

Structure, Inhibitors, and Biological Function in Nervous System and Cancer of Ubiquitin-Specific Protease 46

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ABSTRACT: Deubiquitinating enzymes (DUBs) prevent ubiquitination by eliminating ubiquitin from their substrates. Deubiquitinating enzymes have important roles in a number of cell biology subfields that are highly relevant to diseases like neurodegeneration, cancer, autoimmune disorders, and long-term inflammation. Deubiquitinating enzymes feature a well-defined active site and, for the most part, catalytic cysteine, which makes them appealing targets for small-molecule drug development. Ubiquitin-specific protease 46 (USP46) is a member of the ubiquitin-specific protease family, the largest subfamily of DUBs. Over the past 10 years, some studies have steadily demonstrated the significance of USP46 in several biological processes, although it was identified later and early research progress was modest. Specifically, in the last few years, the carcinogenic properties of USP46 have become more apparent. In the current review, we provide a comprehensive overview of the current knowledge about USP46 including its characteristics, structure, inhibitors, function in diseases, especially in the nervous system, and the correlation of USP46 with cancers.

KEYWORDS: Ubiquitin-specific protease 46, deubiquitinating enzymes, cancer, inhibitor, nervous system

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Introduction

Deubiquitinating enzymes (DUBs) are proteases that counteract the alteration caused by E3 ubiquitin ligases by removing the ubiquitin moiety from ubiquitylated substrates. Currently, it is known that DUBs are essential for controlling some physiological and pathological processes, including immunological homeostasis, carcinogenesis, and neurodegenerative illnesses. A growing body of research indicates that several DUBs may play unique roles in the development of tumors, including oncogenic, tumor-suppressive, or context-dependent activities.¹ These functions are mostly thought to be mediated by changes in the stability of proteins, enzymatic activity, or subcellular localization of their substrates. Significantly, a number of strong inhibitors that target the enzyme function of carcinogenic DUBs have been created, and preclinical models of their anticancer effectiveness indicate promise.² Therefore, investigating the distinct function of DUBs and their related effectors will offer fresh perspectives on the molecular foundation of cancer development.³

There are about 100 DUBs in humans, and they are categorized into 7 groups based on their sequences and domain conservation including: ubiquitin-specific proteases (USPs), ubiquitin carboxy-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Machado-Joseph disease proteases (MJDs), MIU-containing novel DUB family (MINDY) proteases,

Jab1/MPN/MOV34 metalloenzymes (JAMMs), and Zn-finger and UFSF domain proteins (ZUFSPs), those are cysteine peptidases exception of JAMMs.³⁻⁵ Among them, USPs is the largest group, with about 58 members. The size of the USP domains range from less than 300 to more than 800 amino acids.⁶ It has been suggested that accessory domains, such as terminal extensions and the ubiquitin-associated domain (UBA), ubiquitin-interacting motif (UIM), and zinc finger ubiquitin-specific protease domain (ZnF-UBP), might provide USPs substrate specificity.^{6,7} The importance of several USPs in oncogenesis has been studied, and information about their pathological and physiological functions has recently started to accumulate. These USPs regulate cell cycle progression, injury to DNA repair, and tumor-related cellular signaling.^{6,8-11}

A recently found DUBs called ubiquitin-specific protease 46 (USP46) was shown to catalyze the deubiquitination of H2A and H2B for the initial time and may aid in the cultivation of *Xenopus*.¹² WDR48 controls the catalytic activity of USP46, which has a ubiquitin carboxyl-terminal hydrolase (UCH) domain.¹³ Researches have demonstrated a connection between animal immobility and cell proliferation and USP46.^{14,15} The significance of USP46 in human malignancies has drawn more attention lately. It has been discovered that USP46 is crucial for the growth of tumor cells and is linked to metastasis. However, the existing body of knowledge regarding



this protein is somewhat thin, and the information that is known regarding USP46's function is scarce. Complex investigations are thus needed, especially those that deal with its traits and function. In this review, we provide the most comprehensive information related to USP46, including its characteristics, structure, inhibitors, function in diseases, especially in the nervous system, and the correlation of USP46 with cancers.

Characteristics and Structure of USP46

The human USP46 gene is located on chromosome 4p12 and spans approximately 1111 base pairs. The gene architecture is characterized by a complex exon-intron structure, comprising 11 exons separated by introns. The expected molecular size and theoretical isoelectric point (PI) of human USP46 are about 43 kDa and 6.39, respectively (calculated by EXPASy Compute PI/Mw). Based on an alignment of the amino acid structure of USP46 proteins from humans (*Homo sapiens*), black carp (*Mylopharyngodon piceus*), fathead minnow (*Pimephales promelas*), mice (*Mus musculus*), and zebrafish (*Danio rerio*) bcUSP46 is a molecule that has been genetically maintained and has a UCH region. According to phylogenetic study, the *M. amblycephala* USP46 and the bcUSP46 are the most closely related species. As a result, USP46 has evolved with great conservation.¹⁶

Numerous USPs, as shown by previously published research, have modular architectures that include structural domains for localization and protein-protein interactions alongside the catalytic structural domains.¹⁷ The finger, palm, and thumb domains are the 3 substructural domains that make up the main catalytic structure of USPs. The catalytic core is situated between the "palm" and "thumb" subdomains, whereas the "finger" holds the ubiquitin core, which supports the spherical domain of ubiquitin.¹⁸ The prolonged finger domains, together with the palm and thumb domains, constitute the binding cavity for ubiquitin, which recognizes the extended ubiquitin tail and presents its C-terminus to the active-site cysteine in the catalytic central domain of USPs. This unique cysteine catalytic triad is found in the catalytic core domain, including Cys44, His313, and Asp329 (Figure 1). Jianping Yin et al determined the 1.9Å resolution structure of a resolution structure of a covalently attached USP46 25–366 ubiquitin complex. The structure displays the broader fingers, thumb, and palm subdomains of the standard USP structure. The C-terminal sequence of ubiquitin substrate leads into the cysteine protease active site, where conjugated ubiquitin Gly76 is covalently bonded to the catalytic USP46 Cys44. The substrate is cradled between the fingers and the inner palm/thumb regions.¹³ Moreover, a Zn^{2+} integrated by 4 conserved Cys residues stabilizes the USP46 subdomain of the tip of the fingers, and the active-site adopts a catalytically favorable conformation.¹⁹ The USP46:ubiquitin interface buries 1,825Å² of surface area on each protein. Straightforward electron densities at the catalytic core, ubiquitin-proximal loops, and the

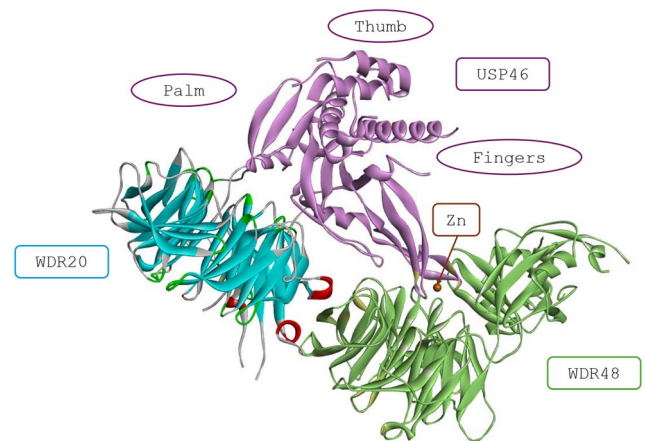


Figure 1. The structure of USP46 catalytic domain was obtained from *Homo sapiens* (PDB ID: 5CVM). Purple is catalytic domain of USP46 (from His33 to Arg365). Yellow is ubiquitin binding to catalytic site of USP46. The blue is catalytic triad of USP46.

