ORIGINAL ARTICLE

Identification of MicroRNAs Binding Site in the 3′Untranslated Region of Long Non-Coding RNA, MIR497HG: A Bioinformatic Prediction

Nursyamila Shamsuddin, Fazleen Haslinda Mohd Hatta, Mizaton Hazizul Hasan, Mohd Shihabuddin Ahmad Noorden

Faculty of Pharmacy, Universiti Teknologi MARA UiTM, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia.

ABSTRACT

Introduction: Prediction and identification of miRNAs target genes are crucial for understanding the biology of miRNAs. Amidst reported long-coding RNA (lncRNA), the microRNA 195-497 cluster host gene (MIR497HG) regulation is mediated by multiple non-coding RNAs (ncRNAs) such as microRNAs (miRNAs). MIR497HG has been implicated as a tumour suppressor in various cancers. However, the impact of MIR497HG and its derived miRNAs is largely unknown and still needs to be further explored. Employing an experimental approach is often challenging since some lncRNAs are difficult to identify and isolate by the current isolation technique. Thus, bioinformatic tools are introduced to aid these problems. This study sought to search and identify the miRNAs targeting the 3′untranslated region (3′UTR) of MIR497HG. **Methods:** Here, bioinformatic tools were adopted to identify a unique list of miRNAs that potentially target the 3′UTR of MIR497HG. **Results:** A total of 57 candidate miRNAs that target the 3′UTR of MIR497HG were extracted using the miRDB. Meanwhile, STarMir predicted 291 miRNAs that potentially target the 3′UTR of MIR497HG. A common list of 36 miRNAs was obtained using the Venny 2.1.0 and further narrowed down using the LogitProb score of StarMir. Finally, a total 4 miRNAs (hsa-miR-3182, hsa-miR-7156-5p, hsa-miR-452-3p and hsa-miR-2117) were identified. The mRNA target of identified miRNAs was identified by TargetScan. Finally, Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of mRNA target was done using Enrichr. **Conclusion:** This finding could be useful in understanding the complex interaction between MIR497HG and its regulatory miRNA. In addition, a comparative analysis of computational miRNA-target predictions is provided in this study would potentially lay the foundations for miRNAs to be used for biomarkers in cancer research.
Malaysian Journal of Medicine and Health Sciences (2024) 20(1):161-167. doi:10.47836/mjmhs.20.1.21

Keywords: microRNA binding site, target prediction, 3′untranslated region, MIR497HG

Corresponding Author:
Mohd Shihabuddin Ahmad Noorden, PhD
Email: shiha@uitm.edu.my
Tel: +603-3258 4843

INTRODUCTION

Long non-coding RNAs (lncRNAs) with more than 200 nucleotides in length are referred to as miRNA host genes and serve as the precursors playing a regulatory role for miRNAs. A microRNA-195-497 cluster host gene (MIR497HG) has been implicated as a tumour suppressor and effective regulators for cancer including bladder cancer (4), glioma (5), colorectal cancer (6) and liver cancer (7). The important roles of MIR97HG in cancer have been documented in recent studies. For example, MIR497HG was reported to be downregulated in glioma tissue and associated with poor prognosis via upregulation of Cyclin E1 (CCNE1) expression as well as upregulation of the miR-588 and tumour suppressor candidate 1 (TUSC1) signalling pathway (5). The biological roles of MIR497HG, miR-3918 and mRNA actin gamma 2 (ACTG2) expressions were found to repress the cell proliferation, migration and invasion of colorectal cancer (6). Despite these documented findings, the exact regulatory role and mechanism of MIR497HG and its significance miRNAs yet remain largely unknown. Therefore, the search for miRNAs that regulate the MIR497HG is important.

miRNA is a class of endogenous short ncRNA with 18-22 nucleotides in length. miRNA functions as post-transcriptional regulators of various diseases and cancers. Interestingly, a single miRNA can regulate hundreds of targets and stimulate multiple signalling pathways and vice versa. miRNA plays a pivotal role in various biological events such as cell growth, progression, proliferation, differentiation and apoptosis (8). To

date, more than 35,000 miRNAs have been identified across 271 organisms. Since miRNAs are involved in regulating various cellular processes, they are proposed as therapeutic targets for cancers including HCC. The miRNAs complementarily bind to target genes resulting in post-transcriptional silencing. A miRNA-target binding pattern and interaction can greatly contribute to a comprehensive understanding of cancer development and progression. The miRNA target is recognised via complementary sequence within the target mRNA and pairing between the miRNA seed region (seed match).

