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REVIEW

The role of E3 ubiquitin ligase HECTD3 in cancer and beyond

Qiuyun Jiang[1,2] · Fubing Li[3] · Zhuo Cheng[1,2] · Yanjie Kong[4] · Ceshi Chen[1,2,5]

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Abstract

Ubiquitin modification plays significant roles in protein fate determination, signaling transduction, and cellular processes. Over the past 2 decades, the number of studies on ubiquitination has demonstrated explosive growth. E3 ubiquitin ligases are the key enzymes that determine the substrate specificity and are involved in cancer. Several recent studies shed light on the functions and mechanisms of HECTD3 E3 ubiquitin ligase. This review describes the progress in the recent studies of HECTD3 in cancer and other diseases. We propose that HECTD3 is a potential biomarker and a therapeutic target, and discuss the future directions for HECTD3 investigations.

Keywords Ubiquitination · HECTD3 · Cancer · Inhibitors

Abbreviations

ACC	Adrenocortical carcinoma
ANGPT1	Angiopoietin 1
BRCA	Breast invasive carcinoma
BRCA1	Breast cancer type 1 susceptibility protein
c-Abl	Abelson murine leukemia viral homolog 1
CHOL	Cholangiocarcinoma
CRAF	RAF proto-oncogene serine/threonine-protein kinase
CRL7	Cullin-RING E3 ubiquitin ligase 7
CUL1	Cullin1
CUL7	Cullin 7
DECL	DNA-encoded compound libraries
DISC	Death-inducing signaling complex
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma
DUB	Deubiquitinating enzyme
E1	Ubiquitin-activating enzyme
E2	Ubiquitin-conjugating enzyme
E3	Ubiquitin ligase
E6-AP	E6-associated protein
EAE	Experimental autoimmune encephalomyelitis
ECT2	Epithelial cell transforming 2
EGFR	Epidermal growth factor receptor
ER	Endoplasmic reticulum
ERBB4	Erb-b2 receptor tyrosine kinase 4
ERK	Mitogen-activated protein kinase 1
ESCC	Esophageal squamous cell carcinoma
FBDD	Fragment-based drug discovery
FBW7	F-box and WD repeat domain containing 7
FP-HTS	Fluorescence polarization assay for high-throughput screening
HECT	Homologous to E6AP C terminus
HECTD3	Homologous to the E6-associated protein carboxyl terminus domain containing 3
HER2	Erb-b2 receptor tyrosine kinase 2
HIF1α	Hypoxia inducible factor 1 subunit alpha
HSP90	Heat shock protein 90
HTS	High-throughput screening technologies
HUWE1	HECT, UBA, and WWE domain containing E3 ubiquitin protein ligase 1
IFN	Interferon
IRE1α	Inositol requiring enzyme 1 alpha

✉ Ceshi Chen
chenc@mail.kiz.ac.cn

1 Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences and Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China

2 Kunming College of Life Science, University of the Chinese Academy of Sciences, Kunming 650204, China

3 Affiliated Cancer Hospital and Institute of Guangzhou Medical University, Guangzhou 510095, China

4 Institute of Translation Medicine, Shenzhen Second People's Hospital, The First Affiliated Hospital of Shenzhen University, Shenzhen 518035, China

5 KIZ-CUHK Joint Laboratory of Bioresources and Molecular Research in Common Diseases, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China

IRF3	Interferon regulatory factor 3
ITCH	Itchy E3 ubiquitin protein ligase
KLF5	Kruppel like factor 5
LATS1	Large tumor suppressor kinase 1
LIHC	Liver hepatocellular carcinoma
LGG	Brain lower grade glioma
LUAD	Lung adenocarcinoma
MALT1	MALT1 paracaspase
MCL1	Myeloid cell leukemia 1
MDM2	Murine double minute 2
miR-153	MicroRNA-153
NEDD4-1	NEDD4 E3 ubiquitin protein ligase
OV	Ovarian serous cystadenocarcinoma
PMA	Phorbol-12-myristate-13-acetate
PML	Promyelocytic leukemia protein
PTEN	Phosphatase and tensin homolog
RBR	RING-IBR-RINGs
RING	Really interesting new genes
RLD	RCC1 like domain
RNF20	Ring finger protein 20
RORγt	Retineic-acid-receptor-related orphan nuclear receptor γ
SCF	SKP1-CUL1-F-box protein
SKP2	S-phase kinase-associated protein 2
SMAD2	SMAD family member 2
SMURF2	SMAD specific E3 ubiquitin protein ligase 2
Stat3	Signal transducer and activator of transcription 3
Tara	Trio-associated repeat on actin
TBK1	TANK binding kinase 1
TGFβ	Transforming growth factor β
TGFβR1	Transforming growth factor β receptor 1
Th17	T helper 17
THCA	Thyroid carcinoma
THYM	Thymoma
TNBC	Triple negative breast cancer
TRAF3	TNF receptor-associated factor 3
TRAF6	TNF receptor-associated factor 6
TRAIL	TNF-related apoptosis-inducing ligand
Ub	Ubiquitin
UCEC	Uterine Corpus Endometrial Carcinoma
UCS	Uterine Carcinosarcoma
UbV	Ub variant
UCS	Uterine carcinosarcoma
VCB-CR	pVHL-elongin C-elongin B-cullin 2-RBX1
VHL	Von Hippel–Lindau disease tumor suppressor
WWP1	WW domain containing E3 ubiquitin protein ligase 1
WWP2	WW domain containing E3 ubiquitin protein ligase 2
XBP1	X-box binding protein 1

Protein ubiquitination

Ubiquitination is a kind of protein posttranslational modification. Sequential reactions catalyzed by ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) result in covalent conjugation of ubiquitin (Ub) molecules to a target protein to serve as a signal label that regulates the fate of a target protein. Therefore, ubiquitination participates in the regulation of numerous fundamental biological processes and is essential for maintenance of normal physiological activities of the organisms [1, 2]. A ubiquitin molecule forms an isopeptide bond to the side chain of a Lys (K) residue of a target protein through its C-terminal Gly residue [3]. Additional ubiquitin molecules can be attached to the previous ones through K residues (6, 11, 27, 29, 33, 48, and 63) or N-terminal Met residue to form a polymeric Ub chain [4, 5]. K48- and K63-linked polyubiquitin chains are the most common [6]. Ubiquitin modification can be removed by the deubiquitinating enzymes (DUBs).

