Insight into the physiological and pathological roles of USP44, a potential tumor target (Review)

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Abstract. Ubiquitin-specific peptidase 44 (USP44) is a member of the ubiquitin-specific proteases (USPs) family and its functions in various biological processes have been gradually elucidated in recent years. USP44 targets multiple downstream factors and regulates multiple mechanisms through its deubiquitination activity. Ubiquitination is, in essence, a process in which a single ubiquitin molecule or a multiubiquitin chain binds to a substrate protein to form an isopeptide bond. Deubiquitination is the catalyzing of the isopeptide bonds between ubiquitin and substrate proteins through deubiquitylating enzymes. These two processes serve an important role in the regulation of the expression, conformation, localization and function of substrate proteins by regulating their binding to ubiquitin. Based on existing research, this paper summarized the current state of knowledge about USP44. The physiological roles of USP44 in various cellular events and its pathophysiological roles in different cancer types are evaluated and the therapeutic potential of USP44 for cancer treatment is evaluated.

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1. Introduction

The ubiquitin proteasome system (UPS) is the main pathway for protein degradation in eukaryotic cells, participating in the degradation of >80% of proteins (1,2). The UPS is highly selective and serves a critical role in maintaining protein homeostasis. The output of the ubiquitin system is mainly manifested in two forms, namely, the control of protein turnover by providing proteasomal and lysosomal targeting signals, and the control of cellular signaling networks by the regulation of protein interactions and activity (3-5).

Ubiquitination is one of the most common and important post-translational modifications, and can alter the stability, localization and activity of target proteins (6,7). Ubiquitination is most commonly demonstrated in the binding of ubiquitin molecules and polyubiquitin chains to lysine residues of substrate proteins. Ubiquitin itself has eight ubiquitination sites, seven lysine residues (K6, K11, K27, K29, K33, K48 and K63) and an amino-terminal methionine (M1), all of which can be involved in the formation of polyubiquitin chains (8). There are various forms of polyubiquitination; together with homotypic and heterotypic chains, the complexity of ubiquitin coding can be enriched via association with ubiquitin-like molecules, such as small ubiquitin-related modifier, neural precursor cell expressed developmentally downregulated 8 and interferon-stimulated gene product 15, and can be modified by phosphorylation and acetylation (8-11). The most dominant and abundant forms of polyubiquitination are K48- and K63-polyubiquitination (12). Ubiquitination requires the sequential action of three enzymes, ubiquitin-activating enzyme (E1), ubiquitin-binding enzyme (E2) and ubiquitin-ligase (E3). E1 catalyzes the ATP-dependent activation of ubiquitin and the formation of a thioester bond between the ubiquitin C-terminus and the catalytic cysteine on E1. Ubiquitin is then transferred to a catalytic cysteine of one of the ~40 E2s and then to the substrate via E3 (13).

Currently, ~100 human deubiquitinating enzymes (DUBs) have been identified and they can be classified into seven subfamilies, including six cysteine protease families [ubiquitin-specific proteases (USPs), ubiquitin C-terminal hydrolases, ovarian tumor proteases, Machado-Joseph disease proteases, zinc finger-containing ubiquitin peptidase 1 and motif interacting with Ub-containing novel DUB family] and one metalloprotease family (JAB1/MPN/Mov34 metalloprotease). DUBs, as key factors in the deubiquitination process, can catalyze the separation of the isopeptide bond between the glycine site of ubiquitin and the lysine site of the target protein to facilitate deubiquitination (14).

Ubiquitin-specific peptidase 44 (USP44) has been extensively studied as a member of the USP family since it was first identified in 2004 (15). The deubiquitination activity of USP44 has been previously reported, as has its cooperation with E3S (a ubiquitin ligase) to regulate the function and stability of target proteins. USP44 has been reported to be involved in the regulation of various physiological functions and pathological processes, including sister chromatid separation, stem cell differentiation and tumor progression (16-18). In the present review, a comprehensive overview of recent advances regarding USP44, focusing on its physiological roles in various cellular activities and its pathophysiological roles in tumor progression, is presented, and its potential therapeutic potential is highlighted.

2. Properties of USP44

USP44 is a USP gene located on human chromosome 12 and composed of five exons. Previous sequence analysis of *USP44* reported that its open reading frame (ORF) was 2,139 bp and it encoded 712 amino acids, with a total molecular weight of ~80 kDa. USP44 consists of ZnF-UBP and USP domains (19,20). The ZnF-UBP domain is located at amino acid residues 29-97, with the conserved catalytic USP domain at amino acid residues 273-678. The catalytic domain of USP44 is similar to that of other family members, consisting of a Cys box, an Asp-containing motif and a His box (15,21) (Fig. 1). These structures contain a highly conserved cysteine residue, an aspartic acid residue and a histidine residue, respectively (22-25). Furthermore, USP44 also has a highly conserved centrin-binding domain that is closely related to the centrosome distribution of USP44 and its function in the prevention of chromosomal hysteresis (26).

USP44 is mainly located in the nucleus, which may be associated with the involvement of USP44 in chromosome-related activities and its close association with chromosomes. For example, USP44 is recruited to nuclear receptor corepressor (N-COR) target loci to participate in gene expression regulation (27) and when DNA double-strand breaks (DSBs) occur, USP44 is recruited to participate in the dynamic regulation of damage repair (28). Furthermore, a low level of USP44 expression in the cytoplasm has also been reported previously (29), and recently, it has been demonstrated that a part of USP44 in the cytoplasm can migrate to transmembrane proteins to participate in the regulation of immune response under the recruitment of viral infection signals (30). In these processes, the binding domain of USP44 appears to be the key, unelucidated element. A previous study reported that when the binding site between USP44 and the target protein was catalytically inactivated, the recruitment of USP44 in the target protein region was significantly reduced (26). This suggests that the binding site of USP44 may largely determine the distribution of USP44.

The activation of catalytic activity is the core role of USP44. Similar to other domain-like DUBs (31,32), USP44 needs to bind to partner proteins to achieve its full enzymatic activity. It has been reported that for the identified downstream factor histone H2B K120 (H2Bub1), the recombinant USP44 protein alone has no catalytic activity and only when USP44 is bound to the N-COR complex can the enzyme activity of USP44 be activated (27). Notably, N-COR can also localize USP44, which suggests that the localization and activation of USP44 may occur simultaneously (27). This also provides a new supplement and explanation to the suggestion that the activation of the catalytic activity of USP44 depends on its exclusive localization. To be precise, the activation of USP44 activity depends on partner proteins, which act as an anchor for localization as well as a switch for catalytic activity. Moreover, it should be noted that the catalytic activity of USP44 for other downstream factors, such as centriole protein centrin 2 (CETN2) has not been reported to be associated with the N-COR complex (26), which suggests that there may be other complexes or mechanisms that mediate the activation of USP44 activity or that CETN2 itself serves three roles, namely, anchor point, activation point and functional protein.

