Computational identification of human miR-802-5p from type I diabetes genome sequence.

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Abstract

Background: Type 1 Diabetes Mellitus (T1DM) is distinguished by the autoimmune destruction of insulin-producing cells in the pancreas by infiltrating CD4+ and CD8+ T cells and macrophages. Several studies have found that there are selective expressions of miRNAs that may be regulated to diabetic conditions. microRNAs (miRNAs) called non-coding RNAs regulate a variety of biological processes including cell proliferation and apoptosis and can serve as diagnostic and therapeutic targets in treating various diseases including T1DM. The current study aims in identifying miRNAs in T1DM from genome sequences found in public genomic databases.

Materials and methods: In this study, we have used the National Centre for Biotechnology Information (NCBI) web portal to identify miR-802-5p for T1DM using a bioinformatics approach and RNA fold was used to create the secondary structure.

Results: hsa-miR-802-5p was identified in the T1DM genome sequence with the minimum folding free energy of the secondary structure was found to be -33.60 kcal.

Conclusion: In conclusion, miR-802-5p, a novel miRNA has been identified from human T1DM through a computational approach. However, more research on miR-802-5p is needed to be studied in suppression and progression of T1DM. The management and treatment strategies for T1DM are elusive and the exact molecular mechanism is not yet clearly studied. This computational approach contributes to a better understanding of miRNAs role as biomarkers and how they can be used for diagnosis, prognosis and as a therapeutic target.

Keywords: MicroRNAs; Type 1 Diabetes Mellitus; miR-802-5p; Biomarker; Innovative technique

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Introduction

Type 1 Diabetes Mellitus (T1DM) is distinguished by the autoimmune destruction of insulin-producing cells in the pancreas by infiltrating CD4+ and CD8+ T cells and macrophages [1]. T1DM has a more complicated etiology, with a number of factors contributing to the disease onset, including due to defective immune system, genetic factors, and virus infections [2]. Although existing treatments cannot prevent or cure T1DM, some research areas provide the rays of hope like islet-cell transplantation, stem cells, primary and secondary disease prevention. Immune tolerance against β-cells can help to halt autoimmune destruction [3]. Early detection of islet cell stress prior to loss of islet function would allow for therapeutic intervention to delay or alleviate diabetes onset. [4]. Emerging technologies for recovering, amplifying and detecting nucleic acids in the blood have enabled sensitive methods to make gene expression profiling correlations with specific states of disease. Several studies have found that there are selective expressions of miRNAs that may be regulated to diabetic conditions [5].

In general, microRNAs (miRNAs) are a type of noncoding single-stranded RNA molecule embedded by endogenous genes of about 19-25 nucleotides length that regulates post-transcriptional gene expression in plants and animals [6]. miRNAs are arising as key regulators of cell proliferation, cell death and physiological conditions [7]. Drosha and Dicer, two RNase III enzymes that catalyze two subsequent processing events, one in the nucleus and other in cytoplasm respectively, are responsible for miRNA biogenesis. Each miRNA can regulate multiple genes, and also several miRNAs can enforce the same gene, resulting in a complex regulatory network that regulates the expression of multiple genes *via* miRNA or a combination of miRNAs [8]. Human miRNAs bind to their target mRNA sequence with perfect or near perfect sequence complementarity, according to the increasing evidence [9]. Thus, miRNAs contribute effective strategies for identifying potential T1DM targets for diagnostic and therapeutic purposes.

Currently, there are no reliable biomarkers for managing and controlling T1DM. As a result, identifying new biomarkers in the early stages of the disease is critical and constitutes a substantial challenge. Our team has extensive knowledge and research experience that has translate into high quality publications [10-28]. Therefore, the current study used a publicly available database to recognize the miRNA from T1DM expressed sequence tags to treat T1DM.

Materials and Methods

We have used a bioinformatics framework to address the miRNA in the T1DM genome sequence in this study, with data collected from publicly accessible databases.

Computational prediction of targets and secondary structure

Human genome sequence data was obtained through the National Center for Biotechnology Information (NCBI) web portal for international nucleotide sequence database consortium. The search term keyword "Type 1 Diabetes Mellitus in *Homo sapiens*" was used to extract the Type 1 Diabetes Mellitus genome sequence using this free search engine.

The search term keyword "Type 1 Diabetes Mellitus in *Homo sapiens*" was extracted using this free search engine. Human mature miRNAs were selected out of many entries from miRbase. After removing the low-quality and redundant sequences, a local nucleotide database was formed for T1DM specific genome sequences. The previously mentioned nucleotide data set was looking for the homolog among the miRNAs dataset.

