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In silico designing of siRNA targeting PB 1 gene of Influenza A virus and *in vitro* validation

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ABSTRACT

Genomic variability makes Influenza A virus (IAV) 'the least susceptible' to existing vaccines or anti-influenza drugs. siRNA targeting viral gene silents the gene by cleaving mRNA. Present study aimed to develop siRNA targeting polymerase basic 1 (PB1) gene and to validate its efficiency *in vitro*. siRNA was designed rationally, targeting the most conserved region of PB1 gene of IAV strains. Total 147 strains worldwide and 42 Indian strains, when aligned, showed seven sets of conserved regions (> 30 bp stretch and < 5% mismatches). To choose the most efficient siRNA, three levels screening method was developed. Finally one pair of siRNA was chosen due to its unique position in conserved region. siRNA efficacy was confirmed in vitro on Madin Darby Canine Kidney (MDCK) cell line propagating two clinical isolates i.e. Influenza A/H3N2 [A/India/LKO864/2011(H3N2)] and Influenza A/pandemicH1N1 [A/India/LKO2151/2012(H1N1)]. The longest ORF was targeted by the selected siRNA, which showed 57 % inhibition in replication of Influenza A/pdmH1N1 and 60.6 % inhibition in replication of Influenza A/H3N2 at 72 hpi and 48 hpi respectively on MDCK cell line. This study shows that siRNA targeting PB1 may be moderately effective in controlling IAV replication so can be used as anti-IAV therapeutic agent.

INTRODUCTION

Influenza A Virus (IAV) has been a continuous global threat to human health for several years with significant mortality. Recently emergence of Influenza A/2009 H1N1 virus has shaken the world and has revealed the poor defense system human has developed to curb this virus. Antivirals are becoming largely ineffective due to the emergence of resistance for these drugs (Calfee and Hayden, 1985). As a prophylactic measure though inactivated seasonal influenza vaccines are formulated every year, yet these are effective only for a short time period as new strains emerge due to the phenomena of genetic shift and drift, shown by IAV. As a result new formulations of vaccine are needed every season. Therefore, there is an urgent requirement for some novel strategy for prevention and management of influenza. RNAi (RNA interfering) mechanism, induced by small interfering RNA (siRNA), is the post transcriptional gene silencing method, which offers great hope in the field of anti-viral drug discovery. siRNA has a potential as therapeutic agent for many viral infections (Coburn and Cullen, 2002, Kapadia *et al.*, 2003, Shlomai and Shaul 2003). SiRNA is about 19-21 nucleotides long dsRNA which initiates the cascade of enzymatic reactions leading to stimulation of RNAi mechanism in living cells.

Its efficiency in silencing any gene depends upon factors like; criteria used to design siRNAs, variations in transfection efficiency, susceptibility of targeted gene and sensitivity of assay etc. Rational designing of siRNA should increase the rate of success as structure of siRNA is the most important determinant of efficacy (Harborth *et al.*, 2003). Shifting the target sequence of siRNA by only few nucleotides affects the activity (Holen *et al.*, 2002).

IAV is a member of Orthomyxoviridae family. Being single stranded RNA virus, IAV becomes an approachable target of anti-influenza RNAi. Its genome is divided into eight segments which encode for eleven proteins. Previous studies have shown efficacy of siRNAs targeting various viral genes of IAV with variable efficacy (Ge *et al.*, 2003, 2004; Hui *et al.*, 2004; Tompkins *et al.*, 2004). PB1 is known to be quite conserved among various strains of IAV and encodes for a protein, which is a major part of polymerase complex.

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Hence, if it can be inhibited effectively, it can control IAV irrespective of frequent strains transformation. With this background, the present study was planned to design siRNA in silico, targeting PB1 gene and validate its efficacy in vitro in inhibiting IAV replication using two clinical isolates of IAV strains; Influenza A/pdm H1N1 [A/India/LKO2151/2012(H1N1)] and Influenza A/H3N2 [A/India/LKO864/2011(H3N2)].

