Targeting neddylation E2s: a novel therapeutic strategy in cancer

Yi-Chao Zheng1, Yan-Jia Guo1, Bo Wang1, Chong Wang2, M. A. A. Mamun1, Ya Gao1* and Hong-Min Liu1*

Abstract
Ubiquitin-conjugating enzyme E2 M (UBE2M) and ubiquitin-conjugating enzyme E2 F (UBE2F) are the two NEDD8-conjugating enzymes of the neddylation pathway that take part in posttranslational modification and change the activity of target proteins. The activity of E2 enzymes requires both a 26-residue N-terminal docking peptide and a conserved E2 catalytic core domain, which is the basis for the transfer of neural precursor cell-expressed developmentally downregulated 8 (NEDD8). By recruiting E3 ligases and targeting cullin and non-cullin substrates, UBE2M and UBE2F play diverse biological roles. Currently, there are several inhibitors that target the UBE2M-defective in cullin neddylation protein 1 (DCN1) interaction to treat cancer. As described above, this review provides insights into the mechanism of UBE2M and UBE2F and emphasizes these two E2 enzymes as appealing therapeutic targets for the treatment of cancers.

Keywords: Neddylation, UBE2M, UBE2F, Therapeutic targets, Anticancer treatment

Introduction
NEDD8 is an ubiquitin-like polypeptide with extensive sequence identity (60%) and homology (80%) with ubiquitin [1–5]. Structurally, NEDD8 exhibits four β-sheets characterized by one α-helix and two 310 helices [1, 6] and has two domains: a flexible carboxy-terminal tail domain and a globular ubiquitin-fold domain (UFD). The Gly–Gly sequence in the tail end conjugates to target proteins and uses different extended structures to combine with neddylation and deneddylation enzymes [7–12].

Protein neddylation, which involves transfer of NEDD8 to a lysine residue of the substrate, is a process that changes the substrate’s activity, conformation, and subcellular localization, not for degradation [13–16]. In many cancers, overactivation of the neddylation pathway causes an increase in the levels of tumor-promoting factors and a decrease in the levels of tumor suppressors, thereby promoting the occurrence of tumors and worsening prognosis (Fig. 1a) [17–24]. In mammalian cells, like ubiquitylation, neddylation starts with ATP-dependent activation of the NEDD8 C-terminus by an E1 NEDD8-activating enzyme (NAE) resulting in the formation a thioester-linked E1-NEDD8 complex. The NAE consists of a heterodimer of NAE1 (APPBP1) and ubiquitin-like modifier activating enzyme 3 (UBA3, NAEβ) subunits [9, 25–28]. Then, activated NEDD8 is transferred to a NEDD8-conjugating enzyme (E2), including the well-studied enzyme UBE2M (also known as UBC12) and the less characterized enzyme UBE2F [29–32], to form another thioester via a transthiolation reaction. Finally, a NEDD8 E3 ligase that binds both the E2-NEDD8 complex and the substrate transfers NEDD8 over to the ε-amino group of the lysine residue in the target protein to form an isopeptide bond. In light of their mechanistic strategy, the majority of the NEDD8 E3 ligases contain interesting novel gene (RING) finger domains, including RING-box protein 1 (RBX1, ROC1) [33–36], RING-box...
protein 2 (RBX2, ROC2) [29, 37–42], murine double minute 2 (MDM2) [43–45], casitas B-lineage lymphoma (c-CBL) [46–49], F-box protein 11 (FBXO11) [50–52], inhibitor of apoptosis (IAP) [53–55], RNA polymerase II transcription factor B subunit 3 (TFB3) [56], tripartite motif 40 (TRIM40) [57], ring finger protein 168 (RNF168) [58], and ring finger protein 111 (RNF111) [59, 60] domains. Interestingly, DCN1, a protein conserved from yeast to mammals, is also a NEDD8 E3 ligase but retains its catalytic activity despite not containing a RING finger domain [61–64]. Furthermore, neddylated substrates can be deneddylated by deneddylases, such as NEDD8 protease 1 (NEDP1) [7, 65–69] and COP9 signalosome (CSN) [70–76]. Hence,
neddylation is a reversible process, as NEDD8 can be recycled [2, 11, 77–79] (Fig. 1b).

