Editorial

Epstein-Barr virus infection of mammary epithelial cells and risk of associated malignancy

Abdel-Rahman N. Zekri, Auhood Nassar, Amira Salah El-Din Youssef

Virology and Immunology Unit, Cancer Biology Department, National Cancer Institute, Cairo University, Cairo, Egypt

Correspondence to: Abdel-Rahman N. Zekri, PhD. Molecular Virology and Immunology Unit, Cancer Biology Department, National Cancer Institute, Cairo University, Kasr Al-Aini st., Fom El-Khaleeg, Cairo 11976, Egypt. Email: ncizekri@yahoo.com.

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The human tumor virus, Epstein-Barr virus (EBV), is a γ-herpesvirus associated with human epithelial and B-cell malignancies. The association of EBV infection with breast cancer has been reported in many recent studies (1). EBV can be divided into two major types, EBV type 1 and EBV type 2. Type 1 is dominant throughout most of the world, but the two types are equally prevalent in Africa (2). The EBV infection pattern is classified as either lytic, or latent 0, I, II and III (3). LMP1 is the well-documented oncoprotein of the EBV latent gene products (4). EBV strongly constitutes a risk factor for malignant transformation because it can infect mammary epithelial cells "MECs" and its DNA fragments can induce immortalization in these cells (5,6). EBV is incriminated as a carcinogenic agent by the World Health Organization (7) so; a childhood immunization against EBV might have a great impact on public health. This editorial is on a research article by Hu *et al*. (8) entitled "Epstein-Barr Virus Infection of Mammary Epithelial Cells Promotes Malignant Transformation". This editorial will focus on the evidence that delayed EBV infection causes latent infection of "MECs" leading to phenotypic changes which is a part of a multistep process of breast carcinogenesis together with other genetic and environmental factors.

EBV infection induces many transcriptional changes, the authors termed EBVness signature, that contains genes related to oncogenesis and this signature correlates with adverse clinicopathological features and most importantly was associated with a significantly lower disease free and overall survival rate. However, in this study only 33% of EBVness positive tumors were positive for EBV DNA suggesting that the presence of EBV is no longer required for tumor growth and explaining why CD21 "EBV receptor" was only expressed on primary and immortalized MECs but not on any breast cancer cell lines. Consistent with prior reports proved that breast cancer cells did not express CD21 (9). Also, the observation that EBV DNA was detected in a subset of human breast cancers reveals that inactive remnant of a previously active EBV infection have been occurred in MECs many years prior to cancer formation supporting the role of EBV in the early steps of malignant transformation and its potential mechanism for oncogenesis. However, using *T*-test to correlate EBVness with the absence or presence of APOBEC signatures was an inappropriate statistical test for categorical data.

Importantly, in this study, Hu *et al*. showed that EBV can infect primary human mammary epithelial cells but not tumor cells leading to the expansion of early MEC progenitor cells with a stem cell phenotype (10). Also, mammosphere cultures were performed to assess the stem cell activity and self-renewal of immortalized EBV infected MECs and there was an increase in the size and the number of spheres derived from EBV-infected MECs. Also, an expansion of CD24 low/CD44 high expressing progenitor cells as a marker of breast cancer cell among the EBV-infected MECs was noticed. This finding opens a new research gate for the possible role of EBV in the induction of stem cell like characteristics in breast cancer.

Also, Hu *et al*. (8) addressed that EBV infection facilitates breast tumor formation *in vivo* evidenced by

decreased the lag time and increased the efficiency as well as the frequency of breast cancers. EBV-related tumors had a high proliferative rate as determined by Ki67.

However, the authors should specify the subtype of EBV infection, as the two subtypes differ in their transforming and reactivation capabilities. As, LMP1 gene was also more strongly induced by type 1 EBNA2 than by type 2.

Interestingly, introduction of activated Ras to previously GFP-EBV infected HMECs increased the CD24 low/CD44 high progenitor cell population and the rate of mammosphere formation. Moreover, when both activated Ras and EBV were present the progenitor cell population increased fivefold while mammosphere formation rate increased up to tenfold indicating that activated Ras cooperate with EBV to increase the proliferative capacity. Surprisingly, HMECs infected with EBV showed features of epithelial mesenchymal transition "EMT" with loss of the epithelioid cobblestone pattern and increased cell mobility. Using RT-PCR, there was loss of E-cadherin expression and gain of Vimentin, N-cadherin and Fibronectin, all consistent with an EMT-like status of MECs infected with EBV.