As part of post-translational modifications, the ultimate biological function of deubiquitinating enzymes depends on the cellular function of downstream proteins (33,34). Therefore, targeting downstream proteins is the main pathway by which USP44 participates in numerous cellular activities. Furthermore, the cellular functions in which USP44 is involved are often redundant and there are numerous deubiquitinating enzymes with similar functions that can compensate, to a certain extent, for the effects caused by USP44 defects (28,35). These points suggest that it is not the USP44 defect, but the change of downstream protein expression caused by the USP44 defect, that is the main cause of specific pathological activity.

3. Roles of USP44 in cellular events

Anaphase initiation. The spindle assembly checkpoint serves a pivotal role in the regulation of the precise separation of sister chromosomes, as well as the initiation of anaphase. The spindle assembly checkpoint monitors the connection of the spindle microtubules to the centromere and the tension between sister chromatids produced by the microtubules (36). When the centromere is not yet bound to the microtubule or the tension threshold is not reached at the checkpoint, the checkpoint is activated and mitotic arrest deficient (MAD)1 interacts with closed (C)-MAD2 to form a stable complex C-MAD2-MAD1. This complex is then used as a template to recruit free open (O)-MAD2. By inducing the conformational transition from O-MAD2 to C-MAD2, the C-MAD2 subunit bound to MAD1 catalyzes the binding of C-MAD2 to cell division cycle 20 (CDC20), which then combines with the budding uninhibited by benzimidazole-related 1 (BUBR1)-BUB3 dimer to form the mitotic checkpoint complex (MCC) (37). When microtubules are properly connected to centromeres, anaphase-promoting

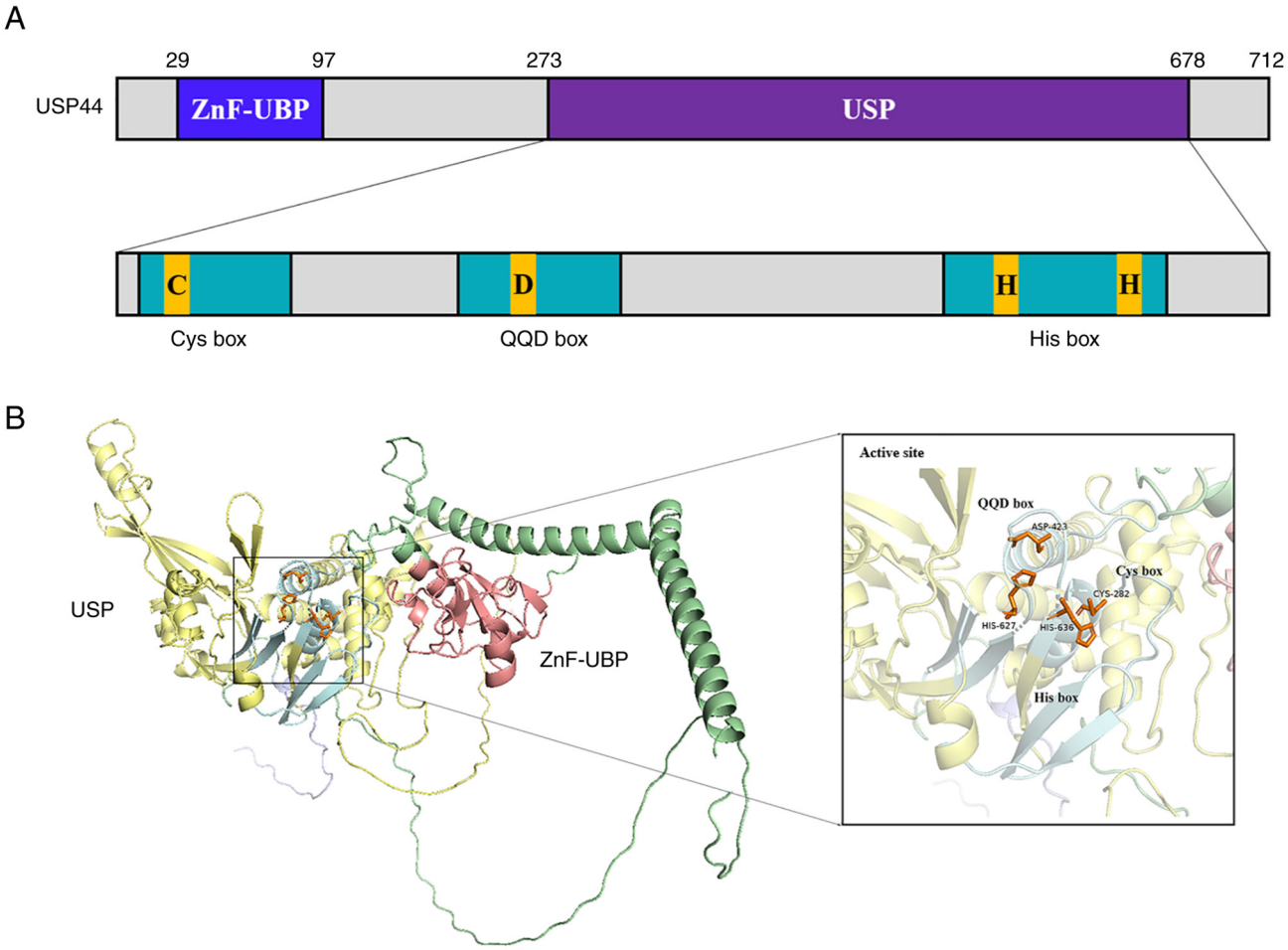

Figure 1. Structure of USP44. (A) Schematic illustration of the domain organization of USP44 in detail. The position of the catalytic residues (residues C, D and H) are indicated in orange. (B) Cartoon representation of the predicted tertiary structure of USP44. USP, ubiquitin-specific peptidase; ZnF-UBP, zinc-finger ubiquitin binding domain.

complex/cyclosome (APC/C) ubiquitinates cyclin B and securin by catalyzing the binding of polyubiquitin chains consisting of Lys11, 48 and 63 to cyclin B and securin (38-40). APC/C is a 1.5-MDa protein complex that is a large ubiquitin ligase consisting of >10 subunits. Like all E3 enzymes, APC/C uses ubiquitin residues that are activated by E1 and then transferred to E2 enzymes [ubiquitin-conjugating enzyme E2 D1 (UBCH5) and UB Ubiquitin-conjugating enzyme E2 C (UBCH10)] (41). An important pathway after ubiquitination is 26S proteasome-mediated degradation (42). APC/C-mediated ubiquitination of cyclin B and securin promotes their rapid destruction by the proteasome, which initiates sister chromatid separation (43,44) (Fig. 2).

