

Review Article

Insights into the Regulatory Roles of E3 Ubiquitin Ligases Associated with VHL-HIF Axis in Clear Cell Renal Cell Carcinoma

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Renal cell carcinoma (RCC) accounts for up to 85% to 90% of all kidney cancers. Clear cell RCC (ccRCC), the major subtype of RCC, is mainly characterized by the inactivation of the tumor suppressor gene VHL. pVHL as an E3 ubiquitin ligase targets the hydroxylated form of HIF- α for proteasomal degradation. The loss of VHL function leads to HIF- α aggregation as the main mechanism of ccRCC. Recently, the regulations of ccRCC through other E3 ubiquitin ligases are emerging. Moreover, most of them are associated with the VHL-HIF axis. In this review, we mainly focus on seven E3 ubiquitin ligases JADE1, SIAH1, CHIP, FBXW7, MDM2, SPOP, and HAF. Based on reported researches of these ligases on ccRCC, they are divided into two groups: JADE1, SIAH1, CHIP, and FBXW7 that negatively regulate the growth of ccRCC; MDM2, SPOP, and HAF that promote ccRCC progression. In addition, we further verify these possible links between these E3 ligases and VHL-HIF axis in ccRCC based on The Cancer Genome Atlas RNA-seq and Clinical data. Understanding the mechanisms by which these ligases regulate ccRCC, especially the interplay between these ligases and VHL-HIF axis may enable the development of novel therapeutic approaches for ccRCC. Overall, the present review reveals the potential mechanism by which seven ligases (JADE1, SIAH1, CHIP, FBXW7, MDM2, SPOP, and HAF) regulate ccRCC progression in detail, especially their relationship to VHL-HIF axis.

1. Introduction

Renal cell carcinoma (RCC) accounts for more than 85% to 90% of all kidney cancers and is considered as the most lethal genitourinary malignancy [1, 2]. RCC is composed of different histologic subtypes with distinct mutational regions and clinical behaviors, and the majority of RCC diagnoses and deaths are due to the clear cell RCC (ccRCC) subtype [3]. ccRCC is characterized by the inactivation of the tumor suppressor gene VHL, which encodes an E3 ubiquitin ligase that cooperates with prolyl hydroxylase (PHD) enzymes to degrade hypoxia inducible factor- α transcription factors (HIF-1 α , HIF-2 α) [4]. The loss of the VHL protein (pVHL) function causes constitutive stabilization of HIF-1 α and HIF-2 α , resulting in the induction of HIF-transcriptional targets [5].

Ubiquitination is a posttranslational modification process that functions in all eukaryotes and is achieved by the consecutive action of three enzymes: ubiquitin (Ub)-activating enzyme (E1), Ub-conjugating enzyme (E2), and Ub ligase (E3). E3 ubiquitin ligases play critical roles in determining the substrate specificity of ubiquitin-proteasome system for various target proteins, which suggests their specific recognition of target substrates [6]. Cullin-RING-type E3 ubiquitin ligases (CRLs), the largest family of E3 ubiquitin ligases, are responsible for ubiquitination of approximately 20% of cellular proteins and are involved in multiple biological processes including cell cycle progression, genome stability, and oncogenesis [7]. They are functional multisubunit complexes including substrate receptors, adaptors, cullin scaffolds, and RING-box proteins. The cullin gene family is evolutionarily conserved and

the mammalian cullin protein family comprises eight members (CUL1 to CUL3, CUL4a, CUL4b, CUL5, CUL7, and the closely related p53-associated Parkin-like cytoplasmic protein (Parc)) that exhibit similar structural architectures and contain cullin homology domains [8]. For instance, CUL2 and CUL5 share an identical adaptor, Elongin C (EloC), known to enhance the rate of RNA polymerase II elongation and utilize either von Hippel–Lindau (VHL) or suppressors of cytokine signaling- (SOCS-) box proteins as distinct substrate receptors [9, 10].

Recently, there is compelling evidence for the existence of other E3 ubiquitin ligases other than pVHL that play crucial roles in ccRCC, such as JADE1, CHIP, HAF, SPOP, and so on. Intriguingly, these E3 ubiquitin ligases are more or less associated with the VHL-HIF axis in ccRCC. The VHL beta domain was sufficient for Jade-1 binding, and both the alpha and beta domains were required for Jade-1 stabilization, which could inhibit the growth of ccRCC [11]; both CHIP [12] and HAF [13] regulated the expression of HIF- α subunits in ccRCC, and even SPOP has been reported as a direct target of HIF- α subunits [14]. These results suggest the potential links between several E3 ligases and VHL-HIF axis. However, the specific correlation between other E3 ligases that regulate ccRCC progression and VHL and/or HIF remains unclear. Therefore, understanding the interaction of other ligases with VHL and/or HIF will contribute to a deeper understanding of the VHL-HIF axis and the development of new ccRCC therapeutic targets.

In this review, we summarized the E3 ubiquitin ligases that are associated with the progression of ccRCC and their regulatory mechanisms and highlighted the relationship between these E3 ubiquitin ligases and the VHL-HIF axis. In addition, we also verified these possible links between these E3 ligases and their downstream molecules based on The Cancer Genome Atlas RNA-seq and Clinical data.

1.1. The VHL-HIF Axis in ccRCC. HIF (hypoxia-inducible factor) is constituted by a heterodimeric complex composed of a hypoxia-inducible alpha subunit (HIF-1 α , HIF-2 α , or HIF-3 α) and a beta subunit (HIF- β), which is constitutively expressed [15, 16]. Under normoxia, specific proline residues on the HIF- α proteins are hydroxylated by the oxygen-dependent dioxygenase HIF- α -specific prolyl-hydroxylases (PHDs) [17]. The hydroxylated form of HIF- α is recognized by the VHL protein (pVHL), which is a substrate recognition part of an ubiquitin complex termed VCB complex containing elongin B/C, cullin-2, and Rbx1 [18, 19]. This complex functions as part of a SCF-like ubiquitin ligase that promotes degradation of target proteins required for growth and vascularization of solid tumors [20, 21]. VHL gene mutation cause inactivation of pVHL; as a result, the HIF- α proteins accumulate and translocate to the nucleus where they dimerize with the continuously expressed aryl hydrocarbon receptor nuclear translocator (ARNT, also known as HIF-1 β) to form an active transcription factor complex. The pseudohypoxic response results in altered transcriptional regulation of numerous HIF target genes, many of which are involved in glycolysis, angiogenesis, and cell life and death pathways and influence

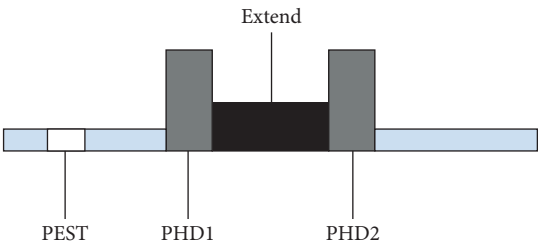
tumor growth, adaptation to the microenvironment, and resistance to chemo- and radiotherapy [16, 22].

It was reported that HIF-1 α acted as a tumor suppressor during further progression of ccRCC by attenuating autonomous VHL-deficient tumor cell proliferation; conversely, HIF-2 α acted as an oncoprotein in ccRCC [23]. HIF-1 α could suppress tumorigenicity of RCC through induction of apoptosis and silencing of HIF-1 α by short hairpin RNA (shRNA) accelerated human renal cancer cell line growth, migration, and invasion [24]. Knockdown for HIF-2 α , but not HIF-1 α , induced cell death related to a reduction in HIF-related gene expression in deprivation-resistant RCC cell [25, 26]. However, the functional relevance of the different HIF-1 α and HIF-2 α in the development and progression of ccRCC remains unclear.

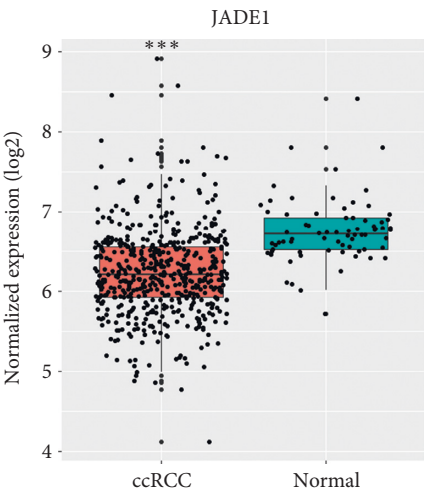
Recently, HIF-2 α antagonists (PT2399 and PT2385) that have been shown to have excellent inhibitory effects against tumors in vivo are currently being developed [27, 28]. Even more, PT2399 has been shown to lead to improved outcomes with regard to progression-free survival in patients with advanced or metastatic ccRCC [28]. Although HIF-2 α antagonists have shown promising potency, ccRCC can still obtain resistance through HIF mutations. Therefore, it is significant to make the upstream regulatory mechanisms of HIF more clearly, and understanding how other E3 ligases other than pVHL regulate HIF may contribute to the development of novel therapeutic approaches for ccRCC.

1.2. JADE1 and SIAH1 Regulate β -Catenin Ubiquitin-Dependent Degradation. JADE family PHD zinc finger 1 (gene for apoptosis and differentiation in epithelia 1; JADE1) was the most studied member of the JADE family protein [29]. It contains a NH₂-terminal candidate PEST degradation domain and two plant homeodomains (PHD) motifs (Figure 1(a)). The PHD was a well-recognized protein-protein interaction motif and may have E3 ubiquitin ligase activity [30]. JADE1 was short lived and mostly highly expressed in proximal tubule cells, which are clear-cell renal cancer precursors, and increased with differentiation [31, 32]. JADE1 could inhibit RCC growth, colony formation, and tumor formation through increasing apoptosis and decreasing the expression of antiapoptotic Bcl-2 [33]. VHL reintroduction into renal cancer cells increased endogenous JADE1 protein abundance up to 10-fold and half-life up to 3-fold [31], indicating JADE1 stabilization by VHL. Moreover, the PHD-extended PHD module may be the major contributor to pVHL binding and VHL-mediated stabilization [32]. Therefore, the VHL stabilization pathway of JADE1 may contribute to the inhibition of ccRCC by JADE1.

