Functional roles of E3 ubiquitin ligases in gastric cancer (Review)

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Abstract. To date, >650 E3 ubiquitin ligases have been described in humans, including >600 really interesting new genes (RINGs), 28 homologous to E6-associated protein C-terminus (HECTs) and several RING-in-between-RINGs. They are considered key regulators and therapeutic targets of many types of human cancers, including gastric cancer (GC). Among them, some RING and HECT E3 ligases are closely related to the proliferation, infiltration and prognosis of GC. During the past few years, abnormal expressions and functions of many E3 ligases have been identified in GC. However, the functional roles of E3 ligases in GC have not been fully elucidated. The present article focuses on the functional roles of E3 ligases related to the proteasome in GC. In this comprehensive review, the latest research progress on E3 ligases involved in GC and elaborate their structure, classification, functional roles and therapeutic value in GC was summarized. Finally, 30 E3 ligases that serve essential roles in regulating the development of GC were described. Some of these ligases may serve as oncogenes or tumor suppressors in GC, whereas the pathological mechanism of others needs further study; for example, constitutive photomorphogenic 1. In conclusion, the present review demonstrated that E3 ligases are crucial tumor regulatory factors and potential therapeutic targets in GC. Therefore, more studies should focus on the therapeutic targeting of E3 ligases in GC.

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Abbreviations: Cbl, casitas B lineage lymphoma; GC, gastric cancer; HECT, homologous to E6-associated protein C-terminus; NEDD, neural precursor cell expressed developmentally downregulated protein; RBR, RING-in-between-RING; RING, really interesting new gene; RNF, RING finger domain; UPS, ubiquitin-proteasome system

Key words: gastric cancer, E3 ligases, targeted therapy, ubiquitin, signaling pathways

Contents

1. Introduction

Gastric cancer (GC) is the fourth most commonly diagnosed cancer and the third leading cause of cancer-related mortality worldwide (1). The pathogenic mechanisms and progression of GC are complex. Currently, well-known pathogenic factors include poor eating habits, chronic *Helicobacter pylori* infection and the misregulation of oncogenes or tumor suppressors (2). The 5-year survival rate of patients with GC is only 27.4% in China (3). The poor prognosis of GC is due to tumor invasion and metastasis (4,5), which are complex processes involving a series of cell biological regulations and requiring multi-step genetic mutations (6). The genes and their products involved in each step of tumor progression are potential prognostic markers and therapeutic targets. Among these biomarkers, E3 ligases play a crucial role in the proliferation, invasion and metastasis of GC (7).

The ubiquitin-proteasome system (UPS) is a common post-translational modification pathway involved in the regulation of cell survival and differentiation (8). The ubiquitination pathway is catalyzed by three types of key enzymes: Ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligases (E3) (9). E3 ligase is the most important component in the UPS owing to its specific ability to recognize target proteins and transfer ubiquitin to substrates for degradation (9,10). To date, >650 E3 ubiquitin ligases have been described in humans, and they can be subdivided into three different families: Homologous to E6-associated protein C-terminus (HECT), really interesting new gene (RING) and RING-in-between-RING (RBR) E3 ligases (11,12). However, RBR E3 ligases, an emerging group of E3 ligases that feature with characteristics of RING and HECT, have not been discovered in GC (13). Hence, RING and HECT E3 ligases are dysregulated in GC cells. Owing to the notable role of E3 ligases on the ubiquitination of tumor-associated

signaling molecules, such as the AKT pathway, targeting E3 ligases could be an efficient approach in cancer treatment (14). Recently, many new types of E3 ligases have been increasingly detected in GC, such as RNF6 and RNF38 (15,16). Herein, a comprehensive review of studies is presented to summarize the latest progress and treatment prospects.

2. Classification of E3 ubiquitin ligases

As aforementioned, only RING- and HECT-type E3 ligases are involved in GC (Table I). The RING family comprises >600 members (17). The RING finger domain is an important component of RING-type E3 ligases and can be sub-divided into two groups: Typical and atypical. The typical conserved region RING domain harbors a RING fold structure coupled with zinc ions. Another atypical type, called the U-box domain, possesses extremely similar RING folds but lacks cysteine residues, which affects zinc ions coordination (18). Both RING and U-box E3 ligases can function as monomers, homodimers, heterodimers or multiple subunits (17,19). The cullin-RING ligase (CRL) is a kind of multi-subunit RING E3 ligase that can be further divided into the S phase kinase associated protein 1 (SKP1)/cullin 1 (CUL1)/F-box protein complex (SCF), CUL2-elongin B/C-VHL or SOCS proteins (CRL2), CUL3-BTBs, CUL4-DDB1-DCAFs (CRL4), CUL5-elonginB/C-SOCS proteins, and the CUL7/F-box/WD repeat-containing 8 (FBXW8) subfamily of E3 ligases (20,21).

HECTs, the second largest E3 ligase family in humans, comprises 28 members and can be categorized into three subfamilies: Neural precursor cell expressed developmentally downregulated protein (NEDD)4 family, HECT domain-containing protein (HERC) family and 'other' HECT ligases (22). Studies to date have suggested that only the NEDD4 family and a member of the Other HECT ligase families are related to GC. The NEDD4 subfamily is the most characteristic family, including nine members in humans: NEDD4-1 (also known as NEDD4), NEDD4-2, Itchy E3 ubiquitin-protein ligase, SMAD ubiquitin regulatory factor (Smurf)1, Smurf2, WW domain-containing E3 ubiquitin protein ligase (WWP)1, WWP2, NEDD4-like 1 (NEDL1) and NEDL2 (23). The Other HECT ligase family includes 13 members, and they all contain different domains in addition to the HECT domain.

