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CHARACTERISTICS OF MIRNA INTERACTION WITH 5'UTR, CDS, 3'UTR MRNA CANDIDATE GENES OF METABOLIC SYNDROME DISEASE

Studying of the pathogenesis of metabolic syndrome is one of the most topical because of the widespread of this pathology. The study of genetic nature of cardiovascular diseases is one of the most promising areas of molecular medicine worldwide. Identification of a group of individual genetic markers allows early diagnosis and optimization of primary and secondary prevention of metabolic syndrome. Therefore, the process of biomarkers discovery should improve the therapy and assessment of the risk of cardiovascular disease. The search for effective markers is the subject of ongoing research. The miRNAs interaction with mRNAs of candidate genes were predicted using the MirTarget program. The genes responsible for the development of metabolic syndrome, regulated by miRNAs were selected by searching in the PubMed database. ADRA2A and SCAP genes are regulated by 12 and 15 miRNAs through 5'UTR with the highest binding energy is -144 kJ/mole and -151 kJ/mole, respectively. AR, CEBPA, IGFBP2, KL and SIRT1 genes are regulated through CDS by 7, 19, 11, 13 and 6 miRNAs, with the highest binding energy is -134 kJ / mole, -142 kJ/mole, -140 kJ/mole, -142 kJ/mole and -138 kJ/mole, respectively. Clusters of miRNA binding sites with overlapping nucleotide sequences were detected in mRNA of several genes. Several associations of miRNA and genes are proposed as biomarkers for developing methods for diagnosing the metabolic syndrome.

Key words: miRNA, mRNA, metabolic syndrome, candidate genes.

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miRNA-дың метаболитикалық синдром ауруы кандидатты гендерінің mRNA-дың 5'UTR, CDS және 3'UTR-мен өзара әрекетінің сипаттамалары

Метоболитикалық синдромының кең таралғандығынан оның патогенезін зерттеуі ең маңыздысы болып табылады. Әлемдік молекулярлы медицинада перспективті түрде және жан-жақты қарастырылып жатқан аурулардың бірі – жүрек-қан тамыр ауруының генетикасын зерттеу. Бөлек генетикалық маркерлерді анықтау – метаболитикалық синдромның біріншілік және екіншілік профилактикасының оптимизациясы мен ерте диагностикасына мүмкіндік береді. Осылайша, биомаркерлер арқылы жүрек-қан тамыр ауруларының қауіп-қатерлерін бағалау және терапиясын жақсартуға болады. Жұмыстың мақсаты – эффективті маркерлерді табу болып табылады. miRNA-лар және кандидатты гендер mRNA-дың арасындағы өзара әрекеттесулері MirTarget бағдарлама арқылы табылды. miRNA-мен реттелінетін метаболитикалық синдромға жауапты гендер PubMed деректер қорынан іздестірілді. ADRA2A және SCAP гендері 5'UTR-де 12 және 15 miRNAs-мен байланысады, жоғары байланысу энергиясы 144 кДж/моль және 151 кДж/моль құрайды. AR, CEBPA, IGFBP2, KL және SIRT1 гендері CDS-те, 7, 19, 11, 13 және 6 miRNAs-мен байланысады, жоғары байланысу энергиясы -134 кДж/моль, -142 кДж/моль, -140 кДж/моль, -142 кДж/моль және 138 кДж/мольді құрайды. Бұл гендерде miRNA-мен байланысатын көптеген

сайттары бар және де осы байланысу кластерлер метаболитикалық синдром диагностикасында биомаркерлер ретінде бола алады.

Түйін сөздер: miRNA, mRNA, метаболитикалық синдром, кандидатты гендер.

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Характеристики взаимодействия miRNA с 5'UTR, CDS и 3'UTR mRNA кандидатных генов метаболического синдрома

Изучение генетической природы метаболического синдрома является одной из наиболее перспективных областей молекулярной медицины во всем мире. Выявление генетических маркеров позволяет разработать раннюю диагностику заболевания и оптимизировать профилактику метаболического синдрома. Обнаружение биомаркеров должно улучшить терапию и оценку риска сердечно-сосудистых заболеваний. Поиск эффективных маркеров метаболического синдрома является предметом текущих исследований. Взаимодействие miRNA с mRNA кандидатных генов было предсказано с помощью программы MirTarget. Гены, ответственные за развитие метаболического синдрома, регулируемые микроРНК, были отобраны путем поиска в базе PubMed. Гены ADRA2A и SCAP регулируются 12 и 15 miRNAs в 5'UTR, наибольшая энергия связывания составляет -144 кДж/моль и -151 кДж/моль соответственно. В CDS mRNA генов AR, CEBPA, IGFBP2, KL и SIRT1 связываются с 7, 19, 11, 13 и 6 miRNA со свободной энергией взаимодействия равной -134 кДж/моль, -142 кДж/моль, -140 кДж/моль, -142 кДж/моль и -138 кДж/моль, соответственно. В mRNA нескольких генов выявлены кластеры сайтов связывания miRNA с наложением нуклеотидных последовательностей. Несколько ассоциаций miRNA и генов предлагаются в качестве биомаркеров для разработки методов диагностики метаболического синдрома.