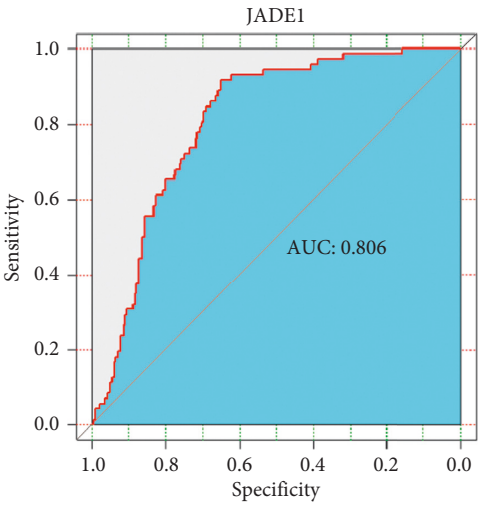
Wnt/ β -catenin signaling is now known to be crucial for numerous developmental processes and is required for regeneration in response to injury. It was activated in various kinds of tumors and played a pivotal role in regulating cancer cell proliferation, invasion, and survival [34]. β -catenin, a transcriptional coactivator, is required for canonical Wnt signal transduction. Study showed that endogenous β -catenin protein was reduced by 50–60% after JADE1 overexpression, and JADE1 silencing by shRNAs led



JADE1
(a)



(b)



(c)

FIGURE 1: Continued.

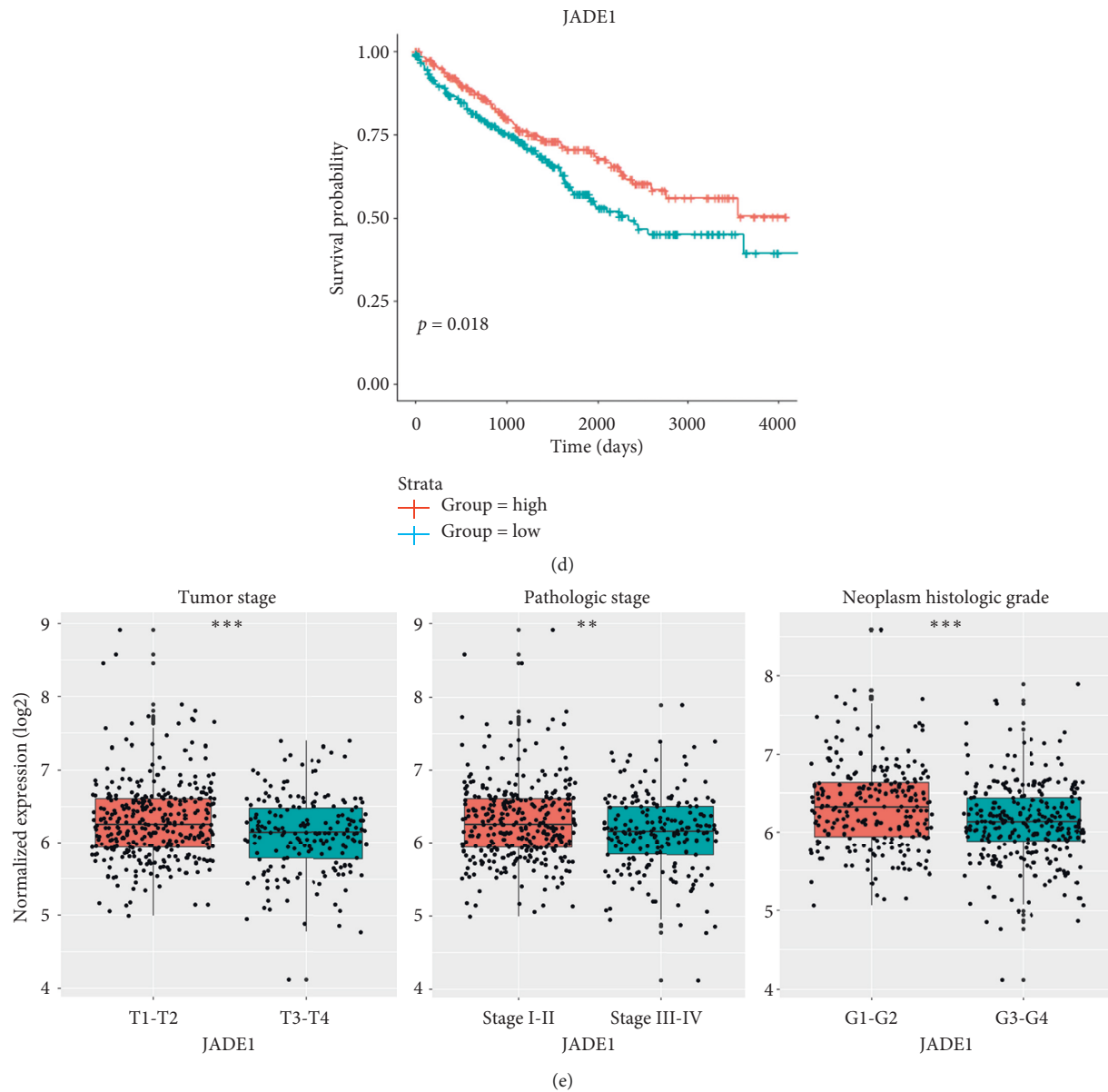


FIGURE 1: The expression of JADE1 mRNA closely related to ccRCC. (a) Structure of JADE1 protein. (b) The expression level of JADE1 mRNA in ccRCC tissues and in normal renal tissues. (c) ROC curve of JADE1 mRNA in ccRCC. (d) K-M curves of JADE1 mRNA in ccRCC. (e) Statistically significant differences of JADE1 mRNA were noted in clinicopathological features: tumor stage (T1/T2 vs. T3/T4), pathologic stage (Stage I-II vs. Stage III-IV), and neoplasm histologic grade (G1/G2 vs. G3/G4). ** $p < 0.01$, *** $p < 0.001$.

to a 100–150% increase in endogenous β -catenin protein levels [11]. Low expression of β TrCP, the canonical β -catenin E3 ubiquitin ligase, did not interfere with JADE1-mediated β -catenin destabilization, indicating that JADE1 acted in downstream or parallel of β TrCP [11]. Moreover, JADE1 could be ubiquitinated by both phosphorylated and non-phosphorylated β -catenin, and β TrCP ubiquitinated only phosphorylated β -catenin [35], which highlights the importance of JADE1 in ccRCC. Taken together, these results suggested that JADE1 as an E3 ubiquitin ligase regulated β -catenin ubiquitin-proteasome-dependent degradation in response to canonical Wnt signaling.

In addition, VHL/JADE1 exhibited significantly decreased expression in ccRCC tissues and was closely related to the tumor size and tumor grade, which further confirmed their tumor suppressor roles in ccRCC [36]. Based on cancer genomic map RNA-seq and clinical data analyses, we also found that the mRNA level of JADE1 in ccRCC was remarkably lower than normal renal tissues (Figure 1(b)) and JADE1 showed diagnostic value to distinguish ccRCC from normal renal tissues with AUC 0.806 (Figure 1(c)). Survival analysis showed that JADE1 was significantly related to the survival time of ccRCC (Figure 1(d)). Furthermore, JADE1 was closely related to some clinical parameters of ccRCC

(Figure 1(e)). All these results demonstrated the inhibitory effect of JADE1 on ccRCC.

SIAH1, the seven-in-absentia homologue (SIAH) RING-type E3 ubiquitin ligase, also has a link with β -catenin besides JADE1 in RCC. The expression of calcyclin-binding protein/SIAH1-interacting protein (CacyBP/SIP) was low or absent in human RCC [37, 38], and upregulation of CacyBP/SIP could inhibit the proliferation and delay cell cycle progression of RCC cell line through destructing β -catenin that always exists in various tumors [38]. In addition, SIAH1 has been reported to regulate the expression of PHD1 and PHD3 proteins in breast cancer cell line and human glioma cells [39, 40]. However, there has been no report on the regulation of PHDs by SIAH1 in ccRCC.

1.3. CHIP Regulates HIF-1 α Ubiquitin-Dependent Degradation.

Heat shock proteins (HSPs) are a group of molecules induced by a variety of environmental and pathologic stresses, including cancer. HSPs function as molecular chaperones to facilitate protein folding, suppress protein aggregation, and adapt to proteotoxic stress within cells. The E3 ubiquitin ligase CHIP (carboxyl terminus of Hsc70-interacting protein), also known as STUB1 (STIP1 homology and U-box containing protein 1), regulated the proteasomal degradation of HIF-1 α in an Hsp70-dependent manner. Disruption of Hsp70-CHIP interaction blocked HIF-1 α degradation, inhibition of Hsp70 or CHIP synthesis by RNA interference, increased protein levels of HIF-1 α , and attenuated the decay of HIF-1 α [12]. However, the expression of HIF-2 α was not affected during prolonged hypoxia. Moreover, decreased expression of CHIP could lead to increased angiogenesis via VEGF-VEGFR2 pathway and poor prognosis in human RCC [41]. Taken together, this regulation is mediated by the interaction of Hsp70 with HIF-1 α and the subsequent recruitment of CHIP (Figure 2(a)). Based on cancer genomic map RNA-seq data analyses, we also found that the mRNA level of STUB1 (the encoding gene of CHIP) was significantly negatively correlated with HIF1A (the encoding gene of HIF-1 α) (Figure 2(b)). In addition, CHIP-mediated degradation of TG2 (transglutaminase 2) negatively regulated tumor growth and angiogenesis in renal cancer [42]. The increased TG2 expression levels during initial tumorigenesis could predict the increased risk of metastasis and the decreased disease-free and cancer-specific survivals in RCC [43] and TG2 inhibitor GK921 abrogated RCC growth in xenograft tumor models [44]. Interestingly, TG2 was a negative regulator of VHL tumor-suppressor protein [45]. It seems that TG2 mediated regulation of VHL-HIF-1 α -VEGF pathway by CHIP in ccRCC. Understanding the roles of TG2 in ccRCC may contribute to exploring a new regulatory mechanism of VHL-HIF-1 α -VEGF pathway.

