Advancements in colorectal cancer research: Unveiling the cellular and molecular mechanisms of neddylation (Review)

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Abstract. Neddylation, akin to ubiquitination, represents a post-translational modification of proteins wherein neural precursor cell-expressed developmentally downregulated protein 8 (NEDD8) is modified on the substrate protein through a series of reactions. Neddylation plays a pivotal role in the growth and proliferation of animal cells. In colorectal cancer (CRC), it predominantly contributes to the proliferation, metastasis and survival of tumor cells, decreasing overall patient survival. The strategic manipulation of the NEDD8-mediated neddylation pathway holds immense therapeutic promise in terms of the potential to modulate the growth of tumors by regulating diverse biological responses within cancer cells, such as DNA damage response and apoptosis, among others. MLN4924 is an inhibitor of NEDD8, and its combined use with platinum drugs and irinotecan, as well as cycle inhibitors and NEDD activating enzyme inhibitors screened by drug repurposing, has been found to exert promising antitumor effects. The present review summarizes the recent progress made in the understanding of the role of NEDD8 in the advancement of CRC, suggesting that NEDD8 is a promising anti-CRC target.

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Abbreviations: Bcl-2, B-cell lymphoma 2; Cifs, cycle inhibitory factors; CRC, colorectal cancer; CRL, cullin-ring ligase; CSN, COP9 signalosome; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal-regulated kinase; FASN, fatty acid synthase; HIF, hypoxia-inducible factor; HuR, Hu protein R; MDM2, murine double minute 2; MSI, microsatellite instability; NAE, NEDD activating enzyme; NEDD8, neural precursor cell expressed developmentally downregulated 8; PCNA, proliferating cell nuclear antigen; PTEN, phosphatase and tensin homolog; pVHL, Von Hippel-Lindau tumor suppressor protein; RhoA, rat sarcoma homolog gene family A; RRP9, ribosomal RNA processing 9; Skp2, s-phase kinase associated protein 2; Smurf, SMAD ubiquitination regulatory factor; UBC12, ubiquitin-conjugating enzyme C12; UBE, ubiquitin-conjugating enzyme; XIAP, X-linked inhibitor of apoptosis protein

Key words: NEDD8, colorectal cancer, neddylation, MLN4924, oxaliplatin, irinotecan, cycle inhibitor

Contents

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed type of cancer globally and ranks second in the cause of cancer-related mortality (1). It has a momentous effect on the lives and health of individuals. The 5-year survival rates are related to the stage at which the disease is detected; patients with stage I disease have a 5-year survival range >90%, whereas for patients

with stage IV disease, the survival rate slightly exceeds 10% in clinical observations. Screening has been shown to reduce CRC morbidity and mortality (2). However, for patients in which the disease is not detected in time, follow-up treatment remains the sole method which allows them to continue to survive.

The majority of surgical treatments for patients with stage I and II CRC are based on surgical resection and the dissection of the surrounding lymph nodes. Apart from a small number of patients who develop post-operative intestinal knotting and wound dehiscence, the majority have a good prognosis and 5-year survival rates. At present, the majority of patients who are eligible for surgical treatment, based on health status and the stage of the disease (3,4), receive open surgery (5). Simultaneously, minimally invasive surgeries, such as laparoscopic surgeries (6,7) and robotic surgeries (8) have gradually gained a place in surgical treatment options due to their characteristics of diminished hemorrhagic tendencies, expedited post-operative recuperation, and lighter immune and inflammatory reactions than open surgeries.

Patients with stage III CRC frequently undergo chemotherapy to mitigate the risk of recurrence. In tandem, individuals with stage II or III CRC for whom surgical interventions prove intolerable may resort to a combined approach, where chemotherapy is complemented by radiation therapy. For patients with stage IV disease, the main treatment option is chemotherapy. Furthermore, adjuvant chemotherapy, peri-operative chemotherapy (9), and neoadjuvant chemoradiotherapy (10,11) not only reduce the disease stage, but also curtail the likelihood of recurrence and elevate the overall survival prospects for these patients. However, hepatotoxicity caused by chemotherapeutic drugs (12), particularly in frail or susceptible elderly patients (13), limits their application.

An increasing number of targeted therapies are also being applied for the treatment of CRC, which significantly enhance the prognosis of patients with malignant tumors compared to other treatments, particularly in individuals diagnosed with metastatic CRC (3,13). Currently, the most frequently used targeted therapies include angiogenesis inhibitors, such as bevacizumab and epidermal growth factor (EGF) inhibitors, such as panitumumab and cetuximab, alongside BRAF inhibitory therapy and HER2 inhibitory therapy (14). Neural precursor cell-expressed developmentally downregulated 8 (NEDD8) has attracted widespread attention for its function in CRC. CRC tissues exhibit an elevated expression of NEDD8, and the participation of NEDD8 in neddylation plays a crucial role in the migration, proliferation and survival of cancer cells. The present review summarizes and discusses the influence and potential of targeted NEDD8 therapy in CRC.

2. Overview of neddylation

NEDD8 was first discovered in mouse neural precursor cells (15) encoding a protein characterized by an arrangement of 81 amino acid residues. The molecular structure of NEDD8 is similar to that of ubiquitin, with up to 60% shared identity (16) and 80% homology (17). Furthermore, it is worth highlighting that the C-terminus of the protein encoded by NEDD8 comprises an uninterrupted quartet of Gly residues, with remote residues such as Gly-75 and Gly-76 bearing the hallmark of evolutionary conservation. The study by Kamitani *et al* (17) confirmed that only Gly-76 is related to the formation of NEDD8 conjugates, and this characteristic exhibits a resemblance to ubiquitin. Moreover, both ubiquitin and NEDD8 share conserved amino acid residues, such as Lys-48. The process of combining NEDD8 with other proteins is similar to ubiquitination, although the mechanism of binding markedly diverges from that of ubiquitin. Furthermore, unlike ubiquitin, NEDD8 assumes a critical role in orchestrating the modulation of linked signal transduction, transcription factors, tumorigenesis and other biological processes.

The neddylation process. As a post-translational modification mechanism for proteins, neddylation shares a number of similarities with ubiquitination. There are four main enzymes involved in the ubiquitin-like process: A precursor processing enzyme, NEDD activating enzyme (E1), NEDD conjugating enzyme (E2) and NEDD ligase (E3). The NEDD8 molecule is synthesized in a precursor form. To expose the Gly-76 residue, the C-terminus of the protein undergoes cleavage (17), where the precursor processing enzymes activate, including ubiquitin carboxyl-terminal hydrolase isozyme L3 (18), a common precursor processing enzyme for NEDD8 and ubiquitin, and the NEDD8-specific precursor processing enzyme NEDD8 protease 1 (19).

After processing the precursor, NEDD8 still needs to be activated to function, similar to ubiquitin, and this process is completed by E1. Functioning as a heterodimer, it comprises both ubiquitin-like modifier activating enzyme 3 and amyloid beta precursor protein binding protein 1. E1 then transfers NEDD8 to the thiol lipid coupling intermediate through a transesterification reaction (20). This forms the E2-NEDD8 conjugates. The E2s discovered thus far mainly include ubiquitin-conjugating enzyme (UBE)2M [also known as ubiquitin-conjugating enzyme C12 (UBC12)] (21) and UBE2F (22). Finally, E3, with the function of determining the specificity of the substrate, is required to localize the NEDD8 molecule to the target molecule. Compared with ubiquitination, neddylation has fewer types of E3s (Fig. 1).

