COMMENTARY

Targeting early EBV-driven IDO1-NAD metabolism as a potential therapeutic strategy for EBV-related diseases

Ling-Jie Luo,[2,3,5] Xu-Ying Pei,[1,5] Chun-Li Zhang,[4,5] Liang Chen,[2,3,*] and Xiao-Dong Mo[1,*]

[1]Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Beijing 100044, China

[2]School of Medicine, Shanghai University, Shanghai 200444, China

[3]State Key Laboratory of New Targets Discovery and Drug Development for Major Diseases, Xi'an 710032, Ganzhou 341000, China

[4]Department of Hematology, Beijing Hospital, National Center of Gerontology, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing 100730, China

[5]These authors contributed equally

*Correspondence: moxiaodong@pkuph.edu.cn (X.M.); lchen1@shu.edu.cn (L.C.)

Received: July 7, 2024; Accepted: August 28, 2024; Published Online: August 29, 2024; https://doi.org/10.59717/j.xinn-med.2024.100084

Citation: Luo L., Pei X., Zhang C., et al., (2024). Targeting early EBV-driven IDO1-NAD metabolism as a potential therapeutic strategy for EBV-related diseases. The Innovation Medicine **2(3)**: 100084.

Epstein-Barr virus (EBV) is a gamma-herpes virus that can establish life-long latency in over 90% of adults, and is associated with almost 2% of all human cancers worldwide. While EBV infection is usually asymptomatic in immunocompetent individuals, EBV infection can trigger a spectrum of pathologies, with a high risk for EBV-driven B-cell lymphoid proliferations and lymphomas in patients with immune deficiency and dysregulation. EBV poses a serious threat in solid organ or stem cell transplant recipients, causing potentially life-threatening post-transplant lymphoproliferative disease (PTLD). The current management of EBV-related diseases primarily relies on chemotherapy, CD20-directed monoclonal antibodies or adoptive T cell therapy.[1] However, a significant proportion of patients experience treatment failure, highlighting the need for more targeted and effective therapies.

The underlying mechanisms driving EBV-mediated B-cell transformation have been continuously investigated.[2] EBV infection in B cells triggers activation of host cell pathways, leading to increased cell proliferation and transformation. In acute EBV infection, adaptive immunity is activated, which induces EBV-specific antibodies and T cells. However, EBV infection becomes latent in the B cells and evades immune surveillance. During the latency phase, EBV episomes bind to the origin of plasmid replication (oriP) in host B cells via EBV nuclear antigen 1 (EBNA1). During B cell division, the EBV genome is replicated via the host B cell oriP and distributed to nascent B cells and into different latency phases. During the growth-promoting latency phase, EBV-

