**REVIEW****Ubiquitin Specific Protease 2: Structure, Isoforms, Cellular Function, Related Diseases and Its Inhibitors**Hao Luo^{1,*,#}, Yanjie Ji^{2,#}, Xinrong Gao¹, Xinying Liu¹, Yunzhao Wu³ and Yingli Wu^{3,*}¹School of Basic Medicine, Weifang Medical University, Weifang, 261053, China²Central Laboratory, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai, 200030, China³Hongqiao International Institute of Medicine, Shanghai Tongren Hospital, Faculty of Basic Medicine, Chemical Biology Division of Shanghai Universities E-Institutes, Key Laboratory of Cell Differentiation and Apoptosis of the Chinese Ministry of Education, Shanghai Jiao Tong University School of Medicine, Shanghai, 200025, China

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ABSTRACT

The ubiquitin-proteasome system (UPS) is an important pathway for cellular protein degradation. The components of this pathway, including the proteasome, ubiquitinase, and deubiquitinase, are highly specialized and strictly regulated. The ubiquitin-specific protease 2 (USP2) belongs to the ubiquitin-specific proteases, a subgroup of deubiquitinating enzymes. USP2 plays essential roles in regulating cell survival, cell cycle, circadian rhythm, cell metabolism, inflammatory response, antiviral response, and metastasis by interacting with certain proteins such as Cyclin D1, PER1, CRY1, HDM2/p53, FASN, LDLR, TRAF6, TBK1, and TGFBR1-TGFBR2 complex. Elevation of USP2 has been observed in a variety of cancers, including glioma, testicular cancer, breast cancer, prostate cancer, and some inflammatory diseases. Moreover, USP2 also plays an important role in many non-neoplastic diseases. At present, there is no officially approved USP2 inhibitor in clinic. A few existing inhibitors targeting USP2 have shown certain effects in the treatment of colorectal cancer, but their mechanism of action and binding site information are not clear. Moreover, the efficacy and selection specificity need to be further optimized. The catalytic centers of USP family are relatively conserved, so the design of compounds targeting allosteric site is expected to improve the specificity and inhibitory activity. In this review, we summarize the latest advances of USP2 in its cellular function, related diseases, and small-molecule inhibitors targeting USP2.

KEYWORDS

USP2; structure; isoforms; cellular function; related diseases; inhibitors

1 Introduction

The ubiquitin-proteasome system (UPS) executes the majority of protein degradation in eukaryotic cells. Specifically, the UPS-mediated protein degradation comprises two steps: conjugation and degradation. Meanwhile, the UPS also includes a large group of proteases, i.e., deubiquitinases (DUBs), which cleave ubiquitin from the substrate proteins and prevent them from being degraded. By regulating ubiquitination and deubiquitination, the UPS is widely involved in various critical cellular activities, such as cell



growth, division, differentiation, and apoptosis [1]. Hence, the UPS is closely associated with various diseases and has become the target of many therapeutic strategies. For example, a series of proteasome inhibitors, such as bortezomib, ixazomib, have been developed and approved for cancer treatment. However, since proteasome is the converging point of lots signaling pathways, inhibiting proteasome usually suffers from drawbacks such as distant toxicity and drug resistance. To circumvent these problems, recent studies have turned to seek new targets from the upstream enzymes in the UPS, especially from DUBs, which possesses vast heterogeneity in both structure and function.

DUBs can be classified into six main superfamilies by structure, the largest one of which is the ubiquitin-specific protease (USP) family that has around 56 members in humans. While the substrates and functions of many USPs still remain unknown, several USPs have been intensely investigated as they play vital roles in disease-related signaling pathways. USP2, a member of the USP family, has three major isoforms and its special structure. Through interacting with and stabilizes a large number of substrates including HDM2/p53, Cyclin D1, PER1, Cry1, FASN, LDLR, TRAF6, TBK1, SKP2 and TGFBR1-TGFBR2 complex, USP2a affects diverse cellular functions such as cell survival, cell cycle, metastasis, metabolism and immune response [2,3]. The dysregulation of USP2 has also been observed in a variety of diseases, including glioma, some inflammatory diseases and testicular cancer. Furthermore, several USP2 inhibitors have been found to exhibit significant anticancer activity [4]. Therefore, USP2 has great potential to become a novel target that brings hope to the treatment of many diseases.

In this review, we aim to summarize recent advances in USP2 research from four aspects, including its structure and isoforms, cellular function, related diseases, and inhibitors. We will first introduce the basic information of USP2, such as its cellular localization and tissue distribution, and then we will show the different function of its isoforms. Subsequently, we will illustrate the essential cellular functions of USP2 with regard to its diverse substrates, followed by an in-depth discussion about how USP2 is associated with different diseases or pathological processes. Finally, we will review the inhibitors of USP2 and their effects *in vitro* and *in vivo*. We hope to provide a comprehensive picture of USP2 that inspires more exciting discoveries in both fundamental research and clinical application.