Here, the EBV-gene expression in infected MECs and the tumors derived from these MECs showed a type II latent pattern. The authors addressed that they found high expression levels of EBNA-1, LMP1, LMP2A, LMP2B, BXLF2 and BFRF3 but they did not perform PCR of LMP1, EBER2 genes as well as immunoblotting of EBNA1 as markers of type II latent pattern.

One of the most interesting aspects of this article is the finding that EBV induces a switch from virus-induced inflammation to transformation through activation of MET signaling. It was reported by Iliopoulos *et al*. that activation of NF-κB and STAT3 signaling pathways is required for this programmatic switch (11). In this study, using a receptor tyrosine kinase array shows an increase in MET and STAT3 phosphorylation in EBV-infected MECs not in GFP-control MECs. The role of STAT3 in c-MET signaling is controversial and tissue-dependent. However, other reports found that, the direct binding of STAT3 to c-MET results in STAT3 phosphorylation, dimerization, translocation to the nucleus, transformation and invasion (12). Also, it was reported that infection of mammalian cells with EBV leads to systematic perturbations of the host cell signaling networks through the interaction of viral proteins with specific host proteins (13). The experimental data provided here show that LMP1 was abundantly expressed in all EBV infected MECs and its depletion with siRNA decreased MET phosphorylation. On the other hand, treatments with Cabozantinib, a met-specific receptor-tyrosine kinase inhibitor, strongly reduced MET phosphorylation, the mammosphere-forming ability of EBV-infected MECs and the number of CD24low/CD44high cells suggesting that MET phosphorylation is induced by EBV infection through the expression of LMP1. So, LMP1 is the key player that induces this switch in infected MECs via c-MET activation.

Finally, data presented by Hu *et al*. (8) was compelling for anyone interested in understanding the sequence of EBV infection of mammary epithelial cells and the risk of associated malignancy. They deserve to be fully vetted. However, a further cohort study with a big sample size will be needed to address the following points:

- If the EBV exists in the tumor tissue or its surrounding tissues or peripheral blood, as it has been addressed that EBV as a part of herpes family has the ability to be latent in different types of cells in the human body;
- The transforming and reactivation capabilities of EBV type 1 and EBV type 2 in mammary epithelial cells and the risk of associated malignancy;
- EBV infection of mammary epithelial cells in relation to different age, race and type of tumor;
- The possible role of EBV in the induction of stem cell like characteristics in breast cancer.

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Footnote

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References

1. Cohen JI, Fauci AS, Varmus H, et al. Epstein-Barr virus: an important vaccine target for cancer prevention. Sci Transl Med 2011;3:107fs7.
2. Odumade OA, Hogquist KA, Balfour HH Jr. Progress and problems in understanding and managing primary Epstein-Barr virus infections. Clin Microbiol Rev 2011;24:193-209.
3. Kutok JL, Wang F. Spectrum of Epstein-Barr virus-associated diseases. Annu Rev Pathol 2006;1:375-404.
4. Ersing I, Bernhardt K, Gewurz BE. NF-κB and IRF7 pathway activation by Epstein-Barr virus Latent Membrane Protein 1. Viruses 2013;5:1587-606.
5. Gao Y, Lu YJ, Xue SA, et al. Hypothesis: a novel route for immortalization of epithelial cells by Epstein-Barr virus. Oncogene 2002;21:825-35.
6. Glenn WK, Heng B, Delprado W, et al. Epstein-Barr virus, human papillomavirus and mouse mammary tumour virus as multiple viruses in breast cancer. PLoS One 2012;7:e48788.
7. Proceedings of the IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Epstein-Barr Virus and Kaposi's Sarcoma Herpesvirus/Human Herpesvirus 8. Lyon, France, 17-24 June 1997. IARC Monogr Eval Carcinog Risks Hum 1997;70:1-492.
8. Hu H, Luo ML, Desmedt C, et al. Epstein-Barr Virus Infection of Mammary Epithelial Cells Promotes Malignant Transformation. EBioMedicine 2016;9:148-60.
9. Speck P, Longnecker R. Infection of breast epithelial cells with Epstein-Barr virus via cell-to-cell contact. J Natl Cancer Inst 2000;92:1849-51.
10. Huo Q, Zhang N, Yang Q. Epstein-Barr virus infection and sporadic breast cancer risk: a meta-analysis. PLoS One 2012;7:e31656.
11. Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. Cell 2009;139:693-706.
12. Syed ZA, Yin W, Hughes K, et al. HGF/c-met/Stat3 signaling during skin tumor cell invasion: indications for a positive feedback loop. BMC Cancer 2011;11:180.
13. Rozenblatt-Rosen O, Deo RC, Padi M, et al. Interpreting cancer genomes using systematic host network perturbations by tumour virus proteins. Nature 2012;487:491-5.

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