EBioMedicine 45 (2019) 3–4

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

EBioMedicine
Published by THE LANCET

Commentary

Targeting neddylation E2 for anticancer therapy, putting new wine into new bottles?

Jianqiu Zou, Huabo Su *

Vascular Biology Center, Medical College of Georgia, Augusta University, Augusta, GA, United States

The cancer cell is capable of hijacking normal protective mechanisms to adapt to the extremely harsh intracellular and extracellular stresses it has created. Neddylation is a protein post-translational modification by which the ubiquitin-like protein NEDD8 conjugates to protein targets *via* an E1-E2-E3 multi-enzymatic cascade. As a vital survival mechanism in maintaining cell and tissue homeostasis in all eukaryotes, neddylation appears to be upregulated in multiple types of cancers, therefore targeting neddylation has become a promising anticancer strategy [1]. To this end, a small molecule, MLN4924 (Pevonedistat) has been developed to specifically inhibit NEDD8 activating enzyme (NAE) (E1), the apex of NEDD8 E1-E2-E3 cascade [2]. NAE is the only NEDD8 E1 identified and a heterodimer of the regulatory subunit NAE1 and the catalytic subunit UBA3. MLN4924 forms a tight, irreversible NEDD8-MLN4924 covalent adduct that resembles NEDD8-AMP and prevents the docking of NEDD8-AMP to the catalytic pocket in UBA3, a process that is required for NEDD8 activation, thereby effectively blocking neddylation [3]. Increasing evidence from pre-clinical studies and clinical trials have consistently demonstrated the effectiveness and potency of MLN4924 in inhibition of tumourigenesis and metastasis in diverse cancers ranging from hematological malignancies to solid tumours [4], highlighting its therapeutic potential. However, similar to other target-based chemotherapies, mutations on UBA3 that diminish the binding affinity of NEDD8-MLN4924 to UBA3 render tumour cells resistant to MLN4924 treatment and may limit its clinical application [5]. Therefore, there is an emergent need to identify additional drug targets in the neddylation pathway in order to develop the next generation of neddylation inhibitors.

In an article in *EBioMedicine*, Li and colleagues explored the possibility of targeting the E2 neddylation conjugation enzyme to suppress tumour growth [6]. In mammalian cells, two NEDD8 E2s have been identified, the well-characterized UBC12 (also known as UBE2M) and the less-studied UBE2F. These two E2s appear to show distinct function: UBC12 interacts with RBX1 to mediate neddylation of Cullin 1–4, whereas UBE2F pairs with SAG/RBX2 to facilitate neddylation of Cullin 5. While UBE2F was recently shown to promote the survival of lung cancer cells by sustaining CRL5 to degrade NOXA [7], little is known about the role of UBC12 in lung cancers.

In this study, Li and colleagues first analyzed two published Affymetrix microarray datasets which revealed that UBC12, but not NAE1 and UBA3, is overexpressed in multiple lung cancers and predicts poor survival rate. Targeted deletion of UBC12 in two lung cancer cell lines led to inhibition of Cullin neddylation, accumulation of multiple cell-cycle inhibitors that are known to be degraded by Cullin-based RING ubiquitin ligases (CRLs), and cell cycle arrest. *In vivo*, deficiency of UBC12 also greatly suppressed tumour growth and metastasis. Together, these results confirm a critical role of UBC12 in controlling neddylation and are reminiscent of those observed by others in MLN4924-treated tumour cells, thereby identifying UBC12 as an attractive alternative therapeutic target in cancer treatment.

Further, the authors addressed whether targeting UBC12 could be a potential strategy to resolve MLN4924-induced drug resistance. In an MLN4924-resistant cancer cell line, whose sensitivity to MLN4924 has dropped ~20-fold compared to its parent wild-type cell line, knockdown of UBC12 was able to effectively inhibit Cullin neddylation and thus repress cell growth, suggesting that inhibition of UBC12 could be effective to suppress the growth of tumours resistant to MLN4924. Future studies are warranted to investigate whether deletion of UBC12 has any impact on tumour growth in mice transplanted with MLN4924-resistant tumours, and to develop a pharmacological compound that specifically inhibits UBC12.

