Neddylation modification of ribosomal protein RPS27L or RPS27 by MDM2 or NEDP1 regulates cancer cell survival

Xiufang Xiong1,3 | Danrui Cui2,3 | Yanli Bi2,3 | Yi Sun1,3 | Yongchao Zhao2,3

Abstract
Neddylation plays a distinct role in stabilization of a subset of ribosomal proteins. Whether the family of ribosomal proteins S27 (RPS27 and RPS27-like) is subjected to neddylation regulation with associated biological consequence is totally unknown. Here, we report that both family members are subjected to neddylation by MDM2 E3 ubiquitin ligase, and deneddylation by NEDP1. Blockage of neddylation with MLN4924, a small molecule inhibitor of neddylation-activating enzyme, destabilizes RPS27L and RPS27 by shortening their protein half-lives. Biologically, knockdown of RPS27L and RPS27 sensitizes, whereas ectopic expression of RPS27L and RPS27 desensitizes cancer cells to MLN4924-induced apoptosis. Taken together, our study demonstrates that neddylation stabilizes RPS27L and RPS27 to confer the survival of cancer cells.

KEYWORDS
MDM2, NEDD8, Neddylation, NEDP1, RPS27, RPS27L

Abbreviations: CRL, cullin-RING ligase; CRISPR, clustered regularly interspaced short palindromic repeats; CSN, COP9 signalosome complex; MDM2, mouse double minute 2 homolog; NAE, NEDD8-activating enzyme; NEDD8, neural precursor cell expressed, developmentally downregulated 8; NEDP1, NEDD8-specific protease 1; RPL7, ribosomal protein L7; RPL11, ribosomal protein L11; RPL23, ribosomal protein L23; RPS6, ribosomal protein S6; RPS14, ribosomal protein S14; RPS27, ribosomal protein S27; RPS27L, ribosomal protein S27-like.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. The FASEB Journal published by Wiley Periodicals LLC on behalf of Federation of American Societies for Experimental Biology
1 | INTRODUCTION

Protein neddylation is a process of conjugating NEDD8 onto lysine residue of the substrate protein, not for degradation, but for modulation of protein activity or subcellular localization. Like ubiquitination, neddylation is catalyzed by an E1 NEDD8-activating enzyme (NAE), an E2 NEDD8-conjugating enzyme, and an E3 NEDD8 ligase. The reverse process of protein neddylation named deneddylation is catalyzed by NEDD8 isopeptidase. The well-known physiological substrates of neddylation are cullin family members, whose neddylation leads to activation of cullin-RING ligases (CRLs), the largest family of E3 ubiquitin ligases responsible for ubiquitination and degradation of many key regulatory proteins. Thus, through modulating CRLs, neddylation regulates many biological processes, including cell cycle progression, signal transduction, and tumorigenesis. In addition to cullins, several ribosomal proteins have been identified as substrates of neddylation, including RPL7, RPL11, and RPS14. Neddylation controls their subcellular localization and stabilization, thereby regulating the MDM2-p53 axis.

RPS27L, an evolutionarily conserved 84-amino acid ribosomal protein in ribosome 40 S small subunit, differs from its family member RPS27 by only three amino acids (R5K, L12P, K17R) at the N-terminus. Our previous study showed that (a) in biochemical setting, RPS27L/RPS27 directly binds to the acidic domain of MDM2 and is subjected to MDM2-mediated ubiquitination and proteasomal degradation; (b) in cell culture setting, RPS27L/RPS27 competes with p53 for MDM2 binding, consequently inhibiting MDM2-mediated p53 ubiquitination and degradation in some cancer cell lines; (c) in an in vivo physiological setting, Rps27l knockout (KO) triggers ribosomal stress to stabilize Mdm2, which preferentially degrades its family member Mdm4, leading to reduced Mdm2-Mdm4 complex and impaired p53 ubiquitination, and increased p53 induces apoptotic depletion of hematopoietic stem cells. Thus, Rps27l KO leads to postnatal death, which is rescued by simultaneous p53 deletion; and (d) inactivation of Rps27l confers mouse sensitivity to irradiation via the Mdm2-p53 and Mdm2-MRN-ATM axes. Thus, RPS27L/RPS27 interplays with the MDM2-p53 axis to regulate p53 activity in a manner dependent of cellular context. Given that MDM2 has neddylation E3 ligase activity that promotes p53 neddylation to inhibit p53 function, we hypothesized that MDM2 may also promote the neddylation of RPS27L/RPS27 as yet another mechanism to regulate their functions.