The structure and functional significance of RINGs in GC will be described in the following paragraphs in the order of monomer, dimer and multiple subunit E3 ligases, and the HECTs will be described in the order of the NEDD4 family, HERC family and other HECT ligases.

3. Structure and function of RING-type E3 ligases in GC

Monomeric RING domain E3 ligase

RING finger (RNF) domain subfamily. The RNF protein family contains a large number of members that are associated with several types of digestive system tumors, such as colorectal cancer and hepatocellular carcinoma (24,25). RNFs also play a vital role in the occurrence of GC. RNF6 encodes a 685-amino-acid protein with a coiled-coil domain at the N-terminus and a RING-H2 finger at the C-terminus (26). RNF38 shares a similar structure with RNF6 (27), and previous studies have shown that RNF6 and RNF38 are overexpressed in GC and regulate GC cell growth. Both RNF6 and RNF38 induce polyubiquitination of tyrosine-protein phosphatase non-receptor type 6 and subsequently enhance STAT3 signaling, which promotes the proliferation of GC cells (Fig. 1) (15,16).

RNF26, which is located in the endoplasmic reticulum, is a polypeptide of 433 amino acids with an N-terminal leucine zipper domain and a C-terminal RING finger domain (28,29). The expression level of RNF26 is upregulated in several types of human cancer cell lines, including HL-60, HeLa S3, SW480 and MKN7 cells (28). As the substrate protein of RNF26, the mediator of interferon regulatory factor 3 can be ubiquitinated and regulate the innate antiviral response (30). However, the functional mechanism of RNF26 in GC has not been fully elucidated.

Similar to RNF6, RNF26 and RNF38, RNF185 also acts as an oncogene in GC, but with distinct subcellular localization and a distinct mechanism of action. RNF185 localizes to the mitochondria, contains a C3HC4-type RING domain and two transmembrane domains (31). PRA1 family protein 3 (JWA) is a multifunctional cytoskeleton-binding protein induced by all-trans retinoic acid. The function of JWA involves enhancing intracellular defenses against H_2O_2-induced oxidative stress and reducing cell apoptosis (32). RN F185 can downregulate the expression of JWA and promote GC cell migration (Fig. 1) (33). Generally, higher expression of RNF185 is associated with a worse prognosis of GC.

RNF43 and zinc/RING finger 3 (ZNRF3) are homologous proteins that have antagonistic effects in combination with leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5) (34). The Lgr5 protein, a member of the G-protein coupled receptor family of proteins, was identified as a novel gastrointestinal stem cell marker (35). Moreover, Lgr5-positive gastric stem cells are cancer-initiating cells able to drive GC cell self-renewal, which contributes to malignant progression (35-37). RNF43 negatively regulates the Wnt/β-catenin pathway by recognizing Lgr5 and markedly downregulating the expression of Lgr5 protein (34). A previous study demonstrated that ZNRF3 acts as a tumor suppressor by downregulating the expression levels of β-catenin and transcription factor 4 protein (38). RNF43/ZNRF3 mediates the ubiquitylation of seven transmembrane domains of frizzled receptors and subsequently inhibits the proliferation of GC cells (39). A few large-scale genomic analyses have reported RNF43 mutations in different cancer types, including GC (40). Whole-genome sequencing revealed that RNF43 is mutated in 4.8% of microsatellite-stable and 54.6% of microsatellite-unstable tumors (41). The mutational landscape of RNF43 may provide a new approach to facilitate genome-guided personalized therapy in GC. Overall, RNF43/ZNRF3 is a tumor suppressor and a potential therapeutic target for GC. Therefore, five members of the RNF subfamily participate in regulating the development of GC, where RNF6, RNF26, RNF38 and RNF185 function as carcinogenic factors, while RNF43/ZNRF3 acts as a tumor suppressor.

Membrane-associated RING-CH (MARCH)8. MARCH8 is a member of the MARCH subfamily. A recent study identified 11 E3 ligases that contained the RING-CH domain and

Table I. Characteristics of E3 ligase associated with gastric cancer.

