demonstrated the higher USP1 mRNA levels in a panel of 20 NSCLC cell lines as well as in NSCLC tumor samples compared with normal tissue supporting the association of USP1 overexpression with NSCLC. At the same time, Chen and colleagues (Chen *et al.*, 2011) identified the USP1/UAF1

protein complex as a promising therapeutic target and talked about the feasibility of using the inhibitors, pimozide and GW7647, to reduce its activity. They found that USP1/UAF1 inhibitors sensitize cisplatin-resistant NSCLC cells to cisplatin. The therapeutic potential of using USP1/UAF1 protein complex inhibitors lies in their synergistic action with cisplatin reducing chemoresistance and inhibiting proliferation of NSCLC cells (Chen *et al.*, 2011; Mohanty *et al.*, 2020).

USP1 in B-cell acute lymphoblastic leukemia

In B-cell acute lymphoblastic leukemia (B-ALL), overexpression of USP1 promotes the progression of malignant disease through the ID1/AKT signaling pathway. Genetic and pharmacological inhibition of USP1 correlates with downregulation of ID1 and results in additional inactivation of the PI3K/AKT signaling pathway. In addition, inhibition of the deubiquitinating functions of USP1 by SJB3-019A induces G2/M cell cycle arrest in B-ALL cells (Kuang *et al.*, 2021).

USP1 in T-cell acute lymphoblastic leukemia

In T-cell acute lymphoblastic leukemia (T-ALL), high expression of USP1 is associated with the development of chemoresistance and a poor prognosis for patients. USP1 contributes to the chemoresistance of T-ALL cells by interacting with and deubiquitinating Aurora B, a key cell cycle regulator that ensures correct chromosome segregation and normal mitosis, its dysfunction is associated with tumorigenesis in many types of cancer, including solid tumors and hematological malignancies (Du *et al.*, 2021). USP1 overexpression in T-ALL cells is mediated by ALKBH5, a member of the well-conserved AlkB family of non-heme Fe(II)/α-KG-dependent dioxygenases, which mediates the repair of N-alkylated nucleobases by oxidative demethylation and is involved in the regulation of proliferation, migration, and apoptosis. ALKBH5 reduces m6A levels and stabilizes USP1 mRNA transcript, whereas ALKBH5 inhibition significantly reduces USP1 and Aurora B levels. m6A (N6-methyladenosine) is a reversible and most common type of mRNA modification characteristic of many biological processes including tumorigenesis (Huo *et al.*, 2021). Blocking USP1 restores sensitivity of T-ALL cells to dexamethasone in vitro and in vivo, by facilitating the expression of the glucocorticoid receptor, promotes cell apoptosis and suppresses cell invasion (Gong *et al.*, 2021).

USP1 in multiple myeloma

In multiple myeloma patients, a high level of USP1 expression is an unfavorable prognostic factor associated with poor survival. Blocking the deubiquitinating activity of USP1 by SJB3-019A (a synthetic inhibitor that targets USP1 in an irreversible manner with high selectivity for other deubiquitinases) causes a series of important functional events, reducing the viability of myeloma cells, inhibiting their growth, triggering apoptosis through the activation of caspase-3, caspase-8, and caspase-9, and overcoming resistance to bortezomib, a first-in-class selective and reversible 26S proteasome inhibitor with antiproliferative and antitumor activity (Das *et al.*, 2017; Robak *et al.*, 2019).

USP1 in chronic myeloid leukemia

In chronic myeloid leukemia (CML) cells, USP1 interacts with the Bcr-Abl oncoprotein via its PH domain with the formation of the Bcr-Abl/USP1 nuclear complex. Bcr-Abl has constitutive tyrosine kinase activity and thus uncontrollably phosphorylates its protein partners, suggesting that oncoprotein interactions may deregulate USP1 activity by overactivating the enzyme. It was established that pharmacological inhibition of the USP1/UAF1 complex by ML323 causes a decrease in the Bcr-Abl level in CML cells. It is believed that USP1 deubiquitinates Bcr-Abl, preventing its proteolysis, and leading to the Bcr-Abl accumulation (Antonenko *et al.*, 2016; Antonenko & Telegeev, 2020).

