CYLD Promotes Apoptosis of Nasopharyngeal Carcinoma Cells by Regulating NDRG1

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Purpose: Nasopharyngeal carcinoma (NPC) is among the most common malignancies derived from the epithelium of the nasopharynx. To date, the regulatory networks involved in NPC have not been fully identified. Previous studies revealed multiple loss-of-function mutations in NPC and specifically in cylindromatosis lysine 6 deubiquitinase (CYLD); however, the exact role of CYLD in NPC progression and its potential mechanism remains unclear.

Methods: We performed immunohistochemical (IHC) staining and real-time quantitative polymerase chain reaction (qPCR) to measure CYLD expression in NPC tissues, and Western blot was conducted to determine CYLD levels in NPC cell lines. Cell proliferation was detected by CCK8 assay and colony formation analysis, and apoptosis was determined by Annexin V/propidium iodide staining. Potential targets of CYLD were verified by co-immunoprecipitation and mass spectrometry. Xenograft assay was conducted to confirm the role of CYLD in vivo.

Results: We found that CYLD levels were significantly decreased in both NPC tissues and cell lines, and that CYLD overexpression inhibited NPC cell proliferation and promoted apoptosis. Additionally, we revealed that CYLD bound and upregulated N-Myc downstream regulated 1 (NDRG1), and that silencing NDRG1 abolished the tumor-suppressor effect of CYLD on NPC cells. Furthermore, CYLD suppressed tumor growth in xenograft mice models.

Conclusion: These results suggest CYLD as a tumor suppressor, potential biomarker for diagnosing NPC, and therapeutic target.

Keywords: NPC, CYLD, proliferation, apoptosis, NDRG1

Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial carcinoma derived from the epithelium of the nasopharynx.1 NPC has a unique geographical distribution, with >70% of cases found in East and Southeast Asia.2 Although NPC incidence and mortality rates gradually declined during the previous decade, ~30% of NPC patients still eventually experience disease progression and therapy failure.3,4 Previous studies reveal that genetic susceptibility increases the risk of NPC progression and therapeutic resistance; however, there is a lack of effective biomarkers capable of accurately predicting NPC progression and therapeutic outcomes. Therefore, novel NPC-specific biomarkers need to be identified to develop novel treatment strategies for patients.

Cylindromatosis lysine 6 deubiquitinase (CYLD) encodes a tumor suppressor and was initially identified as a mutated gene in familial cylindromatosis.5 CYLD
deconjugates Lys63-linked polyubiquitin chains from tar- get proteins and protects them from proteosomal degradation. Previous reports reveal that CYLD negatively regulates various signaling pathways, including nuclear factor kappaB (NF-κB), Wnt/β-catenin, c-Jun N-terminal kinase, Hippo, and Notch signaling. Additionally, studies report significant downregulation of CYLD expression in different types of cancer, such as breast cancer, glioblastoma, and hepatocellular carcinoma. In recent years, whole-exome sequencing and whole-genome sequencing have been performed in NPC patients, and especially in CYLD, identifying multiple loss-of-function mutations. However, the molecular function and detailed mechanisms of CYLD in NPC remain elusive.

In this study, we demonstrated that CYLD expression is downregulated in NPC tissues, and that CYLD overexpression inhibits NPC cell proliferation and promotes apoptosis, whereas CYLD knockdown reverses these effects in NPC cells. Moreover, examination of the molecular mechanisms of CYLD revealed its direct interaction with N-Myc downstream regulated 1 (NDRG1). These findings suggest CYLD as a novel therapeutic target in NPC.

Materials and Methods

Clinical Specimens
We used 10 paraffin-embedded NPC specimens and 10 paraffin-embedded normal nasopharyngeal epithelium specimens for immunohistochemical (IHC) analysis of protein levels. Five primary, fresh NPC samples and five non-cancerous, fresh nasopharyngeal samples were used for RNA extraction. All samples were obtained from Nanfang Hospital (Guangzhou, China) and before patients received therapy. Patients providing tissue samples for research purposes provided informed consent, and the experimental protocols were approval by the Ethics Committee of Nanfang Hospital.