Author, year	Gene	Family/ Subfamily	Expression	Substrates	Pathway	Refs
Che *et al*, 2017; Xu *et al*, 2017; Zhang *et al*, 2009	Cbl-b	RING/Cbl	Up/downregulated	IGF-1R; c-Src,	IGF-1R	(67-69)
Kashima *et al*, 2012	CHFR	RING/NA	Downregulated	PARP-1	NA	(74)
Wang *et al*, 2017	MARCH8	RING/MARCH	Downregulated	DR4	JWA/MARCH8/DR4	(43)
Ko A *et al*, 2012	MKRN1	RING/NA	Downregulated	p14ARF	p14ARF-associated	(76)
Yang *et al*, 2017	PIRH2	RING/PIRH	Upregulated	P53	p53	(59)
Zhang *et al*, 2018	RNF6	RING/RNF	Upregulated	SHP-1	SHP-1/STAT3	(15)
Huang *et al*, 2018	RNF38	RING/RNF	Upregulated	SHP-1	SHP-1/STAT3	(16)
Gao *et al*, 2017; Zhou *et al*, 2013	RNF43	RING/RNF	Downregulated	β-catenin; Lgr5	Wnt/β-catenin/TCF	(34,38)
Qui *et al*, 2018	RNF185	RING/RNF	Upregulated	JWA	NA	(33)
Zhou *et al*, 2014	TRIM59	RING/TRIM	Upregulated	P53	p53	(49)
Chi *et al*, 2009	MDM2	RING/MDM	Upregulated	P53; RUNX3	MDM2/ITGB1	(80)
Liu *et al*, 2015	CHIP	RING/NA	Downregulated	TRAF2	TRAF2/NF-κB	(85)
Bai *et al*, 2011	Cullin1	RING/SCF	Upregulated	p27	NA	(112)
Li *et al*, 2016	FBXL2	RING/SCF	Downregulated	FoxM1	NA	(101)
Cen *et al*, 2014; Wu *et al*, 2015	FBXL5	RING/SCF	Downregulated	Cortactin; Snail1	NA	(102,103)
Zou *et al*, 2018	FBXO31	RING/SCF	Downregulated	Snail1	NA	(104)
Huang *et al*, 2018; Kuai *et al*, 2019; Zhou *et al*, 2014	FBXW7	RING/SCF	Downregulated	c-Myc; RhoA; Brg1; GFI1	RhoA	(92-94)
Gao *et al*, 2013; Wang *et al*, 2016	β-TRCP	RING/SCF	Upregulated	PHLPP1; FOX3	AKT	(99,100)
Li *et al*, 2012	COP1	RING/CRL4	Upregulated	p53	NA	(125)
Ding *et al*, 2018	pVHL	RING/CRL2	Downregulated	NEK8	NA	(119)
Kim *et al*, 2008	NEDD4-1	HECT NEDD4	Upregulated	PTEN	AKT	(130)
Tao *et al*, 2017	SMURF1	HECT NEDD4	Upregulated	DAB2IP	PI3K/AKT	(139)
Zhang *et al*, 2015	WWP1	HECT NEDD4	Upregulated	PTEN	AKT	(132)
Yang *et al*, 2016	UBR5	HECT/Other	Upregulated	GKN1	p16/Rb	(140)

NA, not applicable.

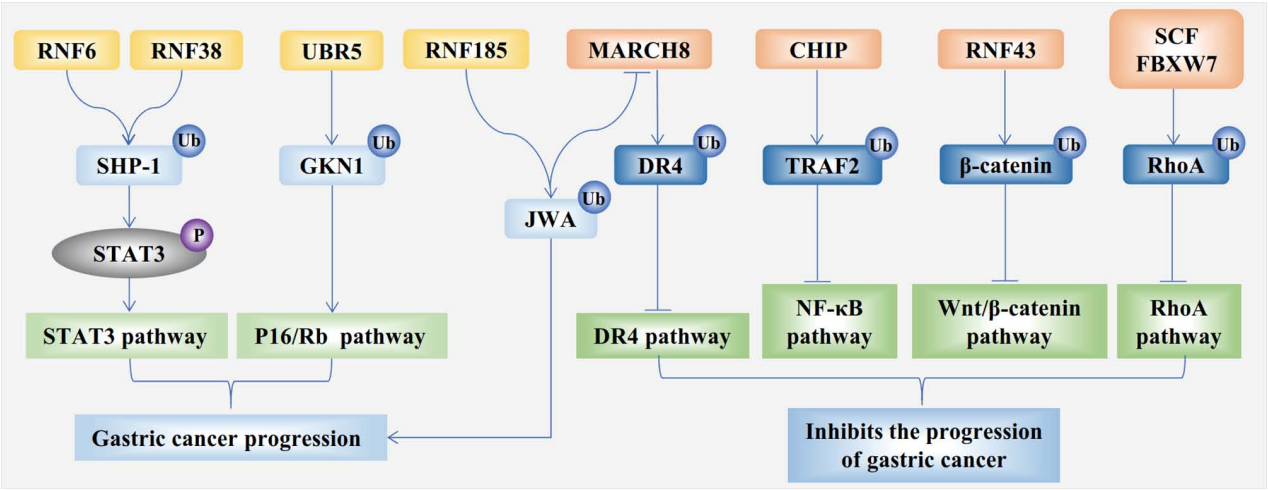

Figure 1. Regulation of important signaling pathways by E3 ubiquitin ligases in gastric cancer. The solid arrows represent activation, and flat-headed arrows represent inhibition. RNF6 and RNF38 induce polyubiquitination of SHP-1 and subsequently enhance STAT3 signaling in GC. GKN1 can be ubiquitinated by UBR5 E3 ligase and then activates the P16/Rb pathway. RNF185 can promote the ubiquitination of JWA and promote GC progression, and loss of JWA decrease the expression of MARCH8 in GC. MARCH8 promotes DR4 ubiquitination for degradation and then inhibites the progression of GC. CHIP facilitates TRAF2 ubiquitination for degradation and then inactivates NF-κB signaling. RNF43 and SCF-FBXW7 inhibit the progression of GC by inactivate Wnt/β-catenin pathway and RhoA pathway. CHIP, C-terminus of Hsp70-interacting protein; DR4, ubiquitination of death receptor 4; FBXW7, F-box/WD repeat-containing protein 7; GKN1, gastrokine 1; JWA, PRA1 family protein 3; MARCH8, membrane-associated RING-CH8; Rb, retinoblastoma protein; RNF, RING finger domain; SCF, Skp1/cullin 1/F-box protein complex; SHP-1, tyrosine-protein phosphatase non-receptor type 6; TRAF2, TNF receptor-associated factor 2; UBR5, ubiquitin protein ligase E3 component n-recognin 5. The solid arrows means activate, and the flat tipped arrows means inhibit.

were named the MARCH1-11 subfamily (42). The structure of MARCH8 contains the RING-CH domain and transmembrane domains. JWA is a downstream protein of RNF185 ligase (33); it promotes the ubiquitination of death receptor 4 by increasing the expression of MARCH8 in GC cells, thereby reducing tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis (Fig. 1) (43). Moreover, MARCH8 induces the apoptosis of GC cell lines by inactivating the PI3K and β-catenin/STAT3 signaling pathways (44). In summary, MARCH8 is a tumor suppressor in GC.