NEDD8 exhibits specificity in binding to substrate proteins. E3 ligases, in conjunction with their respective E2 binding enzymes, typically collaborate in recruiting specific protein substrates to the substrate receptor domain of E3. NEDD8 then undergoes transfer from the independent active site of E3, ultimately facilitating the interaction between NEDD8 and the specific substrate proteins. For instance, ring box protein 1 demonstrates the capacity to use either UBE2M or UBE2F, affecting the binding of NEDD8 to the specific lysine residue 720 within cullin 1 (23). E2 also affects the binding site of E3, which prevents inappropriate modifications from occurring (21).

Neddylation substrate. There are numerous substrates for neddylation, among which the most characterized and well-documented are the cullin family proteins (24). In addition, other typical substrates are p53 (25), the Von Hippel-Lindau tumor suppressor protein (pVHL) (26), proliferating cell nuclear antigen (PCNA) (27), hypoxia-inducible factor (HIF) (28), and murine double minute 2 (MDM2) (25).

Cullin family proteins. Molecular scaffolds of cullin-ring ligase (CRL) E3 ubiquitin ligases involve cullin family proteins (29), which serve as adaptable frameworks,

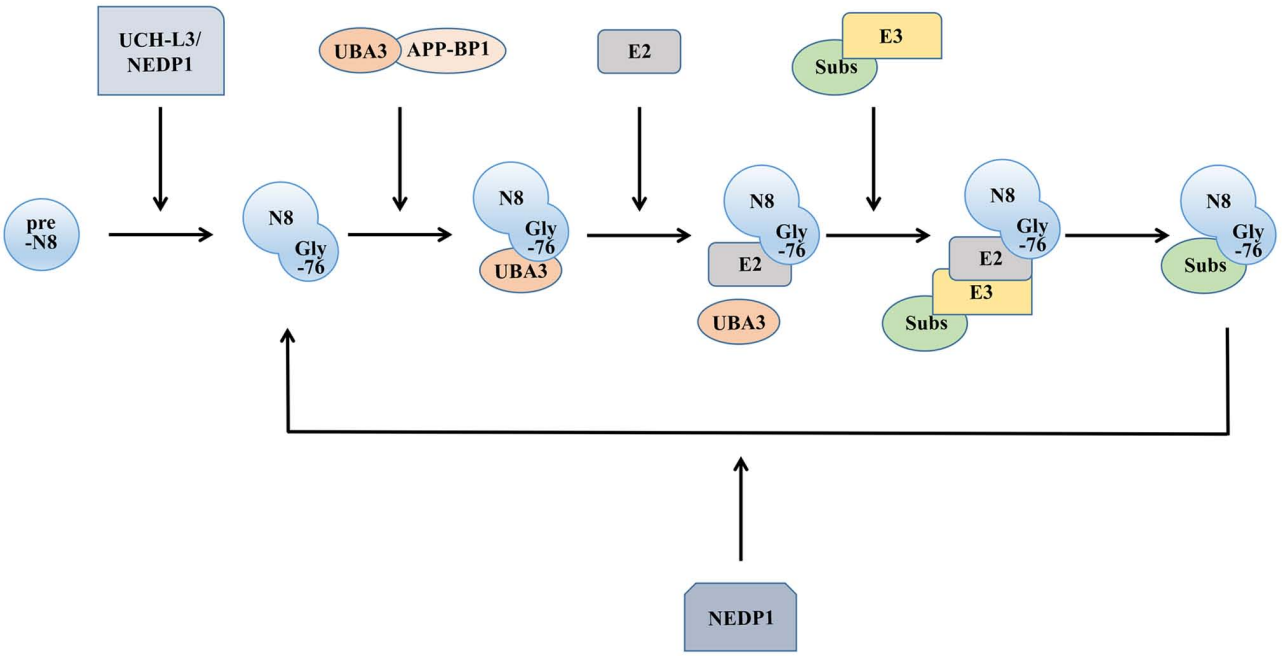

Figure 1. The neddylation and deneddylation process. NEDP1 and other precursor processing enzymes process the NEDD8 precursor molecule, exposing its Gly-76 residue. The UBA3 unit ofE1 binds to NEDD8, activating it. At this juncture, E2 binds to it, while UBA3 departs. Subsequently, E3 associates with the substrate, previously bound to E2, facilitating the interaction between NEDD8 and the substrate molecule. NEDP1 can remove the modification of NEDD8 on the substrate. APP-BP1, amyloid beta precursor protein binding protein 1; E1, NEDD activating enzyme; E2, NEDD conjugating enzyme; E3, NEDD ligase; NEDD8, neural precursor cell expressed developmentally downregulated 8; NEDP1, NEDD8 protease 1; UBA3, ubiquitin-like modifier activating enzyme 3; UCH-L3, ubiquitin carboxyl-terminal hydrolase isozyme L3.

connecting the active site of E2 enzymes with the substrate binding site and facilitating the progression of the ubiquitination process by bridging the spatial gap (30). There are seven types of cullins in mammals: Cullin1, cullin2, cullin3, cullin4A, cullin4B, cullin5 and cullin7 (31), of which cullin4A was the first to be discovered (32). A crucial characteristic of CRL E3 is its dependence on the covalent modification of the cullin protein by the NEDD8 molecule for its activation, resulting in a relatively active conformation of the ring-type E3 ligase (33). Additionally, CRL E3 ubiquitin ligases play crucial roles in coordinating the conjugation of ubiquitin with substrate proteins. They are able to bind to the target proteins and recruit E2 enzymes that are responsible for the transfer of ubiquitin molecules (31). The neddylation of cullin proteins can then instigate a profound transformation in their conformation, promoting neddylation of the CRL substrate.

MDM2/p53. p53 is a tumor suppressor gene with transcription factor activity related to DNA damage repair. In the case that its function is inhibited, the capacity to efficiently mend DNA damage is compromised, leading to its unabated accumulation. This, in turn, paves the way for genome instability, ultimately fostering the emergence of tumorigenesis. MDM2, a ring-type E3 ligase, is mainly amplified in cancer. Notably, it holds the power to catalyze the neddylation of p53, effectively curtailing the transcriptional prowess of this vital transcription factor. At the same time, MDM2 can also undergo neddylation itself, thereby increasing its stability and catalyzing p53 to undergo neddylation (25). Consequently, the functionality of p53 is suppressed, leading to heightened genomic instability within the malignant cells, thereby facilitating the progression of tumor growth.

pVHL/HIF. HIF-1α, which facilitates cancer development by enhancing angiogenesis (34), is an oxygen-regulated transcriptional activator (35). pVHL is one of the components of the E3 ubiquitin ligase that ubiquitinates and degrades hydroxylated HIF-1α. The functions of ubiquitination and degradation of hydroxylated HIF-1α remain unaltered following neddylation, which concurrently affects tumor development by affecting the assembly of the fibrin matrix (26). Under hypoxic conditions, the HIF-1α concentration is maintained by neddylation so that there is oxidative stress in cells, thereby promoting the development of tumor cells (28).

