The emerging role of WWP1 in cancer development and progression

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Emerging evidence demonstrates that WW domain-containing E3 ubiquitin protein ligase 1 (WWP1) participates into carcinogenesis and tumor progression. In this review article, we will describe the association between dysregulated WWP1 expression and clinical features of cancer patients. Moreover, we summarize the both oncogenic and tumor suppressive functions of WWP1 in a variety of human cancers. Furthermore, we briefly describe the downstream substrates of WWP1 and its upstream factors to regulate the expression of WWP1. Notably, targeting WWP1 by its inhibitors or natural compounds is potentially useful for treating human malignancies. Finally, we provide the perspectives regarding WWP1 in cancer development and therapies. We hope this review can stimulate the research to improve our understanding of WWP1-mediated tumorigenesis and accelerate the discovery of novel therapeutic strategies via targeting WWP1 expression in cancers.

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FACTS

- WWP1 mainly targets its substrates for ubiquitination and degradation.
- Targeting WWP1 could be useful for improving therapeutic outcome of cancer patients.
- WWP1 is critically involved in oncogenesis and tumor progression.

OPEN QUESTIONS

- What are the key drivers as the upstream factors to govern the expression of WWP1?
- Does WWP1 have a crosstalk with other NEDD4 family members?
- How to use high-screening approaches to develop the special inhibitor of WWP1 for cancer therapy?

INTRODUCTION

Ubiquitin proteasome system (UPS) plays a critical role in regulating protein homeostasis via targeting protein post-translational modifications (PTM) [1]. Ubiquitination is a normal cellular process that one ubiquitin or multiple ubiquitins are added to the substrates, leading to protein degradation or protein trafficking [2]. This process is performed by ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) [3]. Two E1 enzymes, 38 E2 enzymes, and more than 600 E3 enzymes are reported in humans. Based on their structures, E3 ligases are mainly divided into three groups: RING-type E3s, HECT-type E3s, and RBR E3s [4–7]. HECT-type E3 ligases are classified into three families according to diverse domains of N terminus: well-characterized NEDD4 family with 9 members, HERC family with 6 members, and other E3s with 13 members [8–10]. NEDD4 family displays two–four WW domains, and HERC E3 family has RLD domains, while the other E3s have neither WW nor RLD domains [11]. The WW domains bind to PPXY (phospho-Ser-Pro and Pro-Arg) motifs of the substrates and trigger the degradation [12].

NEDD4 family has nine members: NEDD4-1, NEDD4-2, WWP1, WWP2, ITCH, NEDL1, NEDL2, Smurf1, and Smurf2 [12–14]. WWP1, also called TIUL1 (TGIF-interacting ubiquitin ligase 1) or AIP5 (Atropin-1-interacting protein 5), serves as a multifunction protein, which composes of an N-terminal C2 domain, followed by four WW domains and a C-terminal catalytic HECT domain (Fig. 1) [15]. Genetically, human WWP1, situated in chromosome 8q21, generates more than six isoforms resulting from alternative splicing to exert different functions [16]. Ubiquitin molecules have seven lysine residues, including K6, K11, K27, K29, K33, K48, and K63, which can be polymerized into a diverse range of linkages. K48-based chain is commonly considered as a signal label for proteasomal degradation; however, ubiquitin chains based upon other acceptor lysines, or modulation by single moiety (ies) often have a non-proteolytic effect in various important physiological processes [17]. For instance, a previous study has demonstrated that WWP1-mediated ubiquitylation of

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The chemical constitution of WWP1. WWP1 serves as a potential modulator for the proliferation of epithelial cells [18]. WWP1 mediated PTEN polyubiquitination and repressed its dimerization and membrane recruitment [19]. Accumulating evidence indicates that WWP1 plays a critical role in regulating diverse biological processes, containing regulation of epithelial sodium channels, receptor trafficking and degradation, and viral budding [20, 21]. Moreover, the abnormal regulations of WWP1 are also involved in multiple diseases, like inflammation, neurological disorders, aging, and cancers [22]. In this review article, we will describe the association between WWP1 expression and clinical features of cancer patients. Moreover, we will discuss the various functions of WWP1 as an oncoprotein or a tumor suppressor in a variety of malignancies. Furthermore, we summarize the substrates of WWP1 and its upstream factors regulating the expression of WWP1. We also highlight the compounds as the inhibitors of WWP1 to improve the treatment outcomes of human cancers. Finally, we provide the perspective regarding WWP1 in cancer development and therapies.

**WWP1 EXPRESSION IS ASSOCIATED WITH POOR PROGNOSIS IN CANCER PATIENTS**

Pathologically, several lines of evidence demonstrated that WWP1 expression is aberrantly expressed in a variety of human cancers (Table 1) [22]. For example, WWP1 expression at mRNA and protein levels was reported to be commonly increased in colorectal cancer tissues [23]. High expression of WWP1 was linked to tumor size, T classification, TNM stage, distant metastasis, and poor survival [23]. One group reported that WWP1 expression was associated with single-nucleotide polymorphisms (SNPs) and copy number variants (CNVs) in osteosarcomas [24]. WWP1 mRNA levels and its copy numbers were upregulated in the oral tumor tissues [25]. High expression of WWP1 at mRNA and protein levels was also reported in gastric carcinoma tissues, which was associated with TNM stage, LNM, and invasive depth and poor prognosis in patients with gastric cancer [26]. Poor expression of WWP1 was observed in melanoma cells and melanoma tissues, which was associated with poor prognosis in melanoma patients [27]. WWP1 gene had copy number gain in 44% xenograft and cell lines that were obtained from prostate cancer. Moreover, 60% of these xenografts and cell lines had the overexpression of WWP1. Prostate tumor tissues had 31% of copy number gain of WWP1, but not frequent mutations of WWP1 [28]. Another study also revealed that WWP1 expression was increased in prostate cancer specimens compared with normal prostate specimens and PIN specimens [29]. Moreover, higher expression of WWP1 was observed in metastatic prostate cancer compared with primary prostate cancer [29, 30].