Tripartite motif-containing (TRIM) subfamily. TRIM proteins form a subfamily that regulates multiple cellular processes. TRIMs share a common N-terminal tripartite domain, a RING domain, one or two B-boxes and coiled-coil domains (45). To date, three E3 ligases have been shown to be involved in the development and metastasis of GC. They are TRIM28 (also known as KAP1 or TIF1-β), TRIM29 and TRIM59 (46-49). TRIM28 is a universal transcriptional corepressor (50). The overexpression of TRIM28 causes peritoneal carcinomatosis and poor prognosis in GC (47). Similar to TRIM28, the expression of TRIM29 is also upregulated in GC; the high expression of TRIM29 mRNA may be an independent predictor of lymph node metastasis and depth of invasion (48). TRIM59 has been reported in several human tumors and acts on diverse signaling pathways, such as the focal adhesion kinase/AKT/matrix metalloproteinases pathway in epithelial ovarian cancer (51), the PI3K/AKT/mTOR pathway in human cholangiocarcinoma (52), the Wnt/β-catenin signaling pathway in neuroblastoma (53), the NF-κB pathway in non-small cell lung cancer (54) and the p53 signaling pathway in GC (49). The p53 signaling pathway can be negatively regulated by the E3 ligase TRIM59, which enhances the ubiquitination and subsequent degradation of p53 (Fig. 2). TRIM59 might promote gastric carcinogenesis through this mechanism (53). In summary, TRIM28, TRIM29 and TRIM59 play oncogenic roles in gastric tumorigenesis, but only the regulatory mechanism of TRIM59 has been elucidated.

RING finger and CHY zinc-finger domain containing 1 (PIRH2). PIRH2 E3 ligases are crucial negative regulators of p53 (Fig. 2). In addition to p53, many other proteins, such as p63, p73, c-Myc, p27, DNA polymerase Eta and checkpoint kinase 2, can be ubiquitylated by PIRH2 (55). PIRH2 plays an important role in the regulation of many types of tumors, such as glioma, lung cancer and breast cancer (56-58). In GC, PIRH2 is a key E3 ligase of p53 ubiquitination, and silencing of PIRH2 causes p53 protein accumulation (59). This study also demonstrates that a microRNA (miR)-100-RNF144B-PIRH2-p53-dependent pathway might be a novel mechanism of ubiquitin-mediated p53 degradation in GC cells (59).

CBL subfamily. CBL proteins contain two highly conserved domains: The N-terminal tyrosine kinase-binding domain and the C3HC4 RING finger domain (60). The mammalian CBL family consists of Cbl, Cbl-b and c-Cbl ligases (61). The three members have similar functions, perhaps due to the specific structure. The c-Cbl protein was the first CBL family protein discovered, followed by Cbl-b and Cbl (62). Previous studies have confirmed that all three CBL proteins are closely associated with GC. In 2000, a study reported that the c-Cbl protein was frequently tyrosine phosphorylated in a tumor-specific manner in human GC tissues (63). Subsequently, c-Cbl was found to be involved in stomach carcinogenesis by connecting with the EGFR system (64).

Cbl-b has a significant impact on the prognosis and drug sensitivity of GC, and a previous study showed that Cbl-b is an oncogene (64). However, subsequent studies provide different

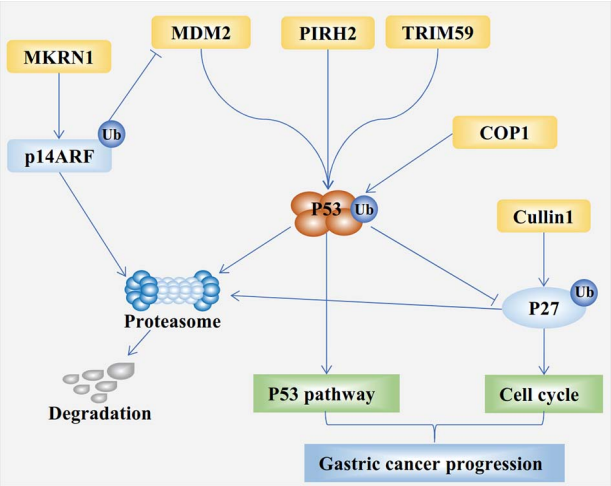

Figure 2. Regulation of the p53 signaling pathway by binding and ubiquitylation of tumor-regulating proteins in GC. The solid arrows represent activation, and flat-headed arrows represent inhibition. MKRN1 induces the ubiquitination and degradation of p14ARF and then downregulates the expression of MDM2. MDM2, PIRH2, TRIM59 and COP1 directly bind to p53, and they promote p53 degradation in the UPS to promote GC progression. Cullin1 facilitates P27 ubiquitination for degradation and then regulates cell cycle of GC. GC, gastric cancer; CRL4, cullin-RING ligase 4; COP1, constitutive photomorphogenic 1; MDM2, MDM2 proto-oncogene; MKRN1, makorin RING finger protein 1; p14ARF, tumor suppressor ARF; PIRH2, RING finger and CHY zinc-finger domain-containing 1; SPA1, suppressor of PHYA-1; TRIM59, tripartite motif; Ub, ubiquitin; UPS, ubiquitin-proteasome system. The solid arrows means activate, and the flat tipped arrows means inhibit.

perspectives, in which Cbl-b is found to enhance the sensitivity to 5-fluorouracil and cetuximab in GC cells through the ubiquitination pathway (65,66). Other studies have also indicated that Cbl-b inhibits tumor metastasis and growth in multiple drug-resistant (MDR) gastric and breast cancer cells, as well as increasing the sensitivity of MDR cells to anticancer drugs (67-69). These findings provide a new research direction for the chemotherapy and targeted therapy of GC.