Ubiquitination regulates protein stability, cellular localization, protein–protein interactions, trafficking, and activity depending on the site, number, linkage, and length of the modifications [3]. For example, degradation-independent functions are associated with monoubiquitination, linear ubiquitination, or K63-linked polyubiquitination. Ubiquitination plays a pivotal role in the regulation of cellular functions, such as cell cycle, apoptosis, DNA damage repair, transcription, endocytosis, and signaling [7–13]. Dysfunction of E3 and DUBs is an important factor contributing to the development and pathogenesis of multiple human diseases and E3 and DUBs are potential therapeutic targets [14–16]. Accumulating evidence indicates importance of ubiquitination in initiation, metastasis, and drug resistance in cancer [17–21].

E3 ubiquitin ligases

There are over 600 E3 ligases in the human body [22]. E3 ligases directly interact with the target proteins and are responsible for specificity of ubiquitination. Several E3 ligases, such as murine double minute 2 (MDM2) [23], SKP1-CUL1-F-box protein (SCF) complex (SCF^{SKP2} [24] and SCF^{FBW7} [25]), pVHL-elongin C-elongin B-cullin 2-RBX1 (VCB-CR) complex [26], and breast cancer type 1 susceptibility protein (BRCA1) [27], are known to play important roles in cancer. Targeting E3 ligases has become a novel avenue for drug development and cancer treatment. Currently, several MDM2 inhibitors have been tested for antitumor activity in clinical trials [28–30].

E3 ligases can be classified into RING (really interesting new genes), HECT (homologous to E6AP C terminus), and RBR (RING- IBR-RINGs) type E3s [3] according to their protein structure and functional mechanisms. RING E3 ligases contain a RING finger domain, but do not possess catalytic activity. They function as adaptor proteins that promote the ubiquitin transfer from E2 to a substrate protein. In contrast, HECT and RBR E3 ligases form an intermediate with ubiquitin through their own catalytic cysteine (Cys) and then transfer the ubiquitin moiety to a substrate protein [31, 32]. RBR family is comprised of a central in-between-RINGs (IBR) domain and two RING finger domains located at both termini [33].

HECT E3 family contains 28 members with common C-terminal HECT domain. Furthermore, HECT E3 ligases can be divided into three subfamilies: Nedd4 subfamily, HERC subfamily, and other HECT E3s [6, 31]. Nedd4 subfamily members contain a C2 domain and 2–4 WW domains. HERC subfamily members contain one or more RCC1 like domain (RLD) domains. Members of the other HECT subfamily are composed of diverse N-terminal domains. Conservative catalytic Cys located in the C-lobe of the HECT domain can form a thioester bond with ubiquitin, while the N-lobe is responsible for E2 enzyme binding [6, 34–36].

HECT-type E3 ligases promote ubiquitination of a large number of substrate proteins. In addition, they are regulated by intricate signals and widely implicated in cancer. E6-associated protein (E6-AP), the first identified HECT-type E3, targets tumor protein p53 for ubiquitin-dependent degradation after interaction with the E6 protein of HPV [37, 38]. Moreover, E6-AP promotes ubiquitination and degradation of tumor suppressor promyelocytic leukemia protein (PML) in B-cell lymphoma and prostate cancer [39–41]. In contrast, Mansour et al. found that E6-AP targets epithelial cell transforming 2 (ECT2) for proteasomal degradation and inhibits breast cancer metastasis [42]. Gene amplification and protein overexpression of WW domain containing E3 ubiquitin protein ligase 1 (WWP1) frequently occur in human prostate cancer and breast cancer [43, 44]. Substrates of WWP1 include tumor protein p63, Kruppel like factor 5 (KLF5), transforming growth factor beta receptor 1 (TGFβR1), and erb-b2 receptor tyrosine kinase 4 (ERBB4) [45–48]. Itchy E3 ubiquitin protein ligase (ITCH) mediates the ubiquitin–proteasome degradation of large tumor suppressor kinase 1 (LATS1) and regulates the Hippo pathway [49]. ITCH also targets H1.2 linker histone for polyubiquitination, which regulates DNA damage response in triple negative breast cancer (TNBC) [50]. SMAD-specific E3 ubiquitin protein ligase 2 (SMURF2) negatively regulates the TGFβ signaling pathway by promoting the degradation of transforming growth factor beta (TGFβ) receptor and SMAD family member 2 (SMAD2) [51, 52]. In addition, SMURF2 regulates monoubiquitination of histones through the ubiquitin-dependent degradation of ring finger protein 20 (RNF20) and is thus involved in maintenance of genomic stability and tumor suppression [53]. NEDD4 E3 ubiquitin protein ligase (NEDD4-1) and WW domain containing E3 ubiquitin protein ligase 2 (WWP2) are suggested to be potential oncoproteins by facilitating the degradation of phosphatase and tensin homolog (PTEN), a distinguished tumor suppressor [54, 55].

HECTD3

Homologous to the E6-associated protein carboxyl terminus domain containing 3 (HECTD3) is classified as the third subfamily of HECT E3s. HECTD3 is comprised of 861 amino acid residues, and contains an N-terminal DOC domain (219-397) and a C-terminal HECT domain (512-857) (Fig. 1). Like other HECT E3s, in its HECT domain, a flexible hinge links the N-lobe which contains E2 binding motif and the C-lobe where catalytic Cys (C832) locates in. The N-terminal DOC domain is responsible for substrate binding. In addition, there are two known phosphorylation sites (S12 and T157) at the N-terminus of HECTD3 (Fig. 1).

