NEDDYLATION: A MODULATOR OF CANCER PROGRESSION AND ITS IMPLICATION IN THERAPEUTICS

Abstract

The maintenance of optimal equilibrium of the intrinsic level of proteins is crucial for various cellular functions, such as the regulation of the transcription of genes and the maintenance of the cell cycle. Protein degradation serves as a prompt and irreversible mechanism that swiftly deactivates crucial regulatory proteins, in contrast to protein synthesis. Alteration in the equilibrium might initiate several facets of cancer growth and advancement. Neddylation refers to a distinct form of post-translational alterations occurring in proteins. The increased significance of neddylation modification, as demonstrated by an elevated level of enzymes responsible for catalyzing this process, not only presents a potential target for cancer therapy but also offers a valuable biomarker for identifying suitable cancer patients who would benefit from treatment with neddylation inhibitors. Our study highlights the process of protein neddylation on the preservation of cancer cell viability, making it an essential pathway for the advancement of innovative cancer treatments. Ultimately, the advancement of novel neddylation-pathway inhibitors, encompassing inhibitors targeting neddylation E2 or E3, holds the promise of augmenting the strategies aimed at inhibiting the overstimulated neddylation pathway. This could potentially address the issue of resistance to E1 inhibitors (such as MLN4924) due to mutations resulting from treatment.

Authors

Monalisa Parija
Cancer Biology Lab, Gene Function & amp; Regulation Group DBT-Institute of Life Sciences, Nalco
Square, Chandrasekharpur Bhubaneswar, Odisha, India.
Regional Centre for Biotechnology, NCR Biotech Science
Cluster3rd Milestone, Faridabad, Gurugram Expressway
Faridabad, Haryana (NCR Delhi), India.

Rakesh Padhan
Cancer Biology Lab, Gene Function & amp; Regulation Group DBT-Institute of Life Sciences, Nalco
Square, Chandrasekharpur Bhubaneswar, Odisha, India.
Regional Centre for Biotechnology, NCR Biotech Science
Cluster3rd Milestone, Faridabad, Gurugram Expressway
Faridabad, Haryana (NCR Delhi), India.

G. Kumari
Cancer Biology Lab, Gene Function & amp; Regulation Group DBT-Institute of Life Sciences, Nalco
Square, Chandrasekharpur Bhubaneswar, Odisha, India.
Regional Centre for Biotechnology, NCR Biotech Science
Cluster3rd Milestone, Faridabad, Gurugram Expressway
Faridabad, Haryana (NCR Delhi), India.

Acknowledgements

DBT-Institute of Life Sciences, Director, core funding.

Keywords: Cancer Cells, EDDylation, Neddylation Modification.

Pritish Samal
Cancer Biology Lab, Gene Function & Regulation Group DBT-Institute of Life Sciences, Nalco
Square, Chandrasekharpur Bhubaneswar, Odisha, India.
Regional Centre for Biotechnology, NCR Biotech Science
Cluster3rd Milestone, Faridabad, Gurugram Expressway
Faridabad, Haryana (NCR Delhi), India

Sanghamitra Dash
Cancer Biology Lab, Gene Function & Regulation Group DBT-Institute of Life Sciences, Nalco
Square, Chandrasekharpur Bhubaneswar, Odisha, India.
Regional Centre for Biotechnology, NCR Biotech Science
Cluster 3rd Milestone, Faridabad, Gurugram Expressway
Faridabad, Haryana (NCR Delhi), India

Surya Prakash
Cancer Biology Lab, Gene Function & Regulation Group DBT-Institute of Life Sciences, Nalco
Square, Chandrasekharpur Bhubaneswar, Odisha, India.
Regional Centre for Biotechnology, NCR Biotech Science
Cluster3rd Milestone, Faridabad, Gurugram Expressway
Faridabad, Haryana (NCR Delhi), India

Dr Sandip K Mishra*
Phd
Cancer Biology Lab, Gene Function & Regulation Group DBT-Institute of Life Sciences, Nalco
Square, Chandrasekharpur Bhubaneswar
Odisha, India.

.

I. INTRODUCTION

Cancer cells rely on signaling mechanisms that facilitate cell cycle progression and inhibit cell death, which would otherwise occur due to the accumulation of abnormal stress. The successful execution of these activities necessitates the carefully monitored degradation of particular intracellular proteins through the ubiquitin-proteasome system (UPS) [1]. Posttranslational modifications (PTMs) refer to the enzymatic and covalent modifications of proteins that occur after their biosynthesis. These modifications are becoming increasingly important in cancer research due to their involvement in various biological processes that are altered in carcinogenesis. These alterations include phosphorylation, acetylation, glycosylation, and ubiquitination, and are governed by various regulatory processes that are frequently deregulated in the context of cancer. These processes include protein assortment, degradation and recycling, gene regulation, tumorigenic pathways, and DNA damage repair. Given the significant importance and extensive dysregulation observed in cancer, there is an increasing focus on the development of therapeutic strategies that can selectively target the ubiquitin and ubiquitin-related pathways, including Nedd8, in neoplasm conditions. Nedd8 shares a similar enzymatic structural framework to ubiquitin modification. This framework studies the involvement of E1-activating, E2-conjugating, and E3-ligating enzymes. Neddylation plays a pivotal role in the stabilization of proteins, regulation of transcriptional processes, facilitation of epithelial-mesenchymal transformation, and induction of apoptosis [2]. The alteration of protein equilibrium is a key characteristic observed in various types of cancer. The breakdown of cellular proteins can be influenced by various pathways. NEDD8 is classified as a ubiquitin-like protein that undergoes conjugation with target proteins, thereby exerting a regulatory influence on their functionality. NEDDylation, a pathway has been demonstrated to exhibit hyperactivation in cancer [3].