The regulation of APC/C is the core component of the spindle assembly checkpoint mechanism. Previous studies by Stegmeier *et al* (16) and Reddy *et al* (45) reported that USP44 may be a key regulatory factor in the physical checkpoint of the spindle and may directly antagonize UBCH10-induced APC/C-driven C-MAD2-CDC20 checkpoint complex decomposition by promoting CDC20 deubiquitination. This pathway leads to C-MAD2 disengagement and APC/C activation. By adjusting this ubiquitination-deubiquitination switch, USP44 prevents premature APC/C activation. Previous studies have reported that APC/C^{MCC} has two CDC20 sites, $CDC20^{APC/C}$ and $CDC20^{MCC}$ (44,46). Alfieri *et al* (47) reported that UBCH10 mediated the catalysis of intramolecular $CDC20^{MCC}$ ubiquitination via cryogenic electron microscopy reconstruction of APC/C^{MCC}. Based on these findings, USP44 is likely to stabilize the binding of C-MAD2 and BUBR1 to CDC20 via the deubiquitination of $CDC20^{MCC}$ (Fig. 2). However, there is no clear evidence that USP44 affects $CDC20^{APC/C}$ in this process.

Centrosome separation. Increased frequency of lagged chromosomes has been reported in USP44-deficient mouse models. Incomplete separation of centrosomes and morphological changes of the spindle have been demonstrated to be the main causes of this phenomenon (26). The ability of USP44 to bind to CETN2 through highly conserved motifs and its deubiquitinating activity are both reported to be key to ensuring accurate chromosome separation (26).

DNA repair. Non-homologous end joining (NHEJ) is the main way to repair DNA DSBs. The cellular response to DSBs is characterized by a rapid accumulation of repair factors and signaling factors in the vicinity of the lesion (48-50). The recruitment of numerous factors in the chromatin region around DSBs requires a ubiquitination cascade. However,

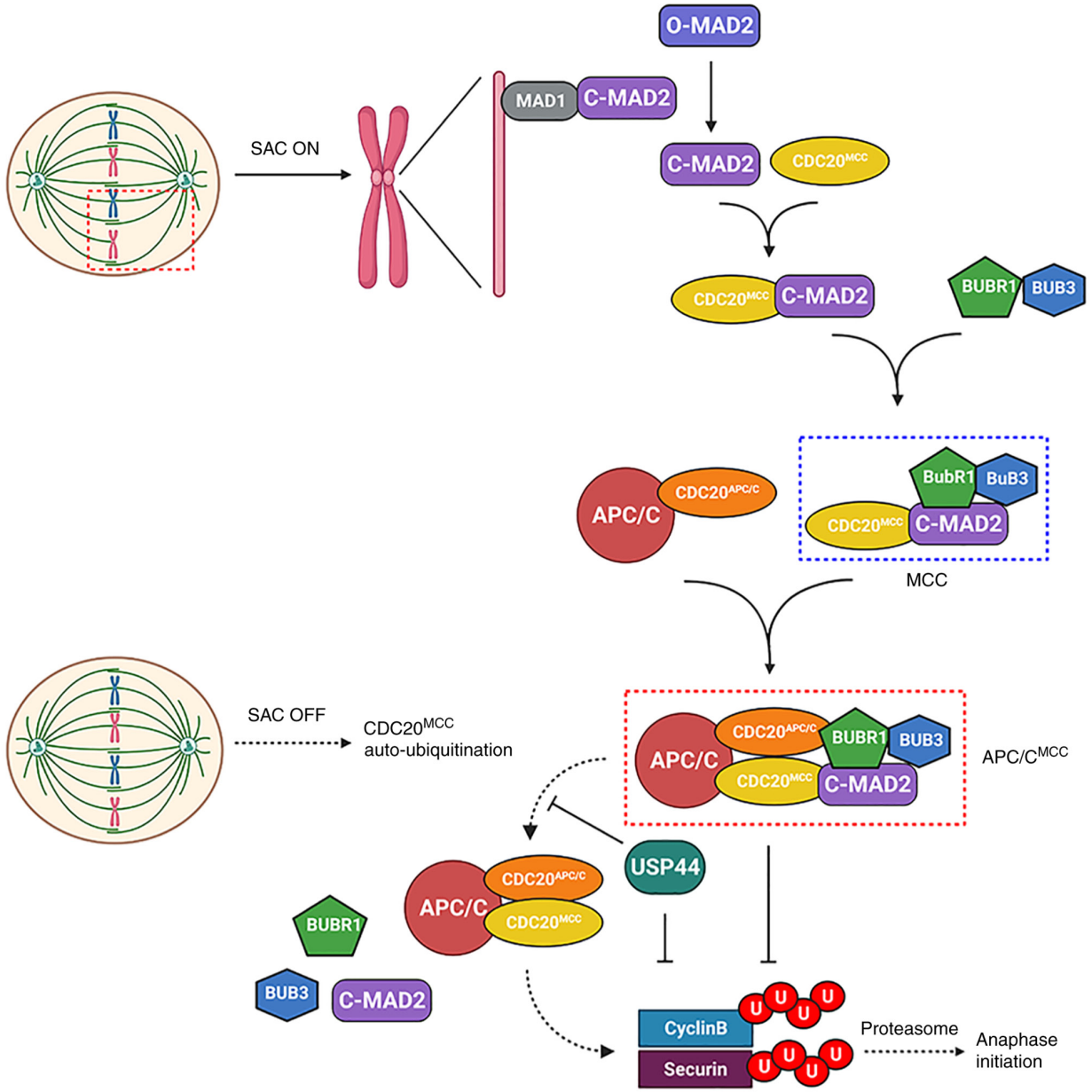

Figure 2. Dynamic balance of APC/C ubiquitination and USP44 deubiquitination controls anaphase initiation. When the centromere is not yet bound to the microtubule or the threshold tension is not reached at the checkpoint, the checkpoint is activated. By inducing the conformational transition from O-MAD2 to C-MAD2, the C-MAD2 subunit bound to MAD1 catalyzes the binding of C-MAD2 to CDC20, which then combines with the BUBR1-BUB3 dimer to form MCC. This complex inhibits the activity of the APC/C through binding to the co-activating subunit CDC20 ($CDC20^{APC/C}$). When microtubules are properly connected to centromeres, the auto-ubiquitination of $CDC20^{MCC}$ leads to the reactivation of APC/C activity, which leads to anaphase initiation through the ubiquitination of cyclin B and securin. In this process, USP44 can delay anaphase initiation not only by direct stabilization of cyclin B and securin, but also by stabilization of the binding of C-MAD2 and BUBR1 to CDC20 by deubiquitination of $CDC20^{MCC}$. MCC, mitotic checkpoint complex; APC/C, anaphase-promoting complex/cyclosome; USP44, ubiquitin-specific peptidase 44; O, open; C, closed; CDC20, cell division cycle 20; MAD2, mitotic arrest deficient 2; BUBR1, budding uninhibited by benzimidazole-related 1; BUB3, budding uninhibited by benzimidazole 3; SAC, spindle assembly checkpoint.

