Roles of USP9X in cellular functions and tumorigenesis (Review)

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Abstract. Ubiquitin-specific peptidase 9X (USP9X) is involved in certain human diseases, including malignancies, atherosclerosis and certain diseases of the nervous system. USP9X promotes the deubiquitination and stabilization of diverse substrates, thereby exerting a versatile range of effects on pathological and physiological processes. USP9X serves vital roles in the processes of cell survival, invasion and migration in various types of cancer. The present review aims to highlight the current knowledge of USP9X in terms of its structure and the possible mediatory mechanisms involved in certain types of cancer, providing a thorough introduction to its biological functions in carcinogenesis and further outlining its oncogenic or suppressive properties in a diverse range of cancer types. Finally, several perspectives regarding USP9X-targeted pharmacological therapeutics in cancer development are discussed.

Contents

1. Introduction

The precise control of cell activity in response to systemic or local signals is largely governed by the regulation of homoeostasis (1). Proteins are known to execute a diverse range of intracellular processes (2). In order to accurately perform various physiological functions, the intracellular protein quality must be precisely and strictly controlled, and this level of control depends on the balance between protein degradation and synthesis (3). The lysosomal systems and the ubiquitin-proteasome system (UPS) form an interconnected protein quality control network for lysosomal and proteasomal protein degradation, respectively (4,5). In the UPS, ubiquitin is conjugated to targeted proteins, which are subsequently recognized by the proteasome for degradation (6,7). Ubiquitinases and deubiquitinases (DUBs) in the ubiquitin proteasome pathway are involved in various pathological and physiological processes, such as DNA repair (8), apoptosis (9) and cancer metabolism (10). DUBs are classified into seven families on the basis of their sequences and structural homology, namely ubiquitin-specific proteases (USPs), ovarian tumor proteases, the JAB1/MPN/MOV34 family of metalloenzymes, ubiquitin carboxy-terminal hydrolases (UCHs), the motif interacting with ubiquitin-containing novel DUB family, Machado-Josephin domain-containing proteases and the zinc finger-containing ubiquitin peptidase 1. The structural or functional abnormalities of these enzymes can lead to numerous diseases, including cancer (11,12). The USP family accounts for the largest proportion of DUBs that have been identified, and share a catalytic domain that comprises 300-800 amino acids (13).

Ubiquitin-specific peptidase 9X (USP9X), a member of the USP family, regulates numerous signaling pathways by deubiquitinating essential proteins, such as myeloid leukemia cell differentiation 1 (MCL-1) (14,15). USP9X is closely associated with neurological disorders (16-18), atherosclerosis (19) and cancers (20). However, to date, USP9X and its precise roles in different types of cancer have not been widely investigated or specifically and systematically reviewed. In addition, the value of USP9X in terms of its clinical and potential application as a cancer target may be underestimated. Therefore, in the present review, the current evidence in support of the cellular functions and underlying regulatory mechanisms of USP9X in tumorigenesis is summarized, as well as the biological mechanisms associated with USP9X in different types of cancer.

2. Structure of USP9X

The highly conserved DUB USP9X is located on chromosome Xp11.4 and was first identified as a human homologue of the

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Drosophila fat facets (*faf*) gene, subsequently named DFFRX, which escapes X-chromosome inactivation and serves vital roles in embryo development (21). The protein sequence of USP9X and its molecular functions are evolutionarily conserved across species (22-25). The two recognizable domains of USP9X are the ubiquitin-like module (UBL) domain (amino acids 886-970) and the catalytic domain with USP-definitive cysteine (amino acid 1,566) and histidine (amino acid 1,879) box catalytic motifs (Fig. 1A) (15). The long non-conserved N- and C-terminal extensions flank the catalytic domains of USP9X (26,27). The homology model of USP9X and its functional domains were constructed from the Alphafold Database (https://alphafold.com/) with PyMOL (The PyMOL Molecular Graphics System; version 2.3; Schrödinger, LLC; Fig. 1B-D). The crystal structure of USP9X has been solved and reported as a conserved and canonical USP-fold consisting of palm, finger and thumb subdomains, and a β-hairpin insertion (28). The catalytic domain of USP9X harbors a zinc finger motif and three ubiquitin binding sites in the fingers subdomain, and the β-hairpin insertion, contributing to polyubiquitin chain processing and cleavage of lysine (Lys)11-, Lys63-, Lys48- and Lys6-linkages, enabling the protein to perform a variety of cellular functions (29-34).

USP9X is predominantly present in the cytoplasm (35,36) and at the cell membrane (37), whereas small amounts of the protein are internalized into the mitochondria (38), the nucleus (39) and the centrosome (40). USP9X regulates apoptosis, mitotic fidelity, inflammation, ribosomal stalling, proliferation, epithelial-mesenchymal transition (EMT), oxidative stress, the stemness of cancer cells, chromatin reprogramming and drug resistance through the precise recognition, recruitment and binding of diverse substrates for targeted deubiquitination and stabilization (41-57) (Table I; Fig. 2). USP9X is also involved in several vital and classical signaling pathways, including the transforming growth factor-β (TGF-β), Hippo, Wnt/β-catenin and Janus kinase (JAK)-STAT signaling pathways (Fig. 3). Moreover, USP9X has been found to be enriched in the majority of cancer samples, and the deleterious genetic variants of USP9X are associated with neurodevelopmental disorders and neurodegeneration (16-18), indicating its essential and potential function in clinical treatment.

3. Cellular and biological functions of USP9X

Regulation of both the expression level of USP9X and its functions could lead to diverse and dynamic biological behavioral changes under a variety of cellular conditions. USP9X has been identified to have a series of substrates that enable the DUB to regulate cell apoptosis and survival, mitotic fidelity and the cell cycle, cell migration and invasion, and DNA damage repair. USP9X exhibits considerable control over these cellular functions and also in the development of a number of diseases.

Regulation of cell apoptosis and survival. The execution of apoptosis is countered by the action of anti-apoptotic proteins (58). Since certain substrates of USP9X, such as MCL-1 and XIAP, are key factors in cellular apoptosis signaling pathways that drive cell apoptosis, targeting these substrates via USP9X might lead to the regulation of cell apoptosis (41-45). USP9X displays both pro- and anti-apoptotic functions, mediated by the deubiquitination of critical components of the apoptotic signaling networks (53,59). Previous studies have reported that the expression of stress-sensing, pro-apoptotic kinases is regulated by USP9X to initiate the apoptotic JNK signaling cascade (59,60). The pro-apoptotic kinase, apoptosis signal-regulating kinase 1 (ASK-1), is activated under conditions of oxidative stress, leading to the selective activation of the JNK signaling pathways (59). USP9X also interacts with ASK-1, protecting it from proteasomal degradation to mediate oxidative stress-induced cell death (59). USP9X also activates and stabilizes the dual leucine zipper kinase in response to extracellular and intracellular stress in neurons, thereby enabling the activation of pro-apoptotic JNK signaling (60).