PCNA. PCNA is involved in DNA synthesis and cell proliferation. The molecular interplay between PCNA and NEDD8 possesses the capacity to counteract the process of ubiquitination. However, PCNA that undergoes neddylation blocks the formation of polη lesions, thereby inhibiting the recruitment of polη and affecting DNA damage repair, which may cause genome instability and lead to tumor occurrence (27).

Role of neddylation in tumorigenesis

Neddylation is related to tumor proliferation. Irregularities within the normal cellular replication process cause the body to lose normal control of its cell cycle and to prompt anomalous cell proliferation, -a decisive sign of tumorigenesis (36). The deficiency of enzymes involved in the neddylation process can result in the inhibition of tumor proliferation. For example, MLN4924, which selectively inhibits the E1 regulatory subunit [NEDD activating enzyme (NAE)], can interfere with neddylation in a number of types of cancer (37-39), particularly CRC (40), leading to cell cycle arrest or the demise of the cell. In addition, SMAD ubiquitination regulatory factor

Table I. Signaling pathways regulated by NEDD8.

Signaling pathway	NEDD8-mediated effects	Roles in tumorigenesis
NEDD8/Smurf	Promotion	Proliferation
NEDD8/Slug/Skp2/E-catenin	Promotion	EMT
NEDD8/MDM2/TAp73	Promotion	Resisting apoptosis
NEDD8/E2F1/TAp73	Promotion	Resisting apoptosis
NEDD8/Cul4/CSN	Promotion	DNA repairing
NEDD8/Cul5	Promotion	Radiotherapy resistance
NEDD8/Cul1/IκB/NF-κB	Promotion	T-cell activation
NEDD8/UBC12/Shc	Promotion	T-cell activation
NEDD8/UBC12/VEGF	Promotion	Angiogenesis
NEDD8/CRL/RhoA	Promotion	Angiogenesis

CRL, cullin-ring ligase; CSN, COP9 signalosome; Cul1, cullin 1; Cul4, cullin 4; Cul5, cullin 5; E2F1, E2F transcription factor 1; EMT, epithelial-to-mesenchymal transition; MDM2, murine double minute 2; NEDD8, neural precursor cell expressed developmentally down-regulated 8; RhoA, rat sarcoma homolog gene family A; Sch, src homology collagen; Skp2, s-phase kinase associated protein 2; UBC12, ubiquitin-conjugating enzyme C12; VEGF, vascular endothelial growth factor.

(Smurf)1, functioning as an E3 ligase, promotes its own neddylation, a process which can subsequently enhance CRC proliferation (41).

Neddylation is related to tumor metastasis. Metastasis encompasses the dissemination of cancer cells from the initial location to neighboring tissues or other organs in the body and their proliferation in the post-metastasis site, which is the crucial cause of tumor occurrence and mortality (36). MLN4924, an inhibitor of neddylation, impedes the metastatic journey of neoplastic cells by thwarting their intravascular persistence and the intricate extravasation process (42). NEDD8, via the s-phase kinase associated protein 2 (Skp2)/Slug pathway, exerts a regulatory effect on the downregulation of E-cadherin (43), consequently triggering the activation of the epithelial-to-mesenchymal transition (EMT) (44).

Neddylation is associated with tumor resistance to cell death. Resisting cell death is one of the abilities of tumors (36), contributing to the enhanced survival of cancer cells under adverse conditions. p73 and its isoforms play a pivotal role in promoting apoptosis in the cell cycle. NEDD8 can attenuate the transactivation of Tap73 through the MDM2/Tap73 pathway (45), as well as reduce the activation of p73 through the E2F transcription factor 1 (E2F1)/p73 route, allowing E2F1 to function in promoting the activation of cell cycle progression genes (46). Double-strand breaks (DSBs) in DNA present the most crucial issue for cells among all DNA damage (47). NEDD8 can facilitate the repair of DSBs (48), with the key player in the repair process being the COP9 signalosome (CSN). DSBs sites recruit CSN through neddylation and mainly interact with cullin4A, which is necessary for DSB repair (49). This DSB repair allows cells to escape death caused by genetic damage. Additionally, NEDD8 is intricately linked to resistance to tumor treatment (50). Resistance to treatment is also a form of tumor resistance to cell death and is closely related to drug resistance and recurrence (51). It has been shown that targeting neddylation enhances the sensitivity of CRC to the topoisomerase I inhibitor in chemotherapy (52). MLN4924 emerges as a novel radiosensitizer, augmenting the sensitivity of CRC to radiotherapy (53). In addition, the interaction between circAFF2 and cullin-associated and neddylation-dissociated protein 1 (CAND1) facilitates the binding of CAND1 to cullin5, suppressing cullin5 neddylation and consequently enhancing radiosensitivity in CRC (54).

Neddylation is related to the tumor microenvironment. Tumor-induced interactions dominate the tumor microenvironment (55). In myeloid-derived suppressor cells, NEDD8 can enhance the enrichment of tumor-infiltrating immune cells, contributing to the creation of an immunosuppressive environment (55,56). NEDD8, by virtue of its role in orchestrating the degradation of IκB via the cullin1-mediated pathway, activates NF-κB, subsequently elevating the release of particular pro-inflammatory cytokines induced by lipopolysaccharides, such as IL-6 and TNF-α (57). This is closely related to the role of tumor-associated macrophages (TAMs) (58). Likewise, the suppression of neddylation significantly inhibits the infiltration of TAMs (59). The anticancer immune response is significantly influenced by the immune response triggered through the activation of T-cells (60). Silencing the expression of UBC12 leads to a marked reduction in neddylation, targeting the adapter protein, src homology collagen, within $CD4^+$ T-cells. This causes a discernible impairment in cytokine production simultaneous to a diminished activation of the extracellular signal-regulated kinase (ERK) (61). At the same time, the targeted inhibition of neddylation can not only reduce NF-κB activity (62), but can also regulate the polarization of T-cells *in vitro* (63). Tumor angiogenesis is a necessary condition for tumor progression (64) and is mainly associated with vascular endothelial growth factor (VEGF) (65). It has been found that the UBC12 concentration increases in accordance with VEGF concentration (66). Furthermore, CRL can be inhibited by MLN4924, culminating in the accumulation of early rat sarcoma homolog gene family A (RhoA) and detrimentally impacting the angiogenic prowess of vascular endothelial cells (67) (Table I).

3. The function of neddylation in colorectal cancer

NEDD8 is inseparable from animal development and plays a crucial regulatory role in cell metastasis and proliferation. In addition, it is related to cell survival and genetic changes. Previous research has confirmed that UBC12, highly expressed in individuals with CRC, is involved in the activation pathway of NEDD8. This upregulation in the expression of NEDD8 is related to tumor cell proliferation and cloning ability (41).

CRC remains a significant contributor to cancer-related mortality. Although patients can achieve certain control of the disease through surgical resection and chemotherapy, the majority of patients eventually succumb to the disease due to tumor invasion and metastasis (68). The role of neddylation in CRC primarily lies in fostering the proliferation and migration of tumor cells, while concurrently protecting these cells from apoptotic processes. It is evident that NEDD8 intricately intertwines with the course of numerous cancers, including CRC, and delving into the association between NEDD8 and CRC illustrates paramount significance.

