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In silico Analysis of Human microRNAs Targeting Influenza a Viruses (subtype H1N1, H5N1 and H3N2)

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***Abstract*—**In this study, three subtypes of influenza A viruses (pH1N1, H5N1 and H3N2) which naturally infected human were analyzed by bioinformatic approaches to find candidate human cellular miRNAs targeting viral genomes. There were 76 miRNAs targeting influenza A viruses. Among these candidates, 70 miRNAs were subtypes specifically targeting each subtype of influenza A virus including 21 miRNAs targeted subtype H1N1, 27 miRNAs targeted subtype H5N1 and 22 miRNAs targeted subtype H3N2. The remaining 6 miRNAs target on multiple subtypes of influenza A viruses. Uniquely, hsa-miR-3145 is the only one candidate miRNA targeting PB1 gene of all three subtypes. Obviously, most of the candidate miRNAs are targeting on polymerase complex genes (PB2, PB1 and PA) of influenza A viruses. This study predicted potential human miRNAs targeting on different subtypes of influenza A viruses which might be useful for inhibition of viral replication and for better understanding of the interaction between virus and host cell.

***Keywords*—**Human miRNAs, Influenza A viruses, H1N1, H5N1, H3N2

I. Introduction

MicroRNAs (miRNAs) are small non-coding RNAs with approximately 22 nucleotides in length which play an important role in regulation of gene expression. [1, 2] The miRNAs biosynthesis transpires originally in nucleus where hundreds and thousands of nucleotides with hairpin structures, called primary miRNAs (pri-miRNAs) were transcribed. Then the primary miRNAs are cropped and trimmed to 60 to 100 nucleotides long with a stem loop structure called precursor miRNAs (pre-miRNAs). The pre-miRNAs are then exported to the cytoplasm by Exportin-5 and then processed by Dicer containing RNaseIII endonuclease activity. [3] The Dicer removes the loop region of the hairpin, and releases the mature miRNA duplexes which approximately 22 nucleotides in length with 2 nucleotides overhanging on both 5' and 3' ends. As soon as the miRNA duplexes assembled with RNA-induced silencing complex (RISC) and then one strand of miRNA is removed by a helicase activity of the RISC, the remaining miRNA strand guides the RISC to a distinctive target mRNA via base pairing. [4] A perfect complementary balancing between miRNA and target mRNA leads to mRNA degradation. However a partial balancing will lead to in translational repression. Therefore, miRNAs play an imperative and foremost undertaking in the regulation of gene expression in terms of gene silencing. [5]

Effective mature miRNAs distinguished their target miRNAs based on specific nucleotide complementary balancing mainly at position 2nd -8th from 5' end of the miRNAs which termed seed region. [6,7] According to previous studies, the binding between miRNAs and target mRNAs can be categorized into 3 distinctive patterns including 5' canonical, 5' seed and 3' compensatory. The 5' canonical pattern encompasses base-parings at least seven nucleotides within a seed region and a supplementary base-pairings in the 3'-end of the miRNAs.

The 5'seed pattern predominantly comprises of only the base-paring within the seed region without any support from the base complement within the 3'-end. The enhanced 3' base pairings in a canonical pattern are likely to be more effective that is attributable to their higher pairing energy. In contrast, the 3' compensatory pattern has no effective base paring within the seed region and requires several base pairing from the middle to 3'-end of miRNA to function. [8]

Human miRNAs implicated in many cellular processes such as cell proliferation, apoptosis and homeostasis. [9] In addition, many reports conjured up that miRNAs also engage in an role of great magnitude in regulation of viral infection and interplay between virus and host cell response. Aforementioned reports described viral encoded miRNAs from DNA and RNA viruses including herpesviruses (HSVs) [10] Epstein–Barr-Virus (EBV) [11], Simian Virus 40 (SV40) [12] and human immunodeficiency virus-1 (HIV-1) [13]. In contrast, host cellular miRNAs can also target viral gene and involve with the replication of many incoming viruses such as primate foamy virus type 1 (PFV-1) [14], vesicular stomatitis virus (VSV) [15] and hepatitis C virus (HCV) [16].