In recent years, the function of HECTD3 has received considerable attention. HECTD3 has been reported to modify a variety of substrate proteins, to be regulated by diverse factors, and to play crucial roles in cellular processes, such as apoptosis, drug resistance, and immunoreaction (Fig. 2, Table 1). HECTD3 may become a therapeutic target in cancer and other diseases.

HECTD3-modified proteins

In 2008, Yu et al. reported HECTD3 as an E3 ligase of trio-associated repeat on actin (Tara) that promotes Tara ubiquitination and degradation [56]. In 2009, Zhang et al. found that syntaxin 8 is another HECTD3 substrate protein and HECTD3 may influence neurodegenerative diseases [57].

Li et al. demonstrated that MALT1 paracaspase (MALT1) is a bona fide HECTD3 substrate protein [58]. The HECTD3 DOC domain interacts with the MALT1 DD domain; therefore, HECTD3 increases MALT1 polyubiquitination and protein stability. This study suggested that the MALT1 ubiquitin chain linkage mediated by HECTD3 is not K48-linked, but is K63-linked and other K-linked. It has been speculated that the stabilizing effect of HECTD3 on MALT1 may antagonize the degradation mediated by other E3s. It is intriguing that an HECTD3 mutant without catalytic activity still promotes MALT1 ubiquitination. A possible explanation for this effect is that HECTD3 promotes ubiquitination of MALT1 by other E3 ligases, such as TNF receptor-associated factor 6 (TRAF6). It has been reported that TRAF6

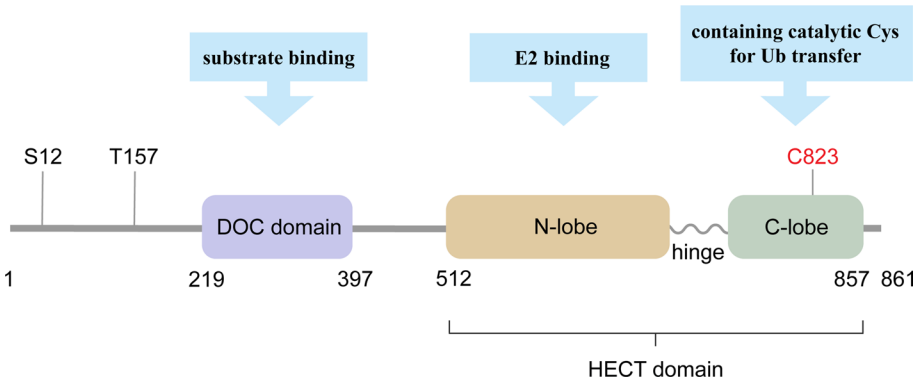


Fig. 1 The diagram of HECTD3 protein structure. HECTD3 belongs to the other HECT E3s subfamily. It is comprised of a DOC domain and an HECT domain. In the HECT domain, a flexible hinge links the N-lobe, which contains the E2 binding site, and the C-lobe, which contains the catalytic Cys for Ub transfer. The DOC domain is responsible for substrate binding. Catalytic Cys and phosphorylation sites are indicated [60, 61, 111]

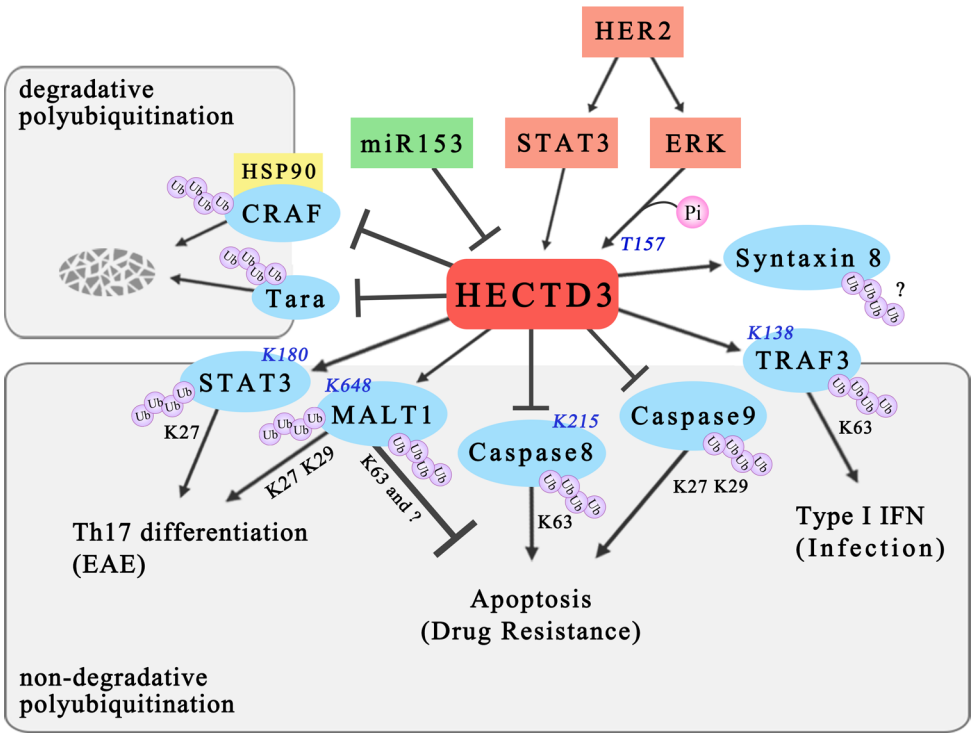


Fig. 2 Functions, mechanisms, and regulation of HECTD3. HECTD3 targets several apoptosis-related proteins (MALT1, caspase-8, and caspase-9) for nondegradative polyubiquitination and confers drug resistance to cancer cell. It also regulates the immune response through nondegradative polyubiquitination of STAT3, MALT1, and TRAF3. In addition to the nondegradative pathway, HECTD3 mediates ubiquitin–proteasome degradation of Tara and CRAF. HER2/ERK and miR-153 regulate the HECTD3 activity and expression. The linkage types of polyubiquitin chain and the modification sites in the substrate proteins are indicated

promotes MALT1 ubiquitination [64]. However, this possibility needs additional supporting evidence.

