In silico prediction of cellular gene targets of herpesvirus encoded microRNAs

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Abstract
Herpesviruses have evolved to encode multiple microRNAs [viral miRNAs (v-miRs)], a unique feature of this family of double stranded DNA (dsDNA) viruses. However, functional role of these v-miRs in host-pathogen interaction remains poorly studied. In this study, we examined the impact of oral disease associated v-miRs viz., miR-H1 [encoded by herpes simplex virus 1 (HSV1)] and miR-K12-3 [encoded by Kaposi sarcoma-associated herpesvirus (KSHV)] by identifying putative targets of viral miRNAs. We used our published microarray data (GSE107005) to identify the transcripts downregulated by the v-miRs. The 3′ untranslated region (UTR) of these genes were extracted using BioMart tool on Ensembl and subjected to RNA:RNA interaction employing RNA Hybrid. We obtained hundreds of potential and novel miR-H1 and miR-K12-3 binding sites on the 3′ UTR of the genes downregulated by these v-miRs. The information can provide likely regulatory mechanisms of the candidate v-miRs through which they can exert biological impact during herpesvirus infection and pathogenesis.

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Specifications Table

| Subject area     | Biology          |
|------------------|------------------|
| More specific subject area | Molecular Virology |
| Type of data     | Text file        |
| How data was acquired | Microarray and Bioinformatics |
| Data format      | Filtered and analyzed |
| Experimental factors | Cells were transfected with v-miRs or control mimics |
| Experimental features | Genes downregulated by v-miRs were scanned for putative miRNA binding sites on the 3'UTR using RNA Hybrid tool. |
| Data source location | NA |
| Data accessibility | Data is presented as supporting file text with this manuscript. Microarray data of transcriptome wide changes in miR-H1 and miR-K12-3 overexpressing human oral keratinocytes compared to control mimics is deposited in the Gene Expression Omnibus public database under Accession Number GSE107005 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE107005). |

Value of the data

The data presented is valuable for the reasons listed below:

- The data provided here enlists human genes that were downregulated by herpesvirus derived miRNAs viz., miR-H1 (Herpes simplex virus 1) and miR-K12-3 (Kaposi sarcoma-associated herpesvirus) and harbor potential v-miR binding sites.
- These genes can provide new avenues to begin focused research on the role of viral miRNAs viz., miR-H1 and miR-K12-3 in the pathogenesis of oral mucosal diseases.
- Due to lack of online tools that can predict viral miRNA binding sites with high confidence, this methodology can provide a starting point to share large datasets examining global impact of v-miRs to identify more reliable candidate targets or facilitate development of algorithms to predict v-miR targets with a high degree of confidence.