Influenza A viruses contain negative single strand RNA genome and are compartmentalized in to the Orthomyxoviridae family. [17] During infection in human, they affect the upper respiratory system and cause either asymptomatic, mild or severe symptoms including high fever, coughing, sneezing, nasal congestion, running nose,

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pneumonia and diarrhea. [18] Predominantly, influenza A virus can be classified into various subtypes based on the antigenic differences between hemagglutinin (HA) and neuraminidase (NA) glycoprotein. However, H1N1 [19,20], H3N2 [21] and H5N1 [22] subtypes were found to naturally infect humans and cause serious global health problems. Therefore, this study focused on these subtypes of influenza A virus and aimed at identification of human cellular miRNAs targeting the genome of influenza A viruses which might be useful for understanding the host defense mechanism in terms of regulating viral infection.

II. Method

A. Viral Nucleotide Sequences

Viral nucleotide sequences were downloaded from NCBI database. Three subtypes of influenza A viruses including A/Thailand/104/2009 (H1N1), A/Thailand/NK165/2005 (H5N1) and A/Thailand/CU-H187/2010 (H3N2) which naturally affect humans were taken account of in this study. Accession numbers of complete coding sequences of each gene (PB2, PB1, PA, HA, NP, NA, M and NS) for each subtype were presented in Table I.

TABLE I
Accession Numbers of Viral Nucleotide Sequences in This Study

subtypes / genes	H1N1	H5N1	H3N2
PB2	GQ205443	DQ372598	CY074963
PB1	GQ259597	DQ372597	CY074964
PA	GQ169383	DQ372596	CY074965
HA	GQ169382	DQ372591	CY074966
NP	GQ169385	DQ372594	CY074967
NA	GQ169381	DQ372593	CY074968
M	GQ169384	DQ372592	CY074969
NS	GQ229379	DQ372595	CY074970

B. Searching for Candidate Human miRNAs

Totally, 1921 human miRNAs sequences are available in the miRBase database [23-26] (http://www.mirbase.org). Based on the average length of miRNA (approximately 22 nucleotides), gene segments of influenza A virus were divided into small fragments with 50 nucleotides (50 bp) in length with 25 nucleotides overlapping between adjacent fragments. Then each small fragment (50 bp) was input and circumspectly examined for nucleotide similarity with all human miRNAs by using "SSEARCH" method in a search tool of the miRBase (www.mirbase.org/search.shtml). In principle, each of the input viral fragment sequence (50 bp) was align with all of the miRNAs in the database and then the miRNAs with highly similar to the viral sequence were identified as candidate miRNAs. Customarily, the mature miRNAs duplex structure consists of two strands of miRNAs that are practically perfect complement to each other. The complementary strand of the candidate miRNAs might complement the inserted viral sequence. Therefore, prediction for hybridization between the viral gene sequence and complementary strand of the candidate miRNA was further analyzed by RNA hybrid.

C. Prediction of Hybridization between miRNAs and Viral RNA

RNA hybrid [27] (http://bibiserv.techfak.unibielefeld.de/ rnahybrid/) was used as a tool to predict the energetically most favorable hybridization between candidate miRNAs and viral RNAs. Subsequently, the results were characterized in terms of hybridization pattern and pairing energy (mfe). The hybridization patterns obtained from RNAhybrid were classified into 4 categories including 5'canonical, 5'seed, 3' compensatory and ineffective hybridization.

Criteria for selection of potential miRNAs

According to the principles of miRNAs target recognition which requires the sufficient base pairing between the miRNAs and their target mRNAs that can be classified into 5'canonical, 5'seed and 3' compensatory [8]. The principle was cogitated to be a foremost criterion for the selection of the potential miRNAs . For 5' dominant classes of target sites that can be divided into 2 subtypes: 5' canonical and 5'seed as described previously, both must indicate the effective base pairing within the 2nd to 8th position from the 5' portion of the miRNAs. For the pattern of 3' compensatory, the candidate miRNAs should show at least half of the sequence from middle to 3' portion of the miRNAs that will perfectly coordinate with the target. Another criterion involved with the pairing energy indicating the stability of the hybridization is the pairing energy or minimum free energy (mfe) at -10 kcal/mol that was utilized for the selection of potential miRNAs. In conclusion, the miRNAs targeting influenza viral gene with effective hybridization patterns (5'canonical, 5'seed or 3' compensatory) and paring energy less than -10 kcal/mol were selected as potential miRNAs. The miRNAs with ineffective hybridization or unsuitable pairing energy were excluded from the study.