Cell Culture
Epstein−Barr virus (EBV)-negative NPC cell lines (CNE2, HONE1, and 5−8F) were generously provided by Professor Musheng Zeng (Sun Yat-sen University Cancer Center, Guangzhou, China). The EBV-positive NPC cell line (HK1-EBV) and two immortalized normal human nasopharyngeal epithelial cell lines (NP460hTert-EBV and NP460hTert) were kindly provided by Professor George S. W. Tsao (University of Hong Kong). The cell lines were confirmed as negative for mycoplasma contamination (Qiagen, Hilden, Germany). All NPC cell lines were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% newborn cow serum (Gibco, Gaithersburg, MD, USA) at 37°C and 5% CO₂. NP460hTert-EBV and NP460hTert cells were cultured in defined keratinocyte serum-free medium (Invitrogen) at 37°C and 5% CO₂. All gifted cell lines were authenticated by STR profile.

IHC Staining
Paraffin sections underwent IHC staining for CYLD, as previously described. All paraffin-embedded tissue specimens were deparaffinized in xylene and rehydrated with an ethanol gradient into distilled water. After treatment with proteinase K (Solarbio, Beijing, China), the tissue sections were pre-incubated with hydrogen peroxide and blocked with bovine serum albumin, followed by incubation with anti-CYLD (1:300; Proteintech, Wuhan, China), labeling with an avidin–biotin peroxidase complex, and diaminobenzidine development (Gene Tech, Shanghai, China). Sections were then counterstained with hematoxylin.

RNA Extraction and Quantitative Polymerase Chain Reaction (qPCR)
Total RNA in tissues and cell lines was extracted using TRIzol reagent (TaKaRa, Shiga, Japan), and complementary DNA (cDNA) was synthesized using the PrimeScript RT reagent kit (TaKaRa). qPCR was performed in triplicate using SYBR Premix ExTaq (TaKaRa), with glyceraldehyde 3-phosphate dehydrogenase used as an endogenous control. Relative gene expression was evaluated as fold change using the 2^−ΔΔCT method. All experiments were performed in triplicate, and primer sequences are shown in Supplementary Table 1.

Lentiviral Infection and RNA Interference (RNAi)
Lentiviral particles carrying the CYLD vector (Ubi-MCS-3FLAG-CBh-gcGFP-ires-puromycin-CYLD) and their randomized flanking control sequence were constructed by GeneChem (Shanghai, China). Harvested virus was then used to infect NPC cells. Stable clones were selected using puromycin and confirmed using qPCR and Western blot assays. Small-interfering (si)RNA oligonucleotides targeting CYLD and NDRG1 were purchased from GenePharma (Jiangsu, China), with the sequences shown
in Supplementary Table 2, siRNA transfection was performed using Lipofectamine 3000 (Invitrogen) for 48 h according to manufacturer instructions.

Protein Extraction and Western Blot Analysis
Proteins from NPC cells were extracted by radioimmunoprecipitation assay buffer (Fdbio Science, Guangzhou, China). Proteins were fractionated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). The blots were probed with specific antibodies against human CYLD (Proteintech), NDRG1 (ZEN-BIO, Shanghai, China), or β-actin (Fdbio Science), followed by incubation with species-matched secondary antibodies (1:10,000; Fdbio Science). After developing the blots using an enhanced chemiluminescence Western blot kit (Wanleibio, Shenyang, China), they were visualized using an auto-exposure system (Tanon-5200; Tanon Science & Technology, Shanghai, China). The antibodies used for IHC and Western blot are shown in Supplementary Table 3.

