

Structural and Functional Conservation of Epstein Barr Virus microRNA Using Phylogenetic Study

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Abstract: MicroRNA (miRNA) are small non coding RNAs of ~22nt in length that are primarily transcribed from primary RNA (pri-miRNA). The signatures of these miRNA are their secondary hairpin structure with bulge in stem region. miRNA molecules are involved in RNA interference mechanism and control gene expression by targeting mRNA. miRNA mostly targets at 3'untranslated region of gene and controls protein production by translational repression or mRNA degradation. The undesirable production of miRNA is substantial factor in many human diseases. Several miRNAs are reported that are associated with human diseases. EBV miRNA is associated with various diseases like Gastric Carcinoma, nasopharyngeal, Lymphoblastoid, lymphoma and other diseases. Identification of conserved EBV miRNA in Rhesus Lymphocryptovirus (RLCV) that infects monkey can be helpful in better understanding of human diseases and its pathological conditions for drug development process for Epstein Barr Virus. In this paper we have done phylogenetic analysis of miRNA among 12 different viruses study shows that there is functional relationship between Epstein Barr Virus and Rhesus Lymphocryptovirus, sharing 21 clades with a confidence bootstrap value from 80-100%. Structural alignment of EBV and RLV miRNA shows functional similarity of EBV miRNAs and a consensus miRNA is designed to study conservational motifs of these miRNA. This study signifies the potential role RLCV miRNA as model molecule to study EBV infection in human and molecular diagnosis of many human diseases associated with EBV miRNA.

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Keywords: miRNA; Epstein Barr Virus; Phylogenetic analysis; Rhesus Lymphocryptovirus, miRNA structure

1. Introduction

RNA interference (RNAi) is a process by which dsRNA molecule of specific length and secondary structure silences its complementary gene, this mechanism is also called as posttranscriptional gene silencing[1]. Molecules of RNAi are small interfering RNA (siRNA) and micro RNA (miRNA) these molecules differ in length of secondary structure they form after processing of parent mRNA and their mechanism of gene silencing[2]. RNA interference is a mechanism used by cells to regulate the expression of genes & replication of viruses and its self defense mechanism in plants for protection against viral infection and foreign molecule[3,4]. Scientist have mimicked this natural mechanism of gene silencing by introducing dsRNA molecule in target cells for sequence specific gene silencing this process of artificial gene silencing have been used in wide field of biotechnology [5], genomics, functional genomics, drug discovery, target validation[6], therapeutics, virus and insect resistant plants and other fields of agriculture[7,8].

miRNA are signature molecules in many human diseases like cancer, differential expression in cancer stages, tumours, Cardiovascular Disease, immunological diseases, neurological diseases, skin diseases etc exploiting the natural mechanism of miRNA gene silencing pathway Drug development industry has tried to synthesize this artificial small

miRNA molecule that can silence disease gene and hence regulate disease prognosis. RNAi molecules are also used in functional genomics study and in drug discovery process like drug target screening and validation and also as therapeutics that are under clinical trials and will revolutionize drug development process by its efficiency to cure many human diseases [9].

miRNA have become an important tool for pathology of many diseases like cancer, stages of cancer, neuronal diseases, muscular dystrophy and viral infection. miRNA have proved a potential molecule in understanding disease prognosis and used for clinical perspectives[10].

1.1. microRNAs in Viruses

microRNAs (miRNA) are double stranded non coding RNA of length 22-23 nt that mediates sequence specific gene silencing of target mRNA. miRNA inhibits expression of mRNA by variety of mechanism like cleavage of targeted mRNA, repression of translation or deadenylation of target mRNA[11]. With advent of next generation sequencing techniques thousands of miRNAs have been identified and sequenced in various organisms from eukaryotes to viruses. Computational techniques and sophisticated softwares have also helped us to identify new miRNAs and unveiling their structure and function[12].

Many viruses encode miRNA but most of them functions are unknown. It has been reported that many virus encoded miRNA are not conserved[13] but some papers report conservation of miRNA structure and function in viruses like human polyomaviruses associated with serious disease in JC virus and BK virus encode miRNAs with the same function as that of

the monkey polyomavirus simian virus 40 miRNAs and are evolutionary conserved[14,15]. Number of databases is there that reports conserved family of miRNAs in viruses. Table 1 list virus miRNA databases detail information of miRNA encoded by viruses and association with diseases can be accessed from these databases.

Table 1. LIST OF VIRUS miRNA DATABASES

s.no	database	url
1	VIRmiRNA	http://crdd.osdd.net/servers/virmirna/
2	Vir-Mir db	http://alk.ibms.sinica.edu.tw .
3	vita	http://vita.mbc.nctu.edu.tw/
4	miRBase	http://www.mirbase.org/
5	mirdb	http://mirdb.org/miRDB/
6	microRNA.org	http://www.microrna.org/microrna/home.do
7	mir2disease database	http://www.mir2disease.org/
8	miRTarBase	http://mirtarbase.mbc.nctu.edu.tw/
9	Tar Base	http://microrna.gr/tarbase .
10	miRNEST	http://rhesus.amu.edu.pl/mirnest/copy/
11	DIANA TOOLS	http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index
12	miREnvironment Database	http://cmbi.bjmu.edu.cn/miren .
13	EpimiR	http://202.97.205.78:8080/EpimiR/index.jsp
14	miRTarBase	http://mirtarbase.mbc.nctu.edu.tw/