2 Structure and Isoforms of USP2

2.1 USP2 Structure Overview

USP2 has three major isoforms, that share a similar structure composed of an N-terminal domain of variable length, and a 347-amino acid long C-terminal domain that contains the characteristic catalytic triad (Cys, His and Asp/Asn) of the USP family [5]. Specifically, USP2a has the largest N-terminal domain with 258 amino acids, while that of USP2b and USP2c has 49 and 15 amino acids, respectively. Note that these isoforms are produced by the alternative splicing of the USP2 mRNA, see Fig. 1 below. The isoform above has been chosen as the “canonical” sequence named USP2a. The sequence of USP2b differs from the canonical sequence as follows: 1-258: MSQLSSTLKR...RSSSPGRDGM→MRTSYTVTLP...FVGLLLNKAK. The sequence of USP2c isoform differs from the canonical sequence as follows: 1-258: MSQLSSTLKR...RSSSPGRDGM→MLVPGSTRPYSKKRQ. The C terminus, shared by USP2a, USP2b and USP2c, contains the canonical isopeptidase CYS and HIS boxes.

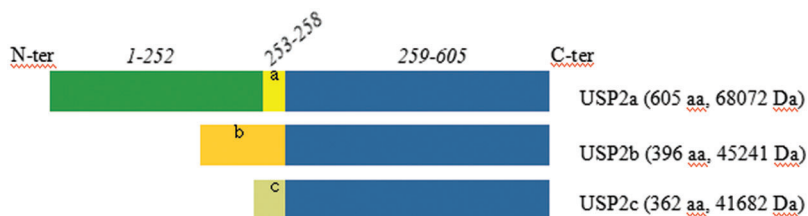


Figure 1: Comparison of amino acid sequences of different variants of human USP2

2.2 The Different Functions of USP2 Isoforms

Although it is not clear yet how the N-terminal domain affects the function of these isoforms, different USP2 isoforms may perform distinct cellular functions [6]. For example, Sendai virus-induced IFN- β activation in HEK293 cells can be inhibited by overexpressing USP2b and USP2c, but not USP2a [7]. Meanwhile, it has been found that specific USP2 isoforms may be selectively expressed in different tissues or organs in mouse and rat models. Further studies suggested that the alternative splicing of USP2 mRNA may be affected by factors including circadian rhythm, nutrition, and androgen. However, different USP2 isoforms may share similar subcellular locations, including cell nucleus, peroxisome, endoplasmic reticulum, and cytoplasm [8].

Despite the difference between the three isoforms, the C-terminal sequence of USP2 is highly conservative and serves as the proteolytic domain. X-ray structure analysis showed that the catalytic domain of USP2 is highly similar to that of USP7, forming a ‘cupped hand’ structure that nicely hosts ubiquitin. In particular, it was demonstrated that the core of ubiquitin interacts with the cupped hand of USP2, which aligns the C-terminus of ubiquitin at the active site cleft of USP2 for hydrolysis. It was also found that the interaction between USP2 and ubiquitin is mediated by an ordered layer of water molecules. Strikingly, SARS-CoV-2 papain-like protease (PLpro) and USP2 share similar structural scaffold and conserved catalytic triplets. The USP folding of these two proteases bears striking similarities to the right-handed thumb-palm-fingers structural scaffold [9]. Overall, USP2 preserves the typical features of the USP family, while its isoforms may differ slightly in structure, cellular function, and tissue distribution.

2.2.1 USP2a and USP2b Play Different Functions in Antiviral Immune Response

pY701-STAT1 was found to be the main form of IFN1-induced STAT1 ubiquitination. pY701-STAT1 is rapidly regulated by the ubiquitin-proteasome system. USP2a plays an important role in antiviral defense by inhibiting the ubiquitination of activated STAT1 in the nucleus and maintaining the antiviral activity of interferon (IFN) [10]. However, USP2b deubiquitinates TBK1 to terminate TBK1 activation and negatively regulate IFN- β signaling, resulting in the decrease of anti Sendai virus reaction in HEK293 cell [11].

2.2.2 USP2a and USP2b Play Different Roles in HCC

Currently, it remains controversial which isoforms of USP2 are expressed in the liver and the isoforms show different function in hepatocellular carcinoma (HCC). Xiong et al. reported that USP2a could stabilize RAB1A by deubiquitinating, which is required for HCC progression [12]. This indicated that targeting USP2a could be a feasible method to treat HCC. However, Christina et al. reported USP2b was significantly down-regulated in HCC and as the notable isoform of the liver [13]. They also found that USP2b exhibited tumorigenic activities through promoting cell proliferation. But on the other hand, USP2b could enhance bile acid-induced apoptosis and necrosis significantly. Whether USP2b act as tumor promoter or suppressor depend on the cell context or status [13].