The research work investigating the involvement of NEDDylation in hepatic cancer cell lines found that neddylation is often significantly increased in cancer cells, indicating a poor outcome for patients. A recent study conducted in 2021 examined the neddylation process in lung cancer cells. Attenuation of NEDDylation in Estrogen receptor (ER) responsive breast cancer cells enhances the susceptibility to Fulvestrant, which may be used in conjunction with endocrine therapy to treat ER-positive breast cancer patients [4]. The findings revealed that lung adenocarcinoma and squamous cell carcinomas exhibited hyperstimulation of the entire neddylation mechanism, including NEDD8 enzyme linkage and the protein NEDDylation [5]. The NEDD8 conjugation pathway presents an intriguing option for the exploration of novel drugs, considering the essential requirements for the development of pharmaceuticals. The significance of protein neddylation in several biological processes is highly important [6].

Notably, a growing body of research sheds light on the pivotal function of neddylation in the modulation of the tumor microenvironment (TME). Current investigations have brought attention to the crucial significance of the neddylation circuits in the realm of tumor biology and the process of immune cell development. The Neddylation pathway has the potency to enhance the progression of tumors by governing various cellular reactions within tumor cells, such as apoptosis and senescence. Additionally, it can also influence the functionality of stromal cells in the tumor microenvironment, such as angiogenesis and immune responses. These findings strengthen the idea that targeting this pathway could serve as an innovative and promising therapeutic approach for anti-cancer therapy. An illustrative

example can be observed in the progress of MLN4924, a compound that is presently under scrutiny in numerous phase I/II/III clinical trials owing to its robust antitumor efficacy and favorable tolerability in terms of toxicity [5].

1. **NEDDylation Mechanism:** NEDDylation is a common biochemical process that encompasses post-translational alteration wherein the activated Neuronal precursor cell-expressed progressively down-regulated protein 8 (NEDD8) conjugates itself to substrate proteins through the sequential enzymatic cascades. NEDDylation plays a pivotal role in maintaining the protein equilibrium for proper functioning of cell metabolism [7,8]. NEDD8 shares 60% homology of the amino acid sequences with ubiquitin. NEDD8 upon activation, covalently binds to the target substrate proteins via generating an iso-peptide link with the lysine residue of the target substrate and glycine residue of NEDD8 [8,9].

2. **NEDDylation Enzymatic Cascade:** The cascade is initiated with the maturation of NEDD8, involving the elimination of 5 amino acid residues from the C terminal tail and parallelly exposing the C terminal of mature NEDD8, is considered as one of the crucial steps for the activation of NEDD8 and its downstream systems. This modification being regulated and executed by specific proteins such as NEDD8-specific protease (NEDP1) or SENP8 [10,11]. NEDD8 activation encompasses a multistep mechanism which is as follows. The first reaction step takes into account the binding of NAE with the NEDD8-MgATP complex forming a high-energy acyl adenylate intermediate NEDD8-AMP releasing Pi [12]. This is followed by the reaction NEDDD8-AMP with catalytic cysteine of NAE, the operative thiol site of the enzyme to form a thioester bond between NAE and NEDD8 and release of AMP. Subsequently another round of NEDD8-AMP along with the first yield a ternary complex NEDD8-NAE-NEDD8-AMP which is able to transfer NEDD8 to one of the E2s [13,14].

   Subsequently, the NAE ternary complex is shifted to one of the two E2s specific for NEDD8, UBC12, and UBE2F via trans thiolation reaction. The specificity for the interaction among NEDD8 charged NAE and its corresponding E2s is provided by this transthiolation reaction [15]. NAE has the ability to identify its various E2s via the combinatorial effect of conformational rigidity and the elasticity of this exact hydrophobic interaction. As a consequence, the NEDDylation cascade integrates specificity with regard to cullin modification which is the major substrate of NEDD8. The 2 Es UBC12 and UBE2F have been shown to neddylate specific cullins. UBC 12 is specifically binds to RING box protein 1 (RBX1) which leading to cullins 1 to 4 NEDDylation in CRL complexes whereas UBE2F pairs with RBX2 and neddylates cullin 5 alone [16]. **Figure-1**