III. Results and Discussion

A. Specific miRNAs Targeting Influenza A Virus Subtype H1N1

From 1,921 mature human miRNAs in miRBase database, 25 miRNAs were predicted as potential miRNAs targeting influenza A virus subtype H1N1 (A/Thailand/104/2009). The details of hybridization patterns and the paring of energy between each miRNA and target viral gene were summarized in Table II. These 25 miRNAs can be divided into 3 groups according to the patterns of hybridization including 5'canonical (16 miRNAs), 5'seed (5 miRNAs) and 3'compensatory (4 miRNAs). In addition, the cellular miRNAs were mostly found to target the polymerase genes of H1N1 influenza A virus (5 miRNAs for PB2, 5 miRNAs for PB1 and 6 miRNAs for PA) whereas a few miRNAs were observed to target other genes (4 miRNAs for NP, 2 miRNAs for NS, 1 miRNAs for HA and only 2 miRNA for NA). No predicted miRNA targeted to the M gene of H1N1 influenza A virus. The numbers of cellular miRNAs targeting each gene of H1N1 influenza A virus were shown in Table V.

TABLE V
AMOUNT OF CELLULAR MIRNAS TARGETING EACH GENE OF INFLUENZA A VIRUS

Segment / Subtype	PB2	PB1	PA	HA	NP	NA	M	NS	Genome
H1N1	5	5	6	1	4	2	0	2	**25**
H5N1	9	9	3	4	2	2	1	1	**31**
H3N2	6	5	7	2	1	3	0	3	**27**
Total	**20**	**19**	**16**	**7**	**7**	**7**	**1**	**6**	**83**

B. References Specific miRNAs targeting influenza A virus subtype H5N1

According to the result of hybridization pattern and pairing energy between human miRNAs and their target viral gene, 31 cellular miRNAs were analyzed as potential miRNAs targeting influenza A virus subtype H5N1 (A/Thailand/NK165/2005). Table III illustrates in details of the hybridization pattern and paring energy between each miRNA and target viral gene. These miRNAs were classified as 5'canonical (23 miRNAs), 5'seed (only 1 miRNA) and 3' compensatory (7 miRNAs) based on their hybridization patterns with target viral gene. Moreover, these 31 miRNAs were found to be predominantly targeted to PB2 and PB1 (9 miRNAs for each gene) of H5N1 influenza A virus. The other genes were significantly less targeted by miRNAs (4 targets in HA, 3 targets in PA, 2 targets in each NP and NA and only 1 target for each M and NS). Table V summarizes the numbers of cellular miRNAs targeting each gene of H5N1 influenza A virus.

C. Abbreviations and Acronyms Specific miRNAs Targeting Influenza A Virus Subtype H3N2

Table IV demonstrates the result of hybridization patterns and pairing energy between potential cellular miRNAs and their target H3N2 influenza viral genes. Based on our analysis and prediction, there were 27 miRNAs targeting influenza A virus subtype H3N2 (A/Thailand/CU-H1817/2010). There were 19 and 8 miRNAs targeting viral gene with 5'canonical and 3'compensatory hybridization pattern, respectively. In spite of this, there was no miRNA targeting the subtype H3N2 influenza viral gene with 5'seed pairing pattern.

Furthermore, these 27 miRNAs were obviously targeted to polymerase genes of H3N2 influenza A virus (6 miRNAs for PB2, 5 miRNAs for PB1 and 7 miRNAs for PA). Instead, only a few of miRNAs were found to target other genes (3 miRNAs for each NA and NS, 2 miRNAs for HA, and only 1 miRNAs for NP). None of the predicted miRNA targeted the M gene of H3N2 influenza A virus. Table V indicates the numbers of potential miRNAs targeting each gene of H3N2 influenza A virus.

