Borneo Journal of Resource Science and Technology (2024), 14(1):30-53
DOI: https:doi.org/10.33736/bjrst.6077.2024

Computational Analysis of Epstein-Barr Virus *Bam*HI A Rightward Transcript (BART) MicroRNAs (miRNAs) Regulation on Messenger RNAs and Long Non-Coding RNAs in Nasopharyngeal Cancer

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Received: 13 September 2023 Accepted: 15 April 2024 Published: 30 June 2024

ABSTRACT

To date, the regulatory framework mediated by Epstein-Barr virus (EBV) *Bam*HI A rightward transcript (BART) microRNAs (miRNAs) via their interaction with long non-coding RNAs (lncRNAs) in the context of nasopharyngeal cancer (NPC) pathogenesis remains partially understood. To derive a more complete insight into this phenomenon, we embarked on a computational study to identify BART miRNAs, mRNAs, lncRNAs, and all associated factors relevant to NPC tumourigenesis and to characterise their interactions. *In silico* integration of multi-level RNA expression and construction of regulatory networks were performed. We found six EBV BART miRNAs (ebv-miR-BART21-3p, ebv-miR-BART19-3p, ebv-miR-BART15, ebv-miR-BART2-5p, ebv-miR-BART20-3p and ebv-miR-BART11-5p) that could interact with four mRNAs (EYA4, EYA1, EBF1 and MACROD2) associated with NPC pathogenesis. These mRNAs can interact with six non-EBV miRNAs (hsa-miR-1246, hsa-miR-93-5p, hsa-miR-16-5p, hsa-miR-135b-5p, hsa-miR-211-5p and hsa-miR-1305), which in turn, could interact with three lncRNAs (CASC2, TPTE2P1 and ARHGEF26-AS1). These findings could shed light on the roles of dysregulated competing endogenous RNA (ceRNA) network in NPC oncogenesis. In addition, we have also predicted the oncogenic and tumour suppressive functions of BART miRNAs and lncRNAs, and more precisely, the involvement of BART miRNAs in DNA repair regulation and apoptosis.

Keywords: BART miRNAs, bioinformatics, EBV, lncRNA, nasopharyngeal cancer

INTRODUCTION

Nasopharyngeal cancer (NPC) is a rare head and neck cancer on a global scale, yet it disproportionately affects populations in southern China and Southeast Asia, including Malaysia (Chang & Adami, 2006). Originating from the fossa of Rosenmüller and lining the nasopharynx epithelial (Tabuchi *et al.,* 2011), NPC is ranked as the sixth most common cancer in Malaysia, where Malaysian males are primarily affected by it, ranking fifth most common cancer affecting males in Malaysia (Global Cancer Observatory, 2022). The correlation of NPC with Epstein-Barr virus (EBV) infection has been well-documented, leading to aberrant RNA regulation within the host (Nakanishi *et al.,* 2017; Tsao *et al.,* 2017). This association is particularly prominent in type II (differentiated non-keratinizing carcinoma) and type III (undifferentiated non-keratinizing carcinoma) NPC (Su *et al.,* 2023), prevalent among Asian populations (Wang *et al.,* 2013).

Epstein-Barr virus (EBV) is a linear, double-stranded DNA virus classified in the family of *Herpesviridae*, a subfamily of *Gammaherpesviridae* (Sarwari *et al.,* 2016). The EBV genome is capable of expressing miRNAs that act similarly to mammalian miRNAs by binding to the 3'UTR of host mRNAs, albeit via imperfect complementary binding mediated at the 5' seed region of EBV miRNAs (Skalsky & Cullen, 2010). The miRNAs clusters encoded by EBV are the *Bam*HI A rightward transcript (BARTs) and *Bam*HI H rightward open reading frame 1 (BHRF1) clusters (Cai *et al.*, 2006).