1.4. Does FBXW7 Lead to HIF-1 α Ubiquitin-Dependent Degradation? F-box/WD repeat-containing protein 7 (FBXW7) is a member of the F-box protein family, which functions as the substrate recognition component of the SCF E3 ubiquitin ligase [46]. The expression of FBXW7 in RCC tissues was much lower than that in paracancerous normal

tissues and closely related to RCC clinical pathologic grade and tumor node metastasis phase [47]. FBXW7 overexpression suppressed RCC cell proliferation, renal cancer metastasis, EMT (epithelial-mesenchymal transition), and induced apoptosis [47], whereas downregulation of FBXW7 facilitated tumor cell migration and invasion via the EMT [48]. In addition, it was reported that FBXW7 was recruited to HIF-1 α through HIF-1 α phosphorylation by glycogen synthase kinase-3 (GSK-3), leading to HIF-1 α proteasomal degradation in ovarian cancer and colorectal cancers cell [49, 50]. We hypothesized that there is a link between FBXW7 and HIF-1 α in ccRCC. Based on cancer genomic map RNA-seq and clinical data analyses, we found that the level of FBXW7 in ccRCC was remarkably higher than in normal renal tissues (Figure 2(c)), and it showed diagnostic value to distinguish ccRCC from normal renal tissues with AUC 0.877 (Figure 2(d)). Survival analysis showed that FBXW7 was significantly related to the survival time of ccRCC (Figure 2(e)), and the mRNA level of FBXW7 was significantly positively correlated with HIF1A (the encoding gene of HIF-1 α) (Figure 2(f)). Although our analysis results were inconsistent with previous studies that evaluated the expression FBXW7 at protein level, these results were still effective to show that FBXW7 plays a significant role in ccRCC. More researches are needed to reveal the specific mechanism by which FBXW7 is associated with HIF-1 α in ccRCC in near future.

1.5. MDM2-Mediated p53 Ubiquitin-Dependent Degradation.

p53 is a well-known tumor suppressor gene, whose activation by hypoxia or DNA damage leads to cell cycle arrest, DNA repair, and apoptosis. It plays a pivotal role in apoptosis of cancer cells, and its inactivation and mutations is a major contributing factor in tumorigenesis. Moreover, p53-regulated apoptotic signaling was one of the factors that resulted in the high resistance to radiation and chemotherapy of VHL-deficient RCC patients [51]. It was reported that the β domain of pVHL was a substrate recognition domain that has been emphasized for tumor formation [52] and had an important function in tumorigenesis [53]. However, pVHL with β -domain mutations could disrupt pVHL/HIF- α interactions without inhibiting tumor formation [53]. In addition, study showed that p53 could bind to the α domain of pVHL and competes with elongin C [54], indicating that α domain is critical for the tumorigenesis of VHL-mutated RCC cells. As a result, p53 binding to the α domain raises the possibility that p53 could be dysregulated in VHL-deficient RCC cells. Moreover, in vitro experiments, compared with VHL-defective (VHL⁻) cells, pVHL-expressing (VHL⁺) RCC cells exhibited elevated levels of polysome-associated p53 mRNA and increased p53 protein levels [55]. Taken together, these evidences indicated a potential tumor suppressive function of pVHL, which may be deeply associated with the regulation of p53 signaling pathway in ccRCC. However, p53 mutations are rarely detected in ccRCC, and DNA damage response pathway seemed to be inhibited by an unknown mechanism in RCC.

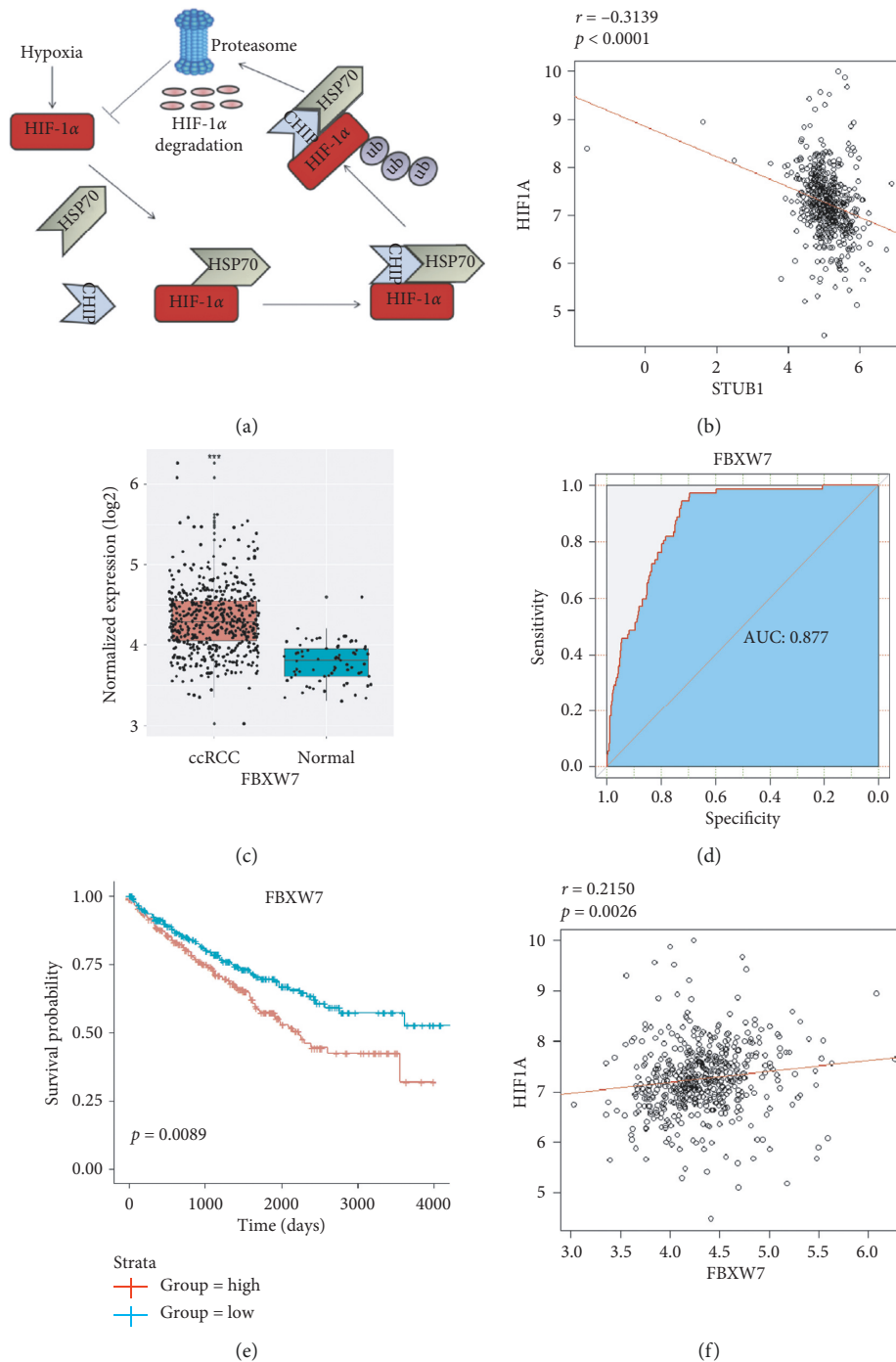


FIGURE 2: CHIP and FBXW7 regulated the expression of HIF-1α in ccRCC. (a) The mechanism of CHIP regulates the proteasomal degradation of HIF-1α. (b) The expression of STUB1 mRNA (the coding gene of CHIP) negatively correlated with HIF1A mRNA (the coding gene of HIF-1α). (c) The expression level of FBXW7 mRNA in ccRCC tissues and normal renal tissues. (d) ROC curves of FBXW7 mRNA in ccRCC. (e) K-M curves of FBXW7 mRNA in ccRCC. (f) The expression of FBXW7 mRNA positively correlated with HIF1A mRNA. *** $p < 0.001$.

The human mouse double minute 2 (MDM2; also called HDM2) gene/protein was associated with accelerated cancer progression, which has been shown to be overexpressed in RCC [56–58]. Based on cancer genomic map RNA-seq data analysis, we found that the level of MDM2 in ccRCC was remarkably higher than in normal renal tissues (Figure 3(a)),

and it showed diagnostic value to distinguish ccRCC from normal renal tissues with AUC 0.906 (Figure 3(b)). Previous studies have indicated that MDM2 mediated the ubiquitination and proteasomal degradation of the HIF-1α via directly binding to HIF-1α under hypoxic conditions [59, 60]. MDM2 could directly bind to and block the

