

miRNAs in Treating Cardiomyopathy

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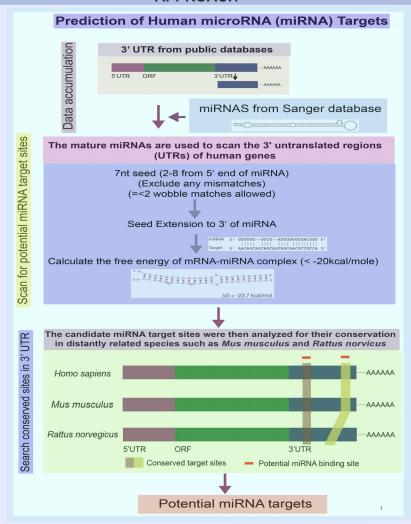
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ABSTRACT

Cardiomyopathy also known as "heart muscle disease" weakens heart muscles and thus interferes with normal functioning of heart, ultimately leading to its failure. Many genes have been proposed to be associated with this disorder, but the regulation of these genes has not yet been analyzed in detail. Recently a few miRNAs were found to play very significant roles in regulating important genes and in the development of heart. The present study aims to explore the role of miRNAs in the genetic regulation of some genes, involved in cardiomyopathy. Sanger miRNA database containing over 6000 reported miRNAs was scanned using a novel algorithm for the potential miRNAs against these genes. The potential candidate miRNAs are being validated by transfecting pre-miRNAs and/or anti-miRNAs into HL-1 cells (cardiomyocytes). In order to validate the results, the confirmed miRNAs will further be subjected to luciferase assay by cloning their target sequences in the reporter construct. The ultimate aim of the study is to construct potential antagomirs and/or miRNA inhibitors which can be used as drugs in order to treat cardiomyopathy that can prove to be better and safe alternatives to the currently available drugs like ACE inhibitors, Digitalis, Beta blockers.

APPROACH



RESULTS

Potential miRNA targets: The following set of genes was found downregulated in cardiomyopathy. These genes were scanned as targets for miRNAs. [Position: binding sites of miRNA with the target; Fold Change: Change in expression level (down/up regulation)]

Gene	Identifier	miRNA	Position	Fold change
Ras-related protein (rab 18)	P35293	miR-19b	93-100	1.7
Elastin (ELN)	M24782	miR-29c	38-45	1.9
Prot-oncogene (BMI-1)	L13689	miR-128a	481-488	1.8
Heat shock protein apg-2	AB02342	miR-549		3.5
Phosphoglucomutase 1	M83088	miR-34, miR-449	150-157	
Human transforming growth factor-beta (tgf-beta)	M60316	miR-489	437-444	2.45
Homo sapiens HMP mRNA for motor protein,	D21094	miR-527	194-201	2.32
Homo sapiens mRNA for ANA	D64110	miR-106b,miR-20a,miR-20b,miR-519d	167-174	2.28
Homo sapiens P2x purinoceptor mRNA,	AF000234	miR-106a	381-388	2.25^
Homo sapiens bone morphogenetic protein-4 (hBMP-4) gene	U43842	miR-363	194-201	1.83
Human galactosyltransferase mRNA,	M13701	miR-424, miR-497, miR-15	393-400	1.83
Homo sapiens KIAA0010 mRNA	D13635	miR-9	300-307	1.70
Human G protein-coupled receptor (EBI 1) mRNA,	L31581	let-7	172-179	1.68
H.sapiens mRNA for IL13 receptor alpha-1 chain	Y09328	miR-22	166-172	1.68
Human putative Cu++-transporting P-type ATPase mRNA	L06133	miR-148b	36-43	1.07
Human APC gene mRNA	M74088	miR-142-3p	310-317	1.03

Multiple target sites on a 3' UTR for one or more miRNAs were also obtained and reflect cooperative regulation of transcription.

