Targeting the deubiquitinase *USP2* for malignant tumor therapy (Review)

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Abstract. The ubiquitin-proteasome system is a major degradation pathway for >80% of proteins *in vivo*. Deubiquitylases, which remove ubiquitinated tags to stabilize substrate proteins, are important components involved in regulating the degradation of ubiquitinated proteins. In addition, they serve multiple roles in tumor development by participating in physiological processes such as protein metabolism, cell cycle regulation, DNA damage repair and gene transcription. The present review systematically summarized the role of ubiquitin-specific protease 2 (USP2) in malignant tumors and the specific molecular mechanisms underlying the involvement of *USP2* in tumor-associated pathways. *USP2* reverses ubiquitin-mediated degradation of proteins and is involved in aberrant proliferation, migration, invasion, apoptosis and drug resistance of tumors. Additionally, the present review summarized studies reporting on the use of *USP2* as a therapeutic target for malignancies such as breast, liver, ovarian, colorectal, bladder and prostate cancers and glioblastoma and highlights the current status of pharmacological research on *USP2*. The clinical significance of *USP2* as a therapeutic target for malignant tumors warrants further investigation.

Contents

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1. Introduction

Proteins, the material basis of life, are essential components of all cells, tissues and organs in the body. Intracellular proteins are predominantly degraded through the lysosomal pathway, cysteine-containing aspartate protease pathway and ubiquitin-proteasome pathway (1-4). The ubiquitin-proteasome system is the primary protein degradation pathway *in vivo*. More than 80% of proteins in the body are degraded through this pathway, which is involved in various metabolic processes in the body (4). The ubiquitin-proteasome pathway can degrade the cell cycle protein cyclin (5,6), spindle-related proteins (7), cell surface receptors such as epidermal growth factor (8), transcription factors (9), the tumor inhibitory factor p53 and oncogenic products (10). In addition, abnormal intracellular proteins are degraded by the ubiquitin-proteasome pathway under stress conditions. Ubiquitin, ubiquitin-activating (E1) enzymes, ubiquitin-binding (E2) enzymes, ubiquitin ligases (E3), protein hydrolases and deubiquitinating enzymes are the main components of the ubiquitin-proteasome pathway (9,11). In the presence of ATP, the glycine (Gly) residue at the C-terminus of ubiquitin forms a high-energy lipid bond with the sulfur group (SH) of the cysteine residue of an E1 enzyme, and the activated ubiquitin is subsequently transferred to an E2 enzyme. In the presence of an E3 ubiquitin ligase, ubiquitin is transferred from the E2 enzyme to the substrate protein, forming an isopeptide bond with the ε-NH2 group of the Lys residue of the substrate protein. Subsequently, the C-terminus of the next ubiquitin molecule is connected to the Lys48 residue of the previous ubiquitin molecule, thus completing polyubiquitination. The ubiquitinated substrate proteins are recognized by cap-shaped regulatory particles of the 19S proteasome and transported into the cylindrical core of 20S, where they are hydrolyzed into oligopeptides and amino acids by various enzymes and are eventually released from the proteasome, thereby completing ubiquitin-mediated degradation (12-14). Ubiquitin molecules involved in ubiquitination are dissociated from substrate proteins and can be reused in the cytoplasm (Fig. 1). E3 ubiquitin ligases, which

serve a key role in ubiquitin-mediated degradation of proteins, specifically mark the substrate proteins and attach ubiquitin to them for degradation (9,15). Deubiquitination is an important mechanism for maintaining intracellular protein stability and is closely associated with the development of cancer. Deubiquitinating enzymes (DUBs) hydrolyze the isopeptide bonds in ubiquitinated substrate proteins, thereby dissociating the ubiquitinated molecules from the substrate proteins and inhibiting ubiquitin-mediated protein degradation. A flowchart demonstrating the mechanism of the ubiquitin-proteasome pathway is shown in Fig. 1 (16-18). DUBs are a large family of proteasomes. It is known that ~100 DUBs are encoded by the human genome, which can be classified as ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs), ovarian tumor-related proteases (OTUs), Machado-Joseph disease (MJD) deubiquitinases and metalloproteases (19-22). Except for the metalloproteinase family, all other deubiquitinases are cysteine proteases; of which, USPs are the most structurally diverse class of deubiquitinases with the largest known membership. USPs inhibit protein degradation by removing ubiquitin from substrate proteins through interaction with a catalytic triplet of residues (cysteine, histidine and aspartate) (23). USPs are involved in regulation of apoptosis (23), protein transport (24), regulation of the cell cycle (25), DNA damage repair (26), chromatin remodeling and protein signaling (27,28). In addition to inhibiting the degradation of ubiquitinated substrate proteins, USPs can regulate related signaling pathways by affecting protein activity. For example, in the TGF-β signaling pathway, USP15 and CYLD lysine 63 deubiquitinase (CYLD) affect the stability of the mothers against decapentaplegic (SMAD) protein in *Drosophila* by antagonizing SMAD protein-specific E3 ligase 2, which in turn negatively regulates the activation of the TGF-β pathway (29). USP is involved in the regulation of multiple cancer-related pathways, including *p53*, Wnt/β-catenin, TGF-β and protein kinase B (Akt) pathways. For example, the overexpression of USP2a stabilizes murine double minute 2 (MDM2) through direct deubiquitination, thereby enhancing the degradation of the tumor suppressor protein p53. The downregulation of p53 eventually leads to tumor progression (30). Overexpression of USP10 directly deubiquitinates and stabilizes Krüppel-like factor 4 protein, which directly binds to the promoter region of tissue inhibitor of metalloproteinase-3 (a tumor suppressor gene) to promote its transcription and exert positive anti-tumor effects (31).

USP2, the second member of the *USP* family, was discovered in chicken muscle in 1997 and was originally defined as UBP41 (32). It is localized on the long arm of human chromosome 11 (11q23.3) and is highly conserved in eukaryotes. According to the Uniprot Protein Data Bank (Accession number: 075604), the mRNA precursor of USP2 can generate four mRNA isoforms, namely, USP2-1, USP2-2, USP2-3 and USP2-4, owing to different splicing patterns. USP2-1 (USP2a/USP2-69) contains 605 amino acids and has a molecular weight of 68,072 Da. USP2-2 (USP2b) contains 353 amino acids and has a molecular weight of 40,638 Da, with 1-252 amino acid residues missing and 253-258 amino acid residues mutated from PGRDGM to MLNKAK (when compared with USP2a). USP2-3 (USP2c) contains 362 amino acids and has a molecular weight of 41,682 Da. USP2-4 contains 396 amino acids and has a molecular weight of 45,241 Da (www.uniprot.org/UniProtKB/O75604/entry). The isomers of USP2 have similar structures, both consisting of an N-terminal structural domain of variable length and a C-terminal structural domain with 347 amino acids, where the C-terminus has the characteristic catalytic triad of the USP family (Cys, His, and Asp/Asn) (33). Specifically, USP2a has the largest N-terminal domain with 258 amino acids, while that of USP2b, USP2c and USP2-4 has 6, 15 and 49 amino acids, respectively (34-37). The basic structure of USP2 is summarized in Fig. 2. Factors involved in isomeric splicing of *USP2* under physiological conditions mainly include changes in the circadian rhythm, nutritional status and androgen levels (38). The circadian rhythm and nutritional status primarily affect the changes in *USP2b*. Pouly *et al* (39) reported that *USP2b* expression continuously increases in mouse kidney tissues during the light phase, reaching the highest value at 12 noon, and then gradually decreases from noon to midnight. Starvation leads to an increase in the mRNA expression of *USP2b* in mice but does not affect the expression of *USP2a* (40). Although androgens can promote alternative splicing of the *USP2a* gene, they do not affect *USP2b* and other isomers (41). In addition, multiple cytokines and signaling pathways are involved in *USP2* splicing. For example, activation of protein kinase C (PKC) signaling can promote alternative splicing of *USP2b* and inhibit alternative splicing of *USP2a* in macrophages (42). The cytokine interleukin-1β (IL-1β) can promote alternative splicing of *USP2a* in mesenchymal stem cells (MSCs) (43). TNF-α can downregulate the mRNA and protein expression of *USP2c* in the liver while promoting the alternative splicing of *USP2a* (44). TGF-β1 and platelet-derived growth factor-BB (PDGF-BB) can both promote alternative splicing of *USP2a*; however, the effects of PDGF-BB are most pronounced (45,46). All isomers have the same structure at the C-terminus, including catalytically active Cys and His residues, whereas the N-terminus can interact with different proteins and perform different physiological functions (33,47). For example, when the N-terminus of USP2 binds to receptor interacting protein-1 (RIP1) protein, it removes the ubiquitinated molecules on RIP1 protein and increases RIP1 expression, which in turn promotes apoptosis (48). The N-terminus binds to and stabilizes the cyclin D1 (CCND1) protein to promote cell cycle progression from the G_1 to the S phase (49). The present review focused on the role and function of *USP2* in cancer-related signaling pathways and its potential application value in cancer therapy.

2. Role of USP2 in the biological behavior of malignant tumors

Initially, *USP2* was thought to be expressed in only human testicular tissue (50); however, with the continuous advancement of detection techniques, *USP2* expression has been observed in various cells, including macrophages, and tissues and organs, including the heart, liver, kidney, breast, brain and skeletal muscle (47,51-56). Previous studies have demonstrated that USP2 promotes tumor cell proliferation by stabilizing proteins such as CCND1 and CCNA1. In addition, USP2 promotes epithelial-mesenchymal transition (EMT) and affects the sensitivity of tumor cells to chemotherapeutic drugs by stabilizing β-catenin protein through deubiquitination (55,57).

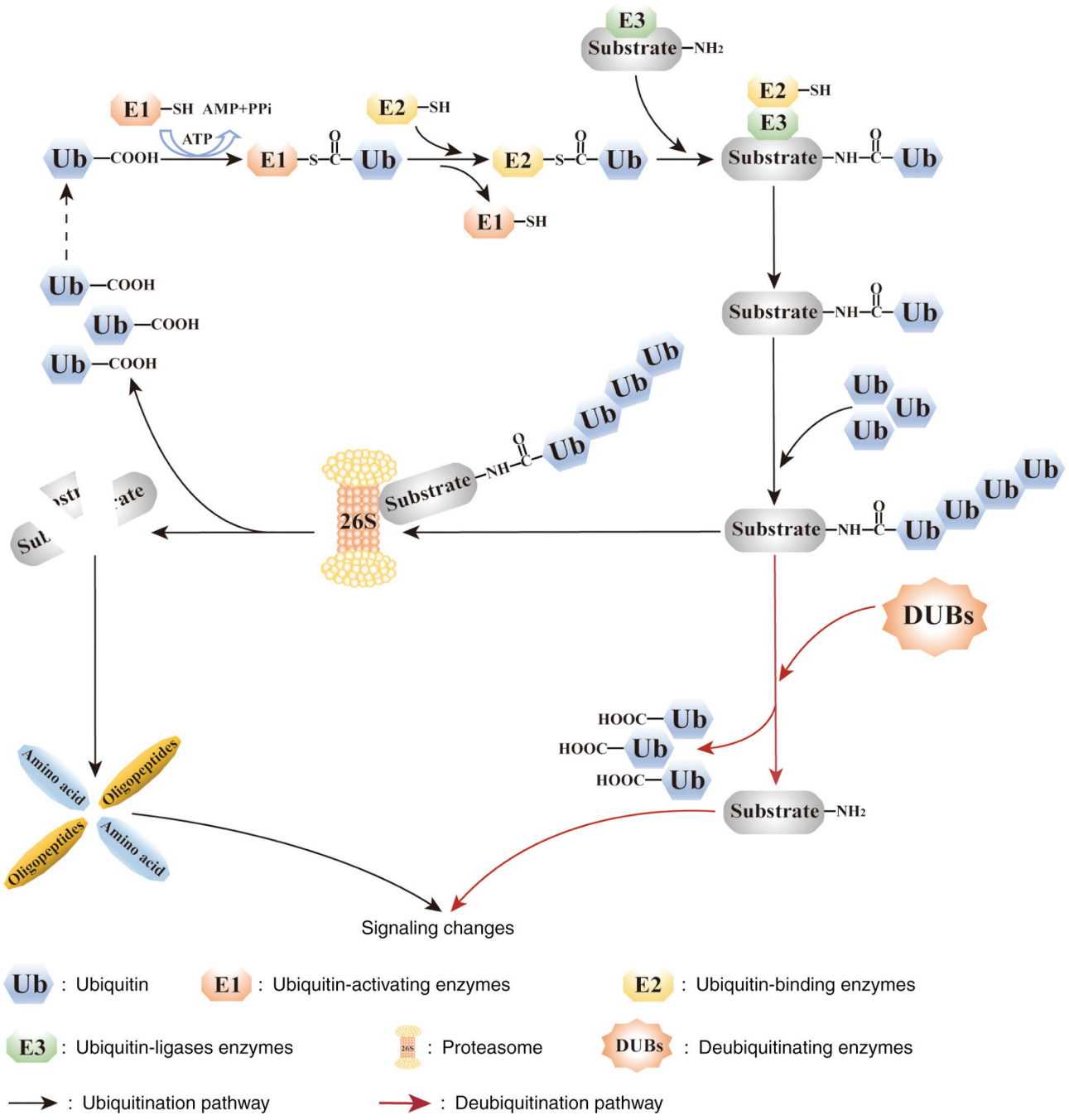

Figure 1. Ubiquitin-proteasome system. In the presence of ATP, the glycine residue at the C-terminus of ubiquitin forms a high-energy lipid bond with the SH of the cysteine residue of an E1 enzyme, and the activated ubiquitin is subsequently transferred to an E2 enzyme. In the presence of an E3 ubiquitin ligase, ubiquitin is transferred from the E2 enzyme to the substrate protein, forming an isopeptide bond with the ε-NH2 group of the Lys residue of the substrate protein. Subsequently, the C-terminus of the next ubiquitin molecule is connected to the Lys48 residue of the previous ubiquitin molecule, thus completing polyubiquitination. The ubiquitinated substrate proteins are recognized by the cap-shaped regulatory particles of the 19S proteasome and transported into the cylindrical core of 20S, where they are hydrolyzed into oligopeptides and amino acids by various enzymes and are eventually released from the proteasome, thereby completing degradation. However, deubiquitinating enzymes can reverse ubiquitination by hydrolyzing the isopeptide bonds in ubiquitinated substrate proteins and dissociating ubiquitin molecules from the substrate proteins. SH, sulfur group.

This section discusses the specific molecular mechanisms through which *USP2* participates in biological processes and highlights the significance of *USP2* as a therapeutic target for tumors.