In this study, we report that RPS27L/RPS27 are indeed neddylated by wild-type MDM2, but not its ligase dead mutant, and deneddylated by NEDP1 in a dose-dependent manner. Nedlylation modulates their protein stability, as evidenced by the observation that neddylation inhibitor MLN4924 destabilized RPS27L and RPS27. Biologically, knockdown of RPS27L or RPS27 sensitized, whereas ectopic expression of RPS27L or RPS27 desensitized cancer cells to apoptosis induced by MLN4924. Collectively, our results demonstrated that RPS27L and RPS27 are novel neddylation substrates of MDM2. Oncogenic activity of MDM2 can be attributable, at least in part, to its neddylation stabilizing RPS27L and RPS27 that confers the survival of cancer cells.

2 | MATERIALS AND METHODS

2.1 | Cell culture and chemicals

HEK293, H1299, SK-BR-3, MDA-MB-231, and MCF7 cells were obtained from American Type Culture Collection (ATCC) and were authenticated by ATCC. Cells were expanded and preserved in liquid nitrogen upon receipt. Cells for experiments were passaged for fewer than 25-30 times. All cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) (11995; Invitrogen), supplemented with 10% (v/v) of fetal bovine serum (10091148; Invitrogen) and 1% (v/v) of penicillin-streptomycin (15140-163; Invitrogen) at 37°C in a humidified incubator with 5% of CO2. MLN4924 (B1036) and CHX (C7698) were obtained from Apexbio and Sigma, respectively.

2.2 | Immunoblotting

Cells were harvested, lysed, and subjected to direct immunoblotting (IB) as previously described. Briefly, cells were lysed in lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.5% sodium deoxycholate, 50 mM NaF, 1 mM Na3VO4, 1% NP-40, 0.1% SDS, 0.5% deoxycholate, 50 mM NaF, 1 mM Na3VO4, 1 mM EDTA] with protease inhibitors (4906837001; Roche) and phosphatase inhibitors (1187358001; Roche) and phosphatase inhibitors (4906837001; Roche). After incubation on ice for 30 minutes, the supernatants were harvested by centrifugation at 14 000 rpm for 25 minutes at 4°C. Protein concentration was measured by the BCA Protein Assay Kit (23225; Thermo) according to the manufacturer’s instructions. About 30-60 μg of protein were separated on SDS-PAGE and transferred to nitrocellulose membranes (10600001; GE). After blocking with 5% of nonfat powdered milk (A600669; Sangon) in TBST buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% Tween 20] for 1 hour at room temperature (RT), the membranes were incubated with primary antibodies (Abs) at 4°C overnight, followed by incubation with corresponding secondary Abs (Jackson ImmunoResearch LABORATORIES, 1:20 000) for 1.5 hours at RT. Finally, bands were visualized using SuperSignal West Pico PLUS Chemiluminescent Substrate (34580; Thermo) with Medical X-ray Processor.
(Carestream). Primary Abs were used as follows: RPS27L or RPS27 polyclonal rabbit antibody was raised and purified as described.\textsuperscript{9,14} NEDD8 (ab81264; Abcam; 1:4000), HA (11867423001; Roche; 1:2000), FLAG (F1804; Sigma; 1:2000), PARP (9542; Cell Signaling Technology; 1:1000), caspase-3 (9665; Cell Signaling Technology; 1:1000), caspase-7 (9492; Cell Signaling Technology; 1:1000), and Actin (A5441; Sigma; 1:10 000).

