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The Epstein Barr Virus miRNA landscape in Hodgkin lymphoma.

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Epstein-Barr virus (EBV) is a key player in the oncogenesis of a subset of classic Hodgkin lymphoma (cHL) cases. The percentage of EBV-associated cases varies by age and ethnicity and is high in patients older than 45 years. Overall infection rates range from 30-40% in Western countries. In EBV-positive cases, infection of B cells with EBV is an early step in pathogenesis, based on the presence of clonal EBV genomes in cHL tumor cells. While the involvement of EBV-derived protein-coding genes is well established, the potential contribution of EBV-derived microRNAs (miRNAs) to the pathogenesis of cHL is still largely unknown. This study aims to characterize the landscape of EBV miRNAs in cHL samples and investigate their potential role in the pathogenesis of cHL.

We established the miRNA expression profiles of 23 EBV-positive cHL (Latency II) samples using small RNA sequencing. Additionally, we included tissue samples with different EBV latency infection types: post-transplant lymphoproliferative disorders (PTLD, Latency III, n=5), Burkitt Lymphoma (BL, Latency I, n=5), and tonsils with primary EBV infection (Lytic, n=3). Preliminary data analysis comparing the EBV-miRNA profile of cHL to those of the EBV-positive control tissues indicated highly similar EBV-miRNA profiles. Specific EBV-miRNAs, including BART2, BART6, BART10, BART11, and BHRF1.1, were selected for functional studies. Expression of these miRNAs was confirmed by RT-qPCR, showing variable expression levels in cHL tissue samples. BART2-5p was the highest expressed miRNA in the full sample set, followed by BHRF1-1.

Next, we generated lentiviral overexpression constructs and confirmed proper overexpression levels in cHL by RT-qPCR. GFP competition assays revealed a growth inhibitory effect for BART11-3p in EBV-negative cHL cell line L1236, while no effect on growth was observed for BART2-5p, BART6-3p, BART10-3p, and BHRF1-1. Experiments to knock down specific EBV miRNAs using CRISPR/Cas9 in EBV-positive cHL cell line L591 are ongoing.

We provide a comprehensive overview of EBV miRNA expression patterns in EBV-positive cHL. The profile is highly similar to those observed in the EBV-positive control tissue samples, indicating possibly limited differences related to the type of latency infection. Overexpression of EBV miRNAs showed an effect in the L1236 cell line for BART11-3p. Knockdown experiments in EBV-positive cHL are ongoing.