In a recent study, Hectd3 was shown to modify Malt1 with K27- and K29-linked polyubiquitin chains while K648 is the ubiquitination site of Malt1, which is related to nuclear translocation of p65 and activation of NF-κB signaling pathway [59]. In addition, Hectd3 binds to the signal transducer and activator of transcription 3 (Stat3) linker region through the DOC domain and modifies Stat3 at K180 with K27-linked polyubiquitin chains; this modification is associated with phosphorylation of Stat3 at Y705 and expression of Retineic-acid-receptor-related orphan nuclear receptor gamma (RORγt) in T helper 17 (Th17) cells [59].

Our previous study suggested that HECTD3 specifically catalyzes K63-linked polyubiquitination of caspase-8 [60]. Ubiquitination prevents the recruitment of caspase-8 into TRAIL (TNF-related apoptosis-inducing ligand)-induced DISC (death-inducing signaling complex) for activation [60]. This study identified HECTD3 C823 as the catalytic Cys and caspase-8 K215 as the ubiquitination site [60]. Later, cullin 7 (CUL7), the scaffold protein of cullin-RING E3 ubiquitin ligase 7 (CRL7), was shown to ubiquitinate

Table 1 HECTD3-mediated protein ubiquitination

Substrate	Ub linkage	Molecular function	Cellular function	References
Tara	–	proteasomal degradation	Cell cycle and formation of multipolar spindle	[56]
Syntaxin 8	–	–	Neurodegenerative diseases	[57]
MALT1	Non K48	Increase in MALT1 protein stability	Antiapoptotic chemoresistance	[58]
	K27, K29	Activation of NF-κB signaling	Promotion of the differentiation of Th17 cells	[59]
Caspase-8	K63	Inhibition of caspase-8 activity	Inhibition of extrinsic apoptosis	[60]
Caspase-9	K27, K29	Inhibition of caspase-9 activity	Inhibition of intrinsic apoptosis	[61]
CRAF	–	Proteasomal degradation	Restriction of MAPK pathway	[62]
TRAF3	K63	Activation of TBK1	Promotion of type I interferon production and bacterial infection	[63]
Stat3	K27	Promotion of Stat3 activation	Induction of Th17 cell differentiation	[59]

caspase-8 at the same site and to have similar antiapoptotic function [65]. In addition to caspase-8, HECTD3 ubiquitinates caspase-9 with K27- and K29-linked polyubiquitin chains and suppresses its activation [61].

HECTD3 was demonstrated to mediate heat shock protein 90 (HSP90) inhibitor-induced degradation of RAF proto-oncogene serine/threonine-protein kinase (CRAF), an HSP90 client kinase. HECTD3 interacts with HSP90 and CRAF and subsequently targets CRAF for degradation [62]. It is well established that chaperones are essential for correct folding of proteins and the ubiquitin–proteasome system is responsible for degradation of incorrectly folded proteins. It is unknown whether HECTD3 targets other chaperon clients.

Most recently, Li et al. reported that HECTD3 catalyzes K63-linked polyubiquitination of TNF receptor-associated factor 3 (TRAF3) at K138 [63]. HECTD3-mediated TRAF3 ubiquitination promotes the interaction between TRAF3 and TANK-binding kinase 1 (TBK1) and subsequent phosphorylation of TBK1 and interferon regulatory factor 3 (IRF3). *Hectd3*-deficient macrophages failed to produce type I interferon (IFN) in response to intracellular bacterial infection [63].

The regulation of HECTD3

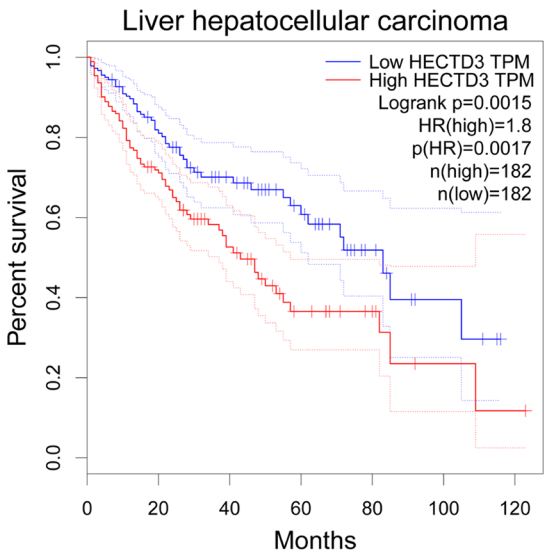
Shu T et al. showed that erb-b2 receptor tyrosine kinase 2 (HER2) increased HECTD3 expression through activation of STAT3 [66]. STAT3 binds to the *HECTD3* gene promoter to induce its transcription. It is well known that STAT3 can be activated by epidermal growth factor receptors (EGFRs) and is usually constitutively activated in cancer [67].

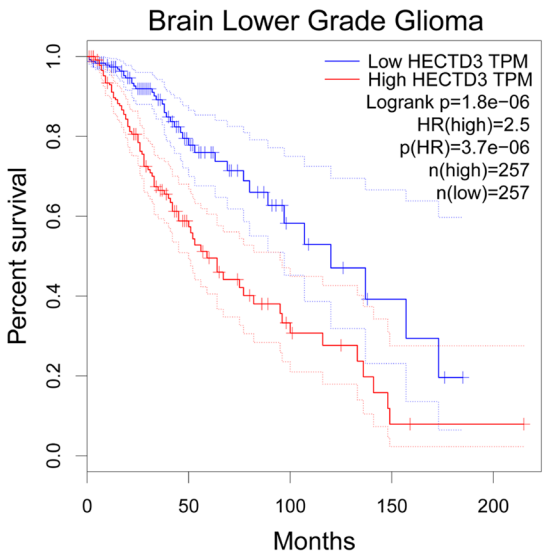
Li Y et al. reported that HECTD3 can be phosphorylated and activated by mitogen-activated protein kinase 1 (ERK, also known as MAPK) [61]. A PKC activator, phorbol-12-myristate-13-acetate (PMA), activates the Raf–MEK–ERK pathway and facilitates the phosphorylation of HECTD3 at Thr157 via ERK. The phosphorylation of HECTD3 increases the binding of HECTD3 and caspase-9 [61].