WWP1 mRNA was overexpressed in 58% of breast tumor cell lines, which was associated with copy number gain of WWP1 [31]. The expression of WWP1 was remarkably higher in breast tumors compared with normal tissues [31–33]. Interestingly, nuclear–cytoplasmic distribution of WWP1 might predict the prognosis of breast cancer patients. Breast cancer patients with only nuclear-localized WWP1 in tumors had favorable prognosis compared with that with both cytoplasmic and nuclear WWP1 expressions [32]. Surprisingly, breast cancer patients with low or absent WWP1 expression had the worst prognosis compared with those patients with middle or high expression of WWP1 [32]. Moreover, this group reported that cytoplasmic WWP1 expression was highly expressed in breast tumor tissues and was linked to estrogen receptor alpha (ERα) and insulin-like growth factor receptor 1 (IGF-1R) expression in breast carcinoma [34]. WWP1 downregulation caused inhibition of ER levels in MCF7 and T47D breast cancer cells [34]. Similarly, WWP1 expression was higher in osteosarcoma tissues compared with matched normal bone tissues [35]. WWP1 expression at both mRNA and protein levels was elevated in hepatocellular carcinoma (HCC) specimens compared with adjacent non-tumor hepatic tissues [36, 37]. The mRNA level of WWP1 was amplified in HCC tissues [37]. Notably, aberrant high expression of WWP1 was associated with poorer prognosis in HCC patients [36]. Moreover, the expression of WWP1 was associated with copy number gain of WWP1 [31]. The expression of WWP1 was remarkably higher in breast tumors compared with normal tissues [31–33]. Interestingly, nuclear–cytoplasmic distribution of WWP1 might predict the prognosis of breast cancer patients. Breast cancer patients with only nuclear-localized WWP1 in tumors had favorable prognosis compared with that with both cytoplasmic and nuclear WWP1 expressions [32]. Surprisingly, breast cancer patients with low or absent WWP1 expression had the worst prognosis compared with those patients with middle or high expression of WWP1 [32].

![Fig. 1](image) The chemical constitution of WWP1.

| Cancer type                        | Expression level of tumor | Clinicopathological features and prognosis values                                                                 | Reference |
|-----------------------------------|---------------------------|-------------------------------------------------------------------------------------------------------------------|-----------|
| Colorectal cancer                 | Increased                 | High expression of WWP1 was related with tumor size, T classification, TNM stage, distant metastasis and poor survival | [23]      |
| Osteosarcomas                     | Upregulated               | WWP1 expression was associated with single-nucleotide polymorphisms and copy number variants                         | [24, 35]  |
| Oral cancer                       | Upregulated               | N/A                                                                                                               | [25]      |
| Gastric cancer                    | Increased                 | High expression of WWP1 was associated with TNM stage, lymph node metastasis, invasive depth and poor prognosis     | [26]      |
| Melanoma                          | Poor expression           | N/A                                                                                                               | [27]      |
| Prostate cancer                   | Upregulated               | N/A                                                                                                               | [28–30]   |
| Breast cancer                     | Upregulated               | Patients with only nuclear-localized WWP1 in tumors had favorable prognosis. And low/absent WWP1 level indicated the worst prognosis | [31–33]   |
| Hepatocellular cancer             | Elevated                  | WWP1 level was linked to tumor size, histological grade, TNM stage, vascular invasion and tumor capsule, poorer prognosis | [36, 37]  |
| Chronic lymphocytic leukemia      | Higher                    | High expression of WWP1 was related with adverse prognostic factors including CD38 and ZAP-70                      | [38]      |
| Cutaneous squamous cell carcinoma | Augmented                 | High expression of WWP1 was associated with histological grade, invasion depth, lymph node metastasis and unfavorable prognosis | [39]      |

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**Table 1.** Expression and prognosis values of WWP1 in human cancers.

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ΔNp63 was based on K63-based polyubiquitin chain, representing nonproteolytic ubiquitination. WWP1 serves as a potential modulator for the proliferation of epithelial cells [18]. WWP1 mediated PTEN polyubiquitination and repressed its dimerization and membrane recruitment [19]. Accumulating evidence indicates that WWP1 plays a critical role in regulating diverse biological processes, containing regulation of epithelial sodium channels, receptor trafficking and degradation, and viral budding [20, 21]. Moreover, the abnormal regulations of WWP1 are also involved in multiple diseases, like inflammation, neurological disorders, aging, and cancers [22]. In this review article, we will describe the association between WWP1 expression and clinical features of cancer patients. Moreover, we will discuss the various functions of WWP1 as an oncoprotein or a tumor suppressor in a variety of malignancies. Furthermore, we summarize the substrates of WWP1 and its upstream factors regulating the expression of WWP1. We also highlight the compounds as the inhibitors of WWP1 to improve the treatment outcomes of human cancers. Finally, we provide the perspective regarding WWP1 in cancer development and therapies.