2.3 In vivo neddylation

HEK293 cells at 70%-80% confluency in a 100 mm dish were transfected with the indicated plasmids with PolyJet (SL100688; SigmaGen Laboratories) for 48 hours. Then, the cells were lysed in denaturing solution [6 M guanidinium-HCl, 0.1 M Na₃HPO₄/NaH₂PO₄, 10 mM Tris-HCl (pH 8.0), 10 mM β-mercaptoethanol] and sonicated, and then, incubated with 70 μL Ni-NTA agarose (1018244; Qiagen) for 4 hours at RT, as described previously.\textsuperscript{15} After centrifugation at 5000 rpm for 1 minute at RT, beads were successively washed once with each of denaturing solution, buffer B [8 M urea, 0.1 M Na₃HPO₄/NaH₂PO₄, 10 mM Tris-HCl (pH 8.0), 10 mM β-mercaptoethanol], buffer C [8 M urea, 0.1 M Na₃HPO₄/NaH₂PO₄, 10 mM Tris-HCl (pH 6.3), and 10 mM β-mercaptoethanol] plus 0.2% of Triton X-100, and buffer C plus 0.1% of Triton X-100 for 5 minutes in each step at RT. Proteins were eluted from beads with 75 μL buffer D [200 mM imidazole, 0.15 M Tris-HCl (pH 6.7), 30% glycerol, 0.72 M β-mercaptoethanol, 5% SDS] for 30 minutes at RT. The eluted proteins were detected by IB using anti-HA Ab.

2.4 siRNA silencing

For a 60 mm dish, cells at 30%-40% confluency were transfected with 200 pmol siRNA oligonucleotides using 10 μL of Lipofectamine 2000 (11668019; Invitrogen), according to the manufacturer's instructions. The siRNA oligonucleotides were used as follows: RPS27L: 5’-AATGATTC AAACAGCTTCTCG-3’; RPS27: 5’-AAGCAGCTCTGAGTAGTC AAGATGA-3’; and siCont: 5’-ATGTATGCGATCGCAGAC-3’.

2.5 ATP-lite cell proliferation assay

Cells were seeded in 96-well plates at 10 000 cells per well, and then, treated with MLN4924 at 1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, and 400 nM for 48 hours, followed by evaluation with the ATP-lite 1 step Luminescence Assay System (6016731; Perkin-Elmer) for cell proliferation, according to the manufacturer’s instructions. The results from three independent experiments, each run in duplicate were plotted.

2.6 FACS analysis

Cells were plated in 60 mm dishes and treated with MLN4924 at 50 and 200 nM for 24 or 48 hours, followed by flow cytometry, as previously described.\textsuperscript{16} Briefly, cells were harvested with 0.05% of trypsin-EDTA (25300-062; Invitrogen), followed by centrifugation at 1000 g for 5 minutes at 4°C. Cells were then washed with cold PBS, and then, fixed in cold 70% of ethanol at −20°C overnight. Cells were washed with cold PBS twice, and then, stained with PI/RNase Staining Buffer (550825; BD Bioscience) for 15 minutes at RT before analysis.

2.7 Statistical analysis

Two-tailed Student's t tests for statistical analyses were performed using SPSS 20.0 (IBM Armonk) with the data from three or two independent biological replicates. \( P \) values less than 0.05 were considered as statistical significance.