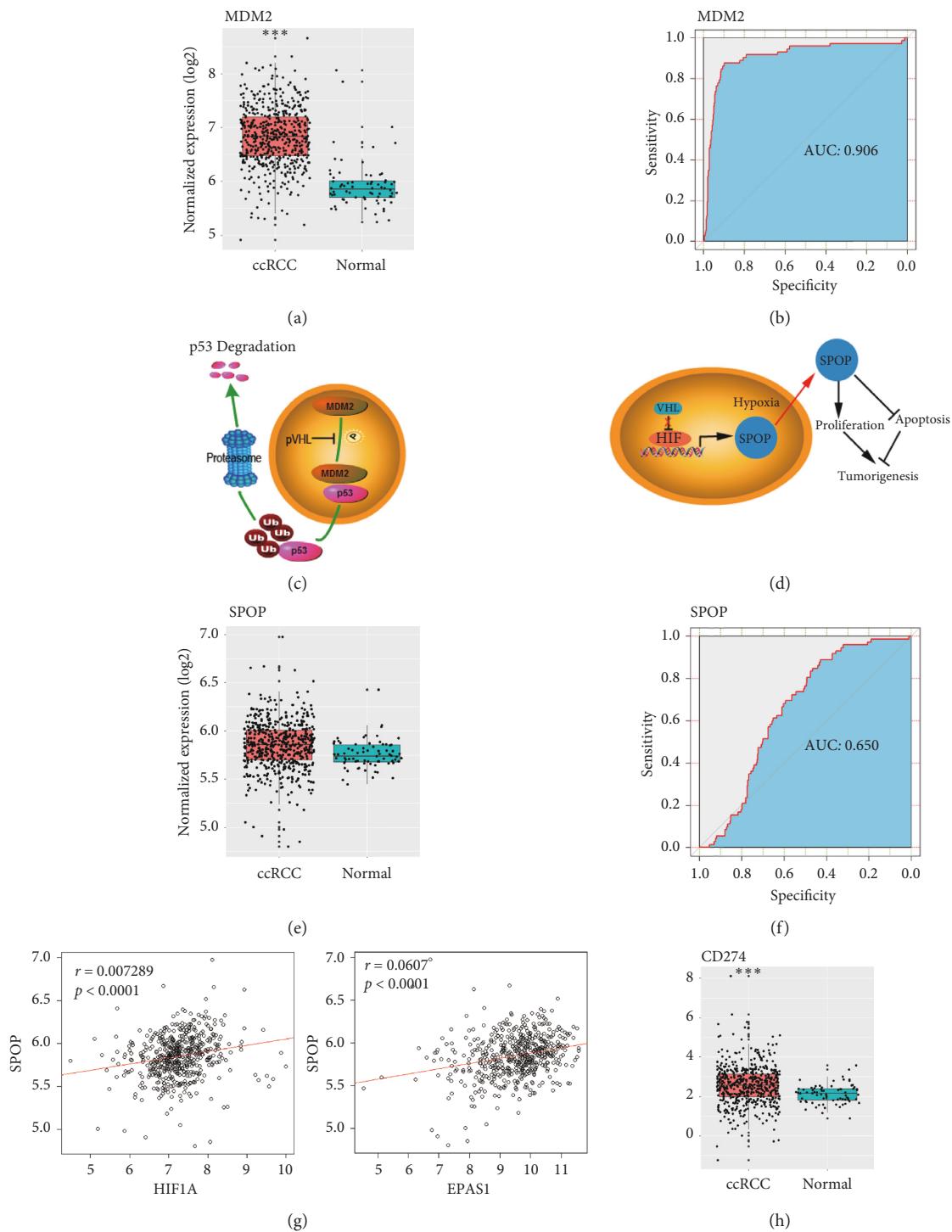


FIGURE 3: Continued.

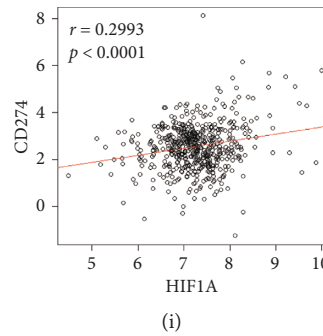


FIGURE 3: Mechanisms of MDM2 and SPOP promoting tumorigenesis. (a) The expression level of MDM2 mRNA in ccRCC tissues and in normal renal tissues. (b) ROC curve of MDM2 mRNA in ccRCC. (c) The mechanism of MDM2-mediated degradation of p53. (d) The mechanism of SPOP promoting tumorigenesis. (e) The expression level of SPOP mRNA in ccRCC tissues and in normal renal tissues. (f) ROC curve of SPOP mRNA in ccRCC. (g) The expression of HIF1A mRNA and EPAS1 mRNA (the coding gene of HIF-2 α) positively correlated with SPOP mRNA. (h) The expression level of CD274 mRNA (the coding gene of PD-L1) in ccRCC tissues and in normal renal tissues. (i) The expression of HIF1A mRNA positively correlated with CD274 mRNA. *** $p < 0.001$.

N-terminal transcriptional activation domain of p53, promote export of p53 from the nucleus to the cytoplasm, and then induce degradation of p53 via ubiquitination through its E3 ligase activity (Figure 3(c)). MDM2 inhibitor MI-319 induced RCC cell apoptosis mainly dependent on p53 overexpression [56]. In addition, the disruption of the MDM2-p53 protein-protein interaction with small molecules has been viewed as an attractive strategy for accelerating p53 levels in cancers [61].

pVHL could indirectly block MDM2-mediated degradation of p53 through mediating ATM-dependent Ser-15 phosphorylation of p53 [54]. In addition, several proteins that were high or low expressed in VHL-deficient RCC cells regulated VHL-mediated p53 translation. For instance, RNA-binding protein HuR could enhance VHL-mediated p53 translation, which was abundant in the cytoplasmic and polysome-associated fractions of VHL⁺ cells and was capable of binding to the 3' untranslated region of the p53 mRNA in a VHL-dependent fashion [55]. Progerin (an altered splicing product of the LMNA gene linked to Hutchinson–Gilford progeria syndrome; HGPS) was responsible for nuclear irregularities, and p53 inactivation was suppressed by pVHL [62].

HIF-2 α could induce Akt-mediated phosphorylation of MDM2 (Ser166), which activated and promoted the nuclear localization of MDM2 resulting in the downregulation of p53 [63]. In addition, HIF-2 α inhibited p53-mediated response by damaging cellular redox homeostasis, thereby allowing reactive oxygen species accumulation and DNA damage [64]. Conversely, inhibiting HIF-2 α expression could enhance p53 activity, increase apoptosis, and reduce colon formation survival of irradiated and nonirradiated cells [64], which further proves the oncoprotein role of HIF-2 α in RCC.

1.6. SPOP as a Target of HIF-1 α . SPOP is the substrate-binding member of the E3 ubiquitin-protein ligase complex that mediates ubiquitination and proteasomal degradation of target proteins. It contains a BTB domain, which serves as

an adaptor for cullin-based E3 ubiquitin ligase, and a MATH domain that is responsible for substrate recognition and CUL3-mediated protein degradation. SPOP mutants reduce wild-type SPOP binding to substrates, resulting in dominant-negative effects on substrate binding, ubiquitination, and degradation. Recently, it has been reported that the miR-520/372/373 family targets the SPOP3'-UTR and suppresses SPOP protein expression, leading to decreased proliferation, migration, and metastasis of RCC cells in vitro and in vivo [65], suggesting that SPOP may be a novel therapy target of RCC.

Compared with the normal kidney tissue, SPOP was upregulated in more than 90% RCC tissues, especially in ccRCC, and located at cytoplasm of ccRCC cells [65, 66]. SPOP was positive in tumors with local invasion or metastasis, and it was associated with poor prognosis of ccRCC patients [66, 67]. SPOP could cause the ubiquitination and degradation of multiple regulators of cellular proliferation and apoptosis, including the tumor suppressor PTEN, ERK phosphatases, the proapoptotic molecule Daxx, and the Hedgehog pathway transcription factor Gli2 [14]. SPOP gene silencing induced cell apoptosis, decreased cell viability, decreased colony formation and migration ability, and elevated drug sensitivity in the RCC cells, which was associated with the downregulation of VEGFR, MMP-9, and VCAM and upregulation of E-cadherin [68]. In addition, study showed that SPOP was a direct target of HIF-1 α in ccRCC, and both HIF-1 α and HIF-2 α regulated the expression of SPOP [14]. Knocking down of HIF-2 α by siRNA in a ccRCC cell that predominantly expresses HIF-2 α resulted in a decrease in the mRNA and protein abundance of SPOP, as well as the known HIF target VEGF [14]. The study also showed that SPOP drove EMT (epithelial-mesenchymal transition) to promote RCC metastasis and upregulated ZEB1 through activating β -catenin/TCF4 signaling to promote RCC invasion [67]. In conclusion, hypoxia resulted in cytoplasmic accumulation of SPOP, which was first identified as a nuclear protein, then induced tumorigenesis through accelerating proliferation and reducing apoptosis (Figure 3(d)). Based on cancer genomic map

RNA-seq data analysis, we also found that the mRNA level of SPOP in ccRCC was remarkably higher than in normal renal tissues (Figure 3(e)), and it showed diagnostic value to distinguish ccRCC from normal renal tissues with AUC 0.650 (Figure 3(f)). Moreover, the expression levels of HIF1A (the encoding gene of HIF-1 α) and EPAS1 (the encoding gene of HIF-2 α) were significantly positively correlated with SPOP (Figure 3(g)).

Recently, study has shown that loss-of-function mutations in SPOP compromised ubiquitination-mediated PD-L1 degradation, leading to increased PD-L1 levels and reduced numbers of tumor-infiltrating lymphocytes in mouse tumors and in primary human prostate cancer specimens, suggesting a crucial role of SPOP in the regulations of PD-L1 protein stability [69]. Although no research shows that SPOP regulates the expression of PD-L1 in RCC, several recent reports, which indicate that PD-L1 may be a target of HIF- α in RCC, have caught our attention.

PD-1 is mainly expressed in activated T cells, B cells, and NK cells, and it is a major immune checkpoint receptor, which mediates the inhibitory interaction between immune cells and target cells. There are two known ligands of PD-1, PD-L1 (also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273). PD-L1 is a 290 amino acid type I transmembrane protein encoded by the CD274 gene on chromosome 9 in human, and it is expressed on antigen-presenting cells and tumor cells and is primarily responsible for the immunosuppressive effects of PD-1. PD-L2 also functions to inhibit T-cell activation. However, PD-L1 is expressed in several cells, including resting T cells, B cells, macrophages, dendritic cells (DCs), and vascular endothelial cells, whereas PD-L2 is only expressed in macrophages and DCs.