Ключевые слова: miRNA, mRNA, метаболический синдром, гены кандидаты.

Introduction

Cardiovascular diseases are the leading cause of death worldwide. Essential hypertension is a major risk factor for the development of other cardiovascular diseases and is caused by a combination of environmental and genetic factors, with up to 50% of blood pressure variance currently attributed to an individual's genetic makeup. [1]. Metabolic syndrome is a cluster of various combinations of metabolic abnormalities associated with an increased risk of cardiovascular disease and type 2 diabetes mellitus (DM) [2, 3]. Efforts to date have identified several candidate genes involved in primary hypertension, including *ADD1, ABCB1, CYP3A5, AGT, GRK4, GNB3* and *NOS3* [4-7]. Genes *ACACB, APOA1, APOA2, APOB, APOC1, APOC3, APOD* and *APOE* are associated with metabolic syndrome [8]. MiRNAs are small noncoding RNAs that have emerged as important regulators of many biological and pathological processes, including those relevant to the development of the heart and cardiovascular disease, such as metabolic syndrome. There are still several interesting reviews about recent progress on miRNAs and cardiovascular disease, which discusses the relationship between miRNAs and cardiac development, myocardial regeneration, and cardiovascular disease [9-21]. The search for effective markers of the risk of metabolic syndrome is the subject of this research.

Materials and methods

The nucleotide sequences of candidate genes of the metabolic syndrome (MS) were downloaded from GenBank [http://www.ncbi.nlm.nih.gov]. 3701 miRNAs were taken from the publication of Londin E. et al. [22]. The miRNAs binding sites in 5'-untranslated regions (5'UTRs), coding domain sequences (CDSs) and 3'-untranslated regions (3'UTRs) of several genes were predicted using the MirTarget program [23]. This program defines the following features of binding: a) the origin of the initiation of miRNA binding to mRNAs; b) the localization of miRNA binding sites in the 5'UTRs, CDSs and the 3'UTRs of the mRNAs; c) the free energy of hybridization (ΔG, kJ/mole); and d) the schemes of nucleotide interactions between miRNAs and mRNAs. The ratio ΔG/ΔGm (%) was determined for each site (ΔGm equals the free energy of miRNA binding with its perfect complementary nucleotide sequence). The miRNA binding sites located on the mRNAs had ΔG/ΔGm ratios of 90% or more. The program identifies the positions of the

binding sites on the mRNA, beginning from the first nucleotide of the mRNA's 5'UTR. The MirTarget program found hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, and A and C. The distances between A and C were equal to those between G and C, A and U, and G and U [24-25]. The numbers of hydrogen bonds in the G-C, A-U, G-U and A-C interactions were found to be 3, 2, 1 and 1, respectively.

Results and Discussion

miRNAs bound with the high ΔG/ΔGm value in 5'UTR, CDS and 3'UTR of mRNAs genes participating in MS. 41 target genes were associated with 70 miRNAs in 5'UTR (Table 1).

mRNA of *ADRA2A* gene had binding sites for miR-19-33623-3p, miR-1-2121-3p, miR-19-21199-3p, miR-17-41168-3p, miR-4-11421-3p, miR-22-16963-5p, miR-8-24509-3p and miR-20-22562-3p located in a cluster from 256 nt to 288 nt. The total length of the miRNAs is 180 nt, which would sequentially take 965 nt of the 5'UTR of this gene. Thus, such arrangement of nine sites in a cluster of 32 nt in length makes sense to reduce the proportion of binding sites.

30 binding sites for miR-19-37933-5p, miR-1-2121-3p, miR-3-8100-5p, miR-19-21199-3p, miR-19-33623-3p, miR-1-155-3p, miR-1-1714-3p, miR-17-40348-5p, miR-2-3313-3p, miR-15-32047-5p, miR-9-28523-5p, miR-2-4453-3p and miR-19-43966-3p were detected in mRNA of *SCAP* gene forming a cluster of 39 nt in length from 96 to 135 nt. Without overlapping sites, their length would be 294 nt, which exceeds the 5'UTR size of 259 nt.

