Targeting neddylation as a novel approach to lung cancer treatment (Review)

ZHAOCHUN TIAN^{1*}, JIAFEI LI^{2*}, RUIJIE MA³, TING LI², ZHIGANG SUN^{3,4} and SHUHONG HUANG^{1,2}

¹Science and Technology Innovation Center, Shandong Provincial Hospital Affiliated to Shandong First Medical University;
²School of Clinical and Basic Medical Sciences, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong 250117; ³Department of Thoracic Surgery, Jinan Central Hospital, Shandong University; ⁴Department of Thoracic Surgery, Central Hospital Affiliated to Shandong First Medical University, Jinan, Shandong 250013, P.R. China

Received January 19, 2023; Accepted April 5, 2023

DOI: 10.3892/ijo.2023.5513

Abstract. As a protein that resembles ubiquitin, neural precursor cell expressed developmentally downregulated 8 (NEDD8) takes part in neddylation, which modifies substrates

Correspondence to: Professor Zhigang Sun, Department of Thoracic Surgery, Central Hospital Affiliated to Shandong First Medical University, Jinan, 105 Jiefang Road, Shandong 250013, P.R. China

E-mail: sunszg@126.com

Professor Shuhong Huang, School of Clinical and Basic Medical Sciences, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, 6699 Qingdao Road, Shandong 250117, P.R. China

E-mail: shuhonghuang@sdfmu.edu.cn

*Contributed equally

Abbreviations: CAFs, cancer-associated fibroblasts; CAND1, cullin-associated and neddylation-dissociated 1; c-CBL, c-casitas B-lineage lymphoma; CCL2, chemotactic cytokine ligand 2; Cdt1, chromatin and DNA replication factor 1; CRL, cullin-ring ligase; CSN, constitutive photomorphogenesis signalosome; DCNL1-5, defective in cullin neddylation 1-like protein 1-5; EGFR, epidermal growth factor receptor; FBXO11, F-box protein 11; HIF, hypoxia-inducible factor; LATS1/2, large tumor suppressor homolog 1/2; LCNEC, large-cell neuroendocrine carcinoma of the lung; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MDM2, murine double minute 2; Mst1/2, mammalian sterile 20-like kinase 1/2; mTOR, mammalian target of rapamycin; NAE, NEDD8-activating enzyme; NEDD8, neural precursor cell expressed developmentally downregulated 8; NEDP1, NEDD8 protease 1; NSCLC, non-small cell lung cancer; PI3K, phosphatidylinositol-3-kinase; RBX1, ring-box 1; ROS, reactive oxygen species; SCF, Skp1-CUL1-F-box protein; Smurf1, SMAD ubiquitylation regulatory factor 1; TGF, transforming growth factor; UBA3, ubiquitin-like modifier activating enzyme 3; UBE2, ubiquitin-binding enzyme E2; YAP, Yes-associated protein

Key words: neddylation, lung cancer, MLN4924, NEDD8, signaling pathway, treatment

in a manner similar to ubiquitination and alters the activity of target proteins. Neddylation may affect the activity of multiple signaling pathways, have a regulatory role in tumor formation, progression and metastasis, and influence the prognosis of cancer treatment. The present review summarizes the regulatory roles of NEDD8 in the MDM2-p53, NF- κ B, PI3K/AKT/mTOR, hypoxia-inducible factor, Hippo and receptor tyrosine kinase signaling pathways, as well as in the development and progression of lung cancer.

Contents

- 1. Introduction
- 2. NEDD8 in post-translational modifications of proteins
- 3. Substrates and signaling pathways associated with NEDD8
- 4. Effects of neddylation on lung cancer
- 5. Lung cancer treatment targeting neddylation
- 6. Conclusion

1. Introduction

Due to environmental pollution and tobacco use, lung cancer has become the most common cause of cancer-related death worldwide, accounting for 18.4% of total cancer mortalities (1). Of note, female patients have a better prognosis than males (2). Nearly 85% of lung cancers are non-small cell lung cancers (NSCLCs). The remainder are SCLCs, of which lung adenocarcinoma is the most common subtype, accounting for 50% of NSCLC cases, followed by squamous cell carcinoma (3). Lung cancer, one of the most common cancer types, is usually detected only at a late stage, as it has no obvious symptoms in its early stages. Early implementation of screening programs is one of the main tools for patients to reduce lung cancer-associated mortality (3). Early-stage lung cancer may be surgically resected, depending on the size and location of the tumor (4). Advanced non-resectable NSCLCs may be treated with dual chemotherapy using cisplatin or carboplatin along with chest radiotherapy (5). However, the overall survival rate of patients with lung cancer is generally low and most lung cancers are metastatic at the time of diagnosis, which in turn contributes to patients' low overall survival rate. There remains an urgent need to explore new biomolecules and pathways involved in lung cancer formation, which may provide new prognostic predictors and therapeutic targets. Doing so may provide a new direction for the treatment of lung cancer.

Recent research has found that in the process of the occurrence and development of lung cancer, neural precursor cell expressed, developmentally down-regulated 8 (NEDD8) is abnormally expressed. NEDD8 is a ubiquitin-like protein that is involved in the post-translational modification of proteins, also known as neddylation. Neddylation regulates not only ubiquitination modification, but also a variety of life activities, thus playing an important role in the formation and prognosis of lung cancer.

2. NEDD8 in post-translational modifications of proteins

The NEDD8 molecule. NEDD8 is a ubiquitin-like, highly conserved protein cloned from mouse embryonic brain tissue, consisting of 81 amino acids and containing one α -helix and three β -lamellar structures. NEDD8 is most highly expressed in cardiac and skeletal tissues and is mainly expressed in the nucleus with relatively low expression in the cytoplasm. The NEDD8 molecule itself is a nonfunctional precursor protein that becomes fully-fledged and functional only when the precursor protein is hydrolyzed by specific proteases of its C-terminal amino acids. The presence of NEDD8 precursors prevents overexpression of neddylation modifications and serves as a reserve pool of NEDD8, ensuring that NEDD8 levels are within normal limits. Following the hydrolysis of amino acids, NEDD8 undergoes regulation in a manner similar to ubiquitination-through a multistage enzymatic reaction and binding to target proteins, but with a specific binding mode unlike that of ubiquitin (6,7).

The neddylation process. The NEDD8 modification process, neddylation, is similar to ubiquitination and requires NEDD8-activating enzyme (NAE) E1, NAE E2 and NAE E3. NAE is the only enzyme found to be able to perform neddylation. NAE consists of β-amyloid precursor protein binding protein 1 APPBP1 (NAE1) and ubiquitin-like modifier activating enzyme 3 (UBA3), which form the N-terminal and C-terminal ends of ubiquitin activating enzymes (8). The two types of E2 ligases that have been identified are ubiquitin-binding enzyme UBE2M (also known as UBC12) and UBE2F. UBE2M is a dual E2 enzyme that may act both as a NEDD8 ligase E2 for neddylation and as a ubiquitination ligase E2 to ubiquitinate and degrade UBE2F (9). The RING class includes ring-box 1 (RBX1), RBX2 (10), murine double minute 2 (MDM2) (11), F-box protein 11 (FBXO11) (12), c-casitas B-lineage lymphoma (c-CBL) (13), inhibitor of apoptosis proteins (14), and defective in cullin neddylation 1-like protein 1-5 (DCNL1-5), also known as DCUN1D1-5 or SCCRO1-5 (15), among others. Defective in cullin neddylation 1 (DCN1) is yeast's E3 ligase. In mammalian cells, however, at least five DCN1-like proteins act as E3 ligases (16). HECT types include SMAD ubiquitylation regulatory factor 1 (Smurf1) and Smurf2 (17). Most of the NEDD8 E3 ligases may also act as ubiquitin ligases (18).

Various specific protease 1 isozymes such as NEDD8 protease 1 (NEDP1), ubiquitin-specific peptidase 21 (USP21)

and ubiquitin C-terminal hydrolase-LC3 (UCH-LC3) expose the NEDD8 precursor's glycine residue at position 76 through hydrolysis. Subsequently, NAE catalyzes the formation of a NAE-S-NEDD8 high-energy thioester bond at the C-terminal in combination with the cysteine active site of UBA3 in the presence of ATP and Mg²⁺. This process may be referred to as the activation of NEDD8. Activated NEDD8 binds to ligase E2 UBE2M or UBE2F to form another thioester bond, while NAE departs. Ligase E3 instantly interacts with E2, which carries NEDD8, to form an isopeptide bond between the glycine at the C-terminal of NEDD8's position 76 and the lysine residue on the substrate. This process involves transferring NEDD8 to the substrate and completing neddylation. After NEDD8 binds to the substrate, ligase E3 releases binding enzyme E2, which rebinds to the next activated NEDD8 for further neddylation (6,8,19) (Fig. 1).

The deneddylation process. Neddylation is a reversible dynamic modification process. Deneddylation occurs when NEDD8 is separated from the substrate by NEDD8 depolymerase. The current study indicated that the zinc metalloprotease COP signalosome [constitutive photomorphogenesis signalosome (CSN)] and the cysteine protease NEDP1, also known as SUMO peptidase family member NEDD8 specific, catalyzes deneddylation (8). *In vivo*, CSN is mainly responsible for cullin family deneddylation, while NEDP1 is responsible for the deneddylation of other proteins (19). Enzymes involved in ubiquitination modifications, such as USP21, Ataxin-3, UCH-L1 and UCH-L3, also display deneddylation activity.

The main depolymerase involved in the deneddylation process is the NEDD8-specific enzyme CSN. CSN has 8 subunits, CSN1 through to CSN8; when the 8 subunits are complete, the main catalytic subunit, CSN5, makes the CSN begin deneddylation to remove NEDD8 from cullin and non-cullin proteins (8). NEDP1 is involved in both the activation of NEDD8 and the separation of NEDD8 from the substrate during deneddylation, meaning that NEDP1 has dual enzymatic activity. However, if the catalytic unit of NEDD1 is mutated into alanine, it is no longer able to remove NEDD8 from non-cullin proteins (20) (Fig. 1).

3. Substrates and signaling pathways associated with NEDD8

Unlike ubiquitination, neddylation mainly relies on interactions with proteins and on signal transduction to exert biological effects. Neddylation also has an important role in signal transduction, participates in DNA repair, promotes cell cycle regulation and protein degradation, and may be used as a key entry point for targeted cancer therapy (Fig. 2).

Regulation of cullin family proteins by neddylation. Cullin family proteins are well-validated neddylation substrates and are important components of ubiquitin cullin-Ring ligase (CRL), having an important role in neddylation. Furthermore, inhibiting neddylation leads to the accumulation of a variety of key CRL substrates, causing apoptosis and aging, in turn inhibiting the development of tumors. Mammalian CRLs contain CUL1, 2, 3, 4A, 4B, 5, 7 and 9, and RBX1 (also known as ROC1) or RBX2 (15). In mammalian cells, the binding enzyme



Figure 1. The neddylation modification process and the deneddylation modification process. First, enzymes such as NEDP1 are involved in the hydrolysis of NEDD8 precursors. NAE is then involved in the activation of NEDD8, thus binding to enzyme E2, at which time NAE leaves. Ligase E3 binds to E2, which carries the substrate and transfers NEDD8 to the substrate. CSN or NEDP1 participates in the depolymerization of NEDD8 from the substrate, known as deneddylation. NEDD8, neural precursor cell expressed developmentally downregulated 8; NAE, NEDD8-activating enzyme; NEDP1, NEDD8 protease 1; CSN, constitutive photomorphogenesis signalosome; USP21, ubiquitin-specific peptidase 21; UCH-LC3, ubiquitin C-terminal hydrolase-LC3.