1.2. Epstein–Barr virus miRNAs.

Human herpesvirus Epstein-Barr virus (EBV) is a first virus in which miRNAs are reported many of which functions are unknown[16]. EBV encodes 23 miRNA various miRNAs are associated with several diseases like nasopharyngeal carcinoma (NPC) [17], gastric carcinoma (GC)[18], lymphoid malignancies, Hodgkin lymphoma, Burkitt lymphoma for example miR-BART21 and miR-BART22 are associated with nasopharyngeal carcinoma [19,20]. Studies also reported differential expression of miR-15a and miR-16 in NPC tissues. miR-155 and miR-146a is highly expressed in various tumors EBV [21] etc. Researches are going on to uncover the function of EBV- miRNA some miRNAs are conserved in other viruses also. In this study we have identified structurally conserved miRNAs of Epstein–Barr virus by phylogenetic analysis of EBV miRNAs with other miRNAs[22,23].

2. Results and Discussion

2.1. Phylogenetic Tree Analysis

miRBase is primary database of miRNA sequence and structure details that include information about miRNA function and gene family also. But miRBase does not contain any information about conservation of miRNA across viruses[31]. miRNA identified and discovered in one virus can be conserved in other viruses also. This is challenge to identify structurally conserved miRNA among viruses as one needs data about all miRNAs encoded by viruses, absence of miRNA in one virus and presence in other virus also sequence information to perform phylogenetic analysis[32].

Phylogenetic analysis for miRNAs from 12 viruses total of 124 miRNA shows the relationship between miRNA among viruses. Figure 1 shows the complete phylogenetic tree to differentiate between different miRNAs of viruses initials are assigned to miRNAs like “ebv” to Epstein Barr virus miRNAs, “rlcv” to Rhesus lymphocryptovirus and so on to other viruses. Phylogenetic tree shows that Epstein Barr virus miRNAs are related to Rhesus lymphocryptovirus miRNAs. 21 EBV miRNAs are identified that are neighbors to Rhesus lymphocryptovirus miRNAs.