The neddylation E2 enzymes UBE2M and UBE2F are only twice as large as NEDD8, and they primarily take part in two types of reactions: transthiolation-transfer from a thioester to a thiol group and aminolysis-transfer from a thioester to an amino group (Fig. 1c). By cooperating with E1 and other E3 enzymes, the E2 enzymes are specific for NEDD8 in catalysis of the neddylation reaction [65, 80]. Therefore, the two E2 enzymes are central players in this enzymatic reaction, in addition to being carriers of NEDD8.

This review discusses the structure of neddylation-related E2 enzymes and summarizes the current understanding of their mechanism and effect in biological processes. UBE2M and UBE2F may become promising targets for cancer treatment.

Structure and basic biology of UBE2M and UBE2F

UBE2M
Full-length UBE2M (human) includes 183 amino acids and consists of two regions: a 26-residue N-terminal docking peptide and an ~150-residue conserved E2 catalytic core domain. And UBE2M functions as a unique E2 ubiquitin-conjugating enzyme in neddylation. Unlike E2s, the 26-residue N-terminal docking peptide of UBE2M is specific for the NEDD8 pathway, as UBE2M’s N-terminal docking peptide is conserved across species and cannot be found in other E2s (Fig. 2a, b) [81]. Furthermore, crystallographic studies [9, 82] of APPBP1-UBA3-UBE2M have revealed that the NAE has three domains: (1) an adenylation domain with an ATP-binding site, (2) a domain surrounding the catalytic cysteine, and (3) a C-terminal domain. The E1–E2 interaction occurs in a bipartite manner: UBE2M’s N-terminal peptide and core domain bind to the NAE to complete the transfer of the NEDD8 from E1 to E2 [81, 83, 84]. The E2’s core domain binds to the C-terminal ubiquitin fold domain, and residues 1–13 of UBE2M’s N-terminal extension and cooperates with a big docking groove in the adenylation domain of E1, which is stabilized via many hydrogen bonds. Although the adenylation domain of UBA3 is conserved among ubiquitin-like protein (UBL)-activating enzymes, the E1–E2 interaction is likely to be unique to the NEDD8 pathway since UBE2M’s N-terminal extension is unique (Fig. 2c) [81].

UBE2F
Full-length human UBE2F includes 185 amino acids and functions as a unique E2 ubiquitin-conjugating enzyme for neddylation. Similar to UBE2M, UBE2F has an N-terminal extension and a conserved catalytic core domain. However, its features are different from those of UBE2M; for example, the N-terminal α1 helix of the UBE2F’s core domain has an offset orientation, the catalytic cysteine is inserted in a loop following the catalytic Cys116 residue, and the C-terminal extension has an α helix instead of a two-stranded β sheet (Fig. 2a, d) [29]. The interaction between UBE2F and the NAE bears great similarity to the UBE2M and NAE interaction: both the N-terminal extension and core domain bind to the NAE. However, the sequence of UBE2M’s N-terminal extension that binds to the UBA3 docking groove is Leu4-Phe5-X-Leu7, whereas the interaction between the UBE2F’s N-terminal extension and UBA3 is through the sequence Met-Leu2-X-Leu4 (Fig. 2e) [29].