D. Equations Potential miRNAs Targeting Multiple Subtypes of Influenza A Virus

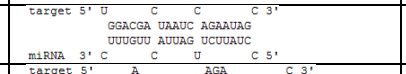
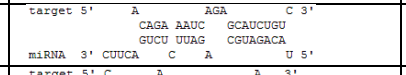
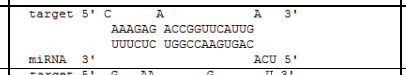
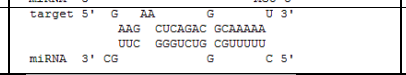
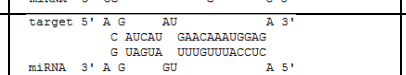
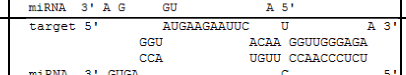
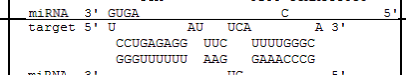
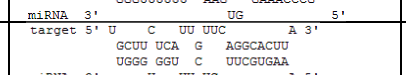
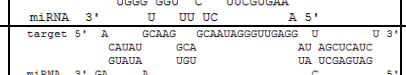
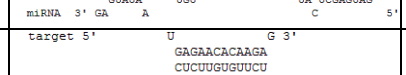
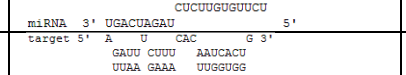
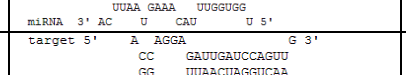
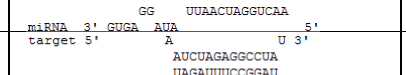
As revealed in the Table II, III and IV, miRNAs targeting multiple subtypes of influenza A virus is being marked with an asterisk (*) sign at the name of each miRNA. There were 6 miRNAs targeting multiple subtypes of influenza A viruses including hsa-miR-4753, hsa-miR-3682, hsa-miR-4513, hsa-miR-216b, hsa-miR-5693 and hsa-miR-3145. The hsa-miR-4753 targeted to PB1 gene of H1N1 subtype and PA gene of H5N1 subtype. The hsa-miR-3682 was anticipated as a potential miRNA for pairing to NA gene of H1N1 subtype and NS gene of H3N2 subtype. The hsa-miR-4513 was analyzed as a potential miRNA to hybridize with PA gene of both H1N1 and H3N2 subtypes. The hsa-miR-216b and hsa-miR-5693 targeted both H5N1 and H3N2 subtypes that complements with NA and PA gene, respectively. Finally, the hsa-miR-3145 was the only potential miRNA targeting all three subtypes (H1N1, H5N1 and H3N2) of influenza A virus. This subtype targeted to PB1 gene of H1N1, H5N1 and H3N2 subtypes with similar paring energy (-18.2, -18.2 and -18.1 kcal/mol, respectively). Interestingly, the 5' portion (the 1st to 12th nucleotides from 5' end) of hsa-miR-3145 (5'-AGAUAUUUUGAG-3') targeted to similar region within PB1 gene for all three viral subtypes. It seemed that this targeting region is highly conserved in the PB1 gene among different subtypes of influenza A viruses. Therefore, hsa-miR-3145 might be the human cellular miRNA targeting PB1 gene of influenza A viruses and might be involved in the inhibition of viral replication.

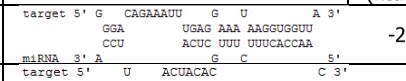
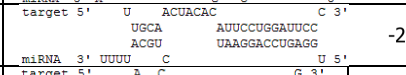
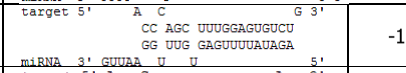
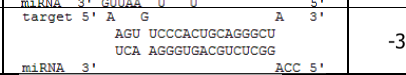
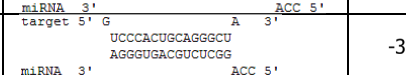
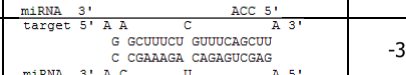
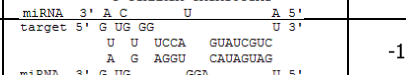
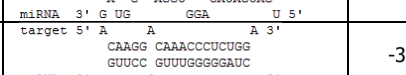
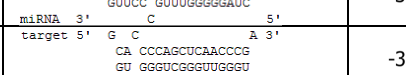
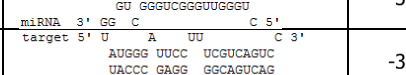
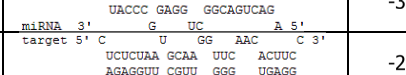
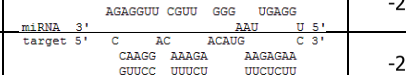
E. Viral Genes Targeted by Human Cellular miRNAs

