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VIRTUAL SCREENING AND BEHAVIOURAL STUDIES OF 2'-O-METHYL OLIGONUCLEOTIDE AGAINST hsa-miR-21 IN PDCD4 CARCINOMAS

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ABSTRACT

MicroRNA (miRNAs), small class of non-coding nucleotides, have critical role in post transcriptional level by targeting mRNA for cleavage or translational repression in human. Selected miRNAs regulates cell proliferation and apoptosis process that is important in cancer is well known fact. The miRNAs located in genomic region amplified in cancer function as oncogenes, whereas some miRNAs located in portions of chromosomes deleted in cancers function as tumour suppressors (1, 2, 3). The microRNA-21 gene (mir-21) functions as oncogene in cell invasion and tumor metastasis by targeting 3' UTR of programmed cell death 4 (PDCD4) genes which control aspects of apoptosis, proliferation, invasion and migration of many carcinomas. Therefore, suppression of mir-21 might provide a novel approach for the treatment of advanced cancers. Hence, the design of 2'-O-methyl oligoribonucleotides for targeting hsa-miR-21 to reduce its expression and hypotheses that these novel ribo-regulators would bring novel insight in the intricate relationship of miRNAs and tumorigenesis in near future.

Keywords: MicroRNA (miRNA), 2'-O-methyl oligoribonucleotides (2' MEO), Programmed cell death 4 (PDCD4)

[I]. INTRODUCTION

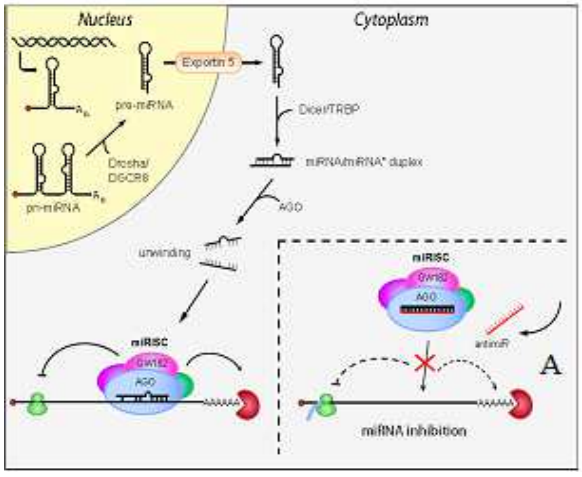
MicroRNAs are a recently discovered class of small, evolutionarily conserved, RNA molecules that negatively regulate gene expression at the post-transcriptional level [1]. Mature microRNAs of approximately 20–22 nucleotides are formed from longer primary transcripts by two sequential processing steps mediated by a nuclear (Drosha) and a cytoplasmic (Dicer) RNAse III endonuclease. In the context of a protein complex, the RNA-induced silencing complex (RISC), microRNAs base-pair with target messenger RNA sequences at "seed" region causes translational repression and/or messenger RNA degradation [2]. MicroRNAs have been implicated in the control of many fundamental cellular and physiological processes such as tissue development, cellular differentiation and proliferation, metabolic and signalling pathways, apoptosis and stem cell maintenance [3].MiRNA expression is

deregulated in cancer by a variety of mechanisms including amplification, deletion, mutation, and epigenetic silencing [4] which correspondence Programmed Cell Death (PCD) 4 (Pdcd4) which is a novel tumour suppressor and originally identified as a neoplastic transformation inhibitor [5]. PDCD4 expression attenuated with progression human tumors of the lung, colon, prostate and breast; diagnostic and prognostic for colon cancer staging with decreased expression in adenomas and a further decrease in stage 1 adenocarcinomas. The hsa-miR-21 binds to 3'UTR of PDCD4 at "seed" region (nt 228-249) by perfect base pairing and negatively regulatedPDCD4, concluded that over expression of hsa-miR-21 provokes invasion/intravasation/metastasis [6, 7, 8, 9, 10].

In vitro analysis has proved that the Ant-miR-21 helps to reduce the expression of hsa-miR-21 in many carcinomas [11]. Anti-miRNA oligonucleotides (AMOs) (or inhibitors) appear to work primarily through a steric blocking mechanism of action; these compounds are synthetic reverse complements that tightly bind and inactivate the miRNA. There are certain factors which are important for functionality of miRNA inhibitors i.e. cross-reactivity of inhibitors for members of same family, effect on similar region of different miRNAs, binding position (Position 13,position18 and position3), "seed" region, superior affinity to target miRNA, high fidelity, low toxicity and improved metabolic stability and locked nucleic acids etc [12].