USP1 in esophageal squamous cell carcinoma

In esophageal squamous cell carcinoma cells, USP1 activity is associated with high levels of c-Myc, cyclin D1, CDK4, and CDK6 proteins, while pharmacological inhibition of USP1 by ML323 significantly affects the viability of malignant cells by blocking cell cycle traverse in G0/G1, interfering with the ability to form colonies and inducing apoptosis by p53-Noxa. Inhibition of the deubiquitinating activity of USP1 causes the accumulation of DNA damage and promotes the development of protective autophagy (Sun *et al.*, 2022).

USP1 in stomach cancer

In gastric cancer cells, USP1 stabilizes ID2 expression by its deubiquitination. Overexpression of USP1 promotes metastasis and correlates with low survival rates, while knockdown of USP1 suppresses proliferation, migration, invasion, and epithelial-mesenchymal transition of gastric cancer cells (Li *et al.*, 2021; Meng *et al.*, 2022).

USP1 in pancreatic ductal adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC) is the most common neoplastic disease of the pancreas, for the development of which BCAT2-mediated branched-chain amino acid (BCAA) catabolism is critical. A key role of USP1 in PDAC development is its ability to regulate BCAT2 levels in cells through its deubiquitinating activity. In turn, the level of USP1 increases under the influence of BCAA through the GCN2-eIF2α signaling pathway. Inhibition of the deubiquitinating activity of USP1 reduces cell proliferation and pancreatic tumor growth in an orthotopic transplant in mice, suggesting USP1-BCAT2-BCAA signaling pathway as a potential target for PDAC therapy (Li *et al.*, 2022b).

USP1 in hepatocellular carcinoma

USP1 overexpression together with the cofactor UAF1 is a poor prognostic factor for survival of hepatocellular carcinoma (HCC) patients. A high level of USP1 promotes metastasis and survival of circulating tumor cells (CTCs) due to deubiquitination and stabilization of transducin β-like 1 X-linked receptor 1 (TBLR1), which is a critical regulator of Wnt (Li *et al.*, 2020). USP1 interacts with 37 ribosomal proteins (RPs), including RPS4X, RPS18, and RPS16, among which the USP1/RPS16 protein complex plays a key role in HCC progression. The binding of USP1 to RPS16 occurs at its C-terminal domain (401–785 aa). USP1 prevents proteasomal degradation of RPS16 by deubiquitination at the K48 site. The USP1-RPS16-Twist1/Snail signaling pathway is believed to be involved in cell proliferation and metastasis in HCC. In addition, USP1 stabilizes c-kit tyrosine kinase, which is upregulated or overexpressed in HCC. Inhibition of USP1 activity increases the sensitivity of HCC cells to lenvatinib treatment by regulating the ex-

pression of c-Kit, and also causes a decrease in the expression of PCNA, cyclin D1, cyclin E1, RPS16, Nanog, Sox2, c-Myc and is accompanied by decreased cancer stemness, including sphere formation ability. Lenvatinib is a multi-targeted tyrosine kinase inhibitor with angiogenic, antitumor, and immunomodulatory effects targeting VEGF receptors (VEGFR) and fibroblast growth factor (FGF) receptors (FGFR), platelet-derived growth factor receptor alpha (PDGFRα), KIT, and RET (Zhong *et al.*, 2021). Restoration of cell sensitivity to doxorubicin after USP1 inhibition is associated with ubiquitination of PCNA, which promotes its proteolysis. PCNA is an important factor in DNA replication and repair, chromatin remodeling, cell cycle regulation, and can be used as a marker of tumor aggressiveness (Zhao *et al.*, 2020; Liao *et al.*, 2021b; Chen *et al.*, 2022; Lu *et al.*, 2022).