The mature miRNAs were used as a source to search for similar T1DM genome sequences. The FASTA formats of all sequences were processed and mature miRNA sequences were aligned against the unique genome sequences. Genome sequences were aligned to reference pre-miRNA sequences. Then the aligned portion was expressed as a candidate pre-miRNA sequence. The secondary structure was then obtained using RNA fold which provided the miRNA expressed in the T1DM genome sequence which helped in target prediction, which was done using target scan.

Results

The computational approach was used to identify miRNA, which is less expensive than other methods. Following the collection of NCBI database and detailed examination of the secondary structure, hsa-miR-802-5p was identified in the T1DM genome sequence.The mature sequence found using RNAfold is GUGGCUGUUAUUUGCAGUCAGUAACAAAGAUUCAU CCUUGUGUCCAUCAUGCAACAAGGAGAAUCUUUGU CACUUAGUGUAAUUAAUAGCUGGAC.The minimum folding free energy of the secondary structure was found to be -33.60 kcal. Figure 1 showing the secondary structure of identified hsa-miR-802-5p. The incorporated pre-miRNAs were confirmed for secondary structure using mRNA Fold.

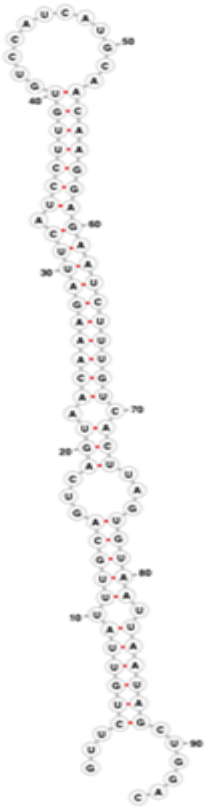
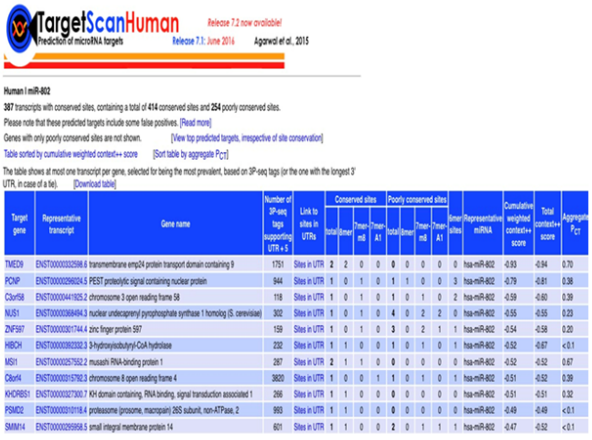


Figure 1. *Showing the secondary structure of identified hsa-miR-802-5p.*

In addition, miRNA target analysis has been analyzed by the target scan online computational tool to identify miR-802-5p targets. The prediction of miR-802-5p targets was determined using Target Scan. Figure 2 representing the target genes of hsa-miR-802-5p and Table 2 representing the characteristics of mature sequence of hsa-miR-802-5p.


TargetScanHuman

Prediction of microRNA targets

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Human | miR-802

387 transcripts with conserved sites, containing a total of **414** conserved sites and **254** poorly conserved sites.

Please note that these predicted targets include some false positives. [Read more]

Genes with only poorly conserved sites are not shown. [View top predicted targets, irrespective of site conservation]

Table sorted by cumulative weighted context++ score [Sort table by aggregate P_{CT}]

The table shows at most one transcript per gene, selected for being the most prevalent, based on 3P-seq tags (or the one with the longest 3' UTR, in case of a tie). [Download table]