1. Data

Human Herpesviruses (HHV) are dsDNA viruses that are highly prevalent worldwide [1]. A key feature of all herpesviruses is their capability to encode microRNAs [2]. These small non-coding RNAs are implicated in wide range of biological functions that govern host-pathogen interaction [2]. Recent evidences show a likely association of herpesvirus in oral diseases, however a role of viral components in the oral pathogenesis remains unknown [3,4]. We recently identified four viral miRNAs that were upregulated in human subjects with inflamed pulps and diseased gingival biopsies compared with healthy tissues [5,6]. Our recent transcriptome and miRNome analysis showed v-miRs can profoundly impact a specific set of genes in oral keratinocytes which are targeted by herpesviruses [6,7]. However, the direct gene targets of these viral miRNAs will shed light on the possible pathways through which viral miRNAs can modulate host cell functions. The data presented here provides a list of potential miR-H1 and miR-K12-3 binding sites on the 3'UTR of host transcripts that were significantly downregulated by these v-miRs in our previously published microarray (GSE107005). Tables 1 and 2 provides list of some representative interaction for miR-H1 and miR-K12-3, respectively, identified in our screening. The remaining interactions are listed as supplementary information in the Supplementary text file 1 (for miR-H1) and Supplementary text file 2 (for miR-K12-3).
| v-miRNA       | Target gene | vmiR and target gene sequence alignment |
|--------------|-------------|----------------------------------------|
| hsv1-miR-H1-5p | PREPL       | Position 2928  
Target 5′ A U U G A 3′  
UCAUUUC GU UCUCUUCAUUU  
GGUGAAG CA GGAAGGUGAAG |
| hsv1-miR-H1-5p | TTC33       | Position 899  
Target 5′ U AA AA A 3′  
CCAUUCUC UCUAUCGUCC  
GGUGAGG GGAAGGUGAAG |
| hsv1-miR-H1-5p | ATG16L1     | Position 1965  
Target 5′ A AG U A A 3′  
CUACU CUG CCUUCAUU  
GGUGAG GGC GGAAGGUAAG |
| hsv1-miR-H1-5p | NOTCH2NL    | Position 2443  
Target 5′ G G G U G 3′  
CAU CCC UCUCUUCAU  
GGUGAGG GGAAGGUGAAG |
| hsv1-miR-H1-5p | ZNF106      | Position 1227  
Target 5′ G A U 3′  
UCGGCUUUC GCUCUUUCGUU  
GGUGAAGGG C GGAAGGUAAG |
| hsv1-miR-H1-5p | CHML        | Position 212  
Target 5′ A AC AU A 3′  
UCACUCUC UCUCUUCAUC  
GGUGAGG GGAAGGUGAAG |
| hsv1-miR-H1-5p | CCDC91      | Position 464  
Target 5′ C A A 3′  
CUCUUUCUC UUUUCGUUCG  
GGUGAAGGG C GGAAGGUGAAG |
| hsv1-miR-H1-5p | RABEP1      | Position 88  
Target 5′ C A 3′  
CCAUUUCUC UCUUCUCUCGUU  
GGUGAAGGG C GGAAGGUGAAG |
| hsv1-miR-H1-5p | TGFBR1      | Position 4034  
Target 5′ A A A A 3′  
UACUUUCUC GCUCUUUCGUU  
GGUGAAGGG C GGAAGGUGAAG |
| hsv1-miR-H1-5p | TRIM52      | Position 453  
Target 5′ G C U U A 3′  
UACUUUCUC UCUCUUUCGUU  
GGUGAAGGG C GGAAGGUGAAG |
| hsv1-miR-H1-5p | DYM         | Position 8446  
Target 5′ A A A A 3′  
UACUUUG UCUCUUUCGUU  
GGUGAAGGG C GGAAGGUGAAG |
| hsv1-miR-H1-5p | NDUFS1      | Position 1742  
Target 5′ A A C A 3′  
GCGUGU UGUUUG CAGAGUGUG  
GGAGG GCGAGG GUCUUACAC |

Table 1  
Predicted miR-H1-5p binding sites on the downregulated host genes. Sequence alignment of selected potential miR-H1-5p binding sites is shown. Only the binding sites with mfe < -20 kcal/mol are shown.
2. Experimental design, materials and methods

2.1. Primary gingival human oral keratinocyte (HOK) culture

Primary HOK (human gingival epithelial cells) were purchased from ScienCell Research Laboratories (Carlsbad, CA). Cultures were tested for HOK markers by immunofluorescent methods using antibodies to cytokeratine-8, -18 and -19 and were negative for Human Immunodeficiency Virus 1 (HIV-1), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), mycoplasma, bacteria, yeast and fungi. Cells were cultured using DermaLife K Keratinocyte Medium Complete Kit (Lifeline Cell Technology, Frederick, MD).

2.2. Transient miRNA transfections and total RNA isolation

Transient viral miRNA (miR-H1 or miR-K12-3) or control mimic transfections in HOK were performed using Lipofectamine 2000 reagent (Life Technologies, San Diego, CA) as described previously [8,9]. Cells were transfected with viral miRNA mimics (Qiagen, Gaithsburg, MD, USA) at a final concentration of 15 nM for 36 h. Total RNA was isolated using the miRNeasy kit (Qiagen).