By contrast, USP9X enhances the activities of a large spectrum of anti-apoptotic factors for cell survival. In the classical mitochondrial apoptosis pathway, the pro-survival B-cell lymphoma-2 family proteins, including MCL-1, preserve mitochondrial integrity and indirectly inhibit the activation of caspase-3 and -7, ultimately limiting the rate of cell apoptosis (17). USP9X stabilizes MCL-1 through its interaction with the protein and the removal of the Lys48-linked polyubiquitin chains that mark a protein for proteasomal degradation (53). A recent study reported that the RNA helicase Asp-Glu-Ala-Asp-box polypeptide 3 interacted with the N-terminus of USP9X and participated in deubiquitination of MCL-1 (61). Therefore, human tumors with a high level of MCL-1 expression may be accompanied by the overexpression of USP9X (53,62). Upregulated expression of USP9X promotes tumorigenicity and cell survival through stabilizing cell death regulators, including X-linked inhibitor of apoptosis protein (XIAP), and inhibiting the induction of apoptosis by specifically stabilizing MCL-1 (53). WP1130 is a small molecule that directly decreases the DUB activity of USP9X, which leads to the downregulation of MCL-1, ultimately facilitating apoptosis (63,64). A previous study reported that apoptosis was induced by the inhibition of USP9X at least partly through oxidative stress, which activated DNA damage responses and stress-associated mitogen-activated protein kinase (MAPK) signaling pathways (43). Therefore, USP9X, when co-expressed with multiple apoptosis-associated proteins, exerts an anti-apoptotic role in cancers, such as oral cancer, prostate cancer, chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) (65-67). Overexpression or depletion of USP9X is therefore an important factor in cell apoptosis and survival.

Regulation of mitotic fidelity and the cell cycle. Regulation of mitosis safeguards cellular integrity and its failure contributes to the progression, maintenance and drug resistance of cancer (68-70). In the cell cycle, the mitotic checkpoint complex (MCC) senses the orientation of sister chromatids on the mitotic spindle and restricts the activity of anaphase-promoting complex/cyclosome (APC/C) ubiquitin ligase to initiate mitotic exit, consequently ensuring that chromosome segregation and anaphase are able to occur (71). In this way, the spindle assembly checkpoint is strengthened and chromosomal stability is made more secure through restricting APC/C-mediated MCC turnover (71). By contrast, downregulation of USP9X contributes to a reduction in the

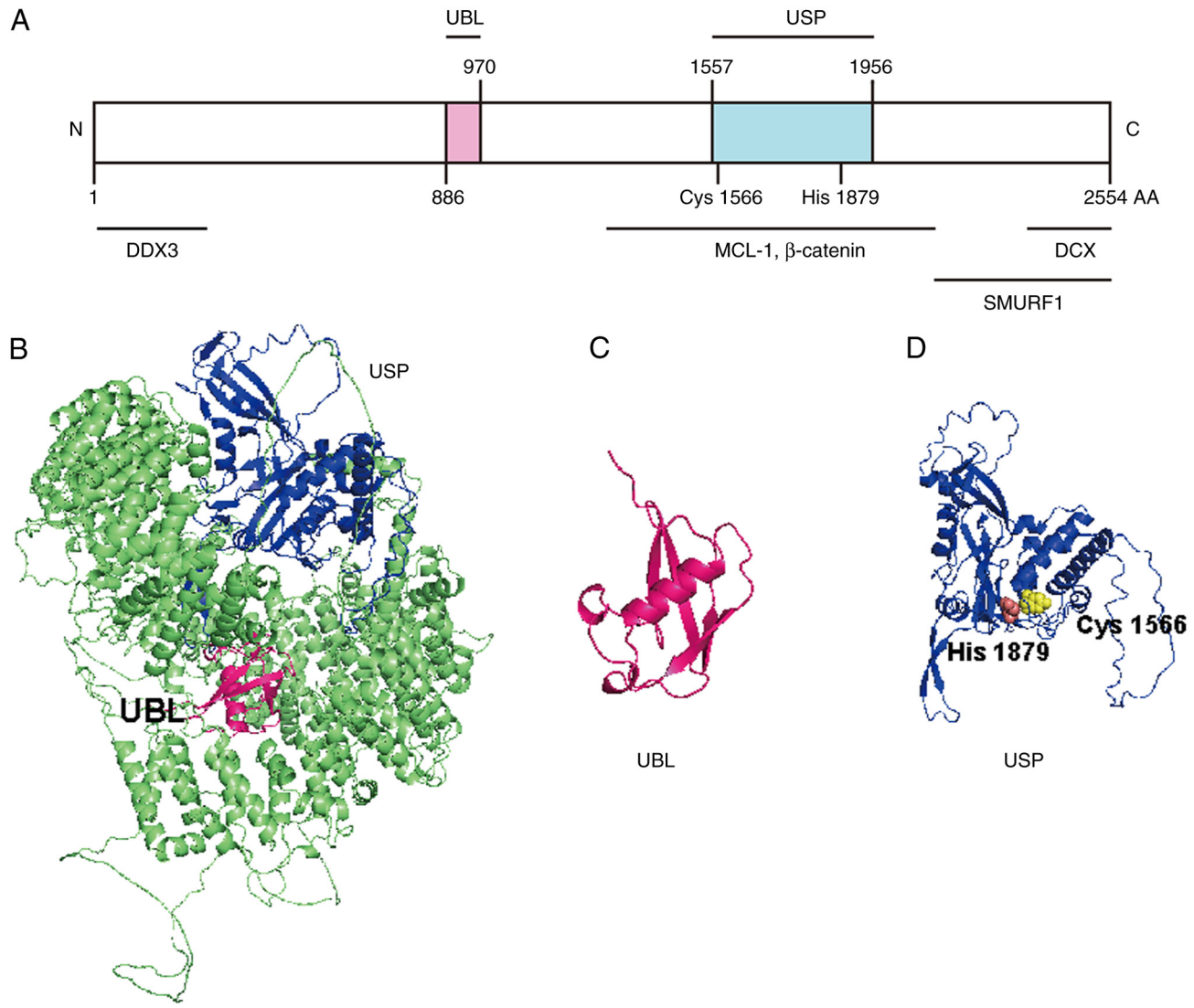

Figure 1. Structure of USP9X. (A) Schematic diagram of USP9X structure showing the functional UBL and USP domains, and the regions of USP9X known to facilitate binding to the interacting proteins. (B) The homology model of USP9X as a cartoon representation of the modeled human USP9X structure. (C) Modeled structure of the UBL domain (amino acids 886-970) and (D) modeled structure of the USP domain (amino acids 1,557-1,956) with the cysteine (amino acid 1,566) and histidine (amino acid 1,879) motifs represented. Structural data were obtained from the AlphaFold Database (https://alphafold.ebi.ac.uk/). His, histidine; cys, cysteine; AA, amino acid; MCL-1, myeloid leukemia cell differentiation 1; UBL, ubiquitin-like module; USP, ubiquitin-specific protease; DCX, doublecortin; DDX3, Asp-Glu-Ala-Asp-box polypeptide 3; SMURF1, specific E3 ubiquitin protein ligase 1; USP9X, ubiquitin-specific peptidase 9X.