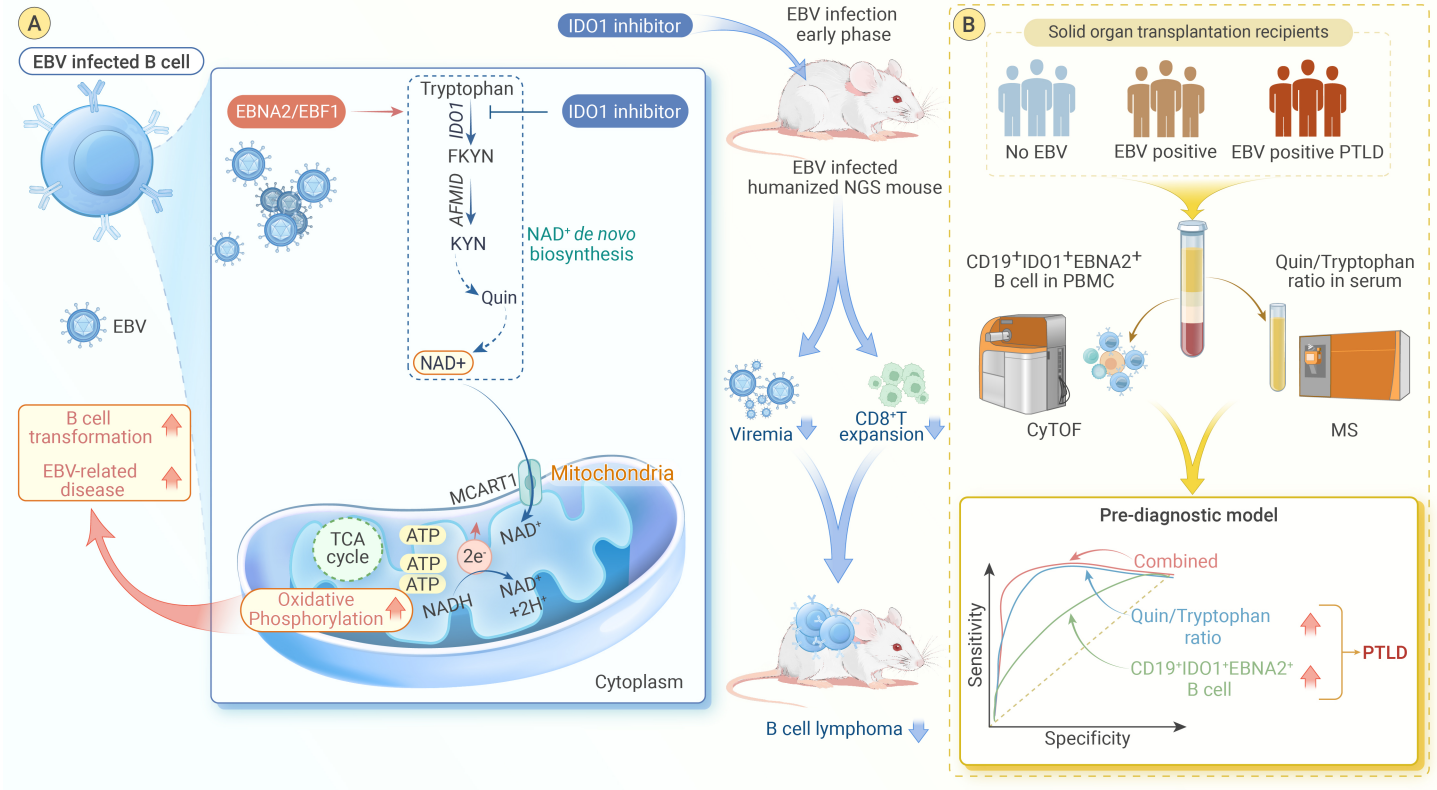

Figure 1. Early EBV-driven IDO1-NAD metabolism for B-cell transformation: a new target for EBV-related diseases (A) In vitro and humanized mouse study: The EBV latent protein EBNA2 induces the expression of IDO1 enzyme in early infected B cells, further increases NAD production and enhances ATP production that supports B-cell proliferation and transformation. Targeting IDO1 activity or NAD de novo synthesis with specific inhibitors significantly impaired EBV-infected B-cell transformation in laboratory models and humanized mice. (B) Clinical study: EBNA2+ IDO1+ B cells and a high IDO1 signature (quinolinic acid/tryptophan ratio) are potential markers for predicting the development of EBV-PTLD.

infected B cells express all latent EBV genes, in which EBNA2 induces the production of the latent membrane protein 1 (LMP1), LMP2, and other EBV-related proteins, resulting in B cell proliferation and transformation. Additionally, EBNA2 and EBNA3 help evade the immune response of EBV-infected B cells via $CD8^+$ T cells. Nonetheless, EBV-related proteins are highly immunogenic and activate both innate and adaptive immunity. In the case of

immunosuppression, such as in post-transplant and HIV-infected patients, the EBV-specific immune response is impaired. Thus, EBNA2 induced transformed B cells escape cytotoxic lymphocytes and constantly divide, which leads to lymphoproliferative diseases. During B cell receptor activation and the differentiation of EBV-infected B cells into plasma cells, the latent EBV could switch to the lytic infection form, by which the EBVs spread in the crowd or infect the other cells in the host. Neo-infected B cells enter a different latent phase, as in growth-promoting latency phase, at which the B cells sustain proliferation and provoke PTLD in post-transplant patients.