2.3. Microarray analysis

We used our published microarray data deposited in the Gene Expression Omnibus public database under Accession Number GSE107005 for the identification of putative viral miRNA target transcripts [6]. Array data were in compliance with Minimum Information About a Microarray Experiment (MIAME) guidelines.

2.4. V-miR target prediction of differentially downregulated genes

To identify miR-H1 and miR-K12-3 gene targets with high confidence, we first selected downregulated genes. The 3’UTR of these genes were extracted using BioMart tool on Ensembl (http://www.ensembl.org/biomart/martview/aa867419c3c6fd64f94af6d4a6549d3c). Briefly, we selected Ensembl Genes 87 database and Human Genes dataset (GRCh38.p7). Next, the "Filters" were selected to match the input genes list. In the "Gene" tab set the "ID list limit" filter to "HGNC symbol(s)". Finally, to procure the 3’UTR sequences "Attributes" were set. In the "Attributes", select "Sequences" and then select 3’UTR start and 3’UTR end, click "Ensembl Gene ID" and "Associated Gene Name". The results were exported to by selecting "File", "FASTA" and "Unique results only". This was done separately for miR-H1 and miR-K12-3 datasets.

Table 1 (continued)

| v-miRNA       | Target gene | vmiR and target gene sequence alignment |
|---------------|-------------|----------------------------------------|
| hsv1-miR-H1–5p| SLC4A7      | Position 1345                           |
|               |             | Target 5’ U G G G 3’                   |
|               |             | UACU UUU GUCUUUUAU                      |
|               |             | GUGA AGG CAGGAAGGUA                     |
| hsv1-miR-H1–5p| PRRC1       | Position 47                             |
|               |             | Target 5’ G C U 3’                     |
|               |             | UACU UUC C UCCUUUGUU                   |
|               |             | GUG AGGG AGGAAGGUAG                     |
| hsv1-miR-H1–5p| IL1RAP      | Position 2670                           |
|               |             | Target 5’ U A U A 3’                   |
|               |             | UACUU UU UCUCUCUU                      |
|               |             | GUGAA GG AGGAAGGUA                     |

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Table 2
Predicted miR-K12-3 binding sites on the downregulated host genes. Sequence alignment of selected potential miR-K12-3 binding sites on the predicted targets is shown. Only the binding sites with mfe $<-20$ kcal/mol are listed.

