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Authors' Affiliation:
Centre for Human Genetics, Hazara University Mansehra, 21130, Pakistan

***Corresponding Author:**
Gohar Rahman
Email:
grkhan117@gmail.com

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An *In-Silico* Approach for the Prediction of miRNAs in Merkel Cell Polyoma Virus and its Target Genes

Gohar Rahman, Bilal Ahmad Mian, Najib Ullah, Huzaifa Khan, Shaker Khan

Abstract

Background: For last five years, as technological advancement occurs, novel kinds of human generation viruses' discovery have been increased. A rare human cancer associated virus Merkel's Cell polyomavirus (MCPyV or MCV) got remarkable attraction among the newly discovered viruses. As a common human virus MCPyV infection frequently found in skin and also occurs at other anatomical sites.

Methods: In this study, the in-silico screening of miRNAs from MCPyV was done, as the computational screening procedures are functionally vital, efficient and inexpensive for the said purpose.

Results: Primarily 52 sequences, possessing possible hairpin-like structures, were extracted from MCPyV genome by searching through VMir software using various filters. 17 nominees were confirmed with real pre-miRNA like hairpin organizations by iMiRNA-SSF program. Further seven nominees were excluded by free energy measurement and other parameters. 10 mature miRNAs were affirmed in 10 impending candidates for pre-miRNAs by MatureBayes web server v1.0, among these 10 candidates 2 were reported already by earlier studies. The homologous miRNA for these candidates were searched in mirBase. To find the target gene and its relevant diseases the best homologous miRNAs were then searched in Target Scan. Among all miRNAs Cancer was found to be common.

Conclusion: These findings open new avenues for researchers to explore the role of these novel miRNAs in viral pathogenesis as well as in developing new antiviral therapy.

Scan me

Introduction

Merkel Cell Polyomavirus (MCPyV) were first identified in 2008 in the tissue from Merkel Cell Carcinoma (MCC) [1]. Natural host of polyomavirus is human and it is the initiating origin of tumor in its host. The virus is reportedly linked with MCC pathogenesis as evident from the monoclonal sequences with evident mutations and expression of T antigens in tumor tissue [2]. Epidemiological studies suggest that most of the adult healthy population is infected with the MCPyV infection during their premature age [3-7]. Therefore, MCPyV infection consequently has a rare incidence of MCC [1-8]. Large and small T antigens (LT and sT-Ag) and structural antigens VP1 and VP2 are coded by MCPyV as of all polyomaviruses. While VP3 encoding by MCPyV is still unknown to us [9]. Polyomavirus T antigens formation occurs due to earlier transcription of an alternatively spliced gene transcript during infection. Thus in MCPyV, a 57K T antigen is formed as a result of alternative splicing of initial transcript [8]. The presence of an alternative open reading frame (ALTO) has been unleashed during recent study, it could be purposely built structure created by T-Ag translational transcripts. The role of ALTO is still unclear though it shows likenesses, with the middle T antigen (mT-Ag) of some other polyomaviruses, in some sequence features. Experimentations unveil that viral DNA replication has not been effected due to existence or nonexistence of ALTO [10]. In addition to the protein products, the MCPyV has been reported with a single encoded microRNA (miRNA) originator which transcribes a couple of mature miRNAs (mcv-miR-M1-3p and mcv-miR-M1-5p) [11]. miRNA is a small fragment of non-coding RNAs (with ~22 nt.) made in result of nucleases Dicer and Drosha sequence processing action on primarily arranged transcripts [12]. After the incorporation of miRNA into RNA-induced silencing complex (RISC), the transcript expression can be destructively regulated by mature miRNA which is identified through complementary sequences. The supposed seed sequence (nt 2-8) pairing of miRNA by flawless Watson - Crick Model, the target site is identified, while in animals sequence complementarity is reduced due to differences in sequences [13]. Partial mRNA pairing can inhibit translational activities of mRNA as a result we can see a minor decrease in translation activity although. In comparison to the rare miRNA activities of animal, plant miRNA affected by animal viruses can also form bonding with target sequences with high precision of complementarity and result into endonucleolytic cleavage of mRNA mediated by RISC.