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was linked to tumor size, histological grade, TNM stage, vascular invasion, and tumor capsule of HCC patients, indicating that WWP1 might be an independent predictor of poor prognosis in HCC patients [36]. The high level of WWP1 mRNA expression was also found in chronic lymphocytic leukemia (CLL) patients and was positively correlated to CD38 and ZAP-70 expressions, indicating that WWP1 might be a potential marker for predicting CLL prognosis [38]. In addition, WWP1 expression was augmented in patients with cutaneous squamous cell carcinoma (CSCC) and was linked to histological grade and lymph node metastasis [39]. In line with these reports, data from Kaplan-Meier plotter (http://kmplot.com) show that dysregulation of WWP1 expression is associated with overall survival in various human cancers (Fig. 2).

**BIOLOGICAL FUNCTIONS OF WWP1 IN CANCER**

Functionally, WWP1 has been demonstrated to play either an oncogenic role or tumor suppressive functions in various types of human tumors [40]. Most studies reveal that WWP1 exerts tumor promotion functions in various types of cancers. WWP1 has been found to promote proliferation, migration and invasion, inhibit apoptosis, and enhance cell cycle in cancer cells [41].

Upregulation of WWP1 promoted proliferation and invasion of AMC-HN-8 laryngeal cancer cells [42]. Overexpression of WWP1 enhanced proliferation and migration in gastric cancer cells and facilitated tumor growth in vivo [43]. Similarly, depletion of WWP1 reduced cell proliferation in vitro and blocked tumor growth in vivo. Moreover, deficiency of WWP1 resulted in G0/G1 phase arrest and apoptosis of MKN-45 and AGS gastric cancer cells via inactivation of the PTEN/Akt pathway [26]. In line with the oncogenic role of WWP1, depletion of WWP1 by siRNA reduced growth and invasiveness of MG63 and HOS osteosarcoma cells [35]. Moreover, deficiency of WWP1 triggered G1 phase arrest and cell apoptosis in osteosarcoma cells. Mechanistically, downregulation of WWP1 regulated the expression of Bcl-2 and Bax to govern apoptosis in osteosarcoma cells. WWP1 also affected the expression of β-catenin, E-cadherin, MMP-2, and MMP-9, leading to regulating invasion of osteosarcoma cells [35]. Knockdown of WWP1 inhibited proliferation of prostate cancer cells and suppressed TGF-β-induced growth [28]. Depletion of WWP1 reduced the migration and invasion of prostate cancer cells [29]. Inactivation of WWP1 impaired MYC-driven prostate oncogenesis in the mice due to activation of PTEN [19]. Deficiency of WWP1 reduced proliferation and elevated apoptosis of oral cancer cells [25].

Similarly, knockdown of WWP1 by siRNA suppressed proliferation, colony formation, migration and invasion of HCC cells, promoted cell apoptosis, and caused cell cycle arrest at the G0/G1 phase in HCC [36]. In agreement with this report, deficiency of WWP1 repressed cell growth and stimulated apoptosis of HCC cells via upregulation of p53 and cleaved caspase 3 expression [37]. Silencing of WWP1 caused cell cycle arrest and apoptotic death of MCF7 and HCC1500 breast cancer cells via activation of caspases expression [31]. Forced upregulation of WWP1 accelerated proliferation of MCF10A and 184B5 cell lines, which are immortalized breast epithelial cells [31]. In line with this finding, another study also showed that overexpression of WWP1 in MCF10A cells promoted cell growth and colony formation, while inhibition of WWP1 repressed colony formation of T47D and MCF7 cells [32]. WWP1 knockdown in combination with tamoxifen inhibited proliferation of T47D and MCF7, and suppressed E2-mediated DNA synthesis [34]. Additionally, WWP1 promoted TRAIL resistance via inhibition of caspase-8-induced apoptosis in ERα-positive breast cancer cells [44]. Depletion of WWP1 reduced proliferation and invasion of colorectal cancer cells, while upregulation of WWP1 led to increased proliferative and invasive ability via regulation of the PTEN/Akt pathway [23]. In addition, WWP1 could mediate the resistance of doxorubicin and cisplatin in human cancer cells [45]. WWP1 expression was augmented in

**Fig. 2** WWP1 expression is associated with overall survival in a variety of human cancers.
acute myeloid leukemia (AML) patients and inactivation of WWP1 inhibited the proliferation of AML cells and tumor growth in mice [46]. WWP1 knockdown led to cell cycle arrest and autophagy, and inhibited survival of AML cells [46]. In CSCC cells, downregulation of WWP1 impaired cell growth, blocked cell migration and invasion, induced cell cycle arrest at the G1/G1 phase and increased apoptosis in CSCC cells via suppressing phosphorylation of STAT3 and inhibiting MMP-2, cyclin D1, and Bcl-2 [39].

Interestingly, two studies exhibited that WWP1 has a tumor-suppressive function in glioma and breast cancer cells [47, 48]. WWP1 overexpression suppressed cell malignant behaviors and tumor growth in glioma xenograft mouse model [47]. Chen et al. [52, 53] reported that WWP1 acted as an E3 ubiquitin ligase for the ubiquitination and degradation of KLF5. WWP1 can bind with KLF5 and its catalytic cysteine residue is required for this degradation. A PY motif in KLF5 domain is important for binding with WWP1 for its degradation [52]. Moreover, this group identified that KLF5 destruction via the proteasome might be governed in a ubiquitin-independent way [57].