Identification of miRNA and its target mRNA is important for characterising miRNA function. Thus, the miRNA-target gene with at least two of algorithms must predict the same miRNA binding site for the validation of these miRNAs (9). The miRNA target prediction employed in this study was based on the predictive (de novo) method; algorithms derived from characteristics of the target mRNA sequence, in this study 3′ untranslated region (3′UTR) of MIR497HG. This approach employs a few features such as sequence features, thermodynamic stability, evolutionary conservation and site accessibility. Locating and identifying miRNA is often experimentally challenging, tedious and costly. On the other hand, computational methods can be conducted by utilising freely available web tools. However, regulation of miRNA based on a single prediction alone in a non-model organism tends to be false (10). Therefore, multiple selections and choices of algorithms are done based on seed pairing, thermodynamic stability, evolutionary conservation and target site accessibility (11). Prediction analysis carried out by miRDB based on support vector machines (SVMs) and high-throughput training dataset (based on statistical learning theory) (12). Meanwhile, prediction analysis carried out by STarMir based on implementation of logistic prediction models developed with miRNA binding data from crosslinking immunoprecipitation (CLIP) studies (13). Using the STarMir, input data is processed by web server to perform prediction of miRNA binding sites, comprehensive sequence, thermodynamic and target structure features and logistic probabilities (LogitProb) as measure of confidence for predicted site. Both miRDB and STarMir are freely available tools without registration or login requirement. Both miRDB and STarMir predicted miRNA targets in five species including human, rat, mouse, rat, dog and chicken. Both computational programs allow the user to enter a specific ''Gene Symbol'' and predicted miRNA target sites within that gene will compute by the algorithm. In this present study, we also utilised mRNA target prediction, TargetScan to identify the most likely functional mRNA targets of putative miRNAs (14).

This present finding could serve as a fundamental step in identifying a miRNA-mRNA target before the experimental validation. Taken together, this bioinformatic analysis may particularly contributed towards in vitro experimental verification.

MATERIALS AND METHODS

Sequence Retrieval and Analysis

The 3′UTR of primary-microRNA-195-497 (MIR497HG) sequence was used for target prediction in this study. Full-length of the MIR497HG sequences was obtained and retrieved (assessed on 18 February, 2022) at the National Centre for Biotechnological Information (NCBI) RefSeq databases (https://www.ncbi.nlm.nih.gov/) (Gene ID: 100506755) (Ensembl: ENSG00000267532). The coding region and open reading frame (ORF) from the nucleotide sequence were predicted and analysed using the ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/). The ORF sequences were analysed using the UTRDB tools (http://utrdb.ba.itb.cnr.it/). The 3′UTR of MIR497HG was determined by the downstream of MIR497HG sequence. Meanwhile, the updated sequences of published miRNAs (2,645 of *Homo sapiens* miRNAs) were obtained from the miRbase (https://www.mirbase.org/). In this present study, the 1.5kb of 3′UTR of MIR497HG was used as the target prediction.

Determination of Potential miRNA Interactions on the 3′UTR of MIR497HG

Bioinformatic target prediction algorithms were applied; miRDB (http://www.mirdb.org/) (12) and STarMir (https://sfold.wadsworth.org/cgi-bin/starmirtest2.pl) for identify and predict the miRNAs that targeting the 3′UTR of MIR497HG (13) . Using the miRDB, target scores was utilised to predict the binding of miRNAs to its target sequence (high score target). The STarMir is a nonlinear logistic prediction based on a miRNA binding database from cross-linking immunoprecipitation (CLIP) sequencing experiment. The STarMir incorporated comprehensive thermodynamic, structural and sequence features for the prediction and comparison of seed and seedless sites. The Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) was operated to inspect the list of common miRNAs and generated the online visualization of the Venn diagram. Then, RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid) was used to find the minimum free energy hybridisation of RNA. Using the Venny 2.1, common miRNAs by the prediction tools were selected for further screening.

Identification of mRNA Target of miRNAs

mRNA targets of putative miRNAs were identified by TargetScan 8.0 (https://www.targetscan.org/vert_80/). TargetScan predicts the mRNA targets by searching for the 6 to 8mer sites that match the seed region of miRNA (14).

Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis of mRNA Targets of miRNAs

The mRNA targets were further submitted to Enrichr (https://maayanlab.cloud/Enrichr/) for Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pthway analysis. Using Enrichr, the

input mRNA targets are compared to 35 gene-set library and the output of enrichment analysis are ranked based on p-value computed using the Fisher exact test (15).

RESULTS

miRDB and STarMir *In Silico* Identification of the miRNAs that Potentially Target 3'UTR MIR497HG

