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Neddylation pathway regulates the proliferation and survival of macrophages

Lihui Li [a,b], Binghong Liu [b], Tiangeng Dong [c], Hyuk Woo Lee [d], Jinha Yu [d], Yijie Zheng [b], Haifeng Gao [b], Yan Zhang [b], Yiwei Chu [b,e], Guangwei Liu [b], Weixin Niu [c], Shimin Zheng [a,*], Lak Shin Jeong [d,*], Lijun Jia [b,e,*]

[a] College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, China
[b] Department of Immunology, Shanghai Medical College, Fudan University, Shanghai 200032, China
[c] Department of General Surgery, Zhongshan Hospital, Fudan University, 180 Fenglin Road, Shanghai, China
[d] Department of Bioinspired Science and College of Pharmacy, Ewha Womans University, Seoul 120-750, Republic of Korea
[e] Biotherapy Research Center of Fudan University, Shanghai 200032, China

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ABSTRACT

Neddylation is a new type of protein post-translational modification which adds the ubiquitin-like molecule Nedd8 to target proteins. The well-identified targets of neddylation are cullins, which serve as essential components of Cullin-RING E3 ligases (CRL). It is reported that inhibition of neddylation repressed NF-κB-mediated proinflammatory cytokine production in macrophages. However, the role of neddylation in the proliferation and survival of macrophages has not been well defined. Here we report that partial inactivation of the neddylation pathway by a specific Nedd8-activating enzyme E1 (NAE) inhibitor MLN4924 reduced LPS-induced production of the proinflammatory cytokines TNF-α and IL-6 without obvious impairment of cell viability. However, persistent and severe inactivation of neddylation by MLN4924 significantly inhibited cell proliferation by inducing G2 phase cell-cycle arrest and further triggered cell death by inducing apoptosis in RAW264.7 macrophages. Mechanistic analysis revealed that inactivation of neddylation blocked cullin neddylation, inhibited CRL E3 ligase activity, and thus led to the accumulation of CRL substrates, resulting in cell-cycle arrest, DNA damage response and apoptosis. The findings revealed that neddylation serves as an important signaling pathway regulating the proliferation and survival of macrophages.

1. Introduction

Neddylation, a process of adding ubiquitin-like molecule Nedd8 to target proteins, is a type of protein post-translational modification which is triggered by the successive action of the Nedd8-activating enzyme E1 (NAE), Nedd8-conjugating enzyme E2 (Ubc12) and Nedd8-E3 ligase [1–4]. Neddylation regulates the localization, stability and function of target proteins [1–4]. The well-characterized targets of neddylation thus far are cullins which function as essential subunits of multiunit Cullin-RING E3 ligases (CRL) [5–8]. CRLs are the largest cellular ubiquitin ligase family and signal degradation of a large numbers of cellular proteins that regulate diverse biological processes [7,9,10]. Neddylation of cullins changes the confirmation of the CRL complex and thus activates its enzymatic function for protein ubiquitination subsequent degradation [7,11].

Recent studies from our and other groups demonstrate that the neddylation pathway is required for cancer-cell survival while the inhibition of neddylation serves as a novel anticancer strategy [7,12–16]. MLN4924, a specific inhibitor of NAE, was initially discovered via high-throughput screening as a first-in-class anticancer agent [7]. MLN4924 inhibits NAE activity by binding to NAE at the active site to form a covalent Nedd8-MLN4924 adduct and thus inactivates the neddylation pathway [17,18]. By doing so, MLN4924 blocks cullin neddylation, inhibits CRL activity, which leads to the accumulation of multiple CRL substrates, including (1) DNA replication licensing protein CDT1 and ORC1, resulting in DNA re-replication stress and DNA damage response; (2) cell-cycle inhibitors such as p21, p27 and Wee1, leading to cell-cycle arrest at different phases in a cell-line-dependent manner; and (3) IκB-α, resulting in the inhibition of NF-κB activity [7,12–14,19]. As a result, MLN4924 suppresses cancer-cell proliferation via cell-cycle arrest and induces cell death via apoptosis, autophagy and/or senescence in cancer cells [7,12–14,20].

