Translational Oncology 14 (2021) 101150

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Commentary

Proteomics identify nuclear export as a targetable pathway in neuroblastoma: Comment on "XPO1 inhibition with selinexor synergizes with proteasome inhibition in neuroblastoma by targeting nuclear export of IκB"

Nilay Shah

Nationwide Children's Hospital, Department of Pediatric Hematology Oncology and Bone Marrow Transplants: Nationwide Children's Hospital Hematology Oncology & Blood and Marrow Transplant, USA

ARTICLE INFO

Keywords:
Neuroblastoma
XPO1
Nuclear export
IkB
NF-kB

ABSTRACT

Neuroblastoma (NBL) is an embryonal malignancy of childhood with poor outcomes for patient with high-risk disease. Multimodal treatment approaches have improved outcomes but at the cost of significant toxicity, and there is no durable therapeutic approach for relapsed disease. As NBL has no singular oncogenic driver, targeted therapeutic options have been limited. Galinski *et al* report the results of a proteomic screen of neuroblastomas and identify the nuclear export protein XPO1 as a protein that is preferentially expressed and located in neuroblast nuclei. XPO1 overexpression is associated with nuclear export of IκB and increased NF-κB activity, both of which can be abrogated in NBL cell lines with the XPO1 inhibitor Selinexor with or without the proteasome inhibitor bortezomib. This work highlights new strategies for therapeutic target identification and the novel identification of nuclear export as a targetable oncogenic pathway across malignancies.

Neuroblastoma (NBL) is an embryonal tumor arising from sympathoadrenal precursors, most commonly in children under 5 years of age [1]. While primary tumors develop most often from the sympathetic chain or adrenal glands, clinical presentation can be diverse, from low-grade tumors that can spontaneously involute in infants to aggressive, widely metastatic disease in children over 18 months of age [1]. Despite numerous clinical advances in multimodal treatment for these patients over the last 25 years, refractory or recurrent disease remains a major problem; while neuroblastoma cases represent 8% of childhood cancers in the US, they represent 14% of childhood cancer-related deaths. Among survivors, toxic treatment approaches leave patients with high-risk disease with significant morbidities, including endocrine dysfunction, growth and developmental delays, and risk of metabolic syndrome [2]. For all these reasons, novel therapeutic approaches must still be developed to improve outcomes without worsening toxicities.

Neuroblastomas generally lack a clear oncogenic driver. While some genetic events are recurrently identified in these tumors, most commonly *MYCN* amplification [3] and mutations or amplification of *ALK* [4], these are either poor therapeutic targets (MYCN) or not sufficient to eradicate disease (ALK) [5]. As such, novel therapeutic approaches must be explored that target other biological pathways upon which the tumor is reliant for viability, while sparing normal tissues.

Researchers have taken many different approaches to identify such key biological pathways. These have included drug screens, relying on serendipity to identify an existing drug with antineoplastic efficacy or a defined phenotypic effect, from which biological effects can then be delineated. Others have used genetic perturbations, most commonly CRISPR-Cas9-mediated screens using established cancer cell lines, to identify genes whose gain or loss of expression affect viability, which can then be further validated. This methods relies on significant work effort, the expectation that the biology of the cell lines sufficiently models human disease biology *in vivo*, that the genes identified will be tractacble therapeutic targets, and that RNA expression correlates sufficiently with biological role (in contrast to protein expression or modification). These approaches certainly have benefits but also rely on models of neuroblastoma as the primary screening material.

In the work by Galinski *et al.* [6], the researchers have taken a different approach to identify relevant targets in neuroblastoma. They have evaluated the proteomes of primary neuroblastoma tumors from patients with high-risk disease, specifically comparing those with durable cures against those who died of disease with rapid progression. This very directly answers the question, "What is different about the

DOI of original article: https://doi.org/10.1016/j.tranon.2021.101114.

https://doi.org/10.1016/j.tranon.2021.101150
Received 1 June 2021; Accepted 2 June 2021

neuroblastomas from survivors versus nonsurvivors?" While there are inherently limitations to any research approach, including proteomic analyses, this method does mitigate concerns regarding the use of models of human disease or effects of gene expression versus presence of protein products. Of the differentially expressed proteins, the researchers identified XPO1 as particularly highly expressed in the tumors of those patients for whom current therapies are ineffective. While they later identify that XPO1 is expressed across neuroblastomas of different clinical risk groups, there is still evidence of consistent expression among high-risk tumors, as validated by assessment of RNA expression datasets.

XPO1, also known as Exportin-1 or CRM1, functions in the cell nucleus. It is the receptor for proteins with leucine-rich nuclear export signal domains [7]; these include ribosomal subunits, RNAs exported from the nucleus as bound by RNA-binding adapters, and hundreds of other proteins. These include a host of tumor suppressors, most notably p53 [8]. In multiple tumors, including multiple myeloma [9] and neuroblastoma [10, 11], overexpression of XPO1 is associated with cell viability through avoidance of apoptosis and chemotherapy resistance. While its role in p53 and surviving biology had been previously studied in neuroblastoma [11], XPO1 had not itself been studied as an oncogenic driver until this report.