Table 1 – Characteristics of miRNAs interaction in the 5'UTR of mRNA of metabolic syndrome candidate gene

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ACSL1	miR-19-42639-3p	81	-115	90	22
ACVR1C	miR-5-15435-5p	9	-108	93	20
	miR-22-46172-3p	207	-121	90	22
ADRA2A	miR-2-3578-5p	36	-110	96	18
	miR-17-38379-3p	496	-119	89	23
	miR-11-29838-3p	871	-121	89	23
	miR-4-13479-5p	913	-106	93	20
ALDH2	miR-21-16482-3p	8	-123	92	21
APLN	miR-6-16211-3p	263	-123	89	23
AR	miR-19-41434-3p	621	-115	95	21
BRAP	miR-X-44865-3p	162	-115	92	20
CAPN10	miR-6-16793-3p	105	-115	95	20
CAV1	miR-19-41383-3p	311	-115	90	23
	miR-16-39450-3p	779	-117	89	23
CXCL16	miR-2-7122-3p	88	-119	93	21
	miR-17-35758-5p	439	-117	93	22
EPO	miR-12-31979-3p	12	-121	89	23
ESR2	miR-17-38870-3p	41	-117	92	22
FAAH	miR-5-15548-3p	19	-129	92	23
FGF19	miR-12-31701-3p	151	-115	90	22
	miR-11-28201-3p	163	-127	88	24
	miR-13-32816-5p	298	-115	90	22
H6PD	miR-6-16847-3p	58	-121	92	22
HMGA1	miR-22-46516-3p	350	-117	92	21
HMOX1	miR-16-22443-3p	75	-113	95	20

Continuation of table 1

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
HNF4A	miR-9-26042-5p	53	-125	92	22
HSD11B1	miR-9-26506-3p	24	-115	93	22
HTR2A	miR-1-2136-3p	600	-104	91	21
HTR2C	miR-4-10151-3p	424	-119	90	22
ICAM1	miR-1-1109-3p	106	-117	89	23
IGFBP2	miR-17-39570-5p	65	-123	91	22
	miR-19-34424-3p	84	-113	95	20
	miR-8-22971-3p	86	-117	92	21
IL6R	miR-19-30988-5p	329	-129	90	23
	miR-10-13751-3p	336	-121	92	21
	miR-7-15849-3p	341	-110	96	18
INPPL1	miR-3-7790-3p	96	-121	93	21
INSIG1	miR-4-11181-3p	5	-115	93	20
	miR-11-14307-3p	53	-115	92	21
MMP2	miR-2-6328-5p	264	-117	89	23
MTMR9	miR-18-41332-3p	281	-125	91	23
	miR-2-6530-5p	288	-119	92	22
	miR-2-5888-3p	290	-125	91	23
NEDD4L	miR-5-12460-5p	56	-129	90	24
NOS3	miR-3-8846-5p	200	-123	88	24
NPY2R	miR-7-20689-3p	903	-96	92	20
PDK4	miR-11-12657-3p	47	-108	91	21
	miR-19-34424-3p	216	-110	93	20
PLTP	miR-17-12804-3p	108	-113	93	20
PTEN	miR-20-43459-5p	75	-115	92	20
	miR-5-15564-3p	486	-125	91	22
	miR-17-41310-3p	708	-110	96	18
	miR-3-9461-3p	790	-121	89	23
SCAP	miR-12-30825-5p	22	-115	92	22
	miR-12-31721-3p	23	-108	91	21
SERPINE1	miR-16-38458-3p	30	-123	88	24
SH2B1	miR-11-23098-5p	225	-110	91	21
STEAP4	miR-14-33186-5p	49	-129	88	24
TGFB1	miR-20-43381-5p	1	-121	92	21
	miR-5-8853-5p	6	-115	92	20
	miR-9-13610-3p	6	-121	92	21
	miR-12-30416-5p	186	-117	92	22
	miR-10-13655-3p	209	-129	95	22
	miR-11-29785-3p	232	-108	91	21
	miR-11-29785-5p	232	-108	91	21
	miR-9-26506-3p	237	-113	91	22
	miR-17-38733-3p	241	-119	89	24
TPM1	miR-19-8151-3p	168	-117	92	21

Continuation of table 1

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
TRIB1	miR-2-3313-3p	233	-142	89	25
	miR-17-40348-5p	239	-127	94	23
	miR-1-155-3p	245	-127	92	22
	miR-2-7252-3p	303	-123	88	24
	miR-4-7472-5p	360	-110	91	21

Binding sites for five miRNAs were identified in mRNA of *MMP2* gene in the region from 110 nt to 125 nt, the miRNAs lengths without sites overlapping would be 114 nt.