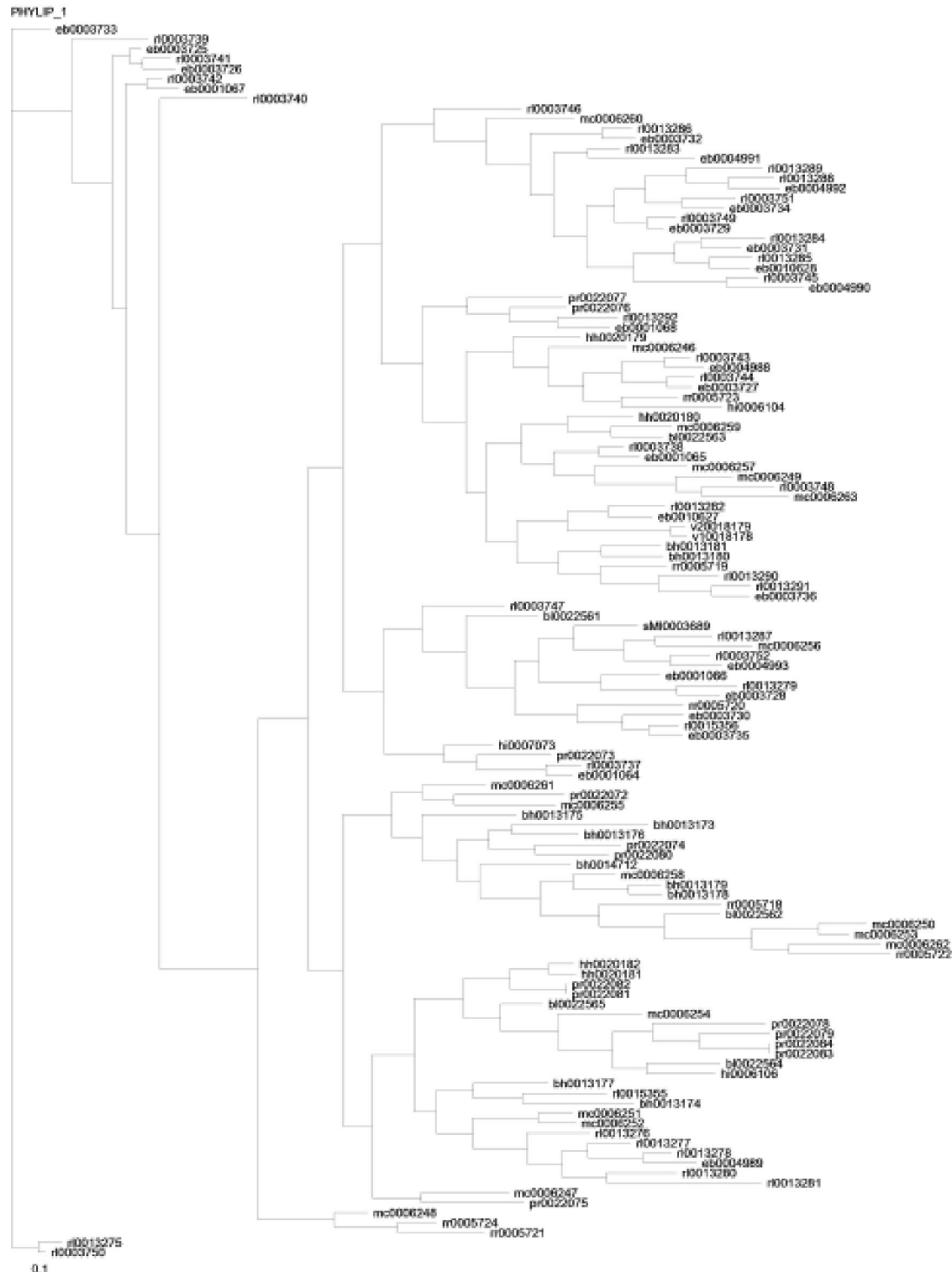


Figure 1. Phylogenetic Tree for Mirna Among 12 Viruses

Epstein Barr virus miRNA leaves are selected in phylogenetic tree to identify neighbors Figure 2 represent the multiple sequence alignment of all miRNA in dataset using NCBI Genome workbench

and Figure 3 shows the Epstein Barr virus miRNA that are marked in red and associated neighbor in phylogenetic tree. Figure 4 shows the branches that are related with miRNAs of EBV and RLCV. Detailed

analysis shows that EBV miRNA are related with Rhesus lymphocryptovirus miRNAs Table 2 list the

clades information of ebv and rlcv miRNAs with confidence score from 60-100%.

