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The Roles of Deubiquitinases in Renal Cell Carcinoma*

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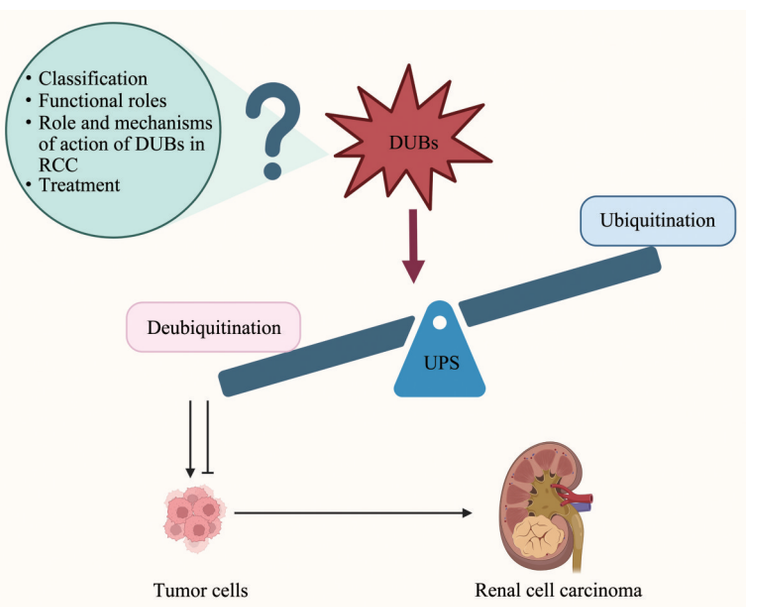
Graphical abstract



Abstract Renal cell carcinoma (RCC) is the primary malignant neoplasm. The ubiquitin-proteasome system (UPS) is crucial to the control of protein level and regulation of physiological and pathological processes. Deubiquitinases (DUBs), key components of UPS, specifically removing ubiquitin chains from the target protein, have showed crucial roles for protein homeostasis and quality control by rigidly regulating the balance between ubiquitination and deubiquitination in normal physiology. Accumulating studies indicate that abnormal function DUBs is associated with the progression and metastasis of RCC. Depending on the substrates, some DUBs may suppress RCC while others promote. Herein, we review recent research advances in RCC-associated DUBs, describe their classification, functional roles, summarize the role and mechanisms of action of DUBs in RCC and discuss the potential of targeting DUBs for cancer treatment.

Key words renal cell carcinoma, ubiquitin-proteasome system, deubiquitinase
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Renal cell carcinoma (RCC) is the primary malignant neoplasm of the adult kidney and accounts for 2.2% of all tumors worldwide, with estimations of 431 288 new cases and 179 368 deaths worldwide in 2020[1-2]. According to pathological classification by the International Society of Urological Pathology (ISUP), RCC mainly includes clear cell (ccRCC), papillary (pRCC), and chromophobe (chRCC) subtypes[3]. ccRCC represents the predominant histologic subtype of RCC and makes up about 70% of all cases, while the remaining subtypes are generally indolent[4-5]. RCC incidence varies geographically, with the highest incidence in developed countries[6]. In current treatment options, surgical resection therapy at the early stage of RCC are the most efficient and curative therapeutic approaches[7]. However, due to the poor diagnosis, the patients with early-stage RCC are not easily detectable, over 30% of patients with RCC are diagnosed with metastasized tumors and many patients eventually recur after surgical treatments[8]. Unfortunately, chemotherapy and radiotherapy are not satisfactory in controlling its progression, tumor cells metastasis and recurrence are the major cause of death and the overall 5-year survival rate of RCC patients remains quite low[8-10].

The ubiquitin-proteasome system (UPS), as a common post-translational modification pathway, is the major proteolytic system that controls protein stability and plays a critical role in many cellular processes, such as DNA repair, stress responses and cell proliferation[11]. UPS is composed of ubiquitin (Ub), ubiquitin-activating enzymes (E1s), the ubiquitin-conjugating enzymes (E2s), ubiquitin ligases (E3s), 26S proteasome and deubiquitinating enzymes (DUBs). The ubiquitination of substrates is a sophisticated post-translational modification cascade. Firstly, E1s utilize adenosine triphosphate (ATP) hydrolysis to activate Ub molecule and generate thioester bond between the C-terminal of Ub and a Cys residue in the active catalytic site of E1s[12]. Then activated Ub is transferred to the E2s, E3 ubiquitin ligases can specifically recognize substrates and transfer the activated Ub to the substrates[13]. The repeated three-step reaction catalyzes the polymerization of Ub to form polyubiquitin chains and the ubiquitin linkage type determines the different fates and functions of the substrates. For example, the K11, K29, and K48 linkages are mainly responsible for 26S proteasome-mediated degradation of targeted substrates. On the other hand, K6, K27, K33, and K63 linkages protect the substrates from proteolysis and involve in virous crucial biological processes such as DNA repair, kinase activation, and transcriptional regulation[14-19] (Figure 1).

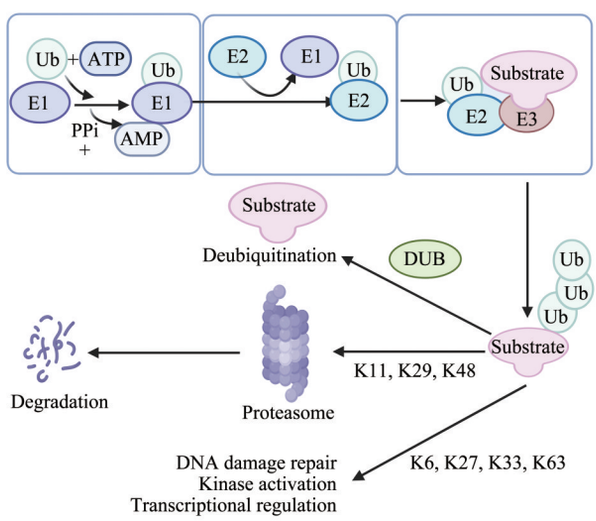

Fig. 1 Ubiquitin ligases and deubiquitinases in the ubiquitination proteasomal system

The UPS consists of ubiquitinating enzymes (E1, E2, and E3), 26S proteasome and deubiquitinating enzymes (DUBs). Firstly, E1 enzyme is activated by ATP and binds to Ub molecule. Then, activated Ub is transferred to the E2s, after which the Ub molecule is transferred to the specific substrates *via* E3 ubiquitin ligases. The ubiquitin linkage type determines the different fates and functions of the substrates: K11, K29, and K48 linkages are mainly responsible for 26S proteasome-mediated degradation of targeted substrates; while K6, K27, K33, and K63 linkages protect the substrates from proteolysis and involve in virous crucial biological processes such as DNA damage repair, kinase activation, transcriptional regulation, or growth response. DUBs reverse this process by cleaving polyubiquitin chains or monoubiquitin from target substrates, and thus prevent proteins from proteasome-dependent degradation or modulate non-proteasomal processes.

Ubiquitination is a dynamic and reversible process, ubiquitination of targeted proteins is reversed by DUBs, a superfamily of metalloproteases and cysteine proteases that cleave ubiquitin-protein bonds[20]. To date, more than one hundred DUBs have been identified in humans. Based on sequence and

conserved domain, they can be classified into six families including ubiquitin-specific proteases (USPs), ubiquitin carboxy-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Machado-Joseph disease protein domain proteases (MJDs), JAMM/MPN domain-associated metallopeptidases (JAMMs), and the monocyte chemotactic protein-induced proteases family (MINDYs) [21-24]. The USP family is the largest and most diverse DUB family, members in this family have a conserved catalytic domain that consists of three subdomains resembling the thumb, fingers, and palm of the right hand[25]. DUBs of UCH family, as the first structurally characterized DUB family, have six or seven β-sheets surrounded by eight α-helices, which act as a gate to preclude large substrates from getting access to the catalytic core located at the bottom of the DUB[26-27]. The OTU domain was initially identified in an ovarian tumor gene, which consists of five β-sheets interspersed between two helical domains[28] . There are four members in MJD family, including the well characterized ATXN3 that is mutated in Machado-Joseph disease, and the other members are ATXN3L, JOSD1, and JOSD2[29-30]. All other DUB families are cysteine peptidases, while JAMMs are zinc metallopeptidases[31-32]. All members of MINDY family consist of an N-terminal ubiquitin association domain, a central CCCH-type zinc-finger domain, and a C-terminal proline-rich domain[33].

1 Functional roles of DUBs in RCC

Growing evidence has shown that DUBs play an important role in RCC *via* regulating the key proteinsstability. In this review, we summarize the aberrant regulation of DUBs in RCC-related signaling pathways and pathological processes, such as NF-κB signaling pathway, PI3K/AKT signaling pathway, RAS/RAF/MEK/ERK pathway, JNK pathway, ECT2-Rho signaling, HIF-2α pathway, p53 pathway, DDR and modulation of VEGF splicing. In the following sections, we will describe and discuss the relationships between some DUBs and RCC in different signal pathways and pathological processes (Table 1, Figure 2−4).