NEDD8 promotes tumor cell proliferation and metastasis through Smurf/E2 and Smurf/ribosomal RNA processing 9 (RRP9). NEDD8 functions by covalently binding to substrates in an ubiquitination-like manner (69). Its E3 not only has a ring scaffold type, but also involves a HECT ligase. Smurf1 emerges as a C2-WW-HECT ligase. Moreover, Smurf1 serves as a ubiquitin ligase E3, whose expression is substantially heightened within CRC tissues (41). Furthermore, in CRC tissues, increased levels of Smurf1, NEDD8, NAE1 and UBC12 expression have been linked to the advancement of cancer and to unfavorable clinical outcomes (41).

Smurf1 can be activated through neddylation, promoting its association with E2. Additionally, Smurf1 possesses the capacity to serve as an E3, thereby catalyzing its own neddylation process (41). The indispensable roles of NEDD8 and Smurf1 are evident in facilitating the ubiquitination of RhoA, a protein mainly related to the migration of tumor cells (70). In breast cancer (71), elevated Smurf1 expression has been substantiated as a consequence of ERK-mediated phosphorylation, which results from the activation of transforming growth factor β1 (TGF-β1) (72). It is worth noting that the overexpression and activation of ERK have also been found in CRC (73). Hence, it is conceivable that a similar mechanism is enacted in CRC. However, some researchers have illustrated that the expression of RhoA is increased in CRC due to EGF (74).

EGF plays a critical role in tumor cell metastasis (75). The downregulation of microRNA (miR)-145, leading to the upregulation of EGF, has been detected in CRC (76). At the same time, it has been confirmed that EGF can promote Smurf1 expression (77), thereby facilitating the neddylation of Smurf1 and prompting TGF-β1-induced ERK to phosphorylate Smurf1, ultimately resulting in the ubiquitination and degradation of RhoA. This appears contradictory to the previously mentioned elevation of RhoA induced by EGF (74). However, during cell migration, RhoA exhibits disparate functionalities in the anterior and posterior regions of tumor cells. The former is mainly inhibited and the latter is mainly activated (78). In CRC, the EFG-induced accumulation of RhoA may be intricately associated with the posterior section of migrating cells. The pathway responsible for diminishing RhoA ubiquitination, mediated by NEDD8, could potentially be linked to the migration of the front of the cell. In addition, the neddylation of Smurf1 can control rRNA preprocessing to promote tumor cell proliferation. As a result, cell proliferation and migration experience severe inhibition in cells, in turn knocking out the Smurf1 gene (79). In addition to the neddylation of Smurf, the mapping of the dynamic signaling network of TGF-β using high-throughput techniques has revealed that RRP9 can interact with Smurf1 (80) as a Smurf1 substrate in neddylation. In human CRC tissue samples and matched adjacent normal tissue samples, significantly upregulated expression levels of RRP9 and Smurf1 have been found in CRC tissues (79).

Substantiated investigations have elucidated the interconnection of RRP9 and NEDD8 through Smurf1 in CRC (79). RRP9, the integral element within the U3 snoRNP complex, augments pre-rRNA processing via its ubiquitination (79,81), an indispensable step in ribosome biogenesis. This augmentation in ribosome biogenesis is frequently associated with the increased proliferation observed in cancer as a high production of ribosomes is necessary to maintain high levels of cell growth and division (82,83). Therefore, Smurf1 exerts its E3 function and not only catalyzes RRP9 neddylation, but also stimulates the proliferation and migration of CRC cells by increasing pre-rRNA processing and upregulating ribosome biogenesis (79). Therefore, Smurf1 exhibits an elevated expression level and is activated through the process of neddylation, promoting the development of colorectal tumors. In the realm of CRC treatment, studies have illuminated that the inhibition of Smurf1 in tumors manifests an amplified anti-tumor efficacy in response to gemcitabine, cisplatin, and the gemcitabine-cisplatin combination (84). This observation suggests that addressing chemotherapy-resistant CRC by therapeutically targeting NEDD8 to suppress Smurf1 activation holds substantial promise.

NEDD8/X-linked inhibitor of apoptosis protein (XIAP)/phosphatase and tensin homolog (PTEN)/fatty acid synthase (FASN) increases tumor cell proliferation by promoting fat synthesis. PTEN encodes a protein phosphatase with strong tumor inhibitory effects (85). It is distributed in both the nucleus and cytoplasm and has complementary mechanisms to inhibit tumor development (86). PTEN has been documented as a substrate susceptible to neddylation, capable of forming a conjugate with NEDD8 by means of XIAP. Moreover, it has been found that, in breast cancer tissue, PTEN neddylation is triggered by increases in glucose levels (87), which is reflected in the process of cancer development. Because the propagation of cancerous cells hinges on the acquisition of extracellular nutrients (88), including glucose (89), research has shown that individuals with hyperglycemia are more susceptible to the onset of CRC. Furthermore, an extreme stimulation of glucose uptake and glycolytic activity stands as a hallmark of CRC. This phenomenon is considered to be intricately linked to the surge in PTEN ubiquitination modification triggered by heightened sugar levels. XIAP is an E3 catalyzing PTEN neddylation that demonstrates an elevated expression level within CRC tissue and may mediate excessive PTEN neddylation.

It has been found that the effect of NEDD8 on PTEN is not derived from stabilizing its molecular structure, but from facilitating the translocation of PTEN into the nucleus in breast cancer cells. NEDD8 interacts with importin α, importin β and importin 5 (IPO5), leading to the nuclear translocation of any PTEN that has interacted with NEDD8 (87). Research on CRC cells has demonstrated that a high expression of IPO5 was associated with tumor development (90), suggesting that NEDD8 promotes PTEN nuclear translocation in CRC cells. NEDD8 stabilizes FASN through the NEDD8/XIAP/PTEN/FASN pathway, thereby reducing FASN ubiquitination and promoting fat synthesis (88). Elevated fat synthesis affected by FASN stabilization is often related to tumor cell proliferation (91). It has also been confirmed that Smurf1 can ubiquitinate and degrade PTEN in glioblastoma (92), which has not yet been proven in CRC. In the domain of breast cancer therapy, the targeted neddylation of PTEN has emerged as a highly promising therapeutic strategy (93). Building upon the analogous PTEN neddylation pathway, targeted interventions along this route hold significant developmental prospects in the context of CRC.

CSN5 promotes tumor cell proliferation by depleting NEDD8 to accumulate β-catenin. Abnormities in the Wnt/β-catenin signaling pathway are prevalent within the majority of CRC cells. The constitutive activation of this pathway promotes the propagation and survival of CRC cells and is a main cause of adenoma formation (94). Principal among these anomalies is the irregular expression of β-catenin, which serves as the primary instigator of the irregular signaling of the Wnt/β-catenin pathway in CRC cells. This perturbation exhibits an intricate association with CSN5. CSN5 mainly affects an alternative degradation pathway of β-catenin (95). In CRC cells, the intricate web of interactions exists among β-catenin, CSN5, and seven in absentia homolog 1 (SIAH-1), in which CSN5 plays a crucial role in cellular processes, such as apoptosis and cell cycle regulation. CRL activity can be regulated by deleting NEDD8 (96) to promote SIAH-1 degradation, causing β-catenin accumulation in CRC and promoting the growth and multiplication of malignant cells (97). Adenoma genesis frequently emerges as a pivotal risk factor in the onset of CRC (98). Consequently, the targeted development of pharmaceutical agents addressing this pathway holds the potential to contribute significantly to the prevention of CRC, thereby mitigating the incidence of this malignancy.