Target gene	Representative transcript	Gene name	Number of 3P-seq tags supporting UTR + 5	Link to sites in UTRs	Conserved sites				Poorly conserved sites				8mer sites	Representative miRNA	Cumulative weighted context++ score	Total context++ score	Aggregate P_{CT}
					total	8mer	7mer-m8	7mer-A1	total	8mer	7mer-m8	7mer-A1					
TMED9	ENST00000332586.6	transmembrane emp24 protein transport domain containing 9	1751	Sites in UTR	2	2	0	0	0	0	0	0	0	hsa-miR-802	-0.93	-0.94	0.70
PCNP	ENST00000296024.5	PEST proteolytic signal containing nuclear protein	944	Sites in UTR	1	0	1	0	1	1	0	0	3	hsa-miR-802	-0.79	-0.81	0.36
C3orf58	ENST00000441925.2	chromosome 3 open reading frame 58	118	Sites in UTR	1	0	1	0	1	0	1	0	2	hsa-miR-802	-0.59	-0.60	0.39
NUS1	ENST00000368494.3	nuclear undecaprenyl pyrophosphate synthase 1 homolog (S. cerevisiae)	302	Sites in UTR	1	0	1	0	4	0	2	2	0	hsa-miR-802	-0.55	-0.55	0.23
ZNF597	ENST00000301744.4	zinc finger protein 597	159	Sites in UTR	1	0	1	0	3	0	2	1	1	hsa-miR-802	-0.54	-0.58	0.20
HIBCH	ENST00000382332.3	3-hydroxyisobutyryl-CoA hydrolase	232	Sites in UTR	1	1	0	0	1	0	1	0	1	hsa-miR-802	-0.52	-0.67	<0.1
MSI1	ENST00000257582.2	musashi RNA-binding protein 1	267	Sites in UTR	2	1	1	0	0	0	0	0	0	hsa-miR-802	-0.52	-0.52	0.67
C8orf4	ENST00000315782.2	chromosome 8 open reading frame 4	3820	Sites in UTR	1	0	0	1	1	0	1	0	1	hsa-miR-802	-0.51	-0.52	0.39
KHDRBS1	ENST00000327300.7	KH domain containing, RNA binding, signal transduction associated 1	266	Sites in UTR	1	1	0	0	0	0	0	0	0	hsa-miR-802	-0.51	-0.51	0.32
PSMD2	ENST00000310118.4	proteasome (prosome, macropain) 26S subunit, non-ATPase, 2	993	Sites in UTR	1	1	0	0	0	0	0	0	0	hsa-miR-802	-0.49	-0.49	<0.1
SMIM14	ENST00000295958.5	small integral membrane protein 14	601	Sites in UTR	1	1	0	0	2	0	1	1	1	hsa-miR-802	-0.47	-0.52	<0.1

Figure 2. *Representing the target genes of hsa-miR-802-5p.*

Source miRNA	Source Organism	PL	MFE ∆.G	MS	ME	Strand	A+U %
hsa-miR-802-5p	Homo sapiens	94	-33.6	23	CAGUAACAAAGAUUCAUCCUUGU	5p	62.76%

Table 1. *Representing the characteristics of mature sequence of hsa-miR-802-5p.*

Discussion

T1DM is characterized by the autoimmune destruction of pancreatic cells, resulting in insufficient insulin production and the development of hyperglycemia [29]. T1DM and its complications are increasing around the world. Evidence gathered suggested that T1DM is caused by a combination of genetic and environmental factors. T1DM is a direct cause of associated complications such as retinopathy, neuropathy and other cardiovascular complications [30]. miRNAs are small RNA molecules that regulate gene expression by post-transcriptional modifications by binding to the complementary sequence in the 3'-untranslated region of their targets. Changes in the expression of miRNA coding genes have been extensively reported in a variety of diseases, including T1DM. The presentation of non-invasive biomarkers for the early detection of T1DM by evaluating miRNA gene expression levels can be a significant step forward in the field of life sciences and medicine [31].

However, a study by Zurawek M et al. suggested that miR-487a-3p may suppress the activity of CTLA4 and FOXO3 genes by binding to their 3' untranslated region contributing to T1DM development [32]. MiR-21, miR-155 and miR-338 expression levels were measured in the Peripheral Blood Mononuclear Cells (PBMCs) of T1DM patients and healthy controls. These findings indicated a significant relationship between miR-21 and miR-155 expression levels in T1DM. Thus, miR-155 can be used as a biomarker to monitor disease progression in T1DM patients [33]. It is also demonstrated that in T1DM patients, miR-21 can be used as an early marker for diagnosing and recognizing diabetic nephropathy [34]. Further development of miRNAs for the treatment of T1DM in both animal models and human clinical trials are urgently needed to be studied.

Conclusion

In conclusion, miR-802-5p, a novel miRNA has been identified from human T1DM through a computational approach. However, more research on miR-802-5p is needed to be studied in suppression and progression of T1DM. The management and treatment strategies for T1DM are elusive and the exact molecular mechanism is not yet clearly studied. This computational approach contributes to a better understanding of miRNAs role as biomarkers and how they can be used for diagnosis, prognosis and as a therapeutic target.

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Conflict of Interest

The authors declare no conflict of interest.

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