Four binding sites in the mRNA of *TGFB1* gene formed a 33 nt long cluster from 232 nt to 265 nt, without overlapping miRNAs lengths would be equal to 88 nt.

mRNAs of *MTMR9* and *TRIB1* genes had three miRNA binding sites formed in the cluster. Four miRNA binding sites were found in mRNAs of *PTEN* and *TGFB1* genes in the cluster from 531 to 558 nt and from 232 to 265, respectively. In the mRNA of *PNPLA3* gene, binding sites of miR-X-13195-3p, miR-1-265-3p, miR-19-33623-3p, miR-17-41168-3p, and miR-1-1714-3p are located in a cluster from 148 nt to 172 nt, the total length of the miRNAs is 109 nt.

The average free energy of miRNAs binding with all target mRNAs in 5'UTR was equal to -122,4 ± -10,5 kJ/mole. There were 69 associations of miRNAs with mRNAs having a free energy of binding of more than -120 kJ/mole.

The following miRNAs and gene pairs were established as effective associations: miR-2-3313-3p and *TRIB1*, miR-2-3313-3p and *SCAP*, miR-1-2121-3p and *SCAP*, miR-19-21199-3p and *SCAP*, miR-1-2121-3p and *ADRA2A*, miR-19-21199-3p and *ADRA2A* with free interaction energy of -140 kJ/mole and more.

Of the 66 miRNAs target genes in CDS, their mRNA was associated with 133 miRNAs (Table 2).

Table 2 – Characteristics of miRNAs interaction in the CDS of mRNA of metabolic syndrome candidate gene

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ABCA1	miR-11-21109-3p	6416	-110	90	23
ACE	miR-10-27065-3p	60	-117	93	21
	miR-11-28656-5p	62	-125	89	23
	miR-2-3313-3p	63	-138	87	25
	miR-3-8100-5p	64	-132	90	24
ACVR1C	miR-17-39672-3p	278	-113	91	21
ADIPOR1	miR-6-16717-3p	1092	-121	93	22
ADRA1A	miR-17-34996-5p	1761	-113	91	23
ADRA2A	miR-7-20411-3p	1064	-123	91	23
	miR-X-44737-3p	1526	-123	89	24
	miR-19-43342-3p	1535	-119	90	22
	miR-5-15733-3p	1536	-132	89	24
	miR-16-38088-5p	1842	-123	89	23
	miR-13-32613-3p	2006	-125	88	24
	miR-19-43736-3p	2049	-119	90	22
ADRA2B	miR-15-36451-5p	455	-121	89	23
	miR-22-44023-3p	465	-121	92	21
	miR-12-32603-3p	882	-115	92	23
	miR-19-43065-3p	889	-113	90	22

Continuation of table 2

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ADRB3	miR-21-45808-3p	1138	-115	95	20
	miR-2-4736-5p	1306	-121	92	21
	miR-1-654-3p	1312	-115	92	20
	miR-5-8853-5p	1312	-115	92	20
AGTR1	miR-6-3109-5p	102	-117	92	22
ALDH2	miR-1-3575-5p	1559	-119	89	23
ANGPTL4	miR-19-43315-5p(2)	258÷259	-121÷-134	90÷100	23
	miR-9-26025-3p	567	-113	90	22
ANGPTL6	miR-19-43342-3p	433	-121	92	22
	miR-7-20142-5p	526	-119	89	23
	miR-19-42224-5p	535	-117	95	21
	miR-6-18047-5p	599	-100	94	19
	miR-16-37839-3p	855	-117	90	23
APOA1	miR-10-13655-3p	841	-123	91	22
APOB	miR-13-36375-5p	179	-119	90	23
	miR-19-25731-3p	2054	-93	92	20
	miR-19-25731-5p	2054	-93	92	20
APOE	miR-X-45440-5p	643	-121	95	22
	miR-9-23547-5p	766	-115	93	20
	miR-9-24355-5p	768	-115	93	20
AR	miR-10-26714-5p	1363	-127	94	24
	miR-17-35260-3p	1517	-115	90	22
	miR-17-40389-5p	1744	-113	95	20
	miR-5-15733-3p	2514	-134	90	24
	miR-1-1819-3p(2)	2515÷2518	-123÷-125	89÷91	23
BTN2A1	miR-10-26815-5p	280	-121	88	24
CAPN10	miR-22-45441-3p	474	-115	89	23
	miR-2-5634-5p	1114	-104	89	24
CDH13	miR-2-7128-3p	1089	-102	89	23
CEBPA	miR-19-28028-5p	233	-132	89	24
	miR-21-23994-3p	236	-113	91	21
	miR-20-40417-3p	777	-113	96	19
	miR-22-16963-5p	782	-127	91	22
	miR-6-16525-3p	885	-119	89	23
CLOCK	miR-3-11123-5p	1181	-98	90	22
CUL7	miR-11-1939-5p	124	-115	92	20
	miR-19-42999-3p	832	-110	90	22
	miR-22-45904-3p	3313	-115	92	22
	miR-17-38391-3p	4439	-119	93	23
	miR-13-35476-3p	4443	-117	90	22
	miR-9-25099-3p	4444	-115	90	22
CYP46A1	miR-1-527-3p	1469	-106	93	20
DYRK1B	miR-5-15058-5p	1562	-113	93	20
	miR-9-25558-3p	1810	-125	88	24