Table 1 RCC−associated DUBs

Pathway	Types	DUBs	Substrates in RCC	Deubiquitination-induced change	Potential role in RCC	Reference
NF-κB signaling pathway	USPs	USP13	ZHX2	Protein stability	Oncogene	［34］
		USP53	IκB	Protein stability	Tumor suppressor	［35］
		USP2	Not reported	Not reported	Tumor suppressor	［36-37］
	OTUs	OTUD1	PTEN	Protein stability	Tumor suppressor	［38］
PI3K/AKT signaling pathway	USPs	USP46	Not reported	Not reported	Tumor suppressor	［39］
		USP39	Not reported	Not reported	Oncogene	［40］
RAS/RAF/MEK/ERK pathway	USPs	USP39	Not reported	Not reported	Oncogene	［40］
		USP19	Not reported	Not reported	Tumor suppressor	［41］
JNK pathway	USPs	USP44	Not reported	Not reported	Tumor suppressor	［42-43］
ECT2-Rho signaling	OTUs	OTUB1	FOXM1	Protein stability	Oncogene	［44］
HIF-2α pathway	USPs	USP37	HIF-2α	Protein stability	Oncogene	［45］
P53 pathway	USPs	USP10	P53	Protein stability and localization	Tumor suppressor	［46］
The DNA damage reponse	USPs	USP38	HDAC1	Protein stability and activity	Tumor suppressor and oncogene	［47］
Modulation of VEGF splicing	USPs	USP39	SRPK1	Not reported	Oncogene	［48］

Continued to Table 1

Pathway	Types	DUBs	Substrates in RCC	Deubiquitination-induced change	Potential role in RCC	Reference
Others	UCHs	BAP1	HCF-1	Not reported	Tumor suppressor	［49］
			MCRS1	Protein stability	Tumor suppressor	［50］
	USPs	USP7	ARMC5	Protein stability	Tumor suppressor	［51］
		USP22	survivin	Protein stability	Tumor suppressor	［52］
	OTUs	OTUD1	Bim	Protein stability	Tumor suppressor	［53］

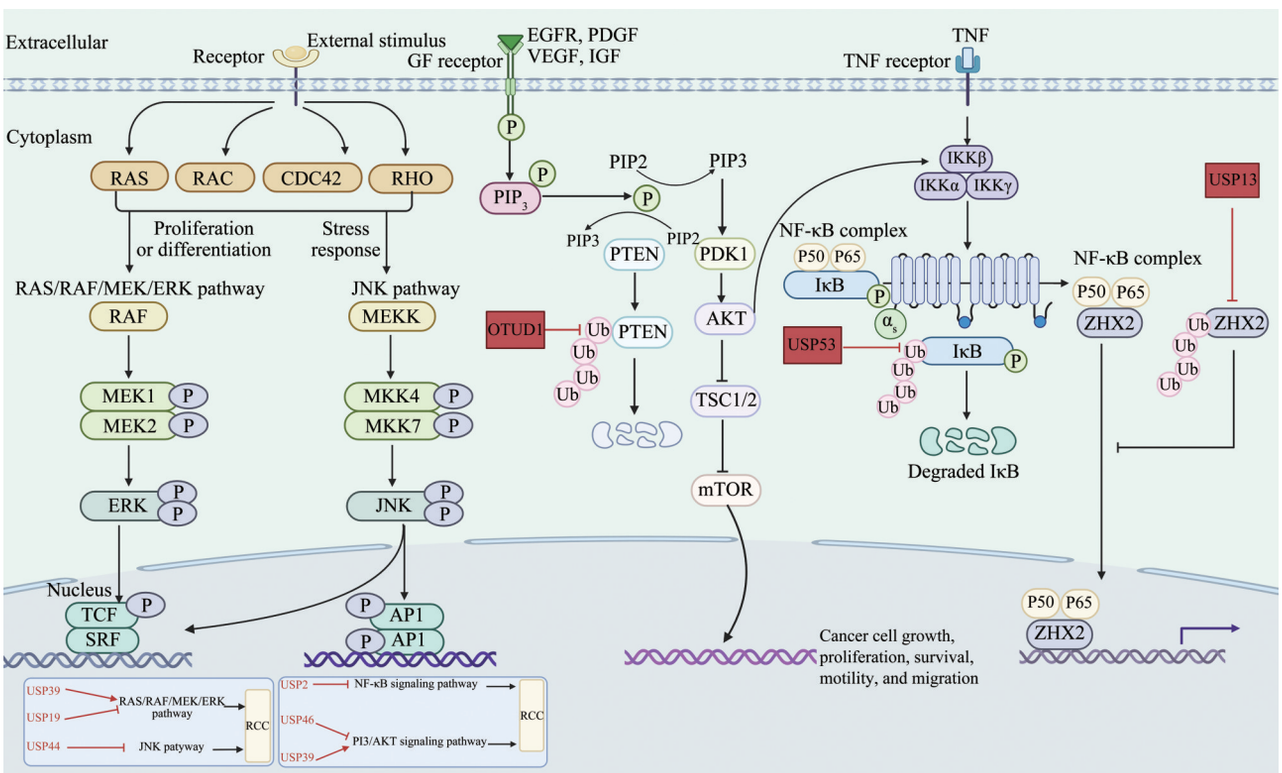

Fig. 2　The role of DUBs in NF−κB，PI3K/AKT，RAS/RAF/MEK/ERK and JNK signaling pathways in RCC

NF-κB signaling pathway: When extracellular or intracellular signals stimulate cell, the IκB kinase β (IKKβ) and IκB would be activated, which subsequently leads to translocation of the NF-κB dimer to the nucleus and transcription of more than 200 genes involved in inflammation, cytokine secretion, chemokine secretion, cell cycle regulation, and angiogenesis. USP13 promotes NF-κB signaling pathway through deubiquitination of ZHX2. USP53 can promote IκB deubiquitination and subsequently inhibits this signaling pathway. OTUD1 and USP2 inhibits the activation of NF-κB signaling pathway. But OTUD1 mediate the deubiquitination of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), which is a negative regulator of both the phosphoinositide 3-kinase/serine/threonine protein kinase AKT (PI3K/AKT) and TNF-α/NF-κB signaling pathways. PI3K/AKT signaling pathway: This signalling pathway is stimulated by assorted oncogenes and growth factor receptors. USP46 is a negative regulator and USP39 is a positive regulator of PI3K/AKT signaling pathway which the molecular mechanism of them regulating PI3K/AKT signaling pathway is not clear yet. RAS/RAF/MEK/ERK pathway: The RAS/RAF/MEK/ERK signal cascade amplification is mainly activated by signals generated *via* membrane-bound receptors. Although USP39 can activate this pathway, while USP19 inhibits the pathway, the mechanism of both regulating RAS/RAF/MEK/ERK pathway is not reported. JNK pathway: The JNK pathway is a kinase cascade which is activated by diverse extracellular stimuliand intracellular stimuliand the stress response signal is transmitted to this cascade by Ras homology (Rho) family GTPase and then MAP3Ks (MEKK1/4, MLK2/3, DLK, TpI-2, TAO1/2) are activated, thus phosphorylating and activating the MAP2K isoforms MKK4 and MKK7, which in turn phosphorylates and activates JNK to drive its nuclear translocation. USP44 inhibits the activation of JNK pathway, the specific mechanism has not been reported.