Table V shows the amount of cellular miRNAs targeting influenza viral genes. PB2 genes became the most targeting sites for 20 miRNAs to bind to. PB1 and PA genes had 19 and 16 targeting sites for miRNAs, respectively. These three genes show the most targeting regions for human cellular miRNAs as 55 miRNAs from total 83 predicted miRNAs (66.67%). These three genes encoded for polymerase enzyme complex which are necessary for viral replication and therefore conserved among different subtypes. Moreover, these genes are the 3 longest genes with 2.2-2.3 kb in length. These may be the reason why most predicted human miRNAs can target these genes of influenza A virus.

Previous study confirmed that PB1 gene of H1N1 influenza A virus (A/WSN/1933) was the specific target for human miRNAs: hsa-miR-323, hsa-miR-491 and hsa-miR-654. [28] However, these 3 miRNAs was not predicted as potential miRNAs targeting H1N1 human pandemic influenza (A/Thailand/104/2009), H5N1 avian influenza (A/Thailand/NK165/2005) and H3N2 seasonal influenza (A/Thailand/CU-H1817/2010) in our study may be due to viral genetic variation among different subtypes (H3N2 and H5N1) and accumulations of point mutations. The viral genome observed in this study was more than 75 years and has different form of the H1N1 influenza A virus (A/WSN/1933) and thus the viral genome became significantly different. Even the "A/WSN/1933" and "A/Thailand/104/2009" are belong to the same subtype but they also contain different viral genome because of the human pandemic influenza subtype H1N1 (A/Thailand/104/2009) that was a new re-assorted virus containing combined genetic materials from human, avian, and swine influenza A viruses. [29] Therefore, the prediction of potential miRNAs targeting multiple subtypes of influenza A virus seems to be more useful than determination of miRNAs targeting individual subtypes.

TABLE II
PREDICTED CELLULAR MIRNAS TARGETING H1N1 INFLUENZA A VIRUS (A/THAILAND/104/2009)

Human miRNAs	Target viral gene	Hybridization	Pairing energy (kcal/mol)	Pairing pattern
hsa-miR-26a-2	NP 196-215		-20.5	5'canonical
hsa-miR-105	NP 104-122		-23.1	5'canonical
hsa-miR-128	PA 364-382		-31.8	3’-compen
hsa-miR-129	PB1 2276-2295		-25.2	5'canonical
hsa-miR-136	NP 58-78		-28.2	5'canonical
hsa-miR-150	PB2 1104-1132		-29.1	5'canonical
hsa-miR-186	PB2 2098-2122		-25.2	5'canonical
hsa-miR-520d	PA 2022-2043		-19.5	5'canonical
hsa-miR-556	PB2 955-993		-20.3	5'canonical
hsa-miR-581	NA 681-692		-23.8	5'seed
hsa-miR-876	PB1 915-933		-14.2	5'canonical
hsa-miR-1243	PB2 1161-1179		-24.3	5'seed
hsa-miR-1245b	HA 1055-1067		-25.3	5'seed

Human miRNAs	Target viral gene	Hybridization	Pairing energy (kcal/mol)	Pairing pattern
hsa-miR-1255b	NS 708-736		-25.4	5'canonical
hsa-miR-1289	PB1 2004-2027		-29.0	5'canonical
hsa-miR-3145*	PB1 1552-1569		-18.2	5'seed
hsa-miR-3155a	PA 1374-1392		-36.9	3'compen
hsa-miR-3155b	PA 1378-1392		-35.4	3'compen
hsa-miR-3160	NS 841-860		-30.7	5'canonical
hsa-miR-3682*	NA 1011-1028		-19.1	5'canonical
hsa-miR-4278	PB2 245-262		-31.2	5'canonical
hsa-miR-4507	PB1 212-228		-34.4	5'seed
hsa-miR-4513*	PA 588-608		-32.3	5'canonical
hsa-miR-4653	NP 529-553		-20.1	3'compen
hsa-miR-4753*	PA 1091-1114		-20.9	5'canonical

* miRNAs targeting multiple subtypes of influenza A viruses; 3’ compen abbreviated for 3’ compensatory