Consistent with our data analysis result (Figure 3(h)), many studies have shown that PD-L1 expression is upregulated in ccRCC compared with paired normal tissues [70, 71] and suggest that RCC patients with intratumoral high PD-L1 expression exhibited aggressive tumors and had increased risk of death [71–75]. Moreover, it was reported that PD-L1 tumor cell expression was strongly associated with increased HIF-2 α expression [76]. In vitro experiments showed that ccRCC cell harboring mutations in VHL, which led to impaired function of pVHL to degrade HIF-2 α , expressed higher levels of PD-L1 than those with mutations that did not affect pVHL's function [77]. Furthermore, cell culture experiment reported that the VHL mutation selectively induced HIF-2 α stabilization; meanwhile, knockdown of HIF-2 α , but not HIF-1 α , led to decreased PD-L1 mRNA and protein levels, suggesting that PD-L1 may be a HIF-2 α target [77, 78]. In addition, we also found that the mRNA level of HIF1A (the encoding gene of HIF-1 α) not EPAS1 (the encoding gene of HIF-2 α) was significantly positively correlated with CD274 (the encoding gene of PD-L1) based on cancer genomic map RNA-seq data analysis (Figure 3(i)), which further proved that PD-L1 was not a target of HIF-1 α . More researches are needed to verify the relationship between HIF-2 α and PD-L1 in near future.

With the US FDA's approval of now five PD-1/PD-L1 inhibitors (atezolizumab, pembrolizumab, nivolumab,

avelumab, and durvalumab) in multiple cancers [79], PD-1/PD-L1 inhibitors are increasingly utilized in treating mRCC (metastatic RCC) patients and have a favorable tolerability profile [80–82]. Although we are unable to determine the specific mechanisms that promote PD-L1 expression in ccRCC, we are convinced that some reactions exist between HIF-2 α and PD-L1. It is significant to understand how HIF-2 α controls PD-L1 protein expression and stability, thus offering a molecular basis to improve the clinical response rate and efficacy of PD-1/PD-L1 blockade in ccRCC patients.

1.7. HAF Mediates the Switching of HIF-1 α to HIF-2 α .

HAF (hypoxia-associated factor), also known as SART1₈₀₀ (squamous cell carcinoma antigen recognized by T cells), has been shown to be overexpressed in a variety of cancer types [83–85]. HAF as an E3 ligase targeted HIF-1 α but not HIF-2 α for degradation irrespective of cellular oxygen tension [13]. It was reported that mutant pVHL protected HIF-1 α from HAF-dependent oxygen-independent degradation via competing for binding site of HIF-1 α [86]. Interestingly, under hypoxia, HAF bound and promoted the transcription of a subset of HIF-2 α target genes, which was dependent upon HAF SUMOylation, whereas HAF-mediated HIF-1 α degradation was SUMOylation independent [83]. It was also demonstrated that HAF induced ubiquitination and proteasome degradation of HIF-1 α protein, which then bound to HIF-2 α and turned on its downstream targets during long-term hypoxia [84]. HAF-mediated switch to HIF-2 α -dependent gene expression promoted the enrichment of the cancer stem cell population [85], resulting in more aggressive tumors in vivo and induced the resistance to antiangiogenesis TKI therapy (antiangiogenic tyrosine kinase inhibitor) in RCC [87]. Based on cancer genomic map RNA-seq and clinical data analyses, we found that the mRNA level of SART1 (the encoding gene of HAF) in ccRCC was remarkably higher than in normal renal tissues (Figure 4(a)), and it showed diagnostic value to distinguish ccRCC from normal renal tissues with AUC 0.690 (Figure 4(b)). Survival analysis showed that SART1 was significantly related to the survival time of ccRCC (Figure 4(c)). Moreover, SART1 was closely related to some clinical parameters of ccRCC (Figure 4(d)). Furthermore, the level of SART1 was significantly negatively correlated with HIF1A and EPAS1 (Figure 4(e)). Although the specific mechanisms of the switching of HIF-1 α to HIF-2 α driven by HAF remain unclear, all these results imply a close correlation between HAF and HIF, which deserves further research.

2. Conclusion and Prospective

This review mainly elucidates the roles of JADE1, SIAH1, CHIP, FBXW7, MDM2, SPOP, and HAF E3 ubiquitin ligases in human ccRCC and the interplay of these ligases with VHL-HIF- α axis. According to these reported studies, JADE1, SIAH1, CHIP, and FBXW7 negatively regulate the growth of ccRCC, whereas MDM2, SPOP, and HAF

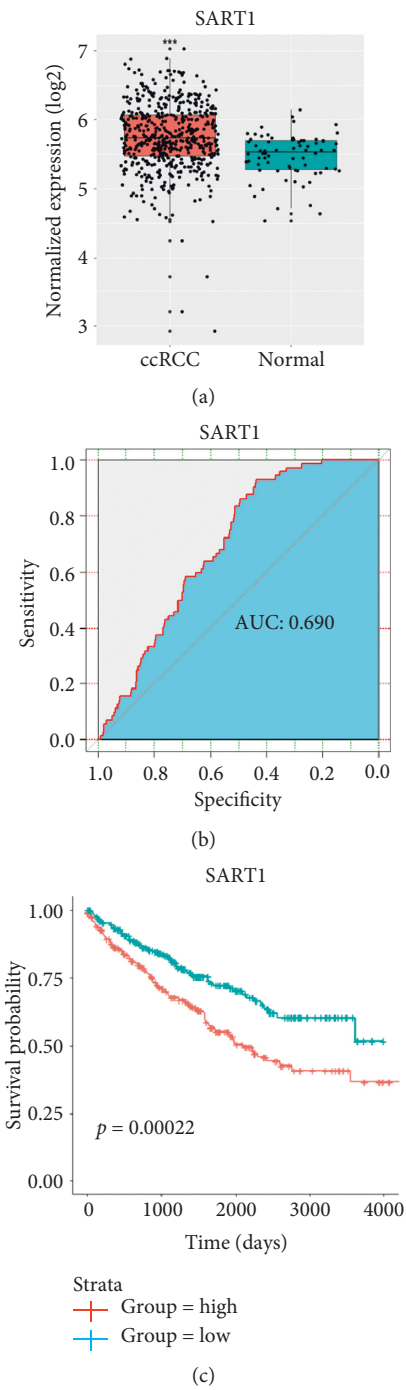


FIGURE 4: Continued.

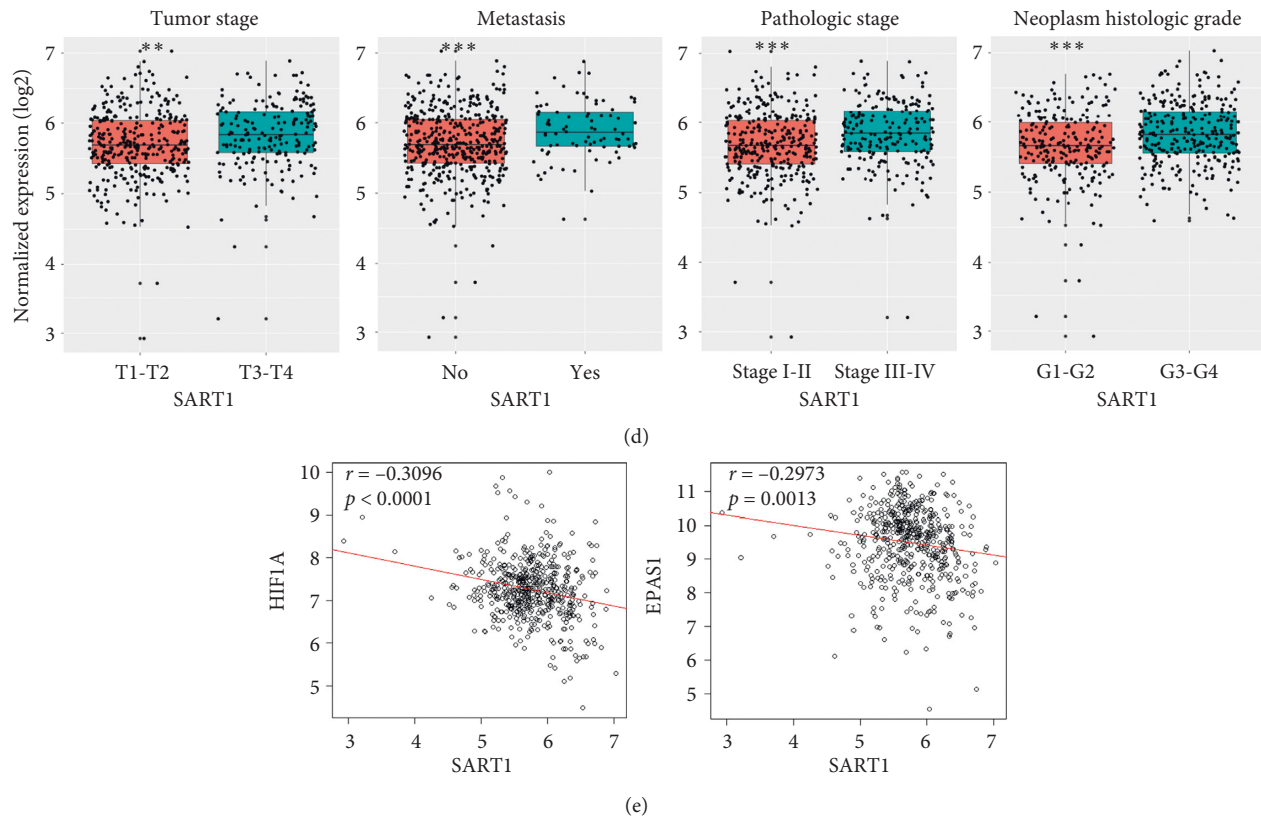


FIGURE 4: The expression of SART1 mRNA closely related to HIF1A and EPAS1 mRNA in ccRCC. (a) The expression level of SART1 mRNA (the coding gene of HAF) in ccRCC tissues and in normal renal tissues. (b) ROC curve of SART1 mRNA in ccRCC. (c) K-M curves of SART1 in ccRCC. (d) Statistically significant differences of SART1 were noted in clinicopathological features: tumor stage (T1/T2 vs. T3/T4), metastasis (no vs. yes), pathologic stage (Stage I-II vs. Stage III-IV), and neoplasm histologic grade (G1/G2 vs. G3/G4). (e) The expression of SART1 mRNA negatively correlated with HIF1A and EPAS1 mRNA. ** $p < 0.01$, *** $p < 0.001$.

promote ccRCC progression. All of these E3 ligases are associated with the VHL-HIF axis in ccRCC, and we summarized the mechanism diagram in Figure 5. pVHL directly stabilizes JADE1 protein to regulate the Wnt/ β -catenin signaling and indirectly blocks MDM2-mediated degradation of p53. CHIP and HAF induce ubiquitination and proteasome degradation of HIF-1 α protein. CHIP regulates the proteasomal degradation of HIF-1 α in a Hsp70-dependent manner. During long-term hypoxia, HAF induces ubiquitination and proteasome degradation of HIF-1 α protein, which then binds to HIF-2 α and turns on its downstream target. HIF-2 α induces Akt-mediated phosphorylation of MDM2, resulting in the downregulation of p53, and both HIF-1 α and HIF-2 α can regulate SPOP expression. However, we were unable to ensure the relationship between FBXW7 and HIF-1 α , and whether SPOP regulates the expression of PD-L1 in ccRCC.