this process is opposed by USP44. USP44 can counteract the ubiquitination of histone H2A mediated by ring finger protein (RNF)168 to inhibit the recruitment of downstream repair factors (28). USP44 can also promote Ku80 degradation by stabilizing E3 ubiquitin ligase tripartite motif-containing protein 25, which inhibits downstream factor recruitment and ultimately inhibits NHEJ-mediated DNA repair (51).

The nucleotide excision repair pathway of DNA repair is responsible for correcting helix-distorting DNA lesions that are caused by chemical damage or exposure to ultraviolet light (52,53). In this process, USP44 stabilizes the accumulation of DNA damage-binding protein 2 on DNA lesions by deubiquitination, allowing sufficient time for the metastasis of the lesions, which guarantees the smooth progress of DNA repair (54).

Immune response. Lin *et al* (35) reported that, in mouse models, USP44 was not necessary for normal lymphoid development and that USP44 deficiency did not significantly affect the B-cell mediated immune response. However, the role of USP44 in T lymphocytes has been reported. FOXP3 is essential for the function of regulatory T cells in immune homeostasis (55).

USP44 has been reported to be a new FOXP3 deubiquitinase and has been demonstrated to stabilize immune function in models of inflammatory disease and cancer. Compared with wild-type regulatory T cells (Tregs), Tregs lacking the USP44 gene had a weaker inhibitory function (56).

Mediator of IRF3 activation (MITA), which is known as a key adaptor protein, is responsible for sensing the second messenger cyclic GMP-AMP, which is synthesized upon DNA virus infection and activation of the induction of type I interferons (IFNs) and proinflammatory cytokines (57). It has been reported that USP44 in the cytoplasm is recruited to MITA to perform deubiquitination after herpes simplex virus 1 (HSV-1) infection and that USP44 inhibits proteasome-mediated degradation of MITA by selectively removing the polyubiquitin chain connected by K48 in MITA. Moreover, gene transcription of IFNs and proinflammatory cytokines in response to HSV-1 was reported to be inhibited in THP-1, bone marrow-derived macrophage and murine lung fibroblast cells with defective USP44 expression (30). These results suggested that USP44 serves an important role in the regulation of the natural immune response to DNA viruses.

Stem cell differentiation. Chromatin modification serves a key role in cell differentiation. It has been reported that changes in histone H2B ubiquitination patterns are essential for the maintenance of stem cell differentiation potential to differentiation progression (58,59). USP44 has recently been reported to be the deubiquitination enzyme involved in this process. USP44, as a regulator of H2Bub1 expression, is downregulated during embryonic stem cell differentiation and, together with RNF20, regulates the dynamics of H2B mono-ubiquitination patterns during stem cell differentiation (17). USP44 affects the ability of embryonic stem cells to differentiate; however, a study has demonstrated that USP44 is not a necessary gene for growth and development, and mice lacking USP44 can still grow and develop normally, which may be related to functional redundancy (26).

Autophagy. Autophagy has been reported to be critical for maintaining cell homeostasis, as it serves a role in clearing abnormal proteins or factors that are no longer needed (60). Previously, H2B ubiquitination regulation has been reported to be one of the mechanisms regulating autophagy: Reduction of H2Bub1 can lead to activation of autophagy (61). As aforementioned, USP44 is responsible for the decrease in H2Bub1, and the autophagy process is indeed inhibited when the interaction between USP44 and H2Bub1 is inhibited. These results suggest that USP44 can indeed affect autophagy by the regulation of the expression of H2Bub1 (62).

4. USP44 in pathophysiological conditions

Aneuploidy. Deletion of USP44 has been reported to lead to chromosome mis-segregation and aneuploidy. USP44 defect-mediated aneuploidy is considered to have two mechanisms, namely, spindle morphology change and mitotic timing change.

Physical changes in spindle morphology are considered to be the main mechanism that affects aneuploidy development. Incomplete centrosome separation and abnormal spindle geometry caused by USP44 deletion are the main causes of mitotic errors and aneuploidy (26).

It is known that the development of aneuploidy in mitosis is closely related to the spindle assembly checkpoint mechanism (63-65). The dynamic balance between APC/C ubiquitination and USP44 deubiquitination regulates the initiation of anaphase (16). The disruption of this balance invalidates the spindle assembly checkpoint mechanism. A weakened spindle assembly checkpoint allows cells with unattached or misaligned kinetochores to proceed from metaphase to anaphase, yielding daughter cells with an abnormal chromosome number. However, in a previous study, in the absence of USP44, there was no evidence reported for the accelerated degradation of cyclin B1, even though there was a significant, moderate deficiency in mitotic checkpoint activity (26). The USP44 defect initiates the process that leads to aneuploidy through the spindle mechanism but stops it partway. This may be associated with the fact that other DUBs take over the basic functions associated with checkpoints after the loss of USP44. Furthermore, USP44 upregulation is also associated with aneuploidy (66). Overexpression of USP44 can also lead to mitotic errors and aneuploidy elevation (67) but there is a lack of research to explain this phenomenon.

According to the aforementioned results, the abnormal expression of USP44 is closely associated with the development of aneuploidy but its complex regulatory mechanism needs to be further evaluated.

Cancer. USP44 is a multifunctional factor in cancer progression; it mediates tumorigenesis and tumor development through different pathways in different tumors and is closely related to the function of substrate proteins (68) (Fig. 3; Table I).

Fructose bisphosphatase 1 (FBP1) is one of the key enzymes in the gluconeogenesis process, which contributes to the conversion of fructose-1,6-bisphosphatase to fructose-6-phosphate and negatively regulates aerobic glycolysis (69). Studies reported that USP44 was downregulated in pancreatic cancer, which was accompanied by the downregulation of FBP1 and changes in glucose metabolism, mediating the chemotherapy resistance to gemcitabine (70). Furthermore, FBP1 loss was accompanied by the upregulation of ERK phosphorylation and changes in cell proliferation (71). Further experiments demonstrated that the FBP1-MAPK pathway was regulated by USP44 and served an important role in the regulation of the growth of the pancreatic cancer (70). These results indicate that USP44 may be a potential therapeutic target for pancreatic cancer.

