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CHARACTERISTICS OF miRNA INTERACTION WITH 5′UTR, CDS AND 3′UTR mRNA CANDIDATE GENES OF MYOCARDIAL INFARCTION AND ISCHEMIC HEART DISEASE

The study of the involvement of miRNAs in the regulation of the expression of candidate genes of myocardial infarction and coronary heart disease will facilitate the development of new effective methods for noninvasive diagnosis of these diseases. Using the MirTarget program to determine the characteristics of the interaction of miRNA with mRNA, the following original results were obtained. The 183 genes involved in the development of MI, 35 genes were associated with 51 miRNAs in the 5′UTR, 53 genes with 94 miRNA in the CDS, and 37 genes with 50 miRNAs in the 3′UTR. 24 genes bind to only one miRNA were arranged in the 5′UTR, 36 genes in the CDS, and 24 genes in the 3′UTR. The remaining mRNAs of the genes involved in the development of MI were associated with two or more miRNAs. Nine genes under the control of miRNAs were bound in the 5′UTR, 21 genes in the CDS, and 15 in the 3′UTR. Most of the interactions occurred at the CDS site. The largest number of binding sites had the mRNA of the CXCR2 and FAIM2 genes in the 3′UTR. Only three genes had binding sites in all regions: AP3D1, GATA2, SEMA3F. MI candidate genes PDED4 was regulated by 13 miRNAs with free energy of binding miRNAs with mRNAs -144 kJ/mole; genes ILF3, GATA2 were regulated by five and three miRNAs respectively with the free energy of binding miRNAs with mRNAs -138 kJ/mole; genes TGFBR1 and AP3D1 were regulated by six and five miRNAs with free energy of binding miRNAs with mRNAs -136 kJ/mole and -140 kJ/mole respectively. The 174 genes participating in the development of IHD, 34 genes were associated with 43 miRNAs in the 5′UTR, 55 genes with 90 miRNAs in the CDS, and 35 genes with 53 miRNAs in the 3′UTR. Of them, 23 genes that bound to only one miRNA were located in the 5′UTR region, 34 genes in the CDS, and 19 genes in the 3′UTR. The mRNAs of genes involved in the development of IHD were associated with two or more miRNAs. Eight genes under the control of miRNAs were bound in the 5′UTR, 20 genes in the CDS, and 15 in the 3′UTR. The mRNA of the THRA gene in 5′UTR, the SMARCA4 gene in the CDS, and the LDLR gene in the 3′UTR had the largest number of interaction sites. The binding sites located at 5′UTR had the strongest interaction. Only two genes had binding sites in all sites: CTCF and F2RL3. IHD candidate genes SMARCA4, TGFB1 and DOCK7 were regulated by seven, five and two miRNAs with free energy of binding miRNAs with mRNAs -140 kJ/mole, -140 kJ/mole and -138 kJ/mole respectively. There were genes characteristic for only one subtype: myocardial infarction – ADRB1, ILF3, GATA2, TGFBR1, AP3D1; ischemic heart disease – SMARCA4, DOCK7, CELSR2, TRIB1. All of the above mentioned ассоциации miRNA с mRNA can be used as the promising markers of myocardial infarction and ischemic heart disease.

Key words: miRNA, mRNA, myocardial infarction, ischemic heart disease, candidate genes.

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miRNA-дың миокард инфарктісі және жүректің ишемиялық ауруы кандидатты гендерінің mRNA-дың 5'UTR, CDS және 3'UTR-мен өзара әрекетінің сипаттамалары