3 Cellular Function of USP2

By deubiquitinating its substrates, USP2 regulates protein stability, intracellular localization, and protein-protein interactions. As USP2 interacts with a wide range of proteins that involve in various critical processes, resolving the fundamental cellular functions of USP2 is the basis of understanding its pathology and related therapeutics. Here, we introduce how USP2 affects different cellular activities, including cell survival, cell cycle, circadian clock, cell metabolism, metastasis, etc., see [Fig. 2](#) below.

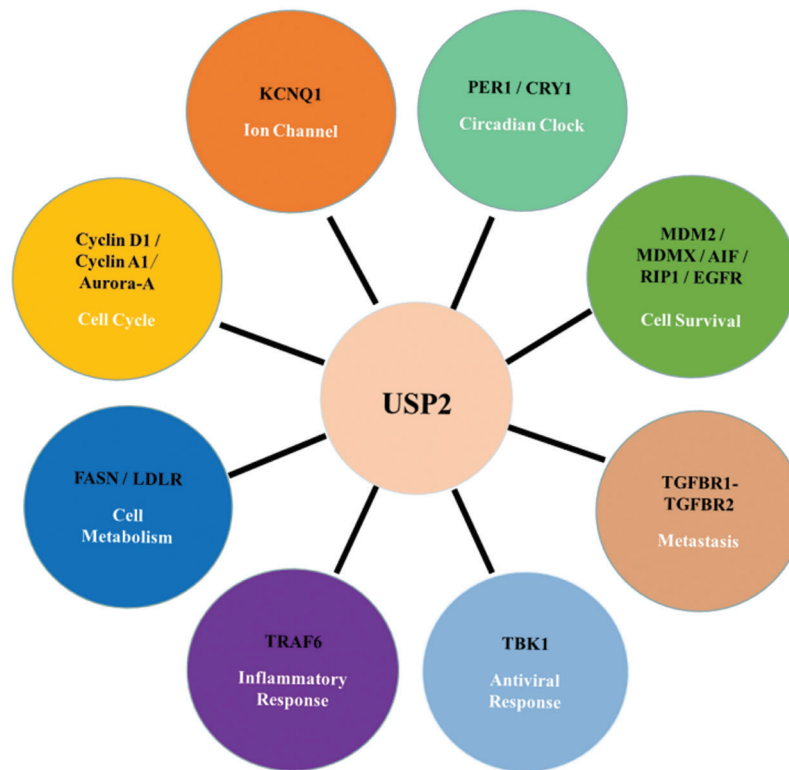


Figure 2: Cellular function of USP2 and its substrates

3.1 Cell Survival

Ectopically expressed USP2 may either promote or inhibit cell survival through different pathways. For example, USP2 can deubiquitinate and stabilize HDM2, which consequently promotes HDM2-mediated p53 degradation hence inhibits the tumor cells apoptosis. Further studies reveal that USP2-induced p53 degradation downregulates a group of miRNAs and hence increases cellular MYC levels, which enhances prostate cancer cell survival [14]. Additionally, USP2a deubiquitinates the internalized EGFR on early lysosome surface and thus improves the survival of EGFR-dependent cancer cells, such as NSCLC cells. In contrast, USP2a and USP2c have been found to deubiquitinate and stabilize RIP1, the accumulation of which will lead to cell apoptosis [15]. In brief, USP2, or its specific isoforms, may play a double-sided role in cell survival via different mechanisms.

3.2 Cell Cycle

USP2 is involved in cell cycle control by interacting with key regulatory proteins. Specifically, USP2a binds to and stabilizes Cyclin D1, an essential regulatory molecule in G1/S cell cycle progression that is highly expressed in various tumor cells. Correspondingly, the inhibition of USP2a by lithocholic acid hydroxyamide induces G0/G1 arrest and the degradation of Cyclin D1 [16]. Studies have shown that Aurora-A, a threonine/serine kinase that regulates mitotic activities including chromosome separation, mitotic spindle formation, and centrosome maturation, is also a substrate of USP2 [17]. Saroj et al. also found that modulation of USP-2 expression plays a key role in cell cycle regulation by adipokines such as adiponectin and leptin [18]. These functions suggest that targeting USP2 may be a novel anti-cancer strategy via cell cycle manipulation.