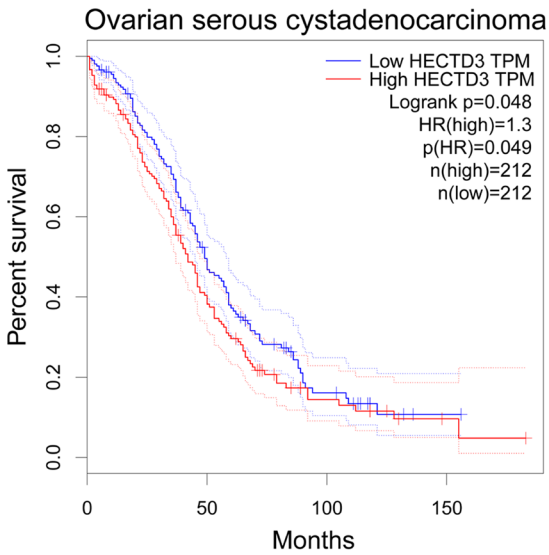
Wu X et al. demonstrated that microRNA-153 (miR-153) targets HECTD3 [68]. MiR-153 inhibits *HECTD3* mRNA expression and enhances the sensitivity of TNBC cells to cisplatin. MiR153 is a well-known tumor suppressor in breast cancer. We reported that miR-153 targets several oncogenes, including *KLF5*, hypoxia inducible factor 1 subunit alpha (*HIF1α)*, angiopoietin 1 (*ANGPT1)*, and myeloid cell leukemia 1 (*MCL1)*, thus boosting stemness, cell growth, and angiogenesis of breast cancer [69–71]. Consistently, the expression level of miR153 is positively associated with the 5-year survival rate and prognosis of breast cancer patients. Interestingly, miR-153 itself is regulated by endoplasmic reticulum (ER) stress. Hypoxia and drug treatment can directly induce *miR-153* transcription through inositol requiring enzyme 1 alpha (IRE1α)/X-box-binding protein 1 (XBP1) [69, 70]. A possibility that ER stress inhibits HECTD3 expression through miR153 has not been tested.

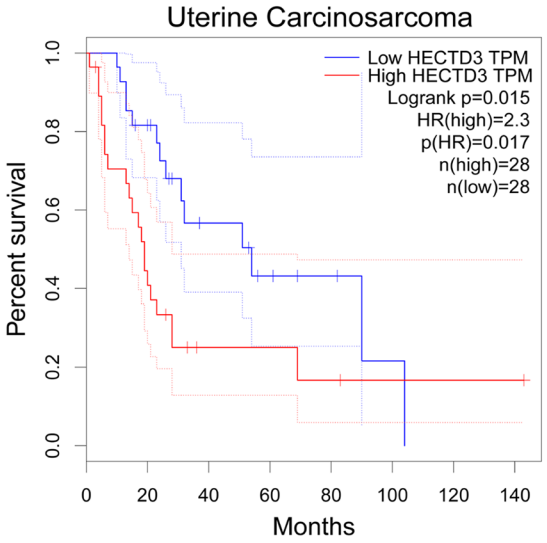
The role of HECTD3 in cancer

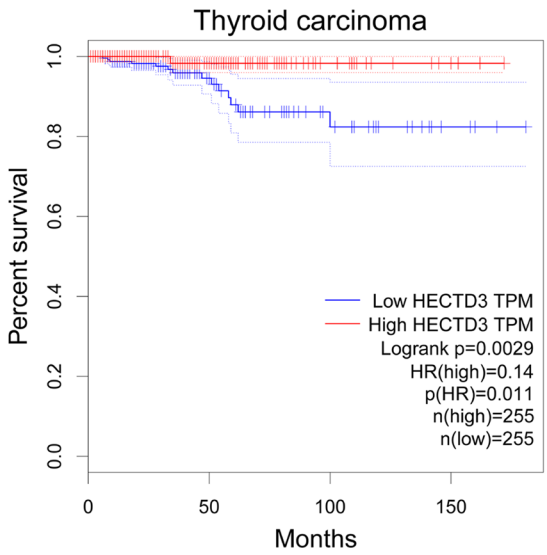
Accumulated evidence suggests that HECTD3 has a prosurvival role in several types of cancer. HECTD3 has become a potential biomarker for cancer diagnosis and prognosis and a therapeutic target. In breast cancer, gene amplification leads to HECTD3 overexpression. The overexpression of HECTD3 was linked to cisplatin resistance through ubiquitination and stabilization of MALT1 [58]. This was the first study to reveal the prosurvival function of HECTD3 and MALT1. Recently, Ekambaram et al. demonstrated that in angiotensin II receptor-positive breast cancer, the activation of the CARMA3–Bcl10–MALT1 pathway promotes cancer cell proliferation and invasion [72]. Moreover, Lin et al. reported that the lack of MALT1 protease activity in Treg cells leads to inhibition of lymphoma growth [73]. Kawadler