"switching" motif equivalent demonstrate their interactions with ubiquitin and its C-terminal tail. Besides, USP46 (residues 143–163) features an insertion at the intersection of the thumb and fingers domains that is disordered, like many other USP homologs. The helix that comes before it (aE) serves as the major shaft for the USP46 design. It descends into this problematic space by making many twists besides the protein's major structure.¹³

Furthermore, USP46 and USP12 are 2 small proteins that have the same active-site domain sequences and have a highly conserved catalytic domain with significant sequence similarity (93%).²⁰ A subfamily of USPs made up of USP1, USP12, and USP46 has a single USP domain and a common WDR partner called WDR48 (also known as USP1-associated factor 1; UAF-1) that, when bound, can increase the activity of these USPs.²¹ The USP46-WDR48-WDR20 and the USP12-WDR48-WDR20 complex have a very comparable overall structure.²² In addition, whereas USP1 cannot interact with WDR20, USP12, and USP46 may; further WDR48 and WDR20 can both independently and cooperatively stimulate the activities of USP12 and USP46.^{23,24} The structures of USP46 demonstrate that WDR48 interacts with the fingers subdomain of USP46. This binding may maintain the substrate binding, hence increasing USP46's activity, without significantly altering the structure around the catalytic core. The USP46-WDR48 interface and the USP46-WDR48-Ub complex share nearly the same characteristics.¹³ The interface between USP46 and WDR20 is facilitated through both hydrophobic and hydrophilic contacts. It involves numerous loops on WDR20's top surface and 2 loops at the base of USP46's palm subdomain. The residues of WDR20 implicated in the interactions with USP46 are rigorously conserved across species, according to sequence alignments. These findings imply that the complex formation and USP46 activity

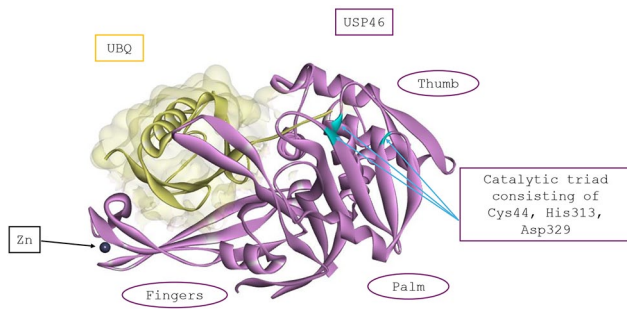


Figure 2. The structure of USP46-WDR48-WDR20 complex (PDB ID: 6JLQ). Blue: WD Repeat Domain 20 (WDR20), Purple: Ubiquitin-specific protease 46, Green: WD Repeat Domain 48 (WDR48).

regulation are significantly influenced by the residues participating in the USP46-WDR20 interaction (Figure 2).²¹

Inhibitors of USP46

In accordance with its physiological and pathological involvement in several significant signaling pathways, USP46 has become a viable target for pharmaceutical intervention in a diverse array of cancers and neurological illnesses. Nonetheless, the creation of strong and focused USP46 inhibitors continues to be an appealing but difficult undertaking, in part because DUBs are so extensively conserved.²⁵ Discovering additional USP46 inhibitors is crucial because there is currently relatively little research on substances that might inhibit USP46.

Salidroside

For myocardial ischemia/reperfusion (I/R) damage, Salidroside exhibits an inhibitory action.²⁶ Myocardial infarction cell model simulation was achieved via I/R therapy of human cardiomyocytes. Cell viability, cell proliferation, and cell apoptosis were evaluated by CCK-8 assay, EdU assay, and flow cytometry analysis, respectively. Using qRT-PCR, the RNA expression levels of circ_0097682, miR-671-5p, F-box, and USP46 were found. The Western blotting test was used to quantify protein expression. Through the application of enzyme-linked immunosorbent assays, the amounts of interleukin (IL)-6, IL-1 β , and TNF- α in the cell supernatant were determined. The administration of Salidroside alleviated the inhibitory impact that I/R had on the proliferation of AC16 cells, while also inducing apoptosis, inflammation, and oxidative damage in the cells. Salidroside reduced the expression of circ_0097682 in AC16 cells administered via I/R. Salidroside-mediated prevention of I/R-induced cellular damage included circ_0097682 expression reduced levels. Furthermore, in AC16 cells treated with I/R, circ_0097682 attached to miR-671-5p, and miR-671-5p inhibitors restored the effects of Salidroside pretreatment. Furthermore, miR-671-5p targeted USP46 in AC16 cells, and the addition of USP46 partly alleviated the effects of Salidroside pretreatment or circ_0097682 depletion in AC16

cells that had received I/R therapy. In conclusion, Salidroside prevented I/R-induced damage to AC16 cells by blocking the circ_0097682/miR-671-5p/USP46 pathway.²⁷

Galeterone

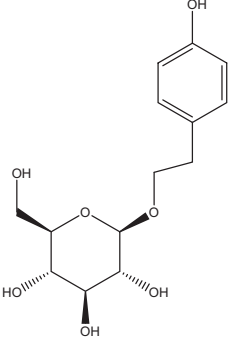
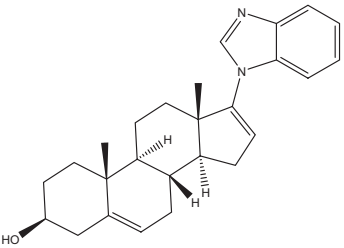
Ubiquitin-specific protease 46 is an enzyme for deubiquitinates androgen receptors (ARs), controlling AR signaling, and the P53 pathway.²⁸ Galeterone has been demonstrated to regulate the stability of the AR and MDM2 proteins in previous studies, while the exact mechanism is still unknown.^{29,30} After screening a wide panel of DUBs, Urszula L. McClurg et al found that Galeterone selectively inhibited USP12 (IC₅₀ 2.1–3.4 μ M) and USP46 (IC₅₀ 3.4–4.2 μ M), but had no impact on any other DUBs. Additional investigation revealed a strong functional relationship between USP12/USP46 silencing and Galeterone therapy.²⁸ Galeterone was able to prevent cell development even in AR-negative prostate cancer (PC) models by blocking the enzymatic activity of USP12 and USP46. This indicates the significance of USP12 and USP46 in controlling the P53 and AKT pathways.^{28,31–34} Their findings suggest that galeterone or other drugs may be a very efficient antitumor therapy by inhibiting USP12 and USP46 activity; however, Galeterone benefits may be restricted in PC patients who express AR V (Table 1).²⁸

Function of USP46 in the Nervous System

Ubiquitin-specific protease 46 is crucial for synaptic function and plasticity in the central nervous system by deubiquitinating and stabilizing key synaptic proteins such as AMPA and GABA receptors. Dysregulation of USP46 has been linked to neurological disorders like major depressive disorder (MDD), intellectual disability, autism spectrum disorder (ASD), and epilepsy. Variants of the USP46 gene affect neurotransmitter systems, contributing to mood disorders, cognitive impairments, and increased seizure susceptibility. As a result, USP46 is a promising therapeutic target, with ongoing research focusing on developing specific inhibitors or modulators for treating these neurological conditions.

To elaborate the mechanism, most immediate excitatory transmission in the brain is accomplished by alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA), and regulating synaptic AMPAR levels is crucial for synapse growth and operation. GluA1–A4 pore-forming subunits may be aggregated into hetero-tetramers to create AMPARs. The biophysical and pharmacological features of the channel are determined by the makeup of its subunits and their associations with accessory subunits.³⁵ Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors can be impacted by several posttranslational modifications, such as ubiquitination, which regulates receptor trafficking and degradation.³⁶ According to a research by Huo et al³⁷ in 2015, USP46 is the AMPARs' DUB. They discovered that K63-type

Table 1. Small molecule inhibitors of USP46.

