



# New mechanistic insights of the pathogenicity of high-risk cytomegalovirus (CMV) strains derived from breast cancer: Hope for new cancer therapy options

Cecilia Söderberg-Nauclér<sup>a,b</sup>

<sup>a</sup>Department of Medicine, Unit of Microbial Pathogenesis, Karolinska Institutet, and Department of Neurology, Karolinska University Hospital, Stockholm, Sweden

<sup>b</sup>MediCity Research Laboratory, University of Turku, and Institute of Biomedicine, University of Turku, Turku, Finland

Breast cancer is the most common cancer type among women, and concerning its prevalence is steadily increasing. Some breast cancers are hereditary, but most are sporadic with an unknown cause. Accumulated evidence over the past two decades implies a very high prevalence of cytomegalovirus (CMV) in breast cancer. Detection of CMV proteins, which signifies an ongoing infection, has been found in 90%-100% of breast, colon, prostate and ovarian cancer, in sarcomas and in neural derived cancers such as glioblastoma, neuroblastoma and medulloblastoma. CMV is also found in lymph node and distant metastases, but viral proteins are not detected in healthy tissues around the tumor. The question therefore arises - does this virus play a role in tumorigenesis or as a tumor promoting virus?

CMV is a common virus infecting 60-90% of the human population and while most have an asymptomatic infection, the immune system is unable to eliminate this virus during primary infection. CMV therefore establishes a life-long latent infection, persisting mainly in myeloid lineage cells. Stress, such as inflammation or genotoxic damage stimulates CMV reactivation, and clinical reactivation and CMV disease are common among organ and stem cell transplanted patients and AIDS patients who are immunosuppressed and unable to control reactivated infection. CMV also resides in cells of the breast and reactivates in breast milk in over 90% of CMV seropositive breastfeeding mothers, which results in 30-40% CMV seropositivity among one year old children.

In a recent issue of *eBioMedicine*, Nehme and colleagues present evidence that further strengthens a role for CMV in breast cancer.<sup>1</sup> Of high importance, they were able to isolate CMV strains from primary breast tumor specimens. The tumor-derived CMV strains

exhibited a slow growing high-risk phenotype that could transform normal mammary epithelial cells (NMEC). Mechanistically, infection promoted the development of a polyploid giant cancer cells (PGCC), which exhibited enhanced expression of the oncogene MyC and downstream activation of enhancer of zeste homologue 2 (EZH2). EZH2 is a histone-lysine N methyl transferase that mediates transcriptional silencing, acting together with EED and SUZ12 in the catalytic subunit of polycomb repressive complex 2 (PRC2) to introduce repressive histone marks (H<sub>3</sub>K27me<sub>3</sub>), but can also methylate non-histone proteins such as STAT3 or RNAs. High EZH2 expression is prominent in the most aggressive breast cancer phenotype triple-negative breast cancer (TNBC),<sup>2</sup> which also has the highest PGCC content among different breast cancer phenotypes. EZH2 is overexpressed in PGCCs, and both EZH2 expression and PGCC content are associated with poor outcome in cancer patients.<sup>3</sup> High expression of EZH2 is associated with aggressive features including high proliferative capacity, induced stemness and accelerated cancer cell growth and metastatic potential. TNBC also has the highest prevalence of CMV among different breast cancer types. CMV infection can induce MyC, promote stemness, and activate pro-EZH2 pathways. EZH2 regulated STAT3 activation can silence the has-mir200 family<sup>4</sup>; this miRNA family controls translation of the CMV IE86 mRNA that is essential for CMV replication and controls cell growth.<sup>5</sup> As EZH2 is implied in cancer initiation, metastasis, drug resistance, cellular metabolism and to control immune functions, viral activation of the MyC/EZH2 axis could have several effects on virus replication and cellular fate.

The current study builds on previous work by this group, where high-risk CMV strains that caused stemness and enhanced epithelial to mesenchymal (EMT) plasticity were shown to induce transformation of NMECs.<sup>6,7</sup> Infection promoted development of aggressive triple negative breast tumors in NSG mice.<sup>6,7</sup> But perhaps this only relates to certain CMV strains, as other investigators observed that CMV infection in vitro with the TB40/E strain induced a mesenchymal to epithelial (MET) rather than an EMT phenotype.<sup>8</sup> In the

**eBioMedicine 2022;81:104103**  
Published online 13 June 2022  
<https://doi.org/10.1016/j.ebiom.2022.104103>

DOI of original article: <http://dx.doi.org/10.1016/j.ebiom.2022.104056>

E-mail addresses: [cecilia.naucler@ki.se](mailto:cecilia.naucler@ki.se), [cesona@utu.fi](mailto:cesona@utu.fi)