Latest studies suggest the miRNA encoding by several animal and human polyomaviruses [11,14-19]. Although detected with in various genomic loci, all of the discovered polyomavirus miRNA are sequence encoded, the same sequences are found to be of antisense orientation to the primary expressing transcript of T antigen. Therefore, flawless complementarity to initial transcript is to be found in mature miRNA species encoded by these loci, while a number of studies demonstrated that all known polyomaviruses are able to regulate translational activity of early gene product negatively [11,15,17-20]. During last decade, various techniques like cloning of cDNA and its confirmation using Northern blotting have been used in common for determination of miRNA in different organisms, including viruses also [21-24]. Still the procedures are cost-effective, time consuming and stressful [25]. While some other techniques with more efficiency, convenience and lower cost, such as computer based prediction strategies for novel miRNAs, are available for this purpose.

Methods

Sequence Retrieval

FASTA format of Merkel Cell Polyomavirus Sequence (accession no NC_010277.2) were retrieved from NCBI http://www.ncbi.nlm.nih.gov. The genome size of Merkel Cell Polyoma virus is about 5387.

Pre-miRNA Extraction

The identification of pre-miRNA candidates was performed by specialized ab initio viral pre-miRNAs prediction software VMir on the basis of comparison to structural characteristics of already recognized pre-miRNA hairpins. In order to extract hairpin-structured miRNA precursors, the viral genome was scanned by VMir software (program version 2.3, scoring algorithm version 1.4) [26,27]. The stringency were kept 10% with minimum score of 80. Primarily, potential hairpin shaped sequences were mined as candidate miRNA precursors (pre-miRNAs).

Real pre-miRNA Confirmation

For filtering real pre-miRNA from pseudo pre-miRNA, iMiRNA-SSF web server was used [28].

Identification of Unique and Potential pre-miRNA Structures

RNAfold web server (http://rna.tbi.univie.ac.at//cgi-bin/RNAWebSuite/RNAfold.cgi) was used to predict secondary structure and minimum free energy (MFE). Sequences with hairpin-like secondary structures, and having lower MFE (equal to or less than –25 kcal/mol) acts as potential miRNA precursors. Sequences having hairpin-like structures and lower MFE were selected and were confirmed to be unique by conducting BLASTn searches.

Mature miRNA Prediction

Prediction of mature miRNAs in MCpV was performed on MatureBayes web server v1.0 (http://mirna.imbb.forth.gr/MatureBayes.html). This computer-based web tool uses a Naive Bayes classifier which is based on secondary structure and sequence features of the pre-miRNAs for the prediction of mature miRNAs in any given pre-miRNA [29]. MatureBayes compute the probabilistic start position of mature miRNA(s) by two alternatives.

Targets of the miRNAs

The mature miRNAs were searched in miRbase [30]. For finding its similar miRNA. The similar miRNA found in miRBase were selected for finding target via Target Scan [31].

Results

VMir is a user friendly software especially designed for viral miRNAs identification [26]. By using RNAfold algorithm, VMir executes structure prediction by minimal folding free energy and detects individual hairpins above a certain size limit (by default 45 nt). VMir assigns score to these hairpins which is based on statistical comparison to a reference set of recognized pre-miRNA hairpins [27]. In this study, the MCPyV genome uploaded to VMir Analyzer was in FASTA format. The parameters for the program was set to window size 1000 and allowed to operate within the parameters and step size was adjusted. To extract hairpin like sequences (pre-miRNAs) the viral genomes were scanned in both orientations. Initially 187 sequences were detected by VMir Analyzer as candidate miRNA precursors (Figure 1). A filter was set to pass these 187 pre-miRNA candidates through it. Only 52 hairpins passed the filter. (Figure 2). Figures 1 and 2 show the location and VMir scores for unfiltered and filtered hairpins, respectively. The real and pseudo pre-miRNAs were differentiated through iMiRNA-SSF online web server by providing the selected 52 pre-miRNA nominees [28]. Among 52 filtered sequences, 17 sequences were confirmed real pre-miRNA with fold-back hairpin shaped structures by IMiRNA-SSF. The number of real pre-miRNA decreased to 10 as lower minimum free energy was measured (equal to or less than –25 kcal/mol) and BLASTn was done to find its unique nature.