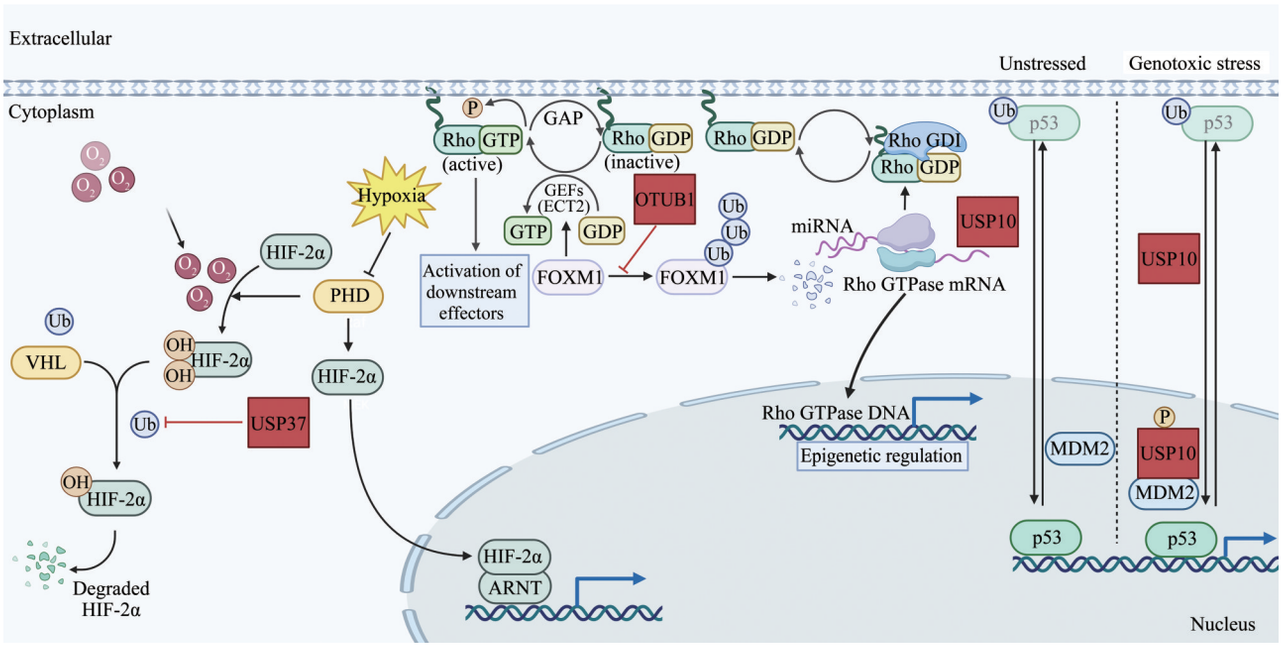

Fig. 3　The role of DUBs in ECT2−Rho，HIF−2α and p53 pathway in RCC

ECT2-Rho signaling: Rho GTPases cycle between an active GTP-bound conformation and an inactive GDP-bound conformation, and the cycle is regulated by three types of protein that Guanine nucleotide exchange factors (GEFs), including ECT2, catalyse the exchange activity of Rho GTPases to stimulate formation of Rho-GTP, whereas GTPase-activating proteins (GAPs) accelerate the intrinsic GTP hydrolysis activity of the Rho GTPase, thereby resulting in the inactive GDP-bound conformation. Guanine nucleotide dissociation inhibitors (GDIs) sequester Rho GTPases in a GDP-bound state in the cytosol and prevent them from localizing to membranes where Rho GTPases can be activated. OTUB1 regulates stabilization of FOXM1 by OTUB1-mediated deubiquitination, and FOXM1 could promote ECT2-Rho signaling whose knockdown decreased ECT2 expression. HIF-2α pathway: Under normoxic conditions, PHD is activated and hydroxylated to modify HIF-2α, which is subsequently degraded by VHL. Under hypoxic conditions, the activity of PHD and VHL was suppressed, leading to accumulation of HIF-2α. USP37 mediated the deubiquitination of HIF-2α and activated HIF-2α signal. p53 pathway: p53 is tightly regulated by inhibitory proteins and is primarily regulated by the E3 ubiquitin ligase murine double minute 2 (MDM2). MDM2 can promote p53 degradation by MDM2-mediated ubiquitination and ubiquitination of p53 by MDM2 could induce p53 translocation from nucleus to cytoplasm. Under unstressed conditions, USP10 can promote p53 deubiquitination in the cytoplasm to counteract the action MDM2 and enable nuclear re-entry. Upon DNA damage, USP10 is phosphorylated by ATM, upregulated and translocates to the nucleus, and subsequently deubiquitinates p53 in the nucleus.

1.1　NF−κB signaling pathway

The nuclear factor-kappa B (NF-κB) is one of the key regulators of inflammation, which is composed of five NF-κB transcription factors: RelA (p65), RelB, c-Rel, p52 and p50. Among them, p52 and p50 are derived from proteolysis of their precursor proteins p100 and p105, respectively[54-55]. NF-κB transcription factors exist as homodimers or heterodimers preferentially bound to the IκB family members, preventing the dimers to transport from cytoplasm to nucleus and thus inhibiting the transcription of genes related to NF-κB signaling[56]. When extracellular or intracellular signals stimulate cell, such as pro-inflammatory cytokines (*e. g.*, tumor necrosis factor (TNF)), the recognition of extracellular or intracellular pathogens (*e. g.*, toll like receptors (TLRs)), and cell stressors (*e. g.*, reactive oxygen species), the IκB kinase β (IKKβ) would be activated. Subsequently, IκB bound to NF-κB dimers is phosphorylated by activated IKKβ, thereby IκB would be degraded by the proteasome, releasing the NF-κB dimer from IκB, which subsequently leads to translocation of the NF-κB dimer to the nucleus and transcription of more than 200 genes involved in inflammation, cytokine secretion, chemokine secretion, cell cycle regulation, and angiogenesis[56-57].

The emerging evidence indicates that NF-κB signaling pathway could promote the progression of RCC[58-60]. Firstly, constitutive NF-κB activation has been observed in numerous RCC cell lines[61-62].

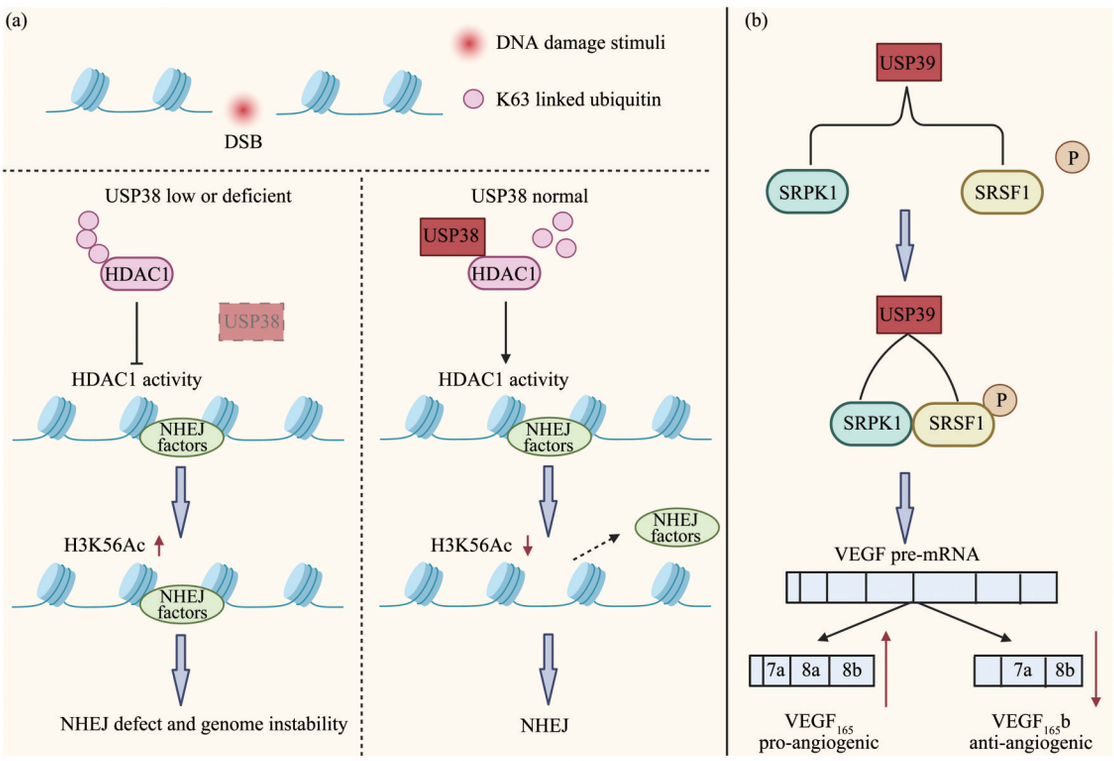

Fig. 4　The role of DUBs in DDR and modulation of VEGF splicing in RCC

(a) The DNA damage response (DDR): For DNA double-strand breaks (DSBs) repair, homologous recombination (HR) and nonhomologous end-joining (NHEJ) are two major pathways. Acetylation regulates transcription of DDR-related proteins, modulating chromatin structure and equilibrating dynamic acetylation level of these proteins. Histone acetylation is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDAC1 and HADC2 regulate the DDR by promoting NHEJ repair through regulation of H3K56 acetylation. USP38 interacts with HDAC1 and specifically removed the K63-linked ubiquitin chain promoting the deacetylase activity of HDAC1. Thereby, HDAC1 is able to deacetylate H3K56 and the depletion of USP38 would enhance H3K56 acetylation. (b) Modulation of VEGF splicing: (H3K56Ac), reduce NHEJ efficiency, causing genome instability and sensitizing cancer cells to genotoxic insults. VEGF can produce different isoforms *via* mRNA splicing, serine/arginine-rich protein specific kinase 1 (SRPK1) could phosphorylate serine/arginine-rich splicing factor 1 (SRSF1) to promote VEGF splicing to generate $VEGF_{165}$ (pro-angiogenesis) and $VEGF_{165b}$ (anti-angiogenesis). USP39 bonds with SRPK1 to promote the phosphorylation and interaction of SRSF1 by SRPK1and USP39 can inhibit $VEGF_{165b}$ alternative splicing *via* regulating SRSF1 and SRPK1.