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TABLE III
PREDICTED CELLULAR MIRNAS TARGETING H1N1 INFLUENZA A VIRUS (A/THAILAND/104/2009)

Human miRNAs	Target viral gene	Hybridization	Pairing energy (kcal/mol)	Pairing pattern
hsa-miR-92a	PB2	target 5' A C G 3' AUGGGCCGG GACAAGUGCA UGUCCGGCC CUGUUCACGU miRNA 3' UAU 5'	-39.2	5'canonical
hsa-miR-127	PB1	target 5' G GCAAC C U 3' GGCCA AGCUCAGAUGG UCU UCGGU UCGAGUCUGCC AGG miRNA 3' U CU 5'	-33.8	3'compen
hsa-miR-146a	PB2	target 5' G A C 3' UCUG GGA UCAGUUCUU GGGU CCU AGUCAAGAG miRNA 3' UU A UA U 5'	-21.7	5'canonical
hsa-miR-216b*	NA	target 5' A G G A 3' UCACAU UGU UGCAGGGAU AGUGUA ACG ACGUCUCUA miRNA 3' A G AA 5'	-31.3	5'canonical
hsa-miR-300	PB1	target 5' G CA CA C 3' GAUGAAGAUUAC GGG GA CUACUUCUAAUG CCC CU miRNA 3' A CC UC 5'	-25.4	3'compen
hsa-miR-367	PB2	target 5' U GCAAU UG G 3' GAG CAU UAGCAAUGGU CUC GUA AUCGUUGUCA miRNA 3' U AAC UA 5'	-21.0	5'canonical
hsa-miR-371b	PB1	target 5' A A A A 3' GAAGUGCUGC AUCU UUUGAG UUUCACGGCG UAGA AAACUC miRNA 3' G A 5'	-30.8	5'canonical
hsa-miR-432	HA	target 5' A A CA C 3' GUGGAG AGCC AUCCAG UACCUC UCGG UAGGUC miRNA 3' UCUG C 5'	-28.6	5'canonical
hsa-miR-520e	PB2	target 5' U G AAGGAA A 3' CC UCAAA GAGGAAGUGCUU GG AGUUU UUCCUUCGUGAA miRNA 3' G A 5'	-27.1	5'canonical
hsa-miR-548c	NS	target 5' A CUUCAG U A 3' GCAAA UG GAUUUUUG CGUUU AC CUAAAAAC miRNA 3' UCAUUA U 5'	-17.6	5'canonical
hsa-miR-548y	PB1	target 5' C A 3' GGCAAAAAUAGUGA CCGUUUUUGUCACU miRNA 3' AAUGAAAA 5'	-25.0	3'compen
hsa-miR-550a	PB2	target 5' A A A G 3' AUGU C CUGAG GGAGUAAGA UACA G GACUC CCUCAUUCU miRNA 3' C GU 5'	-29.7	5'canonical
hsa-miR-550b	PB2	target 5' A AAA CA A G 3' CAG UGU CUGAG GGAGUAAGA GUC ACG GACUC CCUCAUUCU miRNA 3' 5'	-29.0	5'canonical
hsa-miR-576	HA	target 5' A AAUAA UA C 3' GAGGA GUGGAG AAAUUGGAAU UUUCU CACCUC UUUAAUCUUA miRNA 3' G 5'	-25.7	5'canonical
hsa-miR-660	PA	target 5' A A ACC G A 3' AACUUU GA UAUGU GAUGG UUGAGG CU AUACG UUACC miRNA 3' G CAU 5'	-16.9	5'canonical
hsa-miR-670	M	target 5' U G U 3' GCC GC UACG CAGGGG UGG UG AUGU GUCCCU miRNA 3' G U GA G 5'	-21.3	5'canonical