**Smad2 and Smad4**
WWP1 has been identified to inhibit TGF-β signaling via regulating degradation of Smad2 and activated receptor [49, 50]. WWP1 interacted with Smad7 and triggered degradation of the activated type I receptor. WWP1 also can bind with Smad2 and TGF, leading to enhancement of Smad2 degradation. Upregulation of WWP1 attenuated TGF-β-mediated growth arrest, while depletion of WWP1 led to inhibition of Smad2 degradation and promoting TGF-β-induced gene expression [49]. In addition, WWP1 induced nuclear export of Smad7 and suppressed TGF-β-mediated Smad2 phosphorylation, leading to negative regulation of the TGF-β pathway [50]. WWP1, Smurf2, and NEDD4-2 cooperated with Smad7 to downregulate Smad4 via proteasome degradation [58].

**CK2β**
One study has revealed that WWP1 and CHIP are critical E3 ligases for targeting CK2β degradation [59]. CK2 is critically involved in TGFβ-mediated EMT and promoted cancer metastasis. TGF-β increased CK2 activation and inactivation of CK2 blocked TGF-β-induced EMT [59]. Overexpression of WWP1 reduced the CK2β protein levels, while MG132 abolished WWP1-involved degradation of CK2β. WWP1 interacted with CK2β and promoted its ubiquitination and degradation [59]. TGF-β enhanced WWP1-mediated destruction of CK2β, which was abrogated by the absence of WWP1 expression [59]. Moreover, WWP1 was involved in TGF-β-mediated EMT via regulating CK2β degradation.

**CXCR4**
CXCR4 is a chemokine receptor that binds the CXCL12 (also known as SDF-1), which plays a pivotal role in tumorigenesis and cancer
cause degradation of RNF11 and cellular localization [64].

Promoted cell growth. WWP1 ubiquitinated RNF11, but it did not

regulated hepatocyte growth factor receptor activity [15]. WWP1

ubiquitination and degradation, contributing to G0/G1 cell cycle

breast cancer [63]. WWP1 also targeted p27 protein for

expression of ErbB4/HER4 via ubiquitination and degradation in

study, one group also observed that WWP1 suppressed the

migration and bone metastasis in breast cancer.

WWP1 can bind and ubiquitinate and destroy the TAP63 and

membrane recruitment, leading to activation of Akt [19].

Moreover, this group identified that WWP1 K740N and N745S

variants are one of reasons to increase enzymatic activation of

WWP1 and consequent inhibition of PTEN activity [65]. However,

one study did not find the similar results and challenged the

point that K740N and N745S WWP1 variants facilitated tumor-

igenesis via promoting PTEN ubiquitination [66]. Recently, WWP1

was discovered to bind with EGFR and increase its ubiquitination

and enhance EGFR stability, resulting in enhanced lung cancer

progression [67].

**Table 2. Main cancer-related substrates of WWP1.**

| Substrate | Modulation | Roles | Reference |
|-----------|------------|------|-----------|
| KLF2      | Binding KLF2 and inhibiting its transactivation | Not discussed | [51] |
| KLF5      | Degradation in a ubiquitin-independent way | Not discussed | [52, 53] |
| Smad2     | Degradation | Inhibition of TGF-β signaling | [49, 50] |
| Smad4     | Degradation | Attenuated TGF-β signaling | |
| CK2β      | Ubiquitination and degradation | Inhibition of TGF-β-induced EMT | [59] |
| CXCR4     | Limitation of degradation | Enhancement of cell migration and bone metastasis in breast cancer | [48] |
| LATS1     | Ubiquitination and degradation | Promoted proliferation of breast cancer cells | [56] |
| TβRI      | Polyubiquitination and degradation | Inhibited TGF-β cytostatic signaling, and exhibited carcinogenic properties | [54] |
| TAP63     | Ubiquitination and degradation | Restrained apoptosis and sensitivity to doxorubicin and cisplatin in colon cancer cells | [45] |
| DeltaNP63 | Ubiquitination and degradation | Increased doxorubicin-induced apoptosis in breast cancer cells | [45] |
| ErbB4     | Ubiquitination and degradation | Tumor inhibition in breast cancer | [55] |
| p27       | Ubiquitination and degradation | Promoted leukemic cell growth | [46] |
| RNF11     | Ubiquitination, not degradation | Enhanced proliferation and survival of cancer cells | [64] |
| Ezrin     | Ubiquitination, not degradation | Increased Met level and further promoted proliferation of cancer cells | [15] |
| PTEN      | Polyubiquitination | Promotion of cancer development | [19] |
| EGFR      | Ubiquitination and stabilization | Enhanced NSCLC stemness and inhibited its chemosensitivity | [67] |

metastasis [60]. One study showed that knockdown of WWP1

raised the expression of CXCR4 in MDA-MB-231 breast cancer

cells. CXCL12 induced CXCR4 degradation in MDA-MB-231 cells, but

not in WWP1-depletion cells. WWP1 knockdown increased mobility

of MDA-MB-231 cells induced by CXCL12 [48]. Moreover, WWP1

controlled CXCR4 lysosomal localization in response to

CXCL12 [48]. In summary, WWP1 govern CXCL12-mediated

lysosomal degradation of CXCR4, leading to regulation of cell

migration and bone metastasis in breast cancer.