NAME	STRUCTURE	IC50 (UM)	MECHANISM	REFERENCES
Salidroside		No report	Block the circ_0097682/ miR-671-5p/USP46 pathway	Yang et al ²⁷
Galeterone		3.4-4.2 μM	No report	McClurg et al ²⁸

ubiquitination affects AMPARs and that both *in vivo* and *in vitro*, USP46 can deubiquitinate AMPARs. Expression of USP46 causes AMPAR ubiquitination in heterologous cells and neurons to significantly decrease, along with AMPAR decomposition rate diminution and surface AMPAR accumulation increase. In contrast, RNA interference (RNAi)-mediated knockdown of USP46 results in increased AMPAR ubiquitination and decreased surface AMPARs at neuronal synapses. Investigations of tiny stimulating postsynaptic currents repeatedly indicate that neurons expressing USP46-selective RNAi have weaker synapses. These findings show that AMPAR ubiquitination and degradation are regulated by USP46, which could be crucial for neurological activity and synaptic plasticity.³⁷ Ubiquitin-specific protease 46 integrates with GluA1 and PSD95 at synapses in developed astrocytes and is expressed in all regions of the brain, including the hippocampus, amygdala, cerebellum, and prefrontal cortex.^{15,37} Decreased mEPSC amplitudes, lower surface, and total levels of GluA1, and higher amounts of ubiquitinated GluA1 are the outcomes of USP46 knockdown, which is consistent with USP46's function in deubiquitinating mammalian AMPARs.³⁷ All of these findings point to a conserved mechanism in which USP46 deubiquitinates AMPARs at synapses to prevent their breakdown and to encourage their recycling to the cell surface, which in turn impacts synapse function.³⁸

In addition, ubiquitin attaches directly to the cytoplasmic tail of glutamate receptors-1 (GLR-1), triggering clathrin-mediated endocytosis and subsequent destruction. Ubiquitin-specific protease 46 was the first DUB to control GLRs in

Caenorhabditis elegans, as shown by a concentrated RNA interference screen. Ubiquitin-specific protease 46 dysfunctional mutants show reduced amounts of GLR-1 at synapses and elevated levels of ubiquitinated GLR-1.³⁹ Mutants losing USP46 show reduced levels of GLR-1 at synapses and elevated amounts of ubiquitinated GLR-1.⁴⁰ Ubiquitin-specific protease 46 loss-of-function mutants do not alter the abundance of GLR-1(4KR), a mutant receptor that is not ubiquitinated. This suggests that GLR-1's abundance is regulated by USP46 deubiquitinating it. Because USP46 loss-of-function mutants exhibit decreased cell surface levels of GLR-1 and related abnormalities in GLR-1-dependent behaviors, USP46 control of GLR-1 is physiologically relevant.³⁸ Kowalski et al⁴⁰ suggested a hypothesis in which USP46 functions in endosomes to improve GLR-1 stability and recycling to the cell surface, based on evidence demonstrating USP46's partial colocalization with endosomes. According to recent research, vertebrate GluA1(KR), which is not ubiquitinated, eludes lysosomal degradation and returns to the cell surface, supporting this concept.⁴¹

In mice, USP46 is additionally involved in controlling the GABAergic system. Tomida et al¹⁵ found that alterations in behaviors resembling depression, compatible with an antidepressive state, are also present in an inbred strain of mice (CS strain), known to have problems in circadian rhythms. Through quantitative trait locus mapping, a 3 bp deletion in a conserved lysine (Δ K92) in USP46 was found. The mice were CS. Significantly, depression-like behaviors are altered similarly in USP46 knock-out (KO) mice,⁴² and these behaviors were

restored in CS animals by wide expression of a wild-type USP46 transgene.¹⁵ It is possible that USP46 activity is not entirely eliminated by the $\Delta K92$ mutation since the behavioral effects in USP46 ($\Delta K92$) mutant mice were not as pronounced as those in USP46 KO mice.⁴² In fact, studies for *in vitro* deubiquitination showed that USP46($\Delta K92$) still possesses some enzymatic activity.⁴³

Ubiquitin-specific protease 46 is hypothesized to have a role in the regulation of brain activity, synaptic transmission, and synaptic plasticity by regulating glutamatergic AMPARs.³⁷ Ubiquitin-specific protease 46 is also related to the control of the GABAergic system in mice, which preserves rapid inhibitory transmission in the brain.¹⁵ Mice that lack USP46 exhibit depressive-like behaviors⁴⁴ and express less glutamic acid decarboxylase (GAD67), an enzyme involved in the production of GABA.¹⁵ All things considered, USP46's role in controlling a variety of synaptic receptors emphasizes how crucial USP46 is for the development of synapses and the shape of neurons.³⁷ Although the exact mechanism is unknown, several other studies indicate that USP46 influences both presynaptic and postsynaptic components of the GABA system. One theory is that USP46 acts as a compensation mechanism to a basic deficiency in excitatory neurons' AMPAR degradation, which might have an indirect effect on the GABA system. Future research should focus on determining if USP46 acts in GABA neurons and whether it directly deubiquitinates GABA system elements.³⁸

Dysregulation of USP46 in Cancer

Ubiquitin-specific protease 46 is a multifunctional DUB that modulates certain substrates to regulate a variety of biological processes.^{12,40,43,45} Numerous studies have demonstrated the many functions of USP46 in the development and spread of tumors. It acts as a tumor suppressor in hepatocellular carcinoma (HCC),^{46,47} renal cell carcinoma (RCC),⁴⁸ and colon cancer,³² inhibiting the progression of these cancers when highly expressed. However, in esophageal squamous cell carcinoma⁴⁹ and cervical cancer (CC),^{50,51} USP46 increases tumor growth.⁵² The following section focuses on understanding the role of USP46 related to cancers.

Breast cancer

In the world, breast cancer (BC) is the most frequently recognized carcinoma in women and the leading cause of cancer-related deaths. Around the world, the prevalence of BC is steadily rising. Patients with BC make up to 36% of all cancer patients. In 2018, approximately 2.089 million women received a BC diagnosis.^{53,54} All across the world, the number of instances of this malignant tumor is rising, although it is more common in developed nations. Industrialized nations account for nearly half of all cases.⁵⁴ This trend is mainly due to the so-called Western lifestyle, associated with a poor diet, nicotine, excessive stress, and little physical activity.⁵⁵ Estrogen receptor

(ER+) BC represents up to around 70% of the BC subtypes.⁵⁶ Even with the significant therapeutic advantages that endocrine treatment has provided for ER+ BC patients, medication resistance and relapse continue to be significant issues.^{57,58} Thus, to lower the death rate for patients with BC, it is imperative to aggressively seek out novel therapeutic approaches as well as prompt diagnostic and prognostic techniques.

Ubiquitin-specific protease 46 has been demonstrated to be overexpressed in BC cells, and this was associated with a bad prognosis for the patients. Ubiquitin-specific protease 46 upregulation regulated phosphoglycerate mutase 1 (PGAM1)-mediated glycolysis, which in turn promoted the growth, migration, and carcinogenesis of triple-negative breast cancer (TNBC) cells. After USP46 was knocked down, cell death was triggered.⁵⁹

According to a recent study, USP46 is essential for controlling Tamoxifen resistance in BC cells. In particular, USP46 collaborates with PTBP1 to raise the protein levels of PTBP1, which in turn increase the PKM2/PKM1 ratio, improves glycolysis, and encourages BC cells to become resistant to tamoxifen. This pathway is particularly important in BC cells that express a lot of USP46. These results highlight the critical function of the USP46/PTBP1/PKM2 axis in inducing glycolysis and tamoxifen resistance in BC cells, providing a therapeutic approach that shows promise in addressing tamoxifen resistance in BC endocrine treatment.⁵²