NEDD8 downregulates E-cadherin and activates EMT to promote tumor cell metastasis through the Skp2/Slug pathway. Research has illustrated that inhibiting the NEDD8 pathway has a therapeutic effect on clinically invasive CRC and that CRC cells exhibiting heightened sensitivity to NEDD8 inhibition often exhibit EMT transcriptional characteristics (40). EMT is an important prerequisite for CRC cells to metastasize (99), but the mechanisms of NEDD8 in inducing CRC cell metastasis have not been fully elucidated. In prostate cancer, which is also aggressive, it has been found that NEDD8 can down-regulate E-cadherin and activate EMT through the Skp2/Slug pathway to promote tumor cell metastasis (43). As a member of the substrate recognition F-box protein family, Skp2 is a component of CRL1 whose activity is regulated by NEDD8 (100). In other words, Skp2 depends on NEDD8 for its function. NEDD8 combines with Skp2 to perform neddylation, thereby activating the Skp2 downstream molecule Slug. Slug activation may suppress EMT onset by downregulating E-cadherin expression (101). Studies have found that both Skp2 (102) and Slug (103) exhibit a significant upregulation within tissues afflicted by CRC. Moreover, the downregulation of E-cadherin in these tissues also leads to the occurrence of EMT (104). It can be inferred that there is a mechanism in CRC tissues, whereby NEDD8 initiates the Skp2/Slug pathway, culminating in the downregulation of E-cadherin. This, in turn, prompts neoplastic cells to undergo the process of EMT, ultimately facilitating cellular metastasis.

NEDD8 mediates clearance of misfolded aggregates to protect tumor cells with microsatellite instability (MSI). The process of DNA mismatch repair (MMR) is evolutionarily conserved and serves to rectify mismatches arising from DNA replication, evading proofreading mechanisms (105), in a key pathway in DNA repair. The loss of MMR function induces a hypermutational phenotype, and such cells are clinically identified through the genomic scarring of MSI. It has been confirmed that the prognosis of individuals with CRC is associated with the presence of MSI within the tumor (106). Patients with MSI develop intrinsic resistance to chemotherapy, making it difficult to induce cancerous cell apoptosis by chemotherapy drugs, limiting the number of effective treatments for patients (107). There are often a large number of genome instabilities and mutations in MSI, resulting in cellular proteome imbalances and aberrant protein accumulation in the cell. To compensate for these proteins, NEDD8-mediated pathways are indispensable for MSI tumors to clean misfolded aggregates, thereby maintaining tumor cell survival (108).

The NEDD8/UBE2F/cullin5 pathway strengthens NOXA ubiquitination and degradation to protect tumor cells. Defects in apoptosis are the basis of tumorigenesis and the main cause of chemotherapy failure (109). The role of the B-cell lymphoma 2 (Bcl-2) protein family in cellular apoptosis is of utmost importance, particularly in mitochondrial apoptosis (110). Within the cellular proteome, the Bcl-2 family can be segregated into two distinct categories: Pro-apoptotic and anti-apoptotic. The stoichiometry of these two categories determines whether the cell is apoptotic or not. When the subtle balance is altered, a signal is transmitted through the upstream molecule Bcl-2 homology domain only protein (BH3-only), thereby activating Bcl-2-associated X protein (Bax) and Bcl-2-associated K protein (Bak) on the mitochondrial surface, leading to mitochondrial impairment and cell death (111). NOXA is not only a pro-apoptotic protein, but is also a member of the BH3-only family. It plays a key role in Bcl-2-mediated mitochondrial apoptosis, mainly by selectively neutralizing anti-apoptotic Bcl-2 family proteins and altering the balance of pro- and anti-apoptotic signals to cause mitochondrial apoptosis (112). Research has indicated an elevated protein expression of NOXA in CRC; however, it has a short lifespan. The rapid degradation of NOXA could potentially serve as a tactic employed by CRC cells to evade the peril posed by elevated NOXA expression. Peroxiredoxin 1 exhibits

a high expression level within CRC tissues, which enhances cullin5 neddylation through the UBE2F/cullin5 pathway. This neddylation can enhance the ubiquitination and degradation of NOXA mediated by cullin5, resulting in increased tumor cell survival, which is also reflected in the context of tumor cell resistance to the chemotherapy drug etoposide (113). Addressing this pathway through targeted interventions holds great promise in circumventing resistance to etoposide in CRC, thereby presenting a novel perspective for the chemotherapy of colorectal malignancies.

The NEDD8/MDM2/Hu antigen R (HuR) pathway stabilizes HuR to protect tumor cells. A central RNA-binding protein known as HuR plays a crucial role in stabilizing cell proliferation-related mRNA to regulate cell dedifferentiation, proliferation and survival (114,115). A previous study validated the significant association between the elevated expression of HuR and the survival of cancerous cells in tumors (116), while the decreased expression of HuR resulted in cell cycle arrest and promoted cellular apoptosis. A previous study detected the upregulation of HuR and MDM2 expression in colorectal tumor tissues (117). Notably, an intricate correlation has been established between HuR and MDM2. MDM2 has been previously confirmed to be an E3 that inhibits the transcriptional activity of p53 by catalyzing its neddylation (25). Moreover, MDM2 serves as an E3 in CRC tissues. The catalytic substrate of MDM2 is converted into HuR simultaneously, controlling the nuclear localization of HuR through the NEDD8/MDM2/HuR pathway and protecting it from degradation. To help tumor cells to survive, NEDD8 leads to the malignant transformation of tumor cells (118).

NEED8 protects tumor cells through the IκB/NF-κB pathway. Chemoresistance is often related to patient mortality and tumor metastasis in CRC; one form of chemoresistance is resistance to oxaliplatin through the death receptor ligand [Fas and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)] pathway (119). NF-κB plays a pivotal role in the regulation of death receptor signaling pathways (120). In chemoresistant diffuse large B-cell lymphoma, it has been found that neddylation resists tumor cell death by downregulating inhibitory factor IκB and upregulating NF-κB. MLN4924 can reverse such effects (121). The abnormal expression of NF-κB has also been detected in CRC cells (122). In an experiment involving the intraperitoneal injection of mutant IκB colon cells into mice suffering from metastatic CRC, it was discovered that this approach improved the survival rate of the mice, suggesting that NF-κB inhibition mediates the cell-killing effect caused by TRAIL (123). Similarly, NEDD8 is abnormally activated in CRC tissues. It can be inferred that NEDD8's presence in CRC inhibits IκB from activating the NF-κB pathway, thereby inhibiting TRAIL-mediated cell killing and allowing cancer cells to survive in the face of chemical drugs. In light of the deleterious ramifications stemming from cancer cells' resistance to platinum-based drugs, notably oxaliplatin, in the majority of CRCs (123), the targeted development of pharmaceutical agents along this pathway holds immense promise. This endeavor has the potential to furnish novel therapeutic strategies for treatment-resistant CRC (Fig. 2).

4. Exploration of neddylation as a potential molecularly targeted drug and of treatment for colorectal cancer

Neddylation is closely connected to the metastasis, proliferation and survival of CRC cells. Previous research has confirmed that inhibiting neddylation mediated by NEDD8 causes the apoptosis of CRC cells (124). Thus, targeting the neddylation pathway is expected to become a novel treatment method for CRC. Concurrently, such targeted therapy may also find application in combination with CRC treatment drugs to improve the responsiveness of CRC cells to therapeutic agents.