**LATS1**

The large tumor suppressor (LATS1) is a key factor in the Hippo

signaling pathway, which is involved in carcinogenesis and tumor

progression [61, 62]. WWP1 was validated as an E3 ligase to

negatively regulate LATS1 expression. Moreover, WWP1 promoted

LATS1 ubiquitination and degradation, leading to promoting

proliferation of breast cancer cells [56]. Therefore, inhibition of

WWP1 could be a promising approach for activation of LATS1 and

further blocking growth of breast cancer cells.

**Other substrates**

WWP1 was reported to induce the degradation of TβRI in

conjunction with Smad7 [54]. One study demonstrated that WWP1

can bind and ubiquitinate and destroy the TAP63 and

DeltaNP63, two different forms of p63 protein [45]. Another

study reported that WWP1 targeted HER4 and membrane HER4,

but not nuclear HER4, for degradation [55]. In support of this

study, one group also observed that WWP1 suppressed the

expression of ErbB4/HER4 via ubiquitination and degradation in

breast cancer [63]. WWP1 also targeted p27 protein for

ubiquitination and degradation, contributing to G0/G1 cell cycle

arrest in AML cells [46]. WWP1 increased the expression of ErbB2

and EGFR via interacting with RING finger protein 11 (RNF11) and

promoted cell growth. WWP1 ubiquitinated RNF11, but it did not

cause degradation of RNF11 and cellular localization [64].

Therefore, WWP1 exerted oncogenic functions via inhibiting

RNF11-triggered downregulation of ErbB2 and EGFR. Likely,

WWP1 ubiquitinated Ezrin and did not cause the degradation of

Ezrin, but WWP1 upregulated the expression of Met level and

regulated hepatocyte growth factor receptor activity [15]. WWP1

caused PTEN polyubiquitination and blocked its dimerization and

membrane recruitment, leading to activation of Akt [19].

Moreover, this group identified that WWP1 K740N and N745S

alleles were enriched in colon cancer patients. These WWP1

variants are one of reasons to increase enzymatic activation of

WWP1 and consequent inhibition of PTEN activity [65]. However,

one study did not find the similar results and challenged the

point that K740N and N745S WWP1 variants facilitated tumor-

igenesis via promoting PTEN ubiquitination [66]. Recently, WWP1

was discovered to bind with EGFR and increase its ubiquitination

and enhance EGFR stability, resulting in enhanced lung cancer

progression [67].

**UPSTREAM FACTORS OF WWP1**

Accumulating evidence has revealed that WWP1 expression level

is regulated by several factors and noncoding RNAs. In the

following paragraphs, we describe how multiple factors such as

YAP, TAZ [68, 69], BAP1 [27], Notch-1 [70], and noncoding RNAs,

including miR-16-5p [71], miR-21-5p [71], miR-30a-5p [47], miR-

129-3p [43], miR-129-5p [43], miR-452 [29], and

Substrate Modulation Roles Reference

KLF2 Binding KLF2 and inhibiting its transactivation Not discussed [51]

KLF5 Degradation in a ubiquitin-independent way Not discussed [52, 53]

Smad2 Degradation Inhibition of TGF-β signaling [49, 50]

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p27 Ubiquitination and degradation Promoted leukemic cell growth [46]

RNF11 Ubiquitination, not degradation Enhanced proliferation and survival of cancer cells [64]

Ezrin Ubiquitination, not degradation Increased Met level and further promoted proliferation of cancer cells [15]

PTEN Polyubiquitination Promotion of cancer development [19]

EGFR Ubiquitination and stabilization Enhanced NSCLC stemness and inhibited its chemosensitivity [67]

YAP and TAZ

Both Yes-associated protein (YAP) and TAZ antagonized degrada-

tion of KLF5 by WWP1, resulting in enhancement of proliferation of

breast cancer cells [68, 69]. YAP and TAZ, two key factors of

Hippo signaling pathway, blocked the WWP1-mediated KLF5

destruction because YAP and TAZ and WWP1 can bind to the PY

motif of KLF5 [68, 69]. Overexpression of TAZ increased KLF5 and

its target FGF-BP expression, while downregulation of TAZ

and enhance EGFR stability, resulting in enhanced lung cancer

progression [67].

accumulated evidence has revealed that WWP1 expression level

is regulated by several factors and noncoding RNAs.
BAP1 targets KL5 for degradation via K48-linked ubiquitination in melanoma cells. KL5 enhanced malignant phenotypes and suppressed autophagy of melanoma cells via activation of PI3K–AKT–mTOR pathways [27]. BAP1 blocked WWP1-induced degradation of KL5 and led to upregulation of KL5 and promoting melanoma development [27]. In addition, BAP1 as a deubiquitilase enhanced cell proliferation and metastasis via deubiquitilating KL5 in breast cancer cells [73]. BAP1 interacted with KL5 and led to its stability and exerted its oncogenic function in breast cancer. Therefore, the relationships among BAP1, WWP1 and KL5 need to be further investigated.