UBE2M functions via RBX1 to mediate CUL1-4 neddylation, while the binding enzyme UBE2F pairs with RBX2 to control CUL5 neddylation (9,21-23). In this process, the CRL3 complex may be used as ligase E3 in the ubiquitin proteasome degradation pathway; it also has an important regulatory role in neddylation and deneddylation. Furthermore, this indicates that NEDD8-mediated neddylation affects the ubiquitination degradation of proteins (9). Cullin and the RING protein RBX constitute ubiquitin E3 ligase and the binding of the CUL1 C-terminal to RBX1 may promote the accumulation of CUL1 in the nucleus, thereby activating CUL1 ubiquitin ligase activity and promoting CUL1 neddylation in the nucleus. Neddylation promotes CUL1 ubiquitin ligase activity; however, disrupting this process has no effect on RBX1 binding to CUL1, meaning that there is no effect on the nuclear localization of CUL1 (24). CRL controls ~20% of proteasome-regulated proteins to regulate protein degradation (21).

In mammals, neddylation affects CRL activity through at least two mechanisms. On the one hand, CUL1, 2, 3, 4A and 5 bind to the assembly inhibitor cullin associated and neddylation dissociated 1 (CAND1) when the CRL without neddylation is inactivated, thereby inhibiting the assembly of functional CRL (25,26). CRL activation requires to be guided by NEDD8 ligase E3, which separates CAND1 from cullin by substitution and results in conformational changes that regulate CRL activity. The assembly of functional CRL is also promoted and its ubiquitinated ligase is activated. The Skp1-CUL1-F-box protein (SCF) complex is one such CRL (27,28). CUL1 is a scaffold element of the SCF complex and the SCF complex may only be formed through the interaction of CUL1 with the linker protein Skp1 (29). This interaction is involved in proteasomal degradation regulation of various proteins in the cell cycle, such as Wee1 (30), p27 (31), nuclear factor erythroid 2-related factor 2 (32) and SCF, for which expression abnormalities also contribute to tumorigenesis. However, decreased CAND1 levels only have a small effect on the binding of CUL1 to NEDD8, suggesting that the two may be independent processes (33). The dissociation of CAND1 from cullin provides a spatial site for Skp1 and RBX to bind to the cullin so that the substrate recognition protein F-box is able to recognize Skp1 cullin. Subsequently, the RBX protein binds to UBE2 and transfers the ubiquitin tag on UBE2 to the substrate, acting as an E3 ligase for the CRL complex (34). In lung cancer cells, CAND1 regulates proliferation and migration, which may affect CRL neddylation. However, CAND1 overexpression may also have adverse effects, including excessive centrosomal replication and mitotic defects that promote malignant tumor progression (35). On the other hand, after NEDD8 modifies cullin, it changes the β chain of cullin to connect with RBX1, which facilitates RBX1 linkage to ubiquitin ligase E2 (18).

Deneddylation of CRLs is mediated by CSNs (8,19), which specifically bind to NEDD8 on CRLs to control their activity. After the dissociation between NEDD8 and the CRL complex, interferon-induced protein NEDD8 ultimate buster 1 mediates CRL complex proteasomal degradation following NEDD depolymerization and the degradation of the substrate with the ubiquitin tag.

Studies have indicated that the NAE inhibitor MLN4924 completely inhibits the neddylation of cullin, thus inactivating CRL and causing a build-up of CRL substrates, including cell cycle inhibitors p21, p27 and WEE1; NF- κ B inhibitor I κ B- α ; and DNA replication-licensed proteins chromatin and DNA replication factor 1 (Cdt1) and replication initiation recognition complex, subunit 1 (36).

In addition to the cullin family of proteins, proteins associated with the ubiquitin-proteasome pathway that may serve as neddylation substrates include MDM2 and SMAD ubiquitination regulator factor 1 (Smurf1). MDM2 serves as both the ubiquitination ligase E3 and the neddylation ligase E3. Neddylation modification enhances MDM2 stability and promotes ubiquitination degradation of p53 (37). Multiple



Figure 2. NEDD8 regulates the ubiquitin-proteasome degradation pathways related to CRL and MDM2, and it realizes the regulation of proteins by promoting the NF-κB, PI3K/AKT/mTOR, Hippo-YAP and receptor tyrosine kinase signaling pathways. NEDD8 also inhibits MDM2-p53, HIF and other signaling pathways, which in turn affects DNA damage repair, the cell cycle and tumor development. HIF, hypoxia-inducible factor; CRL, cullin-ring ligase; EMT, epithelial to mesenchymal transition; NEDD8, neural precursor cell expressed developmentally downregulated 8; NAE, NEDD8-activating enzyme; NEDP1, NEDD8 protease 1; CSN, constitutive photomorphogenesis signalosome; PTEN, phosphatase and tensin homolog; VHL, von Hippel-Lindau; Mst1/2, mammalian sterile 20-like kinase 1/2; YAP, Yes-associated protein; MDM2, murine double minute 2; PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; LATS1/2, large tumor suppressor homolog 1/2; EGFR, epidermal growth factor receptor; TGFβRII, TGF-β type II receptor.

lysine sites of Smurf1 may enhance both the recruitment of ubiquitin-binding enzyme E2 and its own ubiquitin ligase activity after neddylation (38).

Regulation of the RPL11-MDM2-p53 signaling pathway by neddylation. Nucleoli are at the center of ribosome genesis. Ribosomal proteins generally enter the nucleoli and bind to ribosomal RNA and other proteins to form ribosome-sized subunits (39). p53 is an important tumor suppressor gene that has a role in inhibiting cell cycle progression and inducing cell senescence or apoptosis, responding to a variety of emergency signals, including DNA damage, hypoxia and ribosomal stress. Normally, neddylation inhibits the release of RPL11 from the nucleoli and protects it from degradation. MDM2 then binds to p53 at the promoter site, inhibits the transcription of p53 and promotes p53 degradation to reduce p53 expression. During the nucleolar stress response, the neddylation of L11 in the nucleoli is inhibited. In turn, L11 is released from the nucleolus. L11 released into the nucleoplasm binds to MDM2 at the promoter to inhibit the ubiquitination of MDM2 to p53, which promotes the transcriptional activation of p53. p53 expression increases and promotes p53-dependent cell cycle arrest. L11 is also able to undergo neddylation in the cytoplasm before entering the nucleus, and this process is mediated by MDM2. The cytoplasm contains NEDP1, which mediates the deneddylation of L11 (39,40). In addition, the neddylation of human p53 depends on HDM2, the homologous protein of MDM2. Ribosomal protein S14 may also induce p53 activation during nucleolar stress. The signaling between the two is connected by HDM2, wherein the process is similar to that of RPL11 (41). Mutations in p53 frequently occur in lung cancer and their expression is closely related to lung cancer development and progression. In wild-type p53-preserved tumors, MDM2 overexpression may block the



Figure 3. Several signaling pathways are closely related to NEDD8. L11 is modified by NEDD8 and remains in the nucleolus; in contrast, unmodified L11 enters the nucleoplasm to bind to MDM2, thereby promoting the transcriptional activation of p53. IκB, the inhibitor of NF-κB, is recognized by NEDD8-modified SCF after phosphorylation, thereby degrading it and activating the NF-κB signaling pathway. In the PI3K/AKT/mTOR signaling pathway, PTEN is modified by NEDD8 and moves into the nucleus, promoting the phosphorylation of PIP2 and activating downstream pathways. NEDD8, neural precursor cell expressed developmentally downregulated 8; PTEN, phosphatase and tensin homolog; CSN, constitutive photomorphogenesis signalosome; MDM2, murine double minute 2; SCF, Skp1-CUL1-F-box protein; PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; PDK1, phosphoinositide-dependent kinase 1; RBX, ring-box; NEDP1, NEDD8 protease 1.

transcriptional activity of p53 and thus reduce the expression levels of p53 (39,42).

MDM2 may reduce the expression of p53 in two aspects. First, MDM2 degrades p53 with ubiquitin ligase E3 through the ubiquitin-proteasome pathway; furthermore, MDM2 may be used as NEDD8 ligase E3 to neddylate p53 in order to inhibit nuclear translocation and transcriptional activity. These processes are inhibited by cyclin-dependent kinase inhibitor p14 and histone acetyltransferase TIP60, respectively (11). However, p53 neddylation by MDM2 may be weakened by heat stress, thus reducing the inhibition of p53 activity. Another NEDD8 ligase, FBXO11, has also been found to enable p53 neddylation, thereby inhibiting p53 function and its transcriptional activity without affecting stability (12) (Fig. 3). Regulation of the NF- κ B signaling pathway by neddylation. NF- κ B is a dimer composed of p50 and p65, as well as a transcription factor that inhibits apoptosis. It is also related to immune and inflammatory responses, may induce the expression of a variety of pro-inflammatory mediators and is closely related to tumor occurrence, growth and metastasis. It binds specifically to the enhancer B sequence of the immunoglobulin κ light chain gene GGGACTTTCC, thereby promoting κ light chain gene expression. NF- κ B is widely distributed in mammalian cells, and the Rel homology domains at the N-terminus contained in NF- κ B. The NF- κ B family contains homologous or heterologous dimers-regions specific to the DNA sequence of NF- κ B, including NF- κ B1 (p50 and its precursor p105), NF- κ B2 (p52 and its precursor p100), c-Rel, RelA (p65) and RelB (43).

p100, p105, $I\kappa B\alpha$, $I\kappa B\beta$, $I\kappa B\gamma$, $I\kappa B\epsilon$, Bcl-3 and $I\kappa B-R$ are inhibitory units of the IkB family that both bind to NF-kB and impede its transfer to the nucleus. Thus, NF-KB is usually present in the cytoplasm in an inactive form (43). When IkB is phosphorylated to pIkB by the activated IkB phosphokinase (IKK) complex, it may be recognized by the ubiquitin-binding enzyme SCF, and it may be degraded by ubiquitination and proteases. CUL1 may be activated by enhancing SCF activity after neddylation (44). Activated NF-kB may be transferred to the nucleus, specifically binding to the κB sequence to induce the transcription of related genes, such as tumor necrosis factor- α , interleukin-6 (IL-6) and other pro-inflammatory factors, leading to inflammation and immune response (45). However, it also rapidly encodes inhibitor IkB transcription and the newly synthesized IkB binds to NF-kB to prevent it from moving to the nucleus and performing feedback regulation. In lung cancer, neddylation inactivation inhibits SCF activity and induces substrate IκBα accumulation to block NF-κB transcriptional activity and chemotactic cytokine ligand 2 (CCL2) transactivation, affecting the invasion of tumor-associated macrophages. This in turn affects the development of lung cancer (46). IL-6 also enhances the invasion and metastasis of lung cancer cells via the NF-κB pathway (47).