Human miRNAs	Target viral gene	Hybridization	Pairing energy (kcal/mol)	Pairing pattern
hsa-miR-1248	PA	target 5' G U AA A 3' AGCACA UGC GUAUGAGAAGG UCGUGU ACG UAUGUUCUUCC miRNA 3' AAA C AA A 5'	-32.3	5'canonical
hsa-miR-1282	PB1	target 5' U AAUUGAAGA G 3' GCAGAAAGAGGCA GACGA CGUCUUUUUCCGU UUGCU miRNA 3' UU 5'	-30.1	3'compen
hsa-miR-3145*	PB1	target 5' C UAUGGAGCUGCCC GU G 3' CAAUUUCAG A CUUGGAGUGUCU GUUAAGGUU U GAGUUUUAUAGA miRNA 3' GU 5'	-20.7	5'canonical
hsa-miR-3189	PB2	target 5' G UAUCUCCCGAAGAG CGAA A 3' UACUCU GUCAG ACCCAGGG AUGGGG UAGUC UGGGUUCC miRNA 3' G C 5'	-27.6	5'canonical
hsa-miR-3916	NP	target 5' A AC C 3' ACUGCUGGUCUU UCA UGACGACCAGAA AGU miRNA 3' GUC CCCGGU 5'	-27.9	3'compen
hsa-miR-3925	PB2	target 5' G A 3' AGGCUUCACUUUCA UCCGAGGUGAAAGU miRNA 3' CAAGAGAA 5'	-27.7	3'compen
hsa-miR-3975	PB1	target 5' A A C 3' UAGUG AUUAGCUUC AUCAC UAAUCGGAG miRNA 3' CACUUC G U 5'	-22.1	5'seed
hsa-miR-4260	NA	target 5' C A AC C U 3' GG GAC UCCA GCCCCAA CC CUG AGGU CGGGGUU miRNA 3' A A C 5'	-30.3	5'canonical
hsa-miR-4646	PB1	target 5' A A U C 3' G GAAGGG GAGGGACAA C CUUCCC CUCCCUGUU miRNA 3' GA C U A 5'	-33.7	5'canonical
hsa-miR-4668	NP	target 5' U U A 3' CUGGAGAGGCGAAAA GGA GACCUUUUUGUUUUU CCU miRNA 3' AAAAG 5'	-26.0	3'compen
hsa-miR-4728	HA	target 5' A U C U 3' GUAGGAG GAG UCAGCAUG CGUCCUC CUC AGUCGUAC miRNA 3' GACCC C C 5'	-33.3	5'canonical
hsa-miR-4753*	PB1	target 5' A UUU C G 3' ACACA C AGAGAAAGAGAA UGUGU G UUUCUUUCUCUU miRNA 3' UCC A 5'	-20.9	5'canonical
hsa-miR-4769	HA	target 5' C UUUUAUA G 3' AGG GAGGGAGGAUGGCAGG UCC CUCCCUCCUACCGUCU miRNA 3' CA C 5'	-40.0	5'canonical
hsa-miR-5006	PB2	target 5' U AUACAUGU C 3' UGC UGGAAAGGGAA ACG ACCUUUCCCUU miRNA 3' G GUCCU U 5'	-26.2	5'canonical
hsa-miR-5693*	PA	target 5' C G A 3' CAUU CAGGGCCACUG GUAA GUCUCGGUGAC miRNA 3' CUCAA A G 5'	-28.7	5'canonical

* miRNAs targeting multiple subtypes of influenza A viruses; 3' compen abbreviated for 3' compensatory