3.3 Cell Circadian Clock

USP2 regulates the circadian clock by regulating the internal circadian rhythm and the ability to respond to external signals. USP2 regulates the function of PER1 by controlling the entry and accumulation of its nucleus, thereby regulating the expression of clock-controlled genes. Moreover, USP2 improves the stability of CRY1 protein in serum by de-ubiquitination, so as to inhibit the expression of endogenous PER2 gene and gene promoter activity. In addition, USP2 may regulate the interruption of circadian rhythm by inhibiting CRY1 degradation during inflammation. It depends on USP2 in the process of TNF- α increasing CRY1 protein level and inhibiting rhythmic genes [19]. Scoma et al. [20] have proved that USP2 is associated with the circadian adaptation to environmental day length changes. They identified that USP2 could turnover and regulate the stability of BMAL1, which could alter the CLOCK/BMAL1 related genes [20].

3.4 Cell Metabolism

Recent studies and genetic evidence show that USP2a and USP2b are new regulators of lipoprotein clearance. Because the study found that USP2a is a post-transcriptional and one of the androgen regulator isoenzymes, they can control the ubiquitination dependent degradation and subsequent lysosomal degradation of low-density lipoprotein receptor (LDLR) through IDOL, which as an E3-ubiquitin ligase, and finally play a vital role in the survival of tumors such as prostate cancer through stabilizing fatty acid synthase (FAS) [21]. It was also found that USP2 could regulate glucose metabolism by regulating glucocorticoid signaling and inducing of 11 β -hydroxysteroid dehydrogenase 1 (HSD1) [22].

3.5 Cell Ion Channel

USP2 plays roles in controlling ion channel dynamics. The expression of USP2 is important in sodium homeostasis because aldosterone stimulation has been found to increase the expression of USP2 in the distal nephron. Moreover, studies have shown that USP2 also has a protective effect on Nedd4/Nedd4 like down-regulation of ENaC *in vitro*. KCNQ1 (Kv7.1) together with its KCNE β subunits, plays a pivotal role both in the repolarization of heart tissue and in water and salt transport across epithelial membranes. USP2 may counter Nedd4-2-mediated ubiquitylation by deubiquitylating KCNQ1 as the partial rescue of the membrane localization of the channel [23]. It is also reported that USP2b as the clock output effector acting at the post-translational level at cell membranes, possibly regulates membrane permeability of Ca²⁺ [24].

3.6 Cell Metastasis

It has been demonstrated that TGF- β induces EMT and thereby promotes metastasis. USP2 promotes metastasis by interacting with TGFBR1 and TGFBR2 accompany TGF- β stimulation and can promote the recruitment of SMAD2/3 by removing the K33 linked polyubiquitin chain in lys502 of TGFBR1 [25].

3.7 Cell Antiviral Response

Through activation of various natural receptors downstream to produce IFN- β and IFN regulator 3, one of the essential proteins is TANK-binding kinase 1 (TBK1). Recent studies have shown that USP2b deubiquitinates K63-associated polyubiquitin chains of TBK1 to cease TBK1 activation and controls negative IFN- β signaling and immune responses against viruses [11].

3.8 Cell Inflammatory Response

Recently, USP2 has been shown to inhibit TLR/IL-1 β -induced NF- κ B, which plays an important role in the immune system as a transcription factor activation, in tumor cells by deubiquitination of TRAF6. USP2 promotes T cell activation and TNF- α -induced cell death or inhibits IL-1 β -induced inflammatory responses by deubiquitinating TRAF6, indicating that USP2 might be a tumor suppressor by modulating the tumor micro-environment [26].

4 USP2 and Diseases

USP2 plays an essential role in regulating cell survival, cell cycle, circadian rhythm, cell metabolism, inflammatory response, antiviral response, and metastasis through interacting with many proteins such as Cyclin A1, MDM2/p53, etc., see [Table 1](#). USP2 plays different roles in different tumors. Besides, it also associated with many functional disorders, such as recurrent miscarriage.

Table 1: USP2 and diseases

Disease	Affected pathway or signaling protein	Reference
Bladder cancer	Cyclin A1	[27]
Glioma	FASN / MDM4-p53 / Nrf2	[28–30]
Prostate cancer	FASN / ACDase /MYC	[31–33]
Cutaneous T-cell lymphoma	MDM2-p53	[34]
Breast cancer	p53 / RIP1 / Twist / Bmi1 / ErbB2	[35–39]
Oral squamous cell carcinomas	FASN / ErbB2	[40]
Anti-Thy1.1 nephritis	p27	[41]
Osteoporosis	PTHrP	[42]
Acute stress-induced impairment of spatial memory	GluA1	[43]