© 2022 The Author. 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/>)

present study TB40/E infection showed neither enhanced MyC expression nor oncogenic traits in infected fibroblasts and HMECs, and virus infection did not support soft agar growth, while the high-risk strains did. The CMV IE enhancer exhibited enhanced H3K4Me3 chromatin marks, and viral replication was enhanced with EZH2 inhibitors. Thus, certain viral strains may via high MyC expression and induced EZH2 activation exhibit slow growth properties supporting replication without killing the host cell. Such chronic infections may lead to enhanced genetic instability, accumulation of mutations and alterations in cellular metabolism, which are essential for tumor initiation. If only certain CMV strains are tumor promoting, this would explain why most carriers of CMV do not develop cancer, while most patients with certain tumors concerning are CMV positive. Such CMV strains urgently need to be characterized in further depth, as if they are tumor promoting, these could be added in cancer screening programs and carriers of such strains be eliminated as blood and organ donors.

If some CMV strains can promote tumor aggressiveness and negatively impact on prognosis, this opens for new treatment possibilities and a hope to improve patient outcome in those with CMV positive tumors. We have treated 139 glioblastoma patients with anti-CMV therapy and observed remarkably high extended survival in both primary and recurrent glioblastoma patients treated with antiviral drugs. Other colleagues observed extended survival of glioblastoma patients with CMV targeted immunotherapy approaches. Furthermore, the link between radiation therapy-induced CMV reactivation and potential rapid tumor recurrence provides further evidence of a role of CMV as a tumor promoter<sup>9,10</sup> and should be taken seriously, as it may be preventable. Indeed, Nehme and colleagues have taken an important step by isolating CMV from breast cancer specimens and identifying a viral link to MyC/EZH2 and PGCC, shedding light on an important pathway potentially explaining why only certain CMV strains may promote tumorigenesis and development of more aggressive tumors. Future studies should characterize the high-risk strains and further evaluate if tumor aggressiveness and recurrence can be reduced by

protecting patients from CMV reactivation with anti-viral or viral directed immunotherapy approaches.

## Funding

This study was financed by Swedish Medical Research Council (2017-05807).

## Declaration of interests

The author holds a patent to identify and treat a CMV strain associated with cancer.

## Acknowledgements

The author thanks Kristen Schroeder for helpful comments and editorial assistance.

## References

- 1 Nehme Z, Pasquereau S, Haidar Ahmad S, El Baba R, Herbein G. Polyplloid giant cancer cells, EZH2 and Myc upregulation in mammary epithelial cells infected with high risk human cytomegalovirus. *eBioMedicine*. 2022.
- 2 Gao B, Liu X, Li Z, Zhao L, Pan Y. Overexpression of EZH2/NSD2 histone methyltransferase axis predicts poor prognosis and accelerates tumor progression in triple-negative breast cancer. *Front Oncol*. 2020;10:600514.
- 3 Yu Y, Qi J, Xiong J, et al. Epigenetic co-deregulation of EZH2/TET1 is a senescence-countering, actionable vulnerability in triple-negative breast cancer. *Theranostics*. 2019;9(3):761-777.
- 4 Wang Y, Guo W, Li Z, et al. Role of the EZH2/miR-200 axis in STAT3-mediated OSCC invasion. *Int J Oncol*. 2018;52(4):1149-1164.
- 5 O'Connor CM, Vanicek J, Murphy EA. Host microRNA regulation of human cytomegalovirus immediate early protein translation promotes viral latency. *J Virol*. 2014;88(10):5524-5532.
- 6 Kumar A, Tripathy MK, Pasquereau S, et al. The human cytomegalovirus strain DB activates oncogenic pathways in mammary epithelial cells. *eBioMedicine*. 2018;30:167-183.
- 7 Moussawi FA, Kumar A, Pasquereau S, et al. The transcriptome of human mammary epithelial cells infected with the HCMV-DB strain displays oncogenic traits. *Sci Rep*. 2018;8(1):12574.
- 8 Oberstein A, Shenk T. Cellular responses to human cytomegalovirus infection: induction of a mesenchymal-to-epithelial transition (MET) phenotype. *Proc Natl Acad Sci USA*. 2017;114(39):E8244-E8253.
- 9 Goerig NL, Frey B, Korn K, et al. Frequent occurrence of therapeutically reversible CMV-associated encephalopathy during radiotherapy of the brain. *Neuro Oncol*. 2016;18(12):1664-1672.
- 10 Merchut-Maya JM, Bartek Jr. J, Bartkova J, et al. Human cytomegalovirus hijacks host stress response fueling replication stress and genome instability. *Cell Death Differ*. 2022.