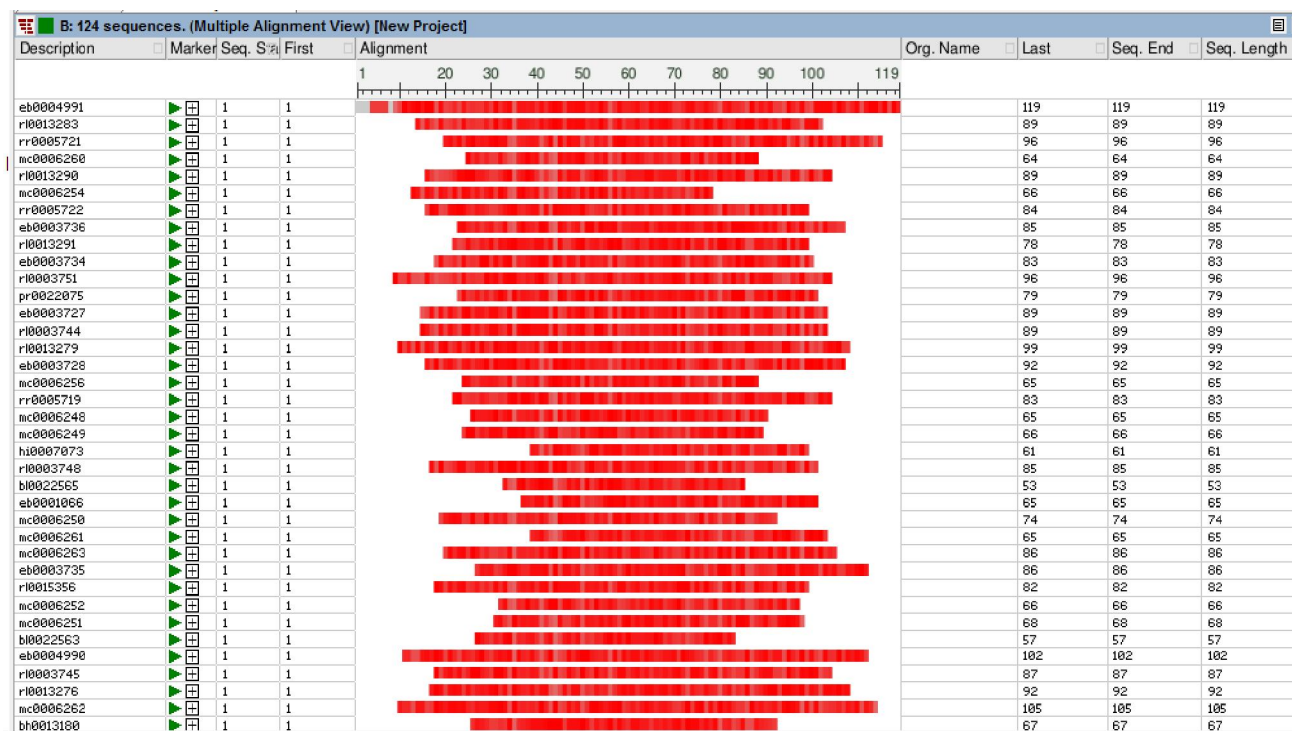


Figure 2. Multiple Sequence Alignment of 124 miRNAs

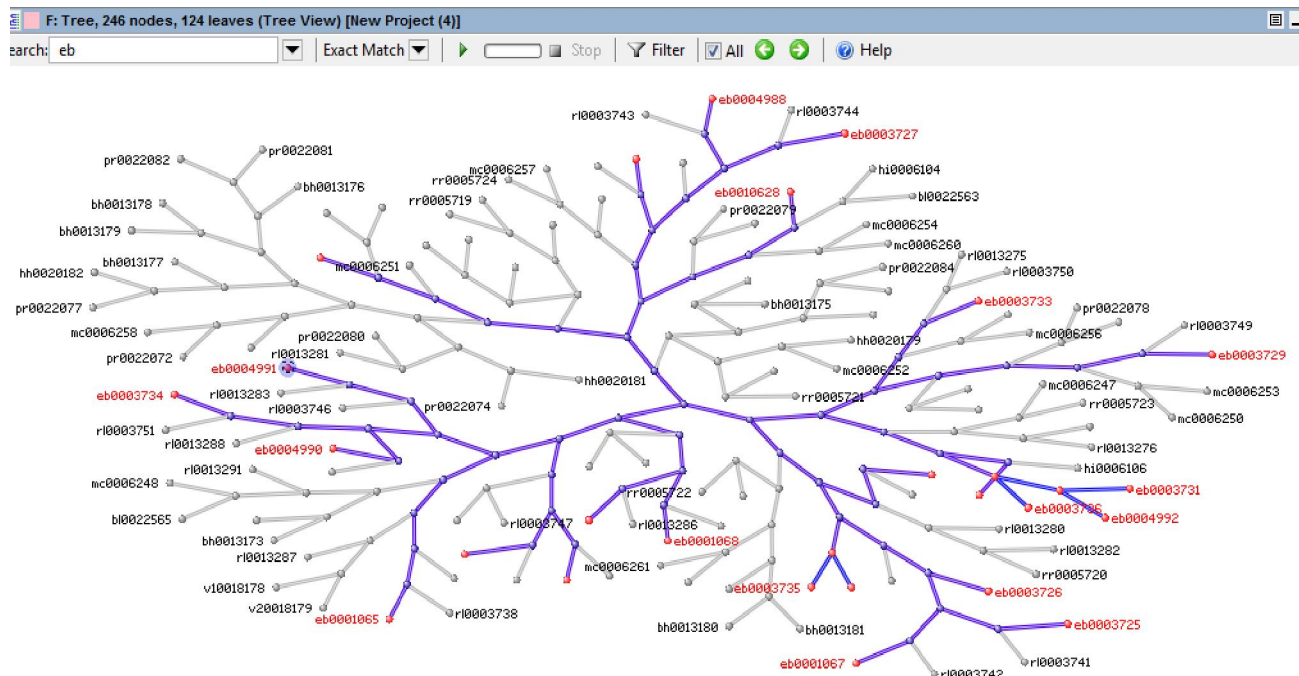


Figure 3. Phylogenetic Tree with Marked in Red Leaves are Epstein Barr Virus miRNA