efficacy of the spindle assembly checkpoint, an increase in chromosome segregation defects and chromosomal instability, which leads to the subsequent promotion of cancer (71). Activating the mitotic phosphorylation of USP9X promotes cell survival through counteracting mitotic ubiquitination and the ensuing proteasomal degradation of Wilms' tumor protein 1, the latter of which modulates the transcription and secretion of CXC motif chemokine ligand 8/interleukin-8 in mitosis (72). In addition, the dephosphorylation of USP9X mediated by cell division cycle 14B (CDC14B) has previously been shown to promote mitotic apoptosis (72). USP9X is an integral component of the centrosome, where it functions to stabilize certain centrosome proteins, centrosomal protein 55 and pericentriolar material 1, thereby promoting centrosome biogenesis (73). Activation of USP9X enhances centrosome amplification and chromosome instability, whereas inactivation of USP9X leads to an impairment of centrosome duplication (40). It has been previously reported that USP9X may stabilize cell cycle-associated proteins to control ribosomal stalling (74), regulate centrosome duplication (40), and antagonize mitotic cell death and chemoresistance (41). Proteins, such as USP9X, that coordinate the normal functioning of the cell cycle may also trigger inappropriate cell divisions, thereby taking on dysfunctional roles in certain pathological disorders, including X-linked intellectual disability (16,18) and malignancies (40).

Regulation of cell migration and invasion. USP9X also serves pivotal roles in cell migration and invasion. Doublecortin (DCX) is a microtubule-associated protein involved in vesicle transport and microtubule dynamics (75). The C-terminus of USP9X binds DCX, which acts as a regulator of neuronal cell migration (76). Loss of USP9X has been shown not only to reduce axon growth, but also to cause a reduction in neuronal cell

Table I. Substrates and mechanisms of ubiquitin-specific peptidase 9X in cancers described in this review.

First author, year	Type of cancer	*In vivo* or *in vitro*	Expression regulation	Target	Effect	Tumor biological behaviors	(Refs.)
Engel *et al*, 2016	Aggressive B-cell lymphoma	*In vivo* and *in vitro*	Up	XIAP	Stabilize	Inhibits apoptosis and increases chemoresistance	(41)
Grou *et al*, 2012	Chronic myelogenous leukemia	*In vitro*	Up	MCL-1	Stabilize	Inhibits apoptosis	(42)
Akiyama *et al*, 2019	AML	*In vivo* and *in vitro*	-	MCL-1	Stabilize	Inhibits apoptosis	(43)
Wang *et al*, 2023	AML	*In vivo* and *in vitro*	Up	ALKBH5	Stabilize	Promotes cell survival	(44)
Akiyama *et al*, 2020	Multiple myeloma	*In vitro*	Up	MCL-1	Stabilize	Promotes cell survival	(45)
Wu *et al*, 2017	Breast cancer	*In vivo* and *in vitro*	Up	SMAD4	Inhibit	Promotes cancer progression and metastasis	(46)
Li *et al*, 2018	Breast cancer	*In vivo* and *in vitro*	Up	YAP1	Stabilize	Promotes cell survival and regulates chemotherapy resistance	(47)
Guan *et al*, 2022	Breast cancer	*In vivo* and *in vitro*	Up	Snail	Stabilize	Promotes metastasis and chemoresistance	(48)
Li *et al*, 2017	Breast cancer	*In vivo* and *in vitro*	Up	CEP131	Stabilize	Promotes centrosome amplification and chromosome instability	(40)
Narayanan *et al*, 2017	Breast cancer	*In vitro*	Up	TDRD3	Stabilize	Inhibits apoptosis	(35)
Lu *et al*, 2021	Breast cancer	*In vitro*	Up	HIF-1α	Stabilize	Improves cancer stem cell self-renewal	(49)
Jie *et al*, 2021	Lung cancer	*In vivo* and *in vitro*	Up	KDM4C	Stabilize	Promotes radioresistance	(50)
Chen *et al*, 2018	NSCLC	*In vivo* and *in vitro*	Up	TTK	Stabilize	Promotes tumorigenesis	(51)
Kushwaha, *et al*, 2015	NSCLC	*In vitro*	Up	MCL-1	Stabilize	Promotes apoptosis	(52)
Sulkshane *et al*, 2021	OSCC	*In vivo* and *in vitro*	Up	MCL-1	Stabilize	Promotes tumorigenesis and inhibits apoptosis	(53)
Cheng *et al*, 2016	Glioblastoma	*In vivo* and *in vitro*	Up	ALDH1A3	Stabilize	Maintains self-renewal and mesenchymal features	(54)
Khan *et al*, 2018	Colorectal cancer	*In vivo* and *in vitro*	Down	FBW7	Stabilize	Suppresses tumor formation	(55)
Potu *et al*, 2017	Melanoma	*In vivo* and *in vitro*	Up	Ets-1	Stabilize	Enhances tumorigenicity	(56)
Chen *et al*, 2021	Cholangiocarcinoma	*In vivo* and *in vitro*	Down	EGLN3	Stabilize	Promotes apoptosis	(57)

XIAP, X-linked inhibitor of apoptosis protein; MCL-1, myeloid cell leukemia 1; AML, acute myeloid leukemia; ALKBH5, AlkB homolog 5; SMAD4, SMAD family member 4; YAP1, yes-associated protein1; CEP131, Centrosomal protein 131; TDRD3, tudor domain-containing protein 3; HIF-1α, hypoxia-inducible factor-1α; KDM4C, histone lysine demethylase 4C; NSCLC, non-small-cell lung cancer; TTK, threonine tyrosine kinase; OSCC, oral squamous cell carcinoma; ALDH1A3, aldehyde dehydrogenase 1A3; FBW7, F-box and WD repeat domain-containing 7; Ets-1, E26 transformation-specific-1; EGLN3, prolyl hydroxylase 3.