In addition to phosphorylating I κ B, IKK may phosphorylate CSN, thereby ubiquitinating and degrading it (48). The main role of CSN is to promote deneddylation. Thus, it is inferred that CSN degradation maintains neddylation such that CRL is activated to promote the NF- κ B pathway (Fig. 3).

Regulation of the PI3K/AKT/mTOR signaling pathway by neddylation. The PI3K/AKT/mTOR signaling pathway is able to regulate cell proliferation, differentiation, apoptosis and migration, and it has an important role in cancer development. Phosphatidylinositol (PI)-3-kinase (PI3K) is a dimer composed of the regulatory subunit p85 and the catalytic subunit p110; furthermore, it is a downstream effector for G protein-coupled receptors and receptor tyrosine kinase (RTK) (49). PI generates PIP and PIP2 by phosphorylation, while RTK activates PI3K. PI3K may phosphorylate PIP2 to generate a second messenger, PIP3, and stay in the plasma membrane to be used as a docking site for intracellular proteins, thereby recruiting protein kinase B (AKT) and phosphoinositide-dependent kinase 1 to the plasma membrane to further activate AKT. In mammals, the mammalian target of rapamycin (mTOR) may be used as a downstream target of AKT, with two complexes, mTORC1 and mTORC2. mTORC1 mainly regulates cell growth and metabolism, and mTORC2 mainly regulates cell proliferation and cytoskeletal remodeling. The downstream transcription factors of mTOR include hypoxia-inducible factor 1 (HIF-1a) and c-Myc. MDM2 is also one of the substrates of AKT and may activate itself by mediating MDM2 phosphorylation, therefore causing MDM2 to enter the nucleus and bind to p53. The tumor suppressor protein phosphatase and tensin homolog (PTEN) may act as a phosphatase to dephosphorylate PIP3 to form PIP2, thereby inhibiting this pathway (49,50). Recent studies have indicated that neddylation may promote the nuclear translocation of PTEN and subsequently reduce cytoplasmic PI3K/AKT/mTOR signaling inhibition. As such, neddylation may enhance the PI3K/AKT/mTOR signaling pathway to promote tumor cell proliferation and infiltration. The neddylation of PTEN relies on the activating enzyme E1 UBA3, the binding enzyme E2 UBE2M and the ligase XIAP, whose deneddylation is mediated by NEDP1. This process is also affected by the glucose concentration; the modification of PTEN by neddylation is promoted under conditions of high glucose concentrations (51).

Activation of the PI3K/AKT/mTOR signaling pathway may promote the morphological transformation of SCLC cells. Adherent SCLC cells with an activated PI3K/AKT/mTOR signaling pathway are more susceptible to chemotherapy resistance (52). This suggests that the PI3K/AKT/mTOR signaling pathway may be inhibited by inhibiting neddylation, thus affecting lung cancer treatment (Fig. 3).

Regulation of the HIF signaling pathway by neddylation. HIF is regulated by oxygen and its own expression; HIF includes HIF-1 and HIF-2, of which HIF-1 is the most important and is composed of two subunits, HIF-1 α and HIF-1 β . In a study of NSCLC, it was found that the HIF pathway is involved in the early stages of tumor progression. Cancer cells grow without restriction when oxygen-supplying blood vessels are not generated, creating a hypoxic environment that induces upregulation of HIF-1 α and its binding to HIF-1 β . The bound heterodimer enters the nucleus and activates the transcriptional expression of related genes. This provides ideal cancerous conditions and the formation of blood vessels for cancer cells growing under hypoxic conditions; it does so by activating glycolytic pathways and increasing glucose transport (53). Activation of cancer-associated fibroblasts (CAFs) is critical for establishing a tumor-promoting microenvironment, and HIF-1 α is highly expressed in lung cancer in hypoxic conditions, inducing the transformation of normal fibroblasts into CAFs (54). Hyperthermia is one of the clinical treatments for lung cancer; however, recurrence is common because the thermal effect at the time of treatment may promote the growth of residual tumors by upregulating the expression of HIF-1 α (55).

HIF-1 α 's main ubiquitin ligase E3 is a complex, the most important component of which is the von Hippel-Lindau (VHL) protein. CUL2 is an upstream protein of VHL that regulates the degradation of HIF-1a by VHL. Both VHL and CUL2 may be neddylated. When CUL2 is modified by neddylation, it may bind to VHL and activate the E3 complex of HIF-1 α ; CUL2 then recognizes and binds to HIF-1 α and multi-ubiquitinates it. The labeled HIF-1 α may be degraded by the 26s proteasome; therefore, inhibition of neddylation with MLN4924 enhances HIF transcription and stabilizes HIF levels, while upregulating the expression of HIF-1 α -mediated UBE2M (9,56). VHL contains three NEDD8 receptor sites at lysines 159, 171 and 196, and it binds to the NEDD8 ligase MDM2 (57,58). When VHL is modified by neddylation, it prevents binding to CUL2. The ubiquitination and degradation of HIF-1 α are inhibited and stabilized, thereby promoting tumorigenesis (57). As such, homeostasis of neddylation and deneddylation may regulate the degradation of HIF-1 α (Fig. 4).

Regulation of the Hippo-YAP signaling pathway by neddylation. The Hippo signaling pathway is involved in cancer cell genesis, invasion, migration and treatment; its core effector Yes-associated protein (YAP) acts as a multifunctional intracellular connexin and transcriptional coactivator that has a role in



Figure 4. Several signaling pathways are closely related to NEDD8. In the HIF signaling pathway, VHL binds to NEDD8-modified CUL2, thereby ubiquitinating HIF-1α; NEDD8-modified VHL inhibits this process. The Hippo-YAP pathway requires NEDD8 for a series of phosphorylation reactions. For instance, NEDD8-modified CUL7 promotes Mst1 degradation and NEDD8-modified CUL4 promotes LATS1/2 degradation. In the RTK signaling pathway, TGFβRII and EGFR are also modified by NEDD8 to remain stable. HIF, hypoxia-inducible factor; NEDD8, neural precursor cell expressed developmentally downregulated 8; Mst1/2, mammalian sterile 20-like kinase 1/2; YAP, Yes-associated protein; RTK, receptor tyrosine kinase; TGFβRII, TGF-β type II receptor; EGFR, epidermal growth factor receptor; TEAD, transcriptional enhanced associate domain; c-CBL, c-casitas B-lineage lymphoma; VHL, von Hippel-Lindau; LATS1/2, large tumor suppressor homolog 1/2.

both intracellular signal transduction and gene transcriptional regulation (59). Hippo kinases include neurofibromatosis 2, mammalian sterile 20-like kinase 1/2 (Mst1/2) and large tumor suppressor homolog 1/2 (LATS1/2). In the cytoplasm, once signaled by extracellular growth inhibition, a series of kinase cascade phosphorylation reactions are activated. This process activates the Hippo kinase and eventually phosphorylates YAP, causing YAP to remain in the cytoplasm and degrade. The YAP signaling pathway is subsequently inhibited. Conversely, when kinase cascade phosphorylation reactions are inhibited, YAP in the cytoplasm is transferred to the nucleus and binds to

transcriptional enhanced associate domain, thereby activating downstream gene transcription and regulating cell proliferation, epithelial-mesenchymal transformation (EMT), metastasis, proliferation and differentiation of cancer stem cells, all of which promote tumor development. NEDD8 substrate CUL7, as a ubiquitin ligase, is able to promote Mst1 ubiquitination, and CUL4, as a ubiquitin ligase, may promote LATS1/2 ubiquitination. Both activate YAP signaling, indicating that neddylation is able to regulate the Hippo-YAP pathway (59-61). Of note, the function of YAP may differ among different lung cancer types. YAP activation promotes the growth of NSCLCs, but inhibits SCLCs; the specific reasons for this require to be further studied (59,62) (Fig. 4).

Regulation of the receptor tyrosine kinase (RTK) signaling pathway by neddylation. RTKs are the largest class of enzyme-linked receptors and are receptors for both growth factors and enzymes to catalyze phosphorylation of downstream target proteins. They have an important role in mediating cell growth, movement, differentiation and metabolism; in addition, they interact with transforming growth factor- β (TGF- β), PI3K/AKT and other signaling pathways. The most common RTKs include epidermal growth factor receptor (EGFR) and fibroblast growth factor receptor (63,64). TGF- β type II receptor (TGF β RII) and EGFR are known examples of RTK signaling regulated by NEDD8 (65).

TGFβRII upregulates the expression of interferon-β in tumor-associated macrophages after radiotherapy; it also facilitates T-cell infiltration. For advanced lung cancer, TGFβRII promotes tumor development; inhibition of TGFβRII may be used to treat lung cancer (66). c-CBL is a proto-oncogene encoding ubiquitin ligase E3 that may bind UBE2M to neddylate TGFβRII, thereby inhibiting the ubiquitination degradation of TGFβRII. In other words, TGFβRII may be stabilized by neddylation to promote TGF-β signaling (prolonging TGFβRII signaling in clathrin-mediated cellular endocytosis). Therefore, c-CBL may be used as the NEDD8 ligase E3 of TGFβRII. At the same time, MLN4924 may block the promotion of TGF-β by c-CBL, promote the ubiquitination and degradation of TGFβRII and induce cell cycle arrest (65,67).

The receptor tyrosine kinase EGFR binds to and is activated by extracellular growth hormone. This process activates a signaling cascade within the cell. c-CBL has dual enzymatic activity that may both ubiquitinate EGFR as a ubiquitin E3 ligase, as well as recruit NEDD8 binding enzyme UBE2M. Neddylation may further enhance the ubiquitination of EGFR, which promotes the degradation and endocytosis of EGFR (65,68). In the treatment of lung cancer, most drugs target EGFR; however, the drug-resistant mutation of EGFR is a significant barrier to treatment (63,69). EGFR gene fusion also affects the therapeutic effect of targeting EGFR in patients with lung cancer (70). Therefore, studying the process and function of EGFR neddylation modification may also help in the exploration of treatments targeting EGFR (Fig. 4).

4. Effects of neddylation on lung cancer

NEDD8-mediated neddylation is closely related to the occurrence and development of tumors. Overexpression of neddylation-related proteins has been seen in lung cancer (30), breast cancer (51), liver cancer (71) and colorectal cancer (72) and may significantly reduce the overall survival rate of patients. To date, it has been found that the expression pattern of NEDD8-related proteins has changed in cancerous lung tissues, including large-cell neuroendocrine carcinoma of the lung (LCNEC), lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) (73).