Hepatocellular carcinoma

The third most frequent cause of cancer-related deaths globally is HCC, one of the most prevalent malignant tumors with a significant morbidity rate.^{60,61} To establish a theoretical foundation for the therapy of this illness, it is therefore very critical to investigate the molecular process underlying HCC growth and metastasis. In HCC tissues, USP46 is downregulated, and low USP46 levels are linked to a worse prognosis for HCC patients. In functional investigations, USP46 knockdown increased cell proliferation and invasiveness *in vitro* and *in vivo* whereas overexpression of USP46 decreased HCC cell proliferation and metastasis. Moreover, we discovered that USP46 inhibits YAP1 to reduce HCC cell growth and metastasis. The suppression of cell growth and metastasis brought on by USP46 overexpression was reversed by ectopic expression of YAP1. Scientifically, USP46 stimulates MST1 expression, which in turn antagonizes YAP1 to inhibit the development of HCC by promoting the breakdown of YAP1. Furthermore, USP46 directly binds to the MST1 protein and lowers its ubiquitination, stabilizing it. When combined, USP46 could represent a potential HCC tumor suppressor. In addition, USP46 functions as an MST1 DUB, enhancing MST1 kinase activity to inhibit tumor development and metastasis. These findings suggest that activating USP46 might be a viable therapeutic approach for HCC.⁴⁶

Through the use of RNA-seq, quantitative polymerase chain response, Western blotting, and luciferase report, USP46 was identified as the main target gene of miR-27a-3p in HCC. Ubiquitin-specific protease 46 mRNA is excessively overproduced, and this might be the primary cause of USP46's poor expression in HCC. Over-expressing miR-27a-3p or down-regulating USP46 in HCC cells resulted in a considerable increase in the proliferation function of HCC cells, whereas on the contrary, these actions dramatically reduced the proliferation of HCC cancer cells. Two potentially successful treatment approaches for HCC were activating USP46 expression or targeting suppression of miR-27a-3p.⁴⁷ Furthermore, it has been found that miR-27a functions as an inducer of tumors and is elevated in a number of human malignancies, including ovarian cancer (OC), lung cancer (LC), endometrial cancer, and colorectal cancer.^{43,62-65} Noncoding RNAs, however, have been demonstrated to be important regulators in malignancies. But as of right now, no treatment approach based on noncoding RNAs is being used in clinical settings.^{63,66-68} As a result, targeting the miR-27a/USP46 axis could represent a useful cancer treatment strategy.

Ovarian cancer

When gynecological malignancies are detected in women, OC is the primary cause of mortality. In general, it ranks as the sixth most common cause of mortality for women. The biggest challenge for treating advanced OC is resistance to clinical chemotherapy, even with the availability of several medications for treating and improving the prognosis of OC. Therefore, there is an urgent need for innovative treatment methods based on a deeper knowledge of the molecular characteristics of this malignancy as well as efficient preventive and detection measures.^{69,70}

Ubiquitin-specific protease 46 expression amounts in DDP-resistant OC were also examined *in vitro* using the OC cell lines, SKOV3, and SKOV3/DDP. Xu et al found that USP46 expression levels were downregulated in OC chemoresistant tissues as compared to chemosensitive tissues, indicating that aberrant expression of USP46 might prove essential to the establishment of resistance in OC. The results showed that overexpression of USP46 increased DDP-mediated apoptosis and made SKOV3/DDP cells more susceptible to cisplatin (DDP). Contrarily, once USP46 expression was knocked down, SKOV3 cells lost their reactivity to chemotherapy. According to these results, USP46 could be a useful marker for identifying OC patients who are undergoing platinum-based chemotherapy and who are both chemoresistant and chemosensitive. In addition, Bcl-2 expression was elevated while Bax, caspase-3, and caspase-9 expression were downregulated upon USP46 suppression. On the contrary, these apoptotic-related factors' expression levels had opposite effects due to the amplification

of USP46. These results showed that USP46 could influence OC cells' chemoresistance through the Bcl-2/caspase-3 signaling system. While USP46 overexpression may reduce phosphorylation levels, downregulation of USP46 expression levels may also encourage phosphorylation of AKT and mTOR. According to the previously reported results, controlling AKT/mTOR phosphorylation had an impact on the proliferative capacity of OC cells, which in turn controlled the chemoresistance of OC. Thus, USP46 might be a potential therapeutic target for DDP-resistant OC.⁷⁰

Lung cancer

With 11.6% of all instances identified, LC is the most often diagnosed kind of cancer worldwide, with growing incidence and mortality rates. There are 236 740 new instances of LC in the United States in 2022, and 130 180 people will die from the disease.⁷¹ Lung cancer continues to be the primary cause of cancer deaths worldwide (18.4% of all cancer deaths), resulting in a large financial loss and social burden.^{71,72} Patients with LC now have a higher survival rate thanks to recent developments in cancer therapy, nevertheless there are still unmet therapeutic requirements. To effectively treat LC, it is essential to understand the molecular pathways that underlie carcinogenesis.

Ubiquitin-specific protease 46 expression has been reported to be downregulated in LC patients, indicating that USP46 might have a tumor suppressor function in LC. This finding is in line with data from research on RCC⁴⁸ and colorectal cancer.³² In LC cells growing normally and in cells exposed to IR, USP46 effectively prevents cell division. On the contrary, radiation therapy combined with an AKT inhibitor inhibits the growth of LC cells, whereas downregulating USP46 promotes the growth of LC cells. These findings have great practical implications because they suggest that a combination of radiation and AKT inhibitors may be a useful treatment option for treating USP46-downregulated LC. The mechanism by which USP46 inhibits LC proliferation involves deubiquitinating Pleckstrin homologous domain leucine-rich repeating protein phosphatases 1 (PHLPP1), which in turn inhibits AKT activation. These results provide a strong basis for *in vivo* studies that will assess USP46's precise function in the evolution of LC in more physiologically realistic mice models. It will be fascinating to look into whether USP46 plays a major role in the development of the main tumor, metastasis, or both. Future research will focus on these inquiries.⁷³

Cervical cancer

Cervical cancer is one of the main causes of cancer-related mortality among women. Over the past 30 years, the increasing proportion of young women affected by CC has ranged from 10% to 40%.⁷⁴ According to the World Health Organization

(WHO) and International Agency for Research on Cancer (IARC) estimates, 529 000 new cases of CC were reported worldwide in 2008.⁷⁵ Cervical cancer is the fourth most common disease globally in terms of diagnoses and the fourth main cause of cancer-related mortality for women in 2018, with an estimated 570 000 cases and 311 000 deaths.⁷² It is critical that we comprehend the pathogenic process and available therapeutic options for this malignancy.

In Human Papillomavirus-induced (HPV-induced) malignancies, E6 oncoproteins from high-risk variants are crucial for cell division and metamorphosis. According to Shashi Kiran's 2018 investigation, the E6 of high-risk HPV specifically recruits USP46, a cellular deubiquitinase, to deubiquitinate and consolidate Cdt2/DTL, but not low-risk HPV. Cell proliferation is stimulated and the amount of Set8, an epigenetic writer, is limited by the stabilization of Cdt2, a component of the CRL4Cdt2 E3 ubiquitin ligase. The growth of HPV-transformed cells requires USP46, but not that of cells lacking HPV. Human cervical malignancies have higher levels of Cdt2, and xenografts with HPV-transformed tumors develop more slowly when USP46 is knocked down. One of the major functions of carcinogenic E6 is the engagement of a physiological deubiquitinase that regulates critical cellular proteins. Because of the significance of the E6-USP46-Cdt2-Set8 pathway in HPV-induced malignancies, USP46 is a potential for treatment of these cancers.⁵⁰