Relationship between UBE2M and UBE2F
UBE2M and UBE2F, which serve as two E2 enzymes, transfer NEDD8 to NEDD8 E3 ligases. Structurally, they are similar to each other and can combine with other enzymes in the same catalytic manner. They both play key roles in the neddylation of cullins to activate cullin-RING ligases (CRLs) [10]. However, they are indeed two independent E2 enzymes and display distinct functions [29]. UBE2M couples with RBX1 to induce the neddylation of cullin1, cullin2, cullin3, cullin4A, and cullin4B and triggers corresponding CRLs, which can influence the levels of its substrates, such as p21, p27, Bim, and WD repeat domain phosphoinositide-interacting protein 2 (WIP12), to take part in autophagy, the cell cycle and DNA repair [29, 85–88]. UBE2F can pair with RBX2 to activate the ligase CRL5 by forming a UBE2F/RBX2/cullin5 complex,
leading to the enrichment of NOXA, thereby participating in apoptosis progression and inhibiting cancer cell growth [89]. UBE2M shows intrinsic specificity for RBX1, but RBX2 shows an intrinsic specificity for UBE2F [3, 29]. Notably, MLN4924 [90–95], which is also known as pevonedistat, is a first-in-class inhibitor of the NAE used in cancer treatment and regulates UBE2M and UBE2F in different ways; MLN4924 causes a dose- and time-dependent increase in UBE2M levels but a decrease in UBE2F levels [89].

In addition, Zhou et al. [96] found that UBE2M is a stress-inducible protein that can be promoted by hypoxia-inducible factor 1α (HIF-1α) and transcription factor AP-1 (AP-1) and plays a dual role as an E2 for ubiquitylation and neddylation to degrade UBE2F (Fig. 3). Under normal physiological conditions, UBE2M serves as a neddylation E2 that can couple with cullin3 and Kelch-like ECH-associated Protein 1 (Keap1) to trigger UBE2F polyubiquitylation and degradation. However, under stressed conditions, UBE2M forms a novel E2–E3 complex, UBE2M/DJ-1/Parkin (DJ-1, also known as Parkinson disease protein 7; Parkin, E3 ubiquitin-protein ligase parkin), with the inducement by HIF-1α and AP-1, and promotes the ubiquitylation and degradation of UBE2F. Then, CRL5 is deactivated, and the substrate NOXA can be enriched, leading to the promotion of apoptosis and cell growth inhibition of lung cancer cells. Taken together, these findings indicate that UBE2M can decrease the amount of UBE2F via two E3 ligases, leading to the inactivation of CRL5 mediated by CRL3, which demonstrates cross-talk between the E2 and E3.

**UBE2M and UBE2F in cancer**

UBE2M and UBE2F play crucial roles in various biological processes by recruiting E3 ligases and targeting the substrates of cullins and non-cullins. Several studies have revealed that UBE2M and UBE2F are both overexpressed in multiple types of cancers, such as hepatocellular carcinoma, lung adenocarcinoma, osteosarcoma, ovarian cancer, and squamous cell carcinoma (Table 1) [97–101], and bioinformatics analysis of The Cancer Genome Atlas (TCGA) datasets has revealed that their expression is upregulated in cancer tissues compared with normal tissues [102–104]. They both act as oncogenes by promoting the neddylation of specific substrates to regulate diverse signaling pathways, regulating several cell biological processes, such as DNA repair, genomic stability, apoptosis, autophagy, and the cell cycle.

**Apoptosis and autophagy**

UBE2M and UBE2F play an essential role in apoptosis and autophagy-mediated by CRLs. UBE2M couples with RBX1 and damage-specific DNA-binding protein 1 (DDB1) to induce cullin4A neddylation and trigger the ubiquitin ligase CRL4A, which can induce the ubiquitination and degradation of WIPI2, an autophagy-associated protein, resulting in cell proliferation [105]. Thus, knockdown of UBE2M can block the autophagy process and inhibit cell proliferation [105]. UBE2F can also take part in the regulation of cell survival and death.