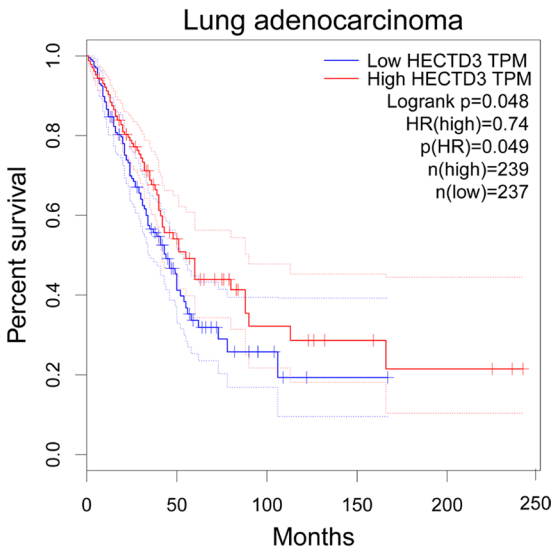

Liver hepatocellular carcinoma
Low HECTD3 TPM
High HECTD3 TPM
Logrank p=0.0015
HR(high)=1.8
p(HR)=0.0017
n(high)=182
n(low)=182
Percent survival
Months


Brain Lower Grade Glioma
Low HECTD3 TPM
High HECTD3 TPM
Logrank p=1.8e−06
HR(high)=2.5
p(HR)=3.7e−06
n(high)=257
n(low)=257
Percent survival
Months


Ovarian serous cystadenocarcinoma
Low HECTD3 TPM
High HECTD3 TPM
Logrank p=0.048
HR(high)=1.3
p(HR)=0.049
n(high)=212
n(low)=212
Percent survival
Months


Uterine Carcinosarcoma
Low HECTD3 TPM
High HECTD3 TPM
Logrank p=0.015
HR(high)=2.3
p(HR)=0.017
n(high)=28
n(low)=28
Percent survival
Months


Thyroid carcinoma
Low HECTD3 TPM
High HECTD3 TPM
Logrank p=0.0029
HR(high)=0.14
p(HR)=0.011
n(high)=255
n(low)=255
Percent survival
Months


Lung adenocarcinoma
Low HECTD3 TPM
High HECTD3 TPM
Logrank p=0.048
HR(high)=0.74
p(HR)=0.049
n(high)=239
n(low)=237
Percent survival
Months

◂**Fig. 3** Prognostic values of *HECTD3* mRNA expression levels in different cancer types. A high *HECTD3* mRNA expression level is associated with poor prognosis in liver hepatocellular carcinoma (LIHC), brain lower grade glioma (LGG), ovarian serous cystadenocarcinoma (OV) and uterine carcinosarcoma (UCS). On the contrary, it is associated with favorable outcomes in thyroid carcinoma (THCA) and lung adenocarcinoma (LUAD)

et al. showed that MALT1 can control the activation of caspase-8 and facilitate lymphocyte proliferation and survival [74]. In addition, HECTD3 promotes the survival of human breast cancer cells under extrinsic apoptotic stimuli via direct ubiquitination of caspase-8 [60]. In esophageal squamous cell carcinoma (ESCC) KYSE30 cells, HECTD3 overexpression results in cisplatin resistance through blockade of activation of caspase-9 [61]. In ovarian cancer cell lines and xenograft mouse models, downregulation of HECTD3 significantly facilitated carboplatin-induced apoptosis [66]. Given that HECTD3 confers apoptosis resistance and chemoresistance, HECTD3 inhibitors in combination with chemotherapeutic drugs may alleviate drug chemoresistance.

However, Li et al. argued that HECTD3 may function as a tumor suppressor, because HECTD3 downregulates an HSP90 protein kinase client CRAF thus inhibiting the activation of MAPK [62]. Yu et al. reported that HECTD3 is the E3 ligase for Tara. Deletion of either Tara or HECTD3 results in the formation of multipolar spindle, indicating that HECTD3 may be important for maintenance of genomic stability [56]. It is possible that HECTD3 plays a context-dependent role.

Moreover, we used GEPIA (Gene Expression Profiling Interactive Analysis, http://gepia.cancer-pku.cn/index.html) to analyze the prognosis values of *HECTD3* mRNA expression levels in different cancer types. HECTD3 appears to act as both tumor-promoting and -suppressing factors in different types of cancer. As shown in Fig. 3, a high *HECTD3* mRNA expression level is associated with poor prognosis in liver hepatocellular carcinoma (LIHC), brain lower grade glioma (LGG), ovarian serous cystadenocarcinoma (OV), and uterine carcinosarcoma (UCS). On the contrary, in thyroid carcinoma (THCA) and lung adenocarcinoma (LUAD), a high *HECTD3* mRNA expression level is associated with favorable prognosis. The context-dependent roles of HECTD3 in different cancers should be investigated in the future.

The role of HECTD3 in other diseases

In addition to cancer, HECTD3 may play significant roles in the immune system. HECTD3 is a potential target for multiple sclerosis and intracellular bacterial infection. First, HECTD3 positively regulates the production of type I IFN via ubiquitination of TRAF3 and activation of TBK1 in response to intracellular bacterial infection [63]. *Hectd3* knockout limits the migration and dissemination of *F. novicida*-carrying macrophages and neutrophils, and promotes stronger defensive behavior in mice [63]. Second, Hectd3 promotes the activation of NF-κB and RORγt expression through ubiquitinating Malt1 and Stat3, respectively, and then facilitates Th17 cell differentiation [59]. Malt1 is an essential component of the Carma1–Bcl10–Malt1 (CBM) complex, which promotes NF-κB activation and Th17 pathogenicity in EAE [75]. Stat3 and RORγt are key transcription factors regulating Th17 cell differentiation. Therefore, *Hectd3* global knockout mice have decreased symptoms of experimental autoimmune encephalomyelitis (EAE) [59]. This phenotype is similar to *Malt1* knockout mice [75]. Finally, HECTD3 may be involved in neurodegenerative diseases by interacting with and ubiquitinating syntaxin 8 [57].

E3 ligase inhibitors

Following the approval of bortezomib, the first proteasome inhibitor, for the treatment of refractory hematologic malignancies [76, 77], great attention has been paid to the studies of the ubiquitin proteasome system. Oncogenic E3s are frequently overactivated in cancer by gene amplification and overexpression [78]. Several strategies can be used to target oncogenic E3s, such as inhibition of E3 expression, inhibition of E3 enzyme activity, disruption of the interaction between E3 and E2 or their substrates, or restraining the assembly of the E3 complex.