**MiRNAs**

One study showed that miR-16-5p was increased in the feces in inflammatory bowel disease (IBD), including ulcerative colitis and Crohn’s disease, whereas miR-21-5p was highly expressed only in ulcerative colitis patients, indicating that these two miRNAs might be biomarkers for predicting IBD [71]. WWP1 could be a potential target of miR-21-5p and miR-16-5p in IBD, and promotes the initiation and progression of IBD-related colorectal cancer [71]. One group demonstrated that WWP1 is a direct target of miR-30a-5p in glioma [47]. WWP1 mRNA expression was negatively associated with miR-30a-5p expression in glioma specimens. Moreover, NF-kappaB p65 upregulated the expression of miR-30a-5p via interaction of NF-kappaB Rela subunit and the miR-30a-5p promoter region, leading to inhibition of WWP1 and promoting glioma malignant phenotype [47]. Interestingly, WWP1 upregulation also reduced miR-30a-5p expression and inhibited p65 expression in glioma cells. Therefore, a miR-30a-5p/WWP1/p65 feedback loop was exhibited to regulate development of glioma [47].

It has been identified that miR-129-5p and miR-129-3p targeted WWP1 in gastric cancer cells [43]. Moreover, miR-129-5p and miR-129-3p can interact with WWP1 mRNA at its CDS region. Furthermore, miR-129-5p and miR-129-3p repressed cell proliferation and migratory ability via suppression of WWP1 in gastric cancer [43]. WWP1 has been reported to be a direct target of miR-452 in prostate cancer cells [29]. Specifically, miR-452 expression was decreased in prostate cancer patients, while WWP1 was highly expressed in patients with prostate cancer. WWP1 expression was negatively correlated with miR-452 expression levels [29]. Moreover, patients with low expression of miR-452 had a poor survival rate in prostate cancer. Overexpression of miR-452 suppressed migratory and invasive capability of prostate cancer cells partly via down-regulation of WWP1 [29]. Targeting miR-452 might be a potential approach to regulating WWP1 for treating prostate cancer.

Overexpression of miR-584-5p suppressed proliferation of gastric cancer cells and increased apoptosis [72]. Moreover, WWP1 was identified as a direct downstream target of miR-584-5p in gastric cancer cells. Upregulation of WWP1 abolished the effects of miR-584-5p overexpression on gastric cancer cells, while depletion of WWP1 impaired the function of miR-584-5p inhibitors [72]. Consistently, WWP1 expression was negatively associated with miR-584-5p expression in gastric cancer specimens. Mechanistically, miR-584-5p decreased WWP1 expression, leading to accelerating senescence and activating TGF-β pathway in gastric cancer [72].

**LncRNA SNGH12**

LncRNA SNGH12 (small nucleolar RNA host gene 12) elevated cell proliferation and invasiveness via acting as a splicer of miR-129-5p and subsequent upregulation of WWP1 in laryngeal cancer cells [42]. WWP1 was positively governed by SNGH12 at the both protein and mRNA levels. In addition, WWP1 was negatively controlled by miR-129-5p in laryngeal cancer cells [42]. This study suggested that WWP1 was regulated by SNGH12/miR-129-5p axis in laryngeal cancer.

**Other upstream factors**

Estrogen promoted the interactions between ERβ, WWP1 and KL5, leading to promotion of KL5 degradation in prostate cancer cells [74]. Activating transcription factor 4 (ATF4) suppressed the expression of WWP1 mRNA under oxidative stress, leading to the stability of LATS1 and inactivation of YAP and promotion of cell death [75]. WWP1 autoinhibition was relieved via interacting with Smad7, leading to promoting TβRI degradation [54]. Upregulation of Smad7 inhibited the abundance of WWP1, whereas knockdown of Smad7 led to an increase of endogenous WWP1 [54]. Moreover, Smad7 expression triggered WWP1 polyubiquitination and degradation via blocking the interaction between C2 or WW and HECT domains [54]. Peptidyl-prolyl isomerase Pin1 interacted with p63a and impaired the binding between p63a and WWP1, resulting in inhibition of WWP1-mediated p63a degradation and promotion of cell proliferation and tumor formation [76]. WWP1 interacted with the cytoplasmic domain of Notch1 and Notch1 regulated the nuclear localization of WWP1 [70]. Moreover, the MYC gene is one of the most commonly deregulated oncogenic genes in the formation, development, and progression of human carcinomas [77]. Recently, Lee et al. [19] discovered that MYC gene significantly increased the expression level of WWP1, and WWP1 depletion markedly reactivated PTEN function in prostate cancer, resulting in the suppression of the PI3K–AKT signal pathway and MYC-mediated carcinogenesis.

**TARGETING WWP1 FOR CANCER THERAPY**

Bortezomib, a proteasome inhibitor, prevented development and bone metastasis via suppression of WWP1, Smurf1, and Smurf2 in prostate cancer [30]. Bortezomib inhibited the mRNA and protein levels of WWP1 in prostate cancer cells, leading to cell growth suppression [30]. Indole-3-carbinol (I3C) was reported to inhibit WWP1 via binding with the WPP1 HECT domain, leading to PTEN plasma membrane accumulation, suggesting that I3C might be a potent inhibitor of WWP1 [19]. DNA damage chemotherapeutic compounds increased the mRNA and protein levels of WWP1 [45]. We believe that more specific inhibitors of WWP1 will be discovered for targeted therapy of human cancer.