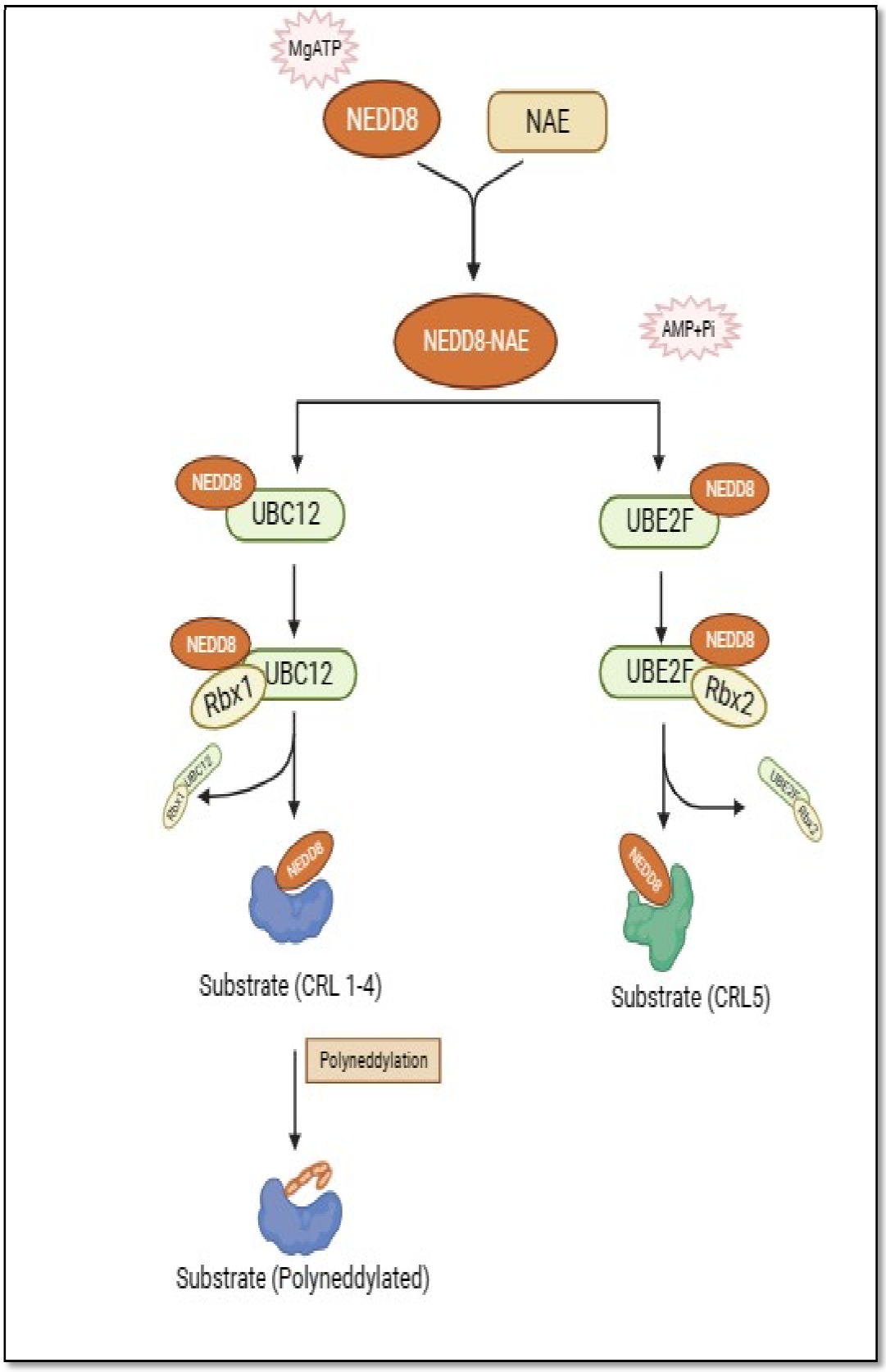

Figure 1: NEDD8 signalling cascade

Cullin scaffold is known to initiate conformational changes in the Cullin-RING E3 ligase (CRL)composite structure wherein the adherence of NEDD8 alternates the closed confirmation of the CRL to an active open state that modulates proper catalytic geometrics for a relatableE2 to transfer ubiquitin to a bound substrate molecule. This involves ubiquitination by CRLs in mediating various cellular developments such as cell cycle progression, cell growth and survival [17]. Deregulation of these NEDD8 regulated CRLs is often associated with neoplastic diseases.

II. INVOLVEMENT OF NEDDYLATION IN CANCER PROGRESSION

Recent advancements emphasize the imperative involvement of the NEDDylation in cancer research and immunology. The NEDD8 pathway follows the ubiquitination by CRLs of proteins and regulates the cellular function involved in cell growth and survival. Few substrate proteins of NEDD8-regulated CRL are Cyclin E, Emil, p27 (cell cycle regulator), NRF2, HIF1α (transcription factors), Cdt-1 (regulator of DNA replication) whose dysregulation is often associated with cancer progression [1]. This in turn influences tumor development and alters the activity of stromal cells with in the tumor microenvironment (e.g., angiogenesis and immuogenic responses) which support the fact that it could lead to promising therapeutic intervention [5]. [**Table 1]** On one side, NEDDylation is required in stromal cells for its activation which aids tumor progression whereas on the other way, it impairs the anti-cancerous action of immune cells such as dendritic cells and T cells [5]. However, one of the studies suggests that the inhibition of NEDDylation promotes migration of cancer cells via upregulation of the ZEB1-HIF1α axis [29]. Further HIF-α stabilization depends on the NEDDylation during the hypoxic and oxidative stress condition which have a significant effect on cell survival [30]. Moreover, an *in vivo* study suggests that inhibition of NEDDylation by inhibitors induces PD-L1 following MEK pathway leading to cancer immunosuppression which rationalizes the combinatory use of anti-PD-L1 antibody with MLN4924 [31]