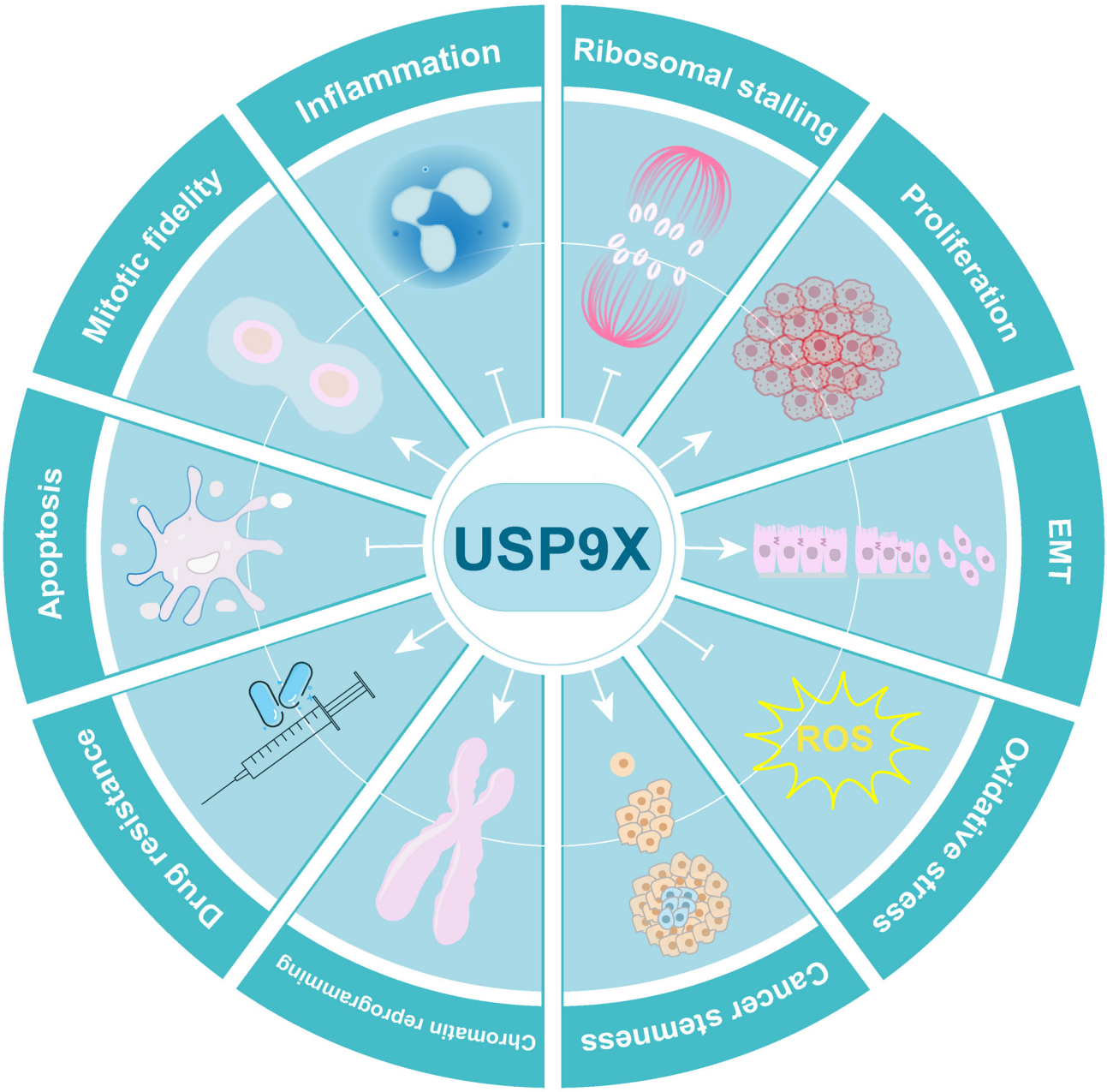

Figure 2. USP9X and hallmarks of cancer. USP9X regulates several fundamental biological processes involved in apoptosis, mitotic fidelity, inflammation, ribosomal stalling, proliferation, EMT, oxidative stress, the stemness of cancer cells, chromatin reprogramming and drug resistance through the precise recognition, recruitment and binding of diverse substrates for targeted deubiquitination and stabilization. The pointed arrows represent the effect of promotion; the flat arrows represent the effect of inhibition. USP9X, ubiquitin-specific peptidase 9X; EMT, epithelial mesenchymal transition.

migration both *in vivo* and *in vitro* via USP9X-mediated disruption of the neuronal cytoskeleton (18). Accordingly, USP9X is required for normal neuronal cell migration. In addition, USP9X-mediated deubiquitination of integrin a5b1 serves a role in a5b1-dependent cell migration (77). A previous study reported that USP9X promoted TGF-β-dependent cancer progression and metastasis through interaction with SMAD4 in the TGF-β signaling pathway, thereby inhibiting the E3 ubiquitin-protein ligase TIF1γ-mediated ubiquitination of SMAD4 (46). SMAD-specific E3 ubiquitin protein ligase 1 (SMURF1) was originally identified to block the TGF/bone morphogenetic protein (BMP) signaling pathway by specifically degrading SMAD1 and SMAD5, as well as TGF/BMP receptors (78,79). The negative regulation of USP9X destabilizes SMURF1 and inhibits SMURF1-dependent cell migration in breast cancer cells (80). USP9X has also been shown to activate the prostaglandin E synthase (PTGES)/prostaglandin E2 (PGE2) pathway, thereby promoting metastatic features of non-small cell lung cancer (NSCLC) cells through the deubiquitination and stabilization of PTGES, which functions as a key enzyme for the process of PGE2 synthesis in the arachidonic acid pathway (81). It has previously been demonstrated that USP9X is able to disrupt neuronal cell migration and growth, which is associated with X-linked intellectual disability; therefore, USP9X may serve roles in both neurodevelopment and the modulation of neural apoptosis (18). Taken together, these findings demonstrate the importance of UPS9X in regulating cell migration and invasion.