Continuation of table 2

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ENPP1	miR-X-44972-5p	32	-119	93	20
EPO	miR-3-8171-3p	741	-110	93	22
ESR1	miR-5-4100-5p	409	-106	91	22
	miR-4-5310-3p	1852	-115	90	23
FAAH	miR-17-38856-3p	108	-115	93	20
	miR-15-36321-3p	901	-115	90	22
FADS2	miR-16-33426-5p	1447	-110	90	22
FGF23	miR-2-7679-5p	301	-102	91	22
GCKR	miR-10-26109-5p	820	-119	92	22
GPX1	miR-5-15733-3p	98	-134	90	24
	miR-5-16634-3p	338	-121	90	22
H6PD	miR-4-12982-5p	519	-110	91	21
	miR-7-20135-3p	1080	-110	90	22
HMGA1	miR-19-43704-3p	550	-115	90	23
HNF1A	miR-4-12346-5p	1463	-108	89	23
	miR-8-24124-3p	1825	-113	90	22
HP	miR-1-3943-5p	1178	-98	92	20
IGFBP2	miR-15-39164-3p	214	-121	97	20
IL6R	miR-2-4533-3p	483	-125	89	23
INPPL1	miR-5-14202-5p	161	-123	91	22
	miR-20-42676-3p	161	-127	91	23
	miR-22-45334-5p	169	-123	92	23
	miR-10-26505-5p	1162	-119	89	23
INS	miR-16-38416-3p	402	-115	90	22
INSIG1	miR-8-23775-5p	339	-117	95	21
	miR-14-14807-5p	414	-110	91	21
	miR-11-28905-3p	601	-117	89	23
INSR	miR-4-11316-5p	127	-132	89	24
IRS1	miR-3-7886-3p	412	-127	88	24
	miR-8-23997-5p	1418	-102	94	19
	miR-22-45452-5p	2084	-102	92	20
	miR-10-26714-5p	2085	-121	89	24
	miR-19-37450-3p	3446	-106	93	21
KL	miR-1-3822-3p	539	-121	92	22
	miR-19-41858-5p	2542	-121	89	23
LDLR	miR-10-26537-5p	2452	-108	96	20
LMNA	miR-16-37015-3p	1392	-113	93	20
LRP5	miR-17-38580-3p	148	-115	90	22
	miR-2-2243-3p	2090	-106	91	21
	miR-19-41684-3p	2469	-121	89	24
	miR-1-3919-5p	4788	-121	88	24
	miR-19-38895-3p	4824	-123	89	24
LRP6	miR-7-20135-3p	3421	-110	90	22

Continuation of table 2

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
MMP2	miR-21-45324-5p	379	-125	91	23
	miR-19-43421-5p	1681	-108	91	21
	miR-17-39037-3p	1691	-113	90	22
MMP9	miR-17-38947-5p(2)	219÷220	-113	90	22
	miR-16-34158-5p	698	-113	93	22
MTRR	miR-12-31626-5p	1751	-108	89	23
NEDD4L	miR-5-14114-5p	853	-123	89	23
	miR-3-9322-5p	2585	-102	91	21
NOS3	miR-15-38767-3p	2946	-123	89	24
	miR-X-45814-5p	3073	-117	89	24
	miR-19-43338-3p	3599	-117	90	22
PNPLA3	miR-22-46211-3p	918	-106	93	22
PRDM16	miR-4-11714-5p	2235	-110	96	20
	miR-11-27530-5p	2876	-119	89	23
	miR-11-29785-3p	3424	-108	91	21
	miR-11-29785-5p	3424	-108	91	21
RLN3	miR-16-33426-5p	46	-110	90	22
	miR-17-39466-3p	55	-110	90	22
	miR-3-7740-5p	304	-113	96	20
SCAP	miR-12-17092-3p	2486	-125	91	22
SERPINE1	miR-2-3962-5p	542	-125	88	24
SH2B1	miR-5-15578-5p	2148	-119	89	23
	miR-4-13219-5p	2834	-106	91	22
	miR-1-1109-3p	2898	-117	89	23
SIRT1	miR-5-13181-3p	232	-123	89	24
	miR-9-25099-3p	435	-115	90	22
SREBF2	miR-1-2002-3p	564	-121	90	22
TNF	miR-20-42898-3p	230	-121	92	23
	miR-20-42898-5p	230	-121	92	23
TPM1	miR-15-35627-5p(2)	332÷333	-117÷-123	95÷100	22
TRIB1	miR-8-24549-5p	756	-127	90	24
UCP3	miR-19-42357-3p	406	-115	89	23
VEGFA	miR-9-26506-3p	775	-113	91	22
	miR-8-21883-3p	887	-123	88	24

