Epstein Barr Virus Encoded BHRF1 miRNA Targets Host Transcription Factor ATF-1 and ATF-2

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Abstract

Epstein Barr-Virus (EBV) miRNAs upregulated during latency may have a role in maintenance of the EBV latency stage, thus contributing to tumorigenesis. Preliminary data from both PITA and miRanda software have found binding sites of BHRF1-1 miRNA to ATF-1 and ATF-2, essential transactivators of EBV lytic reactivation.

Introduction

Epstein Barr virus (EBV) is one of various causative infectious agents associated with about 20% of human cancers (1). A member of the y-herpesvirus family, EBV is associated with approximately 1% of tumors worldwide (2). All EBV tumors are known to express ncRNAs, including 44 different viral microRNAs (miRNAs) (3). Such miRNAs are known to suppress apoptotic responses of the infected cell in order to establish a latent infection. Involved with mRNA target cleavage, EBV miRNAs are predicted to facilitate infection and disease. The targets of EBV miRNAs are unknown. There are two regions within the EBV genome that encode mature miRNAs: BHRF1 and BART1. BHRF1 miRNAs are 22 nucleotides in length and its seed region is 2-8 nucleotides from the 5' end of miRNA. EBV microRNAs miR-BHRF1-1,-2, and -3 are encoded within EBNA transcripts in latency III (4). They are transcribed from an alternative promoter BHRF1p upon lytic reactivation. Although miR-BHRF1-2 and -3 expression are increased early after lytic induction, miR-BHRF1-1 is not increased until after 48 hours post-infection because miR-BHRF1-1 overlaps with the BHRF1 promoter and is not induced until the viral Wp/Cp promoters (employed during lytic and latent infections, respectively) become active (Figure 1).

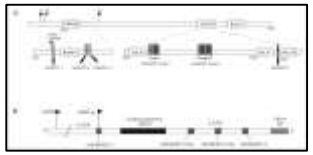


Figure 1. Genomic position of BHRF1 gene and BHRF1 miRNA

miRNA	Sequence
miR-BHRF1-1	UAACCUGAUCAGCCCCGGAGUU
miR-BHRF1-2-3p	UAUCUUUUUCGGCAGAAAUUGA
miR-BHRF1-2-5p	AAAUUCUGUUGCAGCAGAUAGC
miR-BHRF1-3	UAACGGGAAGUGUGUAAGCACA

Figure 2. RNA nt sequence of ebv-miR-BHRF1-1, -2-3p, -5p, and -3 (seed sequence shown in red)

BZLF1 is the early lytic gene mediating the switch from latency to the lytic phase in EBV-infected B cells.

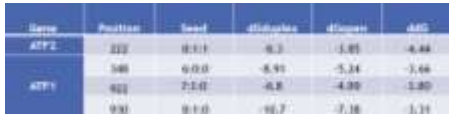
Methods and Materials

MiRanda is an early miRNA target predictor that is available online and must be downloaded. Using a three-step analysis, miRanda considers matching along the entire miRNA sequence to determine probable target sites. However, it weighs matches within the microRNA seed region more heavily than those not in the seed region. Provided miRNA inputs are scanned against provided 3'UTRs to check for WC matches. miRNA: mRNA target pair free energies are calculated by predicting the folding of the hybrid using the Vienna package (6). The miRNA:mRNA target pairs whose free energy do not exceed a certain threshold are passed along to the last step of analysis: conservation of both binding site and position (7).

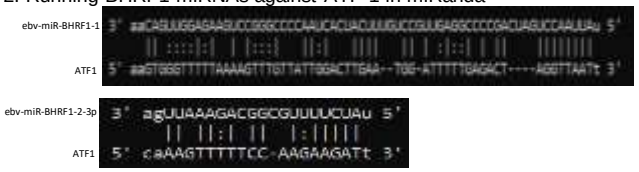
Results

1. Prediction of possible targets of miR-BHRF1-1 by PITA (Probability of Interaction by Target Accessibility) software

Figure 3. PITA analyzes the probability of interaction by target accessibility and predicts the ΔΔG value of each target

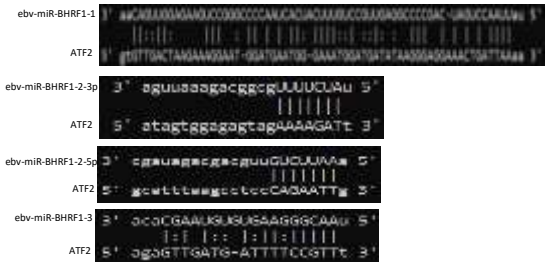


2. Running BHRF1 miRNAs against ATF-1 in miRanda



ebv-miR	# Hits	Score	Energy (kCal/mol)
BHRF1-1	1	182.00	-41.86
BHRF1-2-3p	1	142.00	-12.04
BHRF1-2-5p	No hits were found		
BHRF1-3	No hits were found		

3. Running BHRF1 miRNAs against ATF-2 using miRanda



ebv-miR	# Hits	Score	Energy (kCal/mol)
BHRF1-1	78	143.00	-34.50
BHRF1-2-3p	5	140.00	-6.78
BHRF1-2-5p	10	140.00	-7.07
BHRF1-3	2	141.00	-14.05

Conclusions

- Preliminary data from bioinformatic software PITA suggested the potential target of BHRF1-1 to be sites at the 3'UTR of ATF-1 and ATF-2 mRNA.
- Data from miRNA target site tool miRanda confirmed 1 site at the 3'UTR of ATF-1 mRNA for both ebv-miR-BHRF1-1 and ebv-miR-BHRF1-2-3p. No hits above the default threshold were found for ebv-miR-BHRF1-2-5p and -3.
- Data from miRNA target site tool miRanda confirmed various sites at the 3'UTR of ATF-2 mRNA for all BHRF1-encoded miRNAs.

Future Directions

- Explore other potential cellular and viral targets of BHRF1 miRNAs using the miRanda software package by running the miRNAs against the homo sapiens and EBV genome.
- Further validate targets of ebv-miR-BHRF1-1,-2-3p,-2-5p, and -3 through web-based miRNA target prediction tool Target Scan
- Use *in-vivo* technique Ago HITS-CLIP to simultaneously identify Ago bound miRNAs and the nearby mRNA sites.
- Confirm target predictions by measuring ectopic gene expression of miRNA and potential target in EBV-negative cells.
- Luciferase assay to confirm direct target binding of BHRF1 miRNA to potential targets.

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