MLN4924. Neddylation plays a crucial role in the progression of CRC. Therefore, the process of developing pharmaceutical interventions targeting NEDD8 has prompted notable advancements in the treatment of CRC. MLN4924 is a selective inhibitor of NAE1. It was first confirmed that MLN4924 could affect the activity of E1 to disrupt the cullin-ring ligase-mediated protein turnover as a NEDD8 substrate (124). Subsequently, further research demonstrated that MLN4924 functions as a selective inhibitor by competitively inhibiting the assembly of the NEDD8-MLN4924 compound. When it is deprived of its capacity to partake in subsequent enzymatic reactions, MLN4924 blocks neddylation in CRC cells and ultimately ushers in the onset of cellular death (125). As research on MLN4924 progressed further, its role as a radiosensitizer was demonstrated, illustrating that it could make CRC cells more sensitive to radiation, primarily through p27 accumulation in cells significantly improving the arrest of radiation-induced cell cycle G2/M phases, DNA damage responses and cellular death (53). The method of CRC cell death using MLN4924 was subsequently further studied. MLN4924 stabilizes p53 to induce ribosome stress, leading to the death of tumor cells. Mitoxantrone 1 also has similar action characteristics (126). As a typical p53 target gene death receptor, TRAIL receptor 2 expression is also significantly upregulated during the process (127). On the other hand, MLN4924 induces cell death by activating Bax and Bak on the outer mitochondrial membrane to initiate mitochondrial outer membrane permeabilization (127). During this biological process, there is also the upregulation in the expression of BH3 interacting domain death agonist (BID). As a member of the pro-apoptotic Bcl2 family, BID activates the downstream targets of caspase-8 in the exogenous pathway (128), causing CRC tumor cell death. Currently, the application of MLN4924 in the treatment of CRC is still being studied in the phase I/II clinical stages. These experimental results demonstrate that this drug can control the progression of cancer through the apoptosis of cancer cells (129,130). MLN4924 is additionally being used in synergy with conventional chemotherapy agents to enhance their efficacy significantly. For example, MLN4924 can induce DNA damage response and upregulate cell cycle checkpoint kinase 2 protein expression levels to render CRC cells more sensitive to oxaliplatin (131). At the same time, MLN4924 can also be combined with irinotecan as a chemotherapeutic method to combat chemotherapy-refractory p53 mutant CRC (127).

However, with the use of MLN4924, CRC tissues will inevitably become resistant. This may be related to FLICE inhibitory protein in MLN4924-treated cells, which leads to

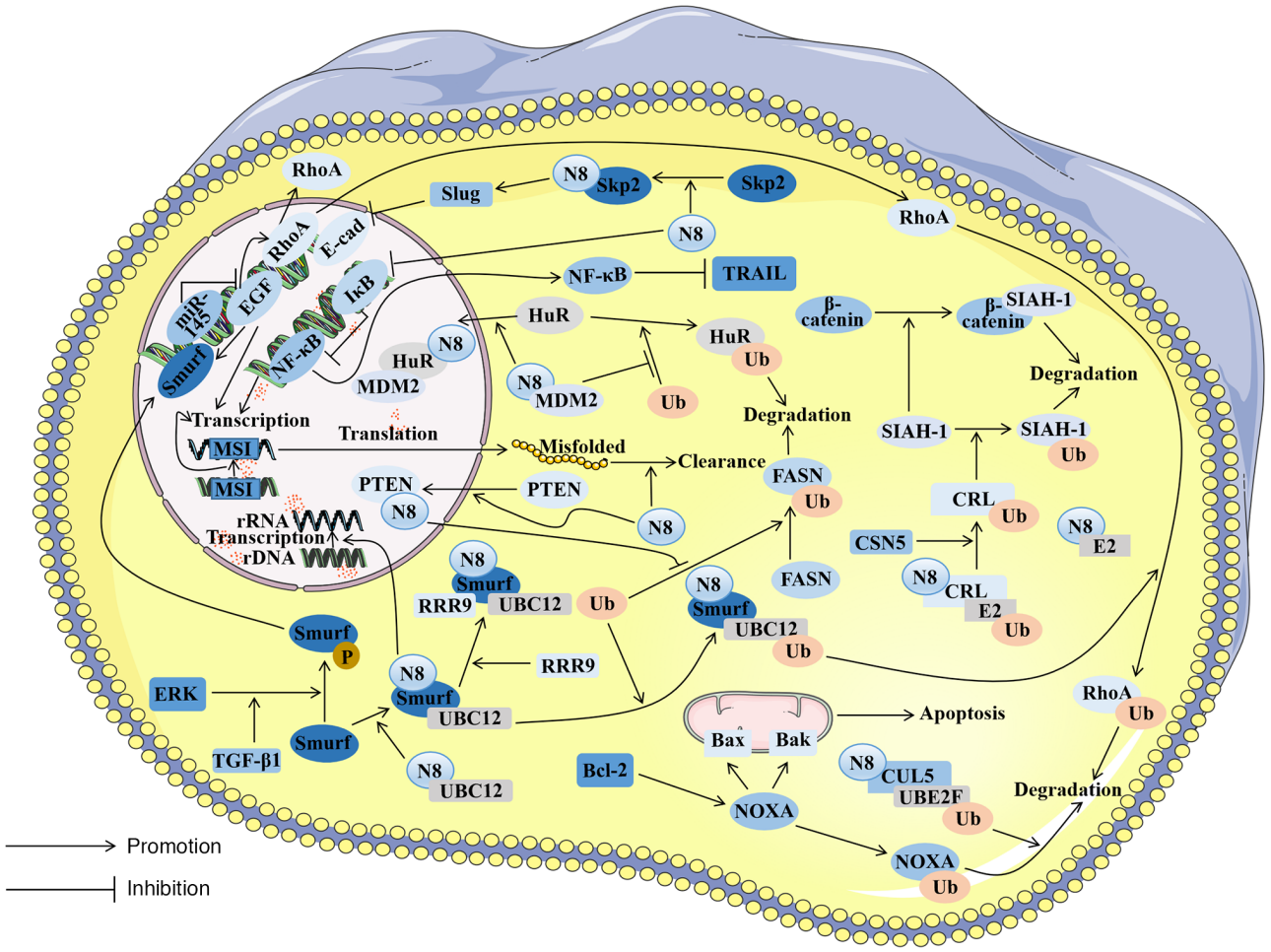

Figure 2. Numerous signaling pathways are associated with NEDD8. NEDD8 promotes the ubiquitination and degradation of RhoA at the leading edge of cells by modifying Smurf. Additionally, the neddylation of Smurf itself and its interaction with RRP9 following neddylation enhance the processing of pre-rRNA. The expression of Smurf and RhoA is stimulated by EGF, while miR-145 inhibits the expression of EGF. NEDD8-mediated neddylation facilitates the nuclear translocation of PTEN, and PTEN, following its translocation, inhibits the ubiquitination and degradation of FASN. CSN5, through its deneddylation action, promotes the ubiquitination and degradation of SIAH-1, consequently leading to the accumulation of β-catenin. NEDD8-modified Skp2 activates the expression of E-cadherin by activating downstream slug. NEDD8-mediated neddylation aids in the clearance of protein aggregates resulting from misfolding due to MSI. NEDD8-modified MDM2 promotes the nuclear translocation of HuR and prevents its ubiquitination and degradation in the cytoplasm. NEDD8 activates NF-κB by inhibiting IκB expression, thereby suppressing the cellular effects mediated by TRAIL. Bak, Bcl-2-associated K protein; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; CRL, cullin-ring ligase; CSN5, COP9 signalosome 5; EGF, epidermal growth factor; ERK, extracellular regulated protein kinases; E-cad, E-cadherin; FASN, fatty acid synthase; HuR, Hu protein R; MDM2, murine double minute 2; MSI, microsatellite instability; NEDD8, neural precursor cell expressed developmentally downregulated 8; PTEN, phosphatase and tensin homolog; RhoA, rat sarcoma homolog gene family A; RRP9, ribosomal RNA processing 9; SIAH-1, seven in absentia homolog 1; Skp2, s-phase kinase associated protein 2; Smurf, SMAD ubiquitination regulatory factor; TGF-β1, transforming growth factor β1; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; UBC12, ubiquitin-conjugating enzyme C12.