For example, a variety of small molecule inhibitors have been identified for MDM2. These inhibitors function through two mechanisms. One mechanism involves repression of the E3 ligase activity of MDM2. Another mechanism is based on interference in interaction between MDM2 and p53. For example, nutlins occupy the p53-binding site of MDM2, while RITA binds to the N-terminal region of p53 thus interfering with the interaction [79–81]. To date, several small molecule inhibitors of MDM2 are in clinical trials. In addition to RG7112 [82], multiple compounds, including MI-219, MI-319, MI-888, MI-77301, and APG-115, have been developed. MI-77301 and APG-115 have undergone phase I clinical trials [28, 29, 83–89]. RG7388 (idasanutlin) has entered a phase III clinical trial for the treatment of acute myeloid leukemia [30, 90].

In addition to traditional small molecule inhibitors of E3 ligases, emergence of several novel strategies provides more possibilities for cancer treatment. Ub variants (UbVs) can be developed as inhibitors or activators of E3s. Several studies screened specific UbVs against 20 HECT E3s and other E3s (Fig. 4). UbV inhibitors can hinder the E2-E3 binding or the assembly of the cullin1 (CUL1)-based E3 complexes [91–93]. These results indicate that UbVs may become a

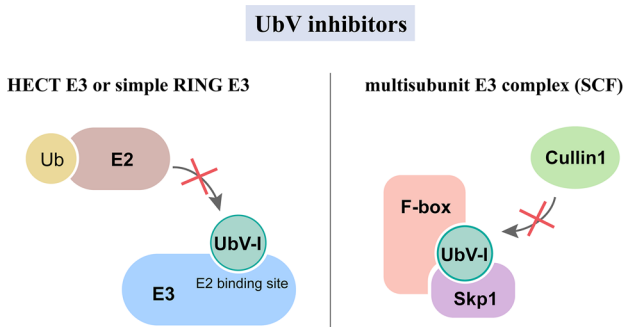


Fig. 4 The functional mechanisms of UbV inhibitors. In the case of HECT E3s and simple RING E3s, UbV inhibitors interfere with the E2–E3 binding. In the case of the multisubunit E3 complex (i.e., SCF), UbV inhibitors suppress the assembly of the Cul1 subunit

novel tool for inhibition of abnormal E3s in cancer. The delivery of UbVs in vivo, however, remains a challenge.

Strategies of targeting HECTD3

As described above, HECTD3 inhibition may overcome chemoresistance in cancer, multiple sclerosis, and intracellular bacterial infection. Importantly, specific inhibition of HECTD3 should be safe, because *Hectd3* whole-body knockout mice are normal and do not have detectable defects.

Various studies provide support and inspiration for HECTD3-targeted therapy. Several strategies can be considered for screening and development of HECTD3 inhibitors, including small molecule inhibitors, peptides, and proteins (Fig. 5).

First, the catalytic activity of HECTD3 can be suppressed. It is well known that HECT E3 ligases possess intrinsic catalytic activity. Mund et al. identified two types of HECT E3 inhibitors that have different mechanisms of action and confirmed that targeting HECT domain for drug design is feasible [94]. One of the inhibitors, heclin, a small molecule compound with wider spectrum, suppresses multiple HECT E3 ligases via induction of spontaneous oxidation of a Cys residue of the E3 active site [94]. Possibility that heclin inhibits HECTD3 enzyme activity deserves investigation.

Second, it is possible to hijack the E2 binding site of HECTD3 or block the interaction between HECTD3 and specific substrate proteins. For instance, bicyclic peptides, another kind of inhibitors described in Mund et al.'s study, have high specificity against individual HECT E3 ligases by targeting the E2-binding sites [94].

Third, the ubiquitin transthiolation can be obstructed to halt the ubiquitination process. Rossi et al. demonstrated that clomipramine, an antidepressant, impedes the transthiolation