In human tumor cells, CpG island (CGI) methylation of promoter region 5 is involved in the regulation of the expression of numerous genes (72), and USP44 is no exception. Recently, the use of the combined bisulfite restriction analysis assay demonstrated that transcriptional silencing of USP44 in CRC cell lines was associated with CGI hypermethylation (73). This result was supported by the re-expression of USP44 in four fully methylated CRC cell lines (RKO, SW620, HCT116 and DDD-1) using the DNA methyltransferase inhibitor decitabine (73). The decreased expression of USP44 in colorectal cancer was also reported in the study by Huang *et al* (74), where USP44, as a tumor suppressor, was demonstrated to inhibit the Wnt/β-catenin pathway and promote the apoptosis of colorectal cancer cells through the deubiquitination of axin 1.

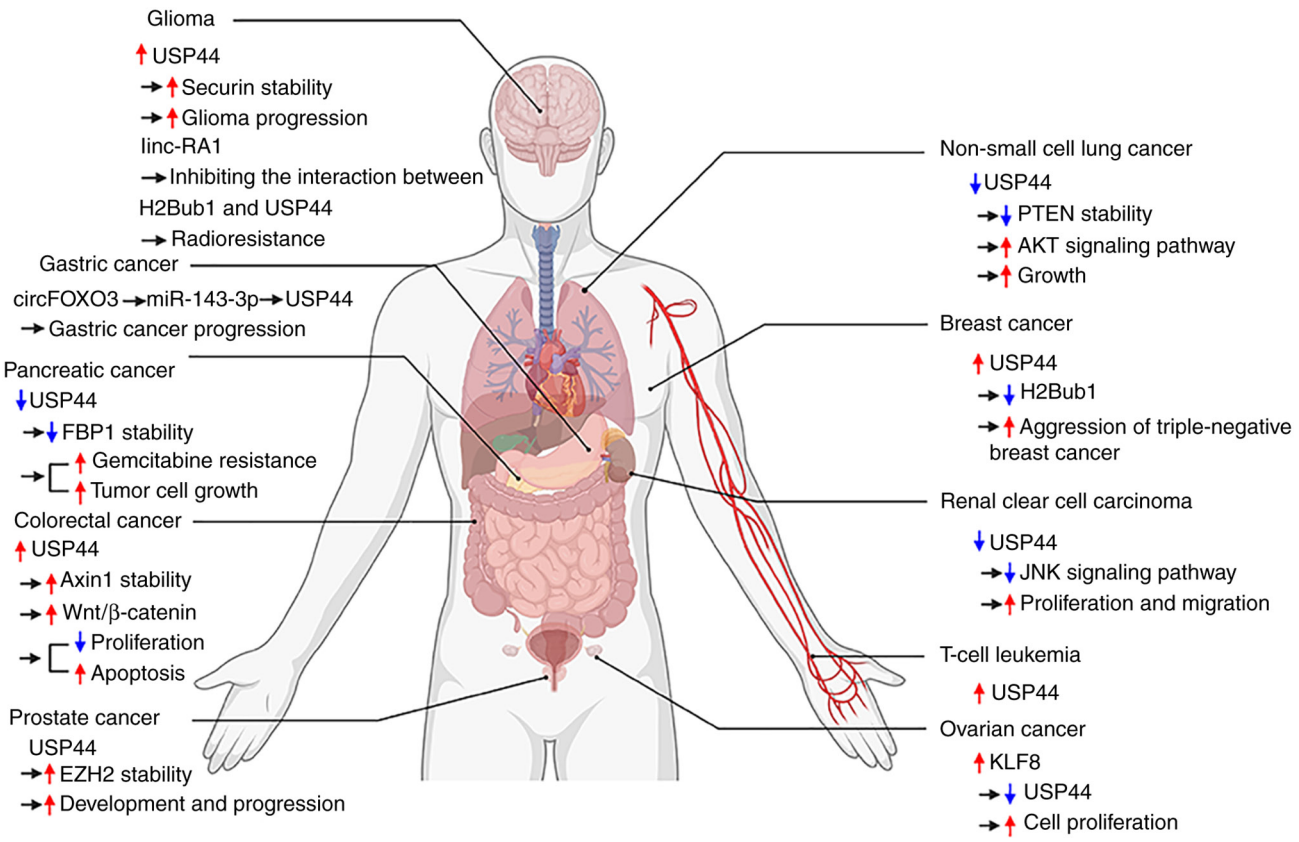

Figure 3. USP44 is closely related to the occurrence and progression of tumors. USP44 is closely related to the occurrence and progression of tumors, including glioma, gastric cancer, pancreatic cancer, colorectal cancer, prostate cancer, non-small cell lung cancer, breast cancer, renal clear cell carcinoma, T cell leukemia and ovarian cancer. Red arrows indicate increased protein expression, pathway activation, increased capacity or disease progression. The blue arrow indicates the opposite to the red arrow. Black arrows indicate process progression. USP44, ubiquitin-specific peptidase 44; EZH2, zeste homolog 2; H2Bub1, histone H2B K120; FBP, fructose bisphosphatase 1.

Renal clear cell carcinoma is the most common type of renal carcinoma. Zhou *et al* (75) reported that USP44 was downregulated in renal clear cell carcinoma and that its expression level was negatively correlated with the grade and stage of renal cancer. It was also demonstrated that downregulation of USP44 promoted cell proliferation and migration through the JNK pathway in renal clear cancer cells. Furthermore, Tang *et al* (76) constructed a more accurate prognostic model using *USP44* methylation as one of the prognostic variables for renal clear cell carcinoma. These findings suggest an important role for *USP44* methylation in renal clear cell carcinoma.

Among patients with lung cancer, non-small cell lung cancer (NSCLC) is the most common type, accounting for ~80% of all cases, and ~75% of patients are reported to have a poor 5-year survival rate (77,78). A previous study has reported that USP44 expression deficiency can lead to a significant increase in the incidence of lung cancer (26). Subsequently, Zhang *et al* (18) reported that the prognosis of patients with lung cancer and low USP44 expression is poor and the overexpression of USP44 may inhibit the progression of lung cancer by stabilizing PTEN protein by the inhibition of AKT signal transduction in lung cancer cells. These results indicate that USP44 may be a potential therapeutic target for NSCLC.