Table 1: Effect of NEDD8 inhibition in different cancer types

Cancer types	Mechanism	References
Breast Cancer	• Nuclear localization of NEDDylated PTEN uplifts the inhibition effect on Akt signaling, thereby inducing tumor growth and development. • Moreover, NEDDylated PTEN dephosphorylates fatty acid synthase (FASN) which impedes ligation of E3 ubiquitin ligase TRIM21 for FASN, facilitating fatty acid synthesis which together suggests that PTEN NEDDylation augments breast tumorigenesis.	[18]

Squamous cell carcinoma (OSCC) (ORAL) Squamous cell carcinoma (HNSCC) (head and neck)	• An increase in the intracellular NEDD8-mediated protein NEDDylation leads to a decrease in the Akt/mTOR pathway which further helps in the formation of autophagosomes which helps in resistance to radiotherapy in OSCC cells. • Upregulation of NAE1/APP-BP1 in head and neck cell line (UPCI:SCC90), inhibits action of p53 thereby reducing apoptosis.	[19,20]
Acute Myeloid Leukaemia (AML)	• Induce apoptosis via p53 signaling • Contribute to drug resistance by upregulation of HDAC1	[21,22]
Lung cancer	• NEDDylation inhibits chemokine CXCL5 which helps the infiltration of myeloid-derived suppressor cell and modulates tumor development. • NEDDylation E2 UBE2F activates CRL5 to degrade Noxa through K11 linkage, thereby promoting the survival of lung cancer cells.	[23,24]
Colorectal cancer	• Hypersensitize CRC cells to the TOP1 inhibitors extensively used in CRC treatment • Accumulate NOXA by inhibiting its PRDX1-mediated NEDDylation leads to apoptosis	[25,26]
Hepatocellular carcinoma	• Inhibition of the NEDDylation pathway downregulates the NF-κB inhibitor α (IκBα) via E3 ligase β-TrCP, thereby suppressing proliferation and apoptosis in HCC cells.	[27]
Pancreatic Cancer	• Knockdown of mediators of NEDDylation pathway such as NAE1, Ubc12, and RBX 1 leads to the significant accumulation of Wee 1, p27, p21 resulting in induction of apoptosis and cell cycle inhibition in Pancreatic cancer.	[28]

1. **Involvement of NEDDylation in Breast Cancer:** NEDDylation stabilizes HER2 which gets resistance to degradation and enhances breast cancer development [32]. Another study suggests that the proteasomal degradation of Estrogen Related Receptor β (ERRβ)

by NEDDylation is associated with poor prognosis in breast cancer [33]. An *in vivo* report using a chick chorioallantoic membrane (CAM) xenograft model suggests that MLN4924 impedes tumor growth. *In vitro* experiments supplementarily conclude that MLN4924 inhibits the downregulation of ERRβ leading to the accumulation of cell cycle inhibitors p21 and p27. ERRβ enhances the promoter activity of E-cadherin. These ultimately attenuate the migratory capability of breast cancer cells. MLN4924 is reported to hinder cell proliferation by inhibiting miR-1303 which is associated with poor prognosis in breast cancer patients as it suppresses an inhibitor of the cell cycle i.e., $p27^{Kip1}$ [34]. Stabilization of SREBP-1 by UBC12 mediated NEDDylation highly impacts the aggressiveness of breast cancer and the xenograft model indicates that the use of MLN4924 reverses this detrimental effect [35]. XIAP and NEDP1 mediate the ligation of NEDD8 to PTEN which promotes nuclear export where nuclear methylated PTEN stabilizes the enzyme fatty acid synthase which is involved in mediating tumor growth in breast cancer [18]. MLN4924 rescues PTEN from nuclear export and constrains Akt hence suppressing tumor cell proliferation. Moreover, 2-deoxy-D-glucose (2-DG) decreases PTEN NEDDylation via downregulating the mRNA Level of UBA3 in breast cancer [36].

2. **Nedd8 As A Therapeutic Target For Cancer:** The pathway initiating from translation to degradation of protein needs to be balanced, hence influencing vital processes as in cellular pathway, transcription factors. Ubiquitin intermediated proteasomal degradation in cells is vital for upholding protein equilibrium. NEDD8, which balances the turnover of multiple proteins by degrading them is intricately involved in cancer biology. NEDD8 activating enzyme (NAE) is an indispensable protein in the NEDD8 conjugation, influencing cancer cell development and survival through CRLs activity [1]. Targeting the NAE is a leading tactic for the advancement in cancer therapies. By treating MLN4924 (pevonedistat), there is an accumulation of p21 and augmented radiation-induced G2/M arrest in breast cancer cells [37]. This finding of the role of NAE in cancer progression makes it a probable therapeutic option in multiple cancer types, resulting in the advancement into the eary clinical studies [38]. Phase 1 study reports that rifampin can be given in combination with MLN4924 in patients with advanced solid tumors [39].

However, a combination of drugs with MLN4924 synergistically suppresses tumor growth capability in breast cancer. Following are the list of drugs that show synergistic effect with MLN4924 (Table 2)

Table 2: List of a combinatorial drug acting synergistically with MLN4924 in breast cancer

Sl. No.	Combinatorial drug	Mechanism	Reference
1.	MK-2206	Inhibits AKT pathway	[40]
2.	Fulvestrant	Suppress ERα transcription through FOXO3a	[4]
3.	Cisplatin	Increased the DNA damage level	[41]

4.	2-deoxy-D-Glucose (2DG)	Induce apoptosis via activation of caspase-3	[42]

TAS4464 inhibits NAE specifically and effectively, having broad antiproliferative effects on a variety of tumor cell lines as a drug. Preclinical studies revealed that TAS4464's antiproliferative effects grew with dose and duration, reaching a stagnate after 24 hours. The anticancer effect of TAS4464 is shown to occur via nuclear factor κB (NFκB) inhibition, thus exerting influence on both the canonical and noncanonical NFκB pathways, according to various studies of myeloma models [44]. TAS4464's first-in-human phase 1 trial examined the potency of the drug against solid tumors [45].