EBV-driven B cell transformation requires enormous energy and diverse biological components by multiple metabolic pathways, such as glycolysis, oxidative phosphorylation, mitochondrial one-carbon metabolism, and fatty acid metabolism. Karyn McFadden et al.[3] demonstrated that EBV infection elicits the glucose consumption with upregulated glucose transporter 1 on cellular membrane resembling the glycolysis modification in the EBV induced nasopharyngeal carcinoma. EBV infected B cells accelerate the extracellular acidification rate via lactate buildup. Emmanuela N. Bonglack et al. reported that monocarboxylate transporter (MCT) 1- and MCT4-mediated lactate transport participates in B cell oxidative phosphorylation and glycolysis rewriting via EBV infection,[4] which could partially explain the Warburg effect. During the energy-generating process, nicotinamide adenine dinucleotide (NAD+) is the pivot of glycolysis, tricarboxylic acid cycle (TCA) and oxidative phosphorylation. EBV infection may influence the pathway that generates NAD+ from the amino acid tryptophan. Enzymes, such as tryptophan-2,3-dioxygenase and indoleamine-2,3-dioxygenase (IDO), play key roles in this process. EBV may also affect pathways that recycle existing NAD+ precursors back into functional NAD+. The dependence of EBV-transformed B cells on a robust NAD+ pool makes them potential therapeutic targets for therapy.

A recent study by Hess et al.,[5] characterize early EBV-driven metabolic changes required for pre-latent transformation of B cells; and reveals a novel therapeutic avenue (Figure 1). Using a time-resolved systems-biology approach, the researchers identify a critical metabolic adaptation exploited by EBV-infected B cells in the early stages of transformation – the activation of the kynurenine pathway for de novo NAD+ synthesis. The study reveals the following three key findings: i) EBV drives IDO1 activity; The EBV latent protein EBNA2 drives the host cell's transcription machinery, specifically the early B cell factor 1 complexes (EBF1) transcription factor, to induce the expression of IDO1, a key enzyme in the kynurenine pathway. ii) IDO1 is essential for early B-cell transformation; IDO1 activity and subsequent NAD+ de novo synthesis are crucial for sustaining the early proliferation and transformation of EBV-infected B cells. Iii) Therapeutic potential: Targeting IDO1 activity or NAD+ de novo synthesis via specific inhibitors significantly impairs EBV-infected B-cell proliferation and transformation in vitro and in EBV-infected humanized mice, suggesting a promising therapeutic strategy. This study is the first to identify a key mechanism by which EBV drives IDO1 activity to promote NAD+ de novo synthesis, which is essential for early B-cell transformation. This finding opens up great prospects for the development of novel therapeutic strategies for EBV-associated PTLDs.

IDO1 is a heme enzyme that breaks down the essential amino acid tryptophan into metabolites and generates immune-regulatory kynurenines. Overexpressed in a variety of cancer types, IDO1 plays a critical role in immune regulation, including suppression of effector T and natural killer cells, activation of regulatory T cells and myeloid-derived suppressor cells, which can promote tumor immune escape and is associated with poor outcomes. As an essential enzyme of extra NAD+ production via tryptophan catabolism and of anti-viral humoral immunity, IDO1 can expand the cellular NAD+ pool for further B cell proliferation and differentiation. The IDO1-NAD+ axis fulfills antiviral humoral immunity energy demand; however, EBV specificities result in EBV-infected B cell proliferation and transformation, leading to the IDO1-NAD+ axis fueling the EBV-driven B cell transformation. Altogether, the IDO1-NAD+ axis from NAD+ de novo synthesis facilitates the EBV-transformed B cell metabolic reprogramming and the EBV-PTLD development. Given their biological importance in cancer, several IDO1 inhibitors (e.g., Epacadostat, BMS-986205, Indoximod) have advanced into clinical trials for multiple cancer types, including melanoma, breast cancer, and endometrial adenocarcinoma. Furthermore, IDO1 is involved in the development of resistance to immune checkpoint inhibitors. Consequently, combining IDO1 inhibitors with checkpoint inhibitors (e.g., PD-1/PD-L1) holds promise as a new cancer immunotherapy for patients with advanced solid tumors, potentially offering better cooperation. While current data is not definitive, these findings suggest that IDO1 inhibitors might be used to treat various cancers, including refractory EBV-PTLD.