**Table 1** Expression and clinical significance of UBE2F and UBE2M in tumors

| Cancer                                | Expression     | Function                  |
|---------------------------------------|----------------|---------------------------|
| **UBE2M**                             |                |                           |
| Osteosarcoma [98]                     | Overexpressed  | Promotes cell viability   |
| Hepatocellular carcinoma [19, 86]     | Overexpressed  | Associated with poor prognosis |
| Lung cancer [87]                      | Overexpressed  | Associated with poor survival |
| Breast cancer [99]                    | Overexpressed  | Worsens prognosis         |
| Intrahepatic cholangiocarcinoma [101] | Overexpressed  | Associated with prognosis  |
| Osteoarthritis [100]                  | Overexpressed  | Promotes apoptosis         |
| Esophageal squamous cell carcinoma [88]| Overexpressed  | Associated with poor survival |
| Non-small cell lung cancer [89]       | Overexpressed  | Associated with poor survival |
| **UBE2F**                             |                |                           |
| Non-small cell lung cancer [89]       | Overexpressed  | Associated with poor survival |
part in the cell apoptosis pathway by downregulating the expression of the proapoptotic protein NOXA [89]. By recruiting RBX2, UBE2F can trigger the neddylation and activation of cullin5, promoting the ubiquitylation and degradation of its substrate NOXA. Knockdown of UBE2F or its mutant (C116A) can induce the accumulation of NOXA, inducing apoptosis of lung cancer cells (Fig. 4).

Cell cycle
In cancer cells, the expression of cell cycle inhibitor proteins is usually downregulated, whereas proteins that promote cell cycle progression are generally overexpressed. Rescuing the levels of cell cycle inhibitor proteins is the primary strategy for disrupting cell cycle progression [106–108]. As UBE2M and UBE2F have been reported to take part in the progression of the cell cycle, E2 enzyme abrogation can arrest cell cycle progression and inhibit cell growth [86, 87]. In lung cancer, knockdown of UBE2M can promote the expression of cyclin-dependent kinase inhibitor 1 (CDKN1A and CDKN1B) and cyclin-associated proteins (such as G2/mitotic-specific cyclin-B1, Cyclin-A2, G1/S-specific cyclin-D3, and Cyclin-dependent kinase 4 homolog) in cell cycle progression. Then, the cell cycle can be arrested at G2 phase and fail to progress to M phase [87]. In hepatocellular carcinoma (HCC), UBE2M can stabilize β-catenin and inhibit cell growth [86, 87]. In lung cancer, knockdown of UBE2M leads to DNA double-strand break (DSB) repair via 53BP1 and breast cancer susceptibility gene 1 (BRCA1) [59]. It is worth mentioning that UBE2M/RNF111-induced neddylation blocks the interaction between CtIP and BRCA1 and then inhibits the CtIP and BRCA1-mediated DNA end resection process, an essential process in the repair pathway [110]. Coincidentally, RNAi-mediated knockdown of UBE2M sensitizes the hormone-resistant prostate cancer cell line DU145 to radiation-induced DSBs [111] (Fig. 4).

Inhibitors of UBE2M
In various cancers, such as hepatocellular carcinoma, lung adenocarcinoma, ovarian cancer, and osteosarcoma, neddylation is always overactivated [13, 15, 19, 78, 112–114]. MLN4924 is an inhibitor of the NAE and used as an anticancer drug [10, 94, 115, 116]. To date, many studies revealed that MLN4924 plays a significant role in cell cycle arrest and the DNA damage response, inhibiting angiogenesis and tumor growth and inducing apoptosis, autophagy, and senescence [117–119]. Although 38 clinical trials have been performed and five completed phase I clinical trials demonstrated that MLN4924 is safe and feasible to date, there is an issue with the specificity of MLN4924, and cancer cells can develop resistance to MLN4924 [120–122].