III. CONCLUSION

The NEDDylation pathway is substantially preserved in vertebrates and relatively less retained in yeast, showing its prevalence during species evolution. Enhanced NEDD8 expression in multiple malignancies and neurological diseases has been reported in several research studies. NEDDylation contributes to the development of cancer by mediating the ubiquitination through CRLs of proteins associated with cancer cell progression and survival. According to numerous preclinical investigations, several NAE inhibitors and NAE agonists have promising curative properties. NAE inhibitors MLN4924 and TAS4464 are currently being evaluated in clinical trials to treat different tumor types, particularly haematological tumors. MLN4924 suppresses breast cancer cells’s self-renewal and differentiation properties and increases their sensitivity to tamoxifen by inhibiting the initial step of the NEDDylation chain reaction [46].

Post-translational modifications provide promising therapeutic options for inhibiting tumor growth and improving anticancer action. It may be helpful to know how these processes impact the pathways of signaling that regulate cancer development and progression in order to develop specialized medications and treatment plans compatible with the needs of particular individuals. A growing number of possible therapeutic targets for the modification of important cellular processes are emerging as a result of our growing understanding of the ubiquitin protostomes system (UPS) and its individual components. Furthermore, in the last decade, we obtained a deeper knowledge of how NEDDylation can be targeted within the UPS owing to its specific control on CRL activity, making it a candidate target for an effective cancer therapeutic approach. Taking into account NAE inhibitors, focused research on inhibiting the E2 and E3 cascade will help gain better insights into impeding the complete NEDDylation pathway that will provide a more concise and effective therapeutics strategy.

REFERENCES

[1] Soucy, T. A., Dick, L. R., Smith, P. G., Milhollen, M. A., & Brownell, J. E. (2010). The NEDD8 Conjugation Pathway and Its Relevance in Cancer Biology and Therapy. Genes & cancer, 1(7), 708–716. https://doi.org/10.1177/1947601910382898

[2] Fuentes-Antrás, J., Alcaraz-Sanabria, A. L., Morafraile, E. C., Noblejas-López, M. D. M., Galán-Moya, E. M., Baliu-Pique, M., López-Cade, I., García-Barberán, V., Pérez-Segura, P., Manzano, A., Pandiella, A., Győrffy, B., & Ocaña, A. (2021). Mapping of Genomic Vulnerabilities in the Post-Translational Ubiquitination, SUMOylation and NEDDylation Machinery in Breast Cancer. Cancers, 13(4), 833. https://doi.org/10.3390/cancers13040833

[3] Jones, T. M., Carew, J. S., Bauman, J. E., & Nawrocki, S. T. (2021). Targeting NEDDylation as a Novel Approach to Improve the Treatment of Head and Neck Cancer. Cancers, 13(13), 3250. https://doi.org/10.3390/cancers13133250

[4] Jia, X., Li, C., Li, L., Liu, X., Zhou, L., Zhang, W., Ni, S., Lu, Y., Chen, L., Jeong, L. S., Yu, J., Zhang, Y., Zhang, J., He, S., Hu, X., Sun, H., Yu, K., Liu, G., Zhao, H., Zhang, Y., … Shao, Z. M. (2019). NEDDylation Inactivation Facilitates FOXO3a Nuclear Export to Suppress Estrogen Receptor Transcription and Improve Fulvestrant Sensitivity. Clinical cancer research : an official journal of the American Association for Cancer Research, 25(12), 3658–3672. https://doi.org/10.1158/1078-0432.CCR-18-2434

[5] Zhou, L., Jiang, Y., Luo, Q. et al. NEDDylation: a novel modulator of the tumor microenvironment. Mol Cancer 18, 77 (2019). https://doi.org/10.1186/s12943-019-0979-1

[6] Bano, I., Malhi, M., Zhao, M., Giurgiulescu, L., Sajjad, H., & Kieliszek, M. (2022). A review on cullin NEDDylation and strategies to identify its inhibitors for cancer therapy. 3 Biotech, 12(4), 103. https://doi.org/10.1007/s13205-022-03162-x

[7] Zubiete-Franco, I., Fernández-Tussy, P., Barbier-Torres, L., Simon, J., Fernández-Ramos, D., Lopitz-Otsoa, F., Gutiérrez-de Juan, V., de Davalillo, S. L., Duce, A. M., Iruzubieta, P., Taibo, D., Crespo, J., Caballeria, J., Villa, E., Aurrekoetxea, I., Aspichueta, P., Varela-Rey, M., Lu, S. C., Mato, J. M., Beraza, N., … Martínez-Chantar, M. L. (2017). Deregulated NEDDylation in liver fibrosis. *Hepatology (Baltimore, Md.)*, *65*(2), 694–709. https://doi.org/10.1002/hep.28933