USP1 in breast cancer

Elevated expression of USP1 in human breast cancer (BC) cells compared with normal breast tissue correlates with poor patient prognosis, promotes metastasis and disease progression through increased expression of a number of pro-metastatic genes, and stabilization of KPNA2 by its deubiquitination. KPNA2 is an important member of the karyopherin family, which plays a central role in nucleocytoplasmic transport and is overexpressed in malignant neoplasms. In addition, due to its deubiquitinating activity, USP1 regulates ERα and thus affects cell proliferation and invasion *via* estrogen signaling. In triple-negative BC, which is the most aggressive BC form, USP1 interacts with and deubiquitinates TAZ (WWTR1), which promotes the proliferation and migration of malignant cells. Inhibition of the USP1 functional activity causes a decrease in the level and inhibition of the activity of KPNA2, ERα, TAZ proteins, disrupts cell proliferation and migration, suppressing BC metastases and preventing the progression of the disease (Ma *et al.*, 2019a; Mussell *et al.*, 2020; Niu *et al.*, 2020).

USP1 in prostate cancer

Proliferation and survival of prostate cancer cells is regulated by the USP1/KDM4A/AR-c-Myc signaling pathway. A critical event in its activation is the increased expression of USP1, which regulates the stability of KDM4A through K48-linked deubiquitination. Inhibition of USP1 activity significantly reduces cell proliferation and enhances the response of cells to enzalutamide, a second-generation androgen receptor (AR) antagonist. However, the USP1/KDM4A/AR-c-Myc signaling pathway is not the only one involving USP1 in prostate cancer cells. USP1, through deubiquitination, stabilizes the embryonic development transcription factor SIX1 in USP1/SIX1 complex with GRP75 chaperone. High expression of SIX1is associated with a poor prognosis for prostate cancer patients. Blocking the activity of the GRP75-USP1-SIX1 protein complex using SNS-032 inhibits the growth and proliferation of prostate cancer cells, and also restores the sensitivity of cells to AR-targeted therapy (Cui *et al.*, 2020; Liao *et al.*, 2021a; Liao *et al.*, 2022)

USP1 in colorectal cancer

A high level of USP1 expression is associated with the proliferation and survival of colorectal cancer cells and promotes resistance to radio- and chemotherapy. The properties of USP1 are realized due to its effect on the expression of anti-apoptotic proteins Bcl-2, Mcl1, cyclins A1, D1, E1 and DNA-repair related substrates FANCD2 and ID1. Knockdown of USP1 causes cell arrest in the G2/M phase and induces colorectal cancer cell death. Pharmacological inhibition of USP1 using ML323 significantly increases the sensitivity of colorectal cancer cells to DNA-targeting chemotherapy drugs, enhancing in particular, the cytotoxic effect of doxorubicin, PARP inhibitor (Olaparib), topoisomerase I and II inhibitors, and DNA-binding agent etoposide (Xu *et al.*, 2019).

USP1 in ovarian cancer

Inhibition of USP1 opens new opportunities to overcome platinum resistance in ovarian cancer. A critical event in ovarian cancer initiation is the phosphorylation of USP1 by ATM and ATR, which triggers the interaction and deubiquitination of Snail. Stabilization of Snail by USP1 correlates with the development of platinum resistance and metastasis, while knockout or pharmacological inhibition of USP1 restores platinum sensitivity (Sonego *et al.*, 2019).