4.1 USP2 and Tumor

The development of tumor involves the regulation of multi-gene and multi-angle comprehensive. Deubiquitination enzyme as a class of important protein hydrolytic enzymes plays an important role in tumor. USP2 is highly expressed in various cancers such as breast cancer, OSCC, bladder cancer and prostate cancer [27–29]. In hematological tumors, it has been reported that MLL and USP2a form a fusion gene named MLL-USP2a [44]. Moreover, USP2 can promote TGF- β -triggered signals to facilitate tumor metastasis. This implied that USP2 has the potential to be target for the treatment of metastatic cancer. As a deubiquitinating protease, USP2 must function by interacting with other proteins. USP2 can regulate some important tumor-related proteins, such as β -catenin, FASN, MDM2, RAB1A, etc., and plays a part in the development of tumor through influencing tumor-related proteins [45]. USP2 plays different roles in different tumors, which may be related to the different downstream substrates in different tumors or different stages of tumors.

4.1.1 USP2 Acts as a Therapeutic Target for TNBC and ErbB2-Positive Breast Cancer

USP2 is over-expressed in breast invasive ductal carcinoma, and is closely related to proliferation promoting effects [35]. Triple negative breast cancer (TNBC) is an aggressive breast cancer subtype with poor prognosis. USP2 expression can be used as a prognostic biomarker of TNBC, and is significantly associated with the invasion of TNBC cell line and the survival rate of breast cancer patients [36]. In addition, TNBC harbors enriched cancer stem cell (CSC) populations in tumors. USP2 maintains the CSC population by activating self-renewing factor Bmi1 and epithelial-mesenchymal transition through Twist upregulation [37]. The canonical Wnt signaling pathway is activated in multiple types of cancers, including colorectal cancer, breast cancer and hepatocellular carcinoma. Mutation or overexpression of β -catenin often results in tumor formation and metastatic progression [38]. USP2a was found able to deubiquitinate and stabilize β -catenin. Genetic depletion or pharmacological inhibition of USP2 in TNBC cells destabilized β -catenin, suggesting that USP2 can serve as a novel therapeutic target of Wnt/ β -catenin signaling in certain cancers such as TNBC. Zhang et al. found that USP2 protects ErbB2 surface levels in ErbB2-positive breast cancer by antagonizing its ubiquitin mediated endocytosis, which could be used to

design novel therapeutic strategies for ErbB2-driven malignancies as a combination therapy with HSP90 inhibitors [39]. USP2 could elevate the level of RIP1 to promote apoptosis by deubiquitinating Ub-RIP1 in human breast cancer cells [15]. Over-expression of USP2 in TRAF2 gene knocked-out mouse embryonic fibroblast cell can obviously promote the RIP1 level and cell apoptosis.

4.1.2 USP2a is Associated with Tumor Progression and Poor Prognosis in OSCC

FASN and ErbB2 have been found to be overexpressed in oral squamous cell carcinoma (OSCC). At the same time, USP2a is expressed in OSCC and may play a role in FASN accumulation. Sabrina Daniela da Silva and others studied the correlation between FASN, ErbB2, USP2a gene expression and immunohistochemical status and the clinicopathological characteristics of OSCC patients. It was found that the expression of ErbB2, FASN and USP2a showed a strong positive correlation. In OSCC, FASN, ErbB2 and USP2a mRNA and protein levels are associated with tumor progression and poor prognosis, and there is a molecular link between them [40].

4.1.3 USP2 Acts as an Oncoprotein in Bladder Cancer

USP2 binds to cyclin A1 and prevents ubiquitination of cyclin A1, resulting in inhibition of cyclin A1 degradation. This leads to an accumulation of cyclin A1 and an increase in cell proliferation. Kim et al have found that USP2a is an oncoprotein in bladder cancer [27]. USP2 inhibits ubiquitination and stabilizes cyclin A1, an important cell cycle regulator, raising the possibility of USP2 targeting as a combination of chemotherapy for bladder tumors [27]. In bladder cancer, Liu et al. also discovered that tight junction protein 1 (TJP1) regulates TWIST1 expression by recruiting USP2, thereby regulating CCL2 expression and regulating angiogenesis in bladder cancer [46].

4.1.4 USP2 Acts as an Antioncogene and an Independent Factor for ccRCC Prognosis

USP2 mRNA level is significantly lower in renal cancer, relative to normal, and is interrelated to poor prognosis of renal cell carcinoma. USP2 acts as an antioncogene and an independent factor for clear cell renal cell carcinoma (ccRCC) prognosis [47]. However, the specific mechanism of USP2's role in ccRCC remains unclear.