By 2022, Shashi Kiran et al demonstrated that, in CC biopsies, Cdt2 stabilization in HPV-induced cancers results in a reduction in H4K20me1 or H4K20me3, which can be identified through immunohistochemistry, as well as a CRL4-Cdt2 substrate, the histone H4K20 mono-methyltransferase Set8. E6 suppression reduces Cdt2 and raises Set8 *in vitro* in HPV-transformed cancer cell lines. Co-knockdown of Set8 demonstrates that amplification in Set8 is responsible for a portion of the alterations in gene expression brought about by E6 knockdown. This gene collection included both EGFR and EGFR regulated genes. Regarding the mechanism through which E6 stabilizes Cdt2, it could be seen that purified E6: USP46 complex exhibits significantly greater deubiquitinase activity *in vitro* than USP46 alone. This indicates that E6 may engage exclusively with USP46 regardless of other proteins and might serve as a stand-in for UAF1 and WDR20, 2 known activators of USP46. Three distinct but overlapping portions of the substrate are necessary for E6 to stabilize the substrate, according to deletion mapping of Cdt2: USP46. It is implied that interaction with DDB1 (and the remainder of the CRL4 complex) or with the substrate of the CRL4-Cdt2 E3 ligase is not required for E6: USP46 to interact with and stabilize Cdt2 because the helix-loop-helix region or the WD40 repeat motivated beta-propeller configuration of Cdt2 is irrelevant for the stabilization. The particular nature of the mechanism is shown by the discovery that 50 amino acid sequences within the 731 amino

acid Cdt2 protein are crucial for the stabilization by E6. In conclusion, E6 causes degradation of Set8 and modifications in gene regulation in HPV-transformed cells, increases the deubiquitinase activity of USP46, and stabilizes Cdt2 by using numerous sites on Cdt2.⁵¹

Renal cell carcinoma

One of the most common carcinogenic disorders of the urination process RCC is a heterogeneous, metastatic condition that is unresponsive to standard treatment approaches.^{76,77} Renal cell carcinoma causes about 140 000 fatalities yearly and accounts for around 95% of kidney cancers.^{72,76,78,79} Because of the inadequate evaluation, the frequency of RCC is gradually increasing. According to A. Sánchez-Gastaldo et al,⁸⁰ over 30% of patients with RCC are identified with metastasized tumors, and many patients subsequently experience recurrence following surgical therapies. Surgical resection therapy remains an effective and curative therapeutic method for RCC, even with the development of innovative immunotherapeutic techniques.^{81,82} Meanwhile, patients with RCC continue to have a very poor 5-year overall survival rate.^{83,84} It is consequently imperative to uncover the molecular processes underpinning the RCC regulatory system.

Ubiquitin-specific protease 46 has been shown by Dingwen Gui et al to operate as a tumor suppressor in RCC by limiting the proliferation and metastasis of RCC cells. It was evident that the USP46 high-expression group had a favorable prognosis and survival rate. In addition, the RCC patients' USP46 mRNA expression was lower than that of the normal controls, and the tumor malignancy grade was inversely linked with USP46 expression. *In vitro*, ectopic USP46 expression significantly decreased tumor cell motility and cell proliferation, and vice versa. AKT pathway downregulation caused by USP46 contributed to abnormalities in cell migration and proliferation. They revealed a novel method by which USP46 suppresses the AKT signaling pathway, hence impairing RCC survival. Verification of AKT inactivation by USP46 is also employed in different tumor scenarios. These results not only offer a fresh perspective on the molecular control of RCC, but they also point to USP46 as a possible target in the development of RCC. To determine the functional effects of USP46 in RCC and to further understand the regulatory mechanism of USP46-mediated AKT inactivation, more research is required.⁴⁸

It will be fascinating to look into whether USP46 deletion alone or in conjunction with other known mutations is sufficient to cause cancers. In addition, it is yet unknown if cancer patients have recurring mutations in the USP46 gene and whether these changes are associated with other established driver mutations. PI3K/AKT gene alterations will probably be correlated in some way with a variety of cancer types. It is

Table 2. USP46 contribution in various tumors.

DISEASES	SUBSTRATES	BIOLOGICAL FUNCTIONS AND CLINICAL SIGNIFICANCE	REFERENCES
Breast cancer	PGAM1	Stimulates the glycolysis Promotes the growth, migration, and carcinogenesis of triple-negative breast cancer cells	Ke et al ⁵⁹
	PTBP1	Improves glycolysis, and encourages BC cells to become resistant to tamoxifen	Gao et al ⁵²
Hepatocellular carcinoma	MST1	Decreases MST1 ubiquitination, stabilizing MST1; Increases MST1 kinase activity to inhibit tumor development and metastasis by promoting the breakdown of YAP1	Qiu et al ⁴⁶
	miR-27a-3p	Considerable increase in the proliferation function of HCC cells	Wen et al ⁴⁷
Ovarian cancer	None reported	Overexpression of USP46 increased DDP-mediated apoptosis, made SKOV3/DDP cells more susceptible to DDP, reduce phosphorylation levels Downregulation of USP46 expression levels encourage phosphorylation of AKT and mTOR, the chemoresistance of OC When USP46 expression decreased, SKOV3 cells lost their reactivity to chemotherapy	Xu et al ⁷⁰
Lung cancer	PHLPP1	Inhibits AKT activation, which in turn inhibits the growth of lung cancer cells	Wang et al ⁷³
Cervical cancer	Cdt2	Stabilizes Cdt2, degradation of Set8 and modifications in gene regulation in HPV-transformed cells; increases the deubiquitinase activity of USP46	Kiran et al ⁵⁰ Kiran et al ⁵¹
Renal cell carcinoma	AKT	Tumor suppressor in RCC by limiting the proliferation and metastasis of RCC	Gui et al ⁴⁸

imperative that genetic data sets be thoroughly analyzed to offer more precise answers to these significant problems (Table 2).

Discussion

Deubiquitinating enzymes have grown in popularity as cancer therapeutic targets throughout the last 10 years. Ubiquitin-specific protease 46 is a significant DUB that regulates most, if not all, of the characteristics of cancer, including the signaling pathways that promote cell cycle progression, proliferation, apoptosis, and resistance to therapy. Over the last 10 years, a number of initiatives have improved our knowledge about USP46. Studies conducted on USP46 have demonstrated its important and diverse functions in both physiological and pathological conditions, especially in carcinogenesis, highlighting the fact that USP46 is becoming a desirable target for the treatment of some linked illnesses. The discovery that USP46 seems to have 2 roles in a variety of biological processes and clinical situations, however, emphasizes the necessity of assessing USP46's function in many settings and illnesses.

We provided the key details about USP46 in this review, including its properties, structure, inhibitors, and function in various illnesses (Figure 3). More research on the regulation and

function of USP46 in both healthy and pathological conditions will shed light on the protein's specificity and activity, paving the way for the development of selective USP46 inhibitors as an effective treatment for cancer and other illnesses. Ubiquitin-specific protease 46's potential as a therapeutic target is indeed promising, yet it remains largely unexplored in clinical trials or therapeutic development. The enzyme is pivotal in regulating synaptic function and has been linked to several neurological disorders, including MDD, intellectual disabilities, and epilepsy. In addition, USP46 has shown potential relevance in cancer research, where its role in modulating protein stability and cellular signaling pathways could influence tumor progression. Despite these implications, specific inhibitors or modulators of USP46 have not yet advanced to clinical trials. The development of selective USP46-targeted therapies requires further research to understand its mechanisms fully and to identify suitable compounds that can effectively modulate its activity while minimizing potential side effects.

Thus, the goal of future research should be to find USP46-specific inhibitors that are more potent. The research and discovery of USP46 as an appropriate therapy is going to be facilitated by this improved understanding of USP46 functions and modulation for medicinal purposes.