**CONCLUSIONS AND PERSPECTIVES**

In conclusion, a line of evidence has highlighted the significance of WWP1 in tumorigenesis mainly via regulating numerous substrate turnovers (Table 2). It is important to mention that WWP1 displays dual roles to promote or inhibit cancer initiation and progression. Although studies show the functions of WWP1 and underlying mechanisms, some crucial questions need to be answered to fully elucidate the molecular insight into WWP1-involved carcinogenesis. For instance, most studies discovered the substrates of WWP1 in cancer cells. What are the key drivers as the upstream factors to govern the expression of WWP1? Does WWP1 have a crosstalk with other NEDD4 family members? It is better to use WWP1 engineered mouse models to define the functions of WWP1 in oncogenesis. The special inhibitors of WWP1 are not available so far. How to use high-screening approaches to develop the special inhibitor of WWP1 for cancer therapy? Downregulation of WWP1 elevated the expression of DeltaNP63a in the MCF10A and 184B5 breast epithelial cells and caused resistance to doxorubicin-mediated apoptosis, but also upregulated TAP63a levels and caused apoptosis, and reduced resistance to doxorubicin and cisplatin in HCT116 colon cancer cells [45]. This study clearly suggested that WWP1 plays a different role in a context-dependent manner via targeting two types of p63 proteins for destruction [45]. Similarly, membrane HER4 was degraded by WWP1, while nuclear HER4 was destructed by the anaphase-promoting complex, indicating that WWP1 might target its substrates in specific cellular compartments [55]. Therefore, it is
pivotal to design and develop medicines targeting WWP1 in special tissues of cancer patients. These investigations will remarkably improve our understanding of WWP1-mediated tumorigenesis and promote the discovery of novel therapeutic strategies via regulation of WWP1 expression in cancers.

REFERENCES

1. Bedford L, Lowe J, Dick LR, Mayer RJ, Brownell JE. Ubiquitin-like protein conjugation and the ubiquitin-proteasome system as drug targets. Nat Rev Drug Discov. 2011;10:29–46.
2. Ciechanover A. Proteolyis: from the lysosome to ubiquitin and the proteasome. Nat Rev Mol Cell Biol. 2005;6:79–87.
3. Muratani M, Tansey WP. How the ubiquitin-proteasome system controls transcription. Nat Rev Mol Cell Biol. 2003;4:192–201.
4. Lipkowitz S, Weissman AM. RINGs of good and evil: RING finger ubiquitin ligases at the crossroads of tumour suppression and oncogenesis. Nat Rev Cancer. 2011;11:629–43.
5. Wang P, Dai X, Jiang W, Li Y, Wei W. RBR E3 ubiquitin ligases in tumorigenesis. Semin Cancer Biol. 2020;67:131–44.
6. Wang Z, Liu P, Inuzuka H, Wei W. Roles of F-box proteins in cancer. Nat Rev Cancer. 2014;14:233–47.
7. Uchida K, Kitagawa M, RING-, HECT-, and RBR-type E3 ubiquitin ligases: involvement in human cancer. Curr Cancer Drug Targets. 2016;16:157–74.
8. Bemassola F, Chillemi G, Melino G. HECT-type E3 ubiquitin ligases in cancer. Trends Biochem Sci. 2019;44:1057–75.
9. Bemassola F, Karin M, Ciechanover A, Melino G. The HECT family of E3 ubiquitin ligases: multiple players in cancer development. Cancer Cell. 2008;14:10–21.
10. Singh S, Ng J, Sivaraman J. Exploring the “Other” subfamily of HECT-E3 ligases for therapeutic intervention. Pharmacol Ther. 2021;224:107890.
11. Zou X, Levy-Cohen G, Blank M. Molecular functions of NEDD4 E3 ubiquitin ligases. Cell Death Disc. 2011;10:29–39.
12. Bedfor L, Lowe J, Dick LR, Mayer RJ, Brownell JE. Ubiquitin-like protein conjugation and the ubiquitin-proteasome system as drug targets. Nat Rev Drug Discov. 2011;10:29–46.
13. Ciechanover A. Proteolyis: from the lysosome to ubiquitin and the proteasome. Nat Rev Mol Cell Biol. 2005;6:79–87.
14. Martin-Serrano J, Eastman SW, Chung W, Bieniasz PD. HECT ubiquitin ligases link apoptosis and autophagy to promote melanoma progression. Exp Cell Res. 2016;342:163–72.
15. Zaarour RF, Chirivino D, Del Maestro L, Daviet L, Ata A. RING finger ubiquitin ligases. Biochem Biophys Res Commun. 2010;402:425–30.
16. Zou X, Levy-Cohen G, Blank M. Molecular functions of NEDD4 E3 ubiquitin ligases. Cell Death Disc. 2011;10:29–39.
17. de Bie P, Ciechanover A. Ubiquitination of E3 ligases: self-regulation of the ubiquitin-proteasome system. Curr Opin Cell Biol. 2015;36:787–98.
18. Martin-Serrano J, Eastman SW, Chung W, Bieniasz PD. HECT ubiquitin ligases link apoptosis and autophagy to promote melanoma progression. Exp Cell Res. 2016;342:163–72.
19. Zaarour RF, Chirivino D, Del Maestro L, Daviet L, Ata A. RING finger ubiquitin ligases. Biochem Biophys Res Commun. 2010;402:425–30.
20. Wang Z, Wang J, Li X, Xing L, Ding Y, Shi P, et al. Tumour-promoting activity of altered WWP1 expression in breast cancer and its utility as a prognostic indicator. J Pathol. 2008;216:93–102.
21. Shearwin-Whyatt L, Dalton HE, Foot N, Kumar S. Regulation of functional diversity remarkably improves our understanding of WWP1-mediated tumorigenesis and promote the discovery of novel therapeutic strategies via regulation of WWP1 expression in cancers.
22. Chen JJ, Zhang W. High expression of WWP1 predicts poor prognosis and associates with tumor progression in human colorectal cancer. Am J Cancer Res. 2018;8:256–65.
23. Xiong Y, Wu S, Du Q, Wang A, Wang Z. Integrated analysis of gene expression and genomic aberration data in osteosarcoma (OS). Cancer Gene Ther. 2015;22:524–9.
24. Lin JH, Hsieh SC, Chen YH, Tsai TL, Chou CC. C247 gene is a potential molecular target of human oral cancer. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;116:221–31.
25. Chen JJ, Zhang W. High expression of WWP1 predicts poor prognosis and associates with tumor progression in human colorectal cancer. Am J Cancer Res. 2018;8:256–65.
26. Zhang L, Wu Z, Ma Z, Liu H, Wu Y, Zhang Q. WWP1 as a potential tumor oncogene regulates PTEN-Akt signaling pathway in human gastric carcinoma. Tumour Biol. 2015;36:787–98.
52. Chen C, Sun X, Guo P, Dong XY, Sethi P, Cheng X, et al. Human Kruppel-like factor 5 is a target of the E3 ubiquitin ligase WWP1 for proteolysis in epithelial cells. J Biol Chem. 2005;280:41553–61.