4.2 USP2 and Inflammation

USP2 is involved in regulating key transcription factors in the inflammatory response. Increasing evidence shows that USP2 participates in the modulation of inflammatory and immune signaling. He et al. previously reported that USP2a could negatively regulates NF- κ B dependent induction of TNF and IL-6 in colorectal carcinoma cells [26]. Conversely, in macrophages Sun et al. reported that USP2 could modifies degradation of TNF- α protein [48]. In addition, Kitamura et al. found that cytokine expression in macrophage-like cells modified by USP2 was systematically monitored so that USP2 inhibited a huge number of cytokines after LPS induction [49].

It was also reported that miR-125b can activate NF- κ B through USP2, ultimately, resulting in psoriasis phenotype. Having the high-expression USP2 variant also gives individuals a slightly higher lifetime risk of psoriasis, and knocking down USP2 strongly reduces keratinocyte proliferation [50]. In addition, the function of USP2 is associated with chronic inflammation associated with circadian rhythm, and USP2 plays an important role in the regulation of the stability of cryptochrome CRY1 protein. Circadian clocks and inflammation are important regulators of metabolism, and a more in-depth understanding of the role of USP2 in CRY1 protein regulation could shed light on the interaction between the inflammatory response and the biological clock. Therefore, USP2 is also expected to be a drug target for treating physiological and metabolic disorders.

In addition, the expression of USP2 in human nephritis MSc is related to the pathological type of nephritis. Inflammatory factors and immune complexes can induce USP2 expression in mesangial MSc

and up-regulate the expression of P27 protein. Overexpression of USP2 can slow the progression of Thy1.1 nephritis in rats [41].

4.3 *USP2 and Others*

4.3.1 *Increased USP2 Expression is Necessary for Osteoblast Proliferation*

Parathyroid hormone (PTH) plays an anabolic role in bone formation and is the main treatment for severe osteoporosis. USP2a and USP2b are predominantly expressed in osteoblasts. *In vivo* imaging analysis has demonstrated that USP2 is required for PTH1-34 induction of primary osteoblast proliferation in fluorescent ubiquitinated cell cycle indicator (Fucci) transgenic mice. It was found that USP2 may be one of the direct target genes for PTHR signaling in studying the effect of PTH on gene expression in osteoblast [42].

4.3.2 *USP2 Participates in Acute Stress-Induced Impairment of Spatial Memory Retrieval*

Loss of ubiquitin homeostasis in neurons leads to neurological diseases. Correction of ubiquitinase abnormalities has a high regulatory effect on cognitive function improvement. Recent studies have shown that USP2 is highly expressed in the rat hippocampus, and retigabine alleviated acute stress-induced spatial memory retrieval impairment through adjusting the aberrance of USP2. PGC-1 α , E4BP4 and β -catenin are the upstream regulators while mTOR, autophagy and GluA1 are its downstream targets. Stress-induced spatial memory impairment can be enhanced by drugs that reverse the abnormal expression of USP2-related signaling pathways [43].

4.3.3 *USP2 is Involved in Maintaining the Integrity of Mitochondrial Membrane*

USP2 is thought to be involved in the differentiation of myoblasts into myotubes. The USP2-KO cells produced by Cas9 reduce the accumulation of oxygen consumption and adenosine triphosphate (ATP) content in the cells. In USP2-KO cells, mitochondrial membrane permeability was assessed using calcian AM-cobalt staining. The membrane potential of USP2-KO cells was significantly reduced. ML364, which a USP2 selective inhibitor, also increased the level of mitochondrial ROS and regulated the mitochondrial membrane potential and morphology. These results suggest that USP2 may be involved in maintaining mitochondrial membrane integrity. This process ensures a supply of ATP in myoblasts, possibly leading to proliferation and differentiation [51].

4.3.4 *USP2 Plays an Important Role in Spermatogenesis and Sperm Function*

USP2 mRNA expression is very high in mouse testis, but the role of USP2 subtypes in spermatogenesis needs further study. Bedard et al. found that although USP2^{-/-} male testicular sperm count and epididymal sperm count were normal, fertility was also a serious drawback, with significantly reduced sperm motility in these animals, only 12% of USP2^{+/+} litters [52]. Mayuko et al. demonstrated a distinct role of USP2 that directly affect sperm function in organ-specific macrophages [53]. Testicular macrophage USP2 does not affect the structure of the testis or modulate retinoic acid and testosterone synthesis. However, it could promote hyperactivation, motility, and sustain GM-CSF expression in testicular interstitial macrophages.

4.3.5 *USP2a Is Associates with Recurrent Miscarriage*

Wang et al. showed that the placental of recurrent miscarriage patients have lower expression of USP2, which could inhibit human trophoblast-derived cell lines apoptosis and promoted its proliferation [54]. They discovered that TGF- β secreted by M2 macrophages can interact with USP2a in trophoblasts to regulate placental formation. What's more, USP2a could promote nuclear translocation of β -catenin through activated PI3K/Akt/GSK3 β signaling pathway while further activated epithelial-mesenchymal transition (EMT) of trophoblasts.