[8] Stuber, K., Schneider, T., Werner, J., Kovermann, M., Marx, A., & Scheffner, M. (2021). Structural and functional consequences of NEDD8 phosphorylation. *Nature communications*, *12*(1), 5939. https://doi.org/10.1038/s41467-021-26189-9

[9] Jiang, Y., Li, L., Li, Y., Liu, G., Hoffman, R. M., & Jia, L. (2021). NEDDylation Regulates Macrophages and Implications for Cancer Therapy. *Frontiers in cell and developmental biology*, *9*, 681186. https://doi.org/10.3389/fcell.2021.681186

[10] Hemelaar, J., Borodovsky, A., Kessler, B. M., Reverter, D., Cook, J., Kolli, N., Gan-Erdene, T., Wilkinson, K. D., Gill, G., Lima, C. D., Ploegh, H. L., & Ovaa, H. (2004). Specific and covalent targeting of conjugating and deconjugating enzymes of ubiquitin-like proteins. *Molecular and cellular biology*, *24*(1), 84–95. https://doi.org/10.1128/MCB.24.1.84-95.2004

[11] Mendoza, H. M., Shen, L. N., Botting, C., Lewis, A., Chen, J., Ink, B., & Hay, R. T. (2003). NEDP1, a highly conserved cysteine protease that deNEDDylates Cullins. *The Journal of biological chemistry*, *278*(28), 25637–25643. https://doi.org/10.1074/jbc.M212948200

[12] Walden, H., Podgorski, M. S., Huang, D. T., Miller, D. W., Howard, R. J., Minor, D. L., Jr, Holton, J. M., & Schulman, B. A. (2003). The structure of the APPBP1-UBA3-NEDD8-ATP complex reveals the basis for selective ubiquitin-like protein activation by an E1. *Molecular cell*, *12*(6), 1427–1437. https://doi.org/10.1016/s1097-2765(03)00452-0

[13] Souphron, J., Waddell, M. B., Paydar, A., Tokgöz-Gromley, Z., Roussel, M. F., & Schulman, B. A. (2008). Structural dissection of a gating mechanism preventing misactivation of ubiquitin by NEDD8's E1. *Biochemistry*, *47*(34), 8961–8969. https://doi.org/10.1021/bi800604c

[14] Huang, D. T., Ayrault, O., Hunt, H. W., Taherbhoy, A. M., Duda, D. M., Scott, D. C., Borg, L. A., Neale, G., Murray, P. J., Roussel, M. F., & Schulman, B. A. (2009). E2-RING expansion of the NEDD8 cascade confers specificity to cullin modification. *Molecular cell*, *33*(4), 483–495. https://doi.org/10.1016/j.molcel.2009.01.011

[15] Huang, D. T., Miller, D. W., Mathew, R., Cassell, R., Holton, J. M., Roussel, M. F., & Schulman, B. A. (2004). A unique E1-E2 interaction required for optimal conjugation of the ubiquitin-like protein NEDD8. *Nature structural & molecular biology*, *11*(10), 927–935. https://doi.org/10.1038/nsmb826

[16] Wu, D., Li, H., Liu, M., Qin, J., & Sun, Y. (2022). The Ube2m-Rbx1 NEDDylation-Cullin-RING-Ligase proteins are essential for the maintenance of Regulatory T cell fitness. *Nature communications*, *13*(1), 3021. https://doi.org/10.1038/s41467-022-30707-8

[17] Duda, D. M., Borg, L. A., Scott, D. C., Hunt, H. W., Hammel, M., & Schulman, B. A. (2008). Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. *Cell*, *134*(6), 995–1006. https://doi.org/10.1016/j.cell.2008.07.022

[18] Xie, P., Peng, Z., Chen, Y., Li, H., Du, M., Tan, Y., Zhang, X., Lu, Z., Cui, C. P., Liu, C. H., He, F., & Zhang, L. (2021). NEDDylation of PTEN regulates its nuclear import and promotes tumor development. Cell research, 31(3), 291–311. https://doi.org/10.1038/s41422-020-00443-z

[19] Yuan, T. Z., Lin, H. Y., Kuei, C. H., Lin, C. H., Lee, H. H., Lee, H. L., Lu, H. W., Su, C. Y., Chiu, H. W., & Lin, Y. F. (2023). NEDD8 promotes radioresistance via triggering autophagy formation and serves as a

novel prognostic marker in oral squamous cell carcinoma. *Cancer cell international*, *23*(1), 41. https://doi.org/10.1186/s12935-023-02883-0

[20] Guihard, S., Ramolu, L., Macabre, C., Wasylyk, B., Noël, G., Abecassis, J., & Jung, A. C. (2012). The NEDD8 conjugation pathway regulates p53 transcriptional activity and head and neck cancer cell sensitivity to ionizing radiation. *International journal of oncology*, *41*(4), 1531–1540. https://doi.org/10.3892/ijo.2012.1584

[21] Chen, Y., & Sun, L. (2022). Inhibition of NEDD8 NEDDylation induced apoptosis in acute myeloid leukemia cells via p53 signaling pathway. Bioscience reports, 42(8), BSR20220994. https://doi.org/10.1042/BSR20220994