mRNA of *AR* gene contained 21 miRNA binding sites for miR-9-20317-3p and miR-17-39416-3p, whose nucleotide sequences are overlapped.

miR-10-27065-3p, miR-11-28656-5p, miR-2-3313-3p, miR-3-8100-5p binding sites were identified in a cluster in the region from 60 to 88 nt of mRNA of *ACE* gene, the total length of the miRNAs is 93 nt.

The 22 miRNA binding sites in mRNA of *CEBPA* gene formed a cluster with a length of 35 nt, which, without overlapping of sites, would be 308 nt.

18 binding sites for 11 miRNAs were identified in mRNA of *IGFBP2* gene in the region from 139 nt to 177 nt.

There is a 45 nt in length cluster in mRNA of *KL* gene formed by 14 binding sites of 11 miRNAs with a total length of 279 nt, therefore such a compact site arrangement is necessary to reduce the proportion of binding sites.

Six binding sites in mRNA of *SIRT1* gene formed a 60 nt long cluster from 264 nt to 324 nt, without overlapping miRNAs lengths is equal to 142 nt. mRNAs of *CUL7* and *INPPL1* genes contained 3 binding sites of miRNAs, forming a cluster.

The average free energy of binding of miRNAs with all mRNAs in CDS is equal to -118±-9 kJ/mole. There are 120 associations of miRNAs with mRNAs that have a free energy of binding of more than -120 kJ/mole.

50 target genes were associated with 82 miRNAs in 3'UTR (Table 3).

Five miRNAs had binding sites in mRNA of *CD36* gene, forming a cluster with a length of 40 nt. The total length of these miRNAs was 115 nt, which is almost 50% of the 275-nt CDS length. mRNA of *IGF1* gene contains nine miRNA-binding sites for miR-3-5147-5p and miR-101-27078-5p, which nucleotide sequences completely coincided, with a cluster length of 39 nt. mRNA of *JAK2* gene bound with miR-10-29282-3p (ten binding sites) and miR-15-36862-3p (nine binding sites) and the formed clusters coincide.

mRNAs of *CD36*, *IGF1* and *JAK2* genes contained 15, 18 and 19 binding sites, respectively, which miRNA nucleotide sequences overlapped. Four miRNA binding sites formed a cluster with a length of 67 nt from 7321 nt to 7388 nt in mRNA of *IRS1* gene.

Table 3 – Characteristics of miRNAs interaction in 3'UTR of mRNA of metabolic syndrome candidate gene

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ACE	miR-13-28252-3p	4068	-117	90	22
	miR-1-2030-3p	4657	-110	90	22
	miR-X-46577-3p	4792	-106	91	21
ADIPOQ	miR-17-39935-3p	1651	-104	91	21
ADRA1A	miR-6-19858-3p	1975	-108	91	22
ADRB3	miR-10-26254-3p	2347	-121	88	24
	miR-6-17487-3p	2442	-115	92	23
	miR-10-29282-3p	2456	-108	93	23
	miR-15-36862-3p	2456	-113	93	23
	miR-15-36862-3p	2462	-113	93	23
AGTR2	miR-16-40163-5p	2307	-121	90	23
AHI1	miR-22-45902-3p	4645	-113	93	22
	miR-9-21385-3p	5491	-96	90	22
AKT1	miR-10-27065-3p	2864	-117	93	21
	miR-12-5800-5p	2866	-113	93	20
	miR-13-36375-5p	2875	-119	90	23
ANGPT2	miR-7-21133-5p	3064	-121	89	24
	miR-5-18072-3p	3071	-102	91	22
AR	miR-3-10752-3p	4096	-108	89	23
CD36	miR-12-31413-3p	3526	-104	89	23
	miR-15-36862-3p(5)	3529÷3539	-108	89	23
	miR-10-29282-3p(5)	3533÷3539	-104	89	23
	miR-19-42814-5p	3542	-106	91	23
	miR-10-29282-3p	3543	-104	89	23
CEBPA	miR-22-45967-3p	2440	-115	92	22
CYP11B2	miR-17-12514-5p	1982	-104	92	20
CYP46A1	miR-13-33774-5p	1571	-123	88	24