For breast cancer, cancer stem cell (CSC) subclones are often used as models. Liu *et al* (79) generated 'mammospheres' from breast cancer cells to evaluate the role of USP44 in CSCs. Using vasculogenic mimicry (VM), a newly defined tumor blood supply pattern that has been reported to be closely associated with tumor aggressiveness (80-82), as a bridge, the relationship between USP44 and tumor aggressiveness was assessed. According to the results of the study, USP44 inhibited breast CSCs with a centrosomal amplification phenotype to form multipolar spindles, but promoted the formation of a bipolar spindle, which was closely associated with VM. After USP44 knockdown, multipole spindle formation was induced, VM was inhibited and the ability of mammosphere-derived MCF-7 AURKA cells to cross endothelial cells was markedly reduced, which suggested a close relationship between the four (79). Moreover, breast cancer with USP44$^+$ CSC subclones were significantly associated with poor overall survival (OS) and disease-free survival (DFS) times. The mean OS and DFS periods were 70.298 months (95% CI, 61.510-79.086) and 53.206 months (95% CI, 45.624-60.788), respectively, for patients with USP44$^+$ CSC subclones; however, for patients without USP44$^+$ CSC subclones, the mean OS and DFS periods were 117.552 months (95% CI. 109.561-125.544) and 95.087 months (95% CI, 86.446-103.728), respectively (79). These results suggested that USP44 appeared to promote the progression of breast cancer as an oncogenic factor. Furthermore, the downregulation of USP44 in triple-negative breast cancer cells can impair the aggressiveness of breast cancer cells, which also supports the

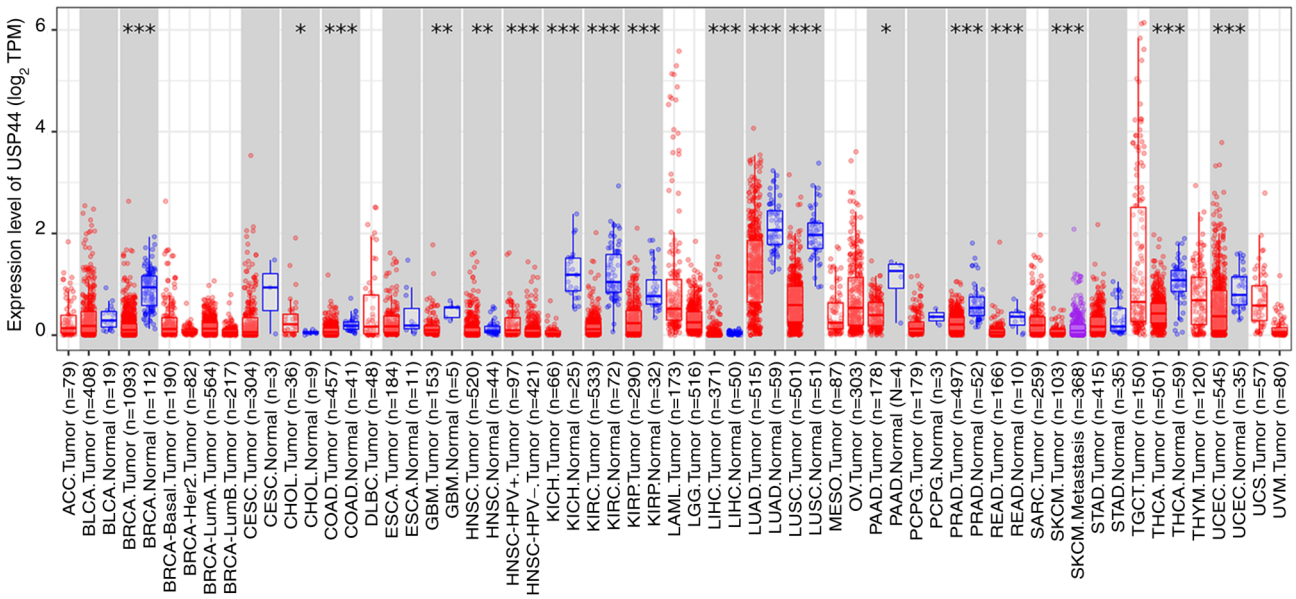

Figure 4. Expression of USP44 in normal tissues and corresponding tumors. *P<0.05, **P<0.01 and ***P<0.001 vs. normal. Gene expression data were provided by the open database TIMER2.0 (http://timer.cistrome.org/). USP44, ubiquitin-specific peptidase 44; TPM, transcripts per million; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; Her2, human epidermal growth factor receptor 2; LumA, Luminal A; LumB, Luminal B; CESC, cervical and endocervical cancer; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; HPV, human papillomavirus; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uterine carcinosarcoma.

carcinogenic effect of USP44 (27). However, Chen *et al* (83) reported contrary results and suggested that USP44 acts as a tumor suppressor in breast cancer to limit tumor progression. The study demonstrated that patients with breast cancer with high expression levels of USP44 had a better prognosis and that the overexpression of USP44 in breast cancer cells inhibited the malignancy of breast cancer, but the reasons for this remain elusive. However, Tarcic *et al* (84) reported new insight into the phenomenon of the aforementioned differential functional expression, with different subtypes of breast cancer cells, which demonstrated the opposite effects on proliferation after knocking down USP44 to increase H2Bub1 levels. These results suggest that USP44 serves different regulatory roles in different breast cancer subtypes. Therefore, we hypothesized that these different results may be caused by phenotypic differences between breast cancer stem cells and breast cancer cells. Further research is needed to evaluate this.

The role of USP44 in glioma, prostate cancer and gastric cancer is different from the aforementioned roles. USP44 is highly expressed in high-grade gliomas with a poor prognosis. Downregulation of USP44 expression can inhibit proliferation, migration and invasion of established glioma cell lines and induce apoptosis (85).

In prostate cancer, USP44 promotes disease progression by stabilizing enhancer of zeste homolog 2 (EZH2). EZH2 is an enzymatic catalytic subunit of polycomb repressive complex 2 (one of the two polycomb group protein core complexes) that can alter gene expression by histone H3 lysine 27 trimethylation (86). EZH2 has been reported to promote the progression of prostate cancer by influencing several factors associated with the cell cycle, autophagy and apoptosis (87-89). The introduction of EZH2 into USP44 knockdown PC3 and DU145 cells significantly rescued USP44 knockdown-induced suppression of wound healing, migration and invasion activity (90).