Cell Viability Assay
A total of $1 \times 10^3$ NPC cells were inoculated per well in 96-well plates, and the viability was determined using a Cell Counting Kit-8 assay (CCK-8; Dojindo, Kumamoto, Japan) every 24 h for 4 consecutive days. Briefly, culture medium was replaced with 100 μL RPMI-1640 containing 10 μL CCK-8 solution, and absorbance was measured on a microplate reader (Bio-Rad) at 450 nm. Five replicates of each treatment were used, and experiments were performed in triplicate.

Colony Formation Assay
Transfected cells (200 cells/well) were plated in 6-well plates and cultured for 10 to 14 days. The colonies were subsequently washed twice with phosphate-buffered saline, fixed with 4% paraformaldehyde, and stained with 0.5% Crystal Violet. Cell colonies with diameters >1.5 mm were counted. The experiment was performed in triplicate.

Apoptosis Assay
Apoptosis of NPC cells following transfection was measured using a FACSCanto II flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and an Annexin V-APC/propidium iodide (PI) apoptosis detection kit (KGA1030; Keygen Biotech, Nanjing, China) according to manufacturer instructions.

Co-Immunoprecipitation (Co-IP) and Mass Spectrometry (MS)
CNE2 cells stably expressing Flag-CYLDD were lysed with immunoprecipitation lysis buffer (Beyotime Biotechnology, Beijing, China). Primary anti-Flag or anti-IgG (negative control) antibodies were incubated with the lysates overnight at 4°C. Protein A/G Sepharose beads (Santa Cruz Biotechnology, Dallas, TX, USA) were added to the immune complexes for recovery, followed by washing and collection. The immune complexes were then eluted with low-pH buffer and separated by SDS-PAGE, followed by silver staining. Candidate bands were subjected to MS analysis for protein identification.

In vivo Xenograft Tumor Models
The animal procedures in this study were approved by the Ethical Committee for Animal Research of Southern Medical University (Guangzhou, China) and were performed to minimize animal suffering. The 4-week-old BALB/c athymic nude mice were purchased from the Guangdong Medical Laboratory Animal Center (Guangzhou, China). CNE2 cells ($1 \times 10^7$ cells in 100 μL PBS) that stably overexpressed the vector or CYLD were injected subcutaneously into the right flank of the mouse. Tumor volume was measured using a caliper for every 2 days. Tumor volume was calculated using the formula: volume = [length *(width)^2]/2. Mice were sacrificed by cervical dislocation on 14th day, and the tumors were collected for hematoxylin-eosin (HE) and IHC staining.

Statistical Analysis
All statistical analyses were performed using SPSS software (v.19.0; IBM Corp., Armonk, NY, USA). Data are presented as the mean ± standard error of the mean (SEM) from at least three independent experiments. Differences were considered statistically significant at a $P < 0.05$ according to Student’s t-tests for two groups or one-way analysis of variance for multiple groups.

Results
CYLD is Downregulated in Clinical NPC Tissues
To determine the role of CYLD in NPC development, we analyzed CYLD protein levels in NPC and normal tissues by IHC. We found that CYLD level was significantly
decreased in NPC tissue samples relative to that in normal tissue samples (Figure 1A and B). Additionally, qPCR analysis of CYLD mRNA levels in five paired, fresh-frozen NPC tissues (T) and normal tissues (N) from patients showed significantly downregulated expression in tumor tissues relative to that in normal tissues (Figure 1C). These findings suggest that CYLD might represent an NPC biomarker according to its downregulation in NPC tissues.

**CYLD Overexpression Inhibits NPC Cell Proliferation**

To investigate the biological function of CYLD, we examined CYLD transcript and protein levels in two normal nasopharyngeal epithelial cell lines and six NPC cell lines. The results showed that both CYLD mRNA (Supplementary Figure 1A) and protein (Figure 2A) levels were significantly downregulated in the NPC cell lines. We then established either

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![Image](https://example.com/image1.png)