Recently, inhibition of neddylation by genetic approaches or NAE inhibitor MLN4924 has been shown to suppress the lipopolysaccharide (LPS)-induced proinflammatory cytokine production in macrophages [21]. Mechanism analysis demonstrated that MLN4924 inhibits cullin neddylation, inactivates CRL E3 ligase and leads to the accumulation of IκB-α, a well-known CRL substrate and NF-κB inhibitor, thereby blocking NF-κB-regulated transcriptional activation of proinflammatory cytokines [21]. These findings demonstrate a critical role of the neddylation pathway in

* Corresponding authors. Department of Immunology, Shanghai Medical College, Fudan University, Shanghai 200032, China (L.Jia).
E-mail addresses: zhengshiminbl@sohu.com (S. Zheng), lakjeong@ewha.ac.kr (L.S. Jeong), jialijun2002@yahoo.com.cn (L. Jia).

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regulation of macrophage function during inflammatory responses and suggest neddylation inhibitors as potential anti-inflammation agents [21]. However, the potential role of neddylation in regulation of the proliferation and survival of macrophages has not been well defined. To address this, in this study, we determined the cell fate of macrophages upon neddylation inhibition by MLN4924. We found that partial inhibition of neddylation regulated the function of macrophages without impairing cell viability, whereas persistent inactivation of neddylation impaired cell survival. The findings in this report demonstrate that neddylation is a cellular pathway regulating the proliferation and survival of macrophages.

2. Materials and methods

2.1. Reagents and cell lines

MLN4924 was synthesized as previously described [22]. MLN4924 was dissolved in DMSO at concentration of 10 mM and stored in −80 °C. LPS (*Escherichia coli*, serotype) was purchased from Sigma (St. Louis, MO, USA). Mouse macrophage cell line RAW264.7 was obtained from the American Type Culture Collection (Manassas, VA). RAW264.7 was cultured in Dulbecco's Modified Eagle's Medium (Hyclone), containing 10% FBS (Biochrom AG) and 1% penicillin–streptomycin solution, at 37 °C with 5% CO_2.

2.2. ATPlite cell viability assay

Cells were seeded in 96-well plates with 5000 cells per well, cultured for 24 h, treated with MLN4924 or DMSO for indicated times. Cell viability was determined using the ATPlite kit according to the manufacturer's instructions [23,24].

2.3. Cell counting kit-8 (CCK8) cell viability assay

Cells were seeded in 96-well plates and treated with MLN4924 or DMSO for the indicated times. CCK8 (10 μL) was added to each well with treated cells and incubated at 37 °C for another 2 h. Optical density (OD) was read at 450 nm [25].

2.4. RNA extraction and RT-PCR

Total RNA was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and treated with RNase-free DNase. Reverse transcription (RT) reaction was performed on 2.5 μg of total RNA per sample using the PrimerScript RT reagent kit (TaKaRa, Shiga, Japan) according to the manufacturer's protocol. After reverse transcription, real-time PCR was performed using the Power SYBR Green PCR Master Mix (Applied Biosystems) on the ABI 7500 thermocycler (Applied Biosystems) following the manual.

Primers used were designed by Primer5.0 and Oligo6.0. The sequences of the primers were as follows:

- mouse β-actin: Forward 5′-CCAGCCTTCCTTCTTGGGTATG-3′,
- Reverse 5′-TGTGTTGGCATAGAGGTCTTTACG-3′;
- mouse TNF-α: Forward 5′-GGCAGGTCTACTTTGGAGTCATTG-3′,
- Reverse 5′-ACATTCGAGGCTCCAGTGAATTCGG-3′;
- mouse IL-6: Forward 5′-ACAACCACGGCCTTCCCTACTT-3′,
- Reverse 5′-CACGATTTCCCAGAGAACATGTG-3′.

2.5. Propidium iodide staining and fluorescence-activated cell-sorting (FACS) analysis

RAW264.7 macrophages treated with DMSO or MLN4924 were harvested and fixed in 70% ethanol at −20 °C overnight, and stained with propidium iodide (PI: 36 μg/mL, Sigma) containing RNase (50 μg/mL, Sigma) for 15 min, then analyzed for apoptosis and cell-cycle profile by CyAn ADP (Beckman Coulter). The percentages of cells in the Sub-G1 population were considered as apoptotic. Data were analyzed with ModFit LT software [14].