A total of 57 miRNAs was predicted by the miRDB web server to target the 3'UTR of MR497HG 1.5kb (in length) in Homo sapiens. Search results were sorted based on the target scores. High target score indicates the accuracy of prediction. Target scores ranged between 51-93. Three miRNAs demonstrated target scores >80 and ranked top including hsa-miR-3165, hsa-miR-7162-3p and hsa-miR-3143. Nine miRNAs displayed target scores between <80 and >70. Meanwhile, the last 45 miRNAs showed target scores between <70 and >50. A list of 57 potential miRNAs predicted by the miRDB is outlined in Table I. By implementing the STarMir program, a total of 291 miRNAs were discovered targeting the 3'UTR of MIR497HG with logitProb >0.5. Integrated comparative analysis of two algorithms unveiled that only 36 miRNAs were in common, targeting the 3'UTR of MIR497HG (Fig. 1). The common miRNAs predicted by the Venny 2.1.0 included hsa-miR-7162-3p, hsa-miR-3143, hsa-miR-7160-3p, hsa-miR-4804-3p, hsa-miR-2117, hsa-miR-4474-5p, hsa-miR-6817-3p, hsa-miR-7156-5p, hsa-miR-6882-3p, hsa-miR-6765-3p, hsa-miR-6763-5p, hsa-miR-3150a-3p, hsa-miR-5193, hsa-miR-6864-5p, hsa-miR-3175, hsa-miR-3918, hsa-miR-3190-5p, hsa-miR-7978, hsa-miR-4267, hsa-miR-16-1-3p, hsa-miR-6075, hsa-miR-942-5p, hsa-miR-921, hsa-miR-6821-3p, hsa-miR-4721, hsa-miR-31-5p, hsa-miR-877-3p, hsa-miR-452-3p, hsa-miR-216a-3p, hsa-miR-7110-3p, hsa-miR-6880-5p, hsa-miR-3182, hsa-miR-6081, hsa-miR-6132, hsa-miR-4270 and hsa-miR-766-3p. Total of 36 miRNAs were selected while validated miRNAs were extracted. The list of 36 predicted miRNAs is shown in Table II. Total of 36 miRNAs were narrowed down using a LogitProb score >0.8 of the STarMir. The list of four miRNAs included hsa-miR-3182, hsa-miR-7156-5p, hsa-miR-452-3p and hsa-miR-2117 as shown in Table III.

miRNA-lncRNA Duplex Target Interaction

miRDB predicted the binding site position of identified miRNAs and the seed sequence on the 3'UTR of MIR497HG. Finding suggested that identified miRNAs possessed the potential binding ability on the 3'UTR of MIR497HG. An illustration of predicted miRNA-miRNA duplex interaction can be seen in Fig. 2.

Prediction of Potential mRNA Targets of Identified miRNAs

Using TargetScan 8.0, total of 5238, 4801, 4006 and 4605 mRNA targets of hsa-miR-3182, hsa-miR-452-3p, hsa-miR-2117 and hsa-miR-7156-5p were found respectively. These mRNA targets were further submitted for enrichment analysis.

Gene Ontology (GO) Enrichment Analysis and KEGG Pathway Functional Analysis of mRNA Targets

Gene Ontology (GO) analysis of the putative mRNA targets of miRNAs (hsa-miR-3182, hsa-miR-452-3p, hsa-miR-2117 and hsa-miR-7156-5p) was done by a web-based enrichment bioinformatic tools Enrichr as in Fig.3. For mRNA targets for hsa-miR-3182, hsa-miR-2117 and hsa-miR-7156-5p the most enriched GO Molecular Function (MF) terms included cis-regulatory and RNA polymerase II cis-regulatory region sequence-specific DNA binding (Fig.3a, Fig.3c and Fig.3d). While, for hsa-miR-452-3p, the most enriched GO MF included ubiquitin conjugating enzyme binding and inward rectifier potassium channel activity (Fig 3b). The most enriched KEGG pathways, by Enrichr of the mRNA targets of miRNAs as in Fig.4. The most enriched KEGG pathways associated with mRNA targets of hsa-miR-3182 included glioma and cell differentiation (Fig.4a). For hsa-miR-452-3p, the most enriched KEGG pathways are included pathways in cancer and signalling pathways regulating pluripotency of stem cells (Fig. 4b). For hsa-miR-2117, the most enriched KEGG pathways are included herpes simplex virus 1 infection and phospholipase D signalling pathway (Fig 4.c). Lastly, for hsa-miR-7156-5p, the most enriched KEGG pathways included ErbB signalling pathway and Herpes simplex virus 1 infection (Fig 4.d).

DISCUSSION

Recent advancements in cancer therapies emerged focusing on the treatment and prognosis of HCC. However, the therapeutic option for HCC remains the major obstacle (16). miRNA regulates gene expression via complementary binding to its target messenger RNA (mRNA). Numerous studies reported that several miRNAs complementary bind to miRNA responsive elements (MREs) on the target mRNAs (17). To date, no studies have yet to be reported in specifically predicting the miRNA targeting the 3' untranslated region (3'UTR) of MIR497HG.