Cbl, in conjunction with the EGFR system, might be related to gastric carcinogenesis and metastasis (70). In summary, Cbl and c-Cbl are oncogenes in GC, whereas Cbl-b can act as an oncogene or tumor suppressor, and only its regulatory mechanism has been well clarified among the three members.

Checkpoint with forkhead and RING finger domains protein (CHFR). Previous studies have reported that the aberrant methylation of CHFR promotes the development of GC (71,72). As an E3 ligase, CHFR contains an N-terminal forkhead-associated domain, a central RING domain and a C-terminal cysteine-rich region (73). PARP-1 may be a substrate by CHFR for ubiquitination and degradation in GC; this process leads to cell cycle arrest before entering mitosis and inhibits the proliferation of GC cells (74). Thus, CHFR functions as a tumor suppressor in GC.

Makorin-1 (MKRN1) and FGF-induced in gastric cancer (FIGC). These two E3 ligases are rarely mentioned in GC. MKRN1 was first identified owing to its interaction with human telomerase reverse transcriptase (TERT) and modulation of telomere length homeostasis (75). The MKRN1-mediated ubiquitination of tumor suppressor ARF (p14ARF) was described in 2011 (76). MKRN1 induces the ubiquitination and degradation of p14ARF and downregulated p14ARF expression (Fig. 2) (76). Furthermore, MKRN1 overexpression was associated with well-differentiated gastric carcinoma, whereas p14ARF overexpression was associated with poorly differentiated gastric carcinoma. FIGC, a novel FGF-induced ubiquitin-protein ligase, consists of an N-terminal RING finger module and proline-rich region at the C-terminus. Only one study has shown that FIGC probably functions as an E3 ligase and is implicated in carcinogenesis through the dysregulation of fibroblast growth modulator (77). In brief, further research is needed to confirm the mechanism of these two E3 ligases in GC.

Dimeric RING domain E3 ligases

MDM2. Dimeric RING domain E3 ligases can be classified into homodimers and heterodimers. MDM2, a heterodimeric RING ligase, was originally identified as a ubiquitin ligase E3 that promotes the degradation of tumor suppressor p53 (Fig. 2) (78). Subsequent studies suggest that MDM2 can ubiquitinate and degrade multiple signaling molecules in GC, including p53, forkhead box protein O3A (FOXO3A) and runt-related transcription factor 3 (RUNX3) (78-81). Human hTERT can interact with MDM2 and dramatically increase the ubiquitination of FOXO3A, resulting in the invasion of GC cells (79). RUNX3 is known as a tumor suppressor (82). MDM2 ligases can recognize Lys94 and Lys148 of RUNX3 and decrease the expression levels of RUNX3 (80). Overall, MDM2 acts as a carcinogenic factor in GC by affecting different signaling proteins.

C-terminus of Hsp70-interacting protein (CHIP). CHIP includes a C-terminal U-box domain and an N-terminal tetratricopeptide repeat domain, which have E3 ubiquitin ligase activity and interact with the molecular chaperones Hsc70-Hsp70 and Hsp90, respectively (83). CHIP is characterized as a homologous dimeric RING ligase and antioncogene in human cancer (15,84). The U-box domain of CHIP facilitates TNF receptor-associated factor 2 ubiquitination for degradation and then inactivates NF-κB signaling (Fig. 1). A previous study also showed that CHIP expression prevents the angiogenesis and metastasis of GC (85). Above all, CHIP overexpression is correlated with good prognosis in GC patients, and targeting CHIP may be a new approach in GC therapy.

Multi-subunit RING domain E3 ligases

SCF subfamily. The CRL1 complex comprises SKP1, CUL1, RING box1 (RBX1) and a member of the F-box protein family (86). The F-box protein family can be further categorized into three subclasses: i) FBXW; ii) F-box and leucine-rich repeat (FBXL); and iii) F-boxes containing other domain motifs (FBXO) proteins. Each subunit of SCF has unique features as follows: i) CUL1 serves as a rigid molecular scaffold protein; ii) RBX1 contains a RING finger domain for the recruitment of E2 enzyme; iii) SKP1 functions as an adaptor; iv) F-box proteins act as a substrate-determining component (86,87), many of them function as E3 ligase and will be discussed in detail below.

FBXW proteins. The WD repeat domain comprises a 44-60 residue sequence that typically contains the GH dipeptide 11-24 residues from its N-terminus and the WD dipeptide at the C-terminus (88). This class of E3 ligases mainly recognizes proteins involved in cell cycle regulation and tumorigenesis, thereby regulating cancerous growth. FBXW7 and β-transducin repeat-containing protein (β-TRCP) are directly correlated with the progression of GC in the form of E3 ligases (89,90).