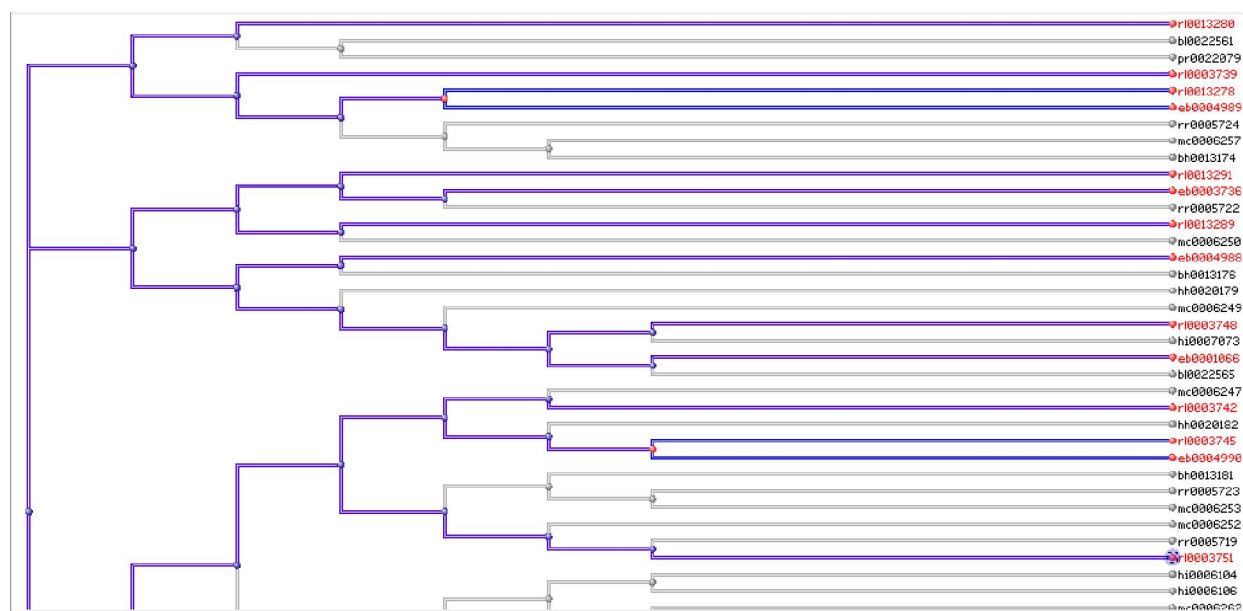


Figure 4. Phylogenetic Tree Showing Mirna of EBV and RLCV as Neighbors

Table 2. Relationship Between EBV and RLCV Mirna with Bootstrap Score

Clade no.	EBV	RLCV	Bootstrap score
1	ebv-mir-BART4 MI0003726	rlcv-mir-rL1-5 MI0003741	60%
2	ebv-mir-BART1 MI0001067	rlcv-mir-rL1-6 MI0003742	100%
3	ebv-mir-BART10 MI0003732	rlcv-mir-rL1-27 MI0013286	80%
4	ebv-mir-BART18 MI0004991	rlcv-mir-rL1-24 MI0013283	80%
5	ebv-mir-BART19 MI0004992	rlcv-mir-rL1-29 MI0013288	80%
6	ebv-mir-BART12 MI0003734	rlcv-mir-rL1-15 MI0003751	100%
7	ebv-mir-BART7 MI0003729	rlcv-mir-rL1-13 MI0003749	80%
8	ebv-mir-BART9 MI0003731	rlcv-mir-rL1-25 MI0013284	80%
9	ebv-mir-BART22 MI0010628	rlcv-mir-rL1-26 MI0013285	80%
10	ebv-mir-BART17 MI0004990	rlcv-mir-rL1-9 MI0003745	100%
11	ebv-mir-BART2 MI0001068	rlcv-mir-rL1-33 MI0013292	100%
12	ebv-mir-BART15 MI0004988	rlcv-mir-rL1-7 MI0003743	60%
13	ebv-mir-BART5 MI0003727	rlcv-mir-rL1-8 MI0003744	100 %
14	ebv-mir-BHRF1-2 MI0001065	rlcv-mir-rL1-2 MI0003738	60 %
15	ebv-mir-BART21 MI0010627	rlcv-mir-rL1-23 MI0013282	100 %
16	ebv-mir-BART14 MI0003736	rlcv-mir-rL1-32 MI0013291	100 %
17	ebv-mir-BART20 MI0004993	rlcv-mir-rL1-16 MI0003752	80 %
18	ebv-mir-BART6 MI0003728	rlcv-mir-rL1-20 MI0013279	80%
19	ebv-mir-BART13 MI0003735	rlcv-mir-rL1-35 MI0015356	100 %
20	ebv-mir-BHRF1-1 MI0001064	rlcv-mir-rL1-1 MI0003737	80 %
21	ebv-mir-BART16 MI0004989	rlcv-mir-rL1-19 MI0013278	80 %

Out of 21 clades 3 clades shows 60 % of confidence score these clades are ebv-mir-BART4 and rlcvmir-rL1-5 ; ebv-mir-BART15 and rlcvmir-rL1-7; ebv-mir-BHRF1-2 and rlcvmir-rL1-2. these miRNAs show no structural similarity. Table 1 shows 10 clades that have confidence score of 80% and 8 clades with confidence score of 100%. Structural similarity between miRNAs of EBV and RLCV is done for 8

clades that show 100 % bootstrap score to identify structural conservation among viruses.

2.2. Structural Similarity of EBV miRNA with RLCV miRNA

Structural similarity between miRNA of EBV and RLCV is done using LocARNA tool. 8 EBV miRNA are structurally conserved in RLCV. Table 3 list the structural similarity of EBV miRNA with RLCV miRNA. These miRNA belongs to same gene family.

Table 3. List of Structural Conserved miRNA of EBV with Associated Disease

S.No	EBV	RLCV	Gene family	Structure conservation	Disease Associated	References
1	mir-BART1	mir-rL1-6	mir-BART1	Figure 5	Gastric Carcinoma, nasopharyngeal carcinoma	33,34
2	mir-BART12	mir-rL1-15	mir-BART12	Figure 6	Gastric Carcinoma, nasopharyngeal carcinoma	33-35
3	mir-BART17	mir-rL1-9	mir-BART17	Figure 7	nasopharyngeal carcinoma	36,37
4	mir-BART2	mir-rL1-33	mir-BART2	Figure 8	Gastric Carcinoma, nasopharyngeal carcinoma	33,34,36
5	mir-BART5	mir-rL1-8	mir-BART5	Figure 9	Gastric Carcinoma, nasopharyngeal carcinoma	37-39
6	mir-BART21	mir-rL1-23	mir-BART21	Figure 10	Lymphoblastoid, B-cell lymphoma	40- 42
7	mir-BART14	mir-rL1-32	mir-BART14	Figure 11	Gastric Carcinoma, nasopharyngeal carcinoma	43,44
8	mir-BART13	mir-rL1-35	mir-BART13	Figure 12	nasopharyngeal carcinoma, myelodysplastic syndromes (MDS), systemic lupus erythematosus	42-44

miRNA are ~22 nt long that are derived from 60–90-nt miRNA precursors. miRNA have specific secondary structure that differentiates it with other siRNA molecule[45]. miRNA have irregular hairpin structures having bulges in stem region that key hallmark of miRNA molecule [46]. Several miRNAs contains mismatches in stem region of hairpin loop, bulges and internal loops [47]. Studies suggest that that miRNA are highly conserved in their hairpin loop secondary structure rather than primary sequences. It is reliable to identify miRNA function on the basis of structural similarity rather than traditional sequence based alignment[48].

mir-BART1 is structurally conserved with mir-rL1-6 at stem region after hairpin loop fig 5 shows the structural similarity. These miRNA belongs to BART1 family members of this family have been reported in cell lines of Gastric Carcinoma and nasopharyngeal carcinoma [49]. No database has reported the function of mir-rL1-6 in RLCV hence according to structural and phylogenetic tree relationship this miRNA can have same function as of human EBV mir-BART1. Hence mir-rL1-6 can be model miRNA molecule for drug development and to understand molecular mechanism of diseases in humans with EBV infection.