Continuation of table 3

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
DIO2	miR-11-28228-3p	4777	-96	92	20
	miR-11-27435-3p	6187	-96	90	22
DYRK1B	miR-11-28385-3p	2463	-104	92	20
ENPP1	miR-10-26483-5p	6274	-110	90	22
	miR-2-4804-5p(2)	6751÷6752	-113÷117	90÷93	24
ESR1	miR-8-24024-3p	3339	-121	88	24
FADS2	miR-19-43386-3p	2407	-117	89	23
	miR-1-1412-5p	2712	-117	90	22
	miR-17-39583-3p	2803	-119	89	23
FTO	miR-17-34996-5p	3710	-110	90	23
	miR-2-5355-3p	3906	-115	90	22
GCKR	miR-6-18764-3p	1956	-121	88	24
H6PD	miR-1-318-5p	3669	-115	92	22
	miR-22-45335-5p	5829	-113	90	23
	miR-X-46030-5p	6616	-110	90	22
HMGA1	miR-15-38620-5p	877	-119	90	22
ICAM1	miR-15-36862-3p	2987	-108	89	23
	miR-17-39935-3p	3022	-104	91	21
	miR-10-26483-5p	3025	-110	90	22
IGF1	miR-3-5147-5p(9)	4042÷4058	-100	90	22
	miR-101-27078-5p(9)	4042÷4058	-108	89	23
IGF2BP3	miR-X-48172-3p	4088	-104	91	22
IL10	miR-17-39466-3p	1200	-110	90	22
IL6R	miR-14-35161-5p	3063	-119	90	24
INPPL1	miR-17-40267-5p	4336	-125	88	24
	miR-10-28609-3p	4512	-104	91	22
INSR	miR-19-42303-3p	5363	-117	90	23
	miR-12-30825-5p	5366	-115	92	22
	miR-12-31721-3p	5367	-115	96	21
IRS1	miR-10-29282-3p(2)	7402÷7404	-104	89	23
	miR-10-29282-3p	7425	-104	89	23
JAK2	miR-10-29282-3p(10)	5184÷5202	-104÷108	89÷91	23
	miR-15-36862-3p(9)	5184÷5200	-108	89	23
LCN2	miR-22-23987-3p	682	-123	94	21
LDLR	miR-17-39466-3p	3887	-110	90	22
	miR-8-11096-5p	3890	-113	90	22
	miR-X-45975-5p	4004	-96	92	22
	miR-4-12245-3p(2)	4559÷4560	-110	90	22
	miR-2-4826-5p(2)	4607÷4608	-113÷115	90÷92	23
	miR-7-20771-3p	4974	-89	91	21
LEP	miR-8-11096-5p	3087	-113	90	22
	miR-17-35758-5p	3092	-113	90	22

Continuation of table 3

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
LMNA	miR-18-41332-3p	2808	-123	89	23
	miR-11-28484-5p	2820	-117	90	22
	miR-19-42375-3p	3033	-113	93	21
LPIN1	miR-17-40078-3p	4485	-113	88	24
LTA	miR-16-9117-3p	1258	-98	92	21
MTHFR	miR-9-24450-5p	3350	-117	89	24
	miR-10-11641-3p	6281	-119	89	23
	miR-X-44909-3p	6342	-108	91	22
	miR-2-4684-5p	6844	-117	93	22
	miR-22-45902-3p	7051	-113	93	22
MTMR9	miR-17-8001-3p	4094	-110	90	23
	miR-X-45975-5p	6064	-96	92	22
	miR-2-4826-5p(2)	6186÷6187	-113	90	23
MTTP	miR-19-42953-5p	3438	-113	91	22
NPY2R	miR-3-9607-3p	2968	-96	90	22
OLR1	miR-3-5147-5p(2)	1504÷1506	-100	90	22
	miR-101-27078-5p(2)	1504÷1506	-108	89	23
PRDM16	miR-19-43175-3p	4675	-113	91	21
	miR-22-44124-5p	4961	-115	89	23
	miR-8-23404-5p	5193	-125	88	24
	miR-X-38664-5p	5199	-110	93	22
PYY	miR-1-252-5p	489	-106	91	21
SH2B1	miR-16-40163-5p	4530	-119	89	23
SLC22A12	miR-7-17280-5p	2874	-119	90	22
SREBF2	miR-17-38738-5p	4196	-117	90	22
	miR-1-2142-3p	4206	-123	92	23
STEAP4	miR-17-34996-5p	3201	-113	91	23
TGFB1	miR-9-13610-3p	2060	-123	94	21
	miR-17-12804-3p	2062	-113	93	20
	miR-8-24549-5p	2066	-125	88	24
	miR-15-38620-5p	2089	-119	90	22
	mir-1-2121-3p	2093	-140	89	25
TRIB3	miR-4-5601-5p	1228	-117	90	22
	miR-16-37914-3p	1540	-121	88	25