Computational identification and prediction of miRNA targeting mRNA is a critical initial step for experimental validation using miRNA target prediction algorithms, common features being used including seed match of miRNAs-mRNA pairing, thermodynamics stability or free energy (MFE) of base-pair probabilities and evolutionary conservation of the target sequence across species (18). Both miRDB and STarMir provided comprehensive sequence, thermodynamic, target structure features and logistic probabilities (LogitProb) scores to measure the confidence for each predicted site. These scores provided a quantitative measure of the overall regulatory effects of multiple seed and seedless sites on the target (12). The miRDB web server was able to correctly identify nine out of ten characterised target genes with a minimum false-

Table I: The list of 57 miRNAs targeting the 3'UTR of MIR497HG as predicted by the miRDB

Target Rank	Target Score (%)	miRNA Name	miRNA Sequence (5'-3')	Seed Location	Seed Match
1	92	hsa-miR-3165	AGGUGGAUGCAAUGUGACCUCA (22 nt)	1058	atccacca
2	85	hsa-miR-7162-3p	UCUGAGGUGGAACAGCAGC (19 nt)	1286	acctcaga
3	80	hsa-miR-3143	AUAACAUUGUAAAGCGCUUCUUUCG (25nt)	477	aatgttaa
4	79	hsa-miR-7160-3p	CAGGGCCCUGGCUUUAGCAGA (21 nt)	1441, 1521	gggccct, gggccct
5	78	hsa-miR-6768-3p	CAAAGGCCACAUUCUCCUGUGCAC (24 nt)	1510	ggccttta
6	77	hsa-miR-6873-3p	UUCUCUCUGUCUUUCUCUCUCAG (23 nt)	445, 1241, 1535, 1564	gagagaa, agagaga, agagagaa,agagaga
7	75	hsa-miR-4804-3p	UGCUUAACCUUGCCCUCGAAA (21 nt)	480	ttaagca
8	74	hsa-miR-2117	UGUUCUCUUUGCCAAGGACAG (21 nt)	287	agagaaca
9	74	hsa-miR-4474-5p	UUAGUCUCAUGAUCAGACACA (21 nt)	1567	gagactaa
10	74	hsa-miR-6780b-3p	UCCCUUGUCUCCUUUCCCUAG (21 nt)	910	acaaggga
11	73	hsa-miR-6817-3p	UCUCUCUGACUCCAUGGCA (19 nt)	1241,1535,1563	agagaga, agagaga, cagagaga
12	71	hsa-miR-7156-5p	UUGUUCUCAAACUGGCUGUCAGA (23 nt)	288	gagaacaa
13	69	hsa-miR-6882-3p	UGCUGCCUCUCCUCUUGCCUGCAG (24 nt)	1311	aggcagca
14	68	hsa-miR-6765-3p	UCACCUGGCUGGCCCGCCCAG (21 nt)	181, 948	ccaggtg, ccaggtg
15	68	hsa-miR-6763-5p	CUGGGGAGUGGCUGGGGAG (19 nt)	963	ctccccaa
16	68	hsa-miR-3150a-3p	CUGGGGAGAUCCUCGAGGUUGG (22 nt)	963	ctccccaa
17	67	hsa-miR-6879-3p	UGUCACCCGCUCCUUGCCCAG (21 nt)	1412, 1453	ggtgaca, gggtgac
18	67	hsa-miR-5193	UCCUCCUCUACCUCAUCCCAGU (22 nt)	416, 713, 988, 991, 1392, 1407	gaggagg, gaggagg, gaggagg, gaggagg, gaggagg
19	66	hsa-miR-6864-5p	UUGAAGGGACAAGUCAGAUAUGCC (24 nt)	1049	cccttcaa
20	65	hsa-miR-4273	GUGUUCUCUGAUGGACAG (18 nt)	288	gagaacaa
21	65	hsa-miR-6887-3p	UCCCCUCCACUUUCCUCCUAG (21 nt)	418, 915, 1394, 1580	ggagggg, ggagggg, ggagggg, gagggga
22	63	hsa-miR-8073	ACCUGGCAGCAGGGAGCGUCGU (22 nt)	179	tgccagg