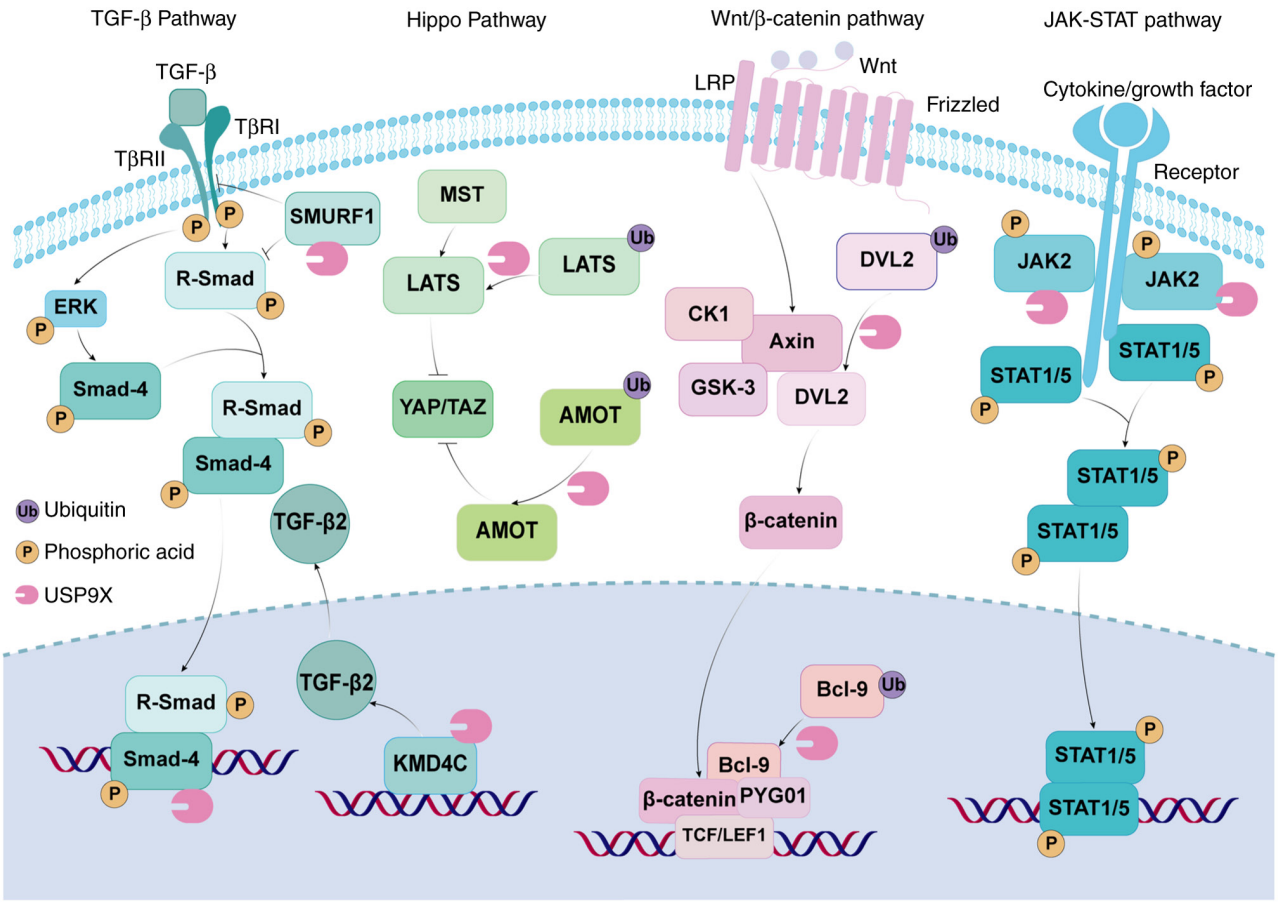

Figure 3. Summary of the role of USP9X in the TGF-β, Hippo, Wnt/β-catenin and JAK-STAT signaling pathways.

Regulation of DNA damage repair. USP9X serves important roles in DNA damage repair (82). Genomic instability is a cancer hallmark, often discovered at early stages of the tumorigenesis process (83). The repair of DNA double-strand breaks may trigger the modification of proteins via polyubiquitin or monoubiquitin around the damage sites (84). During DNA replication, the ataxia telangiectasia-mutated and Rad3-related kinase/checkpoint kinase 1 pathway is activated to coordinate both fork-repair processes and checkpoint responses. In a previous study, USP9X was found to enhance the maintenance of DNA damage checkpoint responses and improve DNA replication fork stability by modulating the checkpoint adaptor and DNA replication factor claspin during S phase, thereby preventing DNA damage accumulation or the DNA replication blockade (85). In addition, USP9X depletion was shown to block the progression of DNA replication forks, which increased sensitivity to ionizing radiation, indirectly impairing genome integrity and leading to excessive endogenous DNA damage (85). Loss of USP9X also affects the radiosensitivity of, and cell survival in, glioblastoma through a number of MCL-independent and -dependent mechanisms (86). The potential involvement of USP9X during radiotherapy remains unknown; however, a previous study reported that it may promote lung cancer radioresistance via epigenetically inducing TGF-β2 transcription, which serves to increase the survival of lung cancer cells (26). Therefore, USP9X serves an important role in the stability of the genome during DNA replication. The positive regulation of the DNA damage repair process may also indicate that USP9X is a potential tumor promoter in cancer, also suggesting its candidacy as a potential clinical target in tumor therapy.

4. Roles of USP9X in human cancer

USP9X expression in different cells and cancer types. USP9X expression level has been shown to differ in tissues and cell lines (Fig. 4A). Furthermore, increased expression levels of USP9X are found in certain cancer types, including breast and lung cancer, and glioblastoma (Fig. 4B; Table I), according to the data from the ProteomicsDB database (87). The therapeutic potential of USP9X in different types of cancer has been demonstrated through considering its deubiquitination capabilities on multiple regulated substrates and critical signaling pathways (88,89). As for the survival and prognosis value of USP9X, high expression of USP9X in adrenocortical, and bladder urothelial carcinoma tissues is associated with lower disease-free survival rates of patients, although it is associated with higher overall survival (OS) rates in cholangiocarcinoma (CHOL; Fig. 5), according to the data from GEPIA (http://gepia.cancer pku.cn/) (90).

USP9X-associated non-coding RNAs in cancer. The available evidence demonstrates that the USP9X-induced aggressive and metastatic phenotypes of cancer are regulated by the abnormal expression of non-coding RNAs (91-97) (Table II). The long non-coding RNA LINC01433 has been shown to enhance

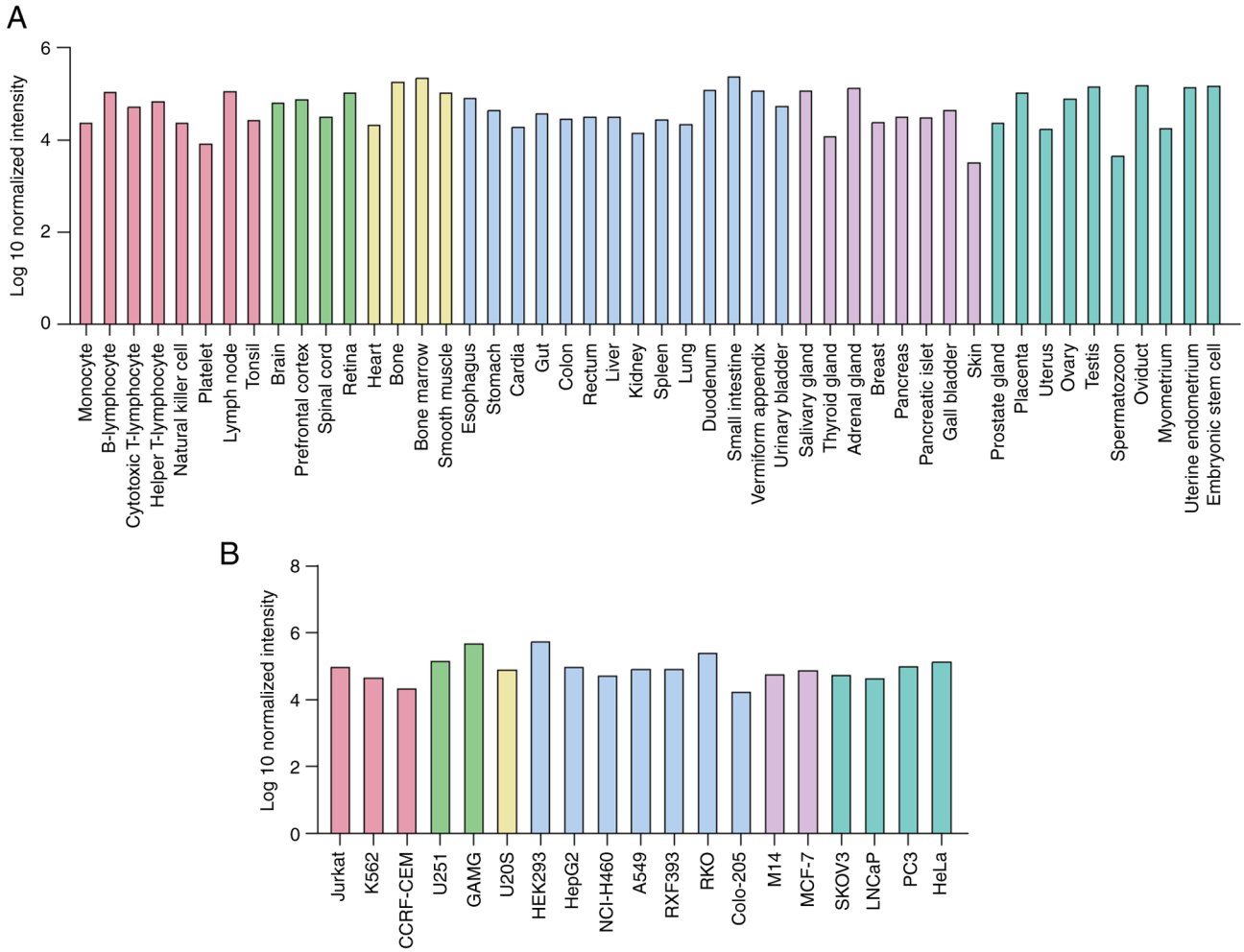

Figure 4. USP9X expression. USP9X expression in (A) healthy tissues and (B) cancer cell lines. Data were obtained from ProteomicsDB (https://www.proteomicsdb.org/) (121). The colors from red to green represent the blood and immune, nervous, musculoskeletal, internal, secretory and reproductive system, respectively. USP9X, ubiquitin-specific peptidase 9X.