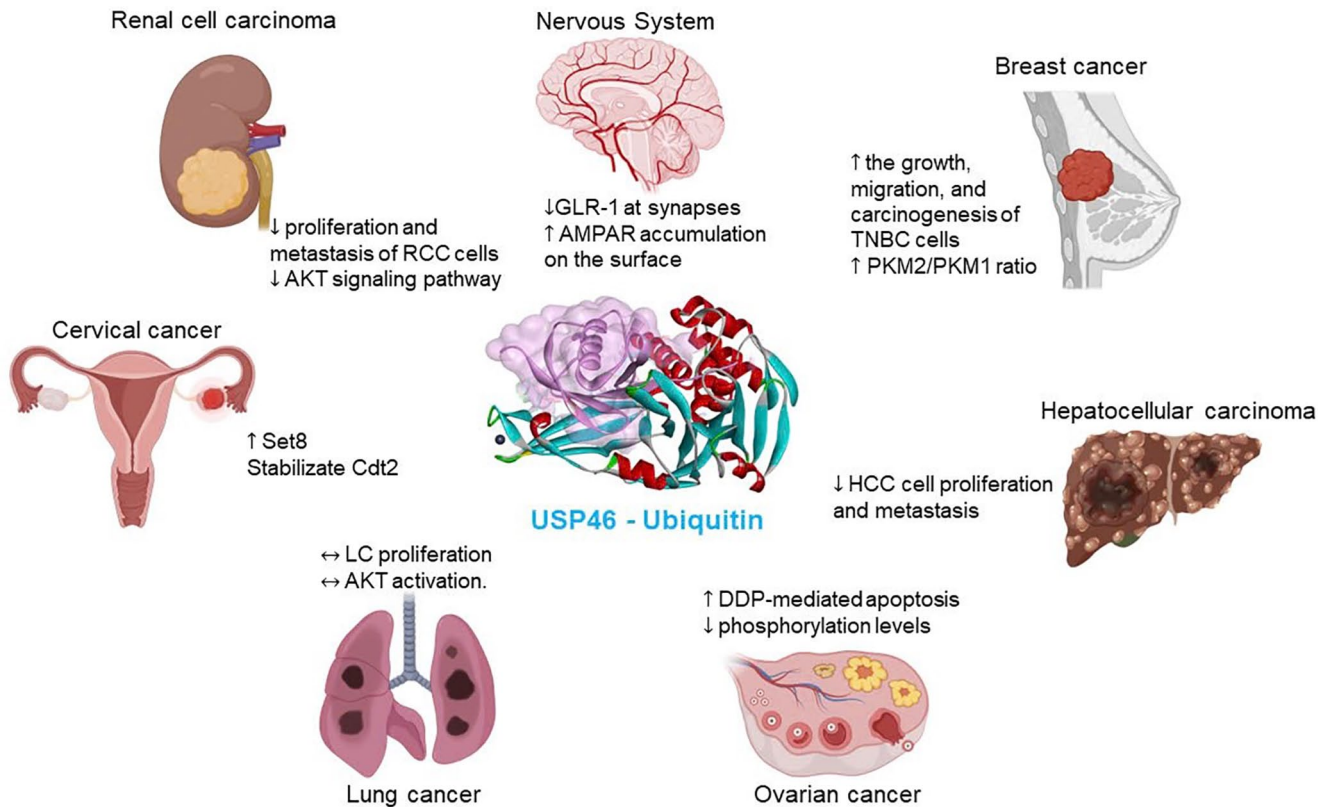


Figure 3. Summary information of USP46 protein.

Author Contributions

KHTP: Conceptualization, Methodology, Validation, Investigation, Writing—Original Draft, Visualization. MHT: Conceptualization, Visualization, Review. LBN: Conceptualization, Visualization, Review. PTVP: Writing—Review & Editing, Supervision. TKN: Writing—Review & Editing, Supervision.

Ethical Approval


Not applicable to this article.

Informed Consent

There are no human subjects in this article and informed consent is not applicable.

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REFERENCES

- Lai KP, Chen J, Tse WKF. Role of deubiquitinases in human cancers: potential targeted therapy. *Int J Mol Sci.* 2020;21:2548. doi:10.3390/ijms21072548
- Tak J, Nguyen TK, Lee K, Kim SG, Ahn HC. Utilizing machine learning to identify nifuroxazide as an inhibitor of ubiquitin-specific protease 21 in a drug repositioning strategy. *Biomed Pharmacother.* 2024;174:116459. doi:10.1016/j.biopha.2024.116459
- Cheng J, Guo J, North BJ, et al. Functional analysis of deubiquitylating enzymes in tumorigenesis and development. *Biochim Biophys Acta Rev Cancer.* 2019;1872:188312. doi:10.1016/j.bbcan.2019.188312
- Hermanns T, Pichlo C, Woiwode I, et al. A family of unconventional deubiquitinases with modular chain specificity determinants. *Nat Commun.* 2018;9:799. doi:10.1038/s41467-018
- Mevisse TE, Komander D. Mechanisms of deubiquitinase specificity and regulation. *Annu Rev Biochem.* 2017;86:159-192. doi:10.1146/annurev-biochem
- Cruz L, Soares P, Correia M. Ubiquitin-specific proteases: players in cancer cellular processes. *Pharmaceuticals.* 2021;14:848. doi:10.3390/ph14090848
- Chen S, Liu Y, Zhou H. Advances in the development ubiquitin-specific peptidase (USP) inhibitors. *Int J Mol Sci.* 2021;22:4546. doi:10.3390/ijms22094546
- Hu B, Zhang D, Zhao K, et al. Spotlight on USP4: structure, function, and regulation. *Front Cell Dev Biol.* 2021;9:595159. doi:10.3389/fcell.2021.595159
- Kitamura H, Hashimoto M. USP2-related cellular signaling and consequent pathophysiological outcomes. *Int J Mol Sci.* 2021;22:1209. doi:10.3390/ijms22031209
- Ning F, Xin H, Liu J, et al. Structure and function of USP5: insight into physiological and pathophysiological roles. *Pharmacol Res.* 2020;157:104557. doi:10.1016/j.phrs.2019.104557
- Wang F, Gao Y, Zhou L, et al. USP30: structure, emerging physiological role, and target inhibition. *Front Pharmacol.* 2022;13:851654. doi:10.3389/fphar.2022.851654
- Joo H-Y, Jones A, Yang C, et al. Regulation of histone H2A and H2B deubiquitination and Xenopus development by USP12 and USP46. *J Biol Chem.* 2011;286:7190-7201. doi:10.1074/jbc.M110.158311
- Yin J, Schoeffler AJ, Wickliffe K, et al. Structural insights into WD-repeat 48 activation of ubiquitin-specific protease 46. *Structure.* 2015;23:2043-2054. doi:10.1016/j.str.2015.08.010
- Ohashi M, Holthaus AM, Calderwood MA, et al. The EBNA3 family of Epstein-Barr virus nuclear proteins associates with the USP46/USP12 deubiquitination complexes to regulate lymphoblastoid cell line growth. *PLoS Pathog.* 2015;11:e1004822. doi:10.1371/journal.ppat.1004822
- Tomida S, Mamiya T, Sakamaki H, et al. Usp46 is a quantitative trait gene regulating mouse immobile behavior in the tail suspension and forced swimming tests. *Nat Genet.* 2009;41:688-695. doi:10.1038/ng.344