Clinicopathological features. Real-time quantitative PCR and microarray testing of mRNA have indicated significantly

elevated NAE1 mRNA expression in LUAD and LUSC compared with adjacent normal tissues (36). The mRNA levels of UBE2M were significantly higher in LUAD, LUSC and LCNEC than in normal tissues, and the expression of UBE2M mRNA was higher in poorly-differentiated tumors within lung adenocarcinoma (30). The ligase E3 DCNL and its paragenes are generally dysregulated in human cancers and are associated with the neddylation of the cullin family, where the most common dysregulated ligase E3 is DCNL5. DCNL5 is overexpressed in LUSC and LUAD (74). This suggests that NEDD8 may be used as a serum metabolic fingerprint (SMF) for the diagnosis and treatment of lung cancer, and there are numerous platforms and methods based on SMF for the early detection of lung cancer (75,76).

Immunohistochemical staining and western blot analysis revealed that primary tumor protein neddylation is overactivated and that NAE1, UBA3, UBE2M and UBE2F are overexpressed in LUAD and LUSC tissues compared with adjacent normal tissues (36,77,78). Particularly in SCLCs, western blot analysis indicated bands with a higher migration form than CUL1 and detected neddylation-modified fragments. This affects the activity of SCF complexes. Immunohistochemical staining suggested a negative correlation with CAND1 protein levels in SCLCs. This, however, is not observed in normal tissues, NSCLCs or carcinoid tissues (29).

In addition, Kaplan-Meier single-gene survival analysis indicated that patients with lung cancer with high UBE2M expression had a poor prognosis and low overall survival. Analysis also indicated that patients with elevated neddylation had lower overall survival than patients with low expression (30,36,73,79); furthermore, patients with high UBE2M and NEDD8 mRNA levels had lower overall survival than patients with low expression. However, there is no significant association between NAE1 - a component of NEDD8-activating enzyme E1 - and UBA3 mRNA levels with the overall survival rates of patients with lung cancer (30).

During the development and progression of lung cancer, related microRNAs (miRNAs/miRs) also change. Efficient, sensitive and specific detection methods and systems for miRNA may improve the accuracy of diagnosis and prognosis of cancers (80). For instance, miR-155 expression is gradually elevated in the development and progression of lung cancer, suggesting a target for early screening and tracking of lung cancer (81). miR-155 is positively regulated by NF- κ B, while NEDD8 promotes the NF- κ B signaling pathway. This results in high miR-155 expression and association with certain aggressive diseases (82).

Experiments at the cellular level. Excessive activation of neddylation in human lung cancer cells (H1299, A549 and H460) compared with normal lung fibroblasts (WI38 and MRC-5) (36) suggests that the development and progression of lung cancer may be closely related to neddylation. After knockout of UBE2M or NEDD8, A549 and H1299 cell proliferation, colony formation, transportation, migration and invasion were all inhibited, thus affecting the malignant phenotype of lung cancer cells (30,73). Propidium iodide staining and fluorescence-assisted cell sorting analysis of NEDD8-knockout cells revealed cell cycle arrest in G_2/M phase. G_2/M phase conversion inhibitor Wee1 accumulation



Figure 5. The NAE inhibitor MLN4924 has utility in the treatment of lung cancer. It may affect the formation of the TME and promote tumor cell death by regulating a variety of proteins and various signaling pathways, such as the HIF pathway and NF-κB pathway. MLN4924 may also be combined with certain drugs to make chemotherapy and radiotherapy more effective. TME, tumor microenvironment; HIF, hypoxia-inducible factor; DSB, double-strand break; NAE, NEDD8-activating enzyme; mTOR, mammalian target of rapamycin; MDM2, murine double minute 2; RBX1, ring-box 1; Cdt1, chromatin and DNA replication factor 1; NOXA, phorbol-12-myristate-13-acetate-induced protein 1; DR5, death receptor 5; PTX, paclitaxel; CRL, cullin-ring ligase.

and M-phase marker phospho-histone h3 downregulation have been discovered by western blot, further indicating that NEDD8 leads to G₂ phase arrest in LUAD cells by inhibiting CRL activation. p21, p27 and Wee1 are substrates for NEDD8-modified CRL; as such, the protein stability and half-life of these cell cycle-associated proteins were analyzed with cycloheximide chase. The protein stability and half-life of these proteins increased significantly in NEDD8-knockdown A549 cells, indicating that they are prevented from degradation and thus, G₂ phase arrest was induced (73). Furthermore, these cells appear enlarged and flattened in shape, suggesting that NEDD8 deletion triggers senescence in A549 cells. This is further confirmed by the β -galactosidase staining associated with senescence (73). It was also indicated that inhibition of NEDD8 expression targeted with small inhibitory siRNA promotes H1299, HCC-44, NCI-H1650 and NCI-H292 cell migration, while having no effect on A549 migration (13). It is important to note that in A549 cells, the degree of neddylation may differ from other cell lines.

5. Lung cancer treatment targeting neddylation

Mouse tumor models established with A549 cells indicated significant inhibition of growth and metastasis in the NEDD8-knockout group (46), and IHC staining suggested that there were fewer Ki67 (tumor proliferation marker)-positive cells in the NEDD8 knockout group (73). This suggests that NEDD8 may be used as a potential target for the treatment of lung cancer. At the same time, this targeted therapy may also be used as an adjunct to radiotherapy (77), chemotherapy (83) and pulsed electromagnetic therapy (84).

Basic information and the mechanism of MLN4924. MLN4924, also known as Pevonedistat, is an AMP-like adenosine sulfamate analog with a similar position in the NEDD8 structure to ATP (85). Therefore, it may specifically bind to the activating enzyme NAE of NEDD8 and inhibit its activity to prevent the formation of thioester bonds between UBA3 and NEDD8 on NAE, which is required to activate NEDD8. Inhibiting NEDD8 inhibits neddylation and CRL activity, resulting in CRL substrate accumulation. Lung metastases in mouse models using wild-type LLC cells have resulted in reduced intrapulmonary metastases in the MLN4924-treated group (46). Clinical trials of MLN4924 in the treatment of solid tumors have also been performed. In the Phase Ib study of MLN4924, it was preliminarily proved that MLN4924 has no additional toxicity for the treatment of solid tumors, which ensures a certain safety. It also has a pharmacodynamic effect in solid tumors such as melanoma, gastric, ovarian, head and neck, adrenal and breast cancer (86,87). *In vivo* and *in vitro* experiments have demonstrated that MLN4924 has an inhibitory effect on lung cancer (36,46,88,89).

MLN4924 induces cell death. MLN4924 effectively inhibits cancer cell growth by inducing three common types of cell death: Apoptosis, senescence and autophagy (Fig. 5).

Apoptosis. MLN4924 inactivates CRL1 and CRL4 by inhibiting neddylation. This results in an increase in substrate Cdt1 levels, inducing DNA re-replication, and triggering a DNA damage response. As such, apoptosis and aging processes that inhibit tumor growth are activated (90-92), and different sensitivities in liver, ovarian, prostate, colon and lymphoma tissues were observed (90).

The activity of the ubiquitin-binding enzyme SCF is also inhibited, and its substrate-NF- κ B inhibitory unit I κ B α -accumulates, thereby blocking NF- κ B activation and inducing apoptosis. MLN4924 also causes the accumulation of CRL substrates such as the pro-apoptotic proteins phorbol-12-myristate-13-acetate-induced protein 1 (NOXA) and activating transcription factor 4, leading to apoptosis (34,36,93).

Senescence. RBX1 may mediate the neddylation of CUL1-4. Studies have indicated that RBX1 is overexpressed in a variety of human tumors, such as lung, liver, breast, colon and ovarian cancers. RBX1 silencing inhibits neddylation, upregulates pro-apoptotic proteins such as the substrate

p53 up-regulated modulator of apoptosis, downregulates anti-apoptotic proteins such as Bcl-2 and triggers a DNA damage response. Accordingly, RBX1 silencing may induce apoptosis and senescence. This aging process is not related to p53 and p16 (94). The percentage of senescence-associated- β -galactose staining may also be increased in cells following brief treatment with MLN4924, suggesting that the same therapeutic effect may be achieved even if the MLN4924 treatment is short in duration (92,95). Another study suggested that p21-mediated aging is associated with growth inhibition induced by low-dose MLN4924. Furthermore, the study suggested that following drug removal, p21 continues to accumulate; DNA damage responses continue to activate, making MLN4924-induced aging irreversible. Thus, it is possible to use low-dose drugs to achieve the desired therapeutic effect (91).

Autophagy. mTOR inactivation and reactive oxygen species (ROS) excess under MLN4924 are the main causes of autophagy.

mTOR is the main regulatory molecule of cell growth and metabolism; it promotes anabolic processes and inhibits catabolic processes such as autophagy. MLN4924 blocks the degradation of the cullin family substrate mTOR inhibitory protein Deptor, resulting in mTORC1 inactivation and autophagy (34,71,96). Furthermore, HIF-1 α is also significantly accumulated due to the inhibition of CUL2 neddylation, which may negatively regulate mTORC1 through the HIF1-regulated in development and DNA damage responses-1-TSC1 axis and in turn trigger autophagy (96). MLN4924 also enhances ROS production and induces oxidative stress in cancer cells; ROS inhibits the activity of the downstream protein mTOR and induces autophagy (71,97).

MLN4924 affects tumor microenvironments and metastasis. Abnormal angiogenesis is an important feature of malignancy (98). In pancreatic cancer, MLN4924 is found to significantly inhibit capillary formation and cell migration, reduce vascular branch points, and inhibit tumor angiogenesis and growth. Mediating the inactivation of RBX2 in CRL, which accumulates substrates the Ras homolog gene family, member A (99), neurofibromatosis type 1 (100) and p27 (101), impairs cell migration and angiogenic processes.

The HIF signaling pathway also affects the tumor microenvironment. The early stages of tumor progression are frequently accompanied by hypoxia, so the expression of HIF-1 α and HIF-2 α is found to be upregulated in most lung cancer cases. Furthermore, their high expression may be closely related to the expression of vascular endothelial growth factor, thymidine phosphorylase and basic fibroblast growth factor. Overexpression of HIF-2 α is associated with a poor prognosis for patients (53). MLN4924 has a negative role here, but it may be degraded by ubiquitination of HIF-1 α to promote tumor development (56).

Immune evasion is considered one of the hallmarks of cancer; inflammation is involved in almost all stages of tumorigenesis and promotes tumor development. Tumor-infiltrating leukocytes include dendritic cells (DCs), T-cells and macrophages (102); these have an important role in generating tumor-promoting immune microenvironments. MLN4924 significantly attenuates the inflammatory response by reducing the expression of pro-inflammatory cytokines and chemokines, such as IL-1β, IL-6 and C-X-C motif chemokine ligand 1, all of which are induced by IL-17A (103-105). MLN4924 accumulates Deptor and inactivates the mTOR signaling pathway, while also inhibiting the biological function of DCs (106). In addition, it inhibits the production of pro-inflammatory factors in DCs by inhibiting the NF-κB signaling pathway (107), further curbing the production of a pro-tumor microenvironment. Tumor-associated macrophages (TAMs) are the most abundant tumor stromal cells and they provide a suitable microenvironment for tumor development by inducing growth factors, angiogenesis regulators and inflammatory mediators. In tumors, TAMs are recruited from the bone marrow primarily by CCL2. In LUAD, NEDD8 expression levels are positively correlated with a high expression of CCL2, leading to a decrease in overall survival. Partial inactivation of neddylation may block transcriptional activation of the NF-KB-regulated CCL2, thereby exerting anticancer effects to inhibit monocyte chemotaxis and TAM invasion, and ultimately, tumor metastasis (46). RBX2-CUL5 may also modulate certain functions of TAMs to control lung inflammation (22,108).