Similarly function of other miRNA in RLCV is identified on the basis of structural and phylogenetic relationship with EBV miRNA and these RLCV miRNA can be used as target molecule to study disease prognosis, disease detection and other pathological condition in monkey as model organism to understand molecular diagnosis of disease in humans RLCV

mir-rL1-15 is structurally conserved with EBV mir-BART12 Fig. 6 shows the structural similarity at stem region of miRNA secondary structure. RLCV mir-rL1-15 can be used to study molecular diagnosis and understanding of Gastric Carcinoma, nasopharyngeal carcinoma

Figure 7 shows the structural conservation of mir-rL1-9 with mir-BART17 and can be used to study nasopharyngeal carcinoma in monkey as model organism to study human diseases. mir-rL1-33 is structurally conserved with mir-BART2 shown in Fig. 8 and can have function in Gastric Carcinoma, nasopharyngeal carcinoma

Figure 9 shows structural similarity of mir-rL1-8 with mir-BART5 that has involvement in Gastric Carcinoma and nasopharyngeal carcinoma. Figure 10 describes the relationship between mir-rL1-23 and mir-BART21 showing structural similarity at various position of miRNA secondary structure and have function in Lymphoblastoid, B-cell lymphoma

Figure 11 and Figure 12 shows structural similarity of mir-rL1-32 and mir-rL1-35 with mir-BART14 and mir-BART13 these miRNA of BART family are involved in various human diseases Gastric Carcinoma, nasopharyngeal carcinoma, nasopharyngeal carcinoma, myelodysplastic, syndromes (MDS), systemic lupus erythematosus.

mir-rL1-23, mir-rL1-35 molecule of RLCV can be used to study molecular understanding of various diseases like Lymphoblastoid, B-cell lymphoma, nasopharyngeal carcinoma, myelodysplastic, nasopharyngeal carcinoma, myelodysplastic syndromes (MDS), systemic lupus erythematosus.

These miRNA of RLCV belongs to mir-BART21 and mir-BART13 family of EBV and also represent structural similarity. Hence these molecules can be potent molecule to study disease prognosis in RLCV virus.

Since miRNA are important molecule that have function in regulation of diseases [50], their presence in different stages of tumors, cancers and diseases [51]

has led revolution in drug discovery in development process[52]. Because of their small size and high sequence specificity these molecules are widely used to study function of genes and their involvement in different biological process and diseases[53]. Hence it is important to identify similar miRNAs in model organism with same structure and function for proper understanding of human diseases.