In gastric cancer, USP44 expression levels were also upregulated. The mean and median expression rates of USP44 in carcinoma were 39.6 and 36.0%, respectively, which were higher than the 14.6 and 11.3%, respectively, in normal mucosa (66). Further studies demonstrated that the upregulation of USP44 in GC was due to the interaction between circFoxO3 and miR-143-3p, which promoted GC proliferation and migration (91). Furthermore, USP44 also has a clinical impact on the induction of DNA aneuploidy and the poor prognosis of gastric cancer. The proportion of DNA aneuploidy in gastric cancer with high USP44 expression levels was significantly higher than that in gastric cancer with low USP44 expression levels. The 5-year OS and progression-free survival rates of gastric cancer with high USP44 expression levels were 36.8 and 32.7%, respectively, which were significantly lower than those of the low USP44 expression levels group (50.5 and 47.4%, respectively) (66). These results suggest that USP44 can not only inhibit tumors as a protective factor, but can also promote tumor progression as an oncogenic factor.

5. Regulation of USP44 expression

As presented in Fig. 4, USP44 is expressed to different degrees in numerous tissues and organs, among which the lungs and kidneys show the highest expression levels, whereas the bile duct and liver show lower expression levels. This indicates that

Table I. Summary of the downstream proteins and functions targeted by USP44.

Downstream protein	Name	Function	(Refs.)
FBP1	Fructose-1,6-bisphosphatase	1. Downregulation of USP44 in pancreatic cancer mediates gemcitabine resistance through FBP1. 2. USP44 inhibits tumor cell growth in pancreatic cancer through the FBP1-MAPK signaling pathway.	(70)
N-COR	Nuclear receptor co-repressor complex	1. USP44 contributes to N-COR functions in regulating gene expression and in modulating invasiveness of triple-negative breast cancer cells. 2. N-COR activates deubiquitination of USP44.	(27)
TBL1X	Transducin β-like 1X	TBL1x is required for USP44 to associate with N-COR.	
TBL1XR1	Transducin β-like 1X-related protein 1	TBL1XR1 is required for USP44 to associate with N-COR.	
EZH2	Enhancer of zeste homolog 2	USP44 promotes the development and progression of prostate cancer by stabilizing EZH2.	(90)
Securin	/	Overexpression of USP44 promotes glioma progression by stabilizing securin.	(85)
Cyclin B1	/	Antagonistic APC/C function.	(16)
Axin 1	/	USP44 suppresses proliferation and enhances apoptosis in colorectal cancer cells by inactivating Wnt/β-catenin signaling pathway via Axin 1 deubiquitination.	(74)
MITA	Mediator of IRF3 activation	USP44 is involved in the regulation of innate immune responses to DNA viruses by deubiquitinating MITA to prevent its degradation by proteasomes.	(30)
CETN2	Centrin-2	Adjusting spindle geometry, interpolar distance and centrosome separation.	(26)
FOXP3	Forkhead box protein P3	USP44 collaborates with USP7 to deubiquitinate and stabilize Foxp3 expression, thereby promoting Treg-mediated immunosuppression.	(56)
PTEN	Phosphatase and tensin homolog	Stabilize PTEN to inhibit the AKT signaling pathway and thereby inhibit the growth of non-small cell cancer cells.	(18)
Unknown	Unknown	USP44 inhibits the JNK signaling pathway in renal carcinoma.	(75)
H2Bub1	Histone H2B K120 mono-ubiquitination	1. Regulates stem cell differentiation. 2. Regulation of H2BuB1-mediated autophagy is involved in the regulation of radiation resistance of glioma. 3. Opposite phenotypes in different subtypes of breast cancer. 4. Aggression of triple-negative breast cancer. 5. USP44-mediated removal of H2Bub1 contributes to inhibition of N-COR target genes.	(17) (62) (84) (27)
CDC20	Cell division cycle 20 homologue	Offsets APC/C-driven decomposition of the MAD2-CDC20 complex and regulates anaphase initiation.	(16)
DDB2	DNA damage-binding protein 2	Participates in nucleotide excision repair.	(54)

USP44, ubiquitin-specific peptidase 44; FBP, fructose bisphosphatase 1; N-COR, nuclear receptor co-repressor complex; EZH2, enhancer of zeste homolog 2; APC/C, anaphase-promoting complex/cyclosome; MITA, mediator of IRF3 activation; Treg, regulatory T cell; H2Bub1, histone H2B K120; CDC20, cell division cycle 20; MAD2, mitotic arrest deficient 2.

USP44 expression not only has certain universality but that it also has certain tissue specificity. However, it has not been clarified whether USP44 is universally expressed in all tissues throughout the life cycle, which demonstrates the need for the elucidation of the macro-regulatory mechanism of USP44. Furthermore, the expression of USP44 also demonstrates a

Table II. Comparison of physiological and pathological functions of USP44.

Physiological function	Causes	Pathological effect
Accurate separation of centrosomes	USP44 downregulation/C281A/W162A	Aneuploidy
Unknown	USP44 upregulation	
Participation in spindle assembly checkpoint mechanism	USP44 downregulation	Accelerated anaphase
Stabilization of immune function	USP44 downregulation/C282S	A weaker immunosuppressive function of Tregs
	USP44 downregulation/C282A	Impair innate immunity to DNA viruses
Involved in stem-cell differentiation	USP44 downregulation/C282A	Impair differentiation and induction of genes
Regulation of autophagy	Inhibited interaction between USP44 and H2Bub1	Radio-resistance of glioma
Involved in DNA repair	USP44 downregulation/C281A	Induce tumors
Regulation of tumor-associated proteins	USP44 downregulation	The tumorigenesis and development of non-small cell carcinoma, colorectal cancer, renal clear cell carcinoma and breast cancer
	USP44 downregulation/C282A	The progression and drug resistance of pancreatic cancer
	USP44 upregulation	The development of glioma and breast cancer
	USP44 upregulation/C282A	The tumorigenesis of prostate cancer

Both 'downregulated' and 'upregulated' in the table refer to changes in USP44 expression. C281A, W162A, C282S and C282A are all mutant forms of USP44: C281A, the Cys at position 281 of USP44 amino acid sequence was mutated to Ala; W162A, the Trp at position 162 of USP44 amino acid sequence was mutated to Ala; C282S, the Cys at position 282 of USP44 amino acid sequence was mutated to Ser; C282A, the Cys at position 282 of USP44 amino acid sequence was mutated to Ala. USP44, ubiquitin-specific peptidase 44; Treg, regulatory T cell; H2Bub1, histone H2B K120.

complex trend in the tumor environment. Compared with those in the corresponding normal tissues, the expression levels of USP44 are significantly downregulated in most tumors, such as lung cancer, kidney cancer and pancreatic cancer, but upregulated in a few tumors, such as cholangiocarcinoma. These results suggest that there may be complex tissue-dependent regulation of USP44 in physiological and pathological conditions. The regulatory mechanisms reported so far are elaborated on next.