USP2 enhances cell cycle and mitosis and promotes abnormal proliferation of tumor cells. Normal cell division, proliferation, differentiation and ageing maintain the self-stability of the body. Cell cycle disturbances can lead to abnormal cell proliferation, which is a common feature of tumor cells (57). The role of *USP2* in cell cycle regulation has been well demonstrated (59,60). CCND1 is abnormally overexpressed in various tumor cells. Shan *et al* (61) screened 76 DUBs *in vitro* to assess their catalytic ability to target CCND1. They identified USP2 as a specific DUB of CCND1, which can directly interact with CCND1, reduce the polymeric ubiquitination-dependent

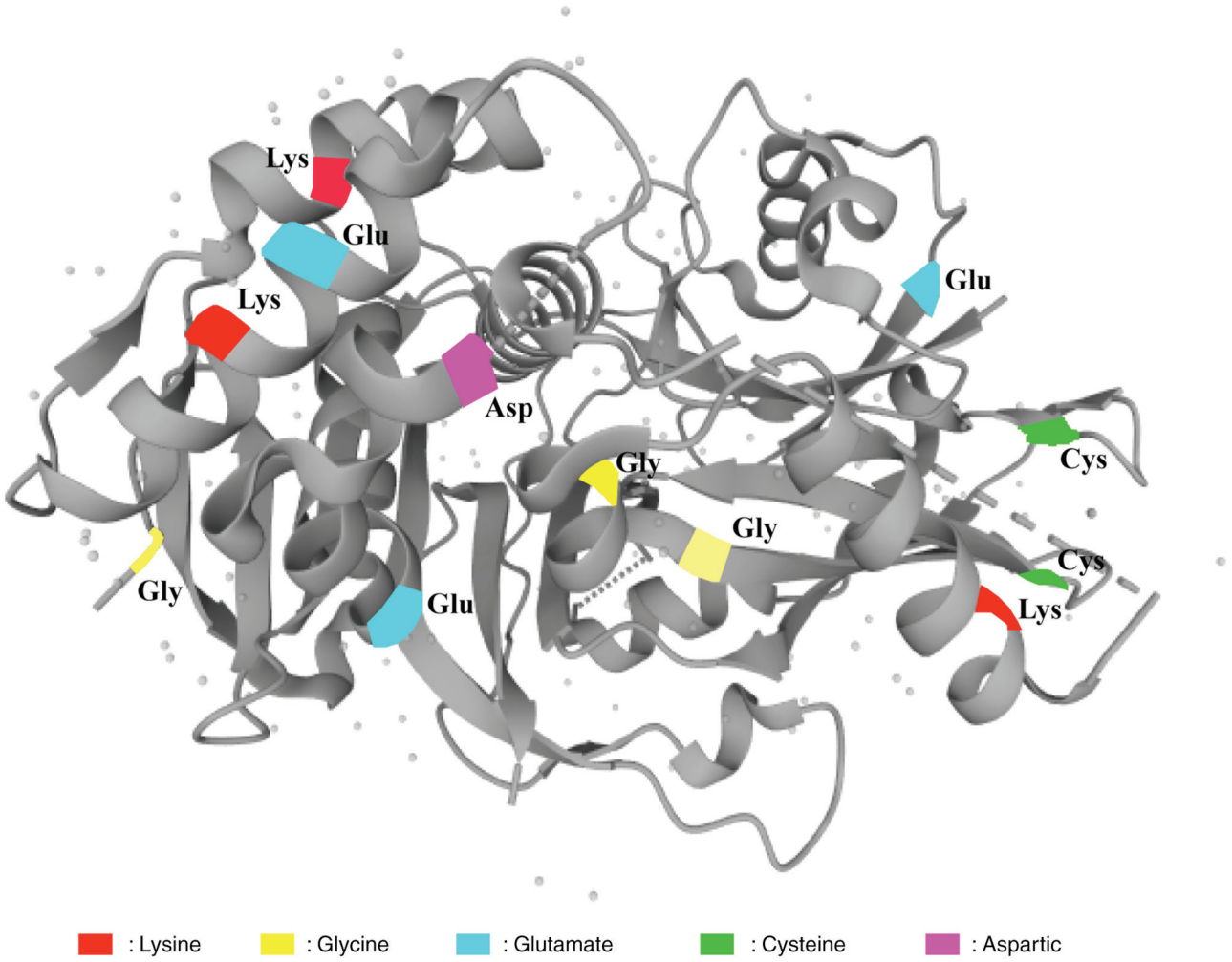

Figure 2. Structure of the deubiquitinating enzyme USP2. USP2, ubiquitin-specific protease 2.

degradation of CCND1 and promote tumor cell proliferation. In the human embryonic kidney cell line 293T, USP2a has been shown to deubiquitinate CCND1, thereby facilitating cell cycle progression from the G_1 to the S phase (38). A study demonstrated that the protein expression of CCND1 is significantly higher in human breast cancer MCF-7 cells and prostate cancer PC3 cells than in normal cells (38). *USP2* knockdown attenuates CCND1 deubiquitination and stability, promotes ubiquitin-mediated degradation, reduces CCND1 expression, inhibits cell progression from the G_1 to the S phase and suppresses cell proliferation (38). In addition, *USP2a* is a downstream target of lithocholic acid (LCA) hydroxyamide (LCAHA), and LCAHA can destabilize CCND1 by inhibiting the expression of the deubiquitinating enzyme USP2a (62). It induces G_0/G_1 phase arrest in colon cancer cells (HCT116), thus exerting an active anti-tumor effect (62). Leptin and adiponectin are two hormones secreted by adipose tissue that have contradictory effects on USP2 expression in tumor cells. Leptin targets *USP2* to upregulate the protein expression of CCND1 to promote cell cycle progression and tumorigenesis(63). Adiponectin targets *USP2* to promote ubiquitin-mediated degradation of CCND1 protein, resulting in cell cycle arrest and inhibition of tumor progression (63). As the intracellular overexpression of CCND1 is a decisive factor in the development of some tumors, CCND1 has been used to assess the potential of USP2 inhibitors as important indicators of the efficacy of antineoplastic drugs (49,64). *USP2* can directly recognize and deubiquitinate CCND1, thereby preventing its degradation, stabilizing its expression and promoting tumor development. Similarly, USP2a can inhibit CCNA1 protein degradation through deubiquitination, stabilize CCNA1 protein expression and promote the progression of bladder cancer T24 cells from G_1 to S phase, which in turn promotes bladder cancer T24 cell proliferation (65).

The oncoprotein *c-Myc* serves a key role in the development, progression and maintenance of cancer, particularly influencing the proliferation of tumor cells (66,67). Inhibition of *c-Myc* expression promotes the senescence of different types of tumor cells, whereas its overexpression inhibits the senescence of melanoma cells and exerts pro-tumor effects (68,69). A recent study demonstrated that *c-Myc* upregulates *USP2-AS1* expression by promoting the transcription of lncRNA USP2-AS1. USP2-AS1 stabilizes E2F1 mRNA and increases E2F1 expression by interacting with the RNA-binding protein G3BP1, which in turn attenuates the senescence of HCT116 and A549 cells and serves a pro-cancer function of *c-Myc* (70).

Aurora-A, a serine/threonine protein kinase, is a mitotic regulator essential for the replication, maturation and

segregation of centrosomes and the subsequent spindle assembly. Overexpression of Aurora-A inhibits Hec1 phosphorylation at serine 55 (Hec1-S55) during metaphase and destabilizes the kinetochore-microtubule attachment, which in turn induces tumor development (71,72). Shi *et al* (73) reported that *USP2a* reverses ubiquitin-mediated degradation of Aurora-A and promotes mitosis in pancreatic cancer MIA PaCa-2 cells. They used small interfering RNAs (siRNAs) to knock down *USP2a* in MIA PaCa-2 cells, which enhanced ubiquitin-mediated degradation of Aurora-A and significantly inhibited the proliferation of tumor cells. Therefore, *USP2a* may promote tumor cell proliferation by stabilizing the Aurora-A protein, and targeting *USP2a* may represent an effective strategy for inhibiting the abnormal proliferation of tumor cells.

USP2 promotes EMT and enhances the migratory and invasive capabilities of tumor cells. EMT is an important biological process in which epithelial cells acquire the ability to migrate and invade (74). TGF-β signaling can induce the transcription of related genes, promote EMT and enhance the migratory and invasive capabilities of tumor cells (75,76). TGF-β binds to two types of transmembrane serine/threonine kinase receptor heterologous complexes to initiate cellular responses (77). Receptor kinases activate the intracellular signaling protein SMAD to form heterologous protein complexes that are transferred to the nucleus, where they regulate the transcription of EMT-related genes, such as Snail, Slug (zinc-finger proteins), Twist, N-cadherin and E-cadherin (78). USP2a promotes the migratory and invasive capabilities of non-small cell lung cancer A549 cells by removing the K33-linked polyubiquitin chain from the TGF-β receptor, thereby promoting the binding of receptor-regulated SMAD (R-SMAD) to the TGF-β receptor and upregulating the expression of Snail (79). A study demonstrated that knockdown of *USP2a* or treatment with the USP2a-specific inhibitor ML364 (10 μM) effectively inhibits the migratory and invasive capabilities of tumor cells. In addition, ML364 significantly prolongs the survival of nude mice injected with hepatocellular carcinoma (HCC) Hep3B cells (via the tail vein) and attenuated lung metastasis (79). Therefore, *USP2a* may serve as a potential therapeutic target for cancer.

Wnt/β-catenin signaling serves a key role in EMT in tumor cells (80-82). Wnt is a secreted glycoprotein that interacts with specific receptors on the cell surface through autocrine and paracrine mechanisms and induces the accumulation of β-catenin through phosphorylation and dephosphorylation of downstream proteins. β-catenin serves an important role in cell adhesion by interacting with E-cadherin at cell junctions and participating in the formation of adhesion bonds. Free β-catenin can enter the nucleus and interact with T-cell Factor/Lymphoid Enhancing Factor DNA-binding proteins to increase the transcription of EMT-related genes (83). In a previous study, a total of 68 DUBs potentially related to β-catenin were analyzed via immunoprecipitation and GST pull-down assays. USP2a was identified as a DUB that directly interacts with β-catenin and positively regulates its levels and activity. Further experiments revealed that USP2a removes the ubiquitin molecules on β-catenin and prevents its degradation, which in turn enhances the activity of the Wnt/β-catenin pathway and promotes the EMT of tumor cells. In addition, knockdown of *USP2a* or treatment with ML364 downregulated the protein expression of β-catenin in human breast cancer BT549 cells and inhibited the migratory and invasive capabilities of tumor cells (84).

USP2 inhibits p53-mediated cell death. The tumor suppressor gene *p53* is a downstream target of *USP2*. It repairs damaged DNA, induces apoptosis and regulates the cell cycle, thereby preventing carcinogenesis (85,86). Loss of *p53* gene is another major cause of tumorigenesis, and >50% of patients with malignant tumors may have *p53* gene mutations (87). MDM2 is an E3 ubiquitin ligase that promotes the degradation of p53 protein through the ubiquitin-proteasome pathway (88). Although *USP2* cannot directly affect the ubiquitin-mediated degradation of p53, it can inhibit the ubiquitin-mediated degradation of MDM2 protein by specifically recognizing and hydrolyzing the isopeptide bond in MDM2. This inhibition increases protein stability and indirectly inhibits *p53* expression in prostate cancer and cutaneous T-cell lymphoma (30,89,90). MDM4 is an important regulator of *p53* upstream and is similar to MDM2 in terms of structure and function. Its high expression inactivates *p53* and induces tumor development. *USP2a* can directly stabilize the protein expression of MDM4 in glioma cells through deubiquitination and promote the ubiquitin-mediated degradation of p53. In glioma cells with *USP2a* knockdown, MDM4 expression is downregulated and p53 protein is transported to mitochondria, promoting cytochrome c-induced apoptosis (91). A study demonstrated that the expression of *p53* is downregulated in hepatoma HepG2 cells and breast cancer MCF-7 cells. The content of *p53* in these cells is significantly higher following leptin treatment than prior to treatment. However, *USP2* knockdown inhibits the leptin-induced increase in intracellular *p53* levels, indicating that the tumor-suppressing effects of leptin rely on the deubiquitinating effects of USP2 on p53 protein (92). Therefore, *USP2* may serve as an effective therapeutic target for malignancies characterized by the loss of function of *p53* gene.

USP2 reduces sensitivity to chemotherapeutic drugs. Drug resistance and metastasis are the major causes of death among patients with cancer. Developing effective strategies for reversing drug resistance and elucidating mechanisms underlying drug resistance constitute the primary focus of modern medical research. Clinical conventional chemotherapeutic drugs mainly exert their cytotoxic effects by inducing apoptosis through the mitochondrial and endoplasmic reticulum stress-mediated autophagic pathways (93-95). The anti-apoptotic regulator cFILP serves an important role in death receptor signaling, and its overexpression is one of the primary mechanisms through which tumor cells acquire drug resistance (96,97). On the one hand, cFILP competes with the precursor caspase-8 to bind to Fas-associated death domain-containing protein (FADD), which inhibits apoptosis and promotes drug resistance in tumor cells (98). On the other hand, cFILP interacts with Akt and enhances the anti-apoptotic function of Akt by regulating the activity of glycogen synthase kinase-3 β (GSK3β) to promote drug resistance in tumor cells (99,100). Previous studies have

reported that *USP2* stabilizes the protein expression of cFILP and promotes the proliferation of HCC Huf7 cells through deubiquitination. Inhibition of *USP2* can reduce cFILP expression in sorafenib-resistant Huf7-SR cells, promote apoptosis and increase sorafenib sensitivity (98). Additionally, *USP2* negatively regulates the expression of miRNA-1915-3P in oxaliplatin-resistant colorectal cancer (CRC) cells; inhibits apoptosis and promotes the proliferative, migratory and invasive capabilities of tumor cells. Knockdown of *USP2* promotes apoptosis and increases the sensitivity of CRC cells to oxaliplatin (101). In addition, knockdown of *USP2* or treatment with ML363 enhances the sensitivity of triple-negative breast cancer cells to doxorubicin (102).

3. Targeting USP2 for cancer therapy

In molecularly targeted therapy, specific oncogenes or gene fragments can be targeted and corresponding targeted drugs can be developed to act at the cellular level. When these targeted drugs enter the human body, they specifically target the cancer-inducing sites, leading to the specific elimination of tumor cells without damaging normal cells (103-105). Therefore, molecularly targeted therapy is considered an effective therapeutic strategy for cancer in modern medicine and is a major focus of cancer research. *USP7*, a member of the DUB family, can promote tumor development by stabilizing the E3 ubiquitin ligase MDM2, promoting p53 degradation and reducing the expression of downstream proteins of p53 (106,107). Elevated *USP7* expression is closely associated with the development of several cancers and *USP7* is an important target for the treatment of prostate cancer (108), malignant melanoma (109), ovarian cancer (110), multiple myeloma (111) and CRC (112). In addition to *USP7*, other USPs such as *CYLD*, *USP1*, *USP6*, *USP8*, *USP9X*, *USP11*, *USP15* and *USP28*, are considered potential therapeutic targets for various cancers (23). Studies have demonstrated that *USP2*, a multifunctional cysteine protease, is a key regulator of ubiquitin-mediated degradation of fatty acid synthase (FAS), MDM2, MDM4, epidermal growth factor receptor (EGFR), the cell cycle proteins A1 and D1 and other oncogenic proteins, and is closely associated with the development of a number of tumors (59,91,106,113). Therefore, targeting *USP2* is a promising strategy for tumor treatment. This section discusses the current research status of *USP2* in cancer therapy and summarizes the targets and related molecular mechanisms of *USP2* (Table I and Fig. 3).