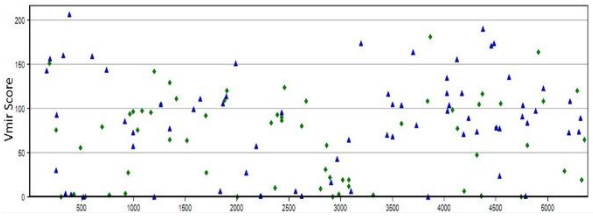

Figure 1: VMir analysis of the MCPyV genome which shows that all hairpins are widely dispersed across the viral genome.

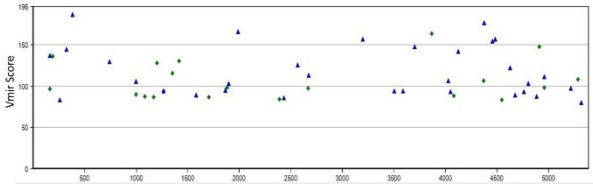

Figure 2: Only those hairpins are shown which passed the filter and attained a VMir score between 85 and 146 and located between nucleotides 200 and 3132. Hairpins are plotted according to genomic position and VMir score.

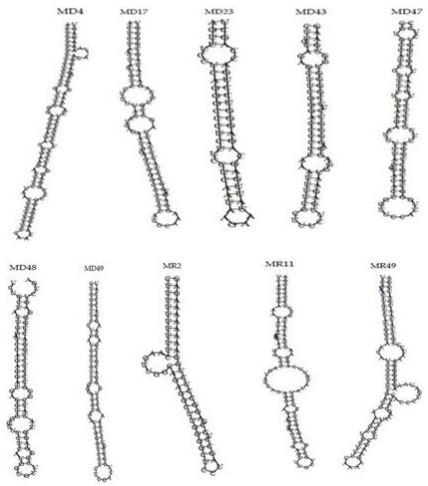

Figure 3: Structures of the miRNAs predicted through RNAFold

>Precursor hairpin:MD4		
Sequence	ACUUUUUUUCUUUCUGUUUGGGAGGGAGACGGAA GACUCUUAACUUUUUUUCAACAAGGGAGGCCCGGA GGCUUUUUUUUCUCUUACAAAGGGAGGAGGACAU UAAAAGAGU	
Duplex	Position 11 (5' stem)	Sequence: UUCUGUUUGGGAGGGAGACGGA
	Position 75 (3' stem)	Sequence: UUUUUCUCUUACAAAGGGAGGA
Mature 5'stem	Position 48	Sequence: UUUCAACAAGGGAGGCCCGGAG
Mature 3'stem	Position 75	Sequence: UUUUUCUCUUACAAAGGGAGGA
>Precursor hairpin: MD17		
Sequence	**ACUCUAACUACUGUCCUUUUAGAUGAGAAUG GAGUGGGCCCUCUAUGCAAAGGAGAUGGCCU AUUUAUUAGCUGUGCAGACAUAGUGGGGU**	
Duplex	Position 21 (5' stem)	Sequence: GAUGAGAAUGGAGUGGGCCCUC
	Position 55 (3' stem)	Sequence: AUGGCCUAUUUAUUAGCUGUGC
Mature 5'stem	Position 25	Sequence: AGAAUGGAGUGGGCCCUCUAUG
Mature 3'stem	Position 55	Sequence: AUGGCCUAUUUAUUAGCUGUGC
>Precursor hairpin: MD23		
Sequence	**AGUUUCCUCAGGGCCCUCUUCCUCAAUAAGAAUA UUGAGCAGAGGGUCCUGACCAGCU**	
Duplex	Position 3 (5' stem)	Sequence: UUCCUCAGGGCCCUCUUCCUCA
	Position 34 (3' stem)	Sequence: UUGAGCAGAGGGUCCUGACCAG
Mature 5'stem	Position 25	Sequence: AUAAGAAUAUUGAGCAGAGGGU
Mature 3'stem	Position 34	Sequence: UUGAGCAGAGGGUCCUGACCAG
>Precursor hairpin: MD43		
Sequence	**CUUGCAGAGCUUCGGGACCCCCCAAAUUUUCGCU UUCUUGAGAAUGGAGGAGGGGUCUUCGGGGUGGU GAAG**	
Duplex	Position 12 (5' stem)	Sequence: CGGGACCCCCCAAAUUUUCGCU
	Position 40 (3' stem)	Sequence: AGAAUGGAGGAGGGGUCUUCGG
Mature 5'stem	Position 5	Sequence: AGAGCUUCGGGACCCCCCAAAU
Mature 3'stem	Position 40	Sequence: AGAAUGGAGGAGGGGUCUUCGG