Additionally, inhibition of NF-κB sensitizes RCC cells to tumor necrosis factor alpha (TNF-α) and TNF-α-related apoptosis-inducing ligand[63]. The clinical evidence for the role of NF-κB in RCC was highlighted in a study which reported that enhanced NF-κB activation is associated with the occurrence and progression of RCC in actual patients[58]. Moreover, NF-κB activation is not only a frequent observation among RCC patients but also correlates with primary tumor size[61].

Thus, understanding the signal molecules that can regulate the NF-κB signaling pathway is of great importance for the treatment of RCC.

1.1.1　USP13

Numerous studies found that von Hippel-Lindau (VHL) can be used as one of the upstream signal molecules that regulates the NF-κB signaling pathway[62, 64-65]. As an E3 ubiquitin ligase protein, *VHL* is the most frequently mutated gene in RCC[63]. Numerous researches seem to focus on its substrate: hypoxia-inducible factor α (HIF-α), a key protein that is related to the hypoxia signaling[66]. However, more and more studies have found that VHL also plays other HIF-α-independent roles in RCC[56]. In addition to being associated with hypoxic signaling, VHL has also been found to be an upstream signal molecule of the NF-κB signaling pathway, but the exact substrates that regulate the NF-κB signaling pathway in cancer have not been much studied[56]. However, Zhang *et al.*[67] have found that VHL indirected regulation of

NF-κB signaling pathway *via* ubiquitinating zinc fingers and homeoboxes 2 (ZHX2). They found that ZHX2 can be regulated by VHL-mediated ubiquitination, and the depletion of ZHX2 contributes to inhibition of the translocation of p65 into the nucleus, resulting in the inactivation of NF-κB signaling pathway.

Ubiquitin specific protease 13 (USP13), early known as Isopeptidase T (ISOT-3), is a DUB enzyme and belongs to the USPs family. It plays an important role in diverse cellular processes, such as mitochondrial energy metabolism, autophagy, DNA damage response, endoplasmic reticulum (ER)-associated protein degradation (ERAD) and other processes by regulating the deubiquitination of diverse key substrate proteins[68]. Xie *et al*.[34] found that USP13 is a potential DUB for ZHX2 in ccRCC. USP13 can increase the protein stability of ZHX2 by inducing the deubiquitination of ZHX2, meanwhile the depletion of USP13 leads to ZHX2 down-regulation in ccRCC, suggesting that USP13 is a potential therapeutic target in ccRCC[34].

Previous study has found that Spautin-1, a potent small molecule inhibitor of autophagy, was an inhibitor for both USP10 and USP13 by promoting the degradation of vps34[69]. Thus, the further investigation of the role of Spautin-1 as an USP10/13 inhibitor is needed. The development of USP13-specific inhibitors may provide great therapeutic potential for ccRCC.

1.1.2 OTUD1

In addition to USP13 as the upstream molecule of NF-κB signaling pathway, it has been found that another deubiquitinase OTU domain-containing 1 (OTUD1 also known as DUBA7) can also indirectly affect the upstream of this pathway[38]. OTUD1 is a DUB that controls the progression of various diseases through involvement in inflammatory homeostasis[70-71]. It is reported that OTUD1 inhibits the activation of NF-κB signaling pathway by inducing the deubiquitination of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), which is a negative regulator of both the phosphoinositide 3-kinase/serine/threonine protein kinase AKT (PI3K/AKT) and TNF-α/NF-κB signaling pathways[38]. Previous studies have shown that the PI3K/AKT signaling pathway can activate TNF-α mediated NF-κB activation through AKT-induced phosphorylation of the inhibitor of NF-κB kinases (IKKs) [72]. In the activation of the PI3K/AKT signaling pathway, the indispensable step is that the activated PI3K is recruited to the plasma membrane and phosphorylates phosphatidylinositol 4, 5-diphosphate (PIP2), thus producing the second messenger phosphatidylinositol 3, 4, 5-triphosphate (PIP3) which can activate the serine/threonine kinase AKT through 3-phosphoinositide dependent protein kinase-1 (PDPK1) [73-75]. Interestingly, this signal transduction process can be inhibited by PTEN[76]. Therefore, the deubiquitination and stability of PTEN mediated by OTUD1 may exert anticancer activity by inhibiting NF-κB signal. At present, tyrosine kinase inhibitors (TKIs) are recognized as the first-line treatment for ccRCC and abnormal activation of AKT and TNF/NF-κB signaling contributes to TKI resistance in ccRCC[77-79]. The researchers also found that OTUD1 sensitizes ccRCC cells to the TKIs *via* PTEN[38]. These findings consistently point out that OTUD1 is a potential tumor suppressor in ccRCC.

1.1.3 USP53

It is reported that ubiquitin-specific peptidase 53 (USP53) plays as a tumor suppressive role in various cancers[80-82]. Notably, USP53 was also found to be a new potential therapeutic target for patients with ccRCC[35]. Gui *et al.*[35] discovered that the expression of deubiquitinating enzyme USP53 is significantly down-regulated in ccRCC, and its expression is negatively correlated with tumor progression and clinical prognosis. The down-regulation of USP53 promoted the growth and proliferation of ccRCC cells *in vitro* and *in vivo*[35]. Besides, they found that high levels of USP53 increased the expression of IκB and proved that USP53 can promote IκB deubiquitination and subsequently inhibit the p65 and p50 complex[35]. As mentioned previously, the activity of the NF-κB signaling pathway can be inhibited when the p65 and p50 complex is bounded by IκB and cannot enter the nucleus to regulate gene transcription[83]. Collectively, these results indicate that USP53 plays a tumor suppressive effect in ccRCC.

1.1.4 USP2

To date, the role of ubiquitin specific protease 2 (USP2) had been reported in various cancers, including prostate cancer, hepatoma, bladder carcinoma, and glioma[84-88]. However, Meng *et al.*[36] discovered that USP2 is a tumor suppressor in ccRCC. They found that USP2 is significantly downregulated

in ccRCC patients and cell lines, and low USP2 mRNA level is associated wtih poor prognosis of ccRCC[36]. These findings consistently point out that downregulation of USP2 possesses prognostic and diagnostic value[36]. In another study, Duan *et al.* [37] discovered the up-regulation of USP2 inhibited the proliferation, migration and invasion of ccRCC cells *in vitro* and *in vivo*. They further proved that USP2 inhibits epithelial-mesenchymal transition (EMT) in ccRCC metastasis by downregulating the NF-κB signaling pathway[37]. However, the molecular mechanism of USP2 regulating NF-κB signaling pathway is not clear yet, and further research is needed.

1.2 PI3K/AKT signaling pathway

1.2.1 USP46

PI3K/AKT signaling pathway plays a crucial role in cell survival and proliferation by regulating the downstream signaling pathways and is frequently disturbed in most cancers, including RCC[89-91]. As mentioned earlier, OTUD1 could inhibit the PI3K/AKT and TNF-α/NF-κB signaling pathways by stabilizing PTEN in ccRCC[38]. Interestingly, another previous study reported that ubiquitin-specific peptidase 46 (USP46) suppresses RCC tumorigenesis through AKT pathway inactivation[39]. USP46, which belongs to a large family of cysteine proteases, is related to circadian clock system and depressive behavior disorders[92-96]. Meanwhile, it's reported that USP46 acts as a tumor promoter or a tumor suppressor in various tumors, including colorectal cancer, breast cancer (BC) and so on[97-99] . In CRC, USP46 functions as a tumor suppressor by controlling PH domain leucine-rich-repeats protein phosphatase (PHLPP)-dependent attenuation of AKT signaling[98]. Gui *et al.* [39] first analyzed The Cancer Genome Atlas (TCGA) database and found that downregulation of USP46 correlated with poor prognosis in primary RCC patients. Also, USP46 expression was negatively correlated with tumor malignancy grading in RCC[39]. Functionally, USP46 overexpression reduced cell proliferation, viability and tumor cell migration of RCC *in vitro* and *vice versa*[39]. In addition, they further found that the overexpression of USP46 leads to reduction of the AKT and its downstream molecule the 70 ku ribosomal protein S6 kinase 1 (S6K1) phosphorylation in RCC[39]. At the same time, in the *USP46* depleted RCC cells treated with AKT inhibitor, the cell growth and migration advantages were obviously diminished[39]. Taken together, as a tumor suppressor, USP46 inhibits RCC survival *via* the downregulation of the AKT signaling pathway. However, it is not clear that the regulation mechanism of USP46 mediated AKT inactivation and further studies are needed.