Targeting USP2 for breast cancer. USP2 expression is low in invasive ductal carcinoma (114) but high in estrogen receptor-positive, progesterone receptor-positive and triple-negative breast cancers and distant metastatic sites. High expression of *USP2* is significantly associated with a poor prognosis in breast cancer (57). *USP2* promotes distant metastasis and invasion in triple-negative breast cancer. Its overexpression upregulates MMP2 to promote the migratory and invasive capabilities of breast cancer cells, whereas its silencing significantly attenuates these capabilities (57). Therefore, *USP2* may be used as a prognostic biomarker and therapeutic target for triple-negative breast cancer.

The Twist protein is a highly conserved basic helix-loop-helix transcription factor that is repressed in normal tissue cells but overexpressed in triple-negative breast cancer and various metastatic tumors (115,116). It serves a key role in the self-renewal and EMT of tumor stem cells (117,118). *USP2* is associated with the upregulation of Twist protein in clinical tumor specimens. Inhibition of *USP2* expression promotes the ubiquitin-mediated degradation of Twist, thereby inhibiting tumor stem cell properties *in vitro* and tumorigenicity *in vivo* (102). The *USP2* inhibitor ML364 inhibits tumor growth and enhances the sensitivity to Adriamycin (102). In addition, the molecular chaperone function of heat shock protein 90 (HSP90) serves a critical role in maintaining the stability of various intracellular proteins and is closely associated with the development of several tumors (119,120). Clinical trials have demonstrated the anticancer effects of multiple HSP90 inhibitors, both as monotherapy and combination therapy with ErbB2-targeting agents (121,122). A preliminary clinical trial of tanespimycin (17-AAG) provides additional evidence for the use of HSP90 inhibitors in the treatment of ErbB2-positive breast cancer (123). In a recent study, HSP90 inhibitors were found to promote the ubiquitin-mediated degradation of ErbB2; however, these effects were reversed by *USP2*. Additionally, ML364 not only enhanced the degradation of ErbB2 by HSP90 inhibitors but also inhibited the growth of ErbB2-positive breast cancer cells and transplanted tumors in mice *in vivo* (56). Therefore, *USP2* may serve as a prognostic biomarker and therapeutic target for breast cancer.

Targeting USP2 for hepatocellular carcinoma. USP2 exerts pro-carcinogenic effects in malignant tumors such as breast and lung cancers; however, its role in liver cancer remains unclear. The expression of different isoforms of *USP2* in the liver is controversial. In one study, USP2c was identified as the major isoform of USP2 protein in the liver (~89% of total USP2), whereas USP2b protein was not detected in the liver (44). However, other studies have reported that USP2b is the major isoform of USP2 in the liver (33,41). Nadolny *et al* (55) used an isoform-specific probe technique to detect USP2 in normal human and mouse liver tissues and identified USP2b as the major isoform of USP2 in the liver. The mRNA and protein expression of USP2 is significantly lower in clinical primary HCC tumor tissues than in para-carcinoma and healthy liver tissues, and the expression of USP2b is consistent with the total USP2 expression (55). Furthermore, *USP2b* can exert both pro- and anti-cancer effects. On the one hand, overexpression of *USP2b* promotes bile acid-induced apoptosis and necrosis of tumor cells to exert anti-tumor effects; on the other hand, overexpression of *USP2b* promotes tumor cell proliferation, colony formation and wound healing to exert pro-cancer effects. Therefore, the ability of *USP2b* to act as a tumor suppressor or initiator depends on the cell state and the specific underlying molecular mechanisms warrant further investigation.

Antisense RNAs refer to RNA molecules that are complementary to mRNAs. They inhibit the translation of mRNAs and block gene function by specifically and complementarily binding to mRNAs (124). Given the medicinal value of antisense RNAs, their role in cell growth and differentiation needs to be intensively investigated (125). The expression

Table I. Reported targets of USP2.

First author, year	USP2 target	Tumor	Mode of action of USP2	Pathway involved	(Refs.)
Magiera *et al*, 2017 Nepal *et al*, 2015	CCND1	Colorectal, breast and hepatoma cancer	Deubiquitination stabilizes CCND1 protein and promotes cell cycle progression	Cell cycle	(62,63)
Kim *et al*, 2012	CCNA1	Bladder cancer	Deubiquitination stabilizes CCNA1 protein and promotes cell cycle progression	Cell cycle	(65)
Shi *et al*, 2011	Aurora-A	Pancreatic cancer	Deubiquitination stabilizes Aurora-A protein and promotes cell mitosis	Cell mitosis	(73)
Kim *et al*, 2018	β-catenin	Breast cancer	Deubiquitination stabilizes β-catenin protein and promotes the migratory and invasive abilities of cells	Wnt/β-catenin pathway	(84)
Bonacci *et al*, 2020 Stevenson *et al*, 2007 Wang *et al*, 2014	MDM2/4	Testicular Cancer/ glioblastoma	Direct stabilization of MDM2/4 activity and promotion of ubiquitin-mediated degradation of p53, the downstream target of MDM2/4	p53 pathway	(30,89,91)
Liu *et al*, 2022	TWIST1	Bladder cancer	Deubiquitination stabilizes TWIST1 protein and promotes vascular remodeling	Epithelial-mesenchymal transition	(53)
Tu *et al*, 2022	SMAD7	Glioblastoma	Deubiquitination stabilizes SMAD7 protein, reduces SMAD7 recruitment of the E3 ligase HERC3 and inhibits the TGF-β signaling pathway	TGF-β signaling pathway	(54)
Qu *et al*, 2015	MMP2	Breast cancer	Deubiquitination stabilizes MMP2 protein and promotes the migratory and invasive abilities of cells	Epithelial-mesenchymal transition	(57)
Xiao *et al*, 2022	E2F4	Gastric cancer	Deubiquitination stabilizes E2F4 protein and promotes cell proliferation	Cell proliferation	(59)
Liu *et al*, 2018	cFILP	T-cell lymphoma	Deubiquitination stabilizes cFILP protein and promotes drug resistance in tumor cells	Endoplasmic reticulum stress	(98)
Liu *et al*, 2018	ITCH	T-cell lymphoma	Direct stabilization of ITCH protein and degradation of the downstream target cFILP	Endoplasmic reticulum stress	(98)
Graner *et al*, 2004	FAS	Prostate cancer	Deubiquitination stabilizes FAS expression and inhibits cell apoptosis	Cell apoptosis	(142)
Zhang *et al*, 2021	Skp2	Lung cancer	Direct stabilization of Skp2 expression and promotion of substrate protein degradation	Cell cycle	(155)

USP2, ubiquitin-specific protease 2; CCND1, cyclin D1; MDM2, murine double minute 2; SMAD, mothers against decapentaplegic; E2F4, E2F transcription factor 4; FAS, fatty acid synthase.

of ubiquitin-specific peptidase 2 antisense RNA 1 (lncRNA USP2-AS1), a USP2-specific antisense RNA, is significantly higher in HCC tissues than in paraneoplastic tissues. The high expression of USP2-AS1 is significantly associated with a poorer prognosis. Knockdown of USP2-AS1 promotes the ubiquitin-mediated degradation of Y-box binding protein 1-mediated hypoxia-inducible factor 1α; inhibits the proliferative, migratory and invasive abilities of HCC cells and reduces the tumorigenicity of HCC cells in mice (126).

cFILP is an important regulator of death receptor signaling that inhibits tumor cell apoptosis by competing with caspase-8 to bind to the junction protein FADD (127). *cFILP* overexpression is one of the major causes of resistance to death receptor-mediated apoptosis and chemotherapy (128).

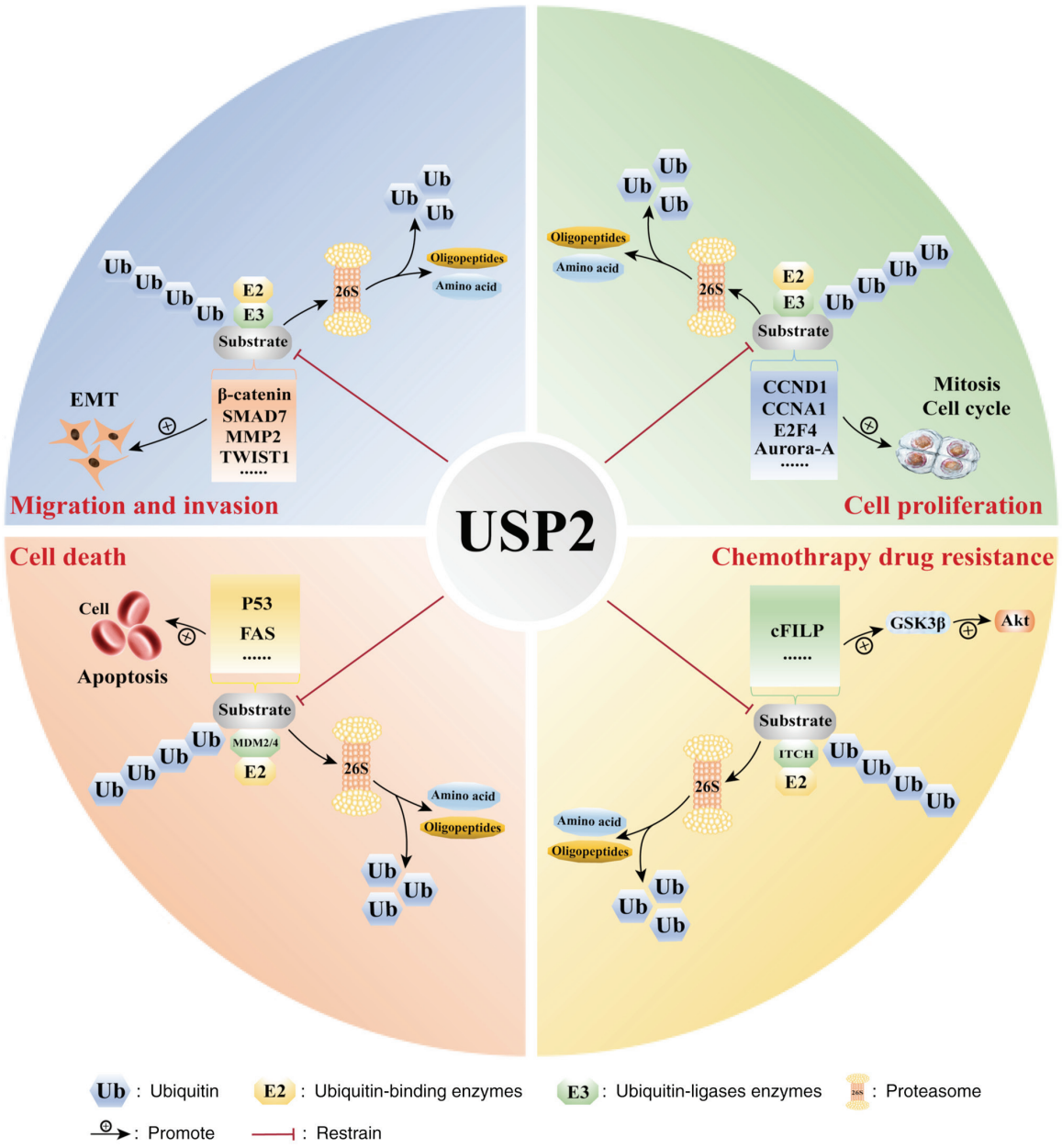

Figure 3. Mechanisms through which USP2 participates in cancer-related pathways. Upper right quadrant: USP2 stabilizes the expression of CCND1, CCNA1 and Aurora-A proteins through deubiquitination and promotes cell mitosis and cell cycle progression, which in turn promotes abnormal proliferation of tumor cells. Upper left quadrant: USP2 inhibits the ubiquitin-mediated degradation of β-catenin, SMAD7 and MMP2 proteins through deubiquitination and promotes epithelial-mesenchymal transition, which in turn enhances the migratory and invasive abilities of tumor cells. Lower left quadrant: USP2 directly interacts with and stabilized MDM2/4, promoting the ubiquitin-mediated degradation of the substrate protein p53 and inhibiting p53-dependent cell death. Lower right quadrant: USP2 can stabilize cFILP protein through deubiquitination, regulate GSK3β activity to enhance the anti-apoptotic function of Akt and induce chemotherapy resistance in tumor cells. USP2, ubiquitin-specific protease 2.

Liu *et al* (98) reported that *cFILP* expression is significantly elevated in sorafenib-resistant HCC cells, and overexpression of *USP2* can promote ubiquitin-mediated degradation of cFILP protein by stabilizing the E3 ubiquitin ligase ITCH to enhance the sensitivity of HCC cells to sorafenib. Therefore, *USP2* and *cFILP* may serve as potential targets for reversing sorafenib resistance in HCC cells.

Targeting USP2 for ovarian cancer. Yang *et al* (129) examined tumor specimens from 40 patients with ovarian plasmacytoid cystic adenocarcinoma and found that the expression of *USP2*, *USP14* and UBE4A (an ubiquitin-related factor) is significantly higher in ovarian cancer tissues than in peri-cancerous tissues and normal ovarian tissues. These findings suggest that the ubiquitin-proteasome pathway is involved in the development of ovarian cancer. USP2-AS1, an antisense RNA of *USP2*, is significantly upregulated in ovarian cancer tissues, and its knockdown can inhibit the proliferative, migratory and invasive abilities of ovarian cancer cells. USP2-AS1 can compete for endogenous RNAs to regulate the expression of downstream genes by sponging miRNAs (126). Guo *et al* (130) investigated the specific molecular mechanisms through which *USP2-AS1* promotes ovarian cancer progression. USP2-AS1 and miRNA-520d-3P were found to

bind to each other, and silencing of miRNA-520d-3P reversed the *USP2-AS1*-induced proliferative, migratory and invasive abilities of ovarian cancer cells. The specific mechanism is related to the involvement of *USP2-AS1* in the ubiquitinated degradation of miRNA-520d-3P downstream gene *KIAA1522*. Therefore, *USP2* and *USP2-AS1* may serve as potential targets for the treatment of ovarian cancer.

Targeting USP2 for colorectal cancer. USP2, a specific deubiquitinating enzyme of CCND1, is highly expressed in colon cancer cells (HCT116). Treatment of HCT116 cells with ML364 can promote the ubiquitin-mediated degradation of CCND1, inhibit tumor cell proliferation and induce the arrest of tumor cells in the G_1 phase (49). In addition, the expression of USP2-AS1 is significantly elevated in clinical colon adenocarcinoma tumor tissues and is positively correlated with tumor size, grade and TNM stage. Knockdown of *USP2-AS1* can promote phosphorylation and ubiquitin-mediated degradation of Yes-associated protein 1 (YAP1) to inhibit the activation of the Hippo/YAP1 signaling pathway, which in turn inhibits the proliferative, migratory and invasive abilities of tumor cells and reduces the tumorigenicity and distant metastatic ability of cancer cells in mice (131).