Миокард инфарктісі және жүректің ишемиялық ауруларына себеп болатын кандидатты гендері экспрессиясын реттеудегі miRNA қатысуын зерттеу – осы ауралардың жаңа инвазивті емес диагностикаларын жасауға жаңа тиімді әдістерін әзірлеуге ықпал етеді. MirTarget программасын қолдану арқылы miRNA мен mRNA-ның бір-бірімен байланысу сипаттамаларын анықтау барысында келесідей нәтижелер алынды. Миокард инфарктісінің дамуына қатысатын 183 ген, олардың ішіндегі 35 ген 5'UTR-дегі 51 miRNAs-мен байланыста болды, 53 ген CDS-тегі 94 miRNA-мен және 37 ген 3'UTR-дегі 50 miRNAs-мен байланыста болды. 24 ген 5'UTR-дегі тек қана бір miRNA-мен байланысты, 36 ген CDS-те, 24 ген 3'UTR-де. Миокард инфарктісіне қатысатын қалған mRNA ген екі не одан көп miRNA-мен байланыста болды. miRNAs-мен бақыланатын тоғыз ген 5'UTR-де, 21 ген CDS-те, 15 ген 3'UTR-де байланыс құрайды. Осылардың ішінде байланысу сайттарының көбісі CDS-те байқалды. Ең көп CXCR2 және FAIM2 гендерінің mRNA-ларының байланысу сайттары 3'UTR-те болды. Тек мына үш гендерінің байланысу сайттары барлық аймақтарда болды: AP3D1, GATA2, SEMA3F. PDED4 гендері 13 miRNA-мен реттеледі, miRNA-ның mRNA-мен байланысу энергиясы 144кДж/моль тең, ILF3, GATA2 гендері бес және үш miRNA-мен реттеліп отырады, miRNA-ның мРНК-мен байланысу энергиясы 138 кДж/моль; TGFBR1 және AP3D1 гендері алты мен бес miRNA-мен реттеліп отырады, miRNA-ның mRNA-мен байланысу энергиясы 136 кДж/моль және 140 кДж/моль-ге тең. 174 ген жүректің ишемиялық ауруының дамуына қатысады, 34 ген 5'UTR-де 43 miRNA-мен, 55 ген CDS-те 90 miRNA-мен және 3 ген 3'UTR-де 53 miRNA-мен байланысы бар екені анықталды. Оның ішінде 23 ген 5'UTR дегі тек қана бір miRNA-мен байланыста болса, 34 ген CDS-те, 19 ген 3'UTR-де байланысты. Жүректің ишемиялық ауруының дамуына қатысатын mRNA гендер екі немесе одан да көп miRNA-мен байланыс түзейтін болып шықты. miRNA-мен бақыланатын сегіз ген 5'UTR-де, 20 ген CDS-те, 15 ген 3'UTR-де байланыс құрайды. Ең көп байланысу сайттары 5'UTR-де THRA гені, CDS-те SMARCA4 гені және 3'UTR-де LDLR гендерінің mRNA-ларында болды. Тек екі кандидатты гендерінің барлық аймақтарда байланысу сайттары болды: CTCF, F2RL3. Жүректің ишемиялық ауруына кандидатты гендер SMARCA4, TGFB1 және DOCK7 жеті, бес және екі miRNA-мен реттеліп отырады, miRNA-ның mRNA-мен байланысу энергиясы 140 кДж/моль және 138 кДж/моль-ге тең. Гендердің арасында тек қана бір субтипке жататын гендер болады: миокард инфарктісі – ADRB1, ILF3, GATA2, TGFBR1, AP3D1; ишемиялық жүрек ауруы – SMARCA4, DOCK7, CELSR2, TRIB. Барлық жоғарыда көрсетілген miRNA-лар mRNA-мен ассоциациялары миокард инфарктісі мен жүректің ишемиялық ауруының перспективті маркерлері болып қолданылуы мүмкін.

Түйін сөздер: miRNA, mRNA, миокард инфарктісі, ишемиялық жүрек ауруы, кандидатты гендер.

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Характеристики взаимодействия miRNA с 5'UTR, CDS и 3'UTR mRNA кандидатных генов инфаркта миокарда и ишемической болезни сердца

Изучение участия miRNA в регуляции экспрессии кандидатных генов инфаркта миокарда и ишемической болезни сердца будет способствовать разработке новых эффективных методов неинвазивной диагностики этих заболеваний. С применением программы MirTarget определения характеристик взаимодействия miRNA с mRNA получены следующие оригинальные результаты. Из 183 генов, участвующих в развитии инфаркта миокарда, 35 генов были связаны с 51 miRNAs

в 5'UTR, 53 гена – с 94 miRNA в CDS и 37 генов – с 50 miRNAs в 3'UTR. 24 гена связывались только с одной miRNA в 5'UTR, 36 генов – в CDS и 24 гена – в 3'UTR. Оставшиеся mRNA генов, участвующих в развитии инфаркта миокарда, были связаны с двумя или более miRNA. Девять генов, контролируемых miRNAs, были связаны в 5'UTR, 21 генов – в CDS и 15 – в 3'UTR. Большинство взаимодействий наблюдалось в CDS. Наибольшее количество сайтов связывания имели mRNA генов CXCR2 и FAIM2 в 3'UTR. Только три гена имели сайты связывания во всех регионах: AP3D1, GATA2, SEMA3F. Гены-кандидаты инфаркта миокарда PDED4 регулировались 13-ю miRNA со свободной энергией связывания miRNA с mRNA -144 кДж/моль; гены ILF3, GATA2 регулировались пятью и тремя miRNA соответственно со свободной энергией связывания miRNA с mRNA -138 кДж/моль; гены TGFBR1 и AP3D1 регулировались шестью и пятью miRNAs со свободной энергией связывания miRNA с mRNA -136 кДж/моль и -140 кДж/моль соответственно. 174 гена участвовали в развитии ишемической болезни сердца, 34 гена были связаны с 43 miRNAs в 5'UTR, 55 генов – с 90 miRNAs в CDS и 35 генов – с 53 miRNAs в 3'UTR. Из них 23 гена связывались только с одной miRNA в 5'UTR, 34 гена – в CDS и 19 генов – в 3'UTR. mRNA генов, участвующих в развитии ишемической болезни сердца, были связаны с двумя или более miRNA. Восемь генов, находящихся под контролем miRNAs, были связаны в 5'UTR, 20 генов – в CDS и 15 – в 3'UTR. mRNA гена THRA в 5'UTR, гена SMARCA4 в CDS и гена LDLR в 3'UTR имели наибольшее количество сайтов связывания. Наибольшее количество сайтов связывания располагалось в 5'UTR. mRNA только двух генов CTCF и F2RL3 имели сайты связывания miRNA во всех областях. Гены-кандидаты ишемической болезни сердца SMARCA4, TGFB1 и DOCK7 регулировались семью, пятью и двумя miRNA со свободной энергией связывания miRNA с mRNA -140 кДж/моль, -140 кДж/моль и -138 кДж/моль соответственно. Существуют гены, характерные только для одного субтипа: инфаркта миокарда – ADRB1, ILF3, GATA2, TGFBR1, AP3D1; ишемическая болезнь сердца – SMARCA4, DOCK7, CELSR2, TRIB1. Все вышеупомянутые ассоциации miRNA с mRNA могут быть использованы в качестве перспективных маркеров инфаркта миокарда и ишемической болезни сердца.