FBXW7 is a well-characterized SCF in GC and facilitates the destruction of oncogenic proteins, such as c-Myc, transforming protein RhoA (RhoA), transcription activator BRG1 (Brg1) and zinc-finger protein GFI-1 (GFI1) (90-93). These targeted proteins all govern gastric tumorigenesis; for example, Brg1 promotes the metastasis of GC (92), RhoA has been implicated in gastric tumorigenesis (Fig. 1) (94), and GFI1 promotes GC cell proliferation as an oncoprotein (93). FBXW7 is also regulated by several upstream signaling molecules. Previous studies indicated that microRNAs and long noncoding RNAs are involved in the occurrence and development of GC by altering the expression of FBXW7 (95,96). Therefore, FBXW7 is a complex tumor suppressor in GC because of its involvement in numerous upstream and downstream signals.

β-TRCP has two distinct isoforms, β-TRCP1 and β-TRCP2, which share similar biochemical properties (97). It was reported that β-TRCP1 and β-TRCP2 were predominantly expressed in the stomach and the small intestine, respectively. A previous study showed that β-TRCP2 might promote gastric carcinogenesis through the activation of the Wnt signaling pathway (98). Moreover, β-TRCPs participate in the regulation of the AKT signaling pathway (Fig. 3) and epidermal growth factor receptor/glycogen synthase kinase-3β/FOXP3 axis through the ubiquitination of PH domain leucine-rich repeat-containing protein phosphatase 1 and FOX3, respectively (99,100). One previous study has reported that β-TRCP can serve as a tumor suppressor or oncoprotein in the etiology of a variety of cancers depending on the type of tumor tissue (99). Nevertheless, β-TRCP might serve as an oncoprotein in GC.

FBXL and FBXO proteins. FBXL proteins contain leucine-rich repeat sequences. FBXL2 and FBXL5 exhibit similar characteristics in GC (101,102). FBXL2 promotes the ubiquitination and degradation of FOXM1, which then inhibits GC proliferation (101). Similarly, FBXL5 can also suppress GC cell migration by the ubiquitination-mediated destruction of Cortactin and Snail1 (102,103). FBXO31, a member of the third class of the F-box protein family, can also target Snail1 for its ubiquitylation and degradation (104). Hence, FBXL2, FBXL5 and FBXO31 exert tumor inhibitory roles in GC.

CUL1. CUL1 is a member of the CUL family and acts as a scaffold protein of the SCF ubiquitin E3 ligase (105). CUL1 is modified by the ubiquitin-like protein NEDD8 and enhances the activity of SCF ligases to p27 (106). Several studies have shown that diverse types of human malignant tumors are related to CUL1. CUL1 can facilitate cell proliferation in osteosarcoma, breast cancer, prostate cancer and lung cancer *in vitro* and *in vivo* (107-110). In addition, the co-expression of CUL1 and CUL2 induces the initiation of carcinogenesis in colorectal cancer by arresting p53-positive colon cells in the G1 phase of the cell cycle (111). Before these findings, immunohistochemistry results suggested that high expression levels of CUL1 were detected in 60% of all GC tissues, in a study of 792 patients (112). Further *in vitro* studies showed that increased CUL1 expression was correlated with poor patient survival by decreasing p27 expression (112) (Fig. 2). Therefore, CUL1, an oncoprotein, can be regarded as a prognostic biomarker of GC.

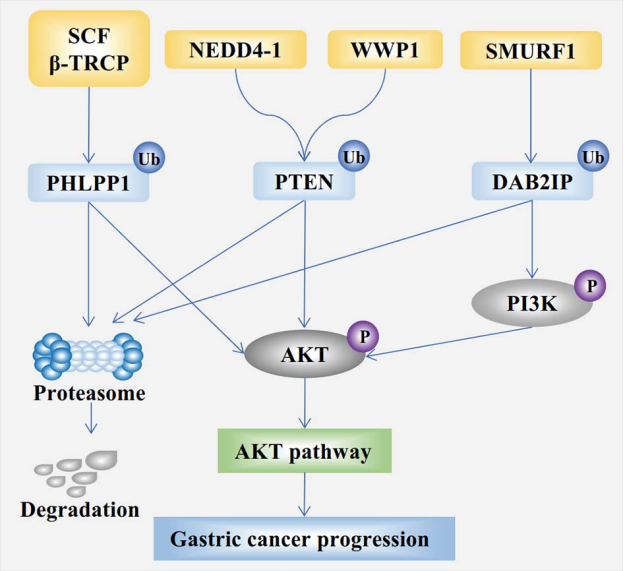

Figure 3. Regulation of the PI3K/AKT pathway by binding and ubiquitylation of tumor-regulating proteins in gastric cancer. The solid arrows represent activation. β-TRCP promotes PHLPP1 ubiquitination for degradation and then regulates the phosphorylation of AKT. NEDD4 and WWP1 have the similar functions by promoting the ubiquitination of PTEN. SMURF1 regulates the phosphorylation of PI3K and AKT by ubiquitinating DAB2IP. β-TRCP, β-transducin repeat-containing protein; DAB2IP, DAB2 interacting protein; NEDD4, neural precursor cell expressed developmentally downregulated protein 4; PHLPP1, PH domain leucine-rich repeat-containing protein phosphatase 1; SCF, Skp1/cullin 1/F-box protein complex; SMURF1, Smad ubiquitin regulatory factor 1; WWP1, WW domain containing E3 ubiquitin protein ligase 1; Ub, ubiquitin.