XPO1 is attractive as a therapeutic target because of the development of Selinexor, a covalent inhibitor of XPO1 through binding in the nuclear export signal groove [12]. Galinski *et al* confirm the efficacy of Selinexor against neuroblastoma *in vitro* against a panel of neuroblastoma cell lines. They identify that this inhibition leads to increased nuclear localization of IκB proteins, inhibiting the function of NF-κB and decreasing viability. This activity is synergistically potentiated when Selinexor is used in combination with the proteasome inhibitor bortezomib. The efficacy of this treatment combination appears to have some *in vitro* dependency on the IκB proteins, an association not yet reported in other malignancies. It will be interesting to see if the XPO1-IκB axis is identified in other malignancies, and conversely which other tumor suppressor pathways are potentiated by XPO1 inhibition in neuroblastoma.

The bedrock of antineoplastic therapy against childhood cancers has been the use of combination treatment regimens for optimal efficacy. Many pediatric cancers lack singular oncogenic drivers that can be attacked directly, likely in part due to the greater role of epigenetic aberrations in oncogenesis. As such, combination chemotherapy regimens lead to the first successes against cancers in children. To this day, even with the advent of more targeted regimens, multimodal therapies are used to attack the numerous "hallmarks of cancer," extending from the traditional attacks on DNA replication and cytokinesis, to immune targeting and modulation through monoclonal antibodies, antibody-drug conjugates and cell therapies, inhibition of angiogenesis via tyrosine kinase inhibitors and antibodies, and metabolic inhibition. Inhibition of nuclear export, with or without proteasome inhibition, may yet serve as another arrow in the quiver against childhood cancers, promoting and augmenting apoptosis induced via other treatment modalities. This may be supported by the recent report by Malone *et al*, demonstrating that NXT1 and NXT2 [13], which promote RNA nuclear export through a parallel mechanism to XPO1, function oncogenically in neuroblastoma and perhaps across cancers. As these studies progress, careful organization of combination therapies may allow for reduced toxicities across the course of treatment with superior outcomes.

CRediT authorship contribution statement

Nilay Shah: Conceptualization, Writing – original draft, Visualization, Writing – review & editing.

Declaration of Competing Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] S. Mahapatra, K.B. Challagundla, Neuroblastoma, StatPearls, Treasure Island (FL), 2021.
[2] D.N. Friedman, T.O. Henderson, Late Effects and Survivorship Issues in Patients with Neuroblastoma, Children (Basel) 5 (8) (2018).
[3] G.M. Brodeur, R.C. Seeger, M. Schwab, H.E. Varmus, J.M. Bishop, Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage, Science 224 (4653) (1984) 1121–1124.
[4] I. Janoueix-Lerosey, D. Lequin, L. Brugieres, A. Ribeiro, L. de Pontual, V. Combaret, et al., Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma, Nature 455 (7215) (2008) 967–970.
[5] Y.P. Mosse, M.S. Lim, S.D. Voss, K. Wilner, K. Ruffner, J. Laliberte, et al., Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group phase 1 consortium study, Lancet Oncol. 14 (6) (2013) 472–480.
[6] B. Galinski, M. Luxemburg, Y. Landesman, B. Pawel, K.J. Johnson, S.R. Master, et al., XPO1 inhibition with selinexor synergizes with proteasome inhibition in neuroblastoma by targeting nuclear export of IkB, Transl. Oncol. 14 (8) (2021), 101114.
[7] J.G. Turner, D.M. Sullivan, CRM1-mediated nuclear export of proteins and drug resistance in cancer, Curr. Med. Chem. 15 (26) (2008) 2648–2655.
[8] L. Golomb, D.R. Bublik, S. Wilder, R. Nevo, V. Kiss, K. Grabusic, et al., Importin 7 and exportin 1 link c-Myc and p53 to regulation of ribosomal biogenesis, Mol. Cell. 45 (2) (2012) 222–232.
[9] J. Schmidt, E. Braggio, K.M. Kortuem, J.B. Egan, Y.X. Zhu, C.S. Xin, et al., Genome-wide studies in multiple myeloma identify XPO1/CRM1 as a critical target validated using the selective nuclear export inhibitor KPT-276, Leukemia 27 (12) (2013) 2357–2365.
[10] S. Vuletic, W. Dong, G. Wolfbauer, J.R. Day, J.J. Albers, PLTP is present in the nucleus, and its nuclear export is CRM1-dependent, Biochim. Biophys. Acta 1793 (3) (2009) 584–591.
[11] S. Mitra, S.V. Muralidharan, M.D. Marco, P.K. Juvvuna, S.T. Kosalai, S. Reischl, et al., Subcellular Distribution of p53 by the p53-Responsive lncRNA NBAT1 Determines Chemotherapeutic Response in Neuroblastoma, Cancer Res. 81 (6) (2021) 1457–1471.
[12] C.J. Walker, J.J. Oaks, R. Santhanam, P. Neviani, J.G. Harb, G. Ferenchak, et al., Preclinical and clinical efficacy of XPO1/CRM1 inhibition by the karyopherin inhibitor KPT-330 in Ph+ leukemias, Blood 122 (17) (2013) 3034–3044.
[13] C.F. Malone, N.V. Dharia, G. Kugener, A.B. Forman, M.V. Rothberg, M. Abdusamad, et al., Selective modulation of a pan-essential protein as a therapeutic strategy in cancer, Cancer Discov. (2021).