16. Shu J, Yang C, Miao Y, et al. USP46 promotes the interferon antiviral signaling in black carp by deubiquitinating TBK1. *Dev Comp Immunol.* 2024;156:105170. doi:10.1016/j.dci.2024.105170
17. Ye Y, Scheel H, Hofmann K, Komander D. Dissection of USP catalytic domains reveals five common insertion points. *Mol Biosyst.* 2009;5:1797-1808. doi:10.1039/b907669g
18. Villamil MA, Liang Q, Zhuang Z. The WD40-repeat protein-containing deubiquitinase complex: catalysis, regulation, and potential for therapeutic intervention. *Cell Biochem Biophys.* 2013;67:111-126. doi:10.1007/s12013-013
19. Hu M, Li P, Li M, et al. Crystal structure of a UBP-family deubiquitinating enzyme in isolation and in complex with ubiquitin aldehyde. *Cell.* 2002;111:1041-1054. doi:10.1016/S0092-8674(02)01199
20. Aron R, Pellegrini P, Green EW, et al. Deubiquitinase Usp12 functions noncatalytically to induce autophagy and confer neuroprotection in models of Huntington's disease. *Nat Commun.* 2018;9:3191. doi:10.1038/s41467-018
21. Zhu H, Zhang T, Wang F, Yang J, Ding J. Structural insights into the activation of USP46 by WDR48 and WDR20. *Cell Discov.* 2019;5:34. doi:10.1038/s41421-019
22. Li H, Lim KS, Kim H, et al. Allosteric activation of ubiquitin-specific proteases by β -propeller proteins UAF1 and WDR20. *Mol Cell.* 2016;63:249-260. doi:10.1016/j.molcel.2016.05.031
23. Dahlberg CL, Juo P. The WD40-repeat proteins WDR-20 and WDR-48 bind and activate the deubiquitinating enzyme USP-46 to promote the abundance of the glutamate receptor GLR-1 in the ventral nerve cord of *Caenorhabditis elegans*. *J Biol Chem.* 2014;289:3444-3456. doi:10.1074/jbc.M113.507541
24. Kee Y, Yang K, Cohn MA, Haas W, Gygi SP, D'Andrea AD. WDR20 regulates activity of the USP12- UAF1 deubiquitinating enzyme complex. *J Biol Chem* 2010;285:11252-11257. doi: 10.1074/jbc.M109.095141
25. Harrigan JA, Jacq X, Martin NM, Jackson SP. Deubiquitylating enzymes and drug discovery: emerging opportunities. *Nat Rev Drug Discov.* 2018;17:57-78. doi:10.1038/nrd.2017.152
26. Tian X, Huang Y, Zhang X, et al. Salidroside attenuates myocardial ischemia/reperfusion injury via AMPK-induced suppression of endoplasmic reticulum stress and mitochondrial fission. *Toxicol Appl Pharmacol.* 2022;448:116093. doi:10.1016/j.taap.2022.116093
27. Yang Y, Liang F, Gao J, et al. Salidroside ameliorates ischemia/reperfusion-induced human cardiomyocyte injury by inhibiting the Circ_0097682/miR-671-5p/USP46 pathway. *Cardiovasc Toxicol.* 2023;23:406-418. doi:10.1007/s12012-023
28. McClurg UL, Azizyan M, Dransfield DT, et al. The novel anti-androgen candidate galeterone targets deubiquitinating enzymes, USP12 and USP46, to control prostate cancer growth and survival. *Oncotarget.* 2018;9:24992. doi:10.18632/oncotarget.25167
29. Kwegyir-Afful AK, Ramalingam S, Purushottamachar P, Ramamurthy VP, Njar VC. Galeterone and VNPT55 induce proteasomal degradation of AR/AR-V7, induce significant apoptosis via cytochrome c release and suppress growth of castration resistant prostate cancer xenografts in vivo. *Oncotarget.* 2015;6:27440. doi:10.18632/oncotarget.4578
30. Yu Z, Cai C, Gao S, Simon NI, Shen HC, Balk SP. Galeterone prevents androgen receptor binding to chromatin and enhances degradation of mutant androgen receptor. *Clin Cancer Res.* 2014;20:4075-4085. doi:10.1158/1078-0432.CCR
31. Brognard J, Sierrecki E, Gao T, Newton AC. PHLPP and a second isoform, PHLPP2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms. *Mol Cell.* 2007;25:917-931. doi:10.1016/j.molcel.2007.02.017
32. Li X, Stevens PD, Yang H, et al. The deubiquitination enzyme USP46 functions as a tumor suppressor by controlling PHLPP-dependent attenuation of Akt signaling in colon cancer. *Oncogene.* 2013;32:471-478. doi:10.1038/onc.2012.66
33. Mayo LD, Donner DB. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc Natl Acad Sci USA.* 2001;98:11598-11603. doi:10.1073/pnas.181181198
34. McClurg UL, Summerscales EE, Harle VJ, Gaughan L, Robson CN. Deubiquitinating enzyme Usp12 regulates the interaction between the androgen receptor and the Akt pathway. *Oncotarget.* 2014;5:7081. doi:10.18632/oncotarget.2162
35. Anggono V, Haganir RL. Regulation of AMPA receptor trafficking and synaptic plasticity. *Curr Opin Neurobiol.* 2012;22:461-469. doi:10.1016/j.conb.2011.12.006
36. Goo MS, Scudder SL, Patrick GN. Ubiquitin-dependent trafficking and turnover of ionotropic glutamate receptors. *Front Mol Neurosci.* 2015;8:60. doi:10.3389/fnfmol.2015.00060
37. Huo Y, Khatri N, Hou Q, Gilbert J, Wang G, Man HY. The deubiquitinating enzyme USP 46 regulates AMPA receptor ubiquitination and trafficking. *J Neurochem.* 2015;134:1067-1080. doi:10.1111/jnc.13194
38. Hodul M, Dahlberg CL, Juo P. Function of the deubiquitinating enzyme USP46 in the nervous system and its regulation by WD40-repeat proteins. *Front Synaptic Neurosci.* 2017;9:16. doi:10.3389/fnpsy.2017.00016
39. Burbea M, Dreier L, Dittman JS, Grunwald ME, Kaplan JM. Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in *C. elegans*. *Neuron.* 2002;35:107-120. doi:10.1016/S0896-6273(02)00749
40. Kowalski JR, Dahlberg CL, Juo P. The deubiquitinating enzyme USP-46 negatively regulates the degradation of glutamate receptors to control their abundance in the ventral nerve cord of *Caenorhabditis elegans*. *J Neurosci.* 2011;31:1341-1354. doi:10.1523/JNEUROSCI.4765-10.2011
41. Widagdo J, Chai YJ, Ridder MC, et al. Activity-dependent ubiquitination of GluA1 and GluA2 regulates AMPA receptor intracellular sorting and degradation. *Cell Rep.* 2015;10:783-795. doi:10.1016/j.celrep.2015.01.015
42. Imai S, Kano M, Nonoyama K, Ebihara S. Behavioral characteristics of ubiquitin-specific peptidase 46-deficient mice. *PLoS ONE.* 2013;8:e58566. doi:10.1371/journal.pone.0058566
43. Zhang W, Tian QB, Li QK, et al. Lysine 92 amino acid residue of USP46, a gene associated with 'behavioral despair' in mice, influences the deubiquitinating enzyme activity. *PLoS ONE.* 2011;6:e26297. doi:10.1371/journal.pone.0026297
44. Imai S, Mamiya T, Tsukada A, et al. Ubiquitin-specific peptidase 46 (Usp46) regulates mouse immobile behavior in the tail suspension test through the GABAergic system. *PLoS ONE.* 2012;7:e39084. doi:10.1371/journal.pone.0039084
45. Fukuo Y, Kishi T, Kushima I, et al. Possible association between ubiquitin-specific peptidase 46 gene and major depressive disorders in the Japanese population. *J Affect Disord.* 2011;133:150-157. doi:10.1016/j.jad.2011.04.020
46. Qiu Y, Huang D, Sheng Y, et al. Deubiquitinating enzyme USP46 suppresses the progression of hepatocellular carcinoma by stabilizing MST1. *Exp Cell Res.* 2021;405:112646. doi:10.1016/j.yexcr.2021.112646
47. Wen M, Xu H, Peng H, Sheng Y, Yang W, Yan J. MiR-27a-3p targets USP46 to inhibit the cell proliferation of hepatocellular carcinoma. *Chem Biol Drug Des.* 2022;100:280-289. doi:10.1111/cbdd.14063
48. Gui D, Peng W, Jiang W, et al. Ubiquitin-specific peptidase 46 (USP46) suppresses renal cell carcinoma tumorigenesis through AKT pathway inactivation. *Biochem Biophys Res Commun.* 2019;519:689-696. doi:10.1016/j.bbrc.2019.09.036
49. Tian M, Zhu R, Ding F, Liu Z. Ubiquitin-specific peptidase 46 promotes tumor metastasis through stabilizing ENO1 in human esophageal squamous cell carcinoma. *Exp Cell Res.* 2020;395:112188. doi:10.1016/j.yexcr.2020.112188
50. Kiran S, Dar A, Singh SK, Lee KY, Dutta A. The deubiquitinase USP46 is essential for proliferation and tumor growth of HPV-transformed cancers. *Mol Cell.* 2018;72:823-835.e5. doi:10.1016/j.molcel.2018.09.019
51. Kiran S, Wilson B, Saha S, Graff JA, Dutta A. HPV E6-USP46 mediated Cdt2 stabilization reduces Set8 mediated H4K20-methylation to induce gene expression changes. *Cancers.* 2021;14:30. doi:10.3390/cancers14010030
52. Gao S, Wang Y, Xu Y, Liu L, Liu S. USP46 enhances tamoxifen resistance in breast cancer cells by stabilizing PTBP1 to facilitate glycolysis. *Biochim Biophys Acta Mol Basis Dis.* 2024;1870:167011. doi:10.1016/j.bbdis.2023.167011
53. Nardin S, Mora E, Varughese FM, et al. Breast cancer survivorship, quality of life, and late toxicities. *Front Oncol.* 2020;10:864. doi:10.3389/fonc.2020.00864
54. Global Cancer Observatory. International Agency for Research on Cancer. Accessed May 10, 2024. <https://gco.iarc.fr/>
55. Bellanger M, Zeinomar N, Tehranifar P, Terry MB. Are global breast cancer incidence and mortality patterns related to country-specific economic development and prevention strategies? *J Glob Oncol.* 2018;4:1-16. doi:10.1200/JGO.17.00207
56. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin.* 2023;73:17-48. doi:10.3322/caac.21763
57. Barua D, Gupta A, Gupta S. Targeting the IRE1-XBP1 axis to overcome endocrine resistance in breast cancer: opportunities and challenges. *Cancer Lett.* 2020;486:29-37. doi:10.1016/j.canlet.2020.05.020
58. Franzoi MA, Agostinotto E, Perachino M, et al. Evidence-based approaches for the management of side-effects of adjuvant endocrine therapy in patients with breast cancer. *Lancet Oncol.* 2021;22:e303-e313. doi:10.1016/S1470-2045(20)30666
59. Ke L, Jia Z, Gao W, Luo L. Ubiquitin specific protease 46 potentiates triple negative breast cancer development by stabilizing PGAM1-mediated glycolysis. *Cell Biol Int.* 2023;47:41-51. doi:10.1002/cbin.11937
60. Cheng H, Sun G, Chen H, et al. Trends in the treatment of advanced hepatocellular carcinoma: immune checkpoint blockade immunotherapy and related combination therapies. *Am J Cancer Res.* 2019;9:1536-1545.
61. Tang T-Y, Zong Y, Shen Y-N, et al. Predicting surgical site infections using a novel nomogram in patients with hepatocellular carcinoma undergoing hepatectomy. *World J Clin Cases.* 2019;7:2176. doi:10.12998/wjcc.v7.i16.2176