the interaction between USP9X and Yes-associated protein (YAP), thereby stabilizing YAP and leading to the promotion of tumor progression and chemotherapy resistance in gastric cancer (GC) cells (91). In addition, the circular RNA hsa_circ_0008434 is highly expressed in GC and serves the role of upregulating the expression of USP9X, thereby promoting the malignant phenotypes of GC cells by serving as a microRNA (miR) sponge for miR-6838-5p (93). Moreover, downregulation of the cellular FADD-like interleukin-1β-converting enzyme-inhibitory protein, which is induced by WP1130, a type of selective USP9X inhibitor that decreases the activity of USP9X, was shown to be regulated by miR-708 through the inhibition of USP9X, which induces the apoptosis of CaSki cells (98). In addition, miR-132 was previously shown to target USP9X-induced EMT, which led to inhibition of the migration and invasive capabilities of NSCLC cells (97).

USP9X and hematological malignancies. USP9X has been shown to exert opposing effects in hematological malignancies. It has been reported that USP9X can promote tumor cell survival in human diffuse large B-cell lymphoma, multiple myeloma and follicular lymphoma through deubiquitinating MCL-1, which protects it from degradation, resulting in poor clinical outcomes (38,45,99,100). In addition, a high level of USP9X in patients with aggressive B-cell lymphoma has been shown to be associated with excessive B-cell proliferation, resulting in an adverse prognosis and resistance to treatment therapies through the deubiquitination and stabilization of XIAP, independent of MCL-1 (41). A BCR-ABL-positive diagnosis is a typical characteristic of patients with CML. The WP1130-mediated inhibition of USP9X is associated with a reduction in MCL-1 levels, followed by blockade of BCR-ABL kinase signaling, which leads to the rapid onset of CML cell apoptosis (21). However, USP9X is not involved in BCR-ABL ubiquitination or cellular localization (101). Moreover, USP9X silencing was shown to lead to lymphoma growth suppression, leading to decreased chemotherapy resistance in B-cell lymphoma (63). Internal tandem duplications of FMS-like tyrosine kinase 3 (FLT3-ITD) occur frequently in AML, and is associated with poor outcomes in patients with AML. USP9X interacts with FLT3-ITD to inhibit its Lys63-linked polyubiquitination (43,102). Furthermore, FLT3-ITD reversely induces the ubiquitination and tyrosine-phosphorylation of USP9X, thereby promoting its proteasomal degradation (102). USP9X also stabilizes RNA m6A demethylase ALKBH5 by removing the K48-linked polyubiquitin chain at K57 and promotes AML

Table II. USP9X-related non-coding RNAs in various types of cancer.

A, LncRNA

Non-coding RNA	Type of cancer	Expression regulation	Mechanisms	Main functions	(Refs.)
lnc01433	Gastric cancer	Up	lnc01433-USP9X-YAP	Promotes proliferation, migration, invasion and chemotherapy resistance	(91)
lnc473	HCC	Up	lnc473-USP9X-survivin	Promotes cell proliferation and invasion	(92)

B, circRNA

Non-coding RNA	Type of cancer	Expression regulation	Mechanisms	Main functions	(Refs.)
hsa_circ_0008434-miR-6838-5p	Gastric cancer	Up	Hsa_circ_0008434-miR-6838-5p-USP9X	Promotes cell proliferation, migration and invasion	(93)

C, miRNA

Non-coding RNA	Type of cancer	Expression regulation	Mechanisms	Main functions	(Refs.)
miR-26b	HCC	Down	miR-26b-USP9X-SMAD4 and TGF-β-EMT	Inhibits EMT	(94)
miR-708	Renal carcinoma	Down	WP1130-USP9X-miR-708-c-FLIP	Promotes apoptosis	(66)
miR-212	Non-small-cell lung cancer	Down	miR-212-USP9X-EMT	Inhibits cell invasion and migration	(95)
miR-212	Pancreatic ductal adenocarcinoma	Down	miR-212-USP9X	Enhances sensitivity to doxorubicin and promotes EMT, apoptosis and autophagy	(96)
miR-132	Lung cancer	Down	miR-132-USP9X-EMT	Inhibits cell migration and invasion	(97)

miR/miRNA, microRNA; lncRNA, long non-coding RNA; circRNA, circular RNA; USP9X, ubiquitin-specific peptidase 9X; YAP, yes-associated protein; HCC, hepatocellular carcinoma; TGF-β, transforming growth factor-β; EMT, epithelial-mesenchymal transition; c-FLIP, cellular FADD-like interleukin-1β-converting enzyme-inhibitory protein.