While aberrant neddylation has been repeatedly reported to associate with tumour malignancy, very little is known about upstream mechanisms regulating neddylation. UBC12 is a stress-responsive protein and is transcriptionally controlled by HIF1α and AP-1 signaling [8]. Thus, it is conceivable that the upregulation of UBC12 in lung cancer cells, as reported in this study, could be consequent to transactivation of HIF1α in the hypoxic tumour microenvironment. Nevertheless, it remains to be determined whether its protein levels are consequently increased and whether such changes are sufficient to enhance the abundance of neddylated proteins in cancer cells.

To pinpoint the underlying mechanism by which UBC12 controls tumourigenesis, Li and colleges performed quantitative proteomic analysis. The results revealed an accumulation of more than 500

DOI of original article: https://doi.org/10.1016/j.ebiom.2019.06.005.
* Corresponding author at: Vascular Biology Center, Medical College of Georgia, Augusta University, 1460 Laney Walker Blvd, Augusta, GA 30912, United States.
E-mail address: HSU@augusta.edu (H. Su).

https://doi.org/10.1016/j.ebiom.2019.07.012
2352-3964/© 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

proteins in cancer cells lacking UBC12 [6] and coincide with the loss of function of CRLs. Yet, UBC12 deficiency also caused significant down-regulation of ~500 proteins in cancer cells, indicating that neddylation is necessary for other cellular processes beyond Cullin-mediated proteolysis. To date, only a dozen of non-Cullin NEDD8 substrates have been identified, which has prevented us from fully understanding the cellular functions of neddylation in not only cancer cells, but also in other tissues. Identification of novel NEDD8 targets is challenging because of the low abundance of neddylated proteins, the transient and reversible nature of the modification, and concerns over the fidelity of targets identified from cells in which NEDD8 is overexpressed. Supported by this study, ectopic expression of UBC12 could a feasible approach to upregulate neddylated proteins, thereby facilitating the search for *bona fide* NEDD8 targets.

Besides cancers, dysregulation of neddylation is implicated in various other diseases. In particular, specific inhibition of neddylation in neurons and cardiomyocytes has been shown to cause defects in synapse formation [9] and cardiac maturation [10], respectively. An important yet unanswered question is whether normal cells/tissues, like cancer cells, could benefit from the global enhancement of neddylation under pathological conditions. Thus far, a valid tool to enhance neddylation *in vivo* is still lacking. As seen in this study and in cases of other ubiquitin-like proteins such as SUMO, overexpression of their E2 enzymes is able to stimulate the respective protein modification. Thus, this strategy could be employed to promote neddylation *in vivo*, ideally in a cell type-specific manner, and to ultimately address this question.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgement

This work was supported by a grant from the US National Institutes of Health (R01HL124248 to H.S.) and a grant from the American Heart Association (17POST33410592 to J.Z.).

References

[1] Watson IR, Irwin MS, Ohh M. NEDD8 pathways in cancer, Sine Quibus Non. Cancer Cell 2011;19(2):168–76.
[2] Soucy TA, Smith PG, Milhollen MA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature 2009;458(7239):732–6.
[3] Brownell JE, Sintchak MD, Gavin JM, 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 2010;37(1):102–11.
[4] Lockhart AC, Bauer TM, Aggarwal C, et al. Phase Ib study of pevonedistat, a NEDD8-activating enzyme inhibitor, in combination with docetaxel, carboplatin and paclitaxel, or gemcitabine, in patients with advanced solid tumors. Invest New Drugs 2019;37(1):87–97.
[5] 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(3):388–401.
[6] Li L, Kang J, Zhang W, et al. Validation of NEDD8-conjugating enzyme UBC12 as a new therapeutic target in lung cancer. EBioMedicine 2019. https://doi.org/10.1016/j.ebiom.2019.06.005.
[7] Zhou W, Xu J, Li H, et al. Neddylation E2 UBE2F promotes the survival of lung cancer cells by activating CRL5 to degrade NOXA via the K11 linkage. Clin Cancer Res 2017;23(4):1104–16.
[8] Zhou W, Xu J, Tan M, et al. UBE2M is a stress-inducible dual E2 for Neddylation and Ubiquitylation that promotes targeted degradation of UBE2F. Mol Cell 2018;70(6):1008–24 e6.
[9] Vogl AM, Brockmann MM, Giusti SA, et al. Neddylation inhibition impairs spine development, destabilizes synapses and deteriorates cognition. Nat Neurosci 2015;18(2):239–51.
[10] Zou J, Ma W, Li J, et al. Neddylation mediates ventricular chamber maturation through repression of hippo signaling. Proc Natl Acad Sci U S A 2018;115(17)(E4101-E10).