3 RESULTS

3.1 RPS27L and RPS27 can be neddylated

Recently, several ribosomal proteins have been identified as substrates of neddylation and neddylation modification alters their stability and functions.\textsuperscript{1-6} To determine whether RPS27L/RPS27 can also be neddylated, we performed a typical in vivo neddylation assay. HEK293 cells were co-transfected with HIS-tagged NEDD8 and HA-tagged RPS27L/ RPS27, followed by Ni-NTA beads pull-down of HIS-tagged NEDD8-conjugated proteins for western blotting detection. We found that neddylated RPS27L and RPS27 were readily detected in Ni-NTA pull-down precipitation (Figure 1A,B, lane 2), which can be inhibited by the treatment of MLN4924, a small molecule inhibitor of neddylation E1 (NAE) \textsuperscript{17} (Figure 1A,B, lanes 3 vs 2). To exclude the possibility that the decrease in the neddylated RPS27L and RPS27 by MLN4924 was also neddylated, we performed a typical in vivo neddylation assay. HEK293 cells were co-transfected with HIS-tagged NEDD8 and HA-tagged RPS27L/ RPS27, followed by Ni-NTA beads pull-down of HIS-tagged NEDD8-conjugated proteins for western blotting detection. We found that neddylated RPS27L and RPS27 were readily detected in Ni-NTA pull-down precipitation (Figure 1A,B, lane 2), which can be inhibited by the treatment of MLN4924, a small molecule inhibitor of neddylation E1 (NAE) \textsuperscript{17} (Figure 1A,B, lanes 3 vs 2). To exclude the possibility that the decrease in the neddylated RPS27L and RPS27 by MLN4924 was due to the reduction of the protein levels of RPS27L and RPS27 in whole-cell extraction (Figure 1A,B, lanes 3 vs 2), we used MG132, a 26S proteasome inhibitor, to block the degradation of RPS27L and RPS27, and found that the neddylated RPS27L and RPS27 was indeed reduced upon MLN4924 treatment, regardless of the treatment of MG132 (Figure 1C,D, lanes 3 vs 2). Thus, RPS27L and RPS27 are the neddylated substrates.
FIGURE 1  RPS27L and RPS27 are NEDD8 substrates. A and B, HEK293 cells were transfected with indicated plasmids for 48 h and treated with 1 μM of MLN4924 for 6 h, lysed under denaturing conditions, and then, pulled down by Ni-NTA beads. Pull-downs (top) and whole-cell extracts (bottom) were subjected to immunoblotting (IB) with indicates antibodies (Abs). C and D, HEK293 cells were transfected with indicated plasmids for 48 h and treated with 20 μM of MG132 or in combination with 1 μM of MLN4924 for 6 h, lysed under denaturing conditions, and then, pulled down by Ni-NTA beads. Pull-downs (top) and whole-cell extracts (bottom) were subjected to IB with indicates Abs. WCE: whole-cell extracts.
3.2 MDM2 mediates the neddylation of RPS27L and RPS27

Given that MDM2 is capable of acting as an E3 for both ubiquitination and neddylation of p53,12,18,19 and that MDM2 promotes ubiquitination of ectopically expressed RPS27L and RPS27,9 we first determined if exogenous RPS27L and RPS27 could bind to MDM2. Indeed, endogenous MDM2 was readily pulled down by FLAG-tagged RPS27L and RPS27, along with RPL23 and RPS6 (Figure 2A), suggesting that 1) ectopically expressed RPS27L and RPS27 can assemble ribosomes with other endogenous ribosomal proteins and 2) MDM2 may bind to RPS27L and RPS27 to mediate neddylation of RPS27L and RPS27. Indeed, wild-type MDM2 dramatically promoted the neddylation of RPS27L and RPS27 (Figure 2B,C, lanes 3 vs 2), whereas the ligase dead mutant MDM2 (K446R/C475S) is completely inactive (Figure 2B,C, lanes 4 vs 2), even with

![Diagram showing the experiment results](image-url)