4.3.6 USP2 Regulate Ubiquitination and Stability of Antithrombin

It was cleared that USP2a overexpression is sufficient to stabilize antithrombin, while it still unclear which role USP2b will play in regulating the expression of antithrombin. It was found that USP2 interacted with antithrombin and regulated the expression of antithrombin, indicating that overexpression of USP2 improve the stability of antithrombin. Cullin 2 E3 ubiquitin ligase and USP2 synergistically regulate antithrombin ubiquitination and degradation. Therefore, targeting USP2 may be a potential strategy for the treatment of hemophilia [55].

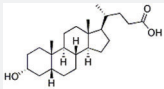
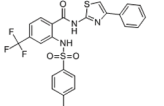
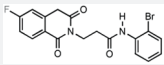
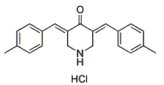
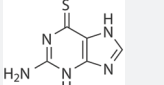
4.3.7 USP2 Has Cardiomyocyte-Specific Function

The expression of USP2 in primary cardiac fibroblasts (CFS) induced by angiotensin II in neonatal rats increased. The inhibition of USP2 restrain the synthesis of collagen, the proliferation of CFS, and the progression of cell cycle. USP2 could stabilize β -catenin and cyclin D1 which regulating Ang II-induced cardiac fibroblasts activation [56]. Xing et al. also demonstrated that USP2 overexpression in the heart would inhibit pressure overload induced cardiac remodeling [57]. USP2 is down regulated in the heart under pressure overload. Overexpression of cardiac USP2 inhibits TAC induced cardiac remodeling by inhibiting cardiac hypertrophy, inhibiting fibrosis and inflammatory response, and reducing oxidative stress.

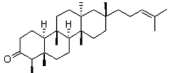
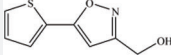
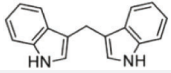
5 USP2 Inhibitors

USP2 is a good anti-tumor drug target, and the discovery of its inhibitors is a hotspot of current research, see Table 2. However, the specificity of existing USP2 inhibitors is not ideal, and the vast majority of inhibitors are at the micromole level of IC₅₀. Currently, USP2 inhibitors are not officially approved for clinical use. Current drug screening methods need to be further optimized, and the discovery of USP2 inhibitors is still in its infancy.

Table 2: Features of USP2 inhibitors

Compound	Structural formula	Binding mode	Activity (IC ₅₀ , μ M)	Selectivity (DUBs tested)	Cell or animal effects	Reference
Lithocholic acid		Non-covalent	9.7	2	Cyclin D1 ↓; Cell proliferation ↓	[16]
ML364		Non-covalent	1.1	3	Cyclin D1 ↓; DNA repair ;	[37,39,58]
Halogen- substituted isoquinoline-1,3-dione-based molecule		Non-covalent	0.25	2	Cyclin D1 ↓; Cell proliferation ↓	[59]
NSC 632839		Covalent	45	2	Not reported	[60]
6-Thioguanine		Covalent	40	1	Not reported	[61]

(Continued)

Table 2 (continued)						
Compound	Structural formula	Binding mode	Activity (IC ₅₀ , μM)	Selectivity (DUBs tested)	Cell or animal effects	Reference
Shionone		Non-covalent	69	1	Cyclin D1 ↓; Cell proliferation↓	[62]
STD1		Non-covalent	1.3	2	Not reported	[63]
3,3'-diindolylmethane		Not clear	< 20	1	Not reported	[64]
Chalcone compounds	Unavailable	Covalent	>10	9	Cyclin D1 ↓; Cell proliferation↓	[65]

5.1 Isoquinoline-1,3-dione Scaffold-Based Inhibitors

Previously reported USP2 inhibitors, such as isoquinoline-1,3-dione scaffold, the introduction of fluorine atom totally changed the selectivity of inhibitors between USP7 and USP2. It was found that USP2 was inhibited by reactive oxygen species independent non-competitive mechanism with IC₅₀ of 250 nm. When treated HeLa cells with 2 and 10 μM of compound 14, the cyclinD1 levels decreased in a time and concentration dependent, which suggests that compound 14 is able to enter cells and trigger USP2 inhibition *in vivo*. The research of isoquinoline 1,3 diketone cores and found of halogenated substituents helps for the development of more highly selective deubiquitinase inhibitors [59].