Oxaliplatin is widely used as the first-line chemotherapeutic agent for the treatment of advanced CRC in clinical settings. The expression of miRNA-1915-3P is reduced in oxaliplatin-resistant CRC cells and overexpression of miRNA-1915-3P downregulates the oncogenes 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 and *USP2*, thus inhibiting tumor proliferation, metastasis and invasion (101). *USP2* is a negative regulator of miRNA-1915-3P. Overexpression of *USP2* restores the proliferative capacity of tumor cells, whereas its knockdown inhibits tumor cell proliferation (101). Therefore, small-molecule inhibitors of *USP2* may be used to induce oxaliplatin sensitivity in advanced CRC.

Targeting USP2 for glioblastoma. Glioblastoma (GBM) is an astrocytic tumor characterized by rapid growth, high malignancy and high mortality rates (132). As a cancer-promoting factor, TGF-β serves an important role in the development of GBM (133,134). SMAD7, a key negative regulator of TGF-β signaling, exerts its inhibitory effects on TGF-β by blocking receptor activity and inducing receptor degradation (135). *USP2* expression is significantly lower in GBM tissues than in normal human brain tissues and the prognosis of patients with lower *USP2* expression is worse. Overexpression of *USP2* can break the isopeptide bond between ubiquitin and the Lys27 and Lys48 residues of SMAD7 protein, reduce the recruitment of SMAD7 protein to the E3 ubiquitin ligase HERC3 and inhibit ubiquitin-mediated degradation of SMAD7, thereby inhibiting the activation of the TGF-β signaling pathway and the progression of GBM (54). Abnormal DNA methylation transferase 3A (DNMT3A)-mediated methylation of *USP2* is the main cause of low expression of *USP2* in GBM tissues, and the DNMT3A inhibitor GSI-1027 can induce *USP2* expression to exert anti-tumor effects against GBM (54).

Previous studies have reported that MDM4 can promote endogenous apoptosis by regulating the expression of oncogene *p53* and that *USP2a* can interact with MDM4 to inhibit its ubiquitin-mediated degradation (91,136,137). The expression of MDM4 and USP2a is significantly lower in GBM tissues than in normal brain tissues and is positively associated with the prognosis of GBM; that is, the higher the expression, the more improved the prognosis. Knockdown of *USP2a* promotes UV irradiation-induced cytochrome c release, p53 protein expression and apoptosis in U87MG glioma cells, whereas simultaneous upregulation of MDM4 can reverse these effects (91). Therefore, *USP2* and *MDM4* may serve as effective targets for the treatment of GBM.

Targeting USP2 for bladder cancer. Bladder cancer is the most common life-threatening tumor of the urinary system. Tight junction protein 1 (TJP1) interacts with TWIST1 to enhance the invasive ability of tumor cells and promotes bladder cancer progression by affecting vascular remodeling (53). TJP1 expression is significantly higher in clinical bladder cancer tissues compared with healthy bladder tissues and is associated with tumor angiogenesis and overall survival of patients (138). *In vitro* studies have demonstrated that overexpression of TJP1 promotes the expression of TWIST1 and chemokine C-C motif ligand 2 (CCL2) in tumor cells, stimulating tumor cells to recruit more macrophages, which secrete VEGF under CCL2 stimulation and enhance tumor angiogenesis. Knockdown of TJP1 inhibits, and overexpression of TWIST1 promotes, vascular remodeling in bladder cancer. TJP1 promotes vascular remodeling by reversing ubiquitin-mediated degradation of TWIST1 by recruiting *USP2*, whereas knockdown of *USP2* promotes ubiquitin-mediated degradation of TWIST1, reduces tumor angiogenesis and exerts positive anti-tumor effects (53). In addition, *USP2a* gene is highly expressed in bladder cancer cells and there is a physical interaction between USP2a and CCNA1. *USP2a* inhibits ubiquitination degradation of CCNA1 protein through deubiquitylation, which in turn increases CCNA1 protein expression and exerts a positive pro-oncogenic effect. Therefore, *USP2* and *TJP1* may serve as effective therapeutic targets for bladder cancer.

Targeting USP2 for prostate cancer. The positive regulation of *FAS* by *USP2* serves a key role in influencing the malignant behavior of prostate cancer (139-141). *USP2* is significantly upregulated in prostate cancer tissues; however, it is either not expressed or is downregulated in healthy prostate tissues. The protein expression of USP2a is directly associated with the malignant behavior of prostate cancer. Overexpression of USP2a promotes the proliferation of LNCaP cells. Following *USP2a* knockdown, ubiquitin-mediated degradation of FAS is enhanced and the protein expression of FAS is significantly downregulated, resulting in apoptosis of tumor cells, which can be reversed by the proteasome inhibitor MG-132 (142). Previous studies have reported that acid ceramidase (ACD), which metabolizes ceramide to sphingomyelin, is upregulated in prostate cancer (143). Mizutani *et al* (144) reported that treatment with the androgen receptor antagonist bicalutamide (Casodex) decreased the protein expression of adrenocortical dysplasia homologue (ACD) in LNCaP cells, whereas treatment with MG132 restored the activity of ACD protein. These findings suggest that the ubiquitin-proteasome pathway is involved in the modification of ACD protein. The oncogenic role of *USP2* in prostate cancer has been demonstrated in previous studies (139,144). The protein expression of ACD

is promoted upon *USP2* overexpression and inhibited upon *USP2* silencing in LNCaP cells. However, silencing or overexpression of *SKP2*, an E3 ubiquitin ligase, does not alter the activity of ACD protein in LNCaP cells, suggesting that ACD activity is affected by deubiquitination of *USP2*, independent of *SKP2* (144). Therefore, *USP2*, *FAS* and *ACD* may influence the malignant behavior of prostate cancer and serve as potential therapeutic targets.

Targeting USP2 for cutaneous T-cell lymphoma. Cutaneous T-cell lymphoma (CTCL) is caused by the clonal proliferation of T lymphocytes originating in the skin and is a type of extranodal non-Hodgkin lymphoma. Psoralen with ultraviolet A (PUVA) phototherapy is a common treatment strategy for CTCL in clinical settings (145,146). A study demonstrated that *USP2* is expressed in both quiescent and activated T lymphocytes, and its expression is significantly reduced in advanced CTCL. Treatment of MyLa2000 cells with PUVA or the *p53* agonist nutlin3a significantly increases the protein expression of USP2 and p53 and promoted apoptosis (90). Silencing of *USP2*, which acts as a tumor suppressor, reduces the protein expression of MDM2 and enhances the transcriptional activity of *p53*, thereby promoting apoptosis and enhancing the sensitivity of MyLa2000 cells to PUVA and nutlin3a. In addition, *p53* induced *USP2* expression and stabilized MDM2 protein via deubiquitination, which in turn inhibited the pro-apoptotic activity of *p53*, forming a negative feedback loop (90). Therefore, small-molecule inhibitors of *USP2* may serve as sensitizing agents in CTCL.

Targeting USP2 for gastric cancer. E2F transcription factor 4 (E2F4), a key factor regulating cell cycle progression, binds to DNA to promote the progression of cells from the G_0 to the G_1 and S phases and is involved in tumor progression (147). *E2F4* can directly regulate the transcription of ATG2A and ULK2 proteins, leading to the autophagic degradation of metallothionein; it can maintain zinc homeostasis in tumor cells and promote the proliferative, migratory and invasive abilities of gastric cancer cells (59). High expression of *USP2* and *E2F4* in gastric cancer tissues is associated with a poor prognosis. Emetine, an autophagy inhibitor, can block the interaction between USP2 and E2F4 and promote E2F4 degradation for an oncogenic effect, which can be reversed upon *USP2* overexpression (59). Therefore, *USP2* and *E2F4* may serve as potential biomarkers for maintaining zinc homeostasis in the treatment of gastric cancer.

Targeting USP2 for lung cancer. Previous studies have demonstrated the involvement of multiple USPs in the tumorigenesis and chemotherapy resistance of lung cancer (148,149). USPs may serve as therapeutic targets for lung cancer. For instance, inhibition of *USP1* and *USP51* can increase cisplatin sensitivity in lung cancer (150,151); inhibition of *USP5* and *USP28* can promote apoptosis of tumor cells (152,153) and promotion of USP52 and USP7 can inhibit the proliferative, migratory and invasive abilities of lung cancer cells (107,154). However, the role of USP2 in lung cancer remains elusive. Zhang *et al* (155) reported that USP2 expression is upregulated in the lung cancer cell lines H1229 and H1270. Knockdown of USP2 promotes ubiquitin-mediated degradation of SKP2 and inhibits the growth of tumor cells. Mechanistically, USP2 interacts with SKP2 and stabilizes its expression to promote lung cancer progression. Therefore, *USP2* and *SKP2* may serve as potential therapeutic targets for lung cancer.

Targeting USP2 for renal clear cell carcinoma. Renal clear cell carcinoma is a common malignant tumor of the urinary system. The proliferation and apoptosis of cancer cells cannot be achieved without the participation of USPs (156,157). The clinical significance of *USP2* in renal clear cell carcinoma has been demonstrated (158). The mRNA and protein expression of USP2 is significantly lower in cancer tissues than in para-cancerous and healthy kidney tissues. Studies have verified the low protein expression of *USP2* in most cancer tissues via immunohistochemical analysis (56,159,160). Overexpression of *USP2* inhibits the proliferative, migratory and invasive abilities of kidney cancer cells (A498 and CAKi-1) (158). In addition, the abnormal expression of *USP2* is closely related to the clinical stage, pathological grade and prognosis of patients with renal cancer, and *USP2* has been identified as an independent risk factor for renal clear cell carcinoma (158). Therefore, *USP2* is a potential target for the diagnosis and treatment of renal clear cell carcinoma.

Targeting USP2 for the treatment of hematological tumors. The role of *USP2* in stabilizing CCND1 during cell proliferation is well established. Davis *et al* (49) were the first to reveal the epigenetic regulation mechanism of *USP2* in a nested cell lymphoma model. They found that *USP2* expression is significantly reduced, the ubiquitin-mediated degradation of CCND1 protein is significantly enhanced, the cell cycle is arrested in the G_1 phase and the proliferative capacity of tumor cells is significantly reduced after Mino cells were treated with the USP2-specific small-molecule inhibitor ML364. However, CCND1 degradation was reversed and the proliferative capacity of cells was restored after the cells were treated with the proteasome inhibitor MG132. 6-thioguanine (6-TG), an anti-tumor agent clinically used in the treatment of acute leukemia and chronic granulocytic leukemia, is a potent inhibitor of *USP2* (161,162). 6-TG forms covalent bonds with the Cys276 residue of *USP2* to inhibit *USP2* in a non-competitive and slow-binding manner. Therefore, it can be used in the clinical treatment of tumors characterized by *USP2* upregulation (139). Lin *et al* (163) reported that disulfiram, a clinical therapeutic agent for alcohol dependence, competitively inhibited the protein activity of both *USP2* and *USP21*. Altogether, the combination of 6-TG and disulfiram may be used for the clinical treatment of USP2-associated tumors.

Lysine (K)-specific methyltransferase 2A (*KMT2A*) serves an important role in embryonic development and the hematopoietic system. The translocation of the *KMT2A* gene produces a *KMT2A* fusion protein that directly binds to DNA and upregulates gene transcription, leading to the development of acute myeloid leukemia (AML) in infants and children (164,165). USP2 serves as a chaperone gene for *KMT2A* and the poor clinical prognosis of children with *KMT2A-USP2*-positive AML has been associated with the aberrant expression of USP2 (166-168). In a prospective study, Meyer *et al* (169) reported that a very small number of patients with acute leukemia have rearranged USP2 and

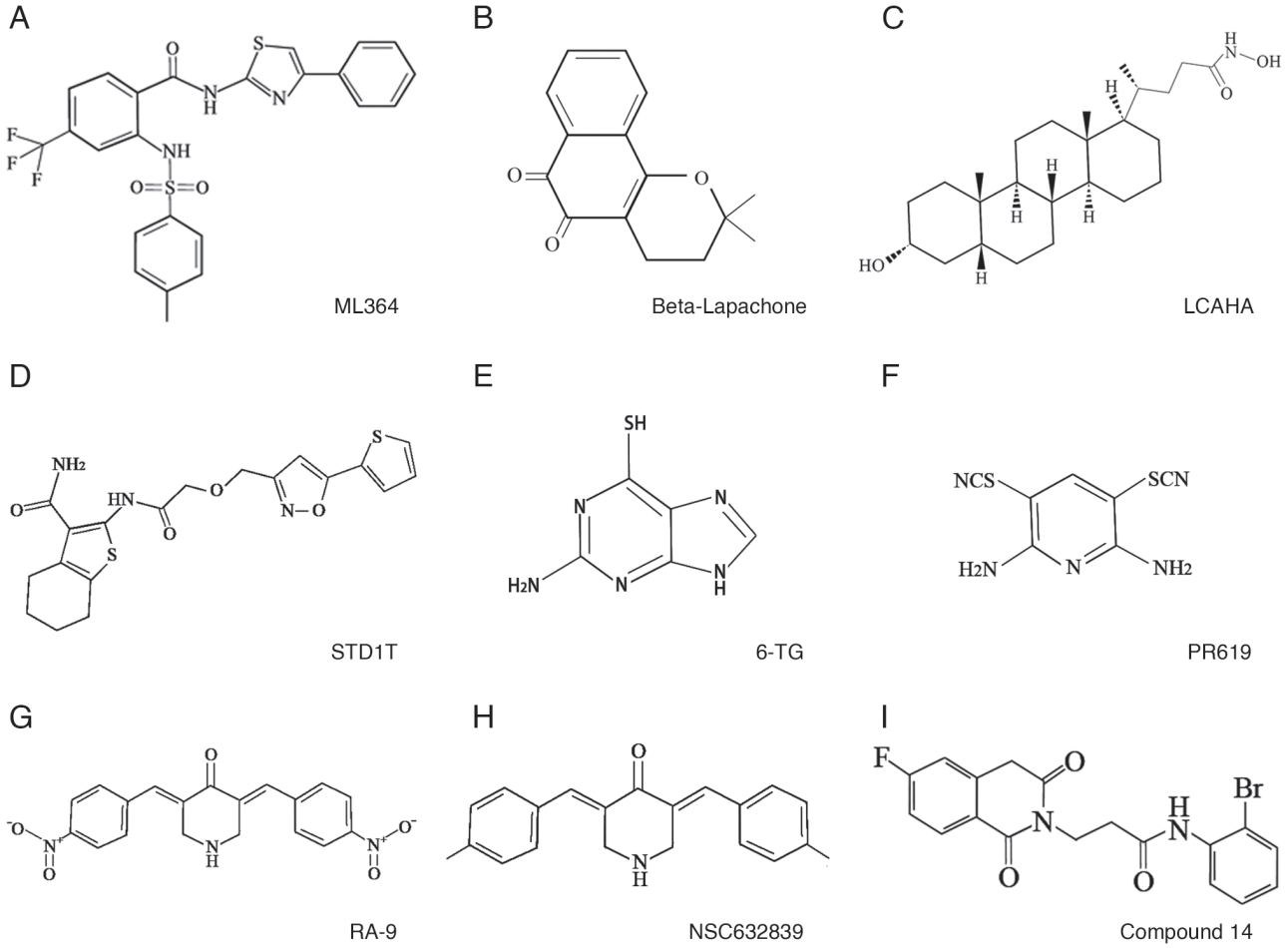

Figure 4. Chemical structural formula of inhibitors. Structural formula of (A) ML364, (B) β-lapachone, (C) LCAHA, (D) STD1T, (E) 6-TG, (F) PR619, (G) RA-9, (H) NSC632839 and (I) compound 14. LCAHA, lithocholic acid hydroxyamide; 6-TG, 6-thioguanine.