**Figure 2** RPS27L and RPS27 are neddylated by MDM2. A, H1299 cells were transfected with mock vector, FLAG-RPS27L, or FLAG-RPS27, and then, selected for stable expression by G418. Cells were lysed for immunoprecipitation with FLAG antibody. Pull-downs (top) and whole-cell extracts (bottom) were subjected to IB with indicated Abs. B and C, HEK293 cells were transfected with indicated plasmids for 48 h, lysed under denaturing conditions, and then, pulled down by Ni-NTA beads. Pull-downs (top) and whole-cell extracts (bottom) were subjected to IB with indicates Abs. D, MDM2-knockout H1299 cells were generated via CRISPR/Cas9 technology. Cells were then transfected with indicated plasmids for 48 h, lysed under denaturing conditions, and then, pulled down by Ni-NTA beads. Pull-downs (top) and whole-cell extracts (bottom) were subjected to IB with indicates Abs.
much higher levels of expression (Figure 2B,C, lanes 4 vs 3). Interestingly, MDM2, but not mutant MDM2 (K446R/C475S), obviously increased the levels of total RPS27L and RPS27 in whole-cell extraction (Figure 2B,C), suggesting that MDM2-mediated neddylation of RPS27L and RPS27 may stabilize them. We further completely depleted MDM2 by CRISPR/Cas9 technology in H1299 cells and found that the neddylation of RPS27L/RPS27 was dramatically reduced in MDM2 null cells, compared to MDM2 wild-type cells (Figure 2D, lanes 2 vs 3, lanes 5 vs 6). Taken together, these results suggest that MDM2 mediates the neddylation of RPS27L and RPS27.

3.3 | NEDP1 mediates the deneddylation of RPS27L and RPS27

Besides CSN (COP9 signalosome complex), a well-known isopeptidase for cullin deneddylation, NEDP1 is another isopeptidase for the deneddylation of noncullin substrates. To test whether NEDP1 would remove NEDD8 from neddylated RPS27L and RPS27, we first transfected MDM2 to establish the neddylated RPS27L and RPS27, and then, transfected different amounts of NEDP1 for potential deneddylation. Indeed, NEDP1 effectively reduced the neddylation of RPS27L and RPS27 in a dose-dependent manner (Figure 3), indicating that NEDP1 is the NEDD8 isopeptidase for RPS27L and RPS27 deneddylation.

3.4 | Neddylation regulates the stability of RPS27L and RPS27

We next investigated the consequence of neddylation of RPS27L and RPS27. Using cycloheximide (CHX) to block new protein synthesis, we found that blocking neddylation by MLN4924 significantly shortened the protein half-lives of RPS27L and RPS27 in both HEK293 (Figure 4A,B) and H1299 cells (Figure 4C,D). Furthermore, we showed that MLN4924 decreased the protein levels of endogenous RPS27L and RPS27 in dose and time-dependent manners, as well as decreased the levels of RPS27L induced by DMSO (Figure 4E,F). Collectively, our data suggest that neddylation modification of RPS27L and RPS27 likely play an important role in their stabilization by reducing the degradation.

3.5 | Knockdown RPS27L and RPS27 sensitizes cancer cells to MLN4924 cytotoxicity

Given that MLN4924 treatment caused significant reduction of RPS27L and RPS27, and at the same time induced apoptosis in cancer cells, we investigated whether the reduction of RPS27L and RPS27 would contribute to apoptosis-inducing effect of MLN4924. To this end, we first generated the cells stably expressing FLAG-tagged RPS27L or RPS27 at the levels similar to endogenous RPS27L and RPS27, and found that ectopic expression of