5.2 NSC 632839

Inhibition of ubiquitin isopeptase by NSC 632839 can be illustrated by the ability of crude lysates to inhibit z-LRGG-AMC cleavage in the range of middle micro-molars. In order to further characterize the inhibitory effect of NSC 632839 on purified enzyme, the inhibitory effect of NSC 632839 on purified USP2, USP7 and SENP2 was determined, and it was proved that NSC 632839 is not only a DUB inhibitor, but also a inhibitor of deSUMOylase. According to the description, the EC₅₀ values of NSC 632839 inhibits USP2, SENP2 and USP7 are 45 ± 4 μM, 9.8 ± 1.8 μM and 37 ± 1 μM, respectively [60].

5.3 6-Thioguanine

In recent studies, 6-thioguanine (6TG), an old drug still in clinical use for the treatment of leukemia and intestinal disease, was identified as an effective USP2 inhibitor. It is reported only 6TG but not 6MP has a significant inhibitory effect of USP2 with an IC₅₀ of 40 ± 2.0 μM [61].

5.4 ML364

ML364 is a non-covalent USP2 inhibitor reported in 2016, IC₅₀ of 1.1 μM [58]. Among the 6 other proteases tested, USP8 was also inhibited. ML364 induces cyclin D1 degradation resulting in cell-cycle arrest, indicating key role in progression of non-Hodgkin's lymphoma and colorectal cancer. He et al. used ML364 in combination with doxorubicin and found that ML364 increased the sensitivity of existing TNBC xenografts to doxorubicin [37]. It can effectively inhibit the *in vivo* growth of ErbB2-positive breast cancer xenograft tumors with the combination of ML364 and HSP90 inhibitors [39].

5.5 Shionone

Domestic researchers found shionone, as a natural compounds, can inhibit USP2 under their screening platform [62]. It was found that shionone can inhibit USP2 substrate Cyclin D1 and inhibit the proliferation of tumor cells. Shionone can provide a lead compound for identification of new USP2 inhibitors.

5.6 3,3'-Diindolylmethane

Indole-3-methanol (I3C) is a derivative of cruciferous vegetables such as cabbage and is known for its many health benefits in chemoprophylaxis and weight loss. It is easily metabolized to more stable 3,3'-diindolylmethane (DIM) under the acidic conditions of the stomach. DIM treatment directly inhibits USP2 deubiquitinase activity, which leads to a decrease in the stability of cyclin D1 and thus increases ubiquitin-mediated proteasome degradation. By inhibiting USP2 to increase the degradation of cyclin D1, USP2 may be responsible for reducing adipose tissue mass and inhibiting HFD-induced obesity in mice [64].

In addition to these, some small molecule inhibitors have also been reported. Lithocholic acid derivatives can inhibit USP2a, but also inhibit the JAMM/MPN+ family in DUBs [16]. A recent study reported small molecules 5-(2-thienyl)-3-isoxazoles (STD1) as the inhibitors of the USP2-ubiquitin protein-protein interaction that bind to USP2 through using NMR-based fragment screening and a biochemical binding assay [63]. Chalcone Compounds (double-digit micro molar potency) could significantly inhibited UCH-L1, UCH-L3, USP2, USP5 and USP8 [65]. The emergence of allosteric modulators provides a new and broader drug development space. The development and design of USP2 allosteric inhibitors will bring a greater prospect for targeted therapy of USP2-related diseases.

6 Conclusions

In recent years, USP2 related research has made progress in many aspects. USP2 is highly expressed in various tumors and is involved in the regulation of immune and inflammatory signals. This indicates that USP2 may be an important therapeutic target in inflammation and tumorigenesis. From our review, we can also see that different subtype of USP2 exhibit different functions. The specific mechanism of why these differences occur needs to be further explored. Furthermore, we conclude that USP2 plays an important role in many other non-neoplastic diseases such as recurrent abortion and osteoporosis. All of these suggest that USP2, as a deubiquitin protease, may play a role in different diseases by regulating and stabilizing different substrates. To further explore the specific interaction protein of USP2 in different diseases has an important role in the targeted treatment of diseases. We believe that as more research progresses, the role of USP2 in other diseases can also be verified.

In addition, specific USP2 inhibitor in clinical has not been officially approved currently. The existing USP2 small molecule inhibitors lack *in vivo* activity tests, and the selectivity is not ideal. Because the catalytic domains of USP are highly homologous, it is important to better understand the cross-reactivity between USP2 and other USP members. In previous studies, the eutectic structure of USP2 and Ub has been reported, but no single crystal structure of USP2 has been analyzed so far. The study reveals its crystal structure will provide important theoretical basis and direction for the development of USP2 specific inhibitors. The summary notes that USP2 is an effective target for cancer and other related diseases. These advances are encouraging because they will provide references and protocols for the clinical application of these diseases. However, the search for USP2 inhibitors is still in its infancy, and huge efforts are needed in the next few years.

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