USP8 genes and that the conserved region of the deubiquitinating enzyme 'UCH-domain' fuses to an extended 5′-MLL portion, which formed the fusion proteins MLL-USP2 and MLL-USP8. Deubiquitination of USP2 stabilizes MDM protein and indirectly enhances the degradation of p53, which may be an important mechanism affecting the development of MLL-USP2 type leukemia. Therefore, *USP2* may serve as a potential therapeutic target for AML.

4. Pharmacological studies of USP2

With the rapid development of structural biology and small-molecule drugs, targeted therapy has emerged as the most promising strategy in clinical tumor treatment. Recent studies have revealed the role of DUBs in life activities and identified DUBs as potential therapeutic targets for tumors (170). *USP2* is closely associated with the development of various tumors, such as breast cancer, liver cancer, CRC, GBM and hematological tumors (49,53,54,56,126). At present, ML364 is the most common small-molecule inhibitor used in clinical trials; other inhibitors include Q29, STD1T and LCAHA (23). The chemical structures of these small-molecule inhibitors are shown in Fig. 4, and key information regarding their mechanism of action and targets is summarized in Table II. The use of *USP2* as a target for tumor treatment has received increasing attention from researchers. Although several *USP2*-targeted agents have shown positive anticancer effects in different cancers, the identified targeting agents are undergoing preclinical investigation at present. Therefore, these agents should be evaluated via complex and comprehensive techniques to provide a theoretical basis for their clinical application.

ML364. ML364 is the most commonly used specific small-molecule inhibitor of *USP2* (IC_{50}=1.1 μM; Fig. 4A). It directly binds to USP2, induces ubiquitin-mediated degradation of cyclin D1, leads to cell cycle arrest in the G_0/G_1 phase, inhibits the proliferation of HCT116 and Mino cells and exerts positive antitumor effects (49). In addition, ML364 enhances ubiquitin-mediated degradation of ErbB2 by HSP90 inhibitors through inhibition of USP2 in the treatment of ErbB2-positive breast cancer (56).

Beta-lapachone. Beta-lapachone (Q29) is a natural naphthoquinone compound (Fig. 4B) that was used in several phase II clinical trials in the early 21st century for the treatment of pancreatic cancer, head and neck tumors and smooth muscle sarcoma (171). In subsequent studies, Q29 was found to exert positive antitumor effects by selectively and irreversibly inhibiting the oxidation of cysteine residues of USP2 and promoting the production

Table II. USP2 inhibitors and their pharmacological mechanisms of action.

First author, year	Inhibitor	Clinical trials	ClinicalTrials.gov ID	USP targets	Tumor	Mechanism	(Refs.)
Davis *et al*, 2016 Zhang *et al*, 2020	ML364	/	/	USP2	Breast cancer/ colorectal cancer/ mantle cell lymphoma	It inhibits USP2 deubiquitination; promotes the degradation of CCND1 and β-catenin; blocks cell cycle progression and inhibits the proliferative, migratory and invasive abilities of tumor cells	(49,56)
Savage *et al*, 2008	β- lapachone	I/II	NCT00075933 NCT00102700 NCT00524524	USP2	/	It selectively and irreversibly inhibits the oxidation of USP2 cysteine residues, interferes with cell cycle progression and promotes apoptosis	(171)
Nguyen *et al*, 2021	LCAHA	/	/	USP2a	Colorectal cancer	It promotes ubiquitin-mediated degradation of CCND1 protein and induces G0/G1-phase progression of tumor cells	(173)
Tomala *et al*, 2018	STD1T	/	/	USP2a	Colorectal cancer/ breast cancer	It binds directly to USP2a, inhibits CCND1 protein expression and blocks cell cycle progression	(64)
Chuang *et al*, 2018	6-TG	I/II	NCT00587873 NCT00504660 NCT00588536	USP2	/	It interacts with the Cys276 residue of USP2 in a non-competitive and slow-binding manner to inhibit USP2 through covalent bonding	(139)
Altun *et al*, 2011	PR619	/	/	USP2/4/ 5/7/8/ 15/20/ 28/47	Colorectal cancer	It induces tumor cell death; however, the exact mechanism remains unclear	(175)
Issaenko *et al*, 2012	RA-9	/	/	USP2/5/8	Breast cancer/ ovarian cancer	It promotes ubiquitin-mediated degradation of CCND1 protein and upregulates the expression of P53, P27 and other tumor suppressor genes, thereby promoting P53-dependent cell death	(178)

Table II. Continued.

First author, year	Inhibitor	Clinical trials	ClinicalTrials.gov ID	USP targets	Tumor	Mechanism	(Refs.)
Nicholson *et al*, 2008	NSC632839	/	/	USP2/7	/	It inhibits USP2 activity and stabilizes Smac protein expression, thereby inducing cell apoptosis	(180)
Vamisetti *et al*, 2019	Compound 14	/	/	USP2/7	/	Non-competitive mechanisms inhibit USP2 protein activity	(181)

USP2, ubiquitin-specific protease 2; LCAHA, lithocholic acid hydroxyamide; 6-TG, 6-thioguanine.

of reactive oxygen species, thereby interfering with cell cycle progression and inducing apoptosis in tumor cells (172).

LCAHA. LCA, a secondary bile acid, serves an important role in lipid metabolism, and several derivatives of LCA have anticancer activity (173,174). The most active, LCAHA (Fig. 4C; IC_{50}=5.8 μM), can directly inhibit the biological activity of *USP2a*, induce G_0/G_1-phase arrest in HCT116 cells and ubiquitously degrade cell cycle protein D1, thereby exerting positive anticancer effects (62).

STD1T. Tomala *et al* (64) used saturation transfer difference nuclear magnetic resonance spectroscopy to screen USP2 protein and found that STD1 could directly bind to USP2a and inhibit its activity. As a derivative of STD1, STD1T (IC_{50}=3.3 μM) has a stronger inhibitory effect on USP2a (Fig. 4D) and can significantly reduce the expression of CCND1 in HCT116 and MCF-7 cells at a concentration of 20 μM, thus exerting a positive oncogenic effect.

6-TG. 6-TG is a clinical agent for the treatment of AML and chronic granulocytic leukemia (161,162) (Fig. 4E). Chuang *et al* (139) used enzyme kinetic and X-ray crystallographic data to verify that 6-TG is a small-molecule inhibitor of *USP2* that forms covalent bonds with the Cys276 residue of *USP2* to inhibit its expression. This finding provides a rationale for the clinical use of 6-TG in the treatment of tumors with *USP2* upregulation.

PR619. Altun *et al* (175) used high-throughput screening and structural optimization to identify PR619 (Fig. 4F) as a broad-spectrum inhibitor of *USP2*, *USP4*, *USP5*, *USP7*, *USP8*, *USP15*, *USP20*, *USP28* and *USP47*. PR619 can inhibit *USP2* in HCT116 cells and induce tumor cell death.

RA-9. Chalcones, members of the flavonoid family, can regulate the malignant behavior of tumors, such as tumor accretion, invasion and metastasis, by targeting the ubiquitin-proteasome system (176,177). Issaenko *et al* (178) found that the chalcone derivative RA-9 (Fig. 4G) inhibits the activity of USP2, USP5 and USP8; downregulates the expression of CCND1 in breast, ovarian and cervical cancer cells, upregulates the expression of oncogenes *p53*, *p27* and *p16* to promote apoptosis and exerts positive anticancer effects.

NSC632839. Aleo *et al* (179) were the first to identify NSC632839 (Fig. 4H) as a DUB inhibitor that can induce apoptosis by stabilizing the second mitochondria-derived activator of caspases. Nicholson *et al* (180) verified the inhibitory effects of NSC632839 and found that it inhibits both *USP2* and *USP7* at the half-maximal effective concentration (EC_{50}) of 45±4 and 37±1 μM, respectively.

Compound 14. Vamisetti *et al* (181) used a unique fluorescence quenching assay and found that Compound 14 (Fig. 4I) inhibited the protein activity of USP2 and USP7 via a non-competitive mechanism (IC_{50}=250 nM). Additionally, the fluorine atom in Compound 14 could reverse the selectivity between USP2 and USP7. Therefore, Compound 14 was identified as a reversible inhibitor of USP2 and USP7.

5. Concluding remarks and potential future directions

USP2 is closely associated with the development of several types of malignant tumors. *USP2a*, which has been more intensively investigated, is upregulated in prostate, gastric and lung cancers and downregulated in bladder cancer, renal clear cell carcinoma and GBM. The expression of *USP2* differs among different types of breast cancers. It is low in invasive ductal carcinoma but high in estrogen receptor-positive and triple-negative breast cancers. *USP2* regulates the stability of key tumor-associated proteins such as CCND1, CCNA1, MDM2, MDM4 and FAS. Therefore, targeting *USP2* represents an effective strategy for the treatment of related malignancies. ML364, a small-molecule inhibitor of *USP2*, can cause cell cycle arrest, promote the expression of *p53* and exert anti-tumor effects *in vitro*. In addition, other inhibitors of *USP2*, such as β-lapachone (a naphthoquinone), chalcone (a flavonoid), 6-TG and stigmasterol acid and its derivatives exert anti-tumor effects in different cancers.

Ubiquitination, one of the post-translational modifications, serves an important role in the development and malignant behavior of several cancers and influences protein expression and signal transduction. DUBs can regulate the stability of substrate proteins by removing ubiquitin tags, thereby regulating the cascade responses of the cell cycle, DNA damage repair, invasion, metastasis and other signaling pathways. Targeting DUBs represents an effective strategy for the treatment of cancer. Some USP-targeted drugs are

undergoing investigation in phase II clinical trials. USP2 primarily influences the expression of CCND1, MDM2, p53 and other proteins by regulating ubiquitin-mediated protein degradation, which in turn affects tumor development. However, the following questions remain to be addressed: i) How do transcription factors recognize *USP2* and regulate its transcription? ii) In addition to affecting the cell cycle and cell death, does *USP2* regulate other biological processes? iii) What are the specific molecular mechanisms through which *USP2* regulates the expression of related factors?

The function of *USP2* may be related to the cell or tissue type, and deletion/overexpression of *USP2* in specific cells/tissues may have different effects on biological processes in tumors. To date, studies on USP2 in tumors have mainly focused on two isomers, namely, USP2a and USP2b. Studies on other isomers in tumors are lacking. An in-depth investigation of the biological effects and specific molecular mechanisms of *USP2* in different malignancies may provide a theoretical basis for the development of safe and effective targeted drugs. In conclusion, *USP2* may serve as a potential therapeutic target for cancer, and the clinical significance of *USP2* in developing targeted drugs should be comprehensively evaluated.

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Availability of data and materials

Data sharing is not applicable to this article, as no data sets were generated or analyzed during the current study.

Authors' contributions

SLZ, YG, and SJZ contributed to the review of data collection, manuscript writing and revision. SZ was involved in the consultation process and article revision. YWZ and ZW participated in the collection and arrangement of materials.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ding Y, Xing D, Fei Y and Lu B: Emerging degrader technologies engaging lysosomal pathways. Chem Soc Rev 51: 8832-8876, 2022.
2. Jiang TY, Shi YY, Cui XW, Pan YF, Lin YK, Feng XF, Ding ZW, Yang C, Tan YX, Dong LW and Wang HY: PTEN deficiency facilitates exosome secretion and metastasis in cholangiocarcinoma by impairing TFEB-mediated lysosome biogenesis. Gastroenterology 164: 424-438, 2023.
3. Karbowski M, Oshima Y and Verhoeven N: Mitochondrial proteotoxicity: implications and ubiquitin-dependent quality control mechanisms. Cell Mol Life Sci 79: 574, 2022.
4. Sinam IS, Chanda D, Thoudam T, Kim MJ, Kim BG, Kang HJ, Lee JY, Baek SH, Kim SY, Shim BJ, *et al*: Pyruvate dehydrogenase kinase 4 promotes ubiquitin-proteasome system-dependent muscle atrophy. J Cachexia Sarcopenia Muscle 13: 3122-3136, 2022.
5. O'Brien S, Kelso S, Steinhart Z, Orlicky S, Mis M, Kim Y, Lin S, Sicheri F and Angers S: SCF ^{FBXW7} regulates G2-M progression through control of CCNL1 ubiquitination. EMBO Rep 23: e55044, 2022.
6. Capecchi MR and Pozner A: ASPM regulates symmetric stem cell division by tuning Cyclin E ubiquitination. Nat Commun 6: 8763, 2015.
7. Zhang L, Li Q, Yang J, Xu P, Xuan Z, Xu J and Xu Z: Cytosolic TGM2 promotes malignant progression in gastric cancer by suppressing the TRIM21-mediated ubiquitination/degradation of STAT1 in a GTP binding-dependent modality. Cancer Commun (Lond) 43: 123-149, 2023.
8. Feng X, Jia Y, Zhang Y, Ma F, Zhu Y, Hong X, Zhou Q, He R, Zhang H, Jin J, *et al*: Ubiquitination of UVRAG by SMURF1 promotes autophagosome maturation and inhibits hepatocellular carcinoma growth. Autophagy 15: 1130-1149, 2019.
9. Li H, Wang N, Jiang Y, Wang H, Xin Z, An H, Pan H, Ma W, Zhang T, Wang X and Lin W: E3 ubiquitin ligase NEDD4L negatively regulates inflammation by promoting ubiquitination of MEKK2. EMBO Rep 23: e54603, 2022.
10. Nan Y, Luo Q, Wu X, Chang W, Zhao P, Liu S and Liu Z: HCP5 prevents ubiquitination-mediated UTP3 degradation to inhibit apoptosis by activating c-Myc transcriptional activity. Mol Ther 31: 552-568, 2023.
11. Cao HJ, Jiang H, Ding K, Qiu XS, Ma N, Zhang FK, Wang YK, Zheng QW, Xia J, Ni QZ, *et al*: ARID2 mitigates hepatic steatosis via promoting the ubiquitination of JAK2. Cell Death Differ 30: 383-396, 2023.
12. Mattiroli F and Penengo L: Histone ubiquitination: An integrative signaling platform in genome stability. Trends Genet 37: 566-581, 2021.
13. Roberts JZ, Crawford N and Longley DB: The role of ubiquitination in apoptosis and necroptosis. Cell Death Differ 29: 272-284, 2022.
14. Wang K, Liu J, Li YL, Li JP and Zhang R: Ubiquitination/de-ubiquitination: A promising therapeutic target for PTEN reactivation in cancer. Biochim Biophys Acta Rev Cancer 1877: 188723, 2022.
15. Liu J, Wei L, Hu N, Wang D, Ni J, Zhang S, Liu H, Lv T, Yin J, Ye M and Song Y: FBW7-mediated ubiquitination and destruction of PD-1 protein primes sensitivity to anti-PD-1 immunotherapy in non-small cell lung cancer. J Immunother Cancer 10: e005116, 2022.
16. Liu Z, Wang T, She Y, Wu K, Gu S, Li L, Dong C, Chen C and Zhou Y: N^6-methyladenosine-modified circIGF2BP3 inhibits $CD8^+$ T-cell responses to facilitate tumor immune evasion by promoting the deubiquitination of PD-L1 in non-small cell lung cancer. Mol Cancer 20: 105, 2021.
17. Wu L, Zhao N, Zhou Z, Chen J, Han S, Zhang X, Bao H, Yuan W and Shu X: PLAGL2 promotes the proliferation and migration of gastric cancer cells via USP37-mediated deubiquitination of Snail1. Theranostics 11: 700-714, 2021.
18. Xie H, Zhou J, Liu X, Xu Y, Hepperla AJ, Simon JM, Wang T, Yao H, Liao C, Baldwin AS, *et al*: USP13 promotes deubiquitination of ZHX2 and tumorigenesis in kidney cancer. Proc Natl Acad Sci USA 119: e2119854119, 2022.
19. Rasaei R, Sarodaya N, Kim KS, Ramakrishna S and Hong SH: Importance of deubiquitination in macrophage-mediated viral response and inflammation. Int J Mol Sci 21: 8090, 2020.
20. Sun T, Liu Z and Yang Q: The role of ubiquitination and deubiquitination in cancer metabolism. Mol Cancer 19: 146, 2020.