1.2.2 USP39

Ubiquitin-specific peptidase 39 (USP39), known as a type of protein associated with the assembly process of spliceosomal snRNP during pre-mRNA maturation, is first found in yeast and directly participates in the pre-mRNA splicing of several oncogenes including Aurora B and RB1[100-102]. It's reported that the expression level of USP39 has a close relationship with cell proliferation in multiple malignancies, including BC, hepatocarcinogenesis, medullary thyroid carcinoma, oral squamous cell carcinoma and so on[103-106]. Also, *USP39* is reported to be an oncogenic factor in RCC and knockdown of *USP39* could inhibit the malignant progression of RCC[40]. Xu *et al.* [40] showed that silencing of *USP39* markedly suppressed RCC cell proliferation, the migratory and invasive capacity of RCC cells as well as induced cell cycle arrest and apoptosis. Mechanistically, knockdown of *USP39* contributed to significant inhibition of AKT phosphorylation at the Ser473 site, indicating that downregulation of USP*39* blocked the activation of AKT signaling pathway[40]. However, the molecular mechanism of USP39 regulating AKT signaling pathway is not clear yet, and further research is needed.

1.3 RAS/RAF/MEK/ERK pathway

Mitogen-activated protein kinases (MAPK), which belong to a large family of serine-threonine kinases, form major cell-proliferation signalling pathways from the cell surface to the nucleus[107]. There are three major subfamilies of MAPK, and the extracellular-signal-regulated kinases (ERK-MAPK, RAS/RAF/MEK/ERK) is one of the most important for cell proliferation, and several key growth factors and proto-oncogenes transduce the signals which promote growth and differentiation *via* this cascade[108-109]. The RAS/RAF/MEK/ERK signal cascade amplification is mainly activated by signals generated *via* membrane-bound receptors such as receptor tyrosine kinases (RTKs), and G protein-coupled receptors (GPCRs). Then GTPase and rat

sarcoma (RAS) are activated, the latter reactivates the core units in the cascade, including a MAPKKK (RAF), a MAPKK (MEK1/2), and a MAPK (ERK). Specifically, RAS firstly recruits rapidly accelerated fibrosarcoma (RAF) kinase to the plasm membrane for activation, then activated RAF kinase phosphorylates downstream mitogen-activated protein kinase kinase (MEK), which then sequentially phosphorylates Tyr and Thr residues of ERK. Activated ERK dimers can regulate target proteins in the cytoplasm or transport into the nucleus, where they phosphorylate various transcription factors that regulate gene expression[110].

1.3.1 USP39

As mentioned above, USP39 may activate the PI3K/AKT signaling pathway, and act as a potential oncogenic protein in RCC[40]. Interestingly, the same group also found that knockdown of USP39 inhibited ERK phosphorylation at the Thr202/Tyr204 obviously, suggesting that expression of USP39 may promote cancer progression by activating the AKT and ERK signaling axis, but the specific mechanism still needs further exploration[40].

1.3.2 USP19

Ubiquitin-specific protease 19 (USP19), belongs to the USPs family, is associated with protein quality control and cellular homeostasis, and affects cellular processes relevant in tumorigenesis such as DNA damage repair, apoptosis, the TGF-β pathway, hypoxia and angiogenesis, immunity, proliferation, ERAD and autophagy[31, 111-119]. Accumulating evidence has demonstrated that USP19 is related with tumor progression, represents a novel prognostic factor for the outcome of several malignant diseases, including ccRCC, in which USP19 may function as a tumor suppressor[41, 120]. Previous studies have demonstrated that USP19 expression was lower in ccRCC tissue compared with in normal kidney tissue by analyzing TCGA KIRC data and uc003cvz.3, as a major isoform of USP19, is mainly localized in the cytoplasm and serves as an indicator of poor outcome in patients with advanced stage ccRCC[112-121]. In another study, according to silico analyses, Hu *et al.* [41] observed that USP19 mRNA levels were significantly lower in ccRCC than normal tissues, furthermore, *USP19* downregulation in ccRCC was associated with disease progression and poor prognostic outcomes in a TCGA cohort of patients. In addition, the same group utilized ccRCC cell lines *in vitro* to reveal that USP19 overexpression negatively affected ccRCC cell proliferation and migration and *vice versa*, meanwhile, they also observed that USP19 knockdown promoted tumor growth *in vivo* as well[41]. The mechanism exploration of the same research showed that USP19 knockdown can promote ERK phosphorylation, thereby activating the ERK signaling pathway, indicating that USP19 inhibits ccRCC cell proliferation and migration may depend on inactivation of the RAS/RAF/MEK/ERK pathway[41]. These results support USP19 as a tumor suppressor in ccRCC, but the corresponding mechanistic studies are still poor, especially the identification of its substrates.

1.4 JNK pathway

Similar to RAS/RAF/MEK/ERK pathway, the c-Jun N-terminal or stress-activated protein kinases (JNK or SAPK) pathway also is a member of the MAPK family and participates in various cellular processes, such as apoptosis, migration, proliferation, and differentiation[122-123]. Besides, it is protumorigenic in many tumor model systems, and it's reported that JNK hyperactivates in ccRCC[124-126]. The JNK pathway is a kinase cascade which is activated by diverse extracellular stimuli (*e.g.*, cytokines, pathogens, morphogenic factors, hormones) and intracellular stimuli (*e.g.*, oxidative stress, DNA damage)[127]. The stress response signal is transmitted to this cascade by Ras homology (Rho) family GTPase and then MAP3Ks (MEKK1/4, MLK2/3, DLK, TpI-2, TAO1/2) are activated, thus phosphorylating and activating the MAP2K isoforms MKK4 and MKK7, which in turn phosphorylates and activates JNK to drive its nuclear translocation.

Ubiquitin-specific protease (USP44), as a member of the USP family, is located at 12q22 and participates in the regulation of various physiological functions and pathological processes, including sister chromatid separation, stem cell differentiation and tumor progression[128-130]. Specifically, aneuploidy caused by chromosomal instability is one of the hallmarks of cancers, and USP44 has a crucial role in cancers for its ability that can stabilize the protein expression of protectin in the cycle of normal cells until all the chromosomes match correctly with spindle fibers and prevent immature mitosis[131-132]. Recently, one study showed that UAP44 serves as a tumor suppressor in ccRCC[42]. Zhou *et al.*[42] reported

that USP44 was downregulated in ccRCC and that its expression level was negatively associated with the grade and stage of ccRCC. Meanwhile, they also demonstrated that UPS44 overexpression inhibited the proliferation and migration of cells *via* inhibition of JNK pathway in ccRCC[42]. Conversely, USP44 knockdown promoted proliferation and migration of ccRCC cells *via* JNK activation which could have been a result of stress-response activation due to chromosome mis-segregation[133]. However, little is known about the catalytic substrates of USP44 and the underlying mechanism that USP44 regulates JNK pathway needs further exploration. In addition, Tang *et al.*[43] constructed a more accurate prognostic model using USP44 methylation as one of the prognostic factors for ccRCC. These results suggest that USP44 plays a tumor-suppressing effect in ccRCC and may be a marker in predicting ccRCC progression.

1.5 ECT2−Rho signaling

Ras homologous (Rho) family proteins (20 human members) comprise a major branch of the Ras superfamily of small GTPases, while RhoA, Rac1 and Cdc42 the most extensively studied and characterized[134-135]. Rho GTPases, as conserved molecular switches that serve as signal transducers in complex biological networks, control fundamental cellular processes such as cytoskeleton organization, cell migration, proliferation, survival and apoptosis, all of which are deregulated in cancers[135-138]. Rho GTPases cycle between an active GTP-bound conformation and an inactive GDP-bound conformation, and the cycle is regulated by three types of protein that Guanine nucleotide exchange factors (GEFs) catalyse the exchange activity of Rho GTPases to stimulate formation of Rho-GTP, whereas GTPase-activating proteins (GAPs) accelerate the intrinsic GTP hydrolysis activity of the Rho GTPase, thereby resulting in the inactive GDP-bound conformation[139-141]. Guanine nucleotide dissociation inhibitors (GDIs) sequester Rho GTPases in a GDP-bound state in the cytosol and prevent them from localizing to membranes where Rho GTPases can be activated.

Rho GTPases are activated most commonly by indirect mechanisms in disease and one prevalent mechanism involves aberrant Rho activation *via* the deregulated expression and /or activity of GEFs[142]. And one of these GEFs, epithelial cell transforming sequence 2 (ECT2) is originally discovered as an oncogene that transformed NIH 3T3 cells, activates RhoA, Rac1 and Cdc42, and as such plays a central role in processes such as cell division and mitotic cell rounding, invasion, proliferation and DNA damage repair[143-145]. ECT2 is overexpressed in various tumors, including ovarian, esophageal and non-small cell lung cancer (NSCLC), and knockdown of *ECT2* could inhibit tumor cell proliferation, migration and invasion[144, 146]. Recently, one study has found a DUB plays a key role in the tumorigenesis of RCC by targeting a molecule related to the ECT2-Rho signaling[44].