USP44 is regulated by differentiation signals. SOX2, Nanog and OCT4 are well known to be pluripotent transcription factors that maintain the state of embryonic stem cells (92,93). USP44 was reported as a direct target of OCT4 by Boyer *et al* (94). The expression of USP44 was downregulated during the differentiation of OCT4 knockdown-induced embryonic cancer cells, which confirmed the regulatory role of OCT4 on USP44 expression at the functional level (95). Moreover, the high expression levels of USP44 in embryonic stem cells, pluripotent stem cells and germinal organs/cells, but low expression levels in differentiation and somatic tissues, is consistent with the aforementioned results (96). Furthermore, upstream signaling that mediates the upregulation of USP44 expression during Treg differentiation has also been reported. TGF-β/SMAD signaling promotes the upregulation of USP44 expression by driving conserved SMAD binding sites on the USP44 promoter (56).

The expression of USP44 is regulated via epigenetic mechanisms. A previous study reported that USP44 not only serves a key role in regulating proteasomal-mediated protein degradation but also self-regulates through K48- and K63-linked polyubiquitination degradation pathways (29). Moreover, cell cycle-dependent changes in USP44 are also associated with ubiquitination. USP44 is mainly localized to the nucleus. With the end of the previous cell division, USP44 expression is rapidly upregulated and reaches a peak in G_1/S phase. After that, USP44 expression begins to be downregulated, and when cells enter mitosis, with nuclear envelope rupture, USP44 is rapidly released into the cytoplasm and USP44 expression is further decreased. Until anaphase, with nuclear envelope recombination, USP44 recovers its tight association with chromosomes and its expression is resumed shortly after mitotic exit (67). During this process, the proteasome inhibitor MG132 stabilized USP44 levels both before and during mitosis, which suggested that USP44 may be ubiquitinated and degraded by the proteasome before and during mitosis (67). However, the E3 ligase mediating ubiquitination of USP44 has not been reported.

APC/C and Skp1-cullin1-F-box (SCF) complex are important E3 enzymes in monomial processes of the cell cycle (97). The possibility of their functioning in the same manner as E3S of USP44 was considered. APC/C is known to be active from mitosis to the subsequent G_1 phase. At the G_1/S boundary, APC/C is forcibly inactivated by numerous mechanisms and remains low until mitosis (98). However, this could not explain the reported trend of USP44 expression peaking and then decreasing in the G_1/S phase (67), so the possibility of SCF functioning in the same manner as E3S of USP44 was

considered. It is known that substrates recognized by SCF are mostly phosphorylated (99), and studies have reported that phosphorylated USP44 does exist in the cell cycle (16,100). Furthermore, USP44 has been reported to interact directly with the WD40 repeat sequence [a specific sequence of the substrate recognition domain of the FBXL family (a sub-family of F-box proteins)] (27). This suggests that USP44 has a structural basis for binding to F-box proteins. Therefore, it is feasible for phosphorylated USP44 to be ubiquitinated by the SCF complex during the cell cycle. Moreover, dephosphorylation of USP44 was reported to be one of the changes that occur at the exit from cell division, which is consistent with the rapid increase in USP44 expression after the exit from cell division (100). In summary, it is hypothesized that the SCF complex, rather than APC/C, mediated the ubiquitination of USP44 during the cell cycle, but further research is needed.

Apart from ubiquitination and the aforementioned phosphorylation, promoter methylation is the most extensively studied epigenetic regulatory mechanism in the regulation of USP44 expression. Tropel *et al* (96) reported that CpG 9 methylation might be involved in USP44 transcriptional regulation and promoter selection, thereby mediating the tissue-specific expression of USP44. Promoter hypermethylation is an important mechanism for the epigenetic silencing of tumor suppressor genes (101,102). A close association between hypermethylation of the USP44 promoter and downregulation of USP44 expression has been reported in colon and breast cancer (73,83). Moreover, Chen *et al* (61) also reported that the DNA methyltransferases DNMT3a and DNMT3b acting on the promoter of USP44 were the transcriptional silencers of the USP44 gene. Finally, the circRNA-miRNA axis is also one of the mechanisms of USP44 expression. CircFOXO3 upregulates USP44 expression by regulating miR-143-3p, which directly targets the USP44 gene (91). However, there is no subsequent study on the mechanism of circRNA regulating USP44 expression, and further research is needed to more comprehensively explore the regulatory mechanisms.

6. Therapeutic potential of targeting USP44

USP44 serves a central role in multiple tumor regulatory networks. Moreover, multiple studies have clearly demonstrated that targeting USP44 is the key to inhibiting the progression of nasopharyngeal, colon and lung cancer (18,51,74), and improve the sensitivity of pancreatic cancer and glioma treatment (62,70). Therefore, according to its expression characteristics, reversing USP44 expression is a promising therapeutic strategy. The exact regulation of USP44 expression by a complex named KRIBB53 (2',4-dihydroxy-3,4',6'-trimethoxychalcone) has been demonstrated. KRIBB53 inhibited the expression of the downstream protein USP44 (IC_{50}, 15 μm) and the progression of teratoma by inducing the proteasome-dependent degradation of OCT4, which confirmed the feasibility of targeting USP44 in the treatment of tumors (103). Furthermore, it can be hypothesized that antagonizing promoter methylation of USP44 (e.g., antagonizing the key enzymes DNMT3a and DNMT3b), limiting ubiquitination of USP44 itself and searching for partner proteins may be the most likely directions for future drug development.

7. Conclusions

In the present study, the basic characteristics, functions and regulatory mechanisms of USP44 were systematically introduced, emphasizing not only its physiological functions in various cellular activities and pathophysiological roles in related diseases, especially tumors (Table II), but also the significant effect of USP44 expression inhibitors on tumor treatment, which fully reflect the feasibility and importance of USP44 as a tumor therapeutic target. However, at present, no substantial progress has been made in the development of drugs that directly target USP44, and numerous problems remain to be resolved. For example, the exact mechanisms by which USP44 achieves specificity for each substrate need to be elucidated, and all possible conformational changes in USP44 and their roles in USP44 activation and substrate specificity need to be further evaluated. These are critical for the development of selective agonists and inhibitors. Therefore, in-depth evaluation of the structure, regulation and dynamics of USP44 is expected to be the key to solve this complex situation.

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Availability of data and materials

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Authors' contributions

YL was involved in the conception of the study and drafted the manuscript. CX and FT reviewed and edited the manuscript. MY performed a literature search and study selection. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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