2.6. Western blot analysis

Cell lysates were prepared for Western bolt analysis, using antibodies against Cullin1 (Santa Cruz Biotechnology), total CHK1 (t-CHK1), total H2AX (t-H2AX) (Epitomics, Inc.), p-CHK1, p-H2AX (Ser139), p21, p27, p53 (BD Bioscience), p-IκB-α (Ser32), t-IκB-α, CDT1, ORC1, cleaved caspase-3, cleaved PARP, Wee1, p-Histone H3 (Ser10) (Cell Signaling) and β-actin (CWBIO) [14].

2.7. Statistical analysis

The results were presented as means ± SEM of three independent experiments. For statistical tests, Prism 5.0 (GraphPad Software, SanDiego, CA, USA) was used. p values less than 0.05 were considered statistically significant.

3. Results

3.1. Partial inhibition of neddylation by MLN4924 inhibited LPS-induced proinflammatory cytokine production by RAW 264.7 macrophages

To determine whether partial inhibition of neddylation by MLN4924 regulates the proinflammatory cytokine-secreting function of RAW264.7 macrophages, we first optimized the doses and duration of treatment. As shown in (Fig. 1A), MLN4924 treatment at 0.1 μM and 0.33 μM for 12 h had no any inhibitory effect on cell viability. However, this treatments inhibited cullin neddylation and blocked the degradation of NF-κB-inhibitory protein and CRL substrate IκB-α, as indicated by the significant accumulation of p-IκB-α (Fig. 1B). Accordingly, the transcriptional activation of proinflammatory cytokines TNF-α and IL-6 upon LPS stimulation was significantly inhibited by MLN4924 at the above doses (Fig. 1C). Consistently, the level of TNF-α and IL-6 in the cell culture supernatant was significantly decreased by MLN4924 (Fig. 1D). The findings suggest that partial inhibition of neddylation regulate the function of macrophages without impairing cell viability.

3.2. Persistent inactivation of neddylation impaired viability of RAW264.7 macrophages

To determine the effect of persistent and severe inactivation of neddylation on cell viability of macrophages, RAW264.7 macrophages were treated with MLN4924 for 24 h and 48 h. As shown in Fig. 2, MLN4924 treatment at a moderate dose (0.33 μM) and high dose (1.0 μM), but not low dose (0.1 μM), significantly decreased cell viability in a dose- and time-dependent manner. The findings indicate that persistent and severe inhibition of neddylation suppressed macrophage viability.

3.3. Neddylation inhibition by MLN4924 induced G2 cell-cycle arrest and apoptosis in macrophages

To address how MLN4924 treatment suppressed cell proliferation, we performed FACS analysis to determine the cell-cycle profile post treatment. As shown in (Fig. 3A), ~51–66% of MLN4924-treated cells were arrested in the G2–M phase of the cell cycle, compared with ~10% of control cells at 12 h and 24 h post treatment. To determine at which phase of the cell cycle (G2 versus