Five miRNAs binding sites were found in mRNA of *TGFB1* gene, from 2060 nt to 2118 nt, forming a cluster with a length of 58 nt, considering a total length of miRNAs sequences is 112. nt

mRNAs of *AKT1* and *TGFB1* genes had three miRNA binding sites formed into clusters, and the mRNA of *ADRB3* gene contains the binding sites of 4 miRNAs from 2442 to 2485 forming the 43 nt length cluster while a total length of the miRNAs sequences is 92 nt.

The average free energy of binding of miRNAs with all mRNAs in 3'UTR was -111 nt. 15 associations of miRNAs and mRNAs were found with a free binding energy of more than -120 kJ/mole.

Among 109 genes participating in the development of MS, 41 genes were associated with

70 miRNAs in 5'UTR, 66 genes with 133 miRNAs in CDS, and 50 genes with 82 miRNAs in 3'UTR.

Consequently, almost half of the miRNAs are bound at the beginning of the nucleotide sequence of mRNA (in the 5'UTR), which makes it possible to stop protein synthesis before the translation stage. This allows you to save energy resources of the cell, since stopping protein synthesis at the late stage of translation may result in the termination of protein synthesis with the formation of defective polypeptides. Binding of miRNA to mRNA at the beginning of the CDS will also help save energy resources. Therefore, the majority of miRNA binding sites in the mRNA of candidate MS genes are located in the CDS immediately after 5'UTR. In addition to the preferential localization of miRNA binding sites at the beginning of mRNA, the free energy of miRNA interaction with mRNA plays an important role in the process of regulating gene expression. As a rule, the free energy of the interaction of miRNA with mRNA for binding sites located in 5'UTR is greater than for binding sites located in CDS. And the free energy of miRNA interaction with mRNA for binding sites in CDS is greater than for binding sites located in 3'UTR.

Of them, 27 genes that bound to only one miRNA were located in 5'UTR region, 33 genes in CDS, and 23 genes in 3'UTR. The remaining mRNAs of genes involved in the development of MS were associated with two or more miRNAs. Thus, 14 genes had miRNA binding sites in 5'UTR, 33 genes in CDS, and 27 in 3'UTR. Consequently, most of miRNA-mRNA associations are located in CDS. mRNAs of following genes interacted with miRNAs with the highest value of free binding energy: *SCAP* gene (ten miRNAs) in 5'UTR, *CEBPA* gene in CDS (seven miRNAs), and *TGFB1* gene in 3'UTR (one miRNAs) The binding sites located in 5'UTR and CDS had the strongest interaction. Only *H6PD, HMGA1, IL6R* and *INPPL1* genes had binding sites in 5'UTR, CDS and 3'UTR. The presence of many binding sites for several miRNAs in mRNA genes indicates a strong dependence of their expression on miRNA.

Identified in the mRNA of some genes, the binding sites of two or more miRNAs, with overlapping nucleotide sequences, lead to competition between these miRNAs for binding to mRNA. The overlap of nucleotide sequences of binding sites reduces their share in the total length of mRNA.

Conclusion

It was established that the genes responsible for the development of metabolic syndrome are regulated by miRNAs. So *ADRA2A*, *PTEN, SCAP* and *TGFB1* genes are regulated by binding of 12, 9, 15 and 8 miRNAs to 5'UTR with the highest binding energy is equal to -144 kJ/mole, -132 kJ/mole, -151 kJ/mole and -129 kJ/mole, respectively. *APRA2A*, *AR*, *CEBPA*, *IGFBP2*, *KL* and *SIRT1* genes are regulated by 7, 7, 19, 11, 13 and 6 miRNAs binding to CDS with the highest binding energy being -132 kJ/mole, -134 kJ/mole, -142 kJ/mole, -140 kJ/mole, -142 kJ/mole and -138 kJ/mole, respectively. *ADRA2A, SCAP, AR, CEBPA, IGFBP2, KL*, and *SIRT1* genes had multiple binding sites of miRNAs, forming clusters. Thus, these genes can be used as potential markers for the diagnosis of the metabolic syndrome.

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