USP1 INHIBITORS

The first identified inhibitors of the USP1/UAF1 protein complex were pimozide and GW7647. Pimozide belongs to the approved antipsychotic drugs, but its pharmacological properties are not limited to the neuroleptic function and it demonstrates an antitumor effect in various types of cancer, including glioma, osteosarcoma, melanoma, myeloproliferative neoplasms, lung cancer, hepatocellular carcinoma, pancreatic cancer, breast cancer, colorectal cancer, prostate cancer, ovarian cancer, etc. (Dakir *et al.*, 2018; Kim *et al.*, 2020; Cui *et al.*, 2020; Ranjan *et al.*, 2020; Vlachos *et al.*, 2021; Li *et al.*, 2022a). The ability to suppress the USP1/UAF1 protein complex is one of the key mechanisms of the effect on malignant cells. Pimozide and GW7647 belong to reversible inhibitors and are characterized by moderate PubChem promiscuity with hit rates of 9.5% and 11.4%, respectively. Both inhibitors bind outside the active site of USP1, which is thought to disrupt the protein-protein interaction between USP1 and the cofactor UAF1. Despite good cellular inhibition of the USP1/UAF1 protein complex, pimozide and GW7647 are able to show activity against unrelated targets. (USP7 for pimozide and USP2, USP7, USP12/USP46 for GW7647) (Table 2). Laboratory studies, which included kidney and liver function tests, showed non-toxicity of intraperitoneal GW6471 at a dose of 20 mg/kg for mice (Chen *et al.*, 2011; Dexheimer *et al.*, 2014; Gonçalves *et al.*, 2019; LaPlante *et al.*, 2021; Morishita *et al.*, 2022; Orozco *et al.*, 2022; Ler *et al.*, 2022). Another known USP1 inhibitor is the compound SJB2-043, which causes downregulation of ID2 and ID3 proteins, growth inhibition and apoptosis, and blocks pancreatic β-cell apoptosis by inhibiting the DNA damage response (Mistry *et al.*, 2013; Gorrepati *et al.*, 2018; Chen *et al.*, 2021). Inhibitor C527 promotes dose-dependent degradation of ID1 in osteosarcoma cells. The mechanisms of action of inhibitors SJB2-043 and C527 have currently been studied *in vitro*. Compounds flupenthixol and trifluoperazine belong to the group of antipsychotic drugs, which are widely used in medical practice for the treatment of mental disorders, in particular schizophrenia, neuroses with anxiety and fear phenomena. Flupenthixol and trifluoperazine have sufficient selectivity for human DUB, however, the mechanisms of their inhibitory effect on USP1 are currently insufficiently studied. Rottlerin is an irreversible inhibitor of USP1/UAF1 with poor selectivity for other DUBs,

Table 2. Selectivity of inhibitors to different USPs.

Compound	USPs						References
	USP1/UAF1	USP2	USP5	USP7	USP8	USP46/ UAF1	
Pimozide	+	–	–	+	–	–	Chen *et al.*, 2011; Dexheimer *et al.*, 2014
GW7647	+	+	–	+	–	+	Dexheimer *et al.*, 2014; Gonçalves *et al.*, 2019
SJB2-043	+	not tested	not tested	not tested	not tested	not tested	Mistry *et al.*, 2013
C527	+	–	+	–	–	+	Mistry *et al.*, 2013; Rennie *et al.*, 2022
Flupenthixol	+	–	–	+	+	–	Chen *et al.*, 2011
Trifluoperazine	+	–	–	+	–	–	Chen *et al.*, 2011
Rottlerin	+	+	+	+	+	+	Chen *et al.*, 2011
ML323	+	–	–	–	–	–	Dexheimer *et al.*, 2014; Rennie *et al.*, 2022
KSQ-4279	+	not tested	not tested	not tested	not tested	not tested	Shenker *et al.*, 2021