OTU domain-containing ubiquitin aldehyde-binding proteins Otubain1 (OTUB1), as a member of OTU domain protease superfamily of DUBs, acts as an important regulatory role in various physiological and pathological processes such as DNA damage repair, apoptosis and inflammatory response[111, 147-149]. Studies have shown that OTUB1 is closely related to the occurrence and progression of numerous cancers, including CRC, PCa and so on[150-151]. Interestingly, it’s reported that OTUB1 could promote deubiquitination of forkhead box M1 (FOXM1) in BC and OC to facilitate tumor progression[152-153]. FOXM1 functions as a proto-oncogenic transcription factor that regulates cell division by activating the expression of genes implicated in the G1/S and G2/M phase transitions and mitotic progression and whose overexpression tightly correlates with poor clinical outcome in many human cancer types, including RCC[154-158]. However, it is not clear whether OTUB1-mediated deubiquitination of FOXM1 also participates in RCC progression, thereby, Zhou *et al.*[44] investigated the effect of OTUB1/FOXM1 axis on RCC progression and even uncovered the underlying mechanism. They firstly found that OTUB1 was elevated in RCC tissues and cell lines and shown to be associated with poor prognosis of RCC[44]. Functionally, they revealed knockdown of *OTUB1* inhibited cell proliferation, migration and invasion of RCC cells *in vitro*. Furthermore, *in vivo* subcutaneous xenograft mouse model also indicated that knockdown of *OTUB1* could suppress *in vivo* tumorigenic ability of RCC[44]. Further mechanism studies have shown that OTUB1 suppressed ubiquitination of FOXM1 in RCC and FOXM1 could regulate ECT2-Rho signaling whose knockdown decreased ECT2 expression in RCC[44]. Taken together, OTUB1 regulates stabilization of FOXM1

by OTUB1-mediated deubiquitination, and OTUB1/FOXM1 axis contributes to RCC tumorigenesis and aggression *via* ECT2-Rho signaling, suggesting a novel insight into the treatment of RCC.

1.6 HIF−2α pathway

As the main regulator of oxygen homeostasis, HIF is composed of an α-regulatory subunit (HIF-1α, HIF-2α, or HIF-3α) and a constitutively expressed HIF-β subunit, also known as the aryl hydrocarbon receptor nuclear translocator (ARNT) that heterodimerizes to form a functional transcriptional complex[159]. The heterodimer can translocate to the nucleus and transcribed genes involved in angiogenesis (*e.g.*, VEGF), glycolysis, glucose transport (*e.g.*, GLUT1), and erythropoiesis (*e.g.*, EPO) [159-160]. The activity of HIF is regulated by oxygen-dependent proteasomal degradation of the HIF-α subunit[161-162]. Under normoxic conditions, members of the prolyl hydroxylase domain (PHD) family of proteins (herein called PHDs) are activated by oxygen and hydroxylate HIF-α at one or both conserved prolyl residues of HIF-α[159]. Then, VHL E3 ubiquitin ligase can recognize the hydroxylated HIF-α and mediate polyubiquitination and subsequent proteasomal degradation of HIF-α[159, 163]. On the contrary, under hypoxia, low oxygen suppresses the activity of PHD and VHL, leading to accumulation of HIF-α[45, 164-165].

It is known that HIF-2α can be degraded by VHL-mediated ubiquitination, but the further exploration is needed to identify the deubiquitination pathway regulating HIF-2α protein stability which is a potential therapeutic target through the development of deubiquitinase inhibitors.

Ubiquitin specific peptidase 37 (USP37) is composed of 979 amino acids harboring three ubiquitin-interacting motifs between the Cys box and His box of the primary sequence[166-167]. USP37 is an effective regulator of the cell cycle, which can accelerate the G1/S transition with abnormally high expression and previous studies have found that USP37 involved in lung cancer and BC progression[166, 168]. Recently, one study has also found that USP37 may be a potential therapeutic target in ccRCC[45]. Hong *et al.* [45] utilized a DUB complementary DNA (cDNA) library, discovering that USP37 as a DUB, interacts with HIF-2α and promotes HIF-2α deubiquitination. In addition, the depletion of USP37 leads to HIF-2α down-regulation in ccRCC and contributes to decreased primary kidney tumorigenesis and spontaneous lung metastasis[45]. In summary, these findings indicate that USP37 may be a therapeutic target in ccRCC and can motivate the development of USP37 inhibitors for potential treatment of ccRCC.

1.7 p53 pathway

p53, referred to the “guardian of the genome”, is a sequence-specific transcription factor activated by diverse stress signals and is identified as crucial tumor suppressor genes[169-171]. p53 exists in non-stressed cells at a very low concentration[172]. Under stress conditions, such as DNA damage, activated oncogenes, hypoxia, ribonucleotide depletion and telomere erosion, p53 is activated and then translocates to the nucleus through a complex network of interactions[172-173]. There it induces the transcription of myriad genes that are involved in cell-cycle control, apoptosis, DNA repair, differentiation and senescence[172]. p53 is tightly regulated by inhibitory proteins and is primarily regulated by the E3 ubiquitin ligase murine double minute 2 (MDM2)[174-175].

It is often remarked that p53 is mutated in 50% of human cancers, however, mutations in ccRCC are rare and wild-type p53 is expressed at lower levels in tumor cells is observed, indicating that this protein might be suppressed by other mechanisms[176-178]. Dell'Atti *et al.* [179] review recent research advances in ccRCC-associated p53 related signaling and numerous findings suggest that in ccRCC, p53 might be inactivated by proteasome degradation in a mTOR-MDM2-dependent manner[179-180]. Specifically, it was observed that in different ccRCC cell lines, the activation of mTOR promotes the expression of E3 ubiquitin-protein MDM2, which, in turn binds and targets p53 for degradation[180].

Ubiquitin-specific peptidase 10 (USP10) in human is 798 amino acids (aa) in length and is expressed in the nucleus and cytoplasm of almost every cell[181]. USP10 participated in diverse cellular processes, including ubiquitin recycling, the DNA damage response, stress granule formation, and recycling of cellular proteins[181]. It’s reported that USP10 is a novel regulator of p53, providing an alternative mechanism of p53 inhibition in ccRCC with wild-type p53[46]. Yuan *et al.* [46] use proteomics methods to determine that USP10 is the interacting partner of p53. Under unstressed conditions, USP10

can promote p53 deubiquitination in the cytoplasm to counteract the action MDM2 and enable nuclear re-entry[181]. Upon DNA damage, USP10 is phosphorylated by ATM, upregulated and translocates to the nucleus, and subsequently deubiquitinates p53 in the nucleus[46]. Furthermore, USP10 suppresses tumor cell growth in cells with wild-type p53, with USP10 expression downregulated in a high percentage of ccRCC[46]. Taken together, these findings consistently point out that USP10 is a potential tumor suppressor in ccRCC.

1.8 The DNA damage response（DDR）

Various sources of endogenous and exogenous damage assault our genomes constantly, among such damage, DNA double-strand breaks (DSBs) are the most cytotoxic DNA lesions[47, 182-183]. To maintain chromatin stability, cells have therefore evolved a complex system of biochemical pathways called DNA damage response (DDR) [184]. For DSB repair, homologous recombination (HR) and nonhomologous end-joining (NHEJ) are two major pathways[184]. The physiological importance of the DDR in humans is highlighted by the fact that defective DNA damage repair causes chromosomal aberrations or mutations and leads to various diseases, including cancers[185]. Also, various post-translational protein modifications (PTMs) modulate the DDR strictly, including ubiquitination and deubiquitination[47, 186-189]. It's reported that DUBs play important role in multiple DDR pathways by either acting directly at DNA damage sites or regulating the activities of key proteins involved in the DDR[190]. Yang *et al.* [47] summarize the role of some DUBs in DDR, and they consider that it is of great importance to identify novel DUBs and elucidate the underlying mechanisms by which they fine-tune the DDR process.

It's also found that acetylation is indispensable in the DDR by regulating transcription of DDR-related proteins, modulating chromatin structure and equilibrating dynamic acetylation level of these proteins[191-193]. Histone acetylation is strongly controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs) [194]. HDACs play an important role in gene expression by regulating the acetylation state of lysine residues located on the amino-terminal tails of histone proteins, and HDACs also play critical roles in cellular growth, apoptosis, DNA damage response, and tumorigenesis by targeting histone or non-histone proteins[195-196]. Notably, it has been found that HDAC1 and HADC2 regulate the DDR by promoting NHEJ repair through regulation of H3K56 acetylation[197]. So many HDACs are implicated in the DDR, it's important to find the precise mechanisms underlying HDAC regulation during DDR, and the latest study showed that ubiquitin-specific peptidase 38 (USP38) is involved in ccRCC tumorigenesis in a HDAC1 regulation -dependent manner[47].