23	63	hsa-miR-221-5p	ACCUGGCAUACAAUGUAGAUUU (22 nt)	179	tgccagg
24	62	hsa-miR-12119	UUCUGAGGGGACGGUAGAUUUGGGG (25 nt)	1287	cctcaga
25	61	hsa-miR-3175	CGGGGAGAGAACGCAGUGACGU (22 nt)	962, 1155, 1194	tctcccca, tctcccc, tctcccc
26	60	hsa-miR-3918	ACAGGGCCGCAGAUGGAGACU (21 nt)	115, 1522	ggccctg
27	59	hsa-miR-3190-5p	UCUGGCCAGCUACGUCCCCA (20 nt)	254, 740	tggccaga, ggccaga
28	59	hsa-miR-1185-2-3p	AUAUACAGGGGGAGACUCUCAU (22 nt)	525	ctgtataa
29	59	hsa-miR-1185-1-3P	AUAUACAGGGGGAGACUCUUAU (22 nt)	525	ctgtataa
30	59	hsa-let-7f-2-3p	CUAUACAGUCUACUGUCUUUCC (22 nt)	525	ctgtataa
31	58	hsa-miR-7978	UCUGGUGUAUAGCGUUGCUCA (21 nt)	315, 1421	acaccaga,acaccaga
32	58	hsa-miR-4267	UCCAGCUCGGUGGCAC (16 nt)	1028, 1402, 1462	agctgga, agctgga,agctgga
33	58	hsa-miR-16-1-3p	CCAGUAUUAACUGUGCUGCUGA (22 nt)	246	aatactga
34	58	hsa-miR-6075	ACGGCCCAGGCGGCAUUGGUG (21 nt)	436, 782	tgggccga, tgggccga
35	58	hsa-miR-942-5p	UCUUCUCUGUUUUGGCCAUGUG (22 nt)	446, 1537	agagaag,agagaaga
36	58	hsa-miR-921	CUAGUGAGGGACAGAACCAGGAUUC (25 nt)	1278	ctcactaa
37	58	hsa-miR-6821-3p	UGACCUCUCCGCUCCGCACAG (21 nt)	679, 726	agaggtca,agaggtca
38	58	hsa-miR-4721	UGAGGGCUCCAGGUGACGGUGG (22 nt)	616, 1326	agccctc, agccctca
39	57	hsa-miR-31-5p	AGGCAAGAUGCUGGCAUAGCU (21 nt)	176	tcttgcca
40	57	hsa-miR-4283	UGGGGCUCAGCGAGUUU (17 nt)	427, 599, 798, 1163	gagcccc, agcccca,gagcccc, agcccca
41	57	hsa-miR-570-3p	CGAAAACAGCAAUUACCUUUGC (22 nt)	519	tgttttc
42	56	hsa-miR-146b-3p	GCCCUGUGGACUCAGUUCUGGU (22 nt)	354, 1228	cacagggacacaggga
43	56	hsa-miR-877-3p	UCCUCUUCUCCCUCCUCCCAG (21 nt)	449, 1540, 1577	gaagagg
44	54	hsa-miR-6782-5p	UAGGGGUGGGGGAAUUCAGGGGUGU (25 nt)	214, 902, 1305	cacccct, acccta, acccta
45	54	hsa-miR-452-3p	CUCAUCUGCAAAGAAGUAAGUG (22 nt)	543	cagatgaa
46	54	hsa-miR-6892-5p	UCCCUCUCCCACCCCUUGCAG (21 nt)	194, 451, 502	gagaggg, agaggga, gagaggga
47	54	hsa-miR-6165	CAGCAGGAGGUGAGGGGAG (19 nt)	9, 467, 844	tcctgct, tcctgcta, cctgcta
48	53	hsa-miR-216a-3p	UCACAGUGGUCUCUGGGAUUAU (22 nt)	31, 48, 70	cactgtg, cactgtg, cactgtg
49	53	hsa-miR-7110-3p	UCUCUCUCCCACUUCCCUGCAG (22 nt)	1241, 1535, 1564	agagaga, agagaga, agagaga
50	53	hsa-miR-5691	UUGCUCUGAGCUCCGAGAAAGC (22 nt)	1091	cagagca
51	52	hsa-miR-6880-5p	UGGUGGAGGAAGAGGGCAGCUC (22 nt)	1059	tccacca
52	51	hsa-miR-3182	GCUUCUGUAGUGUAGUC (17 nt)	1574	acagaaga
53	51	hsa-miR-6081	AGGAGCAGUGCCGGCCAAGGCGCC (24 nt)	11, 387	ctgctcc, ctgctcca
54	51	hsa-miR-6132	AGCAGGGCUGGGGAUUGCA (19 nt)	865, 938	gccctgc, gccctgc
55	51	hsa-miR-4270	UCAGGGAGUCAGGGGAGGGC (22 nt)	831	ctccctga
56	51	hsa-miR-766-3p	ACUCCAGCCCCACAGCCUCAGC (22 nt)	132, 1029, 1403	ctggaga, gctggag, gctggag
57	51	hsa-miR-4319	UCCCUGAGCAAAGCCAC (17 nt)	1369	ctcaggga