![Figure 3](image-url)
RPS27L or RPS27 decreased MLN4924-induced apoptosis in a time-dependent manner, as evidenced by decreased cleavage of PARP and caspase-3, two hallmarks for apoptosis (Figure 5A, lanes 7-8 and 11-12 vs 3-4). We next used siRNA oligos to knock down endogenous RPS27L or RPS27, and found that knockdown of RPS27L or RPS27
significantly sensitized cells to MLN4924-induced growth suppression with the IC_{50} values decreased to approximately fourfold (~200 nM in siCont cells vs ~50 nM in siRPS27L or siRPS27 cells) (Figure 5B). Furthermore, we used FACS analysis and showed that MLN4924 remarkably induced apoptosis in dose and time-dependent manners upon knockdown of RPS27L or RPS27, as reflected by a much higher induction of sub-G1 population in siRPS27L or siRPS27 cells (Figure 5C, a representative FACS profile; and Figure 5D, the percentage of sub-G1 population). Consistently, the levels of the cleaved PARP and caspase-7 induced by MLN4924 were also much higher in siRPS27L or siRPS27 cells than those in siCont cells (Figure 5E, lanes 7-8 and 11-12 vs 3-4). Taken together, these results
indicated that neddylation of RPS27L and RPS27 is an overall survival signal for cancer cells, and depletion of RPS27L or RPS27 in combination with neddylation blockage by MLN4924 significantly suppresses cancer cell growth via enhancing apoptosis.

4 | DISCUSSION

Accumulating lines of evidence have shown that enzymes catalyzing the neddylation reaction (e.g., E1, NAE1/UBA3 and E2, UBE2M/UBE2F) are overactivated in a wide range of human cancer tissues, including lung cancer,23 liver cancer,24 colorectal cancer,25 among other cancers,2 and the overactivated neddylation pathway is correlated with poor prognosis of cancer patients.23-25 Thus, targeting neddylation pathway appears to be an attractive anticancer approach, which was validated by the use of MLN4924, a small molecule inhibitor of NAE, in several Phase I/II clinical trials.17 However, cancer cells can develop resistance to MLN4924. For instance, a point mutation (A171T) of NAEβ, also known as UBA3, a catalytic subunit of NAE, overcomes the inhibitory effects of MLN4924, allowing cancer cell survival.26,27 Recently, we found that MLN4924 treatment significantly induces p53 and its target genes.20 Given that RPS27L is a direct target gene of p5328,29 and ectopic expression of RPS27L reduces the sensitivity of cancer cells to MLN4924-induced apoptosis, the induction of RPS27L by p53 may contribute cell resistance to MLN4924. Thus, disrupting the p53-RPS27L axis may enhance anticancer effects of MLN4924.

Using the proteomics approaches, many ribosomal proteins were identified as potential targets for neddylation modification.30 Subsequent studies characterized RPL7,3 RPL11,5 and RPS146 as neddylation substrates. Generally, neddylation stabilizes ribosomal proteins and alters their subcellular location from the nucleoplasm to the nucleolus to regulate the function of the MDM2-p53 axis.5,6,30 In this study, we further provided multiple lines of evidence to clearly show the stabilization of RPS27L and RPS27 by their neddylation modification (Figure 4). Subcellularly, whether neddylation regulates their subcellular location is a subject for future investigation. Biologically, knockdown of RPS27L or RPS27 sensitized cancer cells to MLN4924 treatment via enhanced apoptosis (Figure 5). To our knowledge, this is the first study to show that ribosomal proteins are involved in MLN4924-mediated growth suppression of cancer cells. Thus, our finding adds another layer of mechanism by which MLN4924 acts as an anticancer agent.

Our previous study showed that under stressed conditions, including DNA damage induced by chemotherapeutic drugs and ribosomal stress, RPS27L and RPS27 are co-localized with MDM2 in the nucleoplasm where they compete with

**FIGURE 6** A model for MDM2- and NEDP1-mediated NEDD8 modification of RPS27/RPS27L in regulation of cancer cell survival. See text for details
p53 for MDM2 binding, thus, releasing p53 to induce apoptosis.9 Interestingly, MDM2 triggers the ubiquitination and proteasomal degradation of RPS27L under both overexpressed and physiological conditions, but promotes RPS27 ubiquitination under overexpressed condition.9 In this study, we found that MDM2 also serves as neddylation E3 to promote the neddylation of both RPS27L and RPS27 to stabilize them. So it is apparently paradoxical, but providing an interesting subject for future investigation to sort out under what physiological and stressed conditions that MDM2 would serve as an ubiquitination E3 or neddylation E3, and whether there is a competition between these two types of posttranslational modifications. Finally, we characterized in this study that NEDP1 as the NEDD8 isopeptidase for RPS27L and RPS27. Interestingly, NEDP1 is a chemotherapy-induced gene, which also deneddylates MDM2, leading to MDM2 destabilization with concomitant p53 activation.31 Thus, our study unveils a balance of RPS27L/RPS27 neddylation vs deneddylation, regulated by MDM2 and NEDP1 to ensure proper stability of these two proteins for cell survival or death (Figure 6).