MATERIALS AND METHODS

Retrieval of sequences

For the purpose of present study, all the sequences of polymerase Basic gene 1 (PB1) of IAV, already present in Influenza Virus Resource of NCBI database (www.ncbi.nlm.nih.gov/genomes/FLU/database) using advanced search, were initially selected. To facilitate the analysis, sequences were shortlisted following the criteria of including one sequence from single country during single year. However, all the strains which were deposited from India in NCBI database after 2009 were included.

Search set in advanced search was defined as sequence type "nucleotide", Type "A", host "human", country/region "Any" and "India" respectively, segment "2(PB1)", subtype "H any" and "N any", sequence length "full length plus" and collection date "from 2009 to 2013" on dated 22.08.2013.

Multiple sequence alignment of PB gene to identify largest ORF (Open reading frame) and largest conserved region

The program MEGA5.05 (clustalw aligner) was used to align these strains. The ORF map of matrix gene of these selected strains identified the largest ORF with the help of DNADynamo software (supplier Blue Tractor Software ltd). The conserved regions of > 30 bp were identified in the largest ORF by clustalw aligner, with mismatches in not more than 5% of strains.

siRNA Designing

Finally using "i score designer" program, list of 2256 pairs of siRNAs targeting the chosen ORF was produced. Thereafter 2nd generation algorithms [i-Score (Ichihara *et al.,* 2007), s-Biopredsi (Huesken *et al.,* 2005) and DSIR (Vert *et al.,* 2006) were taken into account for further screening, based on which the important siRNA selection rules were arranged in three levels of screening. Only those pairs which fulfilled all the criteria of previous level, passed to the next level.

1st level screen included ≥ 50 % value of 2nd generation algorithms (i-Score ≥ 50, DSIR ≥ 50, S-Biopredsi ≥ 0.5), basic Tuscl Rules (1st position of sense strand > 100 nucleotides downstream from start codon, GC% between 30 – 56%, 23-nt sequence motif 5'AA(N19)TT, if not present then 5'AA(N21) and 5'NA(N21) {N, any nucleotide}) (Elbashir *et al.,* 2001), Δ G ≤ -1.5 (free energy value of the most stable secondary structure of siRNA strand). 2nd level screen (Reynolds *et al.,* 2004, Zhou *et al.,* 2005) included following criteria. For sense strand (scoring): nt A at position 19 (score 1), nt A at position 3 (score 1), nt U at position 10 (score 1), Start nt G/C (score 1) and No G at 13 (score 1). For anti sense strand (scoring): nt U at 5' end (score 2), nt A at 5' end (score 1), nt AA at 5' end (score 1) and 1 or 2 U/A between 2nd – 5th position (score 1). Pairs of siRNAs having score of ≥ 6 were selected for further screening.

3rd level screen considered secondary structures of both the strands, determined by Mfold program (Zuker 2003). siRNAs having bonds between nucleotides (< 3 in number) of any type (GC, AU, GU), bond between G and C nucleotides (< 2 in number) and types of secondary structure (circular structure plots) (< 2 in number) passed through this level.

A cross-homology search using NCBI blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was made on the selected 19 nt sequence to screen if the selected siRNA bears homology to any human gene, to avoid gene silencing of any human gene, i.e., off-target cleavage. 19-mer siRNA was then converted to 27-mer Dicer substrate RNA duplex to increase the efficiency of siRNA (Integrated Device Technology Corp. http://eu.idtdna.com/scitools/documents/dicer_substrate_rnai.pdf). At 3' end of the sense strand 6 bases were added corresponding to target mRNA and complementary to these 6 bases were added at 5' end of antisense strand. On the 3' end of the sense strand 2 terminal RNA bases were replaced with DNA bases for ease of processing. To help direct dicing, 2 bases 3'- overhang on one side of the antisense strand was added. Then PO_4 group was added at 5' end of sense strand for better RISC entry.