>Precursor hairpin: MD47		
Sequence	**GGUGCCAUCCGUUCUGGAAGAAUUUCUAGGUACA CUGGUUCCAUUGGGUGUGCUGGAUUCUCUUCCUG AAUUGGUGGUCUCC**	
Duplex	Position 12 (5' stem)	Sequence: UCUGGAAGAAUUUCUAGGUACA
	Position 49 (5' stem)	Sequence: GUGCUGGAUUCUCUUCCUGAAU
Mature 5'stem	Position 30	Sequence: UACACUGGUUCCAUUGGGUGUG
Mature 3'stem	Position 49	Sequence: GUGCUGGAUUCUCUUCCUGAAU
>Precursor hairpin: MD48		
Sequence	**UCUGCUACUGGAUCCAGAGGAUGAGGUGGGUUCC UCAUGGUGUUCGGGAGGUAUAUCGGGUCCUCUGG ACUGGGAGUCUGA**	
Duplex	Position 12 (5' stem)	Sequence: UCCAGAGGAUGAGGUGGGUUCC
	Position 48 (5' stem)	Sequence: AGGUAUAUCGGGUCCUCUGGAC
Mature 5'stem	Position 14	Sequence: CAGAGGAUGAGGUGGGUUCCUC
Mature 3'stem	Position 48	Sequence: AGGUAUAUCGGGUCCUCUGGAC
>Precursor hairpin:MD49		
Sequence	**GUGGGUUCCUCAUGGUGUUCGGGAGGUAUAUCGG GUCCUCUGGACUGGGAGUCUGAAGCCUGGGACGC UGAGAAGGACCCAU**	
Duplex	Position 14 (5' stem)	Sequence: GUGUUCGGGAGGUAUAUCGGGU
	Position 48 (3' stem)	Sequence: GAGUCUGAAGCCUGGGACGCUG
Mature 5'stem	Position 36	Sequence: CCUCUGGACUGGGAGUCUGAAG
Mature 3'stem	Position 48	Sequence: GAGUCUGAAGCCUGGGACGCUG
>Precursor hairpin: MR2		
Sequence	**CUUUUUUUUUUCAAGUUGGCAGAGGCUUGGGGCU CCUAGCCUCCGAGGCCUCUGGAAAAAAAAGAG**	
Duplex	Position 2 (5' stem)	Sequence: UUUUUUUUUCAAGUUGGCAGAG
	Position 43 (5' stem)	Sequence: CGAGGCCUCUGGAAAAAAAAGA
Mature 5'stem	Position 25	Sequence: CUUGGGGCUCCUAGCCUCCGAG
Mature 3'stem	Position 43	Sequence: CGAGGCCUCUGGAAAAAAAAGA
>Precursor hairpin: MR11		
Sequence	**UCAGGAGAAAUAUAUCCACUAAGGCCUAGUACCA GAGGAAGAAGCCAAUCUGGAGUUUGCUGCUGCAG AGUUCCUCCUAUAUGUUCAGGAAUUAAUAUAGCC UCUCCUGA**	
Duplex	Position 2 (5' stem)	Sequence: AGGAGAAAUAUAUCCACUAAGG
	Position 87 (5' stem)	Sequence: GGAAUUAAUAUAGCCUCUCCUG
Mature 5'stem	Position 52	Sequence: GAGUUUGCUGCUGCAGAGUUCC
Mature 3'stem	Position 87	Sequence: GGAAUUAAUAUAGCCUCUCCUG
>Precursor hairpin: MR49		
Sequence	**UCCUAAUUGUUAUGGCAACAUCCCUCUGAUGAAA GCUGCUUUCAAAAGAAGCUGCUUAAAGCAUCACC CUGAUAAAGGGGGAAAUCCUGUUAUAAUGAUGGA**	
Duplex	Position 7 (5' stem)	Sequence: UGUUAUGGCAACAUCCCUCUGA
	Position 76 (5' stem)	Sequence: GGGGGAAAUCCUGUUAUAAUGA
Mature 5'stem	Position 17	Sequence: ACAUCCCUCUGAUGAAAGCUGC
Mature 3'stem	Position 76	Sequence: GGGGGAAAUCCUGUUAUAAUGA