As a member of the USP family, USP38 is a DUB whose function is not fully elucidated and its gene has been reported in a chromosome locus associated with adult asthma in a GWAS study[198-199]. Moreover, the downregulation of USP38 was reported to be associated with numerous diseases, including BC, CRC and so on[200-202]. Recently, Yang *et al.* [47] reported that USP38 is involved in the DDR by regulating the activity of HDAC1. By screening DUBs that translocate to DNA damage sites, they found that USP38 interacted with HDAC1 and specifically removed the K63-linked ubiquitin chain promoting the deacetylase activity of HDAC1[47]. Thereby, HDAC1 was able to deacetylate H3K56 and the depletion of USP38 would enhance H3K56 acetylation(H3K56Ac), reduce NHEJ efficiency, causing genome instability and sensitizing cancer cells to genotoxic insults[47]. What's more, they also found that USP38 was generally expressed at low levels in certain types of cancers including ccRCC, indicating depletion of USP38 expression contributes to genomic instability and may lead to tumorigenesis[47].

In addition, it's well known that the DDR functions have a dual role in tumors. In cancer cells, the survival of DDR can mediate cancer cells resistant to chemotherapy and radiotherapy, on the contrary, in normal cells, depletion of proper DDR functions contributes to the instability of genome and promotes the development of tumors[47]. Thus, for one thing, USP38 acts as a tumor suppressor, regulating genome stability in normal cells to avoid tumorigenesis, for another, USP38 acts as a tumor promoter, mediating cancer cell resistance to DNA-damaging therapy. In short, as a regulator of DDR, USP38 plays a critical role in modulating genome integrity and cancer cell resistance to genotoxic insults by deubiquitinating HDAC1 and regulating its deacetylation activity, implicating USP38 as a potential target for cancer diagnosis.

1.9 Modulation of VEGF splicing

Accumulating evidence has shown that mutations or activities of RNA splicing-related factors participate in the occurrence and progression of various malignant tumors[203-204]. The abnormal pathological process of angiogenesis due to the imbalance of promoter and inhibitor could promote the formation of tumor neovascularization and high expression of vascular endothelial growth factor (VEGF), especially VEGF-A, has been confirmed as a stimulator of tumor angiogenesis[205-206]. VEGF can produce different isoforms *via* mRNA splicing and VEGF-A_{165b}, as one of the isoforms, is believed to be an anti-angiogenetic factor and downregulated in numerous malignancies, including RCC, PCa, CRC and melanoma[207-211]. Interestingly, it's reported that Serine/Arginine-rich protein specific kinase 1 (SRPK1) could phosphorylate Serine/Arginine-rich splicing factor 1 (SRSF1) to promote VEGF-A splicing to generate VEGF-A_{165} (pro-angiogenesis) and VEGF-A_{165b} (anti-angiogenesis) [212-214]. Thereby, hypothesizing that $VEGF_{165b}$ or other anti-angiogenic splicing isoforms may become a promising therapeutic target or mediator of RCC.

As mentioned above, USP39 is an essential component of the spliceosome and knockdown of *USP39* can inhibit RCC progression through blocking AKT/ERK pathways[40]. However, the role of USP39 on splicing complex regulation in RCC progression still remains unclear. Thus, Pan *et al.* [48] investigate the role of USP39 in RCC cell proliferation, malignant progression and angiogenesis and the potential mechanism of VEGF-A alternative splicing to further explore molecular mechanism underlying the development of RCC and provide new clues for exploring molecular targeted therapies of RCC. They firstly confirmed the value of USP39 in predicting survival, recurrence and metastasis in RCC patients, especially in those with low tumor node metastasis (TNM) stage, and that TNM stage combined with USP39 expression was superior to the single index[48]. In addition, knockdown of *USP39* inhibits tubule formation in vascular endothelial cells and *vice versa*[48]. Furthermore, they further confirmed that USP39 bonded with SRPK1 through fragments $_{101-565}$ to promote the phosphorylation and interaction of SRSF1 by SRPK1[48]. Interestingly, previous study has found that cancer cells with knockdown of SRPK1 could increase the expression of VEGF-A165b and reduce the expression of VEGF-A_{165} and Pan *et al.*[48] confirmed that knockdown or overexpression of USP39 either upregulates or downregulates VEGF-A_{165b} expression, indicating that USP39 may promote malignant proliferation and angiogenesis of RCC by inhibiting VEGF-A_{165b} alternative splicing *via* regulating SRSF1 and SRPK1[48, 212]. However, further research is still needed to elucidate the specific mechanisms that how USP39 drives the changes in VEGF-A_{165b}.

1.10 Other DUBs

1.10.1 BAP1

Above we introduced some DUBs that regulate important signal pathways and pathological processes involved in the ccRCC. As mentioned earlier, ccRCC is characterized by *VHL* gene inactivation[63, 215-216], interestingly, it's also found that BAP1 which is also located on chromosome 3p is frequently mutated in ccRCC[217]. BAP1 is a widely expressed DUB that belongs to the UCH domain-containing proteins[218], and the *BAP1* gene has emerged as a major tumor suppressor mutated with various frequencies in numerous human malignancies, including ccRCC[219]. *BAP1* is mutated in 5%−15% of sporadic ccRCC[49, 220]. Increasing evidence shows that *BAP1* plays essential roles in multiple cellular processes including cell proliferation and differentiation, cell metabolism, as well as cell survival and death[219].

Peña-Llopis *et al.* [49] identifies *BAP1* as a candidate two-hit tumor suppressor gene by exome sequencing. Through reciprocal immunoprecipitation experiments, they confirmed that BAP1 binds host cell factor-1 (HCF-1) in renal cancer cell lines, which serves as a scaffold for several chromatin remodeling complexes[49, 221]. In addition, they further identified that BAP1 binding to HCF-1 was indispensable for the suppression of cell proliferation and *BAP1* reintroduction into two different BAP1-deficient ccRCC cell lines reduced cell growth[49]. Taken together, these results indicate that BAP1 binding to HCF-1 is important for its tumor suppressor role in ccRCC. Notably, Peña-Llopis *et al*.[49] also discovered that mammalian BAP1 similarly deubiquitinates monoubiquitinated histone H2A (H2Aub1) in renal cancer cells. However, the role of H2Aub1 in ccRCC requires further study, comparing with *BAP1* binding HCF-1, which is dispensable for H2Aub1 deubiquitination in the suppression of cell

proliferation.

Interestingly, another study identified that the microspherule protein 1 (MCRS1) as a bona fide substrate of BAP1[50]. MCRS1 was originally identified as the interaction partner of the p120 nucleolar protein[222], also it has been confirmed that MCRS1 plays a crucial role in controlling chromosomal microtubule and K-fiber minus-end stability that is essential for spindle assembly and cell division[223]. Loss of MCRS1 caused centrosome aberration, impairment of chromosomal microtubule assembly, high proportion of multipolar spindle formation and aneuploidy, which may further contribute to genomic instability and malignant transformation[223-225]. Peng *et al.* [50] have demonstrated that BAP1 stabilizes MCRS1 by deubiquitination and BAP1 contributes to chromosome stability partially *via* MCRS1. In ccRCC tissues there was a positive correlation between *BAP1* and MCRS1 expression, meanwhile both *BAP1* loss and MCRS1 down-regulation in ccRCC were associated with adverse clinicopathological features[50]. In addition to MCRS1, Peng *et al.*[50] also revealed three potential BAP1 interactors, including thioredoxin (TRX), β-catenin (CTNNB1), and TFIIS (TCEA3). TRX, a small redox-active multifunctional protein, acts as a potent antioxidant and a redox regulator in signal transduction[226]. β-Catenin, as a well-known transcriptional factor of Wnt signaling pathway, plays critical roles in various cancers, including ccRCC[227]. TFIIS is a transcription elongation factor, which directly binds the transcription motor RNA Polymerase II, and allows it to read through various transcription arrest sites[228]. Collectively, it is possible these proteins are molecular mediators of BAP1 activity, and these results indicate that *BAP1* plays a tumor suppressing effect in ccRCC, but the detailed molecular mechanism and clinical relevance in ccRCC remains to be determined in further study[50].

1.10.2 USP7

Ubiquitin-specific processing protease 7 (USP7), belongs to cysteine protease, also serves as one of the well-known tumor-associated DUBs, and participates in the regulation of stability and functions of cellular proteins, including p53, PTEN and involve in the TGF-β and NF-κB signal pathways[229-232]. In addition, a series of studies have proved that USP7 plays an important role in the development and progression of diseases, including PCa, BC, LC, cervical cancer and so on[233-235]. Recently, one study showed that USP7 also serves as a tumour suppressor in RCC[51].