Efficacy of self designed siRNA in inhibiting IAV replication

Cell line and its maintenance

The efficacy of above self designed siRNA to inhibit IAV replication was checked on MDCK (Madin Darby Canine Kidney) cell line. It was maintained in MEM (Gibco by Life technologies) supplemented with 10% heat inactivated Foetal Bovine Serum (FBS) (Gibco by Life technologies), antibiotics (100 U of Penicillin G/ml and 100 µg of Streptomycin/ml) and 3% Glutamine (Himedia).

Viruses and viral load quantification

Two clinical isolates of IAV from our center were used in this study; Influenza A/pdm H1N1 [A/India/LKO2151/2012 (H1N1)] and Influenza A/H3N2 [A/India/LKO864/2011(H3N2)]. These strains were cultured in MDCK cell line and virus titre was determined by standard curve formation by real time PCR (Taqman chemistry) using CDC primers and probes for Matrix gene as per method Ngaosuwankul *et al.,* 2010 (Ngaosuwankul *et al.,* 2010). They were further characterized by sequencing and confirmed by depositing in NCB1, where they had been allotted accession number after validation. (Influenza A/pdm H1N1 [A/India/LKO2151/2012(H1N1)] [GenBank: KC753452] and Influenza A/H3N2 [A/India/LKO864/2011(H3N2)] [GenBank: KF142486])

Test and Control siRNAs

siRNA duplex tagged with fluorescent indicator (siGlo^R Red or siG) was synthesized (ThermoScientific Dharmacon ^{R}) as transfection control. It was chemically synthesized double stranded

RNA tagged with fluorophore which localized to the nucleus, thus permitted visual assessment of uptake into cells. It was used with each experiment to determine optimal siRNA transfection conditions, for tracking individually transfected cells and to monitor relative efficiency of delivery when co-transfected with siRNA.

siRNA targeting one of the internal genes i.e. Cyclophilin B (siGENOME or siC), was used as positive control (ThermoScientific Dharmacon ^{R}) to establish parameters that resulted in successful siRNA delivery without affecting cell viability. Non-targeting siRNA duplex (siN) (ThermoScientific Dharmacon ^{R}) was used as negative control to distinguish sequence specific silencing from nonspecific effects. Untreated cell line (cultured cell without siRNA treatment) was used to determine baseline cell viability and phenotype.

Transfection Optimization (Figure 1)

Transfection Optimization experiments using transfection indicator (siGlo^{R} Red) and transfection reagent DhermaFECT (ThermoScientific Dharmacon ^{R}) was performed. Sixteen different combinations of four concentrations of siGlo^{R} Red (10nM, 25 nM, 37.5 nM and 50 nM) and four concentrations of transfection reagent DhermaFECT (0.05, 0.1, 0.5 and 1.0 µL/ 100 µL of transfection medium) were tested.

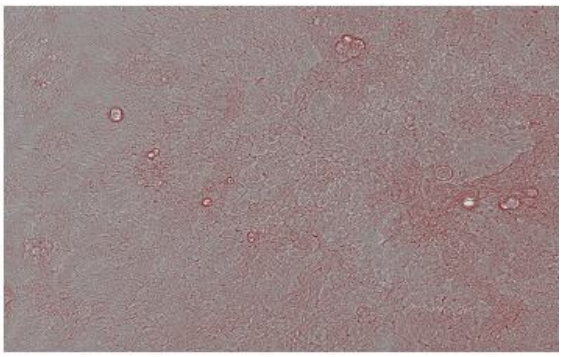

Fig. 1: Transfection of 10 nMsiGlo red with transfecton reagent after 24hours, showing almost 100 % efficiency. MDCK cell in was transfected with optimized concentrations of siGlo Red and transfection teagent, which showed transfection in almost all the cells in 24 hrs and re taired there upto 48hrs.