resistance to standard care therapies (127). It may also be associated with the variation of NAE, which reduces the affinity of the enzyme to MLN4924 and ATP and increases NEDD8 activation (132).

Beyond its anticipated therapeutic effects in targeted therapy, MLN4924 has demonstrated certain unintended consequences that may foster cancer cell proliferation. These outcomes are primarily associated with the activation of intracellular ERK and JNK signaling, leading to activator protein-1 activation (133). Simultaneously, the inhibitory effects of MLN4924-induced neddylation suppression can upregulate T-cell negative regulator programmed death-ligand 1 (PD-L1) expression (134). Cancer cells exhibiting a high expression of PD-L1 can attenuate T-cell cytotoxicity through the activation of the PD-1/PD-L1 axis, inducing immune suppression (135). Combining anti-PD-L1 antibodies or mitogen-activated protein kinase inhibitors with MLN4924 administration offers a potential avenue to address these challenges effectively (133).

Drug repurposing to screen E1 inhibitors. Since developing new drugs remains costly, there is a trend to seek to repurpose existing approved and investigational drugs given their known safety profile and to reduce costs (136). Piperacillin, known as a β-lactam antibiotic, is often used for the management of suspected bacterial infections relying on empirical treatment combined with tazobactam (137). It is frequently used in cancer patients to treat fever attacks following chemotherapy and neutropenia (138,139). Based on the integrated virtual screening method, it has been found that piperacillin 1 (140) can inhibit the degradation of p27, known as a downstream protein substrate of NAE1, the regulatory subunit of E1. It possesses an ATP-competitive inhibitor

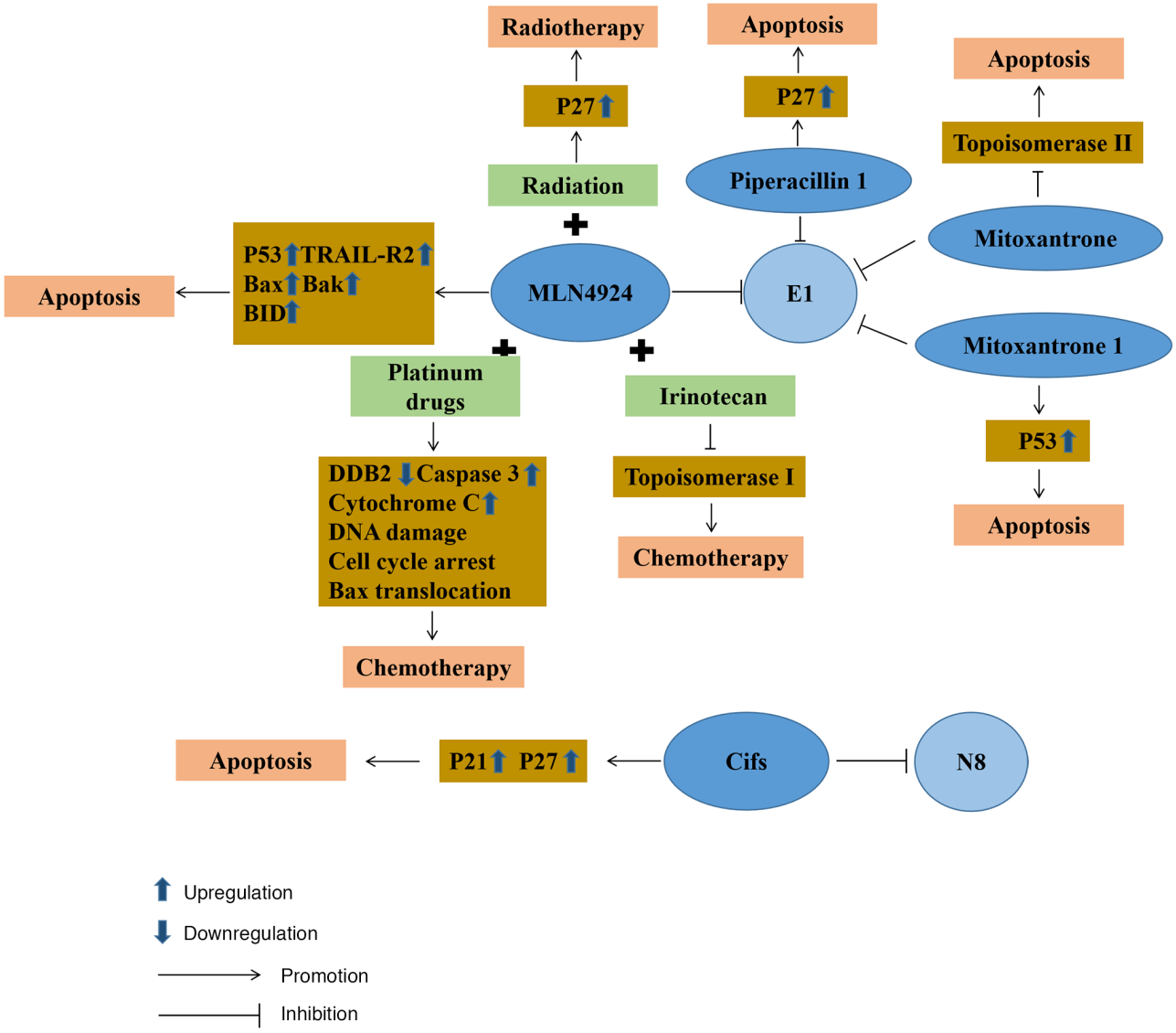

Figure 3. Targeting NEDD8 therapy for colorectal cancer is primarily achieved by inhibiting E1 and directly inhibiting NEDD8. Compounds such as MLN4924, mitoxantrone, mitoxantrone 1 and piperacillin 1 induce apoptosis in tumor cells by inhibiting E1. Additionally, MLN4924 is also combined with radiotherapy, platinum-based drugs, and chemotherapy with irinotecan to enhance its effectiveness. Cycle inhibitory factors induces apoptosis in tumor cells by inhibiting NEDD8. Bak, Bcl-2-associated K protein; Bax, Bcl-2-associated X protein; BID, BH3 interacting domain death agonist; Cifs, cycle inhibitory factors; DDB2, DNA damage-binding protein 2; E1, NEDD activating enzyme; TRAIL-R2, tumor necrosis factor-related apoptosis-inducing ligand receptor 2.

without the ability to form the covalent bonds of E1 in the future. Mitoxantrone, as a DNA topoisomerase II poison, causes tumor cell apoptosis by inhibiting DNA synthesis and delaying cell cycle progression, which can improve the therapeutic properties of anthracyclines (141,142). Through an FDA-approved drug database analysis by virtual screening, mitoxantrone 1 (126) was found to have highly selective NAE activity and to compete with ATP, with great potential as an inhibitor of E1.