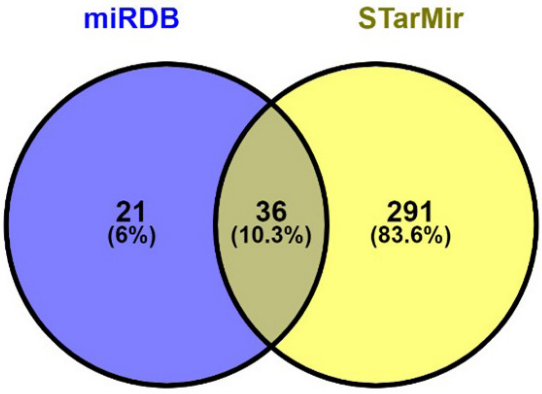

Figure 1: Comparative visualisation of the miRNAs that putatively target the 3′UTR of MIR497HG obtained from the miRDB and STarMir databases. A total of 36 common miRNAs were selected while the validated miRNAs were further removed.

Table II: The list of 36 miRNAs targeting the 3′UTR of MIR497HG obtained from two databases

miRNA	miRNA Sequence (5′-3′)
hsa-miR-7162-3p	UCUGAGGUGGAACAGCAGC (19 nt)
hsa-miR-3143	AUAACAUUGUAAAGCGCUUCUUUCG (25nt)
hsa-miR-7160-3p	CAGGGCCCUGGCUUUAGCAGA (21 nt)
hsa-miR-4804-3p	UGCUUAACCUUGCCCUCGAAA (21 nt)
hsa-miR-2117	UGUUCUCUUUGCCAAGGACAG (21 nt)
hsa-miR-4474-5p	UUAGUCUCAUGAUCAGACACA (21 nt)
hsa-miR-6817-3p	UCUCUCUGACUCCAUGGCA (19 nt)
hsa-miR-7156-5p	UUGUUUCUCAAACUGGCUGUCAGA (23 nt)
hsa-miR-6882-3p	UGCUGCCUCUCCUCUUGCCUGCAG (24 nt)
hsa-miR-6765-3p	UCACCUGGCUGGCCCGCCCAG (21 nt)
hsa-miR-6763-5p	CUGGGGAGUGGCUGGGGAG (19 nt)
hsa-miR-3150a-3p	CUGGGGAGAUCCUCGAGGUUGG (22 nt)
hsa-miR-5193	UCCUCCUCUACCUCAUCCCAGU (22 nt)
hsa-miR-6864-5p	UUGAAGGGACAAGUCAGAUAUGCC (24 nt)
hsa-miR-3175	CGGGGAGAGAACGCAGUGACGU (22 nt)
hsa-miR-3918	ACAGGGCCGCAGAUGGAGACU (21 nt)
hsa-miR-3190-5p	UCUGGCCAGCUACGUCCCCA (20 nt)
hsa-miR-7978	UCUGGUGUAUAGCGUUGCUCA (21 nt)
hsa-miR-4267	UCCAGCUCGGUGGCAC (16 nt)
hsa-miR-16-1-3p	CCAGUAUUAACUGUGCUGCUGA (22 nt)
hsa-miR-6075	ACGGCCCAGGCGGCAUUGGUG (21 nt)
hsa-miR-942-5p	UCUUCUCUGUUUUGGCCAUGUG (22 nt)
hsa-miR-921	CUAGUGAGGGACAGAACCAGGAUUC (25 nt)
hsa-miR-6821-3p	UGACCUCUCCGCUCCGCACAG (21 nt)
hsa-miR-4721	UGAGGGCUCCAGGUGACGGUGG (22 nt)
hsa-miR-31-5p	AGGCAAGAUGCUGGCAUAGCU (21 nt)
hsa-miR-877-3p	UCCUCUUCUCCCUCCUCCCAG (21 nt)
hsa-miR-452-3p	CUCAUCUGCAAAGAAGUAAGUG (22 nt)
hsa-miR-216a-3p	UCACAGUGGUCUCUGGGAUUAU (22 nt)
hsa-miR-7110-3p	UCUCUCUCCCACUUCCCUGCAG (22 nt)
hsa-miR-6880-5p	UGGUGGAGGAAGAGGGCAGCUC (22 nt)
hsa-miR-3182	GCUUCUGUAGUGUAGUC (17 nt)
hsa-miR-6081	AGGAGCAGUGCCGGCCAAGGCGCC (24 nt)
hsa-miR-6132	AGCAGGGCUGGGGAUUGCA (19 nt)
hsa-miR-4270	UCAGGGAGUCAGGGGAGGGC (22 nt)
hsa-miR-766-3p	ACUCCAGCCCCACAGCCUCAGC (22 nt)

Table III: List of four miRNAs targeting the 3′UTR of MIR497HG

miRNA	Sequence	Site Position	Seed Position	Seed Type	Seed Match
hsa-miR-3182	GCUUCUGUAGU-GUAGUC	1569-1580	1574-1580	8mer	acagaag
hsa-miR-7156-5p	UUGUUCU-CAAACUGGCUGU-CAGA	281-295	288-294	8mer	gagaacaa
hsa-miR-452-3p	CUCAUCUG-CAAAGAAGUAAGUG	518-549	543-549	8mer	cagatgaa
hsa-miR-2117	UGUUCUCUUUGC-CAAGGACAG	276-294	287-293	8mer	agagaaca

8mer (a perfect Watson-crick match from nucleotide 2 to nucleotide 8 of the miRNA seed, with an ''A'' in mRNA opposite position 1

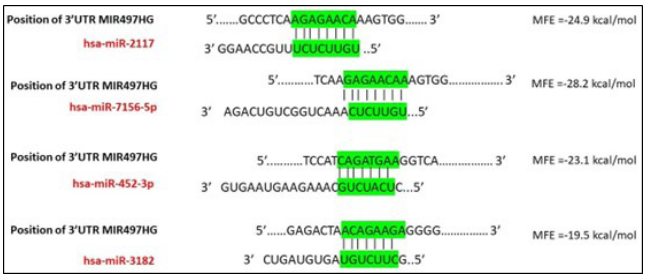

Figure 2: The predicted duplex structure of miRNA-target interaction using the miRDB. The first row (5` to 3`) presents the sequence of the target gene, whereas the second row (3` to 5`) displays the sequence of miRNA. The miRNA seed region is shaded in green as indicated.