ACKNOWLEDGMENTS
This work was supported by the National Key R&D Program of China (2016YFA0501800 to YZ, XX and YS), the National Natural Science Foundation of China (81872728, 81972591, and 81721091 to YZ, 81974429 and 81572708 to XX), and the Natural Science Foundation of Zhejiang Province (LR16C050001 to YZ).

CONFLICT OF INTEREST
The authors declare no competing interests.

AUTHOR CONTRIBUTIONS
X. Xiong designed and performed the experiments, analyzed and interpreted the data, and drafted the manuscript. D. Cui and Y. Bi performed the experiments. Y. Sun analyzed and interpreted the data and revised the manuscript. Y. Zhao designed the study, analyzed and interpreted the data, and revised and finalized the manuscript. All authors have reviewed the manuscript.

REFERENCES
1. Zhao Y, Morgan MA, Sun Y. Targeting neddylation pathways to inactivate cullin-RING ligases for anticancer therapy. Antioxid Redox Signal. 2014;21:2383-2400.
2. Zhou L, Zhang W, Sun Y, Jia L. Protein neddylation and its alterations in human cancers for targeted therapy. Cell Signal. 2018;44:92-102.
3. Enchev RI, Schulman BA, Peter M. Protein neddylation: beyond cullin-RING ligases. Nat Rev Mol Cell Biol. 2015;16:30-44.
4. Maghames CM, Lobato-Gil S, Perrin A, et al. NEDDylation promotes nuclear protein aggregation and protects the Ubiquitin Proteasome System upon proteotoxic stress. Nat Commun. 2018;9:4376.
5. Sundqvist A, Liu G, Mirsaliotis A, Xiromidas DP. Regulation of nuclear signalling to p53 through NEDDylation of L11. EMBO Rep. 2009;10:1132-1139.
6. Zhang J, Bai D, Ma X, Guan J, Zheng X. hCINAP is a novel regulator of ribosomal protein-HDM2-p53 pathway by controlling NEDDylation of ribosomal protein S14. Oncogene. 2014;33:246-254.
7. Zhang Y, Lu H. Signaling to p53: ribosomal proteins find their way. Cancer Cell. 2009;16:369-377.
8. Xu X, Xiong X, Sun Y. The role of ribosomal proteins in the regulation of cell proliferation, tumorigenesis, and genomic integrity. Sci China Life Sci. 2016;59:656-672.
9. Xiong X, Zhao Y, He H, Sun Y. Ribosomal protein S27-like and S27 interplay with p53-MDM2 axis as a target, a substrate and a regulator. Oncogene. 2011;30:1798-1811.
10. Xiong X, Zhao Y, Tang F, et al. Ribosomal protein S27-like is a physiological regulator of p53 that suppresses genomic instability and tumorigenesis. Elife. 2014;3:e02236.
11. Zhao Y, Tan M, Liu X, Xiong X, Sun Y. Inactivation of ribosomal protein S27-like confers radiosensitivity via the Mdm2-p53 and Mdm2-MRN-ATM axes. Cell Death Dis. 2018;9:145.
12. Xiromidas DP, Saville MK, Bourdon JC, Hay RT, Lane DP. Mdm2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. Cell. 2004;118:83-97.
13. Cui D, Dai X, Shu J, et al. The cross talk of two family members of beta-TrCP in the regulation of cell autophagy and growth. Cell Death Differ. 2020;27:1119-1133.
14. Xiong X, Liu X, Li H, He H, Sun Y, Zhao Y. Ribosomal protein S27-like regulates autophagy via the beta-TrCP-DEPTOR-mTORC1 axis. Cell Death Dis. 2018;9:1131.
15. Zhao Y, Xiong X, Sun Y. DEPTOR, an mTOR inhibitor, is a physiological substrate of SCF(betaTrCP) E3 ubiquitin ligase and regulates survival and autophagy. Mol Cell. 2011;44:304-316.
16. Zhao Y, Xiong X, Jia L, Sun Y. Targeting Cullin-RING ligases by MLN4924 induces autophagy via modulating the HIF1-REDD1-TSC1-mTORC1-DEPTOR axis. Cell Death Dis. 2012;3:e386.
17. Soucy TA, Smith PG, Milhollen MA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature. 2009;458:732-736.
18. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. Nature. 1997;387:296-299.
19. Kubbhat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. Nature. 1997;387:299-303.
20. Cui D, Xiong X, Shu J, Dai X, Sun Y, Zhao Y. FBXW7 Confers Radiation Survival by Targeting p53 for Degradation. Cell Rep. 2020;30:497-509.e4.
21. Wei N, Serino G, Deng XW. The COP9 signalosome: more than a physiological regulator of p53. Oncotarget. 2015;6:369-377.
22. Shen LN, Liu H, Dong C, Xiromidas D, Naismith JH, Hay RT. Structural basis of NEDD8 ubiquitin discrimination by the deNEDDylating enzyme NEDP1. EMBO J. 2005;24:1341-1351.
23. Li L, Wang M, Yu G, et al. Overactivated neddylation pathway as a therapeutic target in lung cancer. J Natl Cancer Inst. 2014;106:duj083.
24. Barbier-Torres L, Delgado TC, Garcia-Rodriguez JL, et al. Stabilization of LKB1 and Akt by neddylation regulates energy metabolism in liver cancer. Oncotarget. 2015;6:2509-2523.
25. Xie P, Zhang M, He S, et al. The covalent modifier Nedd8 is critical for the activation of Smurf1 ubiquitin ligase in tumorigenesis. Nat Commun. 2014;5:3733.
26. Milhollen MA, Thomas MP, Narayanan U, et al. Treatment-emergent mutations in NAEbeta confer resistance to the NEDD8-activating enzyme inhibitor MLN4924. *Cancer Cell*. 2012;21:388-401.