**Figure 1** CYLD is downregulated in clinical NPC Tissues. (A) CYLD expression in 10 primary NPC tissues and 10 normal tissues and assessed by IHC. Images are representative of IHC staining results. Scale bar: 100 mm. (B) statistical analysis of CYLD levels. Data represent the mean ± SEM (n=10). ***P < 0.001. (C) qPCR analysis of relative CYLD expression in normal and NPC tissues. Data represent the mean ± SEM (n=3). **P < 0.01, ***P < 0.001.
control CNE2 and HK1-EBV cells or those stably expressing CYLD, as well as HONE1 and 5–8F cells transfected with CYLD-siRNAs or corresponding controls for CYLD-knockdown experiments (Figure 2B). The results of CCK-8 and colony formation assays used to evaluate the effects of CYLD on cell proliferation showed that forced expression of CYLD in NPC cell lines resulted in decreased cell viability. In contrast, CYLD silencing significantly promoted NPC cell proliferation relative to that observed in control cells ($P < 0.001$) (Figure 2C). Consistently, colony formation results showed that CYLD overexpression decreased the colony formation in both CNE2 and HK1-EBV cell lines relative to that observed in control cells, whereas colony formation by HONE1 and 5–8F cells increased following CYLD knockdown (Figure 2D and E). These results indicated that CYLD overexpression exerted an antitumor effect on NPC cells by inhibiting their proliferation.

CYLD Overexpression Promotes NPC Cell Apoptosis

To determine whether CYLD regulates NPC cell apoptosis, we performed Annexin V/PI staining and flow cytometric analysis. We found that CYLD overexpression increased both early and late apoptosis in CNE2 and HK1-EBV cells (Figure 3A and B), whereas CYLD knockdown in HONE1 and 5–8F cells inhibited apoptosis relative to that observed in control cells (Figure C and D). These results indicated that CYLD increased apoptosis in NPC cells, whereas CYLD downregulation inhibited this process.

CYLD Directly Interacts with NDRG1

To elucidate the molecular mechanism underlying the tumor-suppressive effect of CYLD, we co-immunoprecipitated Flag-tagged CYLD from CNE2 cells (Figure 4A) and performed MS analysis to identify potential interacting proteins. The data identified NDRG1 among the group of CYLD-interacting proteins.

Figure 2 CYLD overexpression inhibits NPC cell proliferation. (A) CYLD protein levels measured by Western blot in two immortalized normal human nasopharyngeal epithelial cell lines and six NPC cell lines. β-actin (ACTB) was used as a loading control. (B) CNE2 and HK1-EBV cells stably overexpressed CYLD, whereas HONE1 and 5–8F cells were transfected with scrambled siRNA or CYLD-specific siRNA, and CYLD levels were detected by Western blot. (C) CCK-8 and (D, E) colony formation assays were used to evaluate cell growth and proliferation. Data represent the mean ± SEM (n = 3). ***$P < 0.001$.
proteins. To further study the CYLD–NDRG1 interaction, we performed co-IP experiments in CNE2 cells overexpressing CYLD. We found that the anti-Flag antibody, but not the anti-IgG antibody, pulled down Flag-CYLD, as well as NDRG1, from CNE2 cells. Consistently, the anti-NDRG1 antibody pulled down NDRG1, as well as CYLD, confirming an interaction between CYLD and NDRG1 in CNE2 cells (Figure 4B). We further evaluated the association between CYLD and NDRG1 in NPC cell lines. qPCR analysis confirmed that NDRG1 mRNA levels were not altered by either CYLD overexpression or knockdown in NPC cells (Supplementary Figure 1B). Interestingly, Western blot results indicated that CYLD overexpression increased NDRG1 protein levels, whereas CYLD knockdown decreased NDRG1 levels (Figure 4C). These results indicated that CYLD regulates NDRG1 at the posttranscriptional level. Given that CYLD is a deubiquitinase, we hypothesized that CYLD might inhibit NDRG1 ubiquitination and degradation via the ubiquitin–proteasome pathway.