62. Chae DK, Ban E, Yoo YS, Kim EE, Baik JH, Song EJ. MIR-27a regulates the TGF- β signaling pathway by targeting SMAD2 and SMAD4 in lung cancer. *Mol Carcinog*. 2017;56:1992-1998. doi:10.1002/mc.22655
63. Hao L, Wang J. MiR-27a acts as an oncogene to regulate endometrial cancer progression by targeting USP46. *Int J Clin Exp Pathol*. 2021;14:720-725.
64. Liu W, Qian K, Wei X, et al. miR-27a promotes proliferation, migration, and invasion of colorectal cancer by targeting FAM172A and acts as a diagnostic and prognostic biomarker. *Oncol Rep*. 2017;37:3554-3564. doi:10.3892/or.2017.5592
65. Xu Q, Tong JL, Zhang CP, Xiao Q, Lin XL, Xiao XY. miR-27a induced by colon cancer cells in HLECs promotes lymphangiogenesis by targeting SMAD4. *PLoS ONE*. 2017;12:e0186718. doi:10.1371/journal.pone.0186718
66. Ling H, Fabbri M, Calin GA. microRNAs and other non-coding RNAs as targets for anticancer drug development. *Nat Rev Drug Discov*. 2013;12:847-865. doi:10.1038/nrd414
67. Romano G, Veneziano D, Acunzo M, Croce CM. Small non-coding RNA and cancer. *Carcinogenesis*. 2017;38:485-491. doi:10.1093/carcin/bgx026
68. Slaby O, Laga R, Sedlacek O. Therapeutic targeting of non-coding RNAs in cancer. *Biochem J*. 2017;474:4219-4251. doi:10.1042/BCJ20170079
69. Arora T, Mullangi S, Lekkala MR. Ovarian cancer. In: *StatPearls*. StatPearls Publishing, FL: Treasure Island, 2023.
70. Xu L, Zhang B, Li W. Downregulated expression levels of USP46 promote the resistance of ovarian cancer to cisplatin and are regulated by PUM2. *Mol Med Rep*. 2021;23:263. doi:10.3892/mmr.2021.11902
71. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72:7-33. doi:10.3322/caac.21708
72. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394-424. doi:10.3322/caac.21492
73. Wang W, Chen M, Xu H, Lv D, Zhou S, Yang H. USP46 inhibits cell proliferation in lung cancer through PHLPP1/AKT pathway. *Biomed Res Int*. 2020;2020:2509529. doi:10.1155/2020/2509529
74. Song B, Ding C, Chen W, Sun H, Zhang M, Chen W. Incidence and mortality of cervical cancer in China, 2013. *Chin J Cancer Res*. 2017;29:471-476. doi:10.21147/j.issn.1000-9604.2017.06.01
75. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010;127:2893-2917. doi:10.1002/ijc.25516
76. Capitanio U, Bensalah K, Bex A, et al. Epidemiology of renal cell carcinoma. *Eur Urol*. 2019;75:74-84. doi:10.1016/j.eururo.2018.08.036
77. Linehan WM, Zbar B. Focus on kidney cancer. *Cancer Cell*. 2004;6:223-228. doi:10.1016/j.ccr.2004.09.019
78. Capitanio U, Montorsi F. Renal cancer. *Lancet*. 2016;387:894-906. doi:10.1016/S0140-6736(15)00046
79. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;68:7-30. doi:10.3322/caac.21442
80. Sánchez-Gastaldo A, Kempf E, González Del Alba A, Duran I. Systemic treatment of renal cell cancer: a comprehensive review. *Cancer Treat Rev*. 2017;60:77-89. doi:10.1016/j.ctrv.2017.08.01
81. Tsuchiya N. Molecular-targeted therapy in advanced renal cell carcinoma based on pharmacokinetics, pharmacodynamics and pharmacogenetics: a proposed strategy. *Int J Urol*. 2019;26:48-56. doi:10.1111/iju.13805
82. Zerdes I, Tolia M, Tsoukalas N, et al. Systemic therapy of metastatic renal cell carcinoma: review of the current literature. *Urologia*. 2019;86:3-8. doi:10.1177/0391560318802166
83. Heidenreich A, Wilop S, Pinkawa M, Porres D, Pfister D. Surgical resection of urological tumor metastases following medical treatment. *Dtsch Arztebl Int*. 2012;109:631-637. doi:10.3238/arztebl.2012.0631
84. Tornberg SV, Nisen H, Visapää H, et al. Outcome of surgery for patients with renal cell carcinoma and tumour thrombus in the era of modern targeted therapy. *Scand J Urol*. 2016;50:380-386. doi:10.1080/21681805.2016.1217558