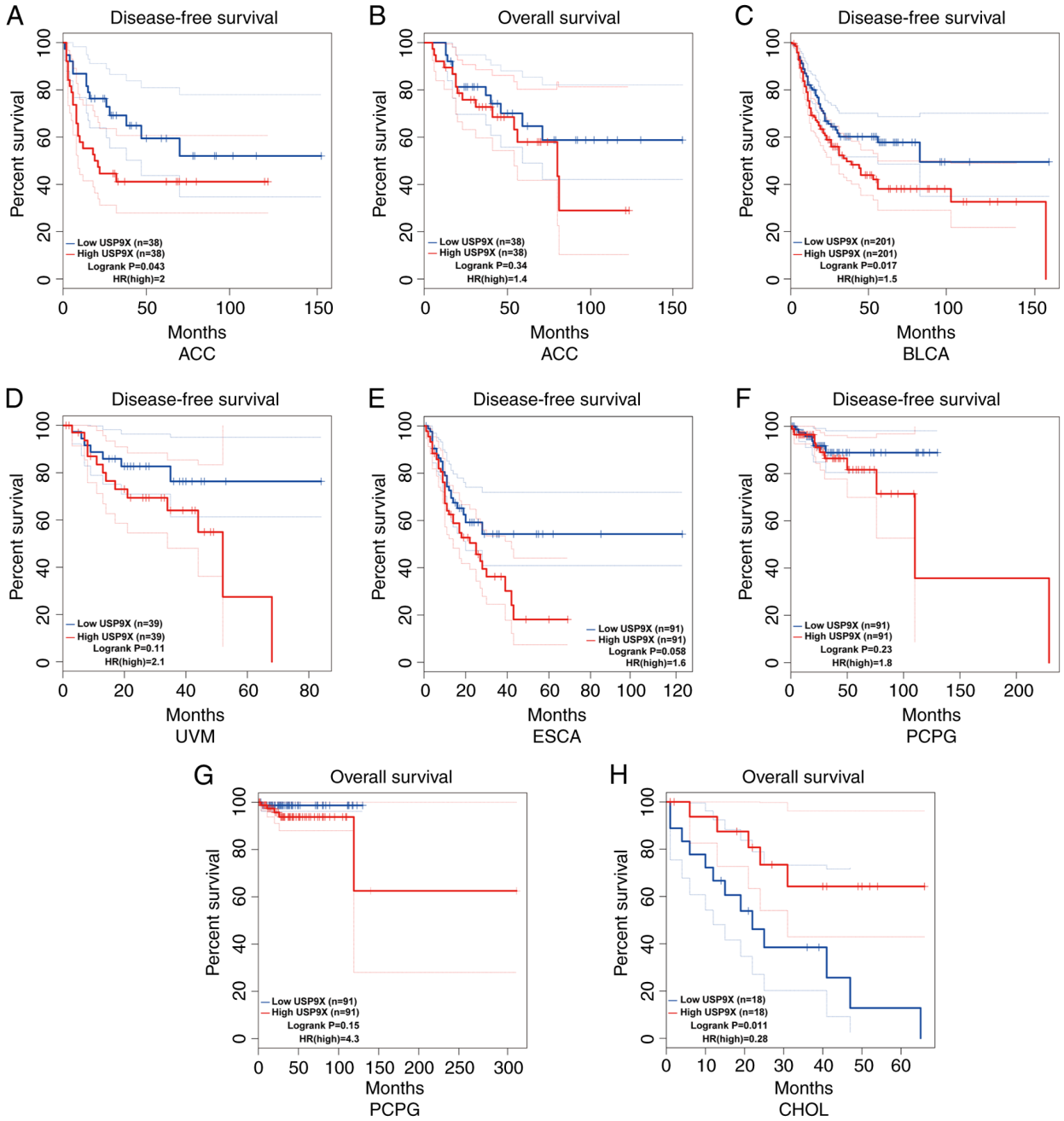

Figure 5. Association between USP9X expression levels and survival rate of patients with cancer. High USP9X expression levels are associated with decreased (A) DFS and (B) OS in patients with ACC. High USP9X expression levels are associated with decreased DFS in patients with (C) BLCA, (D) UVM, (E) ESCA and (F) PCPG. High USP9X expression levels are associated with (G) decreased OS in PCPG and (H) increased OS in patients with CHOL. Dotted lines represent the 95% confidence interval. Data were obtained from GEPIA (http://gepia.cancer-pku.cn/). USP9X, ubiquitin-specific peptidase 9X; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; UVM, uveal melanoma; ESCA, esophageal carcinoma; PCPG, pheochromocytoma and paraganglioma; CHOL, cholangiocarcinoma; OS, overall survival; DFS, disease-free survival; HR, hazard ratio.

cell survival (44). Taken together, these findings identify USP9X as an oncogenic gene that promotes the development of hematological malignancies, and the therapeutic targeting of USP9X may lead to preferential inhibition of certain types of leukemia.

USP9X also exerts negative effects in hematological malignancies by acting as a tumor suppressor gene. USP9X has been identified as a novel leukemia susceptibility gene associated with B-cell acute lymphoblastic leukemia (B-ALL) and multiple neurodevelopmental and congenital abnormalities (103). Low USP9X expression is associated with decreased survival in patients with high-risk B-ALL (103). Genetic or pharmacological inhibition of USP9X can restrict JAK signaling to enhance the survival of cytokine receptor-like factor 2-positive B-ALL in patients with Down syndrome (104), which suggests that USP9X may exert differential effects in different types of leukemia.

USP9X and breast cancer. The underlying mechanisms of USP9X in breast cancer are complex. YAP is an important oncogene that drives cancer progression, and dysregulation of the Hippo/YAP1 signaling pathway is involved in breast cancer development (105). YAP1 is deubiquitinated and

stabilized by USP9X, thereby promoting breast cancer cell survival and resulting in chemotherapy resistance (47). USP9X stabilizes Snail1, a key factor regulating the EMT process, contributing to metastasis and chemoresistance in triple-negative breast cancer (48). Furthermore, downregulation of USP9X renders estrogen receptor α-positive breast cancer resistant to tamoxifen, leading to a poor outcome for patients following adjuvant tamoxifen treatment (89). USP9X can modulate centrosome biogenesis and enhance breast carcinogenesis by deubiquitinating and stabilizing the centriolar satellite protein, CEP131 (40). In a previous study, the arginine methylation of USP9X was shown to improve its interaction with Tudor domain-containing protein 3 (TDRD3), which led to a subsequent enhancement of its anti-apoptotic activities in breast cancer cells (35). The aforementioned study also reported that downregulation of TDRD3 improved the sensitivity of chemotherapeutic drug-induced apoptosis in breast cancer cells, which is likely due to its regulation of USP9X DUB activity on the anti-apoptotic protein MCL-1 and stress granule localization (35).

Obesity may be a risk factor for the development of breast cancer, as it is associated with reduced survival and increases the risk of distant metastasis for female patients diagnosed with breast cancer (106). Plasma free fatty acids further facilitate this biological progression of breast cancer in obese patients (46). USP9X can also be recruited by the transcription factor Nanog to stabilize the hypoxia-inducible factor-1α protein, which leads to an enhancement of self-renewal in breast cancer stem cells (49). Moreover, USP9X selectively promotes activation of the Notch developmental signaling pathway in triple-negative breast cancer, and small molecule EOAI3402143 (G9)-mediated USP9X inhibition specifically inhibits the Notch pathway, which leads to a remodeling of the tumor immune landscape and suppresses tumor growth (107). Taken together, these studies suggest that a diverse range of USP9X-associated mechanisms are involved in breast cancer.

USP9X and lung cancer. Histone lysine demethylase 4C (KDM4C), a substrate of USP9X, is associated with poor clinical outcomes in patients with lung cancer (50). A recent study reported that USP9X activated the TGF-β/SMAD signaling pathway, thereby inducing radioresistance by deubiquitinating and stabilizing KDM4C in lung cancer (50). USP9X is also positively correlated with the dual-specificity protein kinase known as threonine tyrosine kinase, which modulates cell proliferation, migration and tumorigenesis in NSCLC (51). Furthermore, the inhibition of USP9X has been shown to contribute towards MCL-1-mediated proteasomal degradation, radiosensitivity and apoptosis in NSCLC cells (52). Moreover, the chemotherapeutic agent pemetrexed has been shown to induce apoptosis by increasing the expression of the pro-apoptotic protein Noxa, thereby activating the Noxa-USP9X-MCL-1 axis, which demonstrates that USP9X serves a critical role in human lung cancer cells (108). Collectively, these studies have shown that USP9X exerts a range of different functions in lung cancer.