Ключевые слова: miRNA, mRNA, инфаркт миокарда, ишемическая болезнь сердца, гены-кандидаты.

Introduction

Coronary heart disease is the most common cause of death, and the number of individuals at risk is increasing. To better manage this pandemic, improved tool for risk prediction, including more accurate biomarkers are needed. The objective of this study was to assess the utility of circulating miRNAs to predict future fatal acute myocardial infarction (Bye, 2016: 162-8).

Nowadays many papers are dedicated to revealing of main genes related to myocardial infarction (MI), such as *SOCS3, VAPA* and *COL5A2*; transcription factors *FOXO3, MYBL2, CYP4F3, TBL1XR1, GBGT1, USP25, FDFT1, RORA, CCL5, CCL2*, two miRNAs hsa-miR-21-5p и hsa-miR-30c-5p can be involved in these genes expression regulation (Cheng M. 2017: e774). In ischemic heart disease (IHD) development the genes *IL-10, IL6, IL16* (Xu, 2015: 15869-75; Tong, 2013: 1049-56.), *C5L2* (Zheng, 2013: 139), also enzymes participating in fatty acids metabolism (Ravingerova, 2011: 329-41; Ravingerová, 2013: S151-63) can be involved. Consuming, these data help to determine directed set of the genes that play a key role in the IHD and MI pathogenesis and consequently are natural targets of new therapeutic interventions.

Traditional circulating biomarkers play a fundamental role in the diagnosis and prognosis of myocardial infarction. However, they have several limitations. miRNAs, a class of RNA molecules that do not encode proteins, function directly at the RNA level by inhibiting the translation of messenger RNAs. Due to their significant roles in disease development, they can be used as biomarkers. Accumulating evidence has revealed an attractive role of miRNAs as biomarkers of MI and its associated symptoms, including vulnerable atherosclerotic plaques, and their role in disease diagnosis, platelet activation monitoring, and prognostic outcome prediction (Chen, 2018: 140–147). Previously as markers with high sensitivity and specificity for MI and IHD such miRNAs as: miR-208b, miR-499 (Corsten, 2010: 499–506; Gidlöf, 2013: 12; Widera, 2011: 872-5; Li, 2015: 58), miR-1, miR-133a/b, miR-122 (D'Alessandra, 2010: 2765-73; Grabmaier, 2017: 30-36), miR-320a (Devaux, 2015:260-71), miR-221-3p (Coskunpinar, 2016: 90-96), miR-92a (Zhang, 2017: BSR20170047), miR-21, miR-423-5, miR-499-5p (Olivieri, 2013: 531-6; Zhang, 2016: 323–9), miR-26a, miR -191; miR -208b (Li, 2015: 58), miR-181a (Zhu, 2016: 1591-1602), miR-19b-3p, miR-134-5p, miR-186-5p (Wang, 2016: 1015-29), miR-361; miR-519e (Wang, 2014: e105734),

miR-29b (Grabmaier, 2017: 30-36). The best markers for MI diagnostics miR-106a-5p, miR-424-5p, let-7g-5p, miR-144-3p and miR-660-5p were discovered (Bye, 2016: 162-8).

Materials and Methods

For establishment of miRNA interaction with 5'UTR, CDS, 3'UTR mRNA candidate genes of myocardial infarction and ischemic heart disease were used 183 and 174 genes respectively. The nucleotide sequences of candidate genes of the myocardial infarction (MI) and ischemic heart disease (IHD) were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). 3701 miRNA were taken from the publication of Londin E. et al. (Londin, 2015: 1106-1115). 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 (Ivashchenko, 2016: 237-240). 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 the miRNAs and the 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 (Kool, 2001: 1-22; Leontis, 2002: 3497-3531). 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. Table 1 shows sources of information on candidate genes of breast cancer subtypes.

Results and Discussion

Characteristics of miRNA interaction with 5'UTR, CDS and 3'UTR mRNA candidate genes of myocardial infarction.

miRNAs bound with the high ΔG/ΔGm value in 5'UTR, CDS and 3'UTR of mRNAs genes participating in MI genes. 35 target genes were associated with 51 miRNAs in 5'UTR (Table 1).

Two clusters were located in the mRNA of the *ILF3* gene, from 46 nt to 77 nt and from 167 nt to 209 nt, respectively, whose lengths without superimposing of miRNAs equal to 31 nt and 42 nt would occupy 23% of the 5UTR