Table 1: Mature miRNA sequences predicted by MatureBayes web tool. Mature miRNAs are highlighted.

RNAfold program was used to accomplish the confirmation of potential hairpin-like secondary structures in these sequences (Figure 3).

Sr. No	Homologous miRNA in Humans	Target gene	Gene name	Score
1	MD17	NNAT	Neuronatin	-1.08
		LDOC1L	Leucine zipper, down-regulated in cancer 1-like	-0.51
		BCAS4	Breast carcinoma amplified sequence 4	-0.07
		MCTS1	Malignant T cell amplified sequence 1	-0.07
2	MD23	RP11-204N11.1	Uncharacterized protein	-0.81
		BCAS1	Breast carcinoma amplified sequence 1	-0.03
		TSSC1	Tumor suppressing subtransferable candidate 1	-0.35
		COLCA1	Colorectal cancer associated 1	-0.40
3	MD43	GAP43	Growth associated protein 43	-0.80
		COLCA1	Colorectal cancer associated 1	-0.19
		BCAS1	Breast carcinoma amplified sequence 1	-0.06
		TPD52	Tumor protein D52	-0.41
		NAT8	N-acetyltransferase 8 (GCN5-related, putative)	-1.28
		BRMS1	Breast cancer metastasis suppressor 1	-0.24
		LCA10	Putative lung carcinoma-associated protein 10	-0.22
		TNFRSF10B	Tumor necrosis factor receptor superfamily, member 10b	-0.28
4	MR11	HIGD1B	HIG1 hypoxia inducible domain family, member 1B	-0.79
		BRMS1L	Breast cancer metastasis-suppressor 1-like	-0.40
		ST13	Suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein)	-0.33
		TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	-0.34
		KCNJ6	Potassium inwardly-rectifying channel, subfamily J, member 6	-1.06
		PBOV1	Prostate and breast cancer overexpressed 1	-0.50
		BCAS1	Breast carcinoma amplified sequence 1	-0.21
		TNFSF15	Tumor necrosis factor (ligand) superfamily, member 15	-0.35
		MFHAS1	Malignant fibrous histiocytoma amplified sequence 1	-0.07
5	MD4	LINC00632	Long intergenic non-protein coding RNA 632	-1.34
		CASC3	Cancer susceptibility candidate 3	-0.28
		TDGF1	Teratocarcinoma-derived growth factor 1	-0.43
		TP53INP2	Tumor protein p53 inducible nuclear protein 2	-0.85
		MCTS1	Malignant T cell amplified sequence 1	-0.28
6	MD49	TMED7-TICAM2	TMED7-TICAM2 read through	-0.64
		ST5	Suppression of tumorigenicity 5	-0.18
		TMED7-TICAM2	TMED7-TICAM2 read through	-0.64
		ST5	Suppression of tumorigenicity 5	-0.18
		TMED7-TICAM2	TMED7-TICAM2 read through	-0.64