USP9X and pancreatic cancer. Pancreatic cancer is a lethal malignancy, and effective targeted therapies are urgently required to combat its high morbidity rates (2.6%) and mortality (4.7%) rates worldwide in 2020 (109). To date, USP9X has been reported to show tumor-suppressing potential in pancreatic cancer (110-112). USP9X suppressed tumorigenesis in a V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog mouse model of pancreatic ductal adenocarcinoma (PDAC) and its downregulation enhanced malignant transformation, protecting PDAC cells from anoikis (110). However, these properties of USP9X in pancreatic cancer predominantly depend on its intrinsic deubiquitinase activity. USP9X mediates acute MCL-1 stabilization and protection from apoptosis in response to MAPK suppression caused by MEK inhibitors (113), which may provide a promising therapeutic strategy for pancreatic cancer.

USP9X and other types of cancer. The differential expression or genetic alterations of USP9X have been identified in multiple types of human cancer, which suggests that it potentially exerts different roles in tumor progression. Higher expression levels of USP9X are observed in oral squamous cell carcinoma (OSCC) cells, where it drives oral tumorigenesis by deubiquitinating and stabilizing the anti-apoptotic protein MCL-1, which was shown to correlate with poor outcomes in patients with OSCC (53). In addition, aldehyde dehydrogenase 1 family member A3 (ALDH1A3) functions as a key enzyme for maintaining the self-renewal and mesenchymal (MES) features of glioblastoma stem cells (54). Depletion of USP9X leads to a marked downregulation of ALDH1A3, which leads to a loss of the tumorigenic and self-renewal capabilities of MES glioblastoma stem cells. By contrast, a high expression level of USP9X is indicative of potent tumorigenic capability in MES glioblastoma stem cells with enrichment of ALDH1A3 (114). A previous study reported that USP9X-mediated deubiquitination and stabilization of Ets-1 promoted the expression of the N-RAS oncogene and carcinogenesis in melanoma (56).

Although USP9X has been reported to exert oncogenic functions in several different types of cancer, a number of previous studies have also reported the tumor-suppressive properties of USP9X in carcinogenesis. In the murine intestine, USP9X is necessary for tissue homeostasis and regeneration following acute colitis (55); it regulates the function and protein expression levels of the tumor suppressor FBW7, which consequently protects it from proteasomal degradation (55). Therefore, the restricted level of c-Myc that is regulated by USP9X via stabilization of FBW7 reduces the risk of colitis-mediated colorectal cancer (CRC) in mice, whereas the silencing of USP9X is associated with a poor prognosis in human CRC (55). USP9X expression is downregulated in CRC and CHOL (89). Moreover, USP9X is involved in the activation of apoptosis in CHOL, which also suppresses tumor cell proliferation (57). USP9X exerts its tumor-suppressive functions through deubiquitinating Egl-9 family hypoxia inducible factor 3, thereby activating the apoptosis signaling pathway components, kinesin KIF1Bβ and cleaved caspase-3 (57). USP9X also targets and regulates the stability of angiomotin to indirectly inhibit YAP/transcriptional coactivator with PDZ-binding motif activity, and a low level of USP9X is correlated with poor clinical outcome in renal clear cell carcinoma (115). Taken together, these findings suggest that there may be potential for USP9X to be used as a therapeutic target for the treatment of certain types of cancer.

5. USP9X protein as a therapeutic target

Small molecule inhibitors that target DUBs are emerging as a novel form of anticancer therapeutic strategy (82). A previous study reported that USP9X overexpression inhibited apoptosis and promoted tumor cell survival (41,57). Moreover, previous studies have identified USP9X as an oncogene in various types of cancer (47,53), suggesting that it may be a future potential target for therapeutic development. However, no small molecule inhibitors specific to USP9X are currently available for use in a clinical setting. WP1130, previously known as Degrasyn (116), is the best-characterized USP9X inhibitor capable of inhibiting USP9X activity, in addition to the activity of several other DUBs (USP5, USP14, USP24, UCH37 and UCH-L1) (117), thereby highlighting its role as a type of partially selective DUB inhibitor. The proliferation, inhibition and anti-apoptotic effects of WP1130 against diverse tumors have been reported in B-cell malignancies (118), AML (43), NSCLC (51) and glioblastoma (114), mainly through the inhibition of USP9X with the subsequent accumulation of polyubiquitinated proteins and downregulation of antiapoptotic proteins, including MCL-1 and p53. WP1130 has also been shown to increase tumor cell sensitivity to chemotherapy (43). BIX-01294 is an inhibitor developed to inhibit the activity of euchromatic histone-lysine N-methyltransferase 2 and has been shown to promote USP9X downregulation by stimulating both endoplasmic reticulum stress and the expression of phorbol-12-myristate-13-acetate-induced protein 1, resulting in a downregulation of the level of MCL-1, which promotes apoptosis in bladder cancer cells (119). Peterson *et al* (118) reported on a small molecule inhibitor of USP9X/USP24, EOAI3402143(G9), which inhibited the DUB activity of USP9X and USP24 in a dose-dependent manner, increased apoptosis in myeloma and fully regressed or blocked myeloma tumors in mice. G9 was shown to induce apoptosis in lymphoma and myeloma cell lines *in vitro*, inhibiting tumor progression with little overt toxicity (120). BRD0476 selectively inhibits USP9X activity to suppress the JAK-STAT pathway, which protects human pancreatic β-cells and cancer cells from cytokine-induced apoptosis (121). It was also demonstrated that the disruption of USP9X by the CRISPR/Cas9 system and small interfering RNA intervention brought about similar protective effects resulting from BRD0476 treatment, which suggests that BRD0476 may function as a modulator of USP9X (121). Isothiocyanates, such as phenethyl isothiocyanate and benzyl isothiocyanate, have also been shown to exert anticancer activity by inhibiting USP9X and other DUBs in physiologically relevant time scales and concentrations, particularly in hematological malignancies (122). Taken together, these study results highlight the potential of pharmacological inhibitors targeting USP9X, which may be an effective therapeutic target courtesy of its DUB activity. Although inhibitors of USP9X are not at present available for clinical use, novel therapeutic approaches targeting USP9X in human cancers may be developed further in the future.

6. Conclusions and perspectives

USP9X interacts with an extensive range of substrates and has the potential to regulate multiple signaling and survival pathways with cellular responses. USP9X controls a wide variety of cellular pathological and physiological processes through its DUB activity. Previous studies have reported on the significance of USP9X in the cell cycle, apoptosis and survival, and its crucial functions in cell migration and invasion, and DNA damage repair are mediated predominantly via deubiquitination-mediated regulation of the turnover of certain substrates. However, the roles of USP9X in tumor development are complex, and contradictory functions have been identified in different developmental contexts, wherein USP9X displays both oncogenic activity and tumor suppressor functions. In addition to the substrates and downstream signaling pathways regulated by USP9X in tumor progression, the upstream regulatory factors involved in modulating USP9X protein activation in carcinogenesis also merit further exploration. In the future, the development of specific novel inhibitors against USP9X may be a successful strategy for tumor therapy.

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Availability of data and materials

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Authors' contributions

YH conceived and designed the study. YM, YH, ZQ, CH and SY prepared the figures and tables. YH, YM and LY wrote and edited the manuscript. ZQ, CH and SY revised the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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