Metastasis is one of the leading causes of death in cancer patients, and cancer metastasis involves a series of processes. Neddylation may be used as a target for anti-metastatic therapy. In non-small cell carcinomas, MLN4924 has been found to reduce the number of intravascular cancer cells and inhibit their extravasation. MLN4924 inhibits EMT by enhancing p53 activity, inhibiting lung cancer metastasis (109,110) (Fig. 5).

Effect of MLN4924 on chemotherapy. NSCLC is the most common type of lung cancer, and cytotoxic drugs such as cisplatin and carboplatin may be used in the clinical treatment of NSCLC (83). However, resistance to platinum drugs may lead to cancer recurrence and treatment failure, and the prognosis is poor. Upregulation of the NEDD8-binding enzyme UBE2F is an important pathway for lung cancer cells to evade platinum-induced apoptosis. After platinum-based drug treatment, UBE2F as a substrate has a weakened ability to bind to CUL3, resulting in the accumulation of UBE2F. However, the accumulation of UBE2F combined with RBX2 promotes the neddylation of CUL5, which in turn promotes the degradation of substrate NOXA. The oxidative stress capacity of cells and cell survival are subsequently reduced. This also suggests that UBE2F may be used as a new therapeutic target. MLN4924 may inhibit neddylation, thereby indirectly inhibiting UBE2F and allowing NOXA to further promote apoptosis (111).

Conventional chemotherapy also uses paclitaxel (PTX). Resistance to chemotherapy drugs such as PTX is a major cause of chemotherapy failure in patients with NSCLC. MLN4924 significantly inhibits the proliferation of lung cancer cells, as well as tumor formation and metastasis, by increasing CRL substrate levels. The combination of MLN4924 and PTX does not have any synergistic effects in PTX-resistant NSCLC cells, indicating that MLN4924 may be used as a drug for the treatment of PTX-resistant NSCLC (36,112) (Fig. 5).

Effects of MLN4924 on radiotherapy. At present, radiotherapy is the main treatment method for different stages of lung cancer and may significantly prolong the survival time of patients. The use of ionizing radiation and radiation therapy induces

cancer cell death, primarily by producing ROS through water and oxygen reactions; however, high levels of ROS increase the oxidative stress response and induce DNA damage as well as apoptosis. Compared with SCLC, NSCLC exhibited greater tolerance to radiotherapy. Neddylation may be the key to anti-cancer therapy.

After 48 h of irradiation, the expression of UBE2F in A549 and H1299 cells gradually increased. N-acetylcysteine, a typical reactive oxygen species scavenger, completely blocks radiation-induced increases of UBE2F. In cells treated with different concentrations of H_2O_2 , UBE2F increased significantly in a dose-dependent manner, further suggesting that radiation-induced upregulation of UBE2F may be associated with increased ROS levels. Staining with trypan blue after UBE2F knockout indicated that the mortality rate of UBE2F knockout cells in the experimental group increased under irradiation and western blot analysis demonstrated an increase in the concentration of CRL5 substrate pro-apoptotic protein NOXA in cells of the experimental group (77,78). This suggests that the resistance effect may be eliminated by MLN4924.

DNA double strand break (DSB) is a severe form of DNA damage that, if not repaired, may lead to cell carcinogenesis or death. There are two main mechanisms of DSB repair in mammalian cells, namely homologous recombination and non-homologous end joining (NHEJ). FBXW7 is an important tumor suppressor gene and may act as a target protein of the ubiquitin ligase SCF complex, thereby regulating its down-stream genes such as cyclin E. In addition, it may enhance NHEJ by promoting the recruitment of DSB repair factor, which is one of the reasons for cancer cell survival. MLN4924 inhibits the activity of CUL1 and thus inhibits the activity of the SCF complex. This prevents the binding of FBXW7 to the SCF complex, impairing NHEJ and subsequently inducing radio-sensitization of cancer cells (113) (Fig. 5).

Negative effects of MLN4924. In related experiments, it has been found that MLN4924 has different effects depending on serum and drug concentrations, which may inhibit cancer cell survival and may also stimulate tumorigenesis and cell migration (13,114).

In the case of MLN4924 blocking neddylation, c-Myc gradually accumulates as a CRL substrate and c-Myc may induce fibroblasts to transform into pluripotent stem cells (115). EGFR dimerization may be induced to activate and prolong EGFR signaling that is frequently caused by mutation or overexpression of EGFR (114). MLN4924 also activates the PI3K/Akt/mTOR pathway, thereby inducing HIF-1 α expression and promoting cancer cell migration (56,116). Furthermore, neddylation restriction increases cancer cell migration and Slug expression in cells lacking p53, thereby promoting EMT (110). NEDD8 is expressed at low levels in A549 cells, which may also be the reason why the migration of A549 cells after MLN4924 exposure is not affected (13,117).

Other medications. HA-1141 (Ui5-8-11-41), a small molecule compound, may induce autophagy by blocking the neddylation of cullin and triggering endoplasmic reticulum (ER) stress, thus inhibiting cancer cell growth. On the one hand, HA-1141 inactivates the activation enzyme NAE. This blocks neddylation and allows cullin family substrates to accumulate for a short

duration. However, it may trigger atypical ER stress and integration stress response. It may also produce ROS, inactivate mTORC1 and inhibit protein synthesis at the translation level, therefore prompting autophagy (97).

The antihypertensive drug candesartan cilexetil, as a neddylation inhibitor, also competitively inhibits NAE and inhibits tumor growth (118). Candesartan cilexetil is a derivative of benzimidazole and recent studies have evaluated different derivatives of benzimidazole to improve certain shortcomings of candesartan cilexetil by inhibiting neddylation efficacy to fight cancer (119).

Another factor targeting NAE is activity-based probe (ABP) A3. ABP A3 is a dual inhibitor of ubiquitin and NEDD8-activating enzyme NAE and is more effective in inhibiting substrate ubiquitination. It induces the accumulation of intracellular misfolded proteins, stressing the ER, and stimulating apoptosis to inhibit tumor development (120).

Further drugs, including MLN4924, are being used to target NAE. However, during treatment, MLN4924's target UBA3 mutates to result in drug resistance (121-123), as MLN4924 becomes less impactful. Thus, it is necessary to look for other molecules in neddylation as therapeutic targets. Studies have indicated that DI-591 and its derivatives, piperidinyl ureas, may also competitively bind to ligase DCN1 with NEDD8-binding enzyme UBE2M. This blocks the neddylation of CUL1 and CUL3 and achieves therapeutic effects (124-127). However, the flaw of DI-591, compared to MLN4924, is that it cannot halt the neddylation of the entire cullin family.

In addition to drugs targeting NEDD8, there are others that may block the neddylation process by knocking out its substrates. Biomolecules such as DNA, proteins and lipids may be assembled into nanoparticles that may be used to diagnose and treat tumors (128). For instance, polydopamine (PDA) may work to prepare PDA nanoparticles. PDA nanodrugs may be used as carriers of siRNA, targeting tumors and knocking out the neddylation substrate RBX1 according to the pH of tumors and adjacent tissues, thereby promoting tumor cell apoptosis. In liver cancer, it was found that in combination with photothermal therapy, PDA nanodrugs modified by folic acid are able to better target liver cells and improve the therapeutic effect (129). However, there are currently no studies on this in lung cancer. Of note, it may be possible to block neddylation through PDA nanomedicine.

6. Conclusion

The main function of NEDD8 is to mediate the participation of neddylation in a variety of signaling pathways. Neddylation may affect CRL activity and the ubiquitination process. It may also participate in tumorigenesis, growth and metastasis by regulating multiple signaling pathways. There is a strong association between NEDD8 overactivation and cancer progression in lung cancer, breast cancer (51), liver cancer (71) and colorectal cancer (72). NEDD8-mediated upregulation of neddylation has been found in lung cancer, affecting the prognosis and treatment of patients. Numerous studies have revealed a potential link between lung cancer and NEDD8, which encourages the study of the molecular biological mechanisms of lung cancer. *In vivo* and *in vitro* experiments also suggest that NEDD8 as a therapeutic target is a feasible approach. MLN4924 as a selective NEDD8-activating enzyme inhibitor has entered phase III clinical trials in myelodysplastic syndrome, chronic myeloid leukemia and acute myeloid leukemia (130). This suggests that MLN4924 targeting NEDD8 may have therapeutic effects in lung cancer.

In summary, NEDD8 is expected to become an early diagnostic or prognostic index and drug development target for tumors as well as other related diseases.

Acknowledgements

Figures of this review were created with Servier Medical Art (https://smart.servier.com/).

Funding

This research was supported by the National Natural Science Foundation of China (grant no. 81971088) and the Innovation Project of Shandong Academy of Medical Sciences, Shandong Provincial Natural Science Foundation, China (grant no. ZR2018MC008).

Availability of data and materials

Not applicable.

Authors' contributions

ZT and JL contributed equally to this work; they were involved in the conception and design of the study and drafted the manuscript. RM and TL were involved in the analysis of the literature. ZS and SH conceived and supervised the project and directed the writing. All the authors have read and approved the final version of this review. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Oudkerk M, Liu S, Heuvelmans MA, Walter JE and Field JK: Lung cancer LDCT screening and mortality reduction-evidence, pitfalls and future perspectives. Nat Rev Clin Oncol 18: 135-151, 2021.
- Sachs E, Sartipy U and Jackson V: Sex and Survival After Surgery for Lung Cancer: A Swedish Nationwide Cohort. Chest 159: 2029-2039, 2021.
- 3. Thai AA, Solomon BJ, Sequist LV, Gainor JF and Heist RS: Lung cancer. Lancet 398: 535-554, 2021.
- 4. Howington JA, Blum MG, Chang AC, Balekian AA and Murthy SC: Treatment of stage I and II non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 143 (5 Suppl): e278S-e313S, 2013.

- Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL and Paz-Ares L: Lung cancer: Current therapies and new targeted treatments. Lancet 389: 299-311, 2017.
- 6. Watson IR, Irwin MS and Ohh M: NEDD8 pathways in cancer, Sine quibus non. Cancer Cell 19: 168-176, 2011.
- Kamitani T, Kito K, Nguyen HP and Yeh ET: Characterization of NEDD8, a developmentally down-regulated ubiquitin-like protein. J Biol Chem 272: 28557-28562, 1997.
- Chung D and Dellaire G: The Role of the COP9 Signalosome and Neddylation in DNA damage signaling and repair. Biomolecules 5: 2388-2416, 2015.
- 9. Zhou W, Xu J, Tan M, Li H, Li H, Wei W, Sun Y: UBE2M Is a Stress-Inducible Dual E2 for neddylation and ubiquitylation that promotes targeted degradation of UBE2F. Mol Cell 70: 1008-1024.e6, 2018.
- Huang DT, Ayrault O, Hunt HW, Taherbhoy AM, Duda DM, Scott DC, Borg LA, Neale G, Murray PJ, Roussel MF and Schulman BA: E2-RING expansion of the NEDD8 cascade confers specificity to cullin modification. Mol Cell 33: 483-495, 2009.
- Xirodimas DP, Saville MK, Bourdon JC, Hay RT and Lane DP: Mdm2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. Cell 118: 83-97, 2004.
- Abida WM, Nikolaev A, Zhao W, Zhang W and Gu W: FBXO11 promotes the Neddylation of p53 and inhibits its transcriptional activity. J Biol Chem 282: 1797-1804, 2007.
- Lee GW, Park JB, Park SY, Seo J, Shin SH, Park JW, Kim SJ, Watanabe M and Chun YS: The E3 ligase C-CBL inhibits cancer cell migration by neddylating the proto-oncogene c-Src. Oncogene 37: 5552-5568, 2018.
- Broemer M, Tenev T, Rigbolt KT, Hempel S, Blagoev B, Silke J, Ditzel M and Meier P: Systematic in vivo RNAi analysis identifies IAPs as NEDD8-E3 ligases. Mol Cell 40: 810-822, 2010.
- Keuss MJ, Thomas Y, Mcarthur R, Wood NT, Knebel A and Kurz T: Characterization of the mammalian family of DCN-type NEDD8 E3 ligases. J Cell Sci 129: 1441-1454, 2016.
- Meyer-Schaller N, Chou YC, Sumara I, Martin DD, Kurz T, Katheder N, Hofmann K, Berthiaume LG, Sicheri F and Peter M: The human Dcn1-like protein DCNL3 promotes Cul3 neddylation at membranes. Proc Natl Acad Sci USA 106: 12365-12370, 2009.
- He S, Cao Y, Xie P, Dong G and Zhang L: The Nedd8 Non-covalent binding region in the smurf HECT domain is critical to its ubiquitn ligase function. Sci Rep 7: 41364, 2017.
- Deshaies RJ and Joazeiro CA: RING domain E3 ubiquitin ligases. Annu Rev Biochem 78: 399-434, 2009.
- Soucy TA, Dick LR, Smith PG, Milhollen MA and Brownell JE: The NEDD8 conjugation pathway and its relevance in cancer biology and therapy. Genes Cancer 1: 708-716, 2010.
- Mendoza HM, Shen LN, Botting C, Lewis A, Chen J, Ink B and Hay RT: NEDP1, a highly conserved cysteine protease that deNEDDylates Cullins. J Biol Chem 278: 25637-25643, 2003.
 Zhou L, Zhu J, Chen W, Jiang Y, Hu T, Wang Y, Ye X, Zhan M, Ji C, Xu Z, et al: Induction of NEDD8-conjugating enzyme E2
- 21. Zhou L, Zhu J, Chen W, Jiang Y, Hu T, Wang Y, Ye X, Zhan M, Ji C, Xu Z, *et al*: Induction of NEDD8-conjugating enzyme E2 UBE2F by platinum protects lung cancer cells from apoptosis and confers to platinum-insensitivity. Cell Death Dis 11: 975, 2020.
- Zhou L, Jiang Y, Luo Q, Li L and Jia L: Neddylation: A novel modulator of the tumor microenvironment. Mol Cancer 18: 77, 2019.
- Duda DM, Scott DC, Calabrese MF, Zimmerman ES, Zheng N and Schulman BA: Structural regulation of cullin-RING ubiquitin ligase complexes. Curr Opin Struct Biol 21: 257-264, 2011.
- 24. Furukawa M, Zhang Y, McCarville J, Ohta T and Xiong Y: The CUL1 C-terminal sequence and ROC1 are required for efficient nuclear accumulation, NEDD8 modification, and ubiquitin ligase activity of CUL1. Mol Cell Biol 20: 8185-8197, 2000.
- 25. Duda DM, Borg LA, Scott DC, Hunt HW, Hammel M and Schulman BA: Structural insights into NEDD8 activation of cullin-RING ligases: Conformational control of conjugation. Cell 134: 995-1006, 2008.
- 26. Kim AY, Bommelje CC, Lee BE, Yonekawa Y, Choi L, Morris LG, Huang G, Kaufman A, Ryan RJ, Hao B, *et al*: SCCRO (DCUN1D1) is an essential component of the E3 complex for neddylation. J Biol Chem 283: 33211-33220, 2008.
- 27. Goldenberg SJ, Cascio TC, Shumway SD, Garbutt KC, Liu J, Xiong Y and Zheng N: Structure of the Cand1-Cul1-Roc1 complex reveals regulatory mechanisms for the assembly of the multisubunit cullin-dependent ubiquitin ligases. Cell 119: 517-528, 2004.

- 28. Feng S, Shen Y, Sullivan JA, Rubio V, Xiong Y, Sun TP and Deng XW: Arabidopsis CAND1, an unmodified CUL1-interacting protein, is involved in multiple developmental pathways controlled by ubiquitin/proteasome-mediated protein Degradation. Plant Cell 16: 1870-1882, 2004.
- 29. Salon C, Brambilla E, Brambilla C, Lantuejoul S, Gazzeri S and Eymin B: Altered pattern of Cul-1 protein expression and neddylation in human lung tumours: Relationships with CAND1 and cyclin E protein levels. J Pathol 213: 303-310, 2007.
- 30. Li L, Kang J, Zhang W, Cai L, Wang S, Liang Y, Jiang Y, Liu X, Zhang Y, Ruan H, *et al*: Validation of NEDD8-conjugating enzyme UBC12 as a new therapeutic target in lung cancer. EBioMedicine 45: 81-91, 2019.
- Podust VN, Brownell JE, Gladysheva TB, Luo RS, Wang C, Coggins MB, Pierce JW, Lightcap ES and Chau V: A Nedd8 conjugation pathway is essential for proteolytic targeting of p27Kip1 by ubiquitination. Proc Natl Acad Sci USA 97: 4579-4584, 2000.
- 32. Iso T, Suzuki T, Baird L and Yamamoto M: Absolute Amounts and Status of the Nrf2-Keap1-Cul3 Complex within Cells. Mol Cell Biol 36: 3100-3112, 2016.
- Bennett EJ, Rush J, Gygi SP and Harper JW: Dynamics of cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics. Cell 143: 951-965, 2010.
- 34. Żhao Y, Morgan MA and Sun Y: Targeting Neddylation pathways to inactivate cullin-RING ligases for anticancer therapy. Antioxid Redox Signal 21: 2383-2400, 2014.
- 35. Kang M, Li Y, Zhao Y, He S and Shi J: MiR-33a inhibits cell proliferation and invasion by targeting CAND1 in lung cancer. Clin Transl Oncol 20: 457-466, 2018.
- 36. Li L, Wang M, Yu G, Chen P, Li H, Wei D, Zhu J, Xie L, Jia H, Shi J, *et al*: Overactivated neddylation pathway as a therapeutic target in lung cancer. J Natl Cancer Inst 106: dju083, 2014.
- Watson IR, Li BK, Roche O, Blanch A, Ohh M and Irwin MS: Chemotherapy induces NEDP1-mediated destabilization of MDM2. Oncogene 29: 297-304, 2010.
 Xie P, Zhang M, He S, Lu K, Chen Y, Xing G, Lu Y, Liu P,
- 38. Xie P, Zhang M, He S, Lu K, Chen Y, Xing G, Lu Y, Liu P, Li Y, Wang S, *et al*: The covalent modifier Nedd8 is critical for the activation of Smurf1 ubiquitin ligase in tumorigenesis. Nat Commun 5: 3733, 2014.
- Mahata B, Sundqvist A and Xirodimas DP: Recruitment of RPL11 at promoter sites of p53-regulated genes upon nucleolar stress through NEDD8 and in an Mdm2-dependent manner. Oncogene 31: 3060-3071, 2012.
- 40. Sundqvist A, Liu G, Mirsaliotis A and Xirodimas DP: Regulation of nucleolar signalling to p53 through NEDDylation of L11. EMBO Rep 10: 1132-1139, 2009.
- Zhang J, Bai D, Ma X, Guan J and Zheng X: hCINAP is a novel regulator of ribosomal protein-HDM2-p53 pathway by controlling NEDDylation of ribosomal protein S14. Oncogene 33: 246-254, 2014.
- 42. Deben C, Deschoolmeester V, Lardon F, Rolfo C and Pauwels P: TP53 and MDM2 genetic alterations in non-small cell lung cancer: Evaluating their prognostic and predictive value. Crit Rev Oncol Hematol 99: 63-73, 2016.
- Karin M and Ben-Neriah Y: Phosphorylation meets ubiquitination: The control of NF-[Kappa]B activity. Annu Rev Immunol 18: 621-663, 2000.
- 44. Read MA, Brownell JE, Gladysheva TB, Hottelet M, Parent LA, Coggins MB, Pierce JW, Podust VN, Luo RS, Chau V and Palombella VJ: Nedd8 Modification of Cul-1 Activates SCF(beta(TrCP)-Dependent Ubiquitination of IkappaBalpha. Mol Cell Biol 20: 2326-2333, 2000.
- 45. Yu H, Lin L, Zhang Z, Zhang H and Hu H: Targeting NF-κB pathway for the therapy of diseases: Mechanism and clinical study. Signal Transduct Target Ther 5: 209, 2020.
- 46. Zhou L, Jiang Y, Liu X, Li L, Yang X, Dong C, Liu X, Lin Y, Li Y, Yu J, *et al*: Promotion of tumor-associated macrophages infiltration by elevated neddylation pathway via NF-κB-CCL2 signaling in lung cancer. Oncogene 38: 5792-5804, 2019.
- 47. Liu W, Wang H, Bai F, Ding L, Huang Y, Lu C, Chen S, Li C, Yue X, Liang X, *et al*: IL-6 promotes metastasis of non-small-cell lung cancer by up-regulating TIM-4 via NF-κB. Cell Prolif 53: e12776, 2020.
- Orel L, Neumeier H, Hochrainer K, Binder BR and Schmid JA: Crosstalk between the NF-kappaB activating IKK-complex and the CSN signalosome. J Cell Mol Med 14: 1555-1568, 2010.
- Ediriweera MK, Tennekoon KH and Samarakoon SR: Role of the PI3K/AKT/mTOR signaling pathway in ovarian cancer: Biological and therapeutic significance. Semin Cancer Biol 59: 147-160, 2019.