21. Cai J, Culley MK, Zhao Y and Zhao J: The role of ubiquitination and deubiquitination in the regulation of cell junctions. Protein Cell 9: 754-769, 2018.
22. Zhou Y, Park SH and Chua NH: UBP12/UBP13-mediated deubiquitination of salicylic acid receptor NPR3 suppresses plant immunity. Mol Plant 16: 232-244, 2023.
23. Chen S, Liu Y and Zhou H: Advances in the development ubiquitin-specific peptidase (USP) inhibitors. Int J Mol Sci 22: 4546, 2021.
24. Sato Y, Goto E, Shibata Y, Kubota Y, Yamagata A, Goto-Ito S, Kubota K, Inoue J, Takekawa M, Tokunaga F and Fukai S: Structures of CYLD USP with Met1- or Lys63-linked diubiquitin reveal mechanisms for dual specificity. Nat Struct Mol Biol 22: 222-229, 2015.
25. Maertens GN, El Messaoudi-Aubert S, Elderkin S, Hiom K and Peters G: Ubiquitin-specific proteases 7 and 11 modulate Polycomb regulation of the INK4a tumour suppressor. EMBO J 29: 2553-2565, 2010.
26. Cruz L, Soares P and Correia M: Ubiquitin-Specific proteases: Players in cancer cellular processes. Pharmaceuticals (Basel) 14: 848, 2021.
27. Mansilla A, Martin FA, Martin D, and Ferrus A: Ligand-independent requirements of steroid receptors EcR and USP for cell survival. Cell Death Differ 23: 405-416, 2016.
28. An Z, Liu Y, Ou Y, Li J, Zhang B, Sun D, Sun Y and Tang W: Regulation of the stability of RGF1 receptor by the ubiquitin-specific proteases UBP12/UBP13 is critical for root meristem maintenance. Proc Natl Acad Sci USA 115: 1123-1128, 2018.
29. Lim JH, Jono H, Komatsu K, Woo CH, Lee J, Miyata M, Matsuno T, Xu X, Huang Y, Zhang W, *et al*: CYLD negatively regulates transforming growth factor-β-signalling via deubiquitinating Akt. Nat Commun 3: 771, 2012.
30. Bonacci T and Emanuele MJ: Dissenting degradation: Deubiquitinases in cell cycle and cancer. Semin Cancer Biol 67(Pt 2): 145-158, 2020.
31. Wang X, Xia S, Li H, Wang X, Li C, Chao Y, Zhang L and Han C: The deubiquitinase USP10 regulates KLF4 stability and suppresses lung tumorigenesis. Cell Death Differ 27: 1747-1764, 2020.
32. Baek SH, Choi KS, Yoo YJ, Cho JM, Baker RT, Tanaka K and Chung CH: Molecular cloning of a novel ubiquitin-specific protease, UBP41, with isopeptidase activity in chick skeletal muscle. J Biol Chem 272: 25560-25565, 1997.
33. Gousseva N and Baker RT: Gene structure, alternate splicing, tissue distribution, cellular localization, and developmental expression pattern of mouse deubiquitinating enzyme isoforms Usp2-45 and Usp2-69. Gene Expr 11: 163-179, 2003.
34. Moremen KW, Touster O and Robbins PW: Novel purification of the catalytic domain of Golgi alpha-mannosidase II. Characterization and comparison with the intact enzyme. J Biol Chem 266: 16876-16885, 1991.
35. Gerhard DS, Wagner L, Feingold EA, Shenmen CM, Grouse LH, Schuler G, Klein SL, Old S, Rasooly R, Good P, *et al*: The status, quality, and expansion of the NIH full-length cDNA project: The Mammalian Gene Collection (MGC). Genome Res 14 (10B): 2121-2127, 2004.
36. Ota T, Suzuki Y, Nishikawa T, Otsuki T, Sugiyama T, Irie R, Wakamatsu A, Hayashi K, Sato H, Nagai K, *et al*: Complete sequencing and characterization of 21,243 full-length human cDNAs. Nat Genet 36: 40-45, 2004.
37. Luo H, Ji Y, Gao X, Liu X, Wu Y and Wu Y: Ubiquitin specific protease 2: Structure, isoforms, cellular function, relateddiseases and its inhibitors. Oncologie 24: 85-99, 2022.
38. Zhu HQ and Gao FH: The molecular mechanisms of regulation on USP2's alternative splicing and the significance of its products. Int J Biol Sci 13: 1489-1496, 2017.
39. Pouly D, Chenaux S, Martin V, Babis M, Koch R, Nagoshi E, Katanaev VL, Gachon F and Staub O: USP2-45 is a circadian clock output effector regulating calcium absorption at the post-translational level. PLoS One 11: e0145155, 2016.
40. Tong X, Buelow K, Guha A, Rausch R and Yin L: USP2a protein deubiquitinates and stabilizes the circadian protein CRY1 in response to inflammatory signals. J Biol Chem 287: 25280-25291, 2012.
41. Molusky MM, Li S, Ma D, Yu L and Lin JD: Ubiquitin-specific protease 2 regulates hepatic gluconeogenesis and diurnal glucose metabolism through 11β-hydroxysteroid dehydrogenase 1. Diabetes 61: 1025-1035, 2012.
42. Kitamura H, Kimura S, Shimamoto Y, Okabe J, Ito M, Miyamoto T, Naoe Y, Kikuguchi C, Meek B, Toda C, *et al*: Ubiquitin-specific protease 2-69 in macrophages potentially modulates metainflammation. FASEB J 27: 4940-4953, 2013.
43. Wang S, Wu H, Liu Y, Sun J, Zhao Z, Chen Q, Guo M, Ma D and Zhang Z: Expression of USP2-69 in mesangial cells in vivo and in vitro. Pathol Int 60: 184-192, 2010.
44. Haimerl F, Erhardt A, Sass G and Tiegs G: Down-regulation of the de-ubiquitinating enzyme ubiquitin-specific protease 2 contributes to tumor necrosis factor-alpha-induced hepatocyte survival. J Biol Chem 284: 495-504, 2009.
45. Li Y, Kim BG, Qian S, Letterio JJ, Fung JJ, Lu L and Lin F: Hepatic stellate cells inhibit T cells through active TGF-β1 from a cell surface-bound latent TGF-β1/GARP complex. J Immunol 195: 2648-2656, 2015.
46. Mao X, Luo W, Sun J, Yang N, Zhang LW, Zhao Z, Zhang Z and Wu H: Usp2-69 overexpression slows down the progression of rat anti-Thy1.1 nephritis. Exp Mol Pathol 101: 249-258, 2016.
47. Kitamura H, Ishino T, Shimamoto Y, Okabe J, Miyamoto T, Takahashi E and Miyoshi I: Ubiquitin-Specific protease 2 modulates the lipopolysaccharide-elicited expression of proinflammatory cytokines in macrophage-like HL-60 cells. Mediators Inflamm 2017: 6909415, 2017.
48. Mahul-Mellier AL, Datler C, Pazarentzos E, Lin B, Chaisaklert W, Abuali G and Grimm S: De-ubiquitinating proteases USP2a and USP2c cause apoptosis by stabilising RIP1. Biochim Biophys Acta 1823: 1353-1365, 2012.
49. Davis MI, Pragani R, Fox JT, Shen M, Parmar K, Gaudiano EF, Liu L, Tanega C, McGee L, Hall MD, *et al*: Small molecule inhibition of the ubiquitin-specific protease USP2 Accelerates cyclin D1 degradation and leads to cell cycle arrest in colorectal cancer and mantle cell lymphoma models. J Biol Chem 291: 24628-24640, 2016.
50. Bedard N, Yang Y, Gregory M, Cyr DG, Suzuki J, Yu X, Chian RC, Hermo L, O'Flaherty C, Smith CE, *et al*: Mice lacking the USP2 deubiquitinating enzyme have severe male subfertility associated with defects in fertilization and sperm motility. Biol Reprod 85: 594-604, 2011.
51. Xu Q, Liu M, Zhang F, Liu X, Ling S, Chen X, Gu J, Ou W, Liu S and Liu N: Ubiquitin-specific protease 2 regulates Ang II-induced cardiac fibroblasts activation by up-regulating cyclin D1 and stabilizing β-catenin in vitro. J Cell Mol Med 25: 1001-1011, 2021.
52. Hashimoto M, Fujimoto M, Konno K, Lee ML, Yamada Y, Yamashita K, Toda C, Tomura M, Watanabe M, Inanami O and Kitamura H: Ubiquitin-Specific protease 2 in the ventromedial hypothalamus modifies blood glucose levels by controlling sympathetic nervous activation. J Neurosci 42: 4607-4618, 2022.
53. Liu XQ, Shao XR, Liu Y, Dong ZX, Chan SH, Shi YY, Chen SN, Qi L, Zhong L, Yu Y, *et al*: Tight junction protein 1 promotes vasculature remodeling via regulating USP2/TWIST1 in bladder cancer. Oncogene 41: 502-514, 2022.
54. Tu Y, Xu L, Xu J, Bao Z, Tian W, Ye Y, Sun G, Miao Z, Chao H, You Y, *et al*: Loss of deubiquitylase USP2 triggers development of glioblastoma via TGF-β signaling. Oncogene 41: 2597-2608, 2022.
55. Nadolny C, Zhang X, Chen Q, Hashmi SF, Ali W, Hemme C, Ahsan N, Chen Y and Deng R: Dysregulation and activities of ubiquitin specific peptidase 2b in the pathogenesis of hepatocellular carcinoma. Am J Cancer Res 11: 4746-4767, 2021.
56. Zhang J, Liu S, Li Q, Shi Y, Wu Y, Liu F, Wang S, Zaky MY, Yousuf W, Sun Q, *et al*: The deubiquitylase USP2 maintains ErbB2 abundance via counteracting endocytic degradation and represents a therapeutic target in ErbB2-positive breast cancer. Cell Death Differ 27: 2710-2725, 2020.
57. Qu Q, Mao Y, Xiao G, Fei X, Wang J, Zhang Y, Liu J, Cheng G, Chen X, Wang J and Shen K: USP2 promotes cell migration and invasion in triple negative breast cancer cell lines. Tumour Biol 36: 5415-5423, 2015.
58. Liang XR, Liu YF, Chen F, Zhou ZX, Zhang LJ and Lin ZJ: Cell Cycle-Related lncRNAs as innovative targets to advance cancer management. Cancer Manag Res 15: 547-561, 2023.
59. Xiao W, Wang J, Wang X, Cai S, Guo Y, Ye L, Li D, Hu A, Jin S, Yuan B, *et al*: Therapeutic targeting of the USP2-E2F4 axis inhibits autophagic machinery essential for zinc homeostasis in cancer progression. Autophagy 18: 2615-2635, 2022.
60. Zhu M, Wang H, Ding Y, Yang Y, Xu Z, Shi L and Zhang N: Ribonucleotide reductase holoenzyme inhibitor COH29 interacts with deubiquitinase ubiquitin-specific protease 2 and downregulates its substrate protein cyclin D1. FASEB J 36: e22329, 2022.