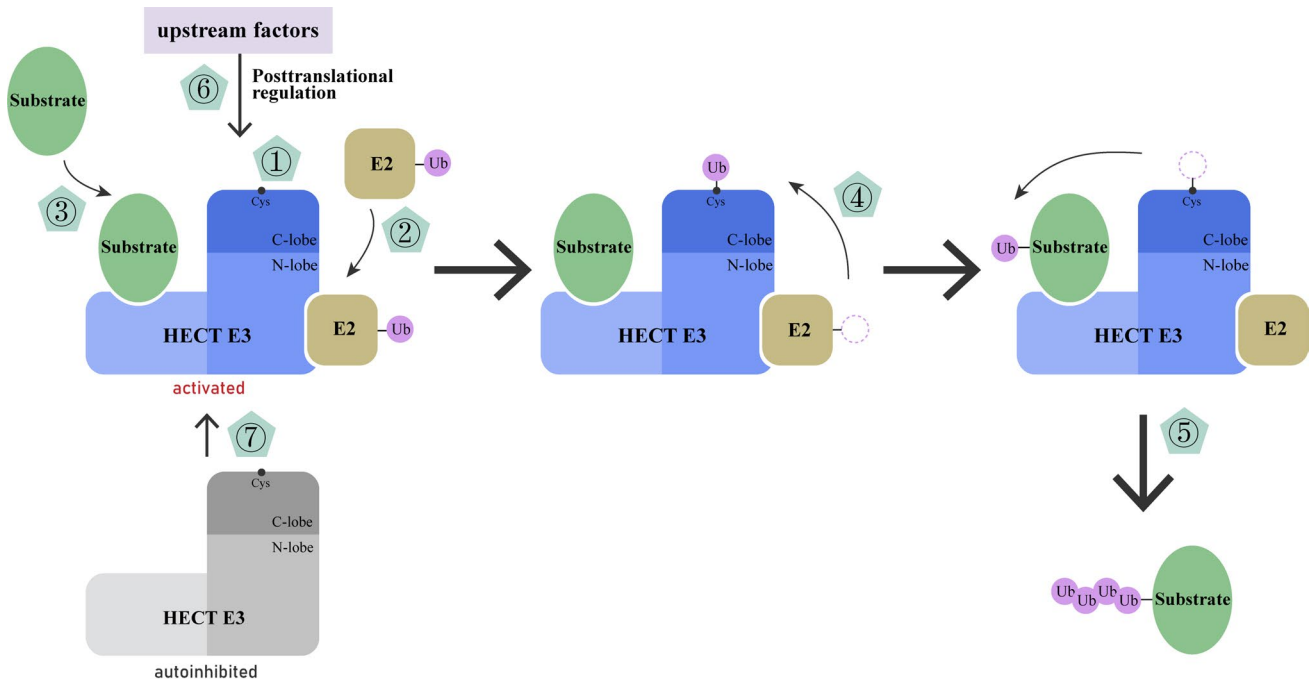


Fig. 5 Strategies to inhibit HECTD3-mediated ubiquitination process. ① Inhibition of the catalytic activity (e.g., heclin). ② Hijacking the E2-binding site (e.g., UbV inhibitor or bicyclic peptides). ③ Interference with the binding of specific substrates. ④ Obstruction of the ubiquitin transthiolation (e.g., clomipramine). ⑤ Interruption of polyubiquitin chain elongation (e.g., occupying the exosite). ⑥ Targeting the HECTD3 posttranslational modification or upstream factors. ⑦ Regulation of the autoinhibition mechanism

of ubiquitin from E2 to HECT E3. Clomipramine irreversibly inhibited the Itch-mediated ubiquitination of p73 and showed anticancer activities in combination with chemotherapy drugs [95].

Fourth, extension of the ubiquitin chain can be blocked. Kathman et al. identified the first covalent inhibitor of Nedd4-1. The compound prevents Nedd4-1 from binding to ubiquitin by reacting with noncatalytic Cys627, which represses E3 progressivity and extension of the ubiquitin chains by occupying the exosite (a processivity site) [96]. This work provides a novel strategy for the development of HECT E3 inhibitors.

Fifth, the upstream positive regulators of HECTD3 can be inhibited. As mentioned above, ERK is one of the upstream factors of HECTD3 and can become a possible target for modulation of HECTD3 activity in cancer [61]. STAT3 may become another possible target for inhibition of HECTD3 expression. Recently, an STAT3 inhibitor, TTI-101, has entered phase I trial (NCT03195699). In addition, it is worthwhile to consider the use of gene therapy based on miRNA to control the transcriptional expression of HECTD3, e.g., by miR153 mimics. In addition to miR153, other miRNAs and regulators should be identified for efficient control of the HECTD3 expression level and activity.

Finally, we can inhibit the HECTD3 activity by regulating the autoinhibition mechanism and targeting its upstream factors. HECT E3 ligases need a "braking system" to appropriately switch between the active and inactive states to ensure proper functioning of E3 ligases and to elaborate regulation of cell signals. For example, HECT, UBA, and WWE domain containing E3 ubiquitin protein ligase 1 (HUWE1) activity is regulated by a conformational switch which causes suppression of the activity through self-dimerization and intramolecular interactions [97]. Several Nedd4 subfamily members lock themselves into a ground state T-shape through the WW2–WW3 linker and this braking effect can be relaxed by tyrosine phosphorylation [98]. For example, Abelson murine leukemia viral homolog 1 (c-Abl) can reduce the E3 ligase activation through phosphorylation of E6-AP at Y636 to alleviate the p53 degradation [99]. Hence, further characterization of HECTD3 protein structure and regulatory mechanism of HECTD3 is required.

Regardless of selected strategy, high-throughput screening technologies (HTS) will dramatically advance the development of HECTD3 inhibitors. The technologies include fragment-based drug discovery (FBDD), phage display technology, alpha screen technology, UbFluor, and fluorescence polarization assay for high-throughput screening (FP-HTS) [100–105]. Natural components from plants and microorganisms and FDA-approved drugs provide rich resources for drug discovery. DNA-encoded compound libraries (DECL) provide unprecedented space for the construction of compound libraries and drug screening [105, 106]. In silico and cell-based assays are important compound screening strategies [107–110]. We have developed an HTS method based on an in vitro HECTD3 self-ubiquitination assay and identified several natural compounds as potential HECTD3 inhibitors. Further experimental investigations are required to validate these HECTD3 inhibitors.

Summary and prospects

The ubiquitin system is crucial for maintenance of normal cellular biological progress. The system can not only target proteins for degradation but also mediate various proteasome-independent functions through the diversity of polyubiquitin chain linkages. E3 ligases have come to the central stage because of their numbers and substrate specificity. Several E3s are attractive therapeutic targets for cancer.

HECTD3 is an under-investigated HECT-type E3 with tremendous research value. Previous studies suggested that HECTD3 has a prosurvival function in cancer. One of the most important features of cancer is resistance to apoptosis and consequent drug resistance in clinic. Further studies are required to determine whether HECTD3 inhibitors have a synergistic effect in combination with chemotherapies in various types of cancer. It is worth investigating whether the expression of HECTD3 in patient specimens can be used as a prognosis biomarker to predict drug sensitivities. Of course, these inhibitors should be evaluated for treatment of multiple sclerosis and intracellular bacterial infection.

In addition to apoptosis and immune regulation, HECTD3 may have other functions depending on the context. A systematic understanding of the roles of HECTD3 remains incomplete. *Hectd3* gene knockout mouse models will play important roles. It has been shown that *Hectd3* gene whole-body knockout mice are resistant to intracellular bacterial infection and EAE [59]. Tissue-specific knockout and knock-in mouse models will be very useful to address the functions and mechanisms of Hectd3 in various organs and diseases.

Moreover, additional substrate proteins and potential upstream regulators need to be identified. Following that, identification of HECTD3 inhibitors with high specificity and strong efficacy is a major direction for future HECTD3 studies.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

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