| v-miRNA       | Target gene | vmiR and target gene sequence alignment                                                                 |
|---------------|-------------|----------------------------------------------------------------------------------------------------------|
| kshv-miR-K12-3| CBX5        | Position 8806                                                                                             |
|               |             | Target 5' U AU C G U 3'                                                                                    |
|               |             | UC UUGUU U UUGGAAUGUGA                                                                                        |
|               |             | AG GACGG G AGUUCUACAU                                                                                        |
|               |             | miRNA 3' CA G 5'                                                                                            |
| kshv-miR-K12-3| GOLGA3      | Position 3592                                                                                              |
|               |             | Target 5' AU GU GA A 3'                                                                                    |
|               |             | GC GU UUCU UAGGAUGUGA                                                                                        |
|               |             | CG CG AGGA GUCUACAU                                                                                        |
|               |             | miRNA 3' AG AGC 5'                                                                                           |
| kshv-miR-K12-3| UIMM21      | Position 55                                                                                                 |
|               |             | Target 5' AU A G A U 3'                                                                                     |
|               |             | GCU U GUCU U GAUGUGA                                                                                        |
|               |             | CGA G CAGGA UCUACAU                                                                                         |
|               |             | miRNA 3' AG G C 5'                                                                                           |
| kshv-miR-K12-3| UBL1X       | Position 2516                                                                                               |
|               |             | Target 5' AA AG A U 3'                                                                                      |
|               |             | GCU U GUCU U GAUGUGA                                                                                        |
|               |             | CGA G CAGGA UCUACAU                                                                                         |
|               |             | miRNA 3' AG C G 5'                                                                                           |
| kshv-miR-K12-3| FKB14       | Position 1178                                                                                               |
|               |             | Target 5' AAA AG A U 3'                                                                                    |
|               |             | CUG GU C GGGUGUGG                                                                                           |
|               |             | GAC CAG G UCUACAU                                                                                            |
|               |             | miRNA 3' AG C G 5'                                                                                           |
| kshv-miR-K12-3| DSUN        | Position 659                                                                                                 |
|               |             | Target 5' A AG A C 3'                                                                                       |
|               |             | UC UUGU UGUCU UC G AAUGUG                                                                                   |
|               |             | AG GACGG GA GG GA UCUACAC                                                                                    |
|               |             | miRNA 3' U C 5'                                                                                             |
| kshv-miR-K12-3| ORC2        | Position 372                                                                                                 |
|               |             | Target 5' G GU A 3'                                                                                          |
|               |             | UGU UUGCUC CAGAGUGUGG                                                                                        |
|               |             | GCCG GCCAGG GUCUACAU                                                                                        |
|               |             | miRNA 3' A AC A 5'                                                                                           |
| kshv-miR-K12-3| COPA        | Position 33                                                                                                  |
|               |             | Target 5' A CC AG U 3'                                                                                      |
|               |             | UGGU CC CC AGAAUGUG                                                                                         |
|               |             | GCGA GG GU UCUACAC                                                                                          |
|               |             | miRNA 3' A CGA AG U 5'                                                                                      |
| kshv-miR-K12-3| POLR3B      | Position 228                                                                                                 |
|               |             | Target 5' AU A AG A U 3'                                                                                    |
|               |             | GCUGGC UG UC A G AAUGUGA                                                                                     |
|               |             | CGACG GC AG G UCUACAC                                                                                        |
|               |             | miRNA 3' AG AG 5'                                                                                           |
| kshv-miR-K12-3| RAB3D       | Position 260                                                                                                 |
|               |             | Target 5' C U U 3'                                                                                           |
|               |             | UUGUCGU UC AGGGUGUGAGAGGCAGG AGG UCUACAC                                                                       |
|               |             | miRNA 3' CAG U 5'                                                                                           |
| kshv-miR-K12-3| SLC1A4      | Position 2435                                                                                               |
|               |             | Target 5' G G C 3'                                                                                           |
|               |             | G UCCU UC AGAGUGUG                                                                                          |
|               |             | C AGCC AGG UCUACAC                                                                                            |
|               |             | miRNA 3' AG G CAG U 5'                                                                                      |
| kshv-miR-K12-3| CCND2       | Position 1742                                                                                               |
|               |             | Target 5' AAA CA C 3'                                                                                       |
|               |             | GCUGU UGUU U CAGAGUGUG AGCAG GCCAGG GUCUACAC                                                                  |
|               |             | miRNA 3' AG A U 5'                                                                                           |
v-miR-target 3′UTR interaction was assessed by target prediction tool RNAHybrid software (https://bibiserv2.cbi.unibe.ch/rnahybrid?id=rnahybrid_view_submission). The procured 3′UTR sequences and miR-H1 and miR-K12-3 sequences (extracted from miRbase v.21) were provided as input for RNA Hybrid analysis. The stringency parameters were set-up for individual sequences and we opted for three hits per target to highlight any probable v-miR binding sequence present on the target.

We considered the following parameters to select putative v-miR regulated genes. (i) There should be high sequence complementarity in the seed region (positions 2–8 nt from 5′ of miRNA), with only 1 mismatch allowed. (ii) For stringency, we picked v-miR-target interactions where more than 11 nts of the v-miR sequence are involved in the interaction. (iii) If there is any mismatch in the seed regions, this should be compensated by strong binding beyond the seed region. (iv) The bulge in the interaction region should not involve more than 3 nucleotides. (v) Entropy of the v-miR-target interaction was set at stringent level with cut-off < 22 kcal/mol.

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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.05.020.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.05.020.
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