Experiment Design/ Transfection of siRNAs

Experiment was setup in 24 wells plate (Figure 2). Once monolayer of MDCK cell line was > 80%, siN, siPB1, siC and siG were transfected in three wells each at 10nM (final optimized concentration). Plates were incubated at 37^{0} C with 5% CO_2. After 16 – 18 hrs plates were inoculated with Influenza A/pdm H1N1 [A/India/LKO2151/2012(H1N1)] and Influenza A/H3N2 [A/India/LKO864/2011(H3N2)] at MOI 1 in their respective wells. Wells transfected with siG and siC were not inoculated with any virus. Three wells were kept untreated, neither virus nor siRNA were put in them.

They served as control for cell lines. Plates were incubated again in same conditions as before. At 24, 48 and 72

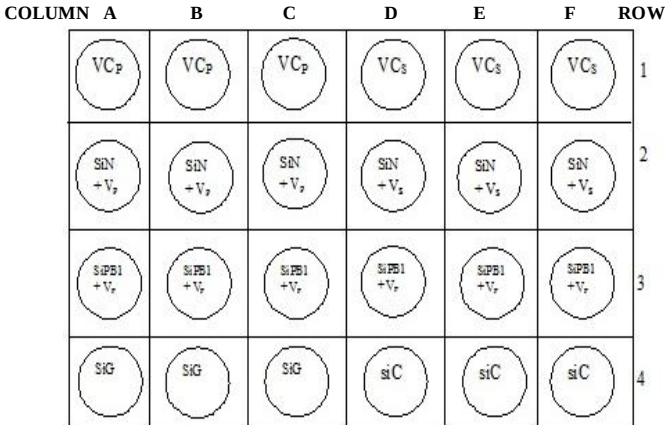

Fig. 2: experiment setup for silencing of NS1 gene of two IAV strains (A/pdm H1N1 and A/H3N2) by siNS1 on MDCK cell line. Experiment was set up in 24 wells plate for MDCK cell line with >80% monolayer. VCs, Influenza A/pdmH1N1 control non-treated with siRNA, VPs, Influezna A/H3N2 control non-treated with siRNA, siN+Vs, scramble siRAN with Influenza A/pdmH1N1, siN+Vs, scrambled siRNA targeting M gene of influenza A/H3N3, siRNA targeting M gene with influenza A/pdmH1M1, siM+Vs, siRNA targrting M gene of Influenza A/H3N2, siG; Transection indicator, siC, siRNA targeting cyclophilin B (internal control) (all tests were performed in triplicate)

hours post infection (hpi) 200µl of culture fluid was taken out from all the wells. Fluid was stored at -20^{0} C till further use. RNA was extracted from all culture fluids together by column based kit (QIAGEN) as per manufacturer's guidelines and real time PCR was done for relative quantification of extracted RNA targeting Matrix gene using primers and probes (CDC) as per method of Ngaosuwankul *et al.*, 2010 (Ngaosuwankul *et al.*, 2010).

Statistical analysis

Result was analyzed statistically to find out silencing efficiency of siRNA. Mean and Standard deviation of triplicate of critical threshold (CT) values and viral load of each set was determined. Percentage inhibition was calculated by finding percentage increase in CT of virus after siPB1 treatment in comparison to the CT value of virus without any siRNA treatment (VC). ΔCT1 (difference in CT values of virus treated with siPB1 and virus not treated with any siRNA) was calculated to show the silencing effect of siPB1 on virus in given conditions. It depicted virtual silencing.

ΔCT2 (difference in CT values of virus treated with siN and not treated with any siRNA) was deciphered to depict any effect of siN on virus replication (Negative silencing). Effect of siRNA against study targets (Actual silencing) ΔΔCT was calculated after normalizing the effect of siN on virus replication. Paired t test was used to calculate the p values between viral loads of both viruses at different time points after treatment with siPB1 on MDCK cell line (GraphPad Prism 6 version 6.02, GraphPad Software, Inc., California, Corporation). Repeated measure ANOVA test was used to calculate the p value of the inhibition of Influenza A/pdm H1N1 and Influenza A/H3N2 strains replication on MDCK cell line by siPB1. P value < 0.05 was taken as significant.

RESULTS

siRNA Designing

The search for IAV PB1 gene full length sequences available on NCBI database from all over the world gave 5567 results, out of which 147 were selected taking only one from those isolated from same place and same year. Along with these 42 sequences deposited from India were also included in the study. The accession no. of 189 sequences used in the study are mentioned in Table 1.

Table. 1: Accession numbers of Polymerase Basic 1 gene of IAV used to decipher most active siRNA, retrieved from NCBI (Influenza virus resource).

Accession Number of Nonstructural 1 gene of IAV				
From India	**From rest of World**			
HM241695	CY039899	CY125781	KF001508	CY093373
CY088672	CY083916	CY125797	CY129836	CY147625
JQ340007	CY129916	CY120721	JQ173146	CY125726
JF764086	GU136025	CY120753	CY121742	GU136041
JX262205	KC781897	CY121798	GU931815	HQ664919
JX262206	CY093261	CY065760	CY098756	CY114507
JF764085	KC883401	CY083549	JQ612505	CY114427
KF280733	GU367337	CY097978	JF764086	CY065798
KF280669	CY071384	GQ329075	JX262205	JF906181
KF280661	CY097946	CY129860	CY069475	CY113027
KF280677	CY073457	CY081492	HM567590	HQ703389
KF280685	CY066829	CY081468	CY041966	CY040040
KF280693	HM569665	JQ714244	GQ251033	CY081059
KF280701	KC881878	GQ359772	CY069315	GU290053
KF280709	HM189318	HM241695	HQ853589	CY071648
KF280717	CY071424	JN703380	GU562464	CY049889
KF280741	CY063596	CY093195	GQ160813	CY093445
KF280749	CY071141	KC488877	CY069323	CY041981
KF280725	CY129668	JF682627	CY129588	CY093421
HM241702	GQ258464	JX913065	CY100818	CY096725
HM460507	CY055532	CY069299	CY093349	KF014996
HQ154078	CY066517	CY083030	CY073156	JX437802
HQ853589	CY071333	CY129756	CY083094	KF015002
HQ853591	CY088767	CY093222	FN423710	JX309436
HM460510	CY083751	CY080270	CY129432	CY124506
HQ154076	CY045501	JQ340007	CY055269	CY079542
HQ853571	GQ183623	JX625636	CY118181	CY079535
HQ154084	JF816660	JX625511	CY090843	CY071632
CY088679	CY073180	CY121758	CY072676	JN187230
CY088686	CY071381	CY065934	CY088905	JN187225
CY088707	GU592903	CY093333	CY092295	CY063001
CY088658	CY044145	CY121066	CY106574	CY063009
CY088665	CY093405	KC473860	JQ927305	CY129556
HM241716	CY129596	CY098735	CY100528	CY129892
CY088630	CY128473	CY080572	CY089461	GQ983546
CY088637	CY056993	CY062809	AB704484	CY074948
HM241723	CY129980	CY062993	CY087688	
CY088693				
CY088700				
CY088651				
CY088644				
HM241709				

Largest ORF from position 7 to 2280 was recognized and multiple sequence alignment of all strains of IAV lead to the identification of following seven conserved regions (> 30 bp long); 109–168nt position, 193 – 257nt position, 281–355nt position, 523–572nt position, 1791–1871nt position, 2175– 2207nt position and 2271–2315nt position. The largest ORF was chosen as "target" for siRNA activity. Out of total 2256 pairs of I score designer predicted siRNAs, only 184 passed through 1st level screen, of which 63 were selected for 2nd level screen, which were in the abovementioned seven conserved regions. 17 sequences scored ≥ 6 in the 2nd level screen.

Among those seven could pass through 3rd level screen and the siRNA finally chosen was the one which was falling in the conserved region with < 5% strains mismatches. The chosen duplex showed < 40 alignment score by NCBI Blast showing least off target effect (similarity with any human gene). The characteristics of chosen siRNA are shown in table 2.

Table. 2: Characteristics of siRNA chosen for the study.

Name of Gene	**PB1**
Target sequence	CCAAGUGGGUAUGCACAAA
Sense strand of siRNA	5'CCAAGTGGGTATGCACAAATT3'
Antisense strand of siRNA	5' UUUGUGCAUACCCACUUGGUU 3'
i-score	73.4
DSIR	91.2
S-Biopredsi	0.853
1st position of sense strand	235
GC%	47.4%
Δ G	-2.2
2nd level score	7
No./Types of bonds (sense strand)	3/2GC,1AU
No./Types of bonds (anti-sense strand)	3/2GC,1AU
No of secondary structures (sense strand)	1
No of secondary structures (anti-sense strand)	2
Human genome alignment score	< 40

Transfection Experiment

Transfection experiment demonstrated that transfection with 10nM (final concentration) of siGlo Red resulted in almost 100% transfection efficiency (% of cells transfected) at cell density of 10^5 cells per ml in about 24 hrs which declined to nil in about 96 hrs.

In vitro replication inhibition of IAV strains by designed siRNA

Viral load of Influenza A/pdm H1N1 and Influenza A/H3N2 on MDCK cell line increased steadily from the time of inoculation to 72 hpi in siN and non-siRNA-treated cells (V_P and V_S). In wells treated with siPB1 and challenged by Influenza A/pdm H1N1 viral load decreased steadily upto 72 hpi (Table 3). In case of wells challenged by Influenza A/H3N2, viral load decreased at 24 hpi, was minimum at 48 hpi and then again increased at 72 hpi. Inhibition of both strains on MDCK cell line in comparison to VC was statistically significant (p value 0.01; paired t test) (Table 3). SiPB1 caused 57% inhibition of Influenza A/pdm H1N1 strain while Influenza A/H3N2 was inhibited 60.6%. Virtual silencing (ΔCT1) of siPB1 before normalization was 7.25 for A/pdm H1N1 while actual silencing (ΔΔCT) after normalization of negative silencing was 6.99. For Influenza A/H3N2 strain virtual silencing (ΔCT1) was 6.82, while actual silencing (ΔΔCT) came out to be 6.73 (Table 3).

Table. 3: siRNA targeting PB1 showing inhibition of Influ A/pdm H1N1 and Influ A/H3N2 replication in MDCK cell line (measured by real time PCR).

CT value; presented as mean ± SD (viral load; log_{10} copies/ml; presented as mean ± SD) of RNA extracted from culture filtrate

	At the time of inoculation	**24 hpi**	**48 hpi**	**72 hpi**
Inf A/pdm H1N1 A/India/LKO2151/2012(H1N1)]				
Virus control (PC)	16.26±0.03 (9.25±0.03)	15.20±0.05	14.53±0.01	12.72±0.05
siN (NC)	16.22±0.06 (9.28±0.06)	15.24±0.02	14.51±0.03	12.98±0.01
SiPB1	16.13±0.04(9.37±0.04)	17.13±0.01	18.34±0.06	19.97±0.02
% inhibition[a]		12.7%	26.2%	**57%**
ΔCT1[b]		1.93	3.81	7.25
ΔCT2[c]		0.04	-0.02	0.26
ΔΔCT[d]		1.89	3.83	6.99
Inf A/H3N2 [A/India/LKO864/2011(H3N2)]				
Virus control (PC)	14.02±0.05	12.84±0.03	11.25±0.01	10.98±0.04
siN (NC)	13.99±0.02	12.87±0.05	11.34 ±0.03	11.01±0.02
SiPB1	14.01 ±0.04	15.25 ±0.03	18.07 ±0.05	15.72 ±0.01
% inhibition[a]		18.8%	**60.6%**	43.2%
ΔCT1 [b]		2.41	6.82	4.74
ΔCT2[c]		0.03	0.09	0.03
ΔΔCT[d]		2.38	6.73	4.71

*P value was **0.045** between the replication inhibition of Influenza A/pdm H1N1 and Influenza A/H3N2 in comparison to VC; calculated by repeated measure ANOVA (Analysis Of Variance)*

[a]% inhibition was calculated by siPB1 CT – PC CT/ PC CT x 100
[b]ΔCT1 was calculated by deducting PC CT from siPB1CT
[c] ΔCT2 was calculated by deducting PC CT from NC CT
[d]ΔΔCT was calculated by deducting ΔCT2 from ΔCT

DISCUSSION

siRNA is highly homology dependent strategy so in present study we planned to delineate the most conserved region of one of the most conserved gene of IAV; PB1 gene by aligning multiple sequences of human pathogenic IAV submitted in NCBI database (Influenza virus resource) from all over the world. This strategy was adopted to develop a siRNA which may work against more or less all IAV strains. siRNAs targeting PB1 gene have demonstrated effective inhibition of Influenza virus replication (Ge *et al.,* 2004; Zhiqiang *et al.,* 2010; Li *et al.,* 2011). Since the most conserved region of PB1 gene has been targeted, it was demonstrated *in silico* that it will inhibit wide range of IAV strains.

To achieve substantial amount of success in siRNA silencing two major steps were considered while designing. First, multiple levels of screening were applied following various stringent criteria of siRNA designing to shortlist the best one from a large pool of putative siRNAs as discussed in methods. Second, newer technology of manufacturing Dicer substrate siRNA was used which is supposed to be highly specific. It is described that 25 – 30 base length long RNA duplex has 100 times more potency in comparison to its 21 mer counterpart at the same location (Kim *et al.,* 2005). Therefore, it has been theorized to provide Dicer (an enzyme required to introduce siRNA into RISC – RNA induced silencing complex) with substrate rather than only a product (19-mer) to improve the efficiency of RNA duplex entry into RISC. siRNA was also looked for having no significant homology to any of human gene as we wanted to avoid cross-silencing of human genes while attempting silencing of viral genes.

Present study showed about 60% inhibition of Influenza A/pdmH1N1 and Influenza A/H3N2 within 72 and 48 hrs of virus inoculation respectively, onto pre-siPB1-transfected MDCK cell line. Li *et al.,* also showed the efficacy of siRNA targeting PB1 in inhibiting IAV propagated on MDCK cell line (Li *et al.,* 2013). The novelty of the study is the method of designing of siRNA. When siRNA was targeted against the most conserved region in PB1 gene, it inhibited IAV efficiently. SiPB1 inhibited not only

Influenza A/H3N2 efficiently but also Influenza A/pdm H1N1. But the inhibition of Influenza A/H3N2 was more in comparison to Influenza A/pdmH1N1, pointing towards the fact that PB1 gene may be having more important role in Influenza A/H3N2 replication rather than in Influenza A/pdmH1N1 replication.

CONCLUSION

IAV infection is known to have limitation of not to be prevented affectively by vaccine or any therapy due to antigenic shift or drift. These results provide a basis for further development of anti IAV – siRNAs. However, as the viral inhibition was only moderate (about 60%), optimization of *in vitro* conditions, siRNA designing conditions and target selection criteria need to be further fine tuned.

Conflict of interest

None of the authors has any conflict of interest.

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REFERENCES

Calfee DP, Hayden FG. New approaches to influenza chemotherapy: neuraminidase inhibitors. Drugs, 1998; 56: 537

Coburn GA, Cullen BR. Potent and specific inhibition of human immunodeficiency virus type 1 replication by RNA interference. J of Virol,2002; 76: 9225

Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature, 2001, 411; 494.

Ge Q, Filip L, Bai A, Nguyen T, Eisen HN et al. Inhibition of influenza virus production in virus-infected mice by RNA interference. PNAS, 2004,101:8676.

Ge Q, McManus MT, Nguyen T, Shen CH, Sharp PA et al. RNA interference of influenza virus production by directly targeting mRNA for degradation and indirectly inhibiting all viral RNA transcription. PNAS, 2003, 100:2718.

Hale BG, Randall RE, Ortı´n J, Jackson D. The multifunctional NS1 protein of influenza A viruses. J of Gen Virol,2008, 89;2359–2376

Harborth J, Elbashir SM, Vandenburgh K, Manninga H, Scaringe SA, Weber K, Tuschl T. Sequence, chemical, and structural variation of small interfering RNAs and short hairpin RNAs and the effect on mammalian gene silencing. Antisense Nucleic Acid Drug Dev, 2003;13:83.

Holen T, Amarzguioui M, Wiiger MT, Babaie E, Prydz H. Positional effects of short interfering RNAs targeting the human coagulation trigger tissue factor, Nucleic Acids Res, 2002;30:757.

Huesken D, Lange J, Mickanin C, Weiler J, Asselbergs F, Warner J, Meloon B, Engel S, Rosenberg A, Cohen D, Labow M, Reinhardt M, Natt F, Hall J. Design of a genome-wide siRNA library using an artificial neural network. Nat Biotechnol, 2005, 23;995-1001.

Hui EK, Yap EM, An DS, Chen IS, Nayak DP. Inhibition of influenza virus matrix (M1) protein expression and virus replication by U6 promoterdriven and lentivirus-mediated delivery of siRNA. J of Gen Virol, 2004, 85:1877

Ichihara M, Murakumo Y, Masuda A, Matsuura T, Asai N, Jijiwa M, Ishida M, Shinmi J, Yatsuya H, Qiao S, Takahashi M, Ohno K. Thermodynamic instability of siRNA duplex is a prerequisite for dependable prediction of siRNA activities. Nucleic Acids Res, 2007, 35:e123.

Kapadia SB, Brideau-Andersen A, Chisari FV. Interference of hepatitis C virus RNA replication by short interfering RNAs. PNAS, 2003, 100:2014

Kim DH, Behlke MA, Rose SD, Chang MS, Choi S, Rossi J. Synthetic dsRNA Dicer substrates enhance RNAi potency and efficacy. Nat Biotechnol, 2005,23:222.

Li G, Zhang J, Tong X, Liu W, Ye X. Heat Shock Protein 70 Inhibits the Activity of Influenza A Virus Ribonucleoprotein and Blocks the Replication of Virus *In Vitro* and *In Vivo*. PLoS ONE 2011; 6(2): e16546. doi:10.1371/journal.pone.0016546

Ngaosuwankul N, Noisumdaeng P, Komolsiri P, Pooruk, Chokephaibulkit PK, Chotpitayasunondh T, Sangsajja C, Chuchottaworn C, Farrar J and Puthavathana P. Infuenza A viral loads in respiratory samples collected from patients infected with pandemic H1N1, seasonal H1N1 and H3N2 viruses. Virology Journal, 2010, 7:75

Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A. Rational siRNA design for RNA interference. Nat Biotechnol, 2004,22:326.

Shlomai A, Shaul Y. Inhibition of hepatitis B virus expression and replication by RNA interference. Hepatology 2003,37:764

Tompkins SM, Lo CY, Tumpey TM, Epstein SL. Protection against lethal influenza virus challenge by RNA interference in vivo. PNAS, 2004 101:8682.

Vert JP, Foveau N, Lajaunie C, Vandenbrouck Y. An accurate and interpretable model for siRNA efficacy prediction. BMC Bioinformatics,2006, 7:520.

Zhiqiang W, Yaowu Y, Fan Y, Jian Y, Yongfeng H, Lina Z, Jianwei W, Qi J. Effective siRNAs inhibit the replication of novel influenza A (H1N1) virus. Antivir Res 2010; 85: 559–561

Zhou H, Zeng X, Wang Y, Seyfarth BR. A Three-Phase Algorithm for Computer Aided siRNA Design. Informatica 2006, **30;**357-364.

Zuker M. M fold web server for nucleic acid folding and hybridization prediction. Nuc Acids Res, 2003, 31:3406

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