Von Hippel-Lindau disease tumor suppressor (pVHL). Von Hippel-Lindau disease was first considered to be a heritable cancer syndrome characterized by retinal and neuronal hemangioblastoma owing to a mutation in the VHL gene (113). Although the pVHL protein itself displays no enzymatic activity, pVHL functions as a substrate recognition subunit in CRL2 E3 ligases after binding with the elongin and CUL proteins (114). pVHL is a tumor suppressor in renal cell carcinoma (RCC) (115,116). Serine/threonine-protein kinase NEK8 is a serine/threonine-specific protein kinase family member that serves a role in the progression of mitosis and carcinogenesis (117). NEK8 is reported to be a novel target of pVHL in the regulation of RCC and GC (118,119); pVHL promotes NEK8 protein degradation via the proteasome-ubiquitin pathway in GC cells (119). In summary, the E3 ligase pVHL targets NEK8 and inhibits the proliferation, colony formation and migration of GC (119).

COP1. COP1 protein structure comprises an N-terminal RING finger region, a coiled-coil domain and seven WD40

repeats at its C-terminus (120). COP1 is well studied in plants, but it has been little studied in humans. COP1 is known as a central repressor in the light signaling pathway and forms a complex with suppressor of PHYA-1 (COP1-SPA1) (121). This complex interacts with the CUL4-DDB1 ligase, which belongs to the CRL4 family, to form CUL4-DDB1$^{COP1-SPA1}$ ligases in plants (122). However, the catalytic mechanism of COP1 has not been fully elucidated in humans. Previous studies revealed that the ubiquitin ligase COP1 promoted the progression of multiple cancer types *in vitro*, including GC, by downregulating the expression of p53 (Fig. 2) (123-125). The role of COP1 in GC is controversial (123-126). One previous study indicates that low COP1 expression resulted in poorer prognoses in patients with GCs (126); however, more studies have suggested that COP1 functions as an oncogene (123-125).

In summary, there are eight multi-subunit RING domain E3 ligases associated with GC, including six SCFs, one CRL2 ligase and one CRL4 ligase. Among these eight CRLs, β-TRCP, CUL1 and COP1 are oncoproteins, whereas FBXW7, FBXL2, FBXL5, FBXO31 and pVHL act as tumor suppressors.

4. Structure and function of HECT-type E3 ligases in GC

NEDD4 subfamily. The E3 ligases of the NEDD4 subfamily are characterized by an N-terminal C2 domain responsible for subcellular localization, between two and four WW domains that recruit substrates and a HECT domain at the C-terminus (127). Three NEDD4 subfamily members might be related to GC, including NEDD4-1, WWP1 and Smurf1, and they will be elaborated below.

NEDD4-1. NEDD4-1 contains four WW domains and is an ancestral member of the NEDD4 family (23). Previous studies have indicated that NEDD4-1 is frequently overexpressed in several types of human cancers, including hepatocellular carcinoma, lung cancer and gastrointestinal cancer (128-130). A range of tumor suppressors can be ubiquitinated by NEDD4-1, including PTEN, c-Myc and large tumor suppressor kinase 1 (128,131). Immunohistochemical analysis conducted by Kim *et al* (130) showed that NEDD4-1 is overexpressed in colorectal and gastric carcinomas. NEDD4-1 was also found to promote GC cell migration and invasion (129). As a carcinogenic factor, the targets of NEDD4-1 remain unclear in human GC, and further studies are needed to explore this research topic.

WW domain-containing 1 (WWP1). WWP1 is another GC-related member of the NEDD4 subfamily that also has four WW domains. Similar to NEDD4-1, WWP1 has been revealed as a versatile E3 ligase with a large repertoire of substrates (23). In GC cell lines, WWP1 is overexpressed, and is closely associated with worse survival by regulating the PTEN-AKT signaling pathway in patients with GC (Fig. 3) (132). The overall survival rate of patients who were positive for WWP1 protein was 25.9%, whereas it was 66.0% in patients who without WWP1 protein in China in 2015 (132). Subsequent studies further confirmed that WWP1 might play a role as an oncogene in GC. miR-584-5p, miR-129-5p and miR-129-3p were found to suppress WWP1 protein expression and inhibit the proliferation of GC *in vivo* and *in vitro* (133,134). These findings suggested that WWP1 might be a valuable prognostic marker and potential target in the treatment of GC.

Smurf1. Smurf1 was first recognized in selective interactions with receptor-regulated SMADs, which led to its initial naming (135). Smurf1 contains two WW domains and negatively regulates the transforming growth factor-β/bone morphogenetic protein signaling (136). DAB2-interacting protein (DAB2IP), a tumor suppressor, is known to be downregulated in GC (137); Smurf1 significantly promotes the ubiquitination-dependent degradation of DAB2IP (Fig. 3) (138). In addition, a subsequent study concluded that the Smurf1/DAB2IP signaling axis has an important impact in GC (139). Overall, Smurf1 might act an oncoprotein in GC.

Other HECT ligases

UBR5. UBR5 is the only member of Other HECT ligase family that serves a role in regulating GC cell growth (140). Structurally, UBR5 is composed of an N-terminal ubiquitin-associated domain, two nuclear localization signals, a ubiquitin recognin box domain, a C-terminal poly(A)-binding domain and a HECT domain at its far C-terminus (141). A previous study showed that UBR5 gene mutations occur in 27.8% of GC and 23.3% of colorectal cancer (142). Gastrokine 1, a gastric tumor suppressor, can be downregulated by UBR5 E3 ligase (Fig. 1), and the overexpression of UBR5 is associated with poor overall and disease-free survival (140). Thus, UBR5 may serve as a carcinogenic agent and a prognostic factor in GC.