Position 167-174 of BTG3 3 UTR 5' UDUUAAAAAAUUAUDGCACUUUA hsa-miR-106b 3' UUAAAAAUUAUAUCACUUUA hsa-miR-20b 3' GAUGGACGUGACAGUCOUGAAAU Position 167-174 of BTG3 3 UTR 5' UDUUAAAAAAUUAUAUCACUUUA hsa-miR-20b 3' GAUGGACGUGACAGUCUUAAAU Position 167-174 of BTG3 3 UTR 5' UUAAAAAUUCAGAAAUUAAUCACUUAAAUUAAAUUAAAU								,
Has-mR-106b 3' UAGAGGUGACAGUCOUGAAAU Has-mR-20b 3' GADGGACGGUGALCCCCOUGAAC Has-mR-20b 3' GADGGACGGUGALCCCCCCUA								
has-mR-166 3' UKAGOGGACAGUCOUDAAAU Position 167-174 of BTG3 T UTR 5'UGUABAAAUUUCGACAGU		Position 167-174 of BTG3 3' UTR	S'	CUUAAAAAAUAUAUGCACUUUA	Position 167-174 of BTG3 3' UTR	5'	.UCUUAAAAAAUAUAUGCACUUUA	
Position 167-174 of BTG3 3' UTR 5' UCUUAAAAAATUAUDCACCUUUA hsa-miR-20s 3' GAUGGACCOUCUACUCUCUCUACUCUCUACUCUCUACUCUCUCUC			1					
		hsa-miR-106b	3' UA	GACGUGACAGUCGUGAAAU	hsa-miR-20b	3'	GAUGGACGUGAUACUCGUGAAAC	
hsa-miR-20a s' GAUGGACGUGGACAGGUCCOCCUA Pestion 393-400 of B4GALT1 YUTR s'DBAAAAUUCGGACAGGUCCOCCUA Pestion 393-400 of B4GALT1 YUTR s'DBAAAAUUCGGACAGGUCCOCCUA Pestion 393-400 of B4GALT1 YUTR s'DBAAAAUUCGGACAGGUCCOCCUA		Position 167-174 of BTG3 3' LITE	8 51 IIC	THIADADADAHAHAHAGCACHHILA	Position 167-174 of BTG3 3' UTR	5'	UCUUAAAAAAUAUAUGCACUUUA	
Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAUUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAAUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*USAAUUCGGAGGUGCUGCUUA Position 393-400 of B4GALT1 3 UTR 5*U								
		hsa-miR-20a	3' GA	UGGACGUGAUAUUCGUGAAAU	hsa-miR-519d	3'	UGUGAGAUUUCCCUCCGUGAAAC	
	DW 202 400 -4 D40 41 T4 22 1TD			D 31 200 400 4 D40 41 T4 D4 17TD				
	Position 393-400 of B4GAL113 UTR			Position 393-400 of B4GAL11 3 UTR			Position 393-400 of B4GALT1 3' UTR 5'	
isa-ilin-457 S GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	han miD 424			han miD 407				
	noo nare 424	- IIIIIII JUUNGUUN - NOO		1130-11111-4-57	o occordance Acadea		nsa-miK-150 31	ACAUUUUGUACUAC-ACGACGAU

Gene Identifier	MFE (Kcal/mole)
P35293	-23.6
M24782	-25.1
L13689	-21.5
AB023420	-22.2
M83088	-22.5
M60316	-24.5
D21094	-27.2
D64110	-29.1
AF000234	-22.6
U43842	-24.1
M13701	-28.6
D13635	-20.4
L31581	-24.5
Y09328	-26.5
L06133	-22.3
M74088	-25.6

selected genes downregulated in Cardiomyopathy					
Position 310-317 of APC 3' UTR 5'GUCUUAAAAUAAUGAACACUACA IIII IIIIIII hsa-miR-142-3p 3' AGGUAUUUCAUCCUUUUGUGAUGU	Position 93-100 of RAB18 3' UTR 5' UUAGGGACCUUGCAGUUUGCACA				
Position 507-514 of PIPSK1B 3' UTR S'ACUAGGUCUGCCUUC-ACUUUAUA	Position 150-157 of PGM1 3' UTR 5' AGUGCAUUUACAAGGCACUGCCA				
Position 226-233 of ST6GALNAC4 3' UTR 5'CUCCCCCCUCCACUC-CCUCAGUA	Position 150-157 of PGM1 3' UTR 5' AGUGCAUUUACAAGGCACUGCCA				
Position 150-157 of PGM1 3' UTR 5' agugcauuuacaaggcacugcca	Position 226-233 of ST6GALNAC4 3' UTR 5' CUCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC				
Position 381-388 of P2RX4 3' UTR 5' GGCGGCUCUGUUCAACACACUUUA IIII IIIIIII	Position 38-45 of ELN 3' UTR 5' CGACCUCAUCAACGUUGGUGCUA IIII IIIIIII hsa-miR-29c 3' UGGCUAAAGGUUCACGGAU				

Detential miDNA target cites in the 21 ITDs of

DISCUSSION AND CONCLUSIONS

A balanced search strategy was chosen to predict the targets in the most efficient manner. To distinguish functional sites from false positives clustering of related genes (from related species) has been done to identify real targets. Multiple miRNA sites were found for single target genes. This can be due to the fact that a single site for a particular miRNA might function in association with independent target sites for other miRNAs in the same UTR. All these facts have to be conseidered while designing antagomirs against miRNAs. This is an ongoing research work and many significant results are pouring in.

ONGOING AND FUTURE WORK

pre-miRNAs and/or anti-miRNAs of the potential candidate miRNAs are being transfected into HL1 (cardiomyocytes) and the results are analyzed by Q-PCR and Western Blot of the respective genes.

In order to validate the results, the confirmed miRNAs are further subjected to luciferase assay by cloning their target sequences in the adjacent reporter construct.



Once validated, probes against the respective miRNAs are planned to be used to quantitate the miRNAs in patient samples. The ultimate aim of the study is to construct potential antagomirs and/or miRNA inhibitors which can be used as drugs in order to treat cardiomyopathy.

REFRENCES

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