In addition, we further confirmed that these ligases are of great significance in ccRCC and associated with the VHL-HIF axis based on cancer genomic map RNA-seq and clinical data analysis. Except CHIP, other E3 ligases showed significant diagnostic value to distinguish ccRCC from normal renal tissues. Survival analyses showed that JADE1, FBXW7, and SART1 were significantly related to the survival time of ccRCC. JADE1, SIAH1, and SART1 were also closely

related to some clinical parameters of ccRCC, respectively. Moreover, the mRNA levels of STUB1, FBXW7, SPOP, and SART1 were significantly negatively or positively correlated with HIF-1 α or/and HIF-2 α . Taken together, all these data analyses demonstrated that these ligases play vital roles in the development and progression of ccRCC.

Previous studies showed that FBXW7 overexpression suppressed RCC cell proliferation and induced apoptosis, and FBXW7 mediated the degradation of HIF-1 α based on the expression levels of protein, whereas our data analyses based on the expression levels of mRNA showed the opposite results. Therefore, more research studies are needed to explore the specific roles of FBXW7 in ccRCC in near future. Previous studies also indicated that both HIF-1 α and HIF-2 α were significantly correlated with SPOP, and PD-L1 acted as a target of HIF-2 α . However, it is still unknown whether SPOP affects the expression of PD-L1 in ccRCC. Recently, it was reported that SPOP as the physiologic E3 ubiquitin ligase regulated PD-L1 in prostate cancer, which first revealed the possible links between SPOP and PD-L1. With PD-L1 as a therapeutic target for RCC, investigating the relationship between SPOP and PD-L1 will contribute to developing a new therapeutic approach. In addition, although some studies indicated that HAF mediated the switching of HIF-1 α to HIF-2 α , the specific switching

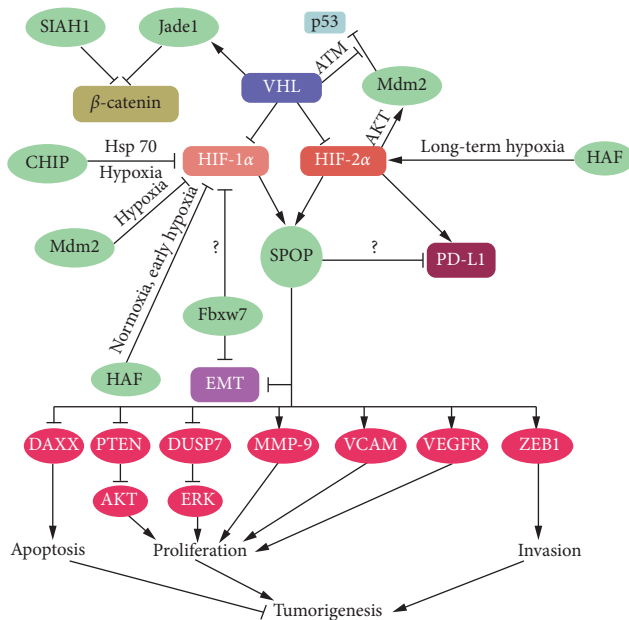


FIGURE 5: Mechanisms of how these E3 ligases are associated with VHL-HIF axis in ccRCC. pVHL directly stabilizes JADE1 protein to regulate the Wnt/ β -catenin signaling and indirectly blocks MDM2-mediated degradation of p53. CHIP and HAF induce ubiquitination and proteasome degradation of HIF-1 α protein. CHIP regulates the proteasomal degradation of HIF-1 α in a Hsp70-dependent manner. During long-term hypoxia, HAF induces ubiquitination and proteasome degradation of HIF-1 α protein, which then binds to HIF-2 α and turns on its downstream target. HIF-2 α induces Akt-mediated phosphorylation of MDM2, resulting in the downregulation of p53, and both HIF-1 α and HIF-2 α can regulate SPOP expression. However, we were unable to ensure the relationship between FBXW7 and HIF-1 α , and whether SPOP regulates the expression of PD-L1 in ccRCC.

mechanisms remain unclear. Moreover, data analyses based on the expression levels of mRNA indicate that HAF was strongly associated with the grade, stage, and metastasis of ccRCCs and survival time of ccRCC patients. Hence, it will make sense to understand how HIF-1 α switches to HIF-2 α during long-term hypoxia.

Although VHL protein inactivation is thought to be the main mechanism promoting the development of ccRCC, the roles of other E3 ligases in ccRCC should not be ignored. This review reveals that at least seven other E3 ubiquitin ligases (JADE1, SIAH1, CHIP, FBXW7, MDM2, SPOP, and HAF) have played crucial roles in ccRCC progression so far and are closely associated with VHL and/or HIF. More researches are needed in the near future to confirm the interaction of these E3 ligases with VHL and/or HIF and explore their specific downstream molecules. Moreover, it is valuable to find novel E3 ligases that contribute to the development and progression of ccRCC. In summary, the extent of the E3 ubiquitin ligase-dependent regulations of ccRCC are only beginning to be unraveled, and understanding the links of other E3 ligases with VHL and/or HIF will lead to a deeper understanding of the VHL-HIF axis and contribute to the development of new ccRCC therapeutic targets.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Wuping Yang and Zhi Li contributed equally to this article.

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References

- [1] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2018," *CA: A Cancer Journal for Clinicians*, vol. 68, no. 1, pp. 7–30, 2018.
- [2] T. K. Choueiri and R. J. Motzer, "Systemic therapy for metastatic renal-cell carcinoma," *New England Journal of Medicine*, vol. 376, no. 4, pp. 354–366, 2017.
- [3] K. Inamura, "Renal cell tumors: understanding their molecular pathological epidemiology and the 2016 WHO classification," *International Journal of Molecular Sciences*, vol. 18, no. 10, 2017.
- [4] J. Zhang, T. Wu, J. Simon et al., "VHL substrate transcription factor ZHX2 as an oncogenic driver in clear cell renal cell carcinoma," *Science*, vol. 361, no. 6399, pp. 290–295, 2018.
- [5] I. J. Frew and H. Moch, "A clearer view of the molecular complexity of clear cell renal cell carcinoma," *Annual Review of Pathology: Mechanisms of Disease*, vol. 10, no. 1, pp. 263–289, 2015.
- [6] A. Sadanandom, M. Bailey, R. Ewan, J. Lee, and S. Nelis, "The ubiquitin-proteasome system: central modifier of plant signalling," *New Phytologist*, vol. 196, no. 1, pp. 13–28, 2012.
- [7] S. M. Jang, C. E. Redon, and M. I. Aladjem, "Chromatin-bound cullin-ring ligases: regulatory roles in DNA replication and potential targeting for cancer therapy," *Frontiers in Molecular Biosciences*, vol. 5, p. 19, 2018.
- [8] A. Sarikas, T. Hartmann, and Z.-Q. Pan, "The cullin protein family," *Genome Biology*, vol. 12, no. 4, p. 220, 2011.
- [9] T. A. F. Cardote, M. S. Gadd, and A. Ciulli, "Crystal structure of the Cul2-Rbx1-EloBC-VHL ubiquitin ligase complex," *Structure*, vol. 25, no. 6, pp. 901–911, 2017.
- [10] J. R. C. Muniz, K. Guo, N. J. Kershaw et al., "Molecular architecture of the ankyrin SOCS box family of Cul5-dependent E3 ubiquitin ligases," *Journal of Molecular Biology*, vol. 425, no. 17, pp. 3166–3177, 2013.
- [11] J. D. Berndt, R. T. Moon, and M. B. Major, " β -catenin gets jaded and von Hippel-Lindau is to blame," *Trends in Biochemical Sciences*, vol. 34, no. 3, pp. 101–104, 2009.
- [12] W. Luo, J. Zhong, R. Chang, H. Hu, A. Pandey, and G. L. Semenza, "Hsp70 and CHIP selectively mediate ubiquitination and degradation of hypoxia-inducible factor (HIF)-1 α but not HIF-2 α ," *Journal of Biological Chemistry*, vol. 285, no. 6, pp. 3651–3663, 2010.
- [13] M. Y. Koh, B. G. Darnay, and G. Powis, "Hypoxia-associated factor, a novel E3-ubiquitin ligase, binds and ubiquitinates hypoxia-inducible factor 1, leading to its oxygen-independent

