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The transcriptomic landscape of Epstein-Barr virus associated tumors at cellular and single-molecule level

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Hörsal Arvid Carlsson, Medicinaregatan 3, den 1 september 2022, klockan 9.00.

av Yarong Tian

Fakultetsopponent: Dr.Weng-Onn Lui, Karolinska Institutet, Sverige

Avhandlingen baseras på följande delarbeten

I. **Yarong Tian***, Guojiang Xie*, Isak Holmqvist, Alan Bäckerholm, Sanna Abrahamsson, Jonas Carlsten, Ka-Wei Tang.
The landscape of Epstein-Barr virus expression in human cancer.
Manuscript

II. Holmqvist I*, Bäckerholm A*, **Tian Y**, Xie G, Thorell K, Tang KW.
FLAME: long-read bioinformatics tool for comprehensive spliceome characterization.
RNA. 2021;27(10):1127-1139.

III. Ziegler P, **Tian Y**, Bai Y, Abrahamsson S, Bäckerholm A, Reznik AS, Green A, Moore JA, Lee SE, Myerburg MM, Park HJ, Tang KW, Shair KHY.
A primary nasopharyngeal three-dimensional air-liquid interface cell culture model of the pseudostratified epithelium reveals differential donor- and cell type-specific susceptibility to Epstein-Barr virus infection.
PLoS Pathogens. 2021; 17(4): e1009041.

IV. 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.
Manuscript

* These authors contributed equally to this work.

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The transcriptomic landscape of Epstein-Barr virus associated tumors at cellular and single-molecule level

Yarong Tian

Avdelningen för infektionssjukdomar, Institutionen för Biomedicin, Sahlgrenska akademin, Göteborgs universitet, Sverige, 2022.

Abstract

Epstein-Barr virus (EBV) was the first oncovirus found in humans. Almost all adults worldwide are asymptomatic carriers of EBV. The latent EBV-infection malignifies in approximately 200,000 individuals each year. The risk of developing certain types of EBV-associated cancer is high in specific regions, for example nasopharyngeal carcinoma in Southeast Asia and Burkitt's lymphoma in Africa. The overall aim of this thesis was to characterize the EBV gene expression patterns in biopsies and elucidate the function of the expressed viral genes.

Bulk transcriptome datasets of 615 tumors from four types of known EBV-associated neoplasms and single-cell transcriptome data from 63 nasopharyngeal samples were screened for EBV expression. The most abundant EBV RNA found at both tissue and single-cell levels, were RPMS1 and the novel co-terminating transcripts which we named BAREs. LMP1/BNLF2a/b and LMP2A/B/BNRF1 were expressed to a lesser extent and large differences were observed between individuals. Single-cell sequencing of B-lymphocytes isolated from the peripheral blood of a patient with a high EBV DNA load showed a similar EBV expression profile as the EBV-positive tumors. Moreover, the highly expressed EBV genes RPMS1 and BAREs were subjected to full-length single-molecule sequencing and all isoforms were characterized using our newly developed bioinformatics tool FLAME.

Our results show that available EBV cell models inadequately portray primary tumors with regard to the viral gene expression and/or the propensity for reactivation. We developed an *in vitro* nasopharyngeal pseudostratified epithelium model which could mimic an EBV infection in the nasopharynx. A donor-dependent susceptibility for EBV infection was observed and both latent and lytic EBV expression patterns were detected in cells from a single donor. Single-cell sequencing data analysis could further distinguish that cells in late lytic stage with virus host shutoff were found amongst the suprabasal cells.

The single-cell data from peripheral EBV-transformed B-lymphocytes identified that EBV induces proliferative pathways. In nasopharyngeal carcinoma tissue the EBV-transformed epithelial cells exists in a microenvironment with lymphocytic infiltration and interferon. Single-cell characterization of the nasopharyngeal cancer cells identified that the EBV expression of RPMS1 along with the miR-BARTs encoded in the introns promotes immune evasion by down-regulation of interferon responsive genes. The findings suggest that EBV contributes to tumorigenesis in two ways, the first is by host cell reprogramming and induction of proliferation by EBNAs and LMP1, and the second is by immune evasion and escape by RPMS1 and BNLF2a.

Keywords: Epstein-Barr virus, carcinogenesis, RPMS1, miRNA, single-cell sequencing, immune evasion

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