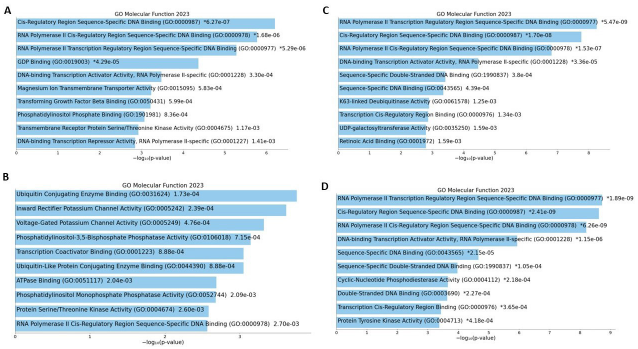

Figure 3: Distribution of differentially expressed mRNA targets based on Gene Ontology (GO). GO analysis of the mRNA targets based on their molecular functions (a) hsa-miR-3182; (b) hsa-miR-452-3p; (c) hsa-miR-2117; (d) hsa-miR-7156-5p.

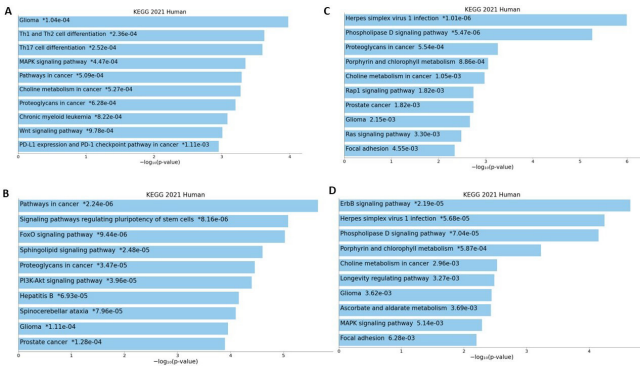

Figure 4: The KEGG pathways of the mRNA targets. (a) hsa-miR-3182; (b) hsa-miR-452-3p; (c) hsa-miR-2117; (d) hsa-miR-7156-5p.

positive rate of around 24% (19). The maximum target score was indicated as the accuracy of the prediction (20). The input data for STarMir was processed to perform the prediction of miRNA binding sites, compute comprehensive sequence, thermodynamic and target structure features and logistic probability as a measure of confidence for each predicted site. In STarMir, any sequences with LogitProb value <0.70 were excluded. In addition, both target prediction tools used in this study utilised full miRNA sequences and any input sequence for searching target genes. This gave a maximum sensitivity to the miRNA target search (21). In this study, we used Enrichr as the most updated databases for GO and KEGG analysis (22).

In this study, 1.5kb of 3'UTR of MIR497HG was used as the gene of interest and as the predicted miRNA binding site since a partial 3'UTR sequence may give imprecise positive results due to the higher accessibility of the miRNA and the incomplete match for the complementary binding (23). The bioinformatic prediction may also generate false positive hits, therefore, multiple bioinformatic target prediction programs were used and the results were further filtered for functional potential targets.

This present study only provides fundamental step-by-step for the identification of regulatory miRNA in the lncRNA. Computational and experimental approaches such as reporter gene assay needs to be done for verifying the authenticity of identified miRNA-mRNA targets in future study.

CONCLUSION

In summary, we employed computational tools to search and identify miRNAs that putatively target the 3'UTR of MIR497HG. Overall, this study concludes that hsa-miR-3182, hsa-miR-7156-5p, hsa-miR-452-3p and hsa-miR-2117 are potential miRNAs of 3'UTR of MIR497HG. Taken together, the miRNAs identified in this study would be discovered as potential biomarkers in cancer research.

ACKNOWLEDGEMENT

The study was supported by the Ministry of Higher Education (MOHE) Malaysia, under the Fundamental Research Grant Scheme, (FRGS/1/2019/SKK10/UITM/02/4).