- degradation," *Molecular and Cellular Biology*, vol. 28, no. 23, pp. 7081–7095, 2008.
- [14] G. Li, W. Ci, S. Karmakar et al., "SPOP promotes tumorigenesis by acting as a key regulatory hub in kidney cancer," *Cancer Cell*, vol. 25, no. 4, pp. 455–468, 2014.
 - [15] W. G. Kaelin Jr. and P. J. Ratcliffe, "Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway," *Molecular Cell*, vol. 30, no. 4, pp. 393–402, 2008.
 - [16] A. J. Majmundar, W. J. Wong, and M. C. Simon, "Hypoxia-inducible factors and the response to hypoxic stress," *Molecular Cell*, vol. 40, no. 2, pp. 294–309, 2010.
 - [17] C. Kuiper, G. U. Dachs, M. J. Currie, and M. C. M. Vissers, "Intracellular ascorbate enhances hypoxia-inducible factor (HIF)-hydroxylase activity and preferentially suppresses the HIF-1 transcriptional response," *Free Radical Biology and Medicine*, vol. 69, pp. 308–317, 2014.
 - [18] C. E. Stebbins, W. G. Kaelin Jr., and N. P. Pavletich, "Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function," *Science*, vol. 284, no. 5413, pp. 455–461, 1999.
 - [19] D. Tarade and M. Ohh, "The HIF and other quandaries in VHL disease," *Oncogene*, vol. 37, no. 2, pp. 139–147, 2018.
 - [20] F. Okumura, K. Uematsu, S. D. Byrne et al., "Parallel regulation of von Hippel-Lindau disease by pVHL-mediated degradation of B-myb and hypoxia-inducible factor α ," *Molecular and Cellular Biology*, vol. 36, no. 12, pp. 1803–1817, 2016.
 - [21] X. Yao, J. Tan, K. J. Lim et al., "VHL deficiency drives enhancer activation of Oncogenes in clear cell renal cell carcinoma," *Cancer Discov*, vol. 7, no. 11, pp. 1284–1305, 2017.
 - [22] B. Keith, R. S. Johnson, and M. C. Simon, "HIF1 α and HIF2 α : sibling rivalry in hypoxic tumour growth and progression," *Nature Reviews Cancer*, vol. 12, no. 1, pp. 9–22, 2011.
 - [23] F. Melendez-Rodriguez, O. Roche, R. Sanchez-Prieto, and J. Aragones, "Hypoxia-inducible factor 2-dependent pathways driving von Hippel-Lindau-Deficient renal cancer," *Frontiers in Oncology*, vol. 8, p. 214, 2018.
 - [24] K. Xu, Q. Ding, Z. Fang et al., "Silencing of HIF-1 α suppresses tumorigenicity of renal cell carcinoma through induction of apoptosis," *Cancer Gene Therapy*, vol. 17, no. 3, pp. 212–222, 2010.
 - [25] T. Isono, T. Chano, J. Yonese, and T. Yuasa, "Hydroxyl-HIF2 α is potential therapeutic target for renal cell carcinomas," *American Journal of Cancer Research*, vol. 6, no. 10, pp. 2263–2276, 2016.
 - [26] S. C. Wong, W. Cheng, H. Hamilton et al., "HIF2 α -Targeted RNAi therapeutic inhibits clear cell renal cell carcinoma," *Molecular Cancer Therapeutics*, vol. 17, no. 1, pp. 140–149, 2018.
 - [27] H. Cho, X. Du, J. P. Rizzi et al., "On-target efficacy of a HIF-2 α antagonist in preclinical kidney cancer models," *Nature*, vol. 539, no. 7627, pp. 107–111, 2016.
 - [28] W. Chen, H. Hill, A. Christie et al., "Targeting renal cell carcinoma with a HIF-2 antagonist," *Nature*, vol. 539, no. 7627, pp. 112–117, 2016.
 - [29] M. V. Panchenko, "Structure, function and regulation of jade family PHD finger 1 (JADE1)," *Gene*, vol. 589, no. 1, pp. 1–11, 2016.
 - [30] L. Coscoy, D. J. Sanchez, and D. Ganem, "A novel class of herpesvirus-encoded membrane-bound E3 ubiquitin ligases regulates endocytosis of proteins involved in immune recognition," *The Journal of Cell Biology*, vol. 155, no. 7, pp. 1265–1274, 2001.
 - [31] M. I. Zhou, H. Wang, J. J. Ross, I. Kuzmin, C. Xu, and H. T. Cohen, "The von Hippel-Lindau tumor suppressor stabilizes novel plant homeodomain protein Jade-1," *Journal of Biological Chemistry*, vol. 277, no. 42, pp. 39887–39898, 2002.
 - [32] M. I. Zhou, H. Wang, R. L. Foy, J. J. Ross, and H. T. Cohen, "Tumor suppressor von Hippel-Lindau (VHL) stabilization of Jade-1 protein occurs through plant homeodomains and is VHL mutation dependent," *Cancer Research*, vol. 64, no. 4, pp. 1278–1286, 2004.
 - [33] M. I. Zhou, R. L. Foy, V. C. Chitalia et al., "Jade-1, a candidate renal tumor suppressor that promotes apoptosis," *Proceedings of the National Academy of Sciences*, vol. 102, no. 31, pp. 11035–11040, 2005.
 - [34] Q. Xu, M. Krause, A. Samoylenko, and S. Vainio, "Wnt signaling in renal cell carcinoma," *Cancers (Basel)*, vol. 8, no. 6, 2016.
 - [35] V. C. Chitalia, R. L. Foy, M. M. Bachschmid et al., "Jade-1 inhibits Wnt signalling by ubiquitylating β -catenin and mediates Wnt pathway inhibition by pVHL," *Nature Cell Biology*, vol. 10, no. 10, pp. 1208–1216, 2008.
 - [36] W. Xiao-Fen, C. Ting, L. Jie, M. Deng-Yang, Z. Qing-Feng, and L. Xin, "Correlation analysis of VHL and Jade-1 gene expression in human renal cell carcinoma," *Open Med (Wars)*, vol. 11, no. 1, pp. 226–230, 2016.
 - [37] H. Zhai, Y. Shi, H. Jin et al., "Expression of calcyclin-binding protein/Siah-1 interacting protein in normal and malignant human tissues: an immunohistochemical survey," *Journal of Histochemistry & Cytochemistry*, vol. 56, no. 8, pp. 765–772, 2008.
 - [38] S. Sun, X. Ning, J. Liu et al., "Overexpressed CacyBP/SIP leads to the suppression of growth in renal cell carcinoma," *Biochemical and Biophysical Research Communications*, vol. 356, no. 4, pp. 864–871, 2007.
 - [39] M. G. Adam, S. Matt, S. Christian et al., "SIAH ubiquitin ligases regulate breast cancer cell migration and invasion independent of the oxygen status," *Cell Cycle*, vol. 14, no. 23, pp. 3734–3747, 2015.
 - [40] H. Shi, B. Zheng, Y. Wu et al., "Ubiquitin ligase Siah1 promotes the migration and invasion of human glioma cells by regulating HIF-1 α signaling under hypoxia," *Oncology Reports*, vol. 33, no. 3, pp. 1185–1190, 2015.
 - [41] C. Sun, H.-L. Li, and H.-R. Chen, "Decreased expression of CHIP leads to increased angiogenesis via VEGF-VEGFR2 pathway and poor prognosis in human renal cell carcinoma," *Scientific Reports*, vol. 5, p. 9774, 2015.
 - [42] B. Min, H. Park, S. Lee et al., "CHIP-mediated degradation of transglutaminase 2 negatively regulates tumor growth and angiogenesis in renal cancer," *Oncogene*, vol. 35, no. 28, pp. 3718–3728, 2016.
 - [43] S. Erdem, G. Yegen, D. Telci et al., "The increased transglutaminase 2 expression levels during initial tumorigenesis predict increased risk of metastasis and decreased disease-free and cancer-specific survivals in renal cell carcinoma," *World Journal of Urology*, vol. 33, no. 10, pp. 1553–1560, 2015.
 - [44] B. M. Ku, S.-J. Kim, N. Kim et al., "Transglutaminase 2 inhibitor abrogates renal cell carcinoma in xenograft models," *Journal of Cancer Research and Clinical Oncology*, vol. 140, no. 5, pp. 757–767, 2014.
 - [45] D.-S. Kim, Y.-B. Choi, B.-G. Han et al., "Cancer cells promote survival through depletion of the von Hippel-Lindau tumor suppressor by protein crosslinking," *Oncogene*, vol. 30, no. 48, pp. 4780–4790, 2011.

