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Characterization of Epstein-Barr virus non-coding RNAs in infected cells

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Åke Göransson, Medicinaregatan 11, den 15 Jun 2023, klockan 09.00

av **Guojiang Xie**

Fakultetsopponent:

Tanel Punga, Universitetslektor

Uppsala Universitet, Sverige

Avhandlingen baseras på följande delarbeten

I. Yarong Tian*, **Guojiang Xie***, Isak Holmqvist, Alan Bäckerholm, Sanna Abrahamsson, Jonas Carlsten, Kathy Ho Yen Shair, Ka-Wei Tang. The landscape of Epstein-Barr virus expression in human cancer. *These authors contributed equally. Submitted manuscript.

II. Alan Bäckerholm*, Yarong Tian*, Isak Holmqvist, **Guojiang Xie**, Diana Vracar, Sanna Abrahamsson, Ka-Wei Tang. Detection of latent Epstein-Barr virus gene expression in single-cell sequencing of peripheral blood mononuclear cells. *These authors contributed equally. bioRxiv2022.05.24.492331; doi: https://doi.org/10.1101/2022.05.24.492331.

III. **Guojiang Xie**, Ka-Wei Tang. The interactome of the Epstein-Barr virus non-coding RNA EBERs in infected cells. Manuscript

IV. Isak Holmqvist, Alan Bäckerholm, Yarong Tian, **Guojiang Xie**, Kaisa Thorell, and Ka-Wei Tang. FLAME: long-read bioinformatics tool for comprehensive spliceome characterization. RNA. 2021 Oct;27(10):1127-1139.

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Characterization of Epstein-Barr virus non-coding RNAs in infected cells

Guojiang Xie

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Abstract

Epstein-Barr virus (EBV) causes multiple types of lymphoid and epithelial malignancies. Unraveling the viral gene expression patterns in tumors and identifying interaction partners between EBV and host elements is crucial for understanding EBV tumorigenesis.

We characterized the EBV RNA in primary tumors by using publicly available bulk and single-cell RNA sequencing data. Our findings show that the dominant polyadenylated RNA transcript in all EBV-associated malignancies originated from the viral long non-coding RNA RPMS1. Additionally, we identified and characterized three novel RNA elements by using full-length single-molecule sequencing together with our own bioinformatics tool, FLAME. These transcripts were co-expressed at high levels with RPMS1 in tumors. Furthermore, the viral immunoevasin BNLF2 was the highest-expressed protein-coding gene in all tumor types. These findings contradict previously proposed EBV latency models.

Single-cell sequencing of primary nasopharyngeal carcinoma tissues and B cells from a splenectomized patient confirmed the EBV expression pattern observed in bulk sequencing, with a dominant RPMS1 expression. Comparative analysis between EBV-positive malignant cells and adjacent healthy epithelium in nasopharyngeal carcinoma revealed a RPMS1 microRNA-mediated downregulation of immune regulatory pathways and tumor suppressor pathways, and induction of oncogene pathways. In a similar manner, EBV-infected B cells displayed a higher propensity for cell proliferation compared with uninfected B cells.

EBV-infected blood cells from immunosuppressed patients expressed RPMS1 as well as the short non-polyadenylated non-coding RNAs EBER1 and EBER2. To investigate the role of these EBV-encoded non-coding RNAs, we utilized chromatin isolation by RNA purification, ChIRP, to identify host interaction partners. RPMS1 was mainly bound by proteins associated with the splicing machinery. Despite the fact that EBERs are neither spliced nor translated, proteins involved in the spliceosome, ribosome, and DNA repair pathway were identified to interact with EBERs. Functionally, EBERs reduced polyribosome formation and inhibited protein translation. Furthermore, EBER2 was found to interact with DNA in a sequence-dependent manner and selectively suppress the cell cycle and cell division.

Our studies provide a comprehensive landscape of EBV expression in latently infected cells, including malignancies, and unveil interactions between the highly expressed viral non-coding RNAs and host elements. The results show that RPMS1 functions as a driver of cancer hallmarks while EBERs induce a cellular state corresponding with cell quiescence observed during latent infection. The findings enhance our comprehension of EBV biology and may guide future research toward identifying drugs for treating EBV-related disease.

Keywords: EBV; EBV-associated malignancies; viral gene expression pattern; RPMS1; EBERs; single-cell sequencing; ChIRP; immune evasion; protein synthesis

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