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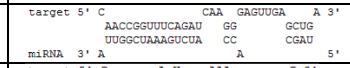
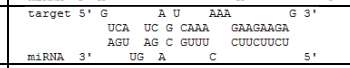
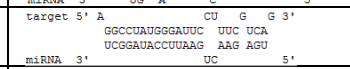
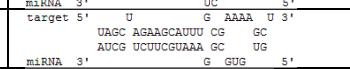
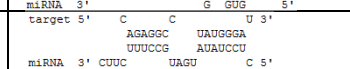
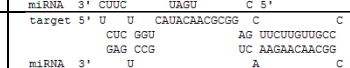
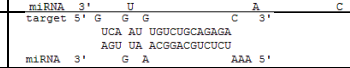
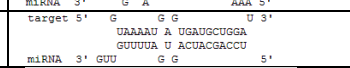
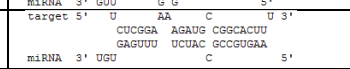
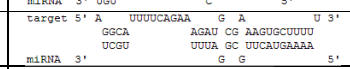
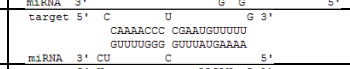
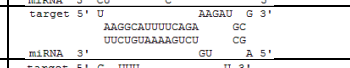
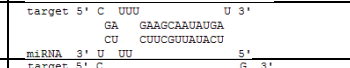
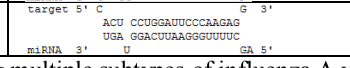
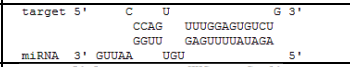
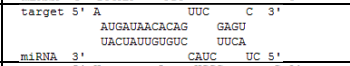
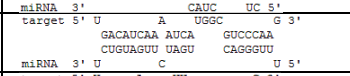
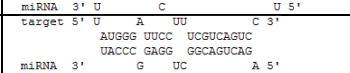
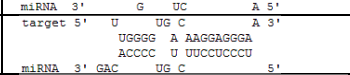
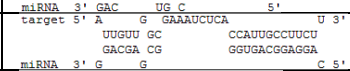
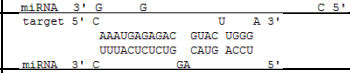
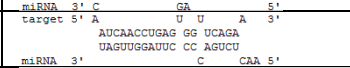
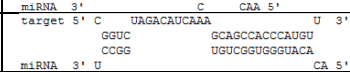
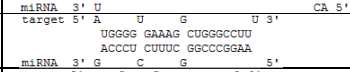
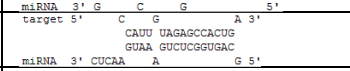
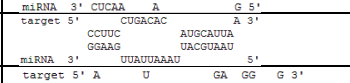
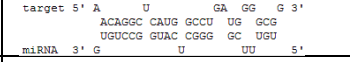
TABLE IV
PREDICTED CELLULAR MIRNAS TARGETING H3N2 INFLUENZA A VIRUS (A/THAILAND/CU-H1817/2010)

Human miRNAs	Target viral gene	Hybridization	Pairing energy (kcal/mol)	Pairing pattern
hsa-miR-29a	HA		-29.0	3'compen
hsa-miR-106a	PB2		-18.4	5'canonical
hsa-miR-146b	PA		-31.7	3'compen
hsa-miR-147b	PB1		-27.1	3'compen
hsa-miR-191	PA		-19.6	5'canonical
hsa-miR-196a	HA		-28.0	5'canonical
hsa-miR-216b*	NA		-30.1	5'canonical
hsa-miR-338	PB2		-28.0	5'canonical
hsa-miR-371a	PB2		-29.3	5'canonical
hsa-miR-548k	PB2		-22.0	5'canonical
hsa-miR-548l	PB1		-22.5	5'canonical
hsa-miR-626	PB2		-25.4	3'compen
hsa-miR-1279	PA		-22.9	5'canonical
hsa-miR-3140	PB1		-33.0	5'canonical

Human miRNAs	Target viral gene	Hybridization	Pairing energy (kcal/mol)	Pairing pattern
hsa-miR-3145*	PB1		-18.1	5'canonical
hsa-miR-3682*	NS		-22.6	3'compen
hsa-miR-3913	NP		-29.9	5'canonical
hsa-miR-4513*	PA		-32.3	5'canonical
hsa-miR-4667	PA		-35.1	5'canonical
hsa-miR-4695	NS		-30.1	5'canonical
hsa-miR-4712	PB2		-27.9	3'compen
hsa-miR-4714	PB1		-30.4	3'compen
hsa-miR-4717	NS		-36.0	5'canonical
hsa-miR-4747	NA		-38.1	5'canonical
hsa-miR-5693*	PA		-27.9	5'canonical
hsa-miR-5700	PA		-17.2	5'canonical
hsa-miR-5705	NA		-33.6	3'compen

* miRNAs targeting multiple subtypes of influenza A viruses; 3' compen abbreviated for 3' compensator

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IV. Conclusion

In conclusion, this study utilizes the information obtained form miRNAs database and using bioinformatic software for the searching and the prediction of candidate potential cellular miRNAs targeting the genes of several subtypes of influenza A virus. The result divulges that hsa-miR-3145 might be the best candidate human cellular miRNA targeting conserved region within PB1 gene of 3 subtypes (H1N1, H5N1 and H3N2) of influenza A viruses. It seems that this miRNA may have a potential for inhibition of viral replication by silencing the function of PB1. However, further in vitro analysis should be performed in order to test for inhibition of influenza viral replication by the effect of hsa-miR-3145.

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