- [46] J. Cao, M.-H. Ge, and Z.-Q. Ling, "Fbxw7 tumor suppressor," *Medicine*, vol. 95, no. 7, p. e2496, 2016.
- [47] Y. Fu, Y. Lin, and Z. Yang, "FBXW7 overexpression suppresses renal cancer cell proliferation and induces apoptosis," *Medical Oncology*, vol. 32, no. 8, p. 215, 2015.
- [48] Y. Cai, M. Zhang, and X. Qiu, "Upregulation of FBXW7 suppresses renal cancer metastasis and epithelial mesenchymal transition," *Disease Markers*, vol. 2017, Article ID 8276939, 7 pages, 2017.
- [49] J. M. Cassavaugh, S. A. Hale, T. L. Wellman, A. K. Howe, C. Wong, and K. M. Lounsbury, "Negative regulation of HIF-1 α by an FBW7-mediated degradation pathway during hypoxia," *Journal of Cellular Biochemistry*, vol. 112, no. 12, pp. 3882–3890, 2011.
- [50] D. Flugel, A. Gorchach, and T. Kietzmann, "GSK-3 regulates cell growth, migration, and angiogenesis via Fbw7 and USP28-dependent degradation of HIF-1," *Blood*, vol. 119, no. 5, pp. 1292–1301, 2012.
- [51] T. Kawasaki, V. Bilim, K. Takahashi, and Y. Tomita, "Infrequent alteration of p53 pathway in metastatic renal cell carcinoma," *Oncology Reports*, vol. 6, no. 2, pp. 329–333, 1999.
- [52] J. K. Maranchie, J. R. Vasselli, J. Riss, J. S. Bonifacio, W. M. Linehan, and R. D. Klausner, "The contribution of VHL substrate binding and HIF1- α to the phenotype of VHL loss in renal cell carcinoma," *Cancer Cell*, vol. 1, no. 3, pp. 247–255, 2002.
- [53] M.-E. Bonicalzi, I. Groulx, N. de Paulsen, and S. Lee, "Role of exon 2-encoded β -domain of the von Hippel-lindau tumor suppressor protein," *Journal of Biological Chemistry*, vol. 276, no. 2, pp. 1407–1416, 2001.
- [54] J.-S. Roe, H. Kim, S.-M. Lee, S.-T. Kim, E.-J. Cho, and H.-D. Youn, "p53 stabilization and transactivation by a von Hippel-Lindau protein," *Molecular Cell*, vol. 22, no. 3, pp. 395–405, 2006.
- [55] S. Galban, J. L. Martindale, K. Mazan-Mamczarz et al., "Influence of the RNA-binding protein HuR in pVHL-regulated p53 expression in renal carcinoma cells," *Molecular and Cellular Biology*, vol. 23, no. 20, pp. 7083–7095, 2003.
- [56] Q. J. Liu, H. L. Shen, J. Lin et al., "Synergistic roles of p53 and HIF1 α in human renal cell carcinoma-cell apoptosis responding to the inhibition of mTOR and MDM2 signaling pathways," *Drug Design, Development and Therapy*, vol. 10, pp. 745–755, 2016.
- [57] C. G. Stoehr, R. Stoehr, A. Wrenners et al., "Homozygous G/G variant of SNP309 in the human MDM2 gene is associated with earlier tumor onset in Caucasian female renal cell carcinoma patients," *Oncogenesis*, vol. 5, p. e205, 2016.
- [58] M. Abolhasani, S. Salarinejad, and M. Asgari, "P53 and MDM2 over-expression and five-year survival of kidney cancer patients undergoing radical nephrectomy—Iranian experience," *Asian Pacific Journal of Cancer Prevention*, vol. 16, no. 12, pp. 5043–5047, 2015.
- [59] D. Chen, M. Li, J. Luo, and W. Gu, "Direct interactions between HIF-1 α and Mdm2 modulate p53 function," *Journal of Biological Chemistry*, vol. 278, no. 16, pp. 13595–13598, 2003.
- [60] R. Ravi, B. Mookerjee, Z. M. Bhujwalla et al., "Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1 α ," *Genes & Development*, vol. 14, no. 1, pp. 34–44, 2000.
- [61] R. P. Wurz and V. J. Cee, "Targeted degradation of MDM2 as a new approach to improve the efficacy of MDM2-p53 inhibitors," *Journal of Medicinal Chemistry*, vol. 62, no. 2, pp. 445–447, 2018.
- [62] Y.-S. Jung, S.-J. Lee, S.-H. Lee et al., "Loss of VHL promotes progerin expression, leading to impaired p14/ARF function and suppression of p53 activity," *Cell Cycle*, vol. 12, no. 14, pp. 2277–2290, 2013.
- [63] A. M. Roberts, I. R. Watson, A. J. Evans, D. A. Foster, M. S. Irwin, and M. Ohh, "Suppression of hypoxia-inducible factor 2 restores p53 activity via Hdm2 and reverses chemoresistance of renal carcinoma cells," *Cancer Research*, vol. 69, no. 23, pp. 9056–9064, 2009.
- [64] J. A. Bertout, A. J. Majmundar, J. D. Gordan et al., "HIF2 inhibition promotes p53 pathway activity, tumor cell death, and radiation responses," *Proceedings of the National Academy of Sciences*, vol. 106, no. 34, pp. 14391–14396, 2009.
- [65] M. Ding, X. Lu, C. Wang et al., "The E2F1-miR-520/372/373-SPOP axis modulates progression of renal carcinoma," *Cancer Research*, vol. 78, no. 24, pp. 6771–6784, 2018.
- [66] O. A. Harb, M. A. Elfeky, B. S. El Shafaay et al., "SPOP, ZEB-1 and E-Cadherin expression in clear cell renal cell carcinoma (Cc-RCC): clinicopathological and prognostic significance," *Pathophysiology*, vol. 25, no. 4, pp. 335–345, 2018.
- [67] W. Zhao, J. Zhou, Z. Deng, Y. Gao, and Y. Cheng, "SPOP promotes tumor progression via activation of β -catenin/TCF4 complex in clear cell renal cell carcinoma," *International Journal of Oncology*, vol. 49, no. 3, pp. 1001–1008, 2016.
- [68] X. Liu, G. Sun, and X. Sun, "RNA interference-mediated silencing of speckle-type POZ protein promotes apoptosis of renal cell cancer cells," *OncoTargets and Therapy*, vol. 9, pp. 2393–2402, 2016.
- [69] J. Zhang, X. Bu, H. Wang et al., "Cyclin D-CDK4 kinase destabilizes PD-L1 via cullin 3-SPOP to control cancer immune surveillance," *Nature*, vol. 553, no. 7686, pp. 91–95, 2018.
- [70] F. Qu, J. Ye, X. Pan et al., "MicroRNA-497-5p down-regulation increases PD-L1 expression in clear cell renal cell carcinoma," *Journal of Drug Targeting*, vol. 27, no. 1, pp. 67–74, 2019.
- [71] R. H. Thompson, M. D. Gillett, J. C. Cheville et al., "Costimulatory molecule B7-H1 in primary and metastatic clear cell renal cell carcinoma," *Cancer*, vol. 104, no. 10, pp. 2084–2091, 2005.
- [72] F. Xu, L. Xu, Q. Wang, G. An, G. Feng, and F. Liu, "Clinicopathological and prognostic value of programmed death ligand-1 (PD-L1) in renal cell carcinoma: a meta-analysis," *International Journal of Clinical and Experimental Medicine*, vol. 8, no. 9, pp. 14595–14603, 2015.
- [73] K. Ueda, S. Suekane, H. Kurose et al., "Prognostic value of PD-1 and PD-L1 expression in patients with metastatic clear cell renal cell carcinoma," *Urologic Oncology*, vol. 36, no. 11, pp. 499.e9–499.e16, 2018.
- [74] K. R. Leite, S. T. Reis, J. Pontes Jr. et al., "PD-L1 expression in renal cell carcinoma clear cell type is related to unfavorable prognosis," *Diagnostic Pathology*, vol. 10, p. 189, 2015.
- [75] R. H. Thompson, S. M. Kuntz, B. C. Leibovich et al., "Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up," *Cancer Research*, vol. 66, no. 7, pp. 3381–3385, 2006.
- [76] H. T. Dogan, M. Kiran, B. Bilgin et al., "Prognostic significance of the programmed death ligand 1 expression in clear cell renal cell carcinoma and correlation with the tumor microenvironment and hypoxia-inducible factor expression," *Diagnostic Pathology*, vol. 13, no. 1, p. 60, 2018.
- [77] M. Ruf, H. Moch, and P. Schraml, "PD-L1 expression is regulated by hypoxia inducible factor in clear cell renal cell

- carcinoma,” *International Journal of Cancer*, vol. 139, no. 2, pp. 396–403, 2016.
- [78] Y. Messai, S. Gad, M. Z. Noman et al., “Renal cell carcinoma programmed death-ligand 1, a new direct target of hypoxia-inducible factor-2 alpha, is regulated by von Hippel-lindau gene mutation status,” *European Urology*, vol. 70, no. 4, pp. 623–632, 2016.
 - [79] D. J. Martini, L. Hamieh, R. R. McKay et al., “Durable clinical benefit in metastatic renal cell carcinoma patients who discontinue PD-1/PD-L1 therapy for immune-related adverse events,” *Cancer Immunology Research*, vol. 6, no. 4, pp. 402–408, 2018.
 - [80] R. J. Motzer, B. Escudier, D. F. McDermott et al., “Nivolumab versus everolimus in advanced renal-cell carcinoma,” *New England Journal of Medicine*, vol. 373, no. 19, pp. 1803–1813, 2015.
 - [81] M. B. Atkins, E. R. Plimack, I. Puzanov et al., “Axitinib in combination with pembrolizumab in patients with advanced renal cell cancer: a non-randomised, open-label, dose-finding, and dose-expansion phase 1b trial,” *The Lancet Oncology*, vol. 19, no. 3, pp. 405–415, 2018.
 - [82] X. Gao and D. F. McDermott, “Ipilimumab in combination with nivolumab for the treatment of renal cell carcinoma,” *Expert Opinion on Biological Therapy*, vol. 18, no. 9, pp. 947–957, 2018.
 - [83] M. Y. Koh, V. Nguyen, R. Lemos et al., “Hypoxia-induced SUMOylation of E3 ligase HAF determines specific activation of HIF2 in clear-cell renal cell carcinoma,” *Cancer Research*, vol. 75, no. 2, pp. 316–329, 2015.
 - [84] Z. Guan, C. Ding, Y. Du et al., “HAF drives the switch of HIF-1 α to HIF-2 α by activating the NF- κ B pathway, leading to malignant behavior of T24 bladder cancer cells,” *International Journal of Oncology*, vol. 44, no. 2, pp. 393–402, 2014.
 - [85] M. Y. Koh, R. Lemos, X. Liu, and G. Powis, “The hypoxia-associated factor switches cells from HIF-1—to HIF-2 -dependent signaling promoting stem cell characteristics, aggressive tumor growth and invasion,” *Cancer Research*, vol. 71, no. 11, pp. 4015–4027, 2011.
 - [86] Á. Ordóñez-Navadijo, E. Fuertes-Yebra, B. Acosta-Iborra et al., “Mutant versions of von Hippel-Lindau (VHL) can protect HIF1 α from SART1-mediated degradation in clear-cell renal cell carcinoma,” *Oncogene*, vol. 35, no. 5, pp. 587–594, 2016.
 - [87] X. M. Lai, S.-Y. Liu, Y.-T. Tsai et al., “HAF mediates the evasive resistance of anti-angiogenesis TKI through disrupting HIF-1alpha and HIF-2alpha balance in renal cell carcinoma,” *Oncotarget*, vol. 8, no. 30, pp. 49713–49724, 2017.