**NDRG1 is a Functional Target of CYLD in NPC**

To determine whether CYLD-regulated NDRG1 levels are associated with the tumor-suppressive effect of CYLD, we knocked down NDRG1 in NPC cells stably overexpressing CYLD (Figure 5A). The results showed that inhibitory effects of CYLD on NPC cell proliferation were significantly abrogated by NDRG1 silencing (Figure 5B and C; Supplementary Figure 1C). Additionally, we observed that the CYLD-specific promotion of NPC cell apoptosis was reversed by NDRG1 silencing (Figure 5D and E). These findings identified NDRG1 as a functional target of CYLD in NPC cells.
Overexpression of CYLD Suppressed Tumor Growth in vivo

To further explore the function of CYLD in vivo, xenograft mice models were established. In the subcutaneous model, the tumor volume and weight were significantly decreased in CYLD-overexpressing group when compared to the control group (Figure 6A and B). Furthermore, IHC staining results showed that CYLD overexpression increased the NDRG1 level in tumor tissue samples relative to that in control tissue samples, whereas Ki-67 and PCNA protein levels decreased following CYLD overexpression (Figure 6C).

Discussion

Despite the 5-year overall survival rate of NPC exceeding 80%, the efficacy of NPC treatments plateaued during the previous decade. Genetic factors play an important role in NPC progression and provide a basis for developing novel therapeutic strategies. Here, we investigated the tumor-suppressive role of CYLD in NPC and revealed an underlying CYLD-specific mechanism according to analyses of results from NPC cell lines and clinical specimens.

IHC and qPCR analyses showed significantly reduced CYLD expression in NPC tissues, suggesting its possible role as a tumor suppressor. This was consistent with previous results identifying inactivating CYLD mutations in primary NPC tissue. In addition to loss-of-function mutations, microRNA-mediated downregulation of CYLD expression has been reported in various types of human malignancies. Future studies should focus on elucidating the underlying mechanism involved in CYLD downregulation in NPC to determine the involvement of epigenetic regulatory mechanisms.

We then determined the molecular function of CYLD in NPC cells, finding that CYLD inhibited NPC cell proliferation but promoted apoptosis according to CYLD-overexpression and -silencing analyses, respectively. A previous study reported that CYLD inhibits cell proliferation and apoptosis resistance in triple-negative breast cancer. Additionally, Sanches et al showed that CYLD suppresses cell migration and invasion in cervical cancer, and Suenaga et al reported that CYLD downregulates induction of cisplatin resistance in oral squamous cell carcinoma. Because therapeutic resistance and distant metastasis are the main causes of therapeutic failure in NPX patients, future studies should focus on determining additional CYLD-specific molecular functions in NPC.

Previous studies report that CYLD negatively regulates NF-κB signaling by removing K63- and M1-linked...
polyubiquitin chains from key signaling molecules, including NF-κB essential modulator, tumor necrosis factor (TNF)-associated factor (TRAF) 2, TRAF6, and Receptor-interacting serine/threonine-protein kinase 1, in familial cylindromatosis tumors. Additionally, Tauriello et al demonstrated that CYLD inhibits the Wnt pathway by deubiquitinating disheveled in familial cylindromatosis tumors. Moreover, CYLD reportedly promotes TNF-α-induced apoptosis and programmed necrosis in human lung cancer cells. However, the detailed molecular mechanism of CYLD in NPC largely remains undetermined. To fully reveal the molecules potentially interacting with CYLD, we performed co-IP and MS analyses, with the results revealing for the first time that CYLD interacts with NDRG1. Furthermore, we found that NDRG1 knockdown reversed CYLD-overexpression-mediated inhibition of cell proliferation and promotion of apoptosis. Further investigation is required to identify the signaling pathways involved in NDRG1-specific regulation of NPC cell proliferation and apoptosis.

NDRG1 is a member of the N-Myc downregulated-gene family and belongs to the α/β hydrolase superfamily.