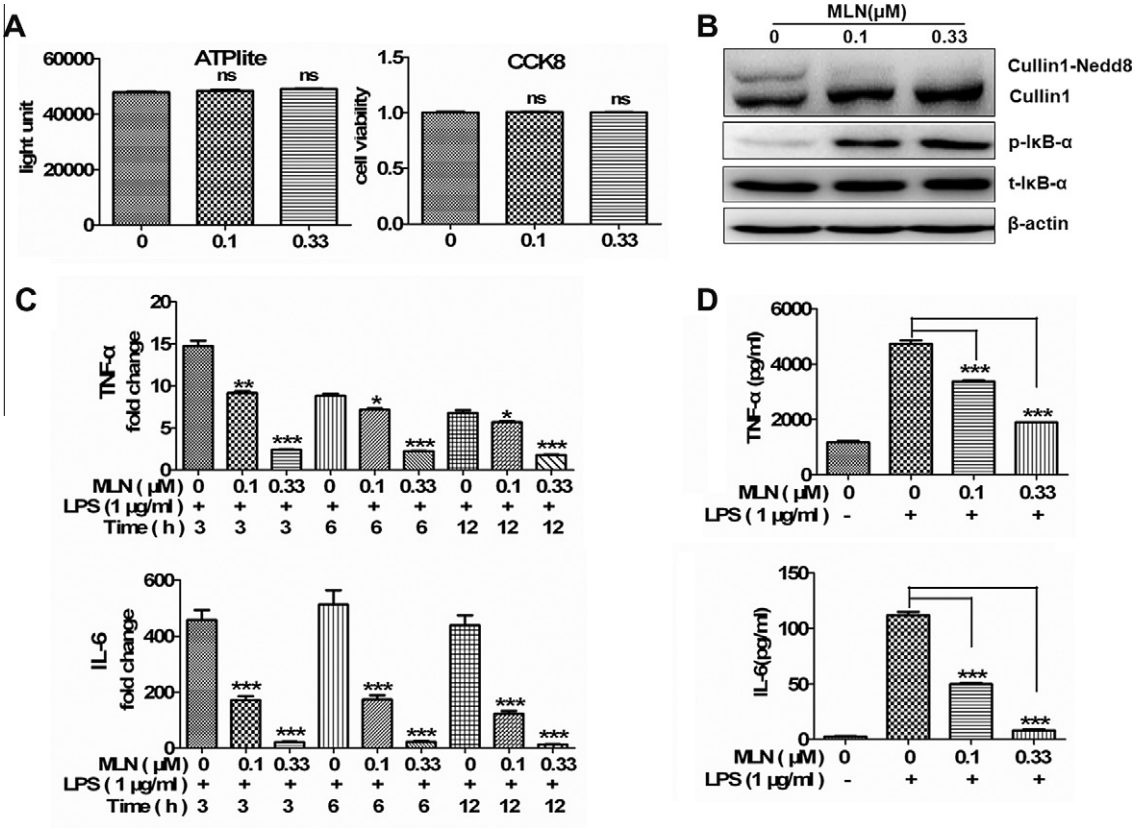


Fig. 1. MLN4924 treatment at non-toxic doses inhibited LPS-induced production of proinflammatory cytokines in RAW 264.7 macrophages. (A) RAW264.7 macrophages seeded into 96-well plates were cultured overnight and treated with MLN4924 at 0, 0.1 and 0.33 μM for 12 h, followed by cell viability analysis with ATPlite and CCK8 assays. (B) RAW264.7 macrophages were treated with MLN4924 at 0, 0.1, and 0.33 μM for 12 h and subjected to Western blot analysis using antibodies against Cullin1, p-IκB-α, t-IκB-α with β-actin as a loading control. (C and D) RAW264.7 macrophages seeded in 24-well plates were cultured overnight and pretreated with MLN4924 at 0, 0.1, and 0.33 μM for 12 h. The treated cells were then stimulated with LPS (1 μg/mL) for the indicated times, followed by expression analysis of TNF-α and IL-6 at the transcriptional level by RT-qPCR (C) and the protein level by ELISA (D). (C) mRNA expression of TNF-α and IL-6 were measured by RT-qPCR. (D) Protein of TNF-α and IL-6 in the cell culture supernatant were determined by ELISA. *ns*: not statistically significant; $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$.