53. Chen C. Regulation of Kruppel-like factor 5 by targeted protein degradation. Methods Mol Biol. 2010;647:267–77.

54. Cournaud T, Ferrand N, Elkhattouti A, Kumar S, Levy L, Ferrigno G, et al. Functional characterization of a WWP1/Tiad1 tumor-derived mutant reveals a paradigm of its constitutive activation in human cancer. J Biol Chem. 2015;290:21007–18.

55. Feng SM, Muralo-Okku RS, Hunter D, Sandahl MA, Caskey LS, Miyazawa K, et al. The E3 ubiquitin ligase WWP1 selectively targets HER4 and its proteolytically derived signaling isoforms for degradation. Mol Cell Biol. 2009;29:892–906.

56. Yeung B, Ho KC, Yang X. WWP1 E3 ligase targets LATS1 for ubiquitin-mediated degradation in breast cancer cells. PLoS ONE. 2013;8:e61027.

57. Chen C, Zhou Z, Guo P, Dong JT. Proteasomal degradation of the KLF5 transcription factor through a ubiquitin-independent pathway. FEBS Lett. 2007;581:1124–30.

58. Moren A, Imamura T, Miyazono K, Helin DH, Moustakas A. Degradation of the tumor suppressor Smad4 by WW and HECT domain ubiquitin ligases. J Biol Chem. 2005;280:22115–23.

59. Kim S, Ham S, Yang K, Kim K. Protein kinase CK2 activation is required for transforming growth factor beta-induced epithelial-mesenchymal transition. Mol Oncol. 2018;12:1811–26.

60. Shi Y, Riese DJ 2nd, Shen J. The role of the CXCL12/CXCR4/CXCR7 chemokine axis in cancer. Front Pharmacol. 2020;11:574667.

61. Dey A, Varelas X, Guan KL. Targeting the Hippo pathway in cancer, fibrosis, wound healing and regenerative medicine. Nat Rev Drug Discov. 2020;19:480–94.

62. Yu FX, Zhao B, Guan KL. Hippo pathway in organ size control, tissue homeostasis, and cancer. Cell. 2015;163:811–28.

63. Li Y, Zhou Z, Alimandi M, Chen C. WW domain containing E3 ubiquitin protein ligase 1 targets the full-length ErbB4 for ubiquitin-mediated degradation in breast cancer. Oncogene. 2005;28:2948–58.

64. Chen C, Zhou Z, Liu R, Li Y, Azmi PB, Seth AK. The WW domain containing E3 ubiquitin protein ligase 1 upregulates ErbB2 and EGF receptor through RING finger protein 11. Oncogene. 2008;27:6845–55.

65. Lee YR, Akaogi K, Suzuki T, Osakabe A, Yamaguchi C, Sunahara N, et al. Estrogen regulates tumor growth through a nonclassical pathway that includes the transcription factors ERbeta and KLF5. Sci Signal. 2011;4:ra22.

66. Rajesh K, Krishnamoorthy J, Gupta J, Kazimierczak U, Papadakis AI, Deng Z, et al. The eIF2alpha serine 51 phosphorylation-ATF4 arm promotes HIPPO signaling and cell death under oxidative stress. Oncotarget. 2016;7:51044–58.

67. Li C, Chang DL, Yang Z, Qi J, Liu R, He H, et al. Pin1 modulates p63alpha protein stability in regulation of cell survival, proliferation and tumor formation. Cell Death Dis. 2013;4:e943.

68. Hu X. The authors declare no competing interests.

69. Duffy MJ, O’Grady S, Tang M, Crown J. MYC as a target for cancer treatment. Cancer Treat Rev. 2021;94:102154.

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AUTHOR CONTRIBUTIONS

X.H., J.Y., Z.L., and RF searched literature regarding to WWP1 and carcinogenesis. X.H. made the figures. X.H., Z.W., and G.C. wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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