Figure 5 NDRG1 silencing abolishes the tumor-suppressor effect of CYLD on NPC cells. (A) siRNA knockdown of NDRG1 in CNE2 and HK1-EBV cells stably overexpressing CYLD. CYLD and NDRG1 protein levels were assessed by Western blot. (B, C) NPC cell proliferation evaluated by CCK-8 and colony formation assays. Data represent the mean ± SEM (n = 3), ***P < 0.001. (D, E) NPC cell apoptosis evaluated by Annexin V/PI staining. Data represent the mean ± SEM (n = 3), *P < 0.05, **P < 0.01, ***P < 0.001.
Previous studies reported that NDRG1 plays different roles in different types of human malignancies, and that NDRG1 works as a tumor suppressor in colorectal, gastric, and prostate cancers, as well as NPC.32–36 While playing an oncogenic role in lung cancer and hepatocellular carcinomas.37,38 There are only two previous reports concerning NDRG1 in NPC. Hu et al.35 reported that NDRG1 suppresses cell invasion and the epithelial–mesenchymal transition in NPC, and Kanda et al.36 demonstrated that NDRG1 expression was decreased in NPC tissue. In the present study, we demonstrated for the first time a direct interaction between CYLD and NDRG1 in NPC cells along with upregulated NDRG1 protein levels but not mRNA levels. A previous study reported NDRG1 degradation via a proteasome-independent mechanism in pancreatic cancer cells.39 Given that CYLD is a lysine deubiquitinase, we hypothesized that CYLD might remove Lys63-linked polyubiquitin chains from NDRG1 and protect it from proteasomal degradation. Future studies should focus on revealing the detailed mechanisms of CYLD in regulating NDRG1.

In conclusion, we demonstrated that CYLD downregulation promoted NPC cell proliferation and apoptosis resistance. Moreover, as a tumor suppressor, CYLD upregulated NDRG1 levels to subsequently suppress NPC progression. And this suppression effect of CYLD could partially release from the NDRG1 knockdown. These findings suggest CYLD as a potential therapeutic target for the treatment of patients with NPC.

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Figure 6 CYLD suppressed tumor growth in vivo. (A) Tumor growth curves. Data represent the mean ± SEM (n = 6). **P < 0.01. (B) Tumor weight was examined after mice were sacrificed. Data represent the mean ± SEM (n = 6). ***P < 0.001. (C) HE staining (left) and IHC staining for CYLD, NDRG1, Ki-67 and PCNA in control and CYLD-overexpressing tumors. Scale bar: 30 μm.
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Disclosure

The authors report no conflicts of interest.

References

1. Chen YP, Chan ATC, Le QT, Blanchard P, Sun Y, Ma J. Nasopharyngeal carcinoma. Lancet. 2019;394(10192):64–80. doi:10.1016/S0140-6736(19)30564-0

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. doi:10.3322/caac.21492

3. Tang LL, Chen WQ, Xue WQ, et al. Global trends in incidence and mortality of nasopharyngeal carcinoma. Cancer Lett. 2016;374(1):22–30.

4. Zhou X, Zheng J, Tang Y, et al. EBV encoded miRNA BART8-3p promotes radiosensitivity in nasopharyngeal carcinoma by regulating ATM/ATR signaling pathway. Biosci Rep. 2019;39(9):BSR20190415. doi:10.1042/BSR20190415

5. Bignell GR, Warren W, Seal S, et al. Identification of the familial cylindromatosis tumour-suppressor gene. Nat Genet. 2000;25(2):160–165. doi:10.1038/76006

6. Massoumi R. Ubiquitin chain cleavage: CYLD at work. Trends Biochem Sci. 2010;35(7):392–399.

7. Komander D, Lord CJ, Scheel H, et al. The structure of the CYLD USP domain explains its specificity for Lys63-linked polyubiquitin and reveals a B box module. Mol Cell. 2008;29(4):451–464. doi:10.1016/j.molcel.2007.12.018

8. Trompouki E, Hatzivassiliou E, Tsichritzis TE, Farmer H, Ashworth A, Mosialos G. CYLD is a deubiquitinating enzyme that negatively regulates NF-kappaB activation by TNFR family members. Nature. 2003;424(6950):793–796. doi:10.1038/nature01803

9. Brummelkamp TR, Nijman SM, Dirac AM, Dernards R. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. Nature. 2003;424(6950):797–801. doi:10.1038/nature01811

10. Kovalenko A, Chable-Bessia C, Cantarella G, Isreal A, Wallach D, Gilles C. The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. Nature. 2003;424(6950):801–805.