61. Shan J, Zhao W and Gu W: Suppression of cancer cell growth by promoting cyclin D1 degradation. Mol Cell 36: 469-476, 2009.
62. Magiera K, Tomala M, Kubica K, De Cesare V, Trost M, Zieba BJ, Kachamakova-Trojanowska N, Les M, Dubin G, Holak TA and Skalniak L: Lithocholic acid hydroxyamide destabilizes cyclin D1 and Induces G (0)/G (1) arrest by inhibiting deubiquitinase USP2a. Cell Chem Biol 24: 458-470 e18, 2017.
63. Nepal S, Shrestha A and Park PH: Ubiquitin specific protease 2 acts as a key modulator for the regulation of cell cycle by adiponectin and leptin in cancer cells. Mol Cell Endocrinol 412: 44-55, 2015.
64. Tomala MD, Magiera-Mularz K, Kubica K, Krzanik S, Zieba B, Musielak B, Pustula M, Popowicz GM, Sattler M, Dubin G, *et al*: Identification of small-molecule inhibitors of USP2a. Eur J Med Chem 150: 261-267, 2018.
65. Kim J, Kim WJ, Liu Z, Loda M and Freeman MR: The ubiquitin-specific protease USP2a enhances tumor progression by targeting cyclin A1 in bladder cancer. Cell Cycle 11: 1123-1130, 2012.
66. Gabay M, Li Y and Felsher DW: MYC activation is a hallmark of cancer initiation and maintenance. Cold Spring Harb Perspect Med 4: a014241, 2014.
67. Stine ZE, Walton ZE, Altman BJ, Hsieh AL and Dang CV: MYC, Metabolism, and Cancer. Cancer Discov 5: 1024-1039, 2015.
68. Wu CH, van Riggelen J, Yetil A, Fan AC, Bachireddy P and Felsher DW: Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation. Proc Natl Acad Sci USA 104: 13028-13033, 2007.
69. Zhuang D, Mannava S, Grachtchouk V, Tang WH, Patil S, Wawrzyniak JA, Berman AE, Giordano TJ, Prochownik EV, Soengas MS and Nikiforov MA: C-MYC overexpression is required for continuous suppression of oncogene-induced senescence in melanoma cells. Oncogene 27: 6623-6634, 2008.
70. Li B, Zhang G, Wang Z, Yang Y, Wang C, Fang D, Liu K, Wang F and Mei Y: c-Myc-activated USP2-AS1 suppresses senescence and promotes tumor progression via stabilization of E2F1 mRNA. Cell Death Dis 12: 1006, 2021.
71. Iemura K, Natsume T, Maehara K, Kanemaki MT and Tanaka K: Chromosome oscillation promotes Aurora A-dependent Hec1 phosphorylation and mitotic fidelity. J Cell Biol 220: e202006116, 2021.
72. Li P, Chen T, Kuang P, Liu F, Li Z, Liu F, Wang Y, Zhang W and Cai X: Aurora-A/FOXO3A/SKP2 axis promotes tumor progression in clear cell renal cell carcinoma and dual-targeting Aurora-A/SKP2 shows synthetic lethality. Cell Death Dis 13: 606, 2022.
73. Shi Y, Solomon LR, Pereda-Lopez A, Giranda VL, Luo Y, Johnson EF, Shoemaker AR, Leverson J and Liu X: Ubiquitin-specific cysteine protease 2a (USP2a) regulates the stability of Aurora-A. J Biol Chem 286: 38960-38968, 2011.
74. Gu Y, Zhang Z, Camps MGM, Ossendorp F, Wijdeven RH and Ten Dijke P: Genome-wide CRISPR screens define determinants of epithelial-mesenchymal transition mediated immune evasion by pancreatic cancer cells. Sci Adv 9: eadf9915, 2023.
75. Chen J, Ding ZY, Li S, Liu S, Xiao C, Li Z, Zhang BX, Chen XP and Yang X: Targeting transforming growth factor-β signaling for enhanced cancer chemotherapy. Theranostics 11: 1345-1363, 2021.
76. He Q, Cao H, Zhao Y, Chen P, Wang N, Li W, Cui R, Hou P, Zhang X and Ji M: Dipeptidyl Peptidase-4 Stabilizes Integrin alpha4β1 complex to promote thyroid cancer cell metastasis by activating transforming growth factor-beta signaling pathway. Thyroid 32: 1411-1422, 2022.
77. Tuersuntuoheti A, Li Q, Teng Y, Li X, Huang R, Lu Y, Li K, Liang J, Miao S, Wu W and Song W: YWK-II/APLP2 inhibits TGF-β signaling by interfering with the TGFBR2-Hsp90 interaction. Biochim Biophys Acta Mol Cell Res: Jul 19, 2023 (Epub ahead of print).
78. Miyazawa K and Miyazono K: Regulation of TGF-β family signaling by inhibitory smads. Cold Spring Harb Perspect Biol 9: a022095, 2017.
79. Zhao Y, Wang X, Wang Q, Deng Y, Li K, Zhang M, Zhang Q, Zhou J, Wang HY, Bai P, *et al*: USP2a supports metastasis by tuning TGF-β signaling. Cell Rep 22: 2442-2454, 2018.
80. Blenman KRM, Marczyk M, Karn T, Qing T, Li X, Gunasekharan V, Yaghoobi V, Bai Y, Ibrahim EY, Park T, *et al*: Predictive markers of response to neoadjuvant durvalumab with nab-paclitaxel and dose-dense doxorubicin/cyclophosphamide in basal-like triple-negative breast cancer. Clin Cancer Res 28: 2587-2597, 2022.
81. Cui Y, Zhao M, Yang Y, Xu R, Tong L, Liang J, Zhang X, Sun Y and Fan Y: Reversal of epithelial-mesenchymal transition and inhibition of tumor stemness of breast cancer cells through advanced combined chemotherapy. Acta Biomater 152: 380-392, 2022.
82. Ahangari F, Becker C, Foster DG, Chioccioli M, Nelson M, Beke K, Wang X, Justet A, Adams T, Readhead B, *et al*: Saracatinib, a selective src kinase inhibitor, blocks fibrotic responses in preclinical models of pulmonary fibrosis. Am J Respir Crit Care Med 206: 1463-1479, 2022.
83. van der Wal T and van Amerongen R: Walking the tight wire between cell adhesion and WNT signalling: A balancing act for beta-catenin. Open Biol 10: 200267, 2020.
84. Kim J, Alavi Naini F, Sun Y and Ma L: Ubiquitin-specific peptidase 2a (USP2a) deubiquitinates and stabilizes β-catenin. Am J Cancer Res 8: 1823-1836. eCollection 2018, 2018.
85. Pichiorri F, Suh SS, Rocci A, De Luca L, Taccioli C, Santhanam R, Zhou W, Benson DM Jr, Hofmainster C, Alder H, *et al*: Retraction notice to: Downregulation of p53-inducible microRNAs 192, 194, and 215 Impairs the p53/MDM2 autoregulatory loop in multiple myeloma development. Cancer Cell 40: 1441, 2022.
86. Wu B and Ellisen LW: Loss of p53 and genetic evolution in pancreatic cancer: Ordered chaos after the guardian is gone. Cancer Cell 40: 1276-1278, 2022.
87. Hassin O and Oren M: Drugging p53 in cancer: One protein, many targets. Nat Rev Drug Discov 22: 127-144, 2023.
88. Dobbelstein M and Levine AJ: Mdm2: Open questions. Cancer Sci 111: 2203-2211, 2020.
89. Stevenson LF, Sparks A, Allende-Vega N, Xirodimas DP, Lane DP and Saville MK: The deubiquitinating enzyme USP2a regulates the p53 pathway by targeting Mdm2. EMBO J 26: 976-986, 2007.
90. Wei T, Biskup E, Gjerdrum LM, Niazi O, Odum N and Gniadecki R: Ubiquitin-specific protease 2 decreases p53-dependent apoptosis in cutaneous T-cell lymphoma. Oncotarget 7: 48391-48400, 2016.
91. Wang CL, Wang JY, Liu ZY, Ma XM, Wang XW, Jin H, Zhang XP, Fu D, Hou LJ and Lu YC: Ubiquitin-specific protease 2a stabilizes MDM4 and facilitates the p53-mediated intrinsic apoptotic pathway in glioblastoma. Carcinogenesis 35: 1500-1509, 2014.
92. Shrestha M and Park PH: p53 signaling is involved in leptin-induced growth of hepatic and breast cancer cells. Korean J Physiol Pharmacol 20: 487-498, 2016.
93. Chen W, Shi K, Liu J, Yang P, Han R, Pan M, Yuan L, Fang C, Yu Y and Qian Z: Sustained co-delivery of 5-fluorouracil and cis-platinum via biodegradable thermo-sensitive hydrogel for intraoperative synergistic combination chemotherapy of gastric cancer. Bioact Mater 23: 1-15, 2023.
94. Wang J, Zhang Y, Zhang G, Xiang L, Pang H, Xiong K, Lu Y, Li J, Dai J, Lin S and Fu S: Radiotherapy-induced enrichment of EGF-modified doxorubicin nanoparticles enhances the therapeutic outcome of lung cancer. Drug Deliv 29: 588-599, 2022.
95. Smith ER, Wang JQ, Yang DH and Xu XX: Paclitaxel resistance related to nuclear envelope structural sturdiness. Drug Resist Updat 65: 100881, 2022.
96. Jang JH, Lee TJ, Sung EG, Song IH and Kim JY: Dapagliflozin induces apoptosis by downregulating $cFILP_L$ and increasing $cFILP_S$ instability in Caki-1 cells. Oncol Lett 24: 401, 2022.
97. Poukkula M, Kaunisto A, Hietakangas V, Denessiouk K, Katajamaki T, Johnson MS, Sistonen L and Eriksson JE: Rapid turnover of c-FLIPshort is determined by its unique C-terminal tail. J Biol Chem 280: 27345-27355, 2005.
98. Liu D, Fan Y, Li J, Cheng B, Lin W, Li X, Du J and Ling C: Inhibition of cFLIP overcomes acquired resistance to sorafenib via reducing ER stress-related autophagy in hepatocellular carcinoma. Oncol Rep 40: 2206-2214, 2018.
99. Iyer AK, Azad N, Talbot S, Stehlik C, Lu B, Wang L and Rojanasakul Y: Antioxidant c-FLIP inhibits Fas ligand-induced NF-kappaB activation in a phosphatidylinositol 3-kinase/Akt-dependent manner. J Immunol 187: 3256-3266, 2011.
100. Quintavalle C, Incoronato M, Puca L, Acunzo M, Zanca C, Romano G, Garofalo M, Iaboni M, Croce CM and Condorelli G: c-FLIPL enhances anti-apoptotic Akt functions by modulation of Gsk3β activity. Cell Death Differ 24: 1134, 2017.
101. Xiao Z, Liu Y, Li Q, Liu Q, Liu Y, Luo Y and Wei S: EVs delivery of miR-1915-3p improves the chemotherapeutic efficacy of oxaliplatin in colorectal cancer. Cancer Chemother Pharmacol 88: 1021-1031, 2021.
102. He J, Lee HJ, Saha S, Ruan D, Guo H and Chan CH: Inhibition of USP2 eliminates cancer stem cells and enhances TNBC responsiveness to chemotherapy. Cell Death Dis 10: 285, 2019.

103. Min HY and Lee HY: Molecular targeted therapy for anticancer treatment. Exp Mol Med 54: 1670-1694, 2022.
104. Assoun S, Lemiale V and Azoulay E: Molecular targeted therapy-related life-threatening toxicity in patients with malignancies. A systematic review of published cases. Intensive Care Med 45: 988-997, 2019.
105. Rosenberg T, Yeo KK, Mauguen A, Alexandrescu S, Prabhu SP, Tsai JW, Malinowski S, Joshirao M, Parikh K, Farouk Sait S, *et al*: Upfront molecular targeted therapy for the treatment of BRAF-mutant pediatric high-grade glioma. Neuro Oncol 24: 1964-1975, 2022.
106. Harakandi C, Nininahazwe L, Xu H, Liu B, He C, Zheng YC and Zhang H: Recent advances on the intervention sites targeting USP7-MDM2-p53 in cancer therapy. Bioorg Chem 116: 105273, 2021.
107. Huang YT, Cheng AC, Tang HC, Huang GC, Cai L, Lin TH, Wu KJ, Tseng PH, Wang GG and Chen WY: USP7 facilitates SMAD3 autoregulation to repress cancer progression in p53-deficient lung cancer. Cell Death Dis 12: 880, 2021.
108. Park SH, Fong KW, Kim J, Wang F, Lu X, Lee Y, Brea LT, Wadosky K, Guo C, Abdulkadir SA, *et al*: Posttranslational regulation of FOXA1 by Polycomb and BUB3/USP7 deubiquitin complex in prostate cancer. Sci Adv 7: eabe2261, 2021.
109. Su D, Wang W, Hou Y, Wang L, Yi X, Cao C, Wang Y, Gao H, Wang Y, Yang C, *et al*: Bimodal regulation of the PRC2 complex by USP7 underlies tumorigenesis. Nucleic Acids Res 49: 4421-4440, 2021.
110. Zhang L, Wang H, Tian L and Li H: Expression of USP7 and MARCH7 is correlated with poor prognosis in epithelial ovarian cancer. Tohoku J Exp Med 239: 165-175, 2016.
111. Liu M, Zhang Y, Wu Y, Jin J, Cao Y, Fang Z, Geng L, Yang L, Yu M, Bu Z, *et al*: IKZF1 selectively enhances homologous recombination repair by interacting with CtIP and USP7 in multiple myeloma. Int J Biol Sci 18: 2515-2526, 2022.
112. Zhu Y, Gu L, Lin X, Cui K, Liu C, Lu B, Zhou F, Zhao Q, Shen H and Li Y: LINC00265 promotes colorectal tumorigenesis via ZMIZ2 and USP7-mediated stabilization of β-catenin. Cell Death Differ 27: 1316-1327, 2020.
113. Ullah S, Junaid M, Liu Y, Chen S, Zhao Y and Wadood A: Validation of catalytic site residues of Ubiquitin Specific Protease 2 (USP2) by molecular dynamic simulation and novel kinetics assay for rational drug design. Mol Divers 27: 1323-1332, 2023.
114. Metzig M, Nickles D, Falschlehner C, Lehmann-Koch J, Straub BK, Roth W and Boutros M: An RNAi screen identifies USP2 as a factor required for TNF-α-induced NF-κB signaling. Int J Cancer 129: 607-618, 2011.
115. Shi J, Wang Y, Zeng L, Wu Y, Deng J, Zhang Q, Lin Y, Li J, Kang T, Tao M, *et al*: Disrupting the interaction of BRD4 with diacetylated Twist suppresses tumorigenesis in basal-like breast cancer. Cancer Cell 25: 210-225, 2014.
116. Peinado H and Cano A: A hypoxic twist in metastasis. Nat Cell Biol 10: 253-254, 2008.
117. Mladinich M, Ruan D and Chan CH: Tackling cancer stem cells via inhibition of EMT transcription factors. Stem Cells Int 2016: 5285892, 2016.
118. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, *et al*: The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133: 704-715, 2008.
119. Kim JY, Cho TM, Park JM, Park S, Park M, Nam KD, Ko D, Seo J, Kim S, Jung E, *et al*: A novel HSP90 inhibitor SL-145 suppresses metastatic triple-negative breast cancer without triggering the heat shock response. Oncogene 41: 3289-3297, 2022.
120. Shih YY, Lin HY, Jan HM, Chen YJ, Ong LL, Yu AL and Lin CH: S-glutathionylation of Hsp90 enhances its degradation and correlates with favorable prognosis of breast cancer. Redox Biol 57: 102501, 2022.
121. Leow CC, Chesebrough J, Coffman KT, Fazenbaker CA, Gooya J, Weng D, Coats S, Jackson D, Jallal B and Chang Y: Antitumor efficacy of IPI-504, a selective heat shock protein 90 inhibitor against human epidermal growth factor receptor 2-positive human xenograft models as a single agent and in combination with trastuzumab or lapatinib. Mol Cancer Ther 8: 2131-2141, 2009.
122. Workman P, Burrows F, Neckers L and Rosen N: Drugging the cancer chaperone HSP90: Combinatorial therapeutic exploitation of oncogene addiction and tumor stress. Ann N Y Acad Sci 1113: 202-216, 2007.
123. Modi S, Stopeck A, Linden H, Solit D, Chandarlapaty S, Rosen N, D'Andrea G, Dickler M, Moynahan ME, Sugarman S, *et al*: HSP90 inhibition is effective in breast cancer: A phase II trial of tanespimycin (17-AAG) plus trastuzumab in patients with HER2-positive metastatic breast cancer progressing on trastuzumab. Clin Cancer Res 17: 5132-5139, 2011.
124. Sesto N, Wurtzel O, Archambaud C, Sorek R and Cossart P: The excludon: A new concept in bacterial antisense RNA-mediated gene regulation. Nat Rev Microbiol 11: 75-82, 2013.
125. Qu X, Alsager S, Zhuo Y and Shan B: HOX transcript antisense RNA (HOTAIR) in cancer. Cancer Lett 454: 90-97, 2019.
126. Chen SP, Zhu GQ, Xing XX, Wan JL, Cai JL, Du JX, Song LN, Dai Z and Zhou J: LncRNA USP2-AS1 promotes hepatocellular carcinoma growth by enhancing YBX1-Mediated HIF1α protein translation under hypoxia. Front Oncol 12: 882372, 2022.
127. Pirnia F, Schneider E, Betticher DC and Borner MM: Mitomycin C induces apoptosis and caspase-8 and -9 processing through a caspase-3 and Fas-independent pathway. Cell Death Differ 9: 905-914, 2002.
128. Wang WD, Shang Y, Wang C, Ni J, Wang AM, Li GJ, Su L and Chen SZ: c-FLIP promotes drug resistance in non-small-cell lung cancer cells via upregulating FoxM1 expression. Acta Pharmacol Sin 43: 2956-2966, 2022.
129. Yang Y, Hou JQ, Qu LY, Wang GQ, Ju HW, Zhao ZW, Yu ZH and Yang HJ: Differential expression of USP2, USP14 and UBE4A between ovarian serous cystadenocarcinoma and adjacent normal tissues. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 23: 504-506, 2007 (In Chinese).
130. Guo B, Yu L, Sun Y, Yao N and Ma L: Long Non-Coding RNA USP2-AS1 accelerates cell proliferation and migration in ovarian cancer by sponging miR-520d-3p and Up-Regulating KIAA1522. Cancer Manag Res 12: 10541-10550, 2020.
131. Li D, Bao J, Yao J and Li J: lncRNA USP2-AS1 promotes colon cancer progression by modulating Hippo/YAP1 signaling. Am J Transl Res 12: 5670-5682. eCollection 2020, 2020.
132. Tatari N, Khan S, Livingstone J, Zhai K, McKenna D, Ignatchenko V, Chokshi C, Gwynne WD, Singh M, Revill S, *et al*: The proteomic landscape of glioblastoma recurrence reveals novel and targetable immunoregulatory drivers. Acta Neuropathol 144: 1127-1142, 2022.
133. Ji YR, Cheng CC, Lee AL, Shieh JC, Wu HJ, Huang AP, Hsu YH and Young TH: Poly (allylguanidine)-coated surfaces regulate TGF-β in glioblastoma cells to induce apoptosis via NF-κB Pathway Activation. ACS Appl Mater Interfaces 13: 59400-59410, 2021.
134. Joseph JV, Magaut CR, Storevik S, Geraldo LH, Mathivet T, Latif MA, Rudewicz J, Guyon J, Gambaretti M, Haukas F, *et al*: TGF-β promotes microtube formation in glioblastoma through thrombospondin 1. Neuro Oncol 24: 541-553, 2022.
135. Yan X, Liao H, Cheng M, Shi X, Lin X, Feng XH and Chen YG: Smad7 protein interacts with receptor-regulated smads (R-Smads) to inhibit transforming growth factor-β (TGF-β)/smad signaling. J Biol Chem 291: 382-392, 2016.
136. Girish V, Lakhani AA, Thompson SL, Scaduto CM, Brown LM, Hagenson RA, Sausville EL, Mendelson BE, Kandikuppa PK, Lukow DA, *et al*: Oncogene-like addiction to aneuploidy in human cancers. Science: Jul 6, 2023 (Epub ahead of print).
137. Mejia-Hernandez JO, Raghu D, Caramia F, Clemons N, Fujihara K, Riseborough T, Teunisse A, Jochemsen AG, Abrahmsén L, Blandino G, *et al*: Targeting MDM4 as a novel therapeutic approach in prostate cancer independent of p53 status. Cancers (Basel) 14: 3947, 2022.
138. Tsai KW, Kuo WT and Jeng SY: Tight junction protein 1 dysfunction contributes to cell motility in bladder cancer. Anticancer Res 38: 4607-4615, 2018.
139. Chuang SJ, Cheng SC, Tang HC, Sun CY and Chou CY: 6-Thioguanine is a noncompetitive and slow binding inhibitor of human deubiquitinating protease USP2. Sci Rep 8: 3102, 2018.
140. Renatus M, Parrado SG, D'Arcy A, Eidhoff U, Gerhartz B, Hassiepen U, Pierrat B, Riedl R, Vinzenz D, Worpenberg S and Kroemer M: Structural basis of ubiquitin recognition by the deubiquitinating protease USP2. Structure 14: 1293-1302, 2006.
141. Kitamura H and Hashimoto M: USP2-Related cellular signaling and consequent pathophysiological outcomes. Int J Mol Sci 22: 1209, 2021.
142. Graner E, Tang D, Rossi S, Baron A, Migita T, Weinstein LJ, Lechpammer M, Huesken D, Zimmermann J, Signoretti S and Loda M: The isopeptidase USP2a regulates the stability of fatty acid synthase in prostate cancer. Cancer Cell 5: 253-261, 2004.