There are several scaffolds possible for generating anti-modified oligonucleotides (AMOs) or antagomiR to reduce the over-expression of miRNA in diseased conditions. The 2'-O-Methyl modification as well as the 2'-O-methoxyethyl (2'-MOE) and 2'- fluoro (2'-F) chemistries are modified at the 2' position of the sugar moiety, whereas locked nucleic acid (LNA) comprises a class of bicyclic RNA analogues in which the furanose ring in the sugar-phosphate backbone is chemically locked in a RNA mimicking N-type (C3'-endo) conformation by the introduction of a 2'- O,4'-C methylene bridge. Nuclease resistance is also improved by backbone modification of the parent phosphodiester linkages into phosphorothioate (PS) linkages in which a sulphuratom replaces one of the non-bridging oxygen atoms in the phosphate group or by using morpholino oligomers, in which a six-membered morpholine ring replaces the sugar moiety. The LNA modification leads to the thermodynamically strongest duplex formation with complementary RNA known and 2'-O-Methoxyethyl (MOE)-modified oligonucleotides have higher affinity and specificity to RNA [13] [Figure 1].

In this work, 2'-O-methyl oligonucleotides were developed against over expressed hsa-miR-21. The 2'-O-methyl-group (OMe) is one of the oldest, simplest and most often used modifications to oligonucleotides. The methyl group contributes a limited amount of nuclease resistance, and improves binding affinity to RNA compared to unmodified sequences. The affinity between the OMe oligonucleotides and a miRNA in a targeted RISC complex was approximately 40-fold stronger than fully complementary mRNA, suggesting that the protein components of the RISC complex greatly enhanced the interaction beyond mere hybridization. The high potency, metabolic stability, high affinity at proper Tm and non toxic activity make OMe competent and superior therapeutic tool for RNA based drug design [14] [Figure 1].



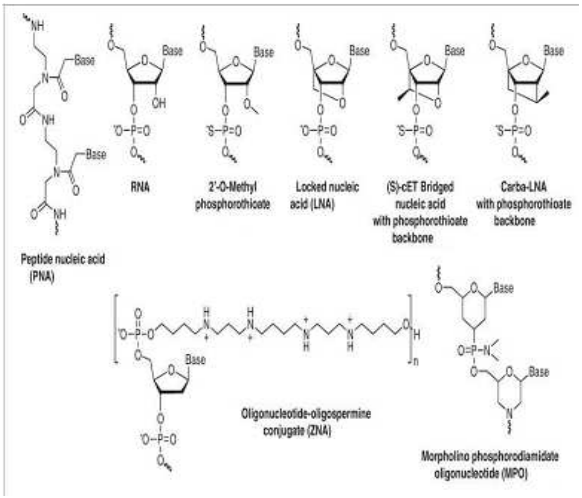


Fig 1: A) The inhibition of miRNA function by antimiR oligonucleotides. Chemically modified antimiR oligonucleotides sequester the mature miRNA in competition with cellular target mRNAs leading to functional inhibition of the miRNA and derepression of the direct targets. B) Different modified oligonucleotides (Antagomir).

II]. COMPUTATIONAL APPROACH

To indentify correct tumor suppressor gene and miRNA, the *human gene, protein database (HGPD)* and *miRBase* was used respectively [15, 16]. Each entry in the miRBase sequence database represented a predicted hairpin portion of a miRNA transcript (termed mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR) [16]. Before going for methylation of each nucleotides of entire sequence of hsa-miR-21 for designing 2'-O-methyl oligonucleotides (2' MEO),the sequence of reverse complimentary of anti-miR-21 into 3D-structure by "MC-Fold web server" was converted [17]. The first structure as it has lowest folding energy was selected. It wasthen designed as anti-miR-21 by swapping 2'O position with methyl group for each nucleotide of hsa-miR-21. This was conserved as PDB format with the help of *YASARA tool* [18] [Figure 2].

The proposed development was achieved at 2'-O-methyl oligonucleotides against hsa-miR-21, and then four different energies were calculated. Structure energy in Kcal, free energy for folding at 37º C at Kcal/mol, hybridization energy and free energy for binding in Kcal/mol by *RNAdraw*, *Mfold web server*, *RNAcofold*, *Hex server* respectively for complex structure of hsa-miR-21-PDCD4 and anti-miR-21-hsa-miR-21 in order. The minimum energy structure prediction algorithm in *RNAdraw* was ported from the Rnafold program included in the Vienna RNA package and analysis of RNA secondary structure by calculating the thermodynamically optimal secondary structure, basepair probability matrix and specific heat capacity for a sequence in specified temperature range [18]. Before calculating hybridization energy, pairwise alignment of coding sequence of PDCD4 (U83908.1) and hsa-miR-21 was undertaken to know the exact position of binding of hsa-miR-21 to target gene using EMBL-EBI (European Molecular Biological Library- European Bioinformatics Institute) online tool.