in particular it is able to bind to USP2, USP7, USP8, USP46, etc. Rottlerin is well tolerated when administered orally or intraperitoneally to mice and does not cause side effects (Chen *et al.*, 2011; Lee *et al.*, 2016; Ma *et al.*, 2018; Xia *et al.*, 2019; Chen *et al.*, 2021). ML323, the potent nanomolecular inhibitor of USP1/UAF1 complex, has excellent selective ability. The mechanism of ML323 inhibitory effect consists in replacing part of the hydrophobic core of USP1, conformational changes of the secondary structure lead to rearrangements in the active center and inhibition of USP1 activity. ML323 belongs to reversible inhibitors, being 5-10-fold more active than C527 (Dexheimer *et al.*, 2014; Rennie *et al.*, 2022). ML323 has shown good antitumor activity in various cancers, including esophageal squamous cell carcinoma, pancreatic ductal adenocarcinoma, chronic myeloid leukemia, breast cancer, colorectal cancer, etc. ML323 reduces DNA repair, increases the sensitivity of cells to cisplatin in osteosarcoma and non-small cell lung cancer. It is promising to use ML323 in combination with compounds that damage DNA, in particular cisplatin, to enhance the cytotoxicity of anticancer drugs. ML323 has low cytotoxicity *in vitro*. In mice ML323 inhibited osteosarcoma progression in the absence of significant cytotoxicity. (Dexheimer *et al.*, 2014; Yu *et al.*, 2017; Song *et al.*, 2022). In 2021, there were reports of a new compound, KSQ-4279, capable of highly selectively inhibiting the activity of USP1 and leading to the accumulation of its monoubiquitinated substrate proteins. Under in vitro conditions, KSQ-4279 performed well in ovarian and triple-negative breast cancer xenograft models, causing dose-dependent inhibition of tumor growth and tumor regression in models insensitive to PARP inhibitors (Shenker *et al.*, 2021). To date, in the available literature there are no reports on the study of KSQ-4279 *in vivo*.

CONCLUSIONS

In malignant cells, there are mechanisms to suppress the degradation of oncoproteins, in which DUBs play a key role. The imbalance between ubiquitination and deubiquitination is a critical event contributing to the accumulation of oncoproteins in the cell and preventing their degradation. Plenty of current research focus on the identification of DUBs associated with different aspects of oncogenesis. Study of their cellular localization, interactions with target proteins and substrate specificity is of importance for developing selective inhibitors and implementing a new cancer treatment strategy. USP1 as an important member of the DUB family with a wide range of cellular substrates is involved in ensuring genetic stability and cell homeostasis. Deregulation of its expression and deubiquitinating activity is revealed in various malignant neoplasms. Recent studies suggest a non-genomic mechanism of protein stabilization mediated by USP1 activity allowing for controlling expression of target genes that play a key role in cancer development and progression. USP1 overexpression is a hallmark of a number of cancers including glioma, osteosarcoma, B-cell and T-cell acute lymphoblastic leukemia, hepatocellular carcinoma, gastric, breast, ovarian, prostate, and colorectal cancers, which correlates with poor survival and unfavorable prognosis. Further studies will help understand the relationship between USP1 expression profiles and cancer, opening up new possibilities for using USP1 as a tumor marker for early detection, staging, and individualized therapy of malignant neoplasms. Inhibition of USP1 in vitro sensitizes cancer cells to radiation and increases their sensitivity to various chemotherapeutic agents, so targeting the USP1/UAF1 complex may be of great benefit in overcoming resistance and widening the use of combination therapy for cancer. The key function of USP1 in the regulation of the substrate ubiquitination allows modulating the level of specific proteins including those contributing to the development of malignant neoplasms.

Despite tremendous progress achieved in the past decade in studying the roles of DUBs in cellular events resulting in malignant transformation, many important questions have not yet been clarified. The exact roles of

different DUBs vary in different forms of cancer and the specific substrates that are ubiquitinated. Moreover, their roles in different cells and tissues of varying histogenesis or different metabolic conditions may not be the same. One should also be aware that DUBs interact with an array of enzymes that sometimes act oppositely defining the fate of cancer cells. The increasing specificity of the substances disrupting or enhancing specific interactions of DUBs with their substrates could be promising in search of more efficient therapeutic strategies.

Although we have a long way to go before the translation of the experimental research into the clinical practice, undoubtedly, DUBs represent putative therapeutic targets for the development of a new strategy for cancer treatment by modulating the levels and the activities of oncoproteins in cancer cell.

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