Yan *et al.* [51] confirmed USP7 as the first DUB that stabilized protein armadillo repeat containing 5 (ARMC5) by inhibiting the degradation of ARMC5. The *ARMC5* gene, as a putative tumor-suppressor gene, is located on chromosome 16p11.2 and belongs to the family of armadillo (ARM) -repeat-containing proteins[236]. Previous researches show that *ARMC5* gene mutation is significantly associated with primary macronodular adrenocortical hyperplasia (PMAH) and meningioma[237-238]. Meanwhile, it has been reported that ARMC5 is participated in the regulation of cell cycle and *in vitro* experiments show missense variants of ARMC5 in human adrenocortical cancer cell line (H295R) leads to loss of apoptosis activity, indicating that these variants affect ARMC5's pro-apoptotic function[239]. Taken together, all the evidence points to ARMC5 defects may contribute to tumorigenesis. Thus, Yan *et al.* [51] explored the expression and significance of ARMC5 in RCC. To begin with, they demonstrated that ARMC5 was decreased at protein levels in the RCC tissues and cell lines, more specifically, they identified that there was an interaction between USP7 and ARMC5 *in vivo* and *in vitro* directly. At the same time, silencing of USP7 enhanced ARMC5 ubiquitination and accelerated cell cycle G1/S, subsequently promoted RCC cell proliferation[51]. In summary, USP7 acts as tumour suppressor in RCC cells and exerts its function *via* stabilizing ARMC5 by directly deubiquitination[51].

1.10.3 USP22

As a member of USP family, ubiquitin-specific protease 22 (USP22) is closely associated with tumor cell cycle regulation, stemness maintenance, invasion and metastasis, chemoresistance, and immune regulation[240]. In multiple malignant tumors, USP22 is highly expressed, including gastric cancer, CRC and PCa[241]. Meanwhile, the expression level of USP22 has a close relationship with metastasis potential, chemotherapeutic resistance and prognosis in patients with cancer, and USP22 also serves as a tumor promoter in RCC[52, 242]

Lin *et al.* [52] confirmed that USP22 promotes proliferation in RCC by stabilizing survivin. Survivin, as a member of the inhibitor of apoptosis protein (IAP) family, serves as a subunit of the chromosomal passenger complex (CPC) to regulate cell division[243].

Functionally, survivin inhibits caspase activation, leading to negative regulation of apoptosis or programmed cell death[244]. Accumulating evidence has shown that survivin expression is closely associated with numerous cancers and its overexpression always suggests a poor prognosis[245-247]. Also, previous studies have demonstrated that overexpression of survivin indicates a poor overall survival and shorter cancer-specific survival in RCC[248-249]. Furthermore, the stability of survivin is regulated by the ubiquitin-proteasome pathway[250]. Thus, Lin *et al.*[52] explored whether USP22 influences the ubiquitination-proteasome pathway-dependent regulation of survivin in the development of RCC. *In vitro* experiments demonstrated that *USP22* knockdown inhibits the proliferation and colony formation abilities of RCC cells and *vice versa*[52]. Besides, the same group revealed that there is a positive correlation between USP22 and survivin expression and both of their protein levels are upregulated in RCC[52]. Meanwhile, through co-immunoprecipitation found that USP22 directly or indirectly binds survivin and stabilizes survivin at the post-transcriptional level *via* deubiquitination, thereby decreasing the cleaved caspase-3 level[52]. Taken together, USP22 decreases the apoptosis in RCC *via* deubiquitinating and stabilizing survivin and USP22 may serve as a novel therapeutic target for patients with RCC.

1.10.4 OTUD1

As mentioned above, OTUD1 may inhibits the PI3K/AKT and NF-κB pathways by up-regulating the expression of PTEN, and acts as a potential tumor suppressor in ccRCC[38]. Notably, it was also found that OTUD1 serves as a crucial role in molecular mechanisms underlying the melatonin-mediated BCL-2-interacting mediator of cell death (Bim) upregulation through post-translational regulation. Melatonin (N-acetyl-5-methoxytryptamine), secreted by the pineal gland, acts as an anticarcinogenic, antioxidant, and anti-inflammatory agent[251-253]. Previous studies have demonstrated that melatonin can increase the expression of Bim, a pro-apoptotic Bcl-2 protein, inducing anticancer effect in renal cancer Caki cells at the transcriptional and post-translational levels[254-255]. Recently, Woo *et al*[53]. have explored the mechanisms underlying melatonin-mediated Bim stabilization, hereby investigate the involvement of DUBs in melatonin-mediated Bim upregulation. In their study, they used various cancer cell lines, including RCC cells, found that melatonin induced Bim upregulation *via* the upregulation of OTUD1 which can induce Bim deubiquitination and *OTUD1* knockdown inhibited melatonin-induced Bim upregulation, thus resulting in attenuation of melatonin-induced apoptosis[53]. Similarly, in patients with RCC, OTUD1 expression levels are positively correlated with Bim levels[53]. In addition, melatonin-induced activation of specificity protein 1 (Sp1) was found to be participated in OTUD1 upregulation at the transcriptional level, and pharmacological inhibition and genetic ablation of *Sp1* (siRNA) interrupted melatonin-induced OTUD1-mediated Bim upregulation[53]. Moreover, in a mouse xenograft model, they identified that melatonin reduced tumor growth and induced upregulation of OTUD1 and Bim[53].

2 Conclusion and perspectives

RCC is the primary malignant neoplasm of the adult kidney, and its incidence varies geographically, with the highest incidence in developed countries. Surgical resection therapy at the early stage of RCC are the most efficient and curative therapeutic approaches, but the early-stage RCC are not easily detectable. With tumor cells metastasis and recurrence are the major cause of death in most patients, targeted therapy is vital for RCC. Recently, growing evidence has demonstrated the vital role of DUBs in RCC progression. Depending on the substrates, some DUBs may suppress RCC while others promote, which may represent novel targets for cancer therapy. To underlie the specific mechanism of DUBs in promoting or suppressing RCC, we may can explore the function and explain the specific mechanism by conditionally knockout of DUBs in animal model.

To date, first-generation DUB inhibitors are now approaching clinical trials. Bortezomib, a broadly acting proteasome inhibitor, has been approved by the US Food and Drug Administration (FDA) for multiple myeloma or mantle cell myeloma treatment, validating the proteasome as a promising target for cancer treatment[256-257]. However, therapeutic strategies targeting specific DUBs, instead of the entire UPS, might be better tolerated[258]. Currently, DUB inhibitors range from pan-DUB inhibitors to specific inhibitors of single DUBs have been

identified. Cyclopentenone prostaglandins, as the first DUB active-site inhibitors, induced accumulation of polyubiquitinated proteins and caused p53-dependent apoptosis in colon cancer cells[259-260]. WP1130, a partially selective DUB inhibitor that inhibits USP5, USP9X, USP14, and UCH37, triggered rapid polyubiquitinated proteins accumulation in aggresomes and induced tumor cell apoptosis[261]. VLX1570, as the most advanced reported DUB inhibitor, was recently in phase I trials (now suspended) for treatment of multiple myeloma and solid tumours and has been considered to target USP14 and UCHL5[262-263]. However, the development of DUB inhibitors is still in the early stages and many questions still remain to be addressed. For example, the substrates and downstream effectors of some DUBs in some pathways are not clear, including RAS/RAF/MEK/ERK, JNK pathways and so on[40-43]. Thus, we need in further study DUBs and their modulation in cells by understanding substrate specificity, which can be determined by biochemistry, yeast two-hybrid interactions, proteomic profiling and genetics[264]. Only by exploring underlying specific mechanisms and substrates, can we promote the development of DUB inhibitors for ultimate clinical use. For example, USP13, as a positive regulator of NF-κB signaling pathway, promotes deubiquitination of ZHX2 and tumorigenesis in RCC[34]. A potent small molecule inhibitor of autophagy, named spautin-1, is confirmed that targets the deubiquitination activity of USP13, the further investigation of the role of Spautin-1 as an USP13 inhibitor is needed, which may provide great therapeutic potential for ccRCC[69].

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去泛素化酶在肾细胞癌中的作用*

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摘要　肾细胞癌（renal cell carcinoma，RCC）是成人肾脏的原发性恶性肿瘤。泛素-蛋白酶体系统（ubiquitin-proteasome system，UPS）对控制蛋白质水平和调节生理病理过程至关重要。去泛素化酶（deubiquitinases，DUBs）是UPS的关键成分，特别是从靶蛋白中去除泛素链，通过严格调节正常生理学中泛素化和去泛素化之间的平衡，对蛋白质稳态和质量控制显示出至关重要的作用。越来越多的研究表明，功能异常的DUBs与RCC的进展和转移有关。根据底物的不同，一些DUB可能会抑制RCC，而另一些则促进。本文综述了RCC相关DUB的最新研究进展，描述了其分类、功能作用，总结了DUB在RCC中的作用和作用机制，并讨论了靶向DUBs用于癌症治疗。

关键词　肾细胞癌，泛素-蛋白酶体系统，去泛素化酶

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