Table 2: The cancer causing top target gene

Mature miRNA sequences inside pre-miRNA hairpin was identified by MatureBayes web server [29]. 10 mature miRNA within the 10 duplexes were predicted by this computational tool (Table 1). To find the similar sequences in human these 10 mature miRNAs were screened in mirBAse. The homologous miRNA sequences were selected for their target recognition. The genes targeted by miRNAs, were found by scanning the sequences in Target scan. The cancer causing top target gene are shown in table 2.

Discussion

In this study, the MCPyV genome was analyzed through several bioinformatics tools. Resulting into the recognition of 10 mature miRNAs. Among these 10 mature mi-RNAs, 2 were reported before and the target genes for these sequences were identified regarding their similarity bases with other miRNAs.

Among the other different causes of cancer, viruses are being reported as the most vital source of cancer development. According to the reports of International Agency for Research on Cancer, up to 20% of cancer cases around the world are due to the infection of viruses [32]. Currently, 10%-15% human cancers are caused by seven human oncogenic viruses. Merkel cell carcinoma

is a rare and highly malignant primary neuroendocrine carcinoma of the skin. Over 90% of cases arise in sun-exposed areas, with half around the cancer near to areas of head and neck. This indicates, in development of MCC, sunlight specifically the ultraviolet radiation play a key role. MCC can also be observed on the torso (trunk of the human body) and genitals with more reduced frequency (<10%) [33]. Numerous polyomaviruses, including John Cunningham Virus (JCV), simian virus 40 (SV40) and BK Virus (BKV) encodes miRNA and control viral transcript level at earlier stage [17]. Therefore, it is predictable that, In addition to the structural proteins, a 22 nucleotide long miRNA, MCV-miR-M1-5p are also encoded by late region [11]. The miRNAs are encoded antisense to the LT coding region. Complementary to a segment of the LT transcript, it regulates the early gene expression by limiting the early gene transcript. In at least half of the MCPyV-positive MCC tumors its expression is conserved, and it can play role in cellular remodeling [34].

In this study, a number of methodologies were applied for the prediction of miRNAs that were previously unidentified. The MCV genome was taken and run on VMir. The resulted 52 pre-miRNAs were obtained after the filters were applied. Pseudo miRNA was eliminated and real pre-miRNA was selected by using iMiRNA-SSF web server. The possible potential miRNA precursors were predicted using RNAfold. For further analysis miRNAs with less than -25 kcal/mol energy were selected. For mature miRNA prediction, MatureBayes web server was used. In previous study on HBV genome, 12 mature miRNAs were predicted in 6 potential pre-miRNA candidates [35]. Through target scan the mature miRNAs were searched for homologous miRNAs in miRBase, the target of the related miRNAs found in miRBase were searched. In most of the patients' current therapies for MCV infection does not work. For novel antiviral therapeutic strategies improved information on MCV-host interaction is compulsory. The biological significance of miRNA can be defined by the relationship of viral encoded miRNAs and pathogenic characteristics of virus, this signifies the role of miRNA in therapeutic activities against a great number of diseases and can triggered a whole new era of treatment with high influence and good mechanism. In silico prediction of miRNAs is just the beginning of miRNA study and should be employed by other investigations such as target and function determination for an extensive understanding of its biological significance.

This study provides an insight to the prediction of miRNAs of MCV and its similar miRNAs in Humans and its target by using several bioinformatics tools.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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