Figure 4. Structural Conservation of EBV-mir-BART1 and rlcv-mir-rL1-6

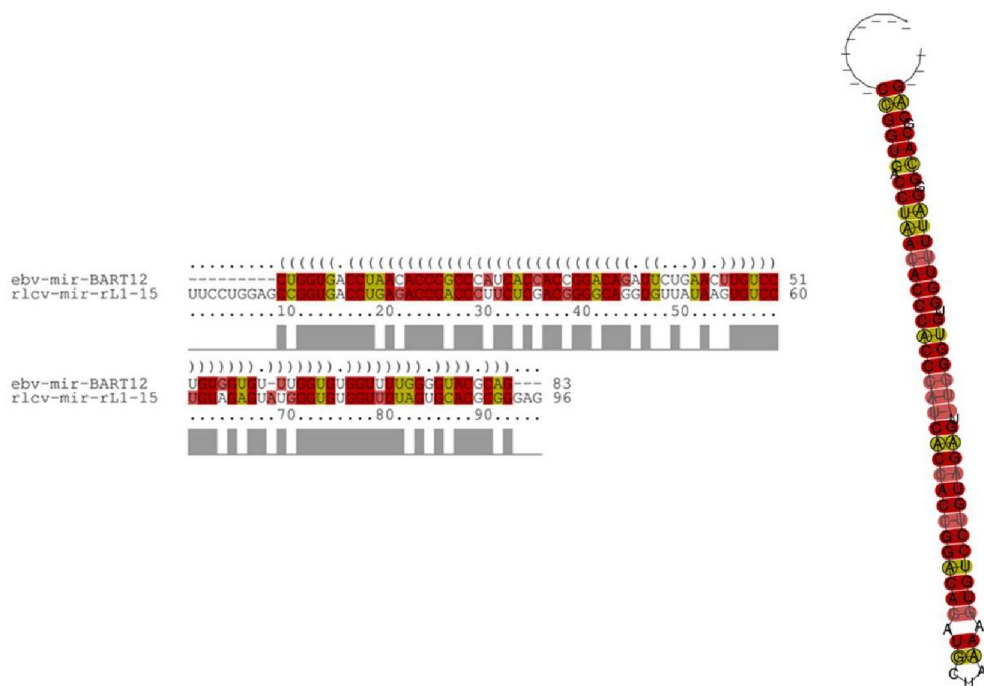


Figure 6. Structural Conservation of EBV-mir- BART12 and rlcv-mir-rL1-15





Figure 9. Structural Conservation of EBV-mir-BART5 and rlcv-mir-rL1-8

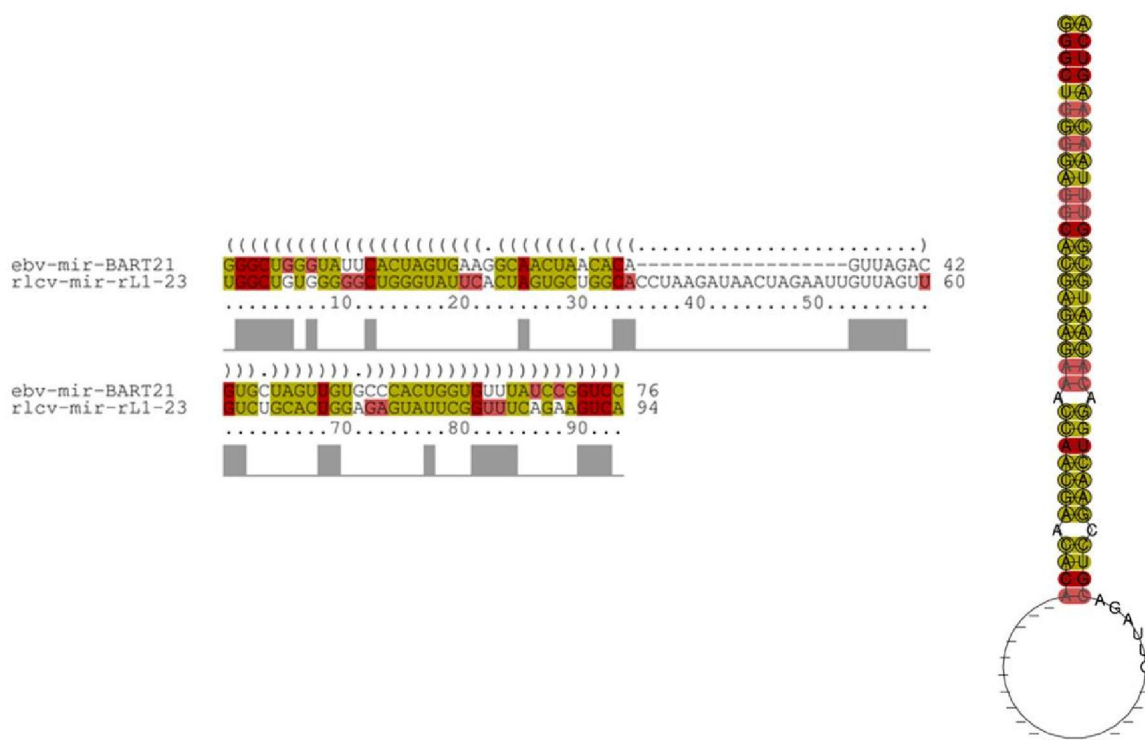


Figure 10. Structural Conservation of EBV-mir-BART21 and rlcv-mir-rL1-23

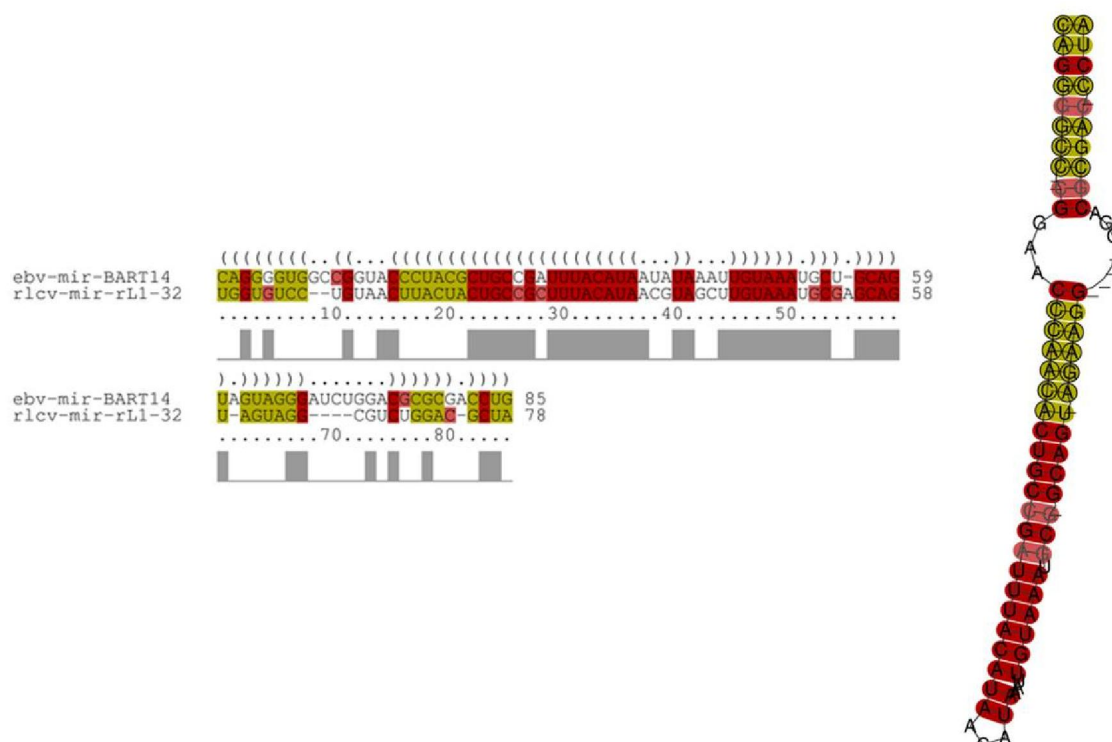


Figure. 11 Structural Conservation of EBV-mir-BART14 and rlcv-mir-rL1-32

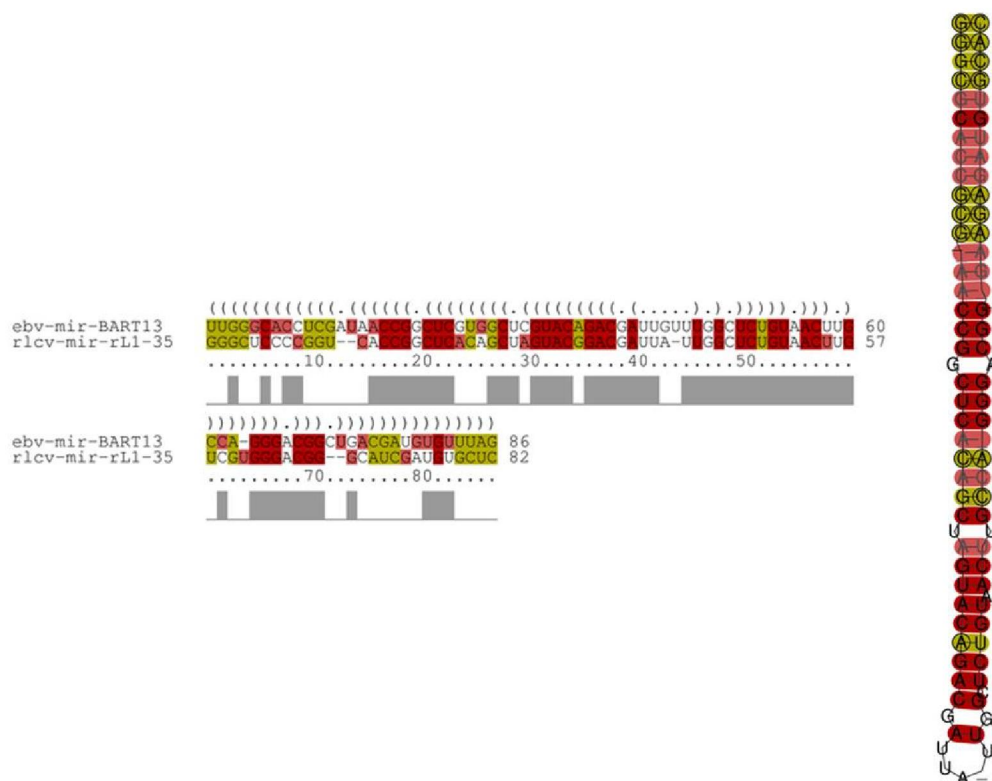


Figure 12. Structural Conservation of EBV-mir-BART14 and rlcv-mir-rL1-32

4. Materials and Methods

4.1. Dataset

Mirbase[24] database was searched for the miRNA sequences of 12 viruses viz, Bandicoot papillomatosis carcinomatosis virus type 1, Bandicoot papillomatosis carcinomatosis virus type 2, Bovine herpesvirus 1, bovine leukemia virus, Epstein Barr virus, Human herpesvirus 6B, Human immunodeficiency virus, Mouse cytomegalovirus, Pseudorabies, Rhesus lymphocryptovirus, rhesus monkey rhadinovirus, Simian virus 40. Total 124 miRNA sequences were selected on the basis of high confidence miRNA in Mirbase database.

4.2. Phylogenetic Tree Construction

Multiple sequence alignment is done using clustal omega [25] and phylogenetic tree is constructed using DNA parsimony method in PHYLIP version 3.695 [26] and phylogenetic tree evaluation is done using Bootstrap method in PHYLIP programs. Phylogenetic tree analysis is done using NCBI Genome Workbench[27]. Clades were identified that are related with EBV miRNA and list of virus encoded miRNA is made that are neighbors of EBV miRNA.

4.3. miRNA Sequence and Structure Alignment