In terms of disease association, EBV infection has been correlated with NPC pathogenesis (Nakanishi *et al.,* 2017; Tsao *et al.,* 2017). The post-infection period involving latency pattern type II is characterised by the presence of the BART cluster and the absence of the BHRF1 cluster, where the BART miRNAs function as oncogenes via down-regulating the apoptotic host mRNAs by binding to their

3'UTR (Kang *et al.,* 2015). Incidentally, lncRNA levels are also affected during EBV infection (Zhang *et al.,* 2020), with a significant negative correlation between lncRNAs and BART miRNAs levels as observed in the down-regulation of LOC553103 following the up-regulation of EBV-miR-BART6-3p (He *et al.,* 2016). Thus far, the intricacies underlying the regulatory network concerning BART miRNAs and lncRNAs in NPC pathogenesis have not been fully and definitively established, although it is apparent that the dysregulation of lncRNAs (He *et al.,* 2017) and the competing endogenous RNAs (ceRNA) network (Xu *et al.,* 2020) may play a role in the disease.

The exploration and understanding of regulatory mechanisms between BART miRNAs and the ceRNA network could unveil potential biomarkers on a preliminary level for early diagnosis, improve survival rates, and develop therapeutic targets for highly metastatic EBV-positive NPC. The deep-seated location of the nasopharynx, coupled with the absence of noticeable clinical symptoms in the early stage, contributes to the high mortality in NPC owing to its invasive and metastatic nature (Tabuchi *et al.,* 2011; Wang *et al.,* 2017; Dwijayanti *et al.,* 2020; Tan *et al.,* 2022).The ceRNA regulatory network involves the binding of miRNAs to both mRNAs and lncRNAs and is based on the hypothesis that lncRNA and mRNAs regulate each other by competing for binding sites on shared miRNAs through partially complementary sequences known as the miRNA recognition elements (MREs) (Salmena *et al.,* 2011). Anomalies in this regulatory network are commonly linked to tumorigenesis (Lee & Young, 2013).

To unravel the interaction among EBV-BART miRNAs, mRNAs, and lncRNAs in NPC pathogenesis, we performed a computational analysis involving constructing and analysing a regulatory network that combines the ceRNA and EBV-miRNAs-mRNAs networks. The outcomes were complemented by the curative and integrative analysis of the expression data of lncRNA, miRNA and mRNA extracted from the Gene Expression Omnibus (GEO) database. Our findings reveal novel conceptual insights into the regulation of lncRNAs by EBV-BART miRNAs with the uncovering of potential biomarkers for therapeutic design and the plausible effects on the modulation of DNA repair and apoptosis in the context of NPC pathogenesis.

MATERIALS AND METHODS

Retrieval of Microarray Data Pertaining to MicroRNAs (miRNAs), Long Non-Coding RNAs (lncRNAs) and Messenger RNAs (mRNAs)

The microarray gene expression profiles of messenger RNAs (mRNAs), microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) from nasopharyngeal cancer (NPC) were retrieved from the Gene Expression Omnibus (GEO) database hosted on the National Centre for Biotechnology Information (NCBI) website (Karagkouni *et al.,* 2020). Specifically, the access year of the datasets was between 2020 and 2023. The keyword "nasopharyngeal carcinoma" was used, and the organism filter was set to *Homo sapiens*. Excluded datasets were from those that are: (i) of cell line samples, (ii) without normal nasopharyngeal controls, and (iii) those submitted before 2014. This filtering is to ensure that the expression datasets follow updated microarray guidelines and protocols. Subsequently, all the datasets were checked for quantile normalization, and quantile normalization was done using an R software called Limma R package in R studio (version 4.0.1).

Identification of Differentially Expressed mRNA (DEmRNA), Differentially Expressed lncRNA (DElncRNA), and Differentially Expressed miRNA (DEmiRNA)

The DEmRNAs, DEmiRNAs, and DElncRNAs were identified using GEO2R (Chen *et al.,* 2015). The cut-off criteria were p-value < 0.05 (Ye *et al.,* 2015) and $|\log2 \text{ fold-change}| \geq 1$ (Liu *et al.,* 2019) to ensure statistically significant. Subsequently, the overlaps of differentially expressed RNAs between two or more datasets were identified and visualised using Venn diagrams via the Venny Tool resource.

Identification of DEmiRNA-Target mRNA Interaction

Gene targets of DEmiRNAs were identified following inverse expression correlation using computationally-predicted functions in miRwalk 2.0 and an experimentally validated database of miRTarBase (Zhu *et al.,* 2020). For miRWalk 2.0,