Cycle inhibitory factors (Cifs). Cifs are capable of impeding the activity of cullin-ring E3 ubiquitin ligases, resulting in cell cycle arrest. At the same time, Cifs also participate in cancer progression by deamidating NEDD8 (143). In CRC cells, cells that regulate Cif expression by doxycycline lead to tumor apoptosis through p21 and p27 accumulation (144). In terms of specific use, attenuated *Salmonella typhimurium* VNP20009 can be used to deliver Cif genes to tumor cells, inducing the expression and intracellular accumulation of proteins p27 and p21, thereby inhibiting the growth of tumor cells (145).

Platinum drugs. Platinum-based pharmaceuticals can be employed in the management of CRC and are amenable to concomitant administration with various classes of anti-neoplastic agents. Commonly used platinum drugs include oxaliplatin, carboplatin and cisplatin, all of which have received global approval (146). Cisplatin is a first-generation platinum drug and interferes with DNA repair by crosslinking with the purine bases of DNA, leading to DNA damage and stimulating tumor cell death (147). Nevertheless, cisplatin lacks the specificity to selectively target CRC cells, consequently leading to diminished accumulative concentrations and a discernible impact on the therapeutic efficacy (148).

During the analysis of tissues from patients with squamous cell carcinoma in the head and neck, it was discovered that MLN4924 possesses the capacity to impede the transcription of DDB2 facilitated by E2F1 (149). Since DDB2 demonstrates a crucial function in modulating sensitivity to cisplatin, the combined application of MLN4924 and cisplatin can increase the activity of cisplatin. Moreover, tissues with a suppressed expression of cullin4A exhibit higher susceptibility to cisplatin, which potentially relates to its connection with DNA repair. In addition, the enhancing effect of MLN4924 on cisplatin has been found in breast cancer (150), pancreatic cancer (151) and cervical tumors (152). It can be concluded that the combined application of MLN4924 and cisplatin in CRC tissues may also enhance the sensitivity of cisplatin to compensate for the impact of its low accumulation concentration. Carboplatin is a cisplatin derivative and a second-generation platinum drug whose mode of action is similar to that of cisplatin. It induces tumor cell apoptosis by damaging DNA. Compared with cisplatin, carboplatin produces inter-chain and intra-chain doublets or single adducts following application (153). In individuals with advanced solid tumors, a clinical phase I trial revealed that E1 inhibitor, MLN4924, combined with carboplatin was well-tolerated and stood as a promising benchmark for forthcoming drug development against CRC (130).

Oxaliplatin, a third-generation platinum therapeutic agent, is a first-line chemotherapy drug. As one of the more commonly used chemotherapeutic drugs following surgical resection, it plays a pivotal role in the management of CRC. It initiates the death of malignant cells by orchestrating a halt in cell cycle progression at the critical juncture of G2/M, thus initiating an apoptotic cascade that includes Bax mitochondrial translocation, the release of cytochrome *c* and caspase-3 catalytic activation (154). In a previous a study on CRC tissues, it was found that MLN4924 combined with oxaliplatin increased oxaliplatin-induced apoptosis (131). Although it has not yet entered clinical trials for CRC, phase I trials have been carried out in the therapeutic management of gastric carcinoma (155). CRC cells have also developed some drug resistance to platinum agents, such as the inhibition of drug accumulation in tumor cells, as well as the acquisition of EMT. A large number of studies on drug resistance have found that exosomes loaded with miR-128-3p (156) and the application of nanoparticles (157) can re-sensitize CRC cells to platinum drugs *in vivo*.

Irinotecan. Irinotecan is used as a second-line chemotherapeutic agent employed in the treatment of patients with advanced stages of CRC in the event that first-line chemotherapy drug treatment fails. It not only selectively inhibits topoisomerase I, causing tumor cell death by inhibiting DNA function, but also has potent anticancer functions following intracellular modification in cells (158). SN38, the metabolite of irinotecan *in vivo*, has a synergistic effect with MLN4924. Apoptosis induced by this pathway proceeds in a manner that does not depend on the presence of p53 and circumvents the effects of TP53 mutations in advanced-stag CRC cells (127). This reveals a novel pathway for managing patients grappling with advanced CRC, particularly those battling chemotherapy-resistant p53 mutant CRC. However, it has not yet entered the clinical trial stage. In terms of drug resistance, epigenetic alterations are likely to cause resistance to irinotecan, such as the acetylation of histones in CRC. Therefore, partially drug-resistant CRC can be combated by combining histone deacetylase inhibitors with irinotecan (159) (Fig. 3).

5. Conclusion and future perspectives

In recent years, post-translational modifications of proteins have gradually attracted wide attention. Owing to this, a more in-depth understanding of the ubiquitin-like modification of neddylation has been obtained. Existing research evidence indicates that NEDD8 is primarily related to the proliferation, migration, survival and genetic alterations of tumor cells, and to the microenvironment of tumorigenesis. In CRC, NEDD8 is of paramount significance in the onset and progression of the disease, particularly within the realms of tumor cell migration, proliferation and viability. It is through these mechanisms that anticancer pharmaceuticals directed toward NEDD8 offer novel insight into therapeutic approaches for CRC. They mainly target NAEs for anticancer treatment. There are also drugs, such as cycle inhibitors that target the NEDD8 substrate cullin-ring E3 ubiquitin ligase for anticancer effects. Simultaneously, amalgamated pharmacological interventions, exemplified by the synergistic deployment of MLN4924 alongside irinotecan or by the combination of MLN4924 and oxaliplatin, represent promising strategies in the battle against partially resistant or recalcitrant CRC.

CRC poses one of the greatest threats to human life and health worldwide. Consequently, relentless exploration into the etiology and therapeutic modalities for CRC is imperative. As NEDD8 emerges as a nascent frontier in the arena of anticancer targets, it necessitates a more profound investigation. At present, treatments mainly target NAEs. Regrettably, limited attention has been devoted to agents targeting E2, E3 and NEDD8 substrates of the NEDD8 pathway. The potential emergence of NEDD8 substrates, including Smurf, IκB, cullin 5 and PTEN, as novel targets for therapeutic intervention, holds promise. Simultaneously, the deneddylation of CSN5 exhibits pharmaceutical design potential. Henceforth, other more effective targeted drugs need to be designed for the pathways that cause CRC. At the same time, for some NEDD8 target drugs that have been used, drug resistance issues should also be paid investigated during the period of treatment, such as the emergence of MLN4924 resistance. In future research, an exploration of novel therapeutic analogs or strategies to combat drug resistance is paramount. Consequently, studying the pathogenesis of CRC based on NEDD8 and unearthing drug targets for CRC are of utmost importance for the prognosis and survival of patients with CRC.

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Authors' contributions

TW and XL contributed equally to the present study; they were involved in the conception and design of the study, and in the drafting of the manuscript. RM and JS were involved in the analysis of the literature. SH, ZS and MW conceived and supervised the study, 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.

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