To overcome the limitations of MLN4924, some studies have focused on discovering specific inhibitors against neddylation-related E2s to achieve more specific modulation of cullin neddylation. Hence, the development of UBE2M-DCN1 protein–protein interaction inhibitors was pursued by medicinal chemists due to the druggable nature of this interaction between UBE2M and DCN1 (Fig. 5a; Table 2) [123]. To discover UBE2M-DCN1 inhibitors, Zhou and colleagues designed potent peptidomimetics by extensively modifying the N-terminal 12-residue peptide of UBE2M, such as DI-404 (Fig. 5b) [124] and DI-591 (Fig. 5c) [125]. At the biochemical level, DI-404 exhibited a high affinity for DCN1 with a Kd value of 6.7 nM and good solubility of 54 µM in PBS at pH 7.4. DCN1 and RBX1 act as co-E3s to promote cullin neddylation [126–128]. A cellular study suggested that DI-404 cannot regulate the levels of UBE2M and DCN1 but can reduce the association between UBE2M and DCN1. Notably, DI-404 can selectively inhibit the neddylation of cullin3 but not the neddylation of other cullins [125]. Due to the peptidic nature of DI-404, although DI-404 showed a high binding affinity for DCN1, it only exhibited moderate cellular activity. Thus, to address this problem and obtain more drug-like compounds, Zhou et al. designed DI-591 through a series of structure-based optimizations [125]. At the biochemical level, DI-591 can bind DCN1 with a Kf value of 12.4 nM and a Kd value of 30.6 nM, as DI-591 uses its bicyclic ring to interact with the subpocket of DCN1, exhibiting extensive hydrophobicity. In addition, the propionyl group

DNA damage response
Neddylation can also contribute to the DNA damage response [58, 59]. For example, UBE2M participates in DNA damage repair and maintains genomic stability via multiple CRLs [85, 101]. As UBE2M can promote the neddylation of cullins and then activate CRLs, the abrogation of UBE2M expression leads to DNA double-strand breaks (DSBs) and increases cell sensitivity to DNA-damaging agents. Scott et al. found that knockdown of UBE2M leads to the blockage of cell cycle progression from G1 to S phase and is related to a delay in the S-phase-dependent DNA damage response [85]. Moreover, UBE2M expression abrogation can also attenuate nonhomologous end-joining (NHEJ) and inhibit cell proliferation [109].

In addition, UBE2M can promote DNA repair via non-cullins. RNF111 together with UBE2M can promote the neddylation of RNF168 and thereby induce the ubiquitylation of histone H4, leading to the activation of DNA repair via 53BP1 and breast cancer susceptibility gene 1 (BRCA1) [59]. It is worth mentioning that UBE2M/RNF111-induced neddylation blocks the interaction between CtIP and BRCA1 and then inhibits the CtIP and BRCA1-mediated DNA end resection process, an essential process in the repair pathway [110]. Coincidentally, RNAi-mediated knockdown of UBE2M sensitizes the hormone-resistant prostate cancer cell line DU145 to radiation-induced DSBs [111] (Fig. 4).
and cyclohexyl group are crucial in forming hydrophobic interactions with DCN1. **DI-591**, which selectively inhibits the neddylation of cullin3 in a dose-dependent manner, shows a remarkable similarity to **DI-404**. Thus, **DI-591** can increase the expression of nuclear factor erythroid 2-related factor 2 (NRF2), the substrate of CRL1 and CRL3, but fails to increase the levels of p21 and Bim, the substrate of CRL1. In addition, **DI-591**
displayed no cytotoxicity in THLE2 human liver epithelial cells [124].