5. Therapeutics targeting E3 ligases in GC

E3 ligases have potent effects on the origin, progression and prognosis of GC through a series of signaling pathways. E3 ligase-targeting molecules and drugs may provide a new approach to GC treatment. Bortezomib was the first proteasome inhibitor approved by the US Food and Drug Administration in multiple myeloma (143). *In vitro*, bortezomib has a significant negative effect on the growth of GC cells (144). It is possible that bortezomib may become a common adjuvant therapeutic target in GC because it has a significant negative effect on the proliferation of GC cells (144). However, only a few E3 ligase-targeting molecules have the ability to suppress the progression of GC.

APG115 has been identified as a novel inhibitor of MDM2 ligase, and its potential for treating GC has been shown *in vitro* and *in vivo* (145). *In vitro*, APG115 inhibited the proliferation of GC cell lines that harbored MDM2 expression by downregulating the mRNA expression of MDM2. In a xenograft mouse model, APG115 contributed to a smaller GC tumor size and enhanced the effect of radiotherapy. As previously mentioned, MDM2 downregulates several tumor suppressors in GC. Consequently, the MDM2 inhibitor APG115 may be applied for GC treatment in the future.

Nutlin proteins were identified in 2004 as the first selective small molecules of MDM2, which could antagonize p53-MDM2 binding (146). Among the nutlins, only nutlin-3 represents a promising therapeutic candidate for drug development in human cancer (147). Over the past decade, it has been confirmed that nutlin-3 can induce cell growth arrest and apoptosis in a number of cancer cell types (148,149). In

p53-defective cancer cells, there is a synergistic effect between nutlin-3 and bortezomib; cotreatment with bortezomib and nutlin-3 significantly induce paraptosis and cell death (150). Nutlin-3 has not been used in the treatment of GC; however, the antitumor activity of nutlin-3 against GC cells has been demonstrated *in vivo* and *in vitro* (151). It has been reported that nutlin-3 induces G1 arrest in MKN-45 and SNU-1 gastric adenocarcinoma cell lines *in vitro*, and the activation of p53 by nutlin-3 effectively increased the incidence of apoptosis in wild-type p53 GC cells. *In vivo*, the combined treatment of nutlin-3 and fluorouracil led to a more potent inhibitory effect on the tumor growth of experimental animals compared with treatment with each agent alone. Overall, nutlin-3 is a broad-spectrum antitumor agent and has the potential to be used in the treatment of GC by targeting the E3 ligase MDM2.

Triptolide is a compound purified from tripterygium wilfordii that exhibits antitumor effects in GC (152-155). In 1991, Chinese scholars found that triptolide had antitumor activity in a variety of cancer cell lines, including GC (152). A subsequent study demonstrated that triptolide treatment of GC cells containing wild-type p53 gene resulted in a significant inhibitory effect on cell growth, whereas GC cells with mutant p53 did not exhibit this effect (153). Another study indicated that this p53-dependent antitumor activity was achieved by inhibiting the overexpression of MDM2 (154). Moutan cortex is another Traditional Chinese Medicine that can also induce apoptosis through the MDM2-p53-dependent pathway in GC cells (155). Therefore, Chinese herbs, such as triptolide and moutan cortex, might be potential anticancer agents for GC.

MLN4924, a neddylation inhibitor, is an indirect inhibitor of CRL E3 ligases. MLN4924 acts as a promoter of apoptosis and is a potential anticancer drug in diverse types of human cancers, including GC (156). It has been reported that MLN4924 downregulates the expression of CRLs and then suppresses the growth of GC cells. There are some other small molecules that can also target E3 ligase. miR-223 can target FBXW7 and downregulate its expression, so drugs that promote degradation of miR-223 may be useful in patients with GC (157). As aforementioned, WWP1 is an oncoprotein in GC, and miR-584-5p, miR-129-5p and miR-129-3p suppress WWP1 protein expression and inhibit the proliferation of GC (133,134). Thus, miRNAs may also be a research direction for E3-targeted therapy for GC. In summary, there are still no drugs targeting E3 used in the clinical treatment of GC, and the effects of the compounds mentioned above are still in the research stage.

6. Conclusions and perspectives

Over the past few years, an increasing number of E3 ligases have been described as tumor regulators in GC. The present review summarizes approximately thirty types of E3 ligases that play essential roles in regulating the development of GC, including RING and HECT ligases. The function and significance of E3 ligases in GC has been well examined, but several E3 ligases, such as COP1, need further studies to elucidate their mechanisms. It has been shown that many synthetic and natural compounds targeting E3 ligases could regulate the level of various signaling proteins through UPS-mediated degradation in human cancers (158). Compounds and small molecules targeting E3 ligases may become underlying templates for the synthesis of targeted therapeutic drugs in GC. However, there are still many obstacles to overcome before the application of compounds targeting E3 ligases in GC, such as the detection and analysis of their complex functional mechanisms and molecular structures. Therefore, further studies should aim to reveal the molecular mechanism of individual E3 ligases in different subtypes of GC, and determine the structure of these targeting compounds to facilitate further synthesis of such targeted therapy drugs. In conclusion, E3 ligases are crucial tumor regulatory factors and potential therapeutic targets in GC.

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Authors' contributions

MW and YL developed the concept of the manuscript. MW, WD and ZK were responsible for writing, reviewing and editing the manuscript. WD participated in revising the manuscript. ZK supervised the project. YL was involved with the project administration. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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