11. Tauriello DV, Hagegebarth A, Kuper I, et al. Loss of the tumour suppressor CYLD enhances Wnt/beta-catenin signaling through K63-linked ubiquitination of Dvl. Mol Cell. 2010;37(5):607–619. doi:10.1016/j.molcel.2010.01.035

12. Reiley W, Zhang M, Sun SC. Negative regulation of JNK signaling by the tumor suppressor CYLD. J Biol Chem. 2004(279(53)):55161–55167. doi:10.1074/jbc.M411049200

13. Chen Y, Wang Z, Wang P, Li D, Zhou J, Wu S. CYLD negatively regulates hippo signaling by limiting Hippo phosphorylation in Drosophila. Biochem Biophys Res Commun. 2014;452(3):808–812. doi:10.1016/j.bbrc.2014.09.005

14. Rajan N, Elliott RJ, Smith A, et al. The cylindromatosis gene product, CYLD, interacts with MIB2 to regulate notch signalling. Oncotarget. 2014;5(23):12126–12140. doi:10.18632/oncotarget.2573

15. Hayashi M, Jono H, Shinriki S, et al. Clinical significance of CYLD downregulation in breast cancer. Breast Cancer Res Treat. 2014;143(3):447–457. doi:10.1007/s10549-013-2824-3

16. Guo J, Shinriki S, Su Y, et al. Hypoxia suppresses cylindromatosis (CYLD) expression to promote inflammation in glioblastoma: possible link to acquired resistance to anti-VEGF therapy. Oncotarget. 2014;5(15):6335–6364. doi:10.18632/oncotarget.2216

17. Kinoshita H, Okabe H, Beppu T, et al. CYLD downregulation is correlated with tumor development in patients with hepatocellular carcinoma. Mol Clin Oncol. 2013;1(2):309–314. doi:10.3989/mco.2013.08

18. You R, Liu YP, Lin DC, et al. Clonal mutations activate the NF-κB pathway to promote recurrence of nasopharyngeal carcinoma. Cancer Res. 2019;79(23):5930–5943. doi:10.1158/0008-5472.CAN-18-3845

19. Li YY, Chung GT, Liu WY, et al. Exome and genome sequencing of nasopharynx cancer identifies NF-κB pathway activating mutations. Nat Commun. 2017;8:14121. doi:10.1038/ncomms14121

20. Zheng H, Dai W, Cheung AK, et al. Whole-exome sequencing identifies multiple loss-of-function mutations of NF-κB pathway regulators in nasopharyngeal carcinoma. Proc Natl Acad Sci U S A. 2016;113(40):11283–11288. doi:10.1073/pnas.1607606113

21. Cai L, Long Y, Chong T, et al. EBV-miR-BART7-3p imposes sternness in nasopharyngeal carcinoma cells by suppressing SMAD7. Front Genet. 2019;10:939.

22. Huang J, Huang W, Liu M, et al. Enhanced expression of SETDB1 possesses prognostic value and promotes cell proliferation, migration and invasion in nasopharyngeal carcinoma. Oncol Rep. 2018;40(2):1017–1025.

23. Zhang K, Guo L. MiR-767 promoted cell proliferation in human melanoma by suppressing CYLD expression. Gene. 2018;641:272–278. doi:10.1016/j.gene.2017.10.055

24. Song H, Li D, Wu T, et al. MicroRNA-301b promotes cell proliferation and apoptosis resistance in triple-negative breast cancer by targeting CYLD. BMC Rep. 2018;51(11):602–607. doi:10.5483/BMCRep.2018.51.11.168

25. Sanches JGP, Xu Y, Yabasin IB, et al. miR-501 is upregulated in cervical cancer and promotes cell proliferation, migration and invasion by targeting CYLD. Chem Biol Interact. 2018;285:85–95.