- 50. Chibaya L, Karim B, Zhang H and Jones SN: Mdm2 phosphorylation by Akt regulates the p53 response to oxidative stress to promote cell proliferation and tumorigenesis. Proc Natl Acad Sci USA 118: e2003193118, 2021.
- Xie P, Peng Z, Chen Y, Li H, Du M, Tan Y, Zhang X, Lu Z, Cui CP, Liu CH, *et al*: Neddylation of PTEN regulates its nuclear import and promotes tumor development. Cell Res 31: 291-311, 2021.
- 52. Li X, Li C, Guo C, Zhao Q, Cao J, Huang HY, Yue M, Xue Y, Jin Y, Hu L and Ji H: PI3K/Akt/mTOR signaling orchestrates the phenotypic transition and chemo-resistance of small cell lung cancer. J Genet Genomics 48: 640-651, 2021.
- 53. Giatromanolaki A, Koukourakis MI, Sivridis E, Turley H, Talks K, Pezzella F, Gatter KC and Harris AL: Relation of hypoxia inducible factor 1 alpha and 2 alpha in operable non-small cell lung cancer to angiogenic molecular profile of tumours and survival. Br J Cancer 85: 881-890, 2001.
- 54. Zhang Y, Bian Y, Wang Y, Wang Y, Duan X, Han Y, Zhang L, Wang F, Gu Z and Qin Z: HIF-1α is necessary for activation and tumour-promotion effect of cancer-associated fibroblasts in lung cancer. J Cell Mol Med 25: 5457-5469, 2021.
- Wan J and Wu W: Hyperthermia induced HIF-1a expression of lung cancer through AKT and ERK signaling pathways. J Exp Clin Cancer Res 35: 119, 2016.
- Curtis VF, Ehrentraut SF, Campbell EL, Glover LE, Bayless A, Kelly CJ, Kominsky DJ and Colgan SP: Stabilization of HIF through inhibition of Cullin-2 neddylation is protective in mucosal inflammatory responses. FASEB J 29: 208-215, 2015.
 Russell RC and Ohh M: NEDD8 acts as a 'molecular switch'
- Russell RC and Ohh M: NEDD8 acts as a 'molecular switch' defining the functional selectivity of VHL. EMBO Rep 9: 486-491, 2008.
- Wolf ER, Mabry AR, Damania B and Mayo LD: Mdm2-mediated neddylation of pVHL blocks the induction of antiangiogenic factors. Oncogene 39: 5228-5239, 2020.
- Hsu PC, Yang CT, Jablons DM and You L: The Crosstalk between Src and Hippo/YAP signaling pathways in non-small cell lung cancer (NSCLC). Cancers (Basel) 12: 1361, 2020.
- 60. Zou J, Ma W, Li J, Littlejohn R, Zhou H, Kim IM, Fulton DJR, Chen W, Weintraub NL, Zhou J and Su H: Neddylation mediates ventricular chamber maturation through repression of Hippo signaling. Proc Natl Acad Sci USA 115: E4101-E4110, 2018.
- Cooper J, Xu Q, Zhou L, Pavlovic M, Ojeda V, Moulick K, de Stanchina E, Poirier JT, Zauderer M, Rudin CM, *et al*: Combined Inhibition of NEDD8-Activating Enzyme and mTOR Suppresses NF2 Loss-Driven Tumorigenesis. Mol Cancer Ther 16: 1693-1704, 2017.
- Maehama T, Nishio M, Otani J, Mak TW and Suzuki A: The role of Hippo-YAP signaling in squamous cell carcinomas. Cancer Sci 112: 51-60, 2021.
- Du Z and Lovly CM: Mechanisms of receptor tyrosine kinase activation in cancer. Mol Cancer 17: 58, 2018.
- 64. Shi Q and Chen YG: Interplay between TGF-β signaling and receptor tyrosine kinases in tumor development. Sci China Life Sci 60: 1133-1141, 2017.
- Enchev RI, Schulman BA and Peter M: Protein neddylation: Beyond cullin-RING ligases. Nat Rev Mol Cell Biol 16: 30-44, 2015.
- 66. Hamon P, Thoré MGD, Classe M, Signolle N, Liu W, Bawa O, Meziani L, Clémenson C, Milliat F, Deutsch E and Mondini M: TGFβ receptor inhibition unleashes interferon-β production by tumor-associated macrophages and enhances radiotherapy efficacy. J Immunother Cancer 10: e003519, 2022.
- 67. Zuo W, Huang F, Chiang YJ, Li M, Du J, Ding Y, Zhang T, Lee HW, Jeong LS, Chen Y, *et al*: c-Cbl-mediated neddylation antagonizes ubiquitination and degradation of the TGF-β type II receptor. Mol Cell 49: 499-510, 2013.
- 68. Oved S, Mosesson Y, Zwang Y, Santonico E, Shtiegman K, Marmor MD, Kochupurakkal BS, Katz M, Lavi S, Cesareni G and Yarden Y: Conjugation to Nedd8 instigates ubiquitylation and down-regulation of activated receptor tyrosine kinases. J Biol Chem 281: 21640-21651, 2006.
- 69. Tumbrink HL, Heimsoeth A and Sos ML: The next tier of EGFR resistance mutations in lung cancer. Oncogene 40: 1-11, 2021.
- Konduri K, Gallant JN, Chae YK, Giles FJ, Gitlitz BJ, Gowen K, Ichihara E, Owonikoko TK, Peddareddigari V, Ramalingam SS, *et al*: EGFR fusions as novel therapeutic targets in lung cancer. Cancer Discov 6: 601-611, 2016.
- 71. Luo Z, Yu G, Lee HW, Li L, Wang L, Yang D, Pan Y, Ding C, Qian J, Wu L, et al: The Nedd8-activating enzyme inhibitor MLN4924 induces autophagy and apoptosis to suppress liver cancer cell growth. Cancer Res 72: 3360-3371, 2012.

- 72. Invrea F, Punzi S, Petti C, Minelli R, Peoples MD, Bristow CA, Vurchio V, Corrado A, Bragoni A, Marchiò C, et al: Synthetic lethality screening highlights colorectal cancer vulnerability to concomitant blockade of NEDD8 and EGFR pathways. Cancers (Basel) 13: 3805, 2021.
- 73. Jiang Y, Cheng W, Li L, Zhou L, Liang Y, Zhang W, Chen W, Wang S, Zhao H, Chen G, et al: Effective targeting of the ubiquitin-like modifier NEDD8 for lung adenocarcinoma treatment. Cell Biol Toxicol 36: 349-364, 2020.
- 74. Bommelje CC, Weeda VB, Huang G, Shah K, Bains S, Buss E, Shaha M, Gönen M, Ghossein R, Ramanathan SY and Singh B: Oncogenic function of SCCRO5/DCUN1D5 requires its Neddylation E3 activity and nuclear localization. Clin Cancer Res 20: 372-381, 2014.
- 75. Wang L, Zhang M, Pan X, Zhao M, Huang L, Hu X, Wang X, Qiao L, Guo Q, Xu W, et al: Integrative serum metabolic fingerprints based multi-modal platforms for lung adenocarcinoma early detection and pulmonary nodule classification. Adv Sci (Weinh) 9: e2203786, 2022.
- 76. Yang J, Yin X, Zhang L, Zhang X, Lin Y, Zhuang L, Liu W, Zhang R, Yan X, Shi L, et al: Defective Fe metal-organic frameworks enhance metabolic profiling for high-accuracy diagnosis of human cancers. Adv Mater 34: e2201422, 2022
- 77. Zhou L, Dong C, Xu Z, Wang X, Zhang L, Chen S, Chen J and Zhu Y: NEDD8-conjugating enzyme E2 UBE2F confers radiation resistance by protecting lung cancer cells from apoptosis.
 J Zhejiang Univ Sci B 22: 959-965, 2021.
 78. Zhou W, Xu J, Li H, Xu M, Chen ZJ, Wei W, Pan Z and Sun Y:
- Neddylation E2 UBE2F promotes the survival of lung cancer cells by activating CRL5 to Degrade NOXA via the K11 Linkage. Clin Cancer Res 23: 1104-1116, 2017.
- 79. Guo ZP, Hu YC, Xie Y, Jin F, Song ZQ, Liu XD, Ma T and Zhou PK: MLN4924 suppresses the BRCA1 complex and synergizes with PARP inhibition in NSCLC cells. Biochem Biophys Res Commun 483: 223-229, 2017. 80. Meng F, Yu W, Chen C, Guo S, Tian X, Miao Y, Ma L, Zhang X,
- Yu Y, Huang L, et al: A versatile electrochemical biosensor for the detection of circulating MicroRNA toward non-small cell lung cancer diagnosis. Small 18: e2200784, 2022.
- 81. Zhu HZ, Hou J, Guo Y, Liu X, Jiang FL, Chen GP, Pang XF, Sun JG and Chen ZT: Identification and imaging of miR-155 in the early screening of lung cancer by targeted delivery of octreotide-conjugated chitosan-molecular beacon nanoparticles. Drug Deliv 25: 1974-1983, 2018.
- Khalife J, Radomska HS, Santhanam R, Huang X, Neviani P, Saultz J, Wang H, Wu YZ, Alachkar H, Anghelina M, et al: Pharmacological targeting of miR-155 via the NEDD8-activating enzyme inhibitor MLN4924 (Pevonedistat) in FLT3-ITD acute myeloid leukemia. Leukemia 29: 1981-1992, 2015.
- 83. Ardizzoni A, Boni L, Tiseo M, Fossella FV, Schiller JH, Paesmans M, Radosavljevic D, Paccagnella A, Zatloukal P, Mazzanti P, et al: Cisplatin-versus carboplatin-based chemotherapy in first-line treatment of advanced non-small-cell lung cancer: An individual patient data meta-analysis. J Natl Cancer Inst 99: 847-857, 2007.
- 84. Xu W, Xie X, Wu H, et al: Pulsed electromagnetic therapy in cancer treatment: Progress and outlook. View 3: 20220029, 2022.
- 85. Brownell JE, Sintchak MD, Gavin JM, Liao H, Bruzzese FJ, Bump NJ, Soucy TA, Milhollen MA, Yang X, Burkhardt AL, et al: Substrate-assisted inhibition of ubiquitin-like protein-activating enzymes: The NEDD8 E1 inhibitor MLN4924 forms a NEDD8-AMP mimetic in situ. Mol Cell 37: 102-111, 2010.
- 86. Lockhart AC, Bauer TM, Aggarwal C, Lee CB, Harvey RD, Cohen RB, Sedarati F, Nip TK, Faessel H, Dash AB, et al: Phase Ib study of pevonedistat, a NEDD8-activating enzyme inhibitor, in combination with docetaxel, carboplatin and paclitaxel, or gemcitabine, in patients with advanced solid tumors. Invest New Drugs 37: 87-97, 2019.
- 87. Sarantopoulos J, Shapiro GI, Cohen RB, Clark JW, Kauh JS, Weiss GJ, Cleary JM, Mahalingam D, Pickard MD, Faessel HM, et al: Phase I study of the investigational NEDD8-Activating enzyme inhibitor pevonedistat (TAK-924/MLN4924) in patients with advanced solid tumors. Clin Cancer Res 22: 847-857, 2016.
- 88. Yin Y, Xie CM, Li H, Tan M, Chen G, Schiff R, Xiong X and Sun Y: The FBXW2-MSX2-SOX2 axis regulates stem cell property and drug resistance of cancer cells. Proc Natl Acad Sci USA 116: 20528-20538, 2019.