Moreover, Guy and Schulman’s group identified non-peptidic and potent small molecule UBE2M-DCN1 inhibitors from over 600,000 compounds through a time-resolved fluorescence energy transfer (TR-FRET) assay (Fig. 5d) [129–131]. One of the identified molecules, NAcM-HIT, can be docked in UBE2M’s N-acetyl-Met-binding pocket in DCN1, breaking the interaction between UBE2M and DCN1. Based on the optimization of NAcM-HIT, an inhibitor, NAcM-OPT, was designed, which displays 100-fold better potency than NAcM-HIT.
NAcM-OPT can selectively reduce cullin1 and cullin3 neddylation by binding DCN1 and DCN2 in cells and induce the expression of the two known substrates of CRLs, NRF2 and p21. Moreover, NAcM-OPT has strong potential in vivo because of its stability and oral bioavailability. In addition, NAcM-COV is another inhibitor that was optimized from NAcM-HIT and can bind to DCN1 irreversibly by targeting the Cys115 residue of DCN1. However, NAcM-COV cannot interact with DCN5 because of its lack a cystine residue corresponding to the Cys115 residue of DCN1, emphasizing the critical role of the Cys115 residue. Consistently, treatment with NAcM-COV reduced the steady-state levels of neddylated cullin1 and cullin3. Compound 27 [133] designed and synthesized compound 27 based on the structure of pyrazolopyridone (Fig. 5e). The researchers suggested that it has a greater degree of three-dimensional structure owing to its two chiral centers, allowing it to more easily dock into the binding pocket of DCN1. Thus, **compound 27** is more potent than NAcM-like inhibitors and can engage cellular DCN1 and selectively decrease the neddylation of cullin1 and cullin3.

In addition, Zhou et al. identified compound **DC-2** from 1000 compounds [132]. Then, via an extensive structure–activity relationship (SAR) study, a novel small molecule inhibitor, **DC-2**, was discovered. DC-2 (Fig. 5f) can inhibit the interaction between UBE2M and DCN1 and disturb the neddylation of cullin3. Wang et al. obtained compound **WS-383** by screening and optimizing a series of compounds [134]. WS-383 (Fig. 5g), a triazolo[1,5-a] pyrimidine-based inhibitor targeting the UBE2M-DCN1 interaction, selectively inhibits the neddylation of cullin1 and cullin3 and increases the expression of p21, p27, and NRF2. All these compounds provide guidance to identify more potent UBE2M-DCN1 protein–protein interaction inhibitors.

### Targeting E2 enzyme for anticancer therapy

The data from preclinical trials and clinical research [14, 92] have revealed the potency, activity, and effectiveness of **MLN4924**, indicating that the neddylation pathway is a potentially powerful therapeutic target. However, resistance can also occur: Mutations in the ATP-binding pocket of UBA3 can inhibit the formation of the MLN4924-NEDD8 adduct [120], which reduces the response to it and limits its clinical application. Hence, other targets in the neddylation pathway are urgently needed as alternative strategies for targeting NAE1.

### Targeting UBE2M for anticancer therapy

In recent years, some studies have indicated that UBE2M might be an attractive alternative therapeutic target. By using two available Affymetrix microarray datasets [135, 136], Li et al. [87] identified that UBE2M, but not NAE1 and UBA3, is overexpressed in multiple types of lung cancers and associated with poor survival outcomes, as UBE2M can induce cell proliferation. In contrast, UBE2M knockdown showed a powerful effect in inhibiting tumor growth and metastasis [85–87]. Moreover, the above-mentioned series of inhibitors have been discovered to target the interaction between UBE2M and DCN1, resulting in the inactivation of the ligases CRL1 and CRL3 and the enrichment of their substrates, such as NRF2 and p21.
as NRF2, p21, and p27. Therefore, these UBE2M-DCN1 inhibitors, which have excellent potencies and pharmacokinetic characteristics, may have therapeutic potential for the treatment of human cancers. In summary, there is enough evidence to prove that UBE2M may be a promising therapeutic target for cancer treatment.

**Targeting UBE2F for anticancer therapy**

Another E2 enzyme, UBE2F, has not been studied extensively. However, Zhou et al. [89] found UBE2F has potential as an anticancer target. First, UBE2F is overexpressed in non-small cell lung cancer (NSCLC) and can be used to predict patient survival outcomes. Overexpression of UBE2F promotes NOXA accumulation, leading to inhibition of apoptosis and thus increasing cell survival. Thus, targeting the UBE2F/RBX2/CRL5 axis either with small-molecule inhibitors such as MLN4924 or by genetic depletion of either component would inactivate CRL5 to cause NOXA accumulation and apoptosis induction, thus antagonizing UBE2F-mediated growth-stimulating processes. Although there has been only one report about the biological role of UBE2F in cancer and while no inhibitors are available to target UBE2F, these results provide evidence that UBE2F may be a potential and novel target for cancer treatment.