[22] Lai, Q. Y., He, Y. Z., Peng, X. W., Zhou, X., Liang, D., & Wang, L. (2019). Histone deacetylase 1 induced by NEDDylation inhibition contributes to drug resistance in acute myelogenous leukemia. Cell communication and signaling : CCS, 17(1), 86. https://doi.org/10.1186/s12964-019-0393-8

[23] Zhou, L., Lin, X., Zhang, L., Chen, S., Chen, J., Zhou, Z., Tang, A., Ruan, J., Wang, X., & Chen, B. (2022). NEDDylation pathway promotes myeloid-derived suppressor cell infiltration via NF-κB-mCXCL5 signaling in lung cancer. International immunopharmacology, 113(Pt A), 109329. https://doi.org/10.1016/j.intimp.2022.109329

[24] Zhou, W., Xu, J., Li, H., Xu, M., Chen, Z. J., Wei, W., ... & Sun, Y. (2017). NEDDylation E2 UBE2F promotes the survival of lung cancer cells by activating CRL5 to degrade NOXA via the K11 linkage. Clinical Cancer Research, 23(4), 1104-1116. https://doi.org/10.1158/1078-0432.CCR-16-1585

[25] Sun, Y., Baechler, S. A., Zhang, X., Kumar, S., Factor, V. M., Arakawa, Y., Chau, C. H., Okamoto, K., Parikh, A., Walker, B., Su, Y. P., Chen, J., Ting, T., Huang, S. N., Beck, E., Itkin, Z., McKnight, C., Xie, C., Roper, N., Nijhawan, D., … Pommier, Y. (2023). Targeting NEDDylation sensitizes colorectal cancer to topoisomerase I inhibitors by inactivating the DCAF13-CRL4 ubiquitin ligase complex. Nature communications, 14(1), 3762. https://doi.org/10.1038/s41467-023-39374-9

[26] Xu, S., Ma, Y., Tong, Q., Yang, J., Liu, J., Wang, Y., Li, G., Zeng, J., Fang, S., Li, F., Xie, X., & Zhang, J. (2021). Correction: Cullin-5 NEDDylation-mediated NOXA degradation is enhanced by PRDX1 oligomers in colorectal cancer. Cell death & disease, 12(4), 369. https://doi.org/10.1038/s41419-021-03656-1

[27] Xiong, H., Zheng, D., Liu, Y., Ma, L., Meng, L., Yang, Z., & Yang, Z. (2022). Activation of the β-TrCP/IκBα/inflammation axis limits the sensitivity of liver cancer cells to NEDDylation inhibition. *Oncology reports*, *48*(5), 201. https://doi.org/10.3892/or.2022.8416

[28] Xu, J., Li, Z., Zhuo, Q., Ye, Z., Fan, G., Gao, H., Ji, S., Yu, X., Xu, X., Liu, W., & Xu, W. (2022). Pevonedistat Suppresses Pancreatic Cancer Growth via Inactivation of the NEDDylation Pathway. Frontiers in oncology, 12, 822039. https://doi.org/10.3389/fonc.2022.822039

[29] Park, J. B., Seo, J., Park, J. W., & Chun, Y. S. (2020). NEDDylation blockade induces HIF-1α driven cancer cell migration via upregulation of ZEB1. Scientific reports, 10(1), 18210. https://doi.org/10.1038/s41598-020-75286-0

[30] Ryu, J. H., Li, S. H., Park, H. S., Park, J. W., Lee, B., & Chun, Y. S. (2011). Hypoxia-inducible factor α subunit stabilization by NEDD8 conjugation is reactive oxygen species-dependent. The Journal of biological chemistry, 286(9), 6963–6970. https://doi.org/10.1074/jbc.M110.188706

[31] Zhang, S., You, X., Xu, T., Chen, Q., Li, H., Dou, L., Sun, Y., Xiong, X., Meredith, M. A., & Sun, Y. (2022). PD-L1 induction via the MEK-JNK-AP1 axis by a NEDDylation inhibitor promotes cancer-associated immunosuppression. Cell death & disease, 13(10), 844. https://doi.org/10.1038/s41419-022-05292-9

[32] Xia, X., Hu, T., He, X., Liu, Y., Yu, C., Kong, W., Liao, Y., Tang, D., Liu, J., & Huang, H. (2023). NEDDylation of HER2 Inhibits its Protein Degradation and promotes Breast Cancer Progression. International journal of biological sciences, 19(2), 377–392. https://doi.org/10.7150/ijbs.75852

[33] Naik, S. K., Lam, E. W., Parija, M., Prakash, S., Jiramongkol, Y., Adhya, A. K., Parida, D. K., & Mishra, S. K. (2020). NEDDylation negatively regulates ERRβ expression to promote breast cancer tumorigenesis and progression. Cell death & disease, 11(8), 703. https://doi.org/10.1038/s41419-020-02838-7

[34] Chen, Y., Du, M., Yusuying, S., Liu, W., Tan, Y., & Xie, P. (2020). Nedd8-activating enzyme inhibitor MLN4924 (Pevonedistat), inhibits miR-1303 to suppress human breast cancer cell proliferation via targeting p27Kip1. Experimental cell research, 392(2), 112038. https://doi.org/10.1016/j.yexcr.2020.112038

[35] Heo, M. J., Kang, S. H., Kim, Y. S., Lee, J. M., Yu, J., Kim, H. R., Lim, H., Kim, K. M., Jung, J., Jeong, L. S., Moon, A., & Kim, S. G. (2020). UBC12-mediated SREBP-1 NEDDylation worsens metastatic tumor prognosis. International journal of cancer, 147(9), 2550–2563. https://doi.org/10.1002/ijc.33113