27. Toth JI, Yang L, Dahl R, Petroski MD. A gatekeeper residue for NEDD8-activating enzyme inhibition by MLN4924. *Cell Rep*. 2012;1:309-316.

28. He H, Sun Y. Ribosomal protein S27L is a direct p53 target that regulates apoptosis. *Oncogene*. 2007;26:2707-2716.

29. Li J, Tan J, Zhuang L, et al. Ribosomal protein S27-like, a p53-inducible modulator of cell fate in response to genotoxic stress. *Cancer Res*. 2007;67:11317-11326.

30. Xi rodimas DP, Sundqvist A, Nakamura A, Shen L, Botting C, Hay RT. Ribosomal proteins are targets for the NEDD8 pathway. *EMBO Rep*. 2008;9:280-286.

31. Watson IR, Li BK, Roche O, Blanch A, Ohh M, Irwin MS. Chemotherapy induces NEDP1-mediated destabilization of MDM2. *Oncogene*. 2010;29:297-304.

How to cite this article: Xiong X, Cui D, Bi Y, Sun Y, Zhao Y. Neddylation modification of ribosomal protein RPS27L or RPS27 by MDM2 or NEDP1 regulates cancer cell survival. *The FASEB Journal*. 2020;34:13419–13429. [https://doi.org/10.1096/fj.20200 0530RRR](https://doi.org/10.1096/fj.20200 0530RRR)