**Conclusion**

This review discusses the current knowledge on the neddylation E2 enzymes UBE2M and UBE2F, including their structures, binding partners, substrates, and roles in the diverse biological processes. In addition, it describes how neddylation-related E2 enzymes function in cells under both normal and pathological conditions. Recent and ongoing investigations have proven that UBE2M and UBE2F are overexpressed in cancer cells and associated with cell proliferation and poor survival rates. UBE2M can facilitate cell cycle progression and autophagy by activating CRLs and reducing the levels of their substrates, leading to cell growth [86, 105]; moreover, UBE2M can also take part in NHEJ, and deletion of UBE2M leads to DSBs [59, 85, 101, 109, 110]. Coincidentally, UBE2F in complex with RBX2 can also inhibit cell apoptosis, resulting in cell proliferation [89]. Therefore, small-molecule inhibitors targeting UBE2M-DCN1 have emerged. Moreover, a cellular biological study showed that UBE2M-DCN1 inhibitors may serve as promising compounds for cancer treatment by blocking the neddylation of cullin1 and cullin3 and augmenting the levels of tumor suppressors. Nevertheless, UBE2M-DCN1 inhibitors, which were identified as discussed in this review, have not entered clinical trials, and it is critical to develop more specific and potent inhibitors.

Taken together, UBE2M may be a novel and appealing target, and UBE2F may become a potential target for cancer treatment. Unquestionably, they deserve to be studied further.

**Abbreviations**

AP-1: Transcription factor AP-1; c-CBL: Casitas B-lineage lymphoma, CDKN1A/B: Cyclin-dependent kinase inhibitor 1; CDT1: DNA replication factor Cdt1; CRLs: Cullin-RING ligases; CSN: COP9 signalosome complex; DDB1: Damage-specific DNA binding protein 1; DSBs: DNA double-strand breaks; HCC: Hepatocellular carcinoma; HIF-1α: Hypoxia-inducible factor 1α; HR: Homologous recombination; IAPs: Inhibitor of apoptosis; LUAD: Lung cancer tissue adenocarcinomas; MDM2: Murine double minute 2; NAE: NEDD8-activating enzyme; NEDD8: Neural precursor cell expressed, developmentally downregulated 8; NEDP1: NEDD8 protease 1; NRF2: Nuclear factor erythroid 2-related factor 2; NSCLC: Non-small cell lung cancer; PTM: Posttranslational modification; RBX1/2: Ring-box protein 1/2; RING: Really interesting new gene; RNF111: Ring finger protein; SAR: Structure–activity relationship; TCGA: The Cancer Genome Atlas; TRIM40: Tripartite motif 40; UBA3: Ubiquitin-like modifier activating enzyme 3; UBE2F/M: Ubiquitin-like protein modifying enzyme 3; UFD: Ubiquitin-fold domain; UBL: Ubiquitin-like protein; WIP12: WD repeat domain, phosphoinositide interacting 2.

**Authors’ contributions**

YCZ, YJG, BW, CW, and MM collected the related papers and drafted the manuscript. LHM and YG revised and finalized the manuscript. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1 State Key Laboratory of Esophageal Cancer Prevention and Treatment, Key Laboratory of Advanced Drug Preparation Technologies, Ministry of Education of China, Key Laboratory of Henan Province for Drug Quality and Evaluation, Institute of Drug Discovery and Development, School of Pharmaceutical Sciences, Zhengzhou University, 100 Kexue Avenue, Zhengzhou 450001, Henan, China. 2 Department of Hematology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China.

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