143. Cheng JC, Bai A, Beckham TH, Marrison ST, Yount CL, Young K, Lu P, Bartlett AM, Wu BX, Keane BJ, *et al*: Radiation-induced acid ceramidase confers prostate cancer resistance and tumor relapse. J Clin Invest 123: 4344-4358, 2013.
144. Mizutani N, Inoue M, Omori Y, Ito H, Tamiya-Koizumi K, Takagi A, Kojima T, Nakamura M, Iwaki S, Nakatochi M, *et al*: Increased acid ceramidase expression depends on upregulation of androgen-dependent deubiquitinases, USP2, in a human prostate cancer cell line, LNCaP. J Biochem 158: 309-319, 2015.
145. Vieyra-Garcia PA and Wolf P: A deep dive into UV-based phototherapy: Mechanisms of action and emerging molecular targets in inflammation and cancer. Pharmacol Ther 222: 107784, 2021.
146. Nakahashi K, Nihira K, Suzuki M, Ishii T, Masuda K and Mori K: A novel mouse model of cutaneous T-cell lymphoma revealed the combined effect of mogamulizumab with psoralen and ultraviolet a therapy. Exp Dermatol 31: 1693-1698, 2022.
147. Hsu J and Sage J: Novel functions for the transcription factor E2F4 in development and disease. Cell Cycle 15: 3183-3190, 2016.
148. Moghadami AA, Aboutalebi Vand Beilankouhi E, Kalantary-Charvadeh A, Hamzavi M, Mosayyebi B, Sedghi H, Ghorbani Haghjo A and Nazari Soltan Ahmad S: Inhibition of USP14 induces ER stress-mediated autophagy without apoptosis in lung cancer cell line A549. Cell Stress Chaperones 25: 909-917, 2020.
149. Liu C, Chen Z, Ding X, Qiao Y and Li B: Ubiquitin-specific protease 35 (USP35) mediates cisplatin-induced apoptosis by stabilizing BIRC3 in non-small cell lung cancer. Lab Invest 102: 524-533, 2022.
150. Chen J, Dexheimer TS, Ai Y, Liang Q, Villamil MA, Inglese J, Maloney DJ, Jadhav A, Simeonov A and Zhuang Z: Selective and cell-active inhibitors of the USP1/UAF1 deubiquitinase complex reverse cisplatin resistance in non-small cell lung cancer cells. Chem Biol 18: 1390-1400, 2011.
151. Zhou F, Du C, Xu D, Lu J, Zhou L, Wu C, Wu B and Huang J: Knockdown of ubiquitin-specific protease 51 attenuates cisplatin resistance in lung cancer through ubiquitination of zinc-finger E-box binding homeobox 1. Mol Med Rep 22: 1382-1390, 2020.
152. Zhang L, Xu B, Qiang Y, Huang H, Wang C, Li D and Qian J: Overexpression of deubiquitinating enzyme USP28 promoted non-small cell lung cancer growth. J Cell Mol Med 19: 799-805, 2015.
153. Zhang Z, Cui Z, Xie Z, Li C, Xu C, Guo X, Yu J, Chen T, Facchinetti F, Bohnenberger H, *et al*: Deubiquitinase USP5 promotes non-small cell lung cancer cell proliferation by stabilizing cyclin D1. Transl Lung Cancer Res 10: 3995-4011, 2021.
154. Zhu M, Zhang H, Lu F, Wang Z, Wu Y, Chen H, Fan X, Yin Z and Liang F: USP52 inhibits cell proliferation by stabilizing PTEN protein in non-small cell lung cancer. Biosci Rep 41: BSR20210486, 2021.
155. Zhang F, Zhao Y and Sun Y: USP2 is an SKP2 deubiquitylase that stabilizes both SKP2 and its substrates. J Biol Chem 297: 101109, 2021.
156. Zhou J, Wang T, Qiu T, Chen Z, Ma X, Zhang L and Zou J: Ubiquitin-specific protease-44 inhibits the proliferation and migration of cells via inhibition of JNK pathway in clear cell renal cell carcinoma. BMC Cancer 20: 214, 2020.
157. Hu W, Su Y, Fei X, Wang X, Zhang G, Su C, Du T, Yang T, Wang G, Tang Z and Zhang J: Ubiquitin specific peptidase 19 is a prognostic biomarker and affect the proliferation and migration of clear cell renal cell carcinoma. Oncol Rep 43: 1964-1974, 2020.
158. Meng X, Xiong Z, Xiao W, Yuan C, Wang C, Huang Y, Tong J, Shi J, Chen Z, Liu C, *et al*: Downregulation of ubiquitin-specific protease 2 possesses prognostic and diagnostic value and promotes the clear cell renal cell carcinoma progression. Ann Transl Med 8: 319, 2020.
159. Yi J, Tavana O, Li H, Wang D, Baer RJ and Gu W: Targeting USP2 regulation of VPRBP-mediated degradation of p53 and PD-L1 for cancer therapy. Nat Commun 14: 1941, 2023.
160. Zhu L, Chen Z, Guo T, Chen W, Zhao L, Guo L and Pan X: USP2 inhibits lung cancer pathogenesis by reducing ARID2 protein degradation via ubiquitination. Biomed Res Int 2022: 1525216, 2022.
161. Estlin EJ: Continuing therapy for childhood acute lymphoblastic leukaemia: Clinical and cellular pharmacology of methotrexate, 6-mercaptopurine and 6-thioguanine. Cancer Treat Rev 27: 351-363, 2001.
162. Vora A, Mitchell CD, Lennard L, Eden TO, Kinsey SE, Lilleyman J and Richards SM; Medical Research Council; National Cancer Research Network Childhood Leukaemia Working Party: Toxicity and efficacy of 6-thioguanine versus 6-mercaptopurine in childhood lymphoblastic leukaemia: A randomised trial. Lancet 368: 1339-1348, 2006.
163. Lin HC, Kuan Y, Chu HF, Cheng SC, Pan HC, Chen WY, Sun CY and Lin TH: Disulfiram and 6-Thioguanine synergistically inhibit the enzymatic activities of USP2 and USP21. Int J Biol Macromol 176: 490-497, 2021.
164. Tsai HK, Gibson CJ, Murdock HM, Davineni P, Harris MH, Wang ES, Gondek LP, Kim AS, Nardi V and Lindsley RC: Allelic complexity of KMT2A partial tandem duplications in acute myeloid leukemia and myelodysplastic syndromes. Blood Adv 6: 4236-4240, 2022.
165. Zhou X, Zhang P, Aryal S, Zhang L and Lu R: UTX loss alters therapeutic responses in KMT2A-rearranged acute myeloid leukemia. Leukemia 37: 226-230, 2023.
166. Ikeda J, Shiba N, Tsujimoto SI, Yoshida M, Nakabayashi K, Ogata-Kawata H, Okamura K, Takeuchi M, Osumi T, Tomizawa D, *et al*: Whole transcriptome sequencing reveals a KMT2A-USP2 fusion in infant acute myeloid leukemia. Genes Chromosomes Cancer 58: 669-672, 2019.
167. Lopes BA, Poubel CP, Teixeira CE, Caye-Eude A, Cave H, Meyer C, Marschalek R, Boroni M and Emerenciano M: Novel Diagnostic and therapeutic options for KMT2A-Rearranged acute leukemias. Front Pharmacol 13: 749472, 2022.
168. Blackburn PR, Smadbeck JB, Znoyko I, Webley MR, Pitel BA, Vasmatzis G, Xu X, Greipp PT, Hoppman NL, Ketterling RP, *et al*: Cryptic and atypical KMT2A-USP2 and KMT2A-USP8 rearrangements identified by mate pair sequencing in infant and childhood leukemia. Genes Chromosomes Cancer 59: 422-427, 2020.
169. Meyer C, Lopes BA, Caye-Eude A, Cave H, Arfeuille C, Cuccuini W, Sutton R, Venn NC, Oh SH, Tsaur G, *et al*: Human MLL/KMT2A gene exhibits a second breakpoint cluster region for recurrent MLL-USP2 fusions. Leukemia 33: 2306-2340, 2019.
170. Liu J, Cheng Y, Zheng M, Yuan B, Wang Z, Li X, Yin J, Ye M and Song Y: Targeting the ubiquitination/deubiquitination process to regulate immune checkpoint pathways. Signal Transduct Target Ther 6: 28, 2021.
171. Savage RE, Tyler AN, Miao XS and Chan TC: Identification of a novel glucosylsulfate conjugate as a metabolite of 3,4-dihydro-2,2-dimethyl-2H-naphtho[1,2-b] pyran-5,6-dione (ARQ 501, beta-lapachone) in mammals. Drug Metab Dispos 36: 753-758, 2008.
172. Ohayon S, Refua M, Hendler A, Aharoni A and Brik A: Harnessing the oxidation susceptibility of deubiquitinases for inhibition with small molecules. Angew Chem Int Ed Engl 54: 599-603, 2015.
173. Nguyen TT, Ung TT, Li S, Sah DK, Park SY, Lian S and Jung YD: Lithocholic Acid Induces miR21, Promoting PTEN Inhibition via STAT3 and ERK-1/2 signaling in colorectal cancer cells. Int J Mol Sci 22: 10209, 2021.
174. Li W, Wang Z, Lin R, Huang S, Miao H, Zou L, Liu K, Cui X, Wang Z, Zhang Y, *et al*: Lithocholic acid inhibits gallbladder cancer proliferation through interfering glutaminase-mediated glutamine metabolism. Biochem Pharmacol 205: 115253, 2022.
175. Altun M, Kramer HB, Willems LI, McDermott JL, Leach CA, Goldenberg SJ, Kumar KG, Konietzny R, Fischer R, Kogan E, *et al*: Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes. Chem Biol 18: 1401-1412, 2011.
176. Gupta SC, Kim JH, Prasad S and Aggarwal BB: Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastasis Rev 29: 405-434, 2010.
177. Yang H, Landis-Piwowar KR, Chen D, Milacic V and Dou QP: Natural compounds with proteasome inhibitory activity for cancer prevention and treatment. Curr Protein Pept Sci 9: 227-239, 2008.

178. Issaenko OA and Amerik AY: Chalcone-based small-molecule inhibitors attenuate malignant phenotype via targeting deubiquitinating enzymes. Cell Cycle 11: 1804-1817, 2012.
179. Aleo E, Henderson CJ, Fontanini A, Solazzo B and Brancolini C: Identification of new compounds that trigger apoptosome-independent caspase activation and apoptosis. Cancer Res 66: 9235-9244, 2006.
180. Nicholson B, Leach CA, Goldenberg SJ, Francis DM, Kodrasov MP, Tian X, Shanks J, Sterner DE, Bernal A, Mattern MR, *et al*: Characterization of ubiquitin and ubiquitin-like-protein isopeptidase activities. Protein Sci 17: 1035-1043, 2008.
181. Vamisetti GB, Meledin R, Gopinath P and Brik A: Halogen Substituents in the Isoquinoline Scaffold Switches the Selectivity of Inhibition between USP2 and USP7. Chembiochem 20: 282-286, 2019.