Hess et al. further investigate the association between EBV-driven IDO1 activity and the development of EBV-PTLD in clinical patients who have received solid organ transplants. Using cytometry with time of light (CyTOF) technology, researchers identify promising markers – EBNA2+ IDO1+ B cells and blood IDO1 activity signature (quinolinic acid/tryptophan ratio), which may be useful for predicting the development of EBV-PTLD. The identification of potential markers for PTLD development holds immense promise the development of improved diagnostic tools. Early detection of PTLD will allow for prompt intervention and potentially improve patient outcomes. While this study presents a significant leap forward, further research is necessary to solidify these findings. Future studies should involve larger patient cohorts and more frequent sample collection to gain a more comprehensive understanding of changes in IDO1 activity throughout the course of EBV infection and PTLD development. Additionally, exploring the efficacy and safety of IDO1 inhibitors in clinical trials for EBV-related diseases is crucial for translating these promising preclinical findings into tangible patient benefits.

Overall, the groundbreaking study by Hess et al. makes an important contribution to our understanding of the metabolism and transformation mechanisms of EBV, B-cell metabolism, and EBV-related disease development. This study offers a new and promising approach for predicting and preventing potentially life-threatening EBV-related diseases. Additionally, the study's findings on IDO1 as a key player in EBV-mediated B-cell transformation open doors for the development of novel therapeutic strategies not only for PTLD but also for a broader range of EBV-associated malignancies.

REFERENCES

1. Lv, M., Shen, M., and Mo, X. (2023). Development of allogeneic hematopoietic stem cell transplantation in 2022: Regenerating "Groot" to heal the world. The Innovation **4**: 100373. DOI: 10.1016/j.xinn.2023.100373.
2. Choi, I.K., Wang, Z., Ke, Q., et al. (2021). Mechanism of EBV inducing anti-tumour immunity and its therapeutic use. Nature **590**: 157–162. DOI: 10.1038/s41586-020-03075-w.
3. McFadden, K., Hafez, A.Y., Kishton, R., et al. (2016). Metabolic stress is a barrier to Epstein–Barr virus-mediated B-cell immortalization. Proc. Natl. Acad. Sci. U. S. A. **113**: E782–790. DOI: 10.1073/pnas.1517141113.
4. Bonglack, E.N., Messinger, J.E., Cable, J.M., et al. (2021). Monocarboxylate transporter antagonism reveals metabolic vulnerabilities of viral-driven lymphomas. Proc. Natl. Acad. Sci. U. S. A. **118**: e2022495118. DOI: 10.1073/pnas.2022495118.
5. Müller-Durovic, B., Jäger, J., Engelmann, C., et al. (2024). A metabolic dependency of EBV can be targeted to hinder B cell transformation. Science **385**: eadk4898. DOI: 10.1126/science.adk4898.

FUNDING AND ACKNOWLEDGMENTS

This work was supported by Major Program of the National Natural Science Foundation of China (grant number 82293630), Being Nova Program (grant number 20220484076), Peking University Clinical Scientist Training Program (grant number BMU2024PYJH012), Beijing Natural Science Foundation (Z230016), and Peking University Medicine Fund for World's Leading Discipline or Discipline Cluster Development (grant number 71003Y3035). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

Luo, L.J. Pei, X.Y. Zhang C.L., Chen L. and Mo X.D. together wrote the commentary and drawn the diagram.

DECLARATION OF INTERESTS

The authors declare no competing interests.