[36] Du, M. G., Peng, Z. Q., Gai, W. B., Liu, F., Liu, W., Chen, Y. J., Li, H. C., Zhang, X., Liu, C. H., Zhang, L. Q., Jiang, H., & Xie, P. (2021). The Absence of PTEN in Breast Cancer Is a Driver of MLN4924 Resistance. Frontiers in cell and developmental biology, 9, 667435. https://doi.org/10.3389/fcell.2021.667435

[37] Yang, D., Tan, M., Wang, G., & Sun, Y. (2012). The p21-dependent radiosensitization of human breast cancer cells by MLN4924, an investigational inhibitor of NEDD8 activating enzyme. PloS one, 7(3), e34079. https://doi.org/10.1371/journal.pone.0034079

[38] Sarantopoulos, J., Shapiro, G. I., Cohen, R. B., Clark, J. W., Kauh, J. S., Weiss, G. J., Cleary, J. M., Mahalingam, D., Pickard, M. D., Faessel, H. M., Berger, A. J., Burke, K., Mulligan, G., Dezube, B. J., & Harvey, R. D. (2016). Phase I Study of the Investigational NEDD8-Activating Enzyme Inhibitor Pevonedistat (TAK-924/MLN4924) in Patients with Advanced Solid Tumors. Clinical cancer research : an official journal of the American Association for Cancer Research, 22(4), 847–857. https://doi.org/10.1158/1078-0432.CCR-15-1338

[39] Zhou, X., Vaishampayan, U., Mahalingam, D., Harvey, R. D., Chung, K. Y., Sedarati, F., Dong, C., Faller, D. V., Venkatakrishnan, K., & Gupta, N. (2022). Phase 1 study to evaluate the effects of rifampin on pharmacokinetics of pevonedistat, a NEDD8-activating enzyme inhibitor in patients with advanced solid tumors. Investigational new drugs, 40(5), 1042–1050. https://doi.org/10.1007/s10637-022-01286-8

[40] Chen, X., Cui, D., Bi, Y., Shu, J., Xiong, X., & Zhao, Y. (2018). AKT inhibitor MK-2206 sensitizes breast cancer cells to MLN4924, a first-in-class NEDD8-activating enzyme (NAE) inhibitor. Cell cycle (Georgetown, Tex.), 17(16), 2069–2079. https://doi.org/10.1080/15384101.2018.1515550

[41] Misra, S., Zhang, X., Wani, N. A., Sizemore, S., & Ray, A. (2020). Both BRCA1-wild type and -mutant triple-negative breast cancers show sensitivity to the NAE inhibitor MLN4924 which is enhanced upon MLN4924 and cisplatin combination treatment. Oncotarget, 11(8), 784–800. https://doi.org/10.18632/oncotarget.27485

[42] Oladghaffari, M., Islamian, J. P., Baradaran, B., Monfared, A. S., Farajollahi, A., Shanehbandi, D., & Mohammadi, M. (2015). High Efficiency Apoptosis Induction in Breast Cancer Cell Lines by MLN4924/2DG Co-Treatment. Asian Pacific journal of cancer prevention : APJCP, 16(13), 5471–5476. https://doi.org/10.7314/apjcp.2015.16.13.5471

[43] Yoshimura, C., Muraoka, H., Ochiiwa, H., Tsuji, S., Hashimoto, A., Kazuno, H., Nakagawa, F., Komiya, Y., Suzuki, S., Takenaka, T., Kumazaki, M., Fujita, N., Mizutani, T., & Ohkubo, S. (2019). TAS4464, A Highly Potent and Selective Inhibitor of NEDD8-Activating Enzyme, Suppresses NEDDylation and Shows Antitumor Activity in Diverse Cancer Models. Molecular cancer therapeutics, 18(7), 1205–1216. https://doi.org/10.1158/1535-7163.MCT-18-0644

[44] Muraoka, H., Yoshimura, C., Kawabata, R., Tsuji, S., Hashimoto, A., Ochiiwa, H., Nakagawa, F., Fujioka, Y., Matsuo, K., & Ohkubo, S. (2019). Activity of TAS4464, a novel NEDD8 activating enzyme E1 inhibitor, against multiple myeloma via inactivation of nuclear factor κB pathways. Cancer science, 110(12), 3802–3810. https://doi.org/10.1111/cas.14209

[45] Yamamoto, N., Shimizu, T., Yonemori, K., Kitano, S., Kondo, S., Iwasa, S., Koyama, T., Sudo, K., Sato, J., Tamura, K., Tomomatsu, J., Ono, M., Fukuda, N., & Takahashi, S. (2021). A first-in-human, phase 1 study of the NEDD8 activating enzyme E1 inhibitor TAS4464 in patients with advanced solid tumors. Investigational new drugs, 39(4), 1036–1046. https://doi.org/10.1007/s10637-020-01055-5

[46] Yin, Y., Xie, C. M., Li, H., Tan, M., Chen, G., Schiff, R., Xiong, X., & Sun, Y. (2019). The FBXW2-MSX2-SOX2 axis regulates stem cell property and drug resistance of cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(41), 20528–20538. https://doi.org/10.1073/pnas.1905973116