26. Suenaga N, Kuramitsu M, Komure K, et al. Loss of tumor suppressor CYLD expression triggers cisplatin resistance in oral squamous cell carcinoma. Int J Mol Sci. 2019;20(20):5194. doi:10.3390/ijms20205194

27. Cai L, Ye Y, Jiang Q, et al. Epstein-Barr virus-encoded microRNA BART1 induces tumour metastasis by regulating PTEN-dependent pathways in nasopharyngeal carcinoma. Nature Commun. 2015;6:7353. doi:10.1038/ncomms8353

28. Cai LM, Luy XM, Luo WR, et al. EBV-miR-BART7-3p promotes the EMT and metastasis of nasopharyngeal carcinoma cells by suppressing the tumor suppressor PTEN. Oncogene. 2015;34(17):2156–2166. doi:10.1038/onc.2014.341

29. Wang L, Du F, Wang X. TNF-alpha induces two distinct caspase-8 activation pathways. Cell. 2008;133(4):693–703. doi:10.1016/j.cell.2008.03.036

30. Lin X, Chen Q, Huang C, et al. CYLD promotes TNF-α-induced cell necrosis mediated by RIP-1 in human lung cancer cells. Mediators Inflamm. 2016;2016:1542786. doi:10.1155/2016/1542786

31. Kovacevic Z, Richardson DR. The metastasis suppressor, Ndrg-1: a new ally in the fight against cancer. Carcinogenesis. 2006;27(12):2355–2366. doi:10.1093/carcin/bgl146

32. Liu J, Shao Y, He Y, et al. MORC2 promotes development of an aggressive colorectal cancer phenotype through inhibition of NDRG1. Cancer Sci. 2019;110(1):135–146.

33. Chang X, Xu X, Xue X, et al. NDRG1 controls gastric cancer migration and invasion through regulating MMP-9. Pathol Oncol Res. 2016;22(4):789–796. doi:10.1007/s12253-016-0071-8
34. Dixon KM, Lui GY, Kovacevic Z, et al. Dp44mT targets the AKT, TGF-β and ERK pathways via the metastasis suppressor NDRG1 in normal prostate epithelial cells and prostate cancer cells. Br J Cancer. 2013;108(2):409–419.
35. Hu ZY, Xie WB, Yang F, et al. NDRG1 attenuates epithelial-mesenchymal transition of nasopharyngeal cancer cells via blocking Smad2 signaling. Biochim Biophys Acta. 2015;1852(9):1876–1886. doi:10.1016/j.bbadis.2015.06.009
36. Kanda T, Miyata M, Kano M, Kondo S, Yoshizaki T, Iizasa H. Clustered microRNAs of the Epstein-Barr virus cooperatively down-regulate an epithelial cell-specific metastasis suppressor. J Virol. 2015;89(5):2684–2697.
37. Azuma K, Kawahara A, Hattori S, et al. NDRG1/Cap43/Drg-1 may predict tumor angiogenesis and poor outcome in patients with lung cancer. J Thorac Oncol. 2012;7(5):779–789.
38. Cheng J, Xie HY, Xu X, et al. NDRG1 as a biomarker for metastasis, recurrence and of poor prognosis in hepatocellular carcinoma. Cancer Lett. 2011;310(1):35–45. doi:10.1016/j.canlet.2011.06.001
39. Sahni S, Park KC, Kovacevic Z, Richardson DR. Two mechanisms involving the autophagic and proteasomal pathways process the metastasis suppressor protein, N-myc downstream regulated gene 1. Biochim Biophys Acta Mol Basis Dis. 2019;1865(6):1361–1378. doi:10.1016/j.bbadis.2019.02.008