miRNAs having bootstrap score of 100% and that are neighbors in phylogenetic tree are used for sequence and structure alignment to identify conservation of miRNA structure among viruses. miRNA sequence and structure alignment is done using LocARNA tool[28-30] that shows structural conservation of EBV miRNA with other virus miRNAs

Conclusions

Phylogenetic analysis for miRNAs among 12 viruses we identified miRNA that are evolutionary related with EBV miRNA. Study shows that there is functional relationship between miRNA of Epstein Barr Virus and Rhesus Lymphocryptovirus. phylogenetic tree analysis shows 21 EBV miRNA that are related with RLCV miRNA out of these 8 EBV miRNAs are identified with confidence score of 100% that are highly related with RLCV miRNA. Structural similarity between related miRNA shows that there is structural conservation between miRNAs of both viruses and these miRNA belong to same gene family. Further through literature survey diseases associated with these EBV miRNA is identified. EBV miRNA are associated with various disease like Gastric Carcinoma, nasopharyngeal carcinoma, Lymphoblastoid, B-cell lymphoma, myelodysplastic, myelodysplastic syndromes (MDS), systemic lupus erythematosus. Identification of similar miRNA with same function in other virus that infects other model organism can help drug development industry for better understanding of molecular diagnosis, disease

prognosis and pathology of human diseases. Hence Rhesus lympho cryptovirus miRNAs can be used as a model system to study molecular mechanism of disease pathogenesis and new drug development process for Epstein Barr Virus.

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