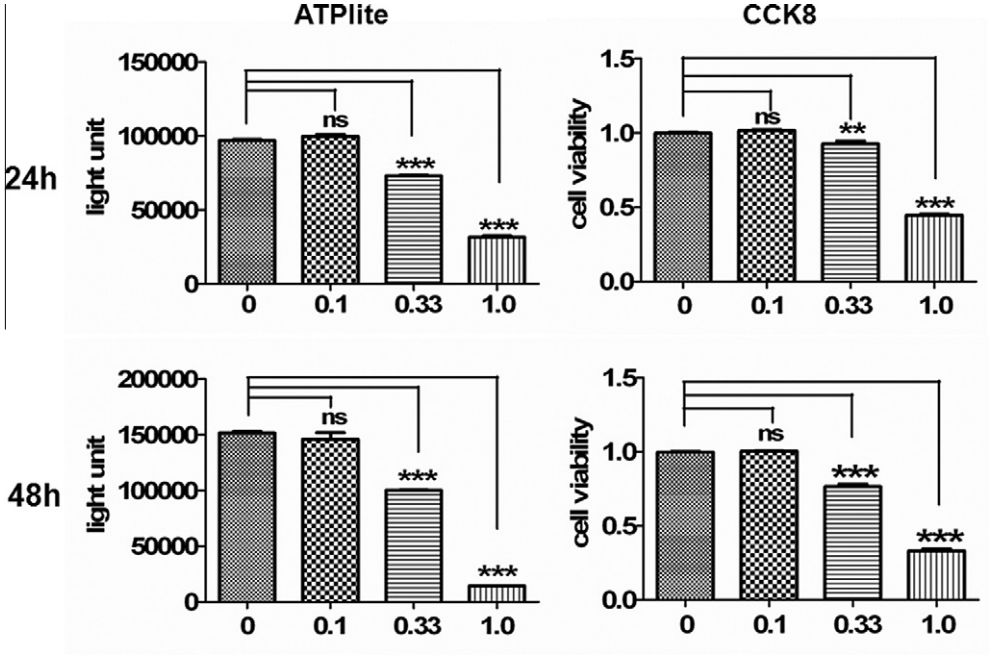


Fig. 2. Persistent inactivation of neddylation by MLN4924 inhibited macrophage proliferation and induced cell death. RAW264.7 macrophages seeded in 96-well plates were cultured overnight and treated with MLN4924 at different concentrations for 24 h (upper panel) and 48 h (bottom panel), followed by ATPlite and CCK8 cell viability assays, respectively.

M) RAW264.7 macrophages were arrested upon neddylation inhibition, we determined the expression status of Wee1, a well-defined CRL substrate and an inhibitor of G2–M phase transition [26], as well as p-Histone H3 (p-H3, ser10), a marker of M phase cells [27]. As shown in Fig. 3(B), MLN4924 induced significant accumulation of Wee1 whereas it decreased the expression of p-H3, indicating that MLN4924-treated cells were arrested at the G2 phase of cell cycle upon neddylation inhibition.

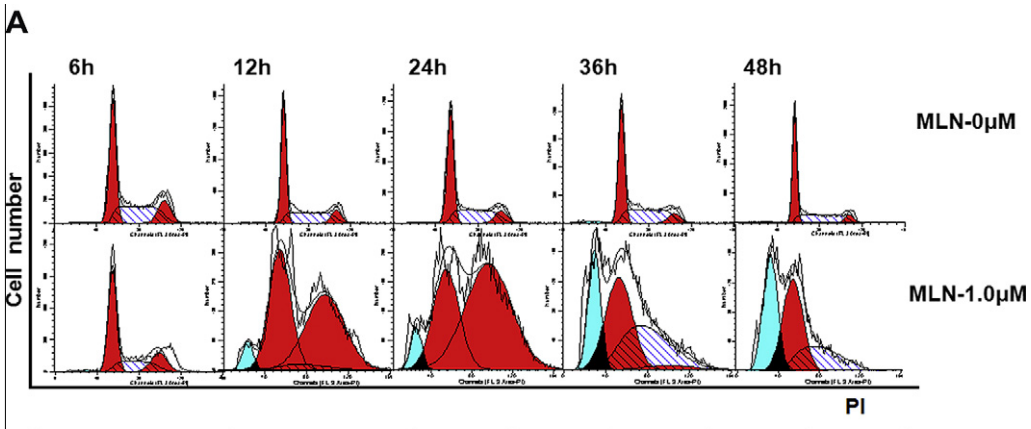
FACS analysis also revealed that neddylation inhibition by MLN4924 triggered apoptosis as indicated by the appearance of a Sub-G1 fraction which occurred at 12 h, increased gradually over time and reached the peak at 48 h post treatment. Apoptotic induction upon neddylation inhibition was further confirmed by the up-regulation of cleaved caspase-3 and cleaved PARP as classical apoptotic markers (Fig. 3B). These findings suggested that neddylation inhibition by MLN4924 sequentially induced G2 phase cell-cycle arrest and apoptosis, G2 phase-arrested cells subsequently died of apoptosis.