- 89. Norton JP, Augert A, Eastwood E, Basom R, Rudin CM and MacPherson D: Protein neddylation as a therapeutic target in pulmonary and extrapulmonary small cell carcinomas. Genes Dev 35: 870-887, 2021.
- 90. Soucy TA, Smith PG, Milhollen MA, Berger AJ, Gavin JM, Adhikari S, Brownell JE, Burke KE, Cardin DP, Critchley S, et al: An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature 458: 732-736, 2009.
- 91. Jia L, Li H and Sun Y: Induction of p21-dependent senescence by an NAE inhibitor, MLN4924, as a mechanism of growth suppression. Neoplasia 13: 561-569, 2011.
- 92. Lin JJ, Milhollen MA, Smith PG, Narayanan U and Dutta A: NEDD8-targeting drug MLN4924 elicits DNA rereplication by stabilizing Cdt1 in S phase, triggering checkpoint activation, apoptosis, and senescence in cancer cells. Cancer Res 70: 10310-10320, 2010.
- 93. Chen P, Hu T, Liang Y, Li P, Chen X, Zhang J, Ma Y, Hao Q, Wang J, Zhang P, et al: Neddylation inhibition activates the extrinsic apoptosis pathway through ATF4-CHOP-DR5 axis in human esophageal cancer cells. Clin Cancer Res 22: 4145-4157, 2016.
- 94. Jia L, Soengas MS and Sun Y: ROC1/RBX1 E3 ubiquitin ligase silencing suppresses tumor cell growth via sequential induction of G2-M arrest, apoptosis, and senescence. Cancer Res 69: 4974-4982, 2009.
- 95. Rizzardi LF and Cook JG: Flipping the switch from g1 to s
- phase with e3 ubiquitin ligases. Genes Cancer 3: 634-648, 2012.
 96.Zhao Y, Xiong X, Jia L and Sun Y: Targeting Cullin-RING ligases by MLN4924 induces autophagy via modulating the HIF1-REDD1-TSC1-mTORC1-DEPTOR axis. Cell Death Dis 3: e386, 2012.
- 97. Li Y, Wang C, Xu T, Pan P, Yu Q, Xu L, Xiong X, Hou T, Cui S and Sun Y: Discovery of a small molecule inhibitor of cullin neddylation that triggers ER stress to induce autophagy. Acta Pharm Sin B 11: 3567-3584, 2021.
- 98.Weis SM and Cheresh DA: Tumor angiogenesis: Molecular pathways and therapeutic targets. Nat Med 17: 1359-1370, 2011.
- 99. Yao WT, Wu JF, Yu GY, Wang R, Wang K, Li LH, Chen P, Jiang YN, Cheng H, Lee HW, et al: Suppression of tumor angiogenesis by targeting the protein neddylation pathway. Cell Death Dis 5: e1059, 2014.
- 100. Tan M, Zhao Y, Kim SJ, Liu M, Jia L, Saunders TL, Zhu Y and Sun Y: SAG/RBX2/ROC2 E3 ubiquitin ligase is essential for vascular and neural development by targeting NF1 for degradation. Dev Cell 21: 1062-1076, 2011.
- 101. Tan M, Li H and Sun Y: Endothelial deletion of Sag/Rbx2/Roc2 E3 ubiquitin ligase causes embryonic lethality and blocks tumor angiogenesis. Oncogene 33: 5211-5220, 2014. 102. Conway EM, Pikor LA, Kung SH, Hamilton MJ, Lam S,
- Lam WL and Bennewith KL: Macrophages, inflammation, and lung cancer. Am J Respir Crit Care Med 193: 116-130, 2016. 103. Deng Q, Zhang J, Gao Y, She X, Wang Y, Wang Y and Ge X:
- MLN4924 protects against bleomycin-induced pulmonary fibrosis by inhibiting the early inflammatory process. Am J Transl Res 9: 1810-1821, 2017.
- 104. Zhou L, Zhang W, Sun Y and Jia L: Protein neddylation and its alterations in human cancers for targeted therapy. Cell Signal 44: 92-102, 2018.
- 105. Hao R, Song Y, Li R, Wu Y, Yang X, Li X, Qian F, Ye RD and Sun L: MLN4924 protects against interleukin-17A-induced pulmonary inflammation by disrupting ACT1-mediated signaling. Am J Physiol Lung Cell Mol Physiol 316: L1070-L1080, 2019.
- 106. Cheng M, Hu S, Wang Z, Pei Y, Fan R, Liu X, Wang L, Zhou J, Zheng S, Zhang T, et al: Inhibition of neddylation regulates dendritic cell functions via Deptor accumulation driven mTOR inactivation. Oncotarget 7: 35643-35654, 2016.
- 107. Mathewson N, Toubai T, Kapeles S, Sun Y, Oravecz-Wilson K, Tamaki H, Wang Y, Hou G, Sun Y and Reddy P: Neddylation plays an important role in the regulation of murine and human dendritic cell function. Blood 122: 2062-2073, 2013.
- 108. Zhu Z, Sun L, Hao R, Jiang H, Qian F and Ye RD: Nedd8 modification of Cullin-5 regulates lipopolysaccharide-induced acute lung injury. Am J Physiol Lung Cell Mol Physiol 313: L104-L114, 2017.
- 109. Jiang Y, Liang Y, Li L, Zhou L, Cheng W, Yang X, Yang X, Qi H, Yu J, Jeong LS, *et al*: Targeting neddylation inhibits intravascular survival and extravasation of cancer cells to prevent lung-cancer metastasis. Cell Biol Toxicol 35: 233-245, 2019.

- 110. Kim Y, Park JB, Fukuda J, Watanabe M and Chun YS: The effect of neddylation blockade on slug-dependent cancer cell migration is regulated by p53 mutation status. Cancers (Basel) 13: 531, 2021.
- 111. Sun Y and Li H: Functional characterization of SAG/RBX2/ROC2/RNF7, an antioxidant protein and an E3 ubiquitin ligase. Protein Cell 4: 103-116, 2013.
- 112. Xu Q, Lin G, Xu H, Hu L, Wang Y, Du S, Deng W, Hu W, Cheng W and Jiang K: MLN4924 neddylation inhibitor promotes cell death in paclitaxel-resistant human lung adenocarcinoma cells. Oncol Lett 15: 515-521, 2018.
- 113. Zhang Q, Karnak D, Tan M, Lawrence TS, Morgan MA and Sun Y: FBXW7 facilitates nonhomologous End-Joining via K63-Linked Polyubiquitylation of XRCC4. Mol Cell 61: 419-433, 2016.
- 114. Zhou X, Tan M, Nyati MK, Zhao Y, Wang G and Sun Y: Blockage of neddylation modification stimulates tumor sphere formation in vitro and stem cell differentiation and wound healing in vivo. Proc Natl Acad Sci USA 113: E2935-2944, 2016.
- 115. Takahashi K and Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663-676, 2006.
- 116. Park JB, Seo J, Park JW and Chun YS: Neddylation blockade induces HIF-1α driven cancer cell migration via upregulation of ZEB1. Sci Rep 10: 18210, 2020.
- 117. Korrodi-Gregório L, Soto-Cerrato V, Vitorino R, Fardilha M and Pérez-Tomás R: From proteomic analysis to potential therapeutic targets: Functional profile of two lung cancer cell lines, A549 and SW900, widely studied in pre-clinical research. PLoS One 11: e0165973, 2016.
- 118. Ni S, Chen X, Yu Q, Xu Y, Hu Z, Zhang J, Zhang W, Li B, Yang X, Mao F, et al: Discovery of candesartan cilexetic as a novel neddylation inhibitor for suppressing tumor growth. Eur J Med Chem 185: 111848, 2020.
- 119. Chen X, Yang X, Mao F, Wei J, Xu Y, Li B, Zhu J, Ni S, Jia L and Li J: Development of novel benzimidazole-derived neddylation inhibitors for suppressing tumor growth invitro and invivo. Eur J Med Chem 210: 112964, 2021.
- 120. An H and Statsyuk AV: An inhibitor of ubiquitin conjugation and aggresome formation. Chem Sci 6: 5235-5245, 2015.
- 121. Milhollen MA, Thomas MP, Narayanan U, Traore T, Riceberg J, Amidon BS, Bence NF, Bolen JB, Brownell J, Dick LR, et al: Treatment-emergent mutations in NAEβ confer resistance to the NEDD8-activating enzyme inhibitor MLN4924. Cancer Cell 21: 388-401, 2012.

- 122. Toth JI, Yang L, Dahl R and Petroski MD: A gatekeeper residue for NEDD8-activating enzyme inhibition by MLN4924. Cell Rep 1: 309-316, 2012.
- 123. Verma S, Singh A and Mishra A: Molecular dynamics investigation on the poor sensitivity of A171T mutant NEDD8-activating enzyme (NAE) for MLN4924. J Biomol Struct Dyn 32: 1064-1073, 2014.
- 124. Hammill JT, Scott DC, Min J, Connelly MC, Holbrook G, Zhu F, Matheny A, Yang L, Singh B, Schulman BA and Guy RK: Piperidinyl ureas chemically control defective in cullin neddylation 1 (DCN1)-Mediated cullin neddylation. J Med Chem 61: 2680-2693, 2018.
- 125. Hammill JT, Bhasin D, Scott DC, Min J, Chen Y, Lu Y, Yang L, Kim HS, Connelly MC, Hammill C, *et al*: Discovery of an orally bioavailable inhibitor of defective in cullin neddylation 1 (DCN1)-Mediated cullin neddylation. J Med Chem 61: 2694-2706, 2018.
- 126. Zhou H, Lu J, Liu L, Bernard D, Yang CY, Fernandez-Salas E, Chinnaswamy K, Layton S, Stuckey J, Yu Q, et al: A potent small-molecule inhibitor of the DCN1-UBC12 interaction that selectively blocks cullin 3 neddylation. Nat Commun 8: 1150, 2017.
- 127. Zhou H, Lu J, Chinnaswamy K, Stuckey JA, Liu L, McEachern D, Yang CY, Bernard D, Shen H, Rui L, *et al*: Selective inhibition of cullin 3 neddylation through covalent targeting DCN1 protects mice from acetaminophen-induced liver toxicity. Nat Commun 12: 2621, 2021.
- 128. Li Y, Bao Q, Yang S, Yang M and Mao C: Bionanoparticles in cancer imaging, diagnosis, and treatment. View 3: 20200027, 2022.
- 129. Zhang Z, Zhang J, Tian J and Li H: A polydopamine nanomedicine used in photothermal therapy for liver cancer knocks down the anti-cancer target NEDD8-E3 ligase ROC1 (RBX1). J Nanobiotechnology 19: 323, 2021.
 130. Deng L, Meng T, Chen L, Wei W and Wang P: The role of ubiq-
- 130. Deng L, Meng T, Chen L, Wei W and Wang P: The role of ubiquitination in tumorigenesis and targeted drug discovery. Signal Transduct Target Ther 5: 11, 2020.

6	0	٢	(
	BY	NC	

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.