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer 2021; 40, 1-41. doi: 10.3322/caac.21660.
2. Wang YC, Li S. 2019. Clinical characteristics and prognosis of 2887 patients with hepatocellular carcinoma. Medicine 2019; 98(4). doi: 10.1097/MD.0000000000014070.
3. Morishita A, Oura K, Tadokoro T, Fujita K, Tani J, Masaki T. MicroRNAs in the pathogenesis of hepatocellular carcinoma: A Review. Cancers 2021; 13(514). doi.org/10.3390/cancers13030514.
4. Zhuang C, Liu Y, Fu S, Yuan C, Luo J, Huang X, Yang W, Zhuang C. Silencing of lncRNA MIR497HG via CRISPR/Cas13d induced bladder cancer progression through promoting the crosstalk between Hippo/Yap and TGF-β/Smad signaling. Frontiers in Molecular Biosciences, 2020;7. doi: 10.3389/fmolb.2020.616768.
5. Ji LY, Wei M, Liu YY, Di ZL, Li SZ. miR-497/MIR497HG inhibits glioma cell proliferation by targeting CCNE1 and the miR-588/TUSC1 axis. Oncology Reports, 2021; 46(225). doi: 10.3892/or.2021.8206.
6. Tang G, Wu D, Guo M, Li H. LncRNA MIR497HG inhibits colorectal cancer progression by the miR-3918/ACTG2 axis. Journal of Genetics, 2022; 101(27). https://dpi.org/10.1007/s12041-022-01367-w.
7. Furuta M, Kozaki KI, Tanimoto K, Tanaka S, Arii S, Shimamura T, Niida A, Miyano S, Inazawa J. The tumour-suppressive miR-497-195 cluster targets multiple cell-cycle regulators in Hepatocellular carcinoma. PLOS ONE, 2013; 8(3). doi:10.1371/journal.pone.0060155.
8. Rossi MN, Antonangeli F. LncRNAs: New players in apoptosis control. International Journal of Cell Biology, 2014. doi:10.1155/2012/473857
9. Kuhn E.D, Martin MM, Feldman DS, Terry Jr AV, Nuovo GJ, Elton TS. Experimental validation of miRNA targets. Methods, 2008; 44(1), 47-54. doi: 10.1016/j.ymeth.2007.09.005
10. Fridrich A, Hazan Y, Moran Y. Too many false targets for microRNAs: Challenges and pitfalls in prediction of miRNA targets and their gene ontology in model and non-model organisms. BioEssays, 2019; 41:1-11. doi: 10.1002/bies.201800169.
11. Riolo G, Cantara S, Marzocchi C, Ricci C. miRNA targets: From prediction tools to experimental validation. Methos Protoc, 2021; 4(1). https://dpi.org/10.3390/mps4010001.
12. Chen Y, Wang X. miRDB: an online database for prediction of functional miRNA targets. Nucleic Acids Res, 2020; 8(48): 127-131. doi: 10.1093/nar/gkz757.
13. Rennie W, Liu C, Carmack CS, Wolenc A, Kanoria S, Lu J, Long D, Ding Y. STarMir: a web server for prediction of microRNA binding sites. Nucleic Acids Research, 2014; 42. doi:10.1093/nar/gku376.
14. Agarwal V, Bell GW, Nam JW, Bartel DP. eLife, 2015, 4(e05005), 1-38. doi: 10.7554/eLife.05005.
15. Chen YE, Tan CM, Kou Y, Duan Q, Wang Z,

Meirelles GV, Clark NR, Ma'ayan A. Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics, 2013; 14(128). doi: 10.1186/1471-2105-14-128.

16. Kumari R, Sahu MK, Tripathy A, Uthansingh K, Behera M. Hepatocellular carcinoma treatment: hurdles, advances and prospects. Hepat.Oncol, 2018; 5(2). doi: 10.2217/hep-2018-0002.
17. Chipman LB, Paquinelli AE. miRNA targeting: Growing beyond the seed. Trends in Genetics, 2019; 35(3). doi:10.1016/j.tig.2018.12.005
18. Peterson SM, Thompson JA, Ufkin ML, Sathyanarayana P, Liaw L, Condon CB. Common features of microRNA target prediction tools. Frontiers in Genetics, 2014; 23(5), 1-10. doi: 10.3389/fgene.2014.00023.
19. Kanoria S, Rennie W, Liu C, Carmack CS, Lu J, Ding Y. STaRMir tools for prediction of microRNA binding sites. Methods Mol Biol, 2016; 1490. 73-82. doi: 10.1007/978-1-4939-6433-8.
20. Min H, Yoon S. Got target? : computational methods for microRNA target prediction and their extension. Exp. Mol. Med, 2010; 42(4). 233-244. doi: 10.3858/emm.2010.42.4.032.
21. Wong N, Wang X. miRDB: An online resource for microRNA target prediction and functional annotation. Nucleic Acids Research, 2015; 43: 146-152. doi: 10.1093/nar/gkul1104.
22. Zahari Sham SY, Azwar S, Yip WK, Ng CT, Abdullah M, Thevandran k, Osman M, Seow HF. Prediction of mRNA targets of miR-101-3p in diabetic kidney disease by bioinformatic tools. Mal J Med Health Sci, 2022; 18(Supp21):67-41. doi:10.47836/mjmhs18.s21.11.
23. Barreda-Manso MA, Nieto-Diaz M, Soto A, Munoz-Galdeano T, Reigada D, Maza RM. In silico and in vitro analyses validate human microRNAs targeting the SARS-CoV-2 3'UTR. Int. J. Mol.Sci, 2021; 22(6094). doi: 10.3390/ijms22116094