3.4. Inactivation of neddylation blocked the degradation of cell-cycle and apoptosis-regulatory CRL substrates and triggered DNA damage responses

To further address the underlying mechanism of MLN4924 action on the induction of cell-cycle arrest and apoptosis in RAW264.7 macrophages, we determined the expression of cell-cycle and apoptosis-triggering CRL substrates in treated cells upon neddylation inhibition [7,12,14]. As shown in Fig. 4, MLN4924 inhibited cullin neddylation and led to the accumulation of well-known cell-cycle inhibitory CRL substrates p21 and p27. Meanwhile, MLN4924 blocked the degradation of IκB-α, as demonstrated by the up-regulation of p-IκB-α, indicating the inhibition of NF-κB signaling. Moreover, MLN4924 induced the accumulation of DNA replication licensing protein CDT1 and ORC1 and triggered DNA damage response, as demonstrated by a significant increase of phosphorylated H2AX and CHK1 as well as the activation of p53. The accumulation of cell-cycle inhibitor, inactivation of NF-κB pathway and induction of DNA damage response upon neddylation inhibition may contribute to cell-cycle arrest and apoptosis after MLN4924 treatment in RAW264.7 macrophages.

4. Discussion

Neddylation is a type of protein post-translational modification, regulating diverse biological processes by affecting subcellular localization, stability, confirmation and function of target proteins [2]. The role of neddylation in development and cancer cell biology has been frequently investigated [2]. Neddylation also affects the function of macrophages and T cells during inflammatory responses [21,28]. In the present study, we showed that partial



	treatments	6h	12h	24h	36h	48h
G2 (%)	MLN-0μM	15.7	10.6	10.6	8.1	7.7
	MLN-1.0μM	18.9	50.6	65.5	5.6	0.0
Sub-G1 (%)	MLN-0μM	0.2	0.1	0.2	2.0	2.5
	MLN-1.0μM	1.9	5.0	5.8	22.7	36.2

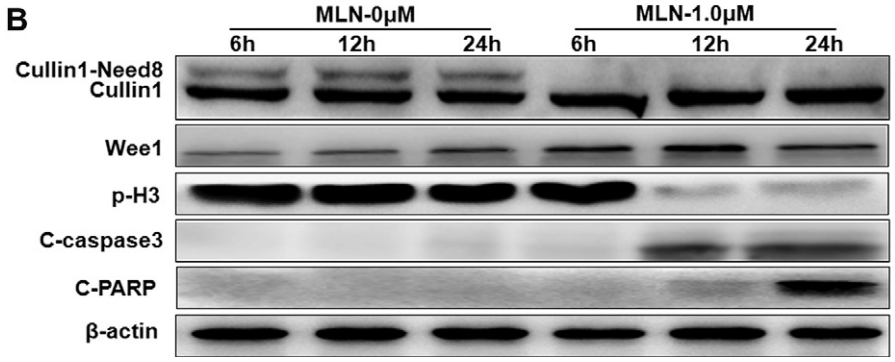


Fig. 3. Neddylation inhibition by MLN4924 induced G2 cell-cycle arrest and apoptosis. (A) RAW264.7 macrophages were treated with MLN4924 for the indicated times and subjected to PI staining and FACS analysis. The percentage of cells in the G2–M phase and Sub-G1 phase was indicated. (B) RAW264.7 macrophages were treated with MLN4924 at 1.0 μM for the indicated times and subjected to Western blot analysis using antibodies against Cullin1, Wee1, p-Histone H3, cleaved caspase-3 (C-caspase3), and cleaved PARP (C-PARP) with β-actin as a loading control.

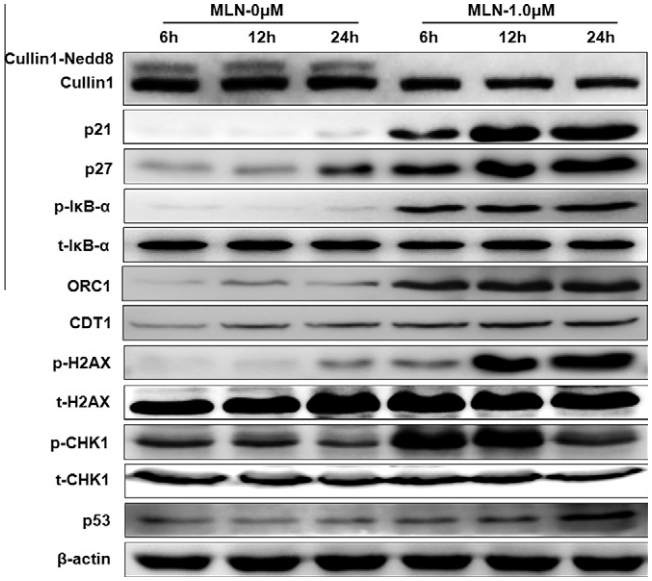


Fig. 4. Persistent inactivation of neddylation blocked the degradation of cell-cycle and apoptosis-regulatory CRL substrates and triggered DNA damage responses. RAW264.7 macrophages were treated with MLN4924 at 1.0 μM for the indicated times and subjected to Western blot analysis using antibodies against p21, p27, p-IκB-α, t-IκB-α, CDT1, ORC1, p-H2AX, p-CHK1, t-H2AX, and t-CHK1 with β-actin as a loading control.

inhibition of neddylation by MLN4924 suppressed LPS-induced production of proinflammatory cytokines in macrophages, mainly resulting from suppression of NF-κB activity due to the accumulation of CRL substrate IκB-α. The findings highlight the neddylation pathway in the regulation of inflammatory responses and suggest that inhibition of neddylation by specific inhibitors such as MLN4924 may serve as novel anti-inflammation strategy [21,28].

The major finding of this study is that neddylation serves as a novel cellular pathway essential for the survival of macrophages. Previous studies from our and other groups have demonstrated that neddylation was required for cancer cell growth, while inactivation of neddylation by MLN4924 inhibited cell proliferation and induced cell death in diverse cancer cell types [7,12–14]. In the present study, we demonstrated that neddylation is not only required for the function of macrophages but also essential for their proliferation and survival as it facilitates cell-cycle progression and prevent apoptosis-induced cell death.

Our findings demonstrate that similar mechanisms of growth suppression are shared by macrophages and cancer cells upon neddylation inhibition. In both macrophages and cancer cells, neddylation inactivation by MLN4924 blocked cullin neddylation, inhibited CRL activity and induced cell-cycle arrest and apoptosis by inducing the accumulation of well-known CRL substrates, including (a) cell-cycle inhibitors p21, p27, and Wee1; (b) NF-κB inhibitor IκB-α; and (c) DNA replication licensing proteins CDT1 and ORC1 which triggers DNA re-replication stress and DNA damage response [7,12–14]. These observations suggest that neddylation is a conversed pathway to control the growth and survival of different cells. The present study thus demonstrates that neddylation is a critical pathway which regulates the proliferation and survival of macrophages.

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References

[1] S.J. Loftus, G. Liu, S.M. Carr, et al., NEDDylation regulates E2F-1-dependent transcription, EMBO Rep. 13 (2012) 811–818.
[2] D.P. Xirodimas, Novel substrates and functions for the ubiquitin-like molecule Nedd8, Biochem. Soc. Trans. 36 (2008) 802–806.
[3] D.P. Xirodimas, M.K. Saville, J.C. Bourdon, et al., Mdm2-mediated Nedd8 conjugation of p53 inhibits its transcriptional activity, Cell 118 (2004) 83–97.
[4] W. Zuo, F. Huang, Y.J. Chiang, et al., C-Cbl-mediated neddylation antagonizes ubiquitination and degradation of the TGF-beta type II receptor, Mol. Cell 49 (2013) 1–12.
[5] A. Saha, R.J. Deshaies, Multimodal activation of the ubiquitin ligase SCF by Nedd8 conjugation, Mol. Cell 32 (2008) 21–31.
[6] E. Sakata, Y. Yamaguchi, Y. Miyauchi, et al., Direct interactions between Nedd8 and ubiquitin E2 conjugating enzymes upregulate cullin-based E3 ligase activity, Nat. Struct. Mol. Biol. 14 (2007) 167–168.
[7] T.A. Soucy, P.G. Smith, M.A. Milhollen, et al., An inhibitor of Nedd8-activating enzyme as a new approach to treat cancer, Nature 458 (2009) 732–736.
[8] J.T. Wu, H.C. Lin, Y.C. Hu, et al., Neddylation and deneddylation regulate Cul1 and Cul3 protein accumulation, Nat. Cell Biol. 7 (2005) 1014–1020.
[9] G. Nalepa, M. Rolfe, J.W. Harper, Drug discovery in the ubiquitin-proteasome system, Nat. Rev. Drug Discovery 5 (2006) 596–613.
[10] M.D. Petroski, R.J. Deshaies, Function and regulation of Cullin-RING ubiquitin ligases, Nat. Rev. Mol. Cell Biol. 6 (2005) 9–20.
[11] E.J. Bennett, J. Rush, S.P. Gygi, et al., Dynamics of Cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics, Cell 143 (2010) 951–965.
[12] L. Jia, H. Li, Y. Sun, Induction of p21-dependent senescence by an NAE inhibitor, MLN4924, as a mechanism of growth suppression, Neoplasia 13 (2011) 561–569.
[13] J.J. Lin, M.A. Milhollen, P.G. Smith, et al., 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 (2010) 10310–10320.
[14] Z. Luo, G. Yu, H.W. Lee, et al., The Nedd8-activating enzyme inhibitor MLN4924 induces autophagy and apoptosis to suppress liver cancer cell growth, Cancer Res. 72 (2012) 3360–3371.
[15] L. Jia, Y. Sun, SCF E3 ubiquitin ligases as anticancer targets, Curr. Cancer Drug Targets 11 (2011) 347–356.
[16] Y. Zhao, Y. Sun, Cullin-RING ligases (CRLs) as attractive anti-cancer targets, Curr. Pharm. Des. 2013. [Epub ahead of print], PMID: 23151137.
[17] J.E. Brownell, M.D. Sintchak, J.M. Gavin, 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 (2010) 102–111.
[18] M.D. Petroski, Mechanism-based neddylation inhibitor, Chem. Biol. 17 (2010) 6–8.
[19] M.A. Milhollen, T. Traore, J. Adams-Duffy, et al., MLN4924, a Nedd8-activating enzyme inhibitor, is active in diffuse large B-cell lymphoma models: rationale for treatment of NF-{kappa}B-dependent lymphoma, Blood 116 (2010) 1515–1523.
[20] Y. Zhao, X. Xiong, L. Jia, et al., Targeting Cullin-RING ligases by MLN4924 induces autophagy via modulating the HIF1-REDD1-TSC1-mTORC1-DEPTOR axis, Cell Death Dis. 3 (2012) e386.
[21] F.M. Chang, S.M. Reyna, J.C. Granados, et al., Inhibition of neddylation represses lipopolysaccharide-induced proinflammatory cytokine production in macrophage cells, J. Biol. Chem. 287 (2012) 35756–35767.
[22] H.W. Lee, S.K. Nam, W.J. Choi, et al., Stereo selective synthesis of MLN4924, an inhibitor of Nedd8-activating enzyme, J. Org. Chem. 76 (2011) 3557–3561.
[23] L. Jia, M.S. Soengas, Y. Sun, ROC1/RBX1 E3 ubiquitin ligase silencing suppresses tumor cell growth via sequential induction of G2–M arrest, apoptosis, and senescence, Cancer Res. 69 (2009) 4974–4982.
[24] L. Jia, J. Yang, X. Hao, et al., Validation of SAG/RBX2/ROC2 E3 ubiquitin ligase as an anticancer and radiosensitizing target, Clin. Cancer Res. 16 (2010) 814–824.
[25] D. Yang, L. Li, H. Liu, et al., Induction of autophagy and senescence by knockdown of ROC1 E3 ubiquitin ligase to suppress the growth of liver cancer cells, Cell Death Differ. 20 (2013) 235–247.
[26] B. Sarcar, S. Kahali, A.H. Prabhu, et al., Targeting radiation-induced G2 checkpoint activation with the Wee-1 inhibitor MK-1775 in glioblastoma cell lines, Mol. Cancer Ther. 10 (2011) 2405–2414.
[27] W. Fischle, B.S. Tseng, H.L. Dormann, et al., Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation, Nature 438 (2005) 1116–1122.
[28] H.S. Jin, L. Liao, Y. Park, et al., Neddylation pathway regulates T-cell function by targeting an adaptor protein Shc and a protein kinase Erk signaling, Proc. Natl. Acad. Sci. USA 110 (2013) 624–629.