Followed by that, the hybridization energy was calculated by *RNAcofold web server*, which supported standard dynamic programming algorithm for computing secondary structure of linear single-stranded RNA molecules, which was extended to the co-folding of two interacting RNAs [19]. Then,the output in (.b) vienna format was recorded from *RNAcofold web server*, free energy for folding calculated by *mfold* which was not only calculated the minimum free energy structure but also a few sub-optimal structure with an energy near minimum free energy [20]. Lastly, free energy for binding was confirmed by *hex web server*, which aimed to provide a relatively large number of putative complexes for re-scoring, the requested number of prediction was presented as a single compressed multi-structure PDB file in which each structure was identified using the standard PDB 'MODEL' and 'ENDMDL' keywords [21].

[III]. OBSERVATIONS

All the energy levels were calculated by different tools and web server, the 2'-O-methyl

oligonucleotides (anti-miR-21) was observed higher affinity and specificity to hsa-miR-21. The modified anti-miR-21 and hsa-miR-21 requires lower energies compare to actual bind of hsa-miR-21 and PDCD4; was determined and confirmed. The proposed anti-miR-21 necessitated -11.03Kcal and -11.70Kcal/mol structure energy and free folding energy (dG) respectively reported with actual capacity.The binding of hsa-miR-21 and PDCD4 complex required -9.15Kcal and -11.70Kcal/mol [Table 1]. The proposed ribo-regulator also required less hybridization energy and ETOTAL than the actual one. The anti-miR-21 and hsa-miR-21 complex required -16.10Kcal/mol and -867.77Kcal/mol whereas complex structure of hsa-miR-21 and PDCD4 needed -10.00Kcal/mol and -618.77Kcal/mol [Table 1]. The difference of these thermodynamic energies indicated that the 2'-O-methyl oligonucleotides might be useful to suppress the expression of hsa-miR-21 in PDCD4 associated malignancies.

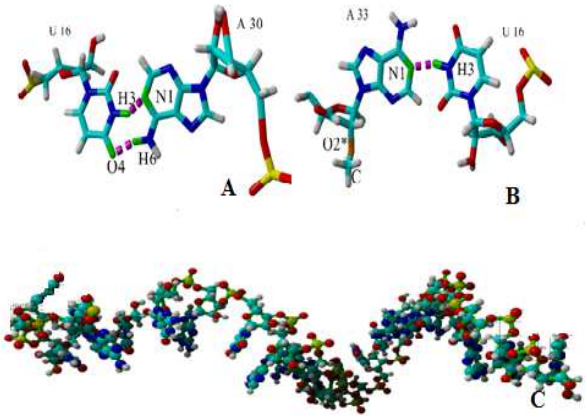


Fig 2: A) Binding of PDCD4 and hsa-miR-21 where U16 represents the nucleotide of PDCD4 which formed hydrogen bonds (Purple color) with A30 of hsa-miR-21. B) The 2'-O-methyl oligonucleotides (Orange color) of inhibitor (A 33) formed bond with hsa-miR-21 (U 16). C) The complete chain of modified anti-miR (2'OME).

Table 1: Energy calculation: anti-miR-21 with hsa-miR-21

Thermodynamic Energies	**hsa-miR-21 and PDCD4 complex**	**Anti-mir-21 and hsa-miR-21 complex**
Structure energy inKCal	-9.15	-11.03
Free folding energy in Kcal/mol (dG)	-8.10	-11.70
Hybridization energy in Kcal/mol	-10.00	-16.10
ETOTAL	-618.54	-867.77

[IV] CONCLUSIONS

Distinct functions of miRNAs in tumor initiation, progression and metastasis in human cancers strongly suggested miRNAs as novel drug targets or therapeutic tools to develop novel strategies for the treatment of cancers in humans. The over expression of hsa-miR-21 lead to progression and metastases of PDCD4 associated cancer and 2'-O-methyl oligonucleotide, which targets the hsa-miR-21, observed strong thermodynamic stability compare to natural binding. Hence, these small molecules could be utilized as potential drug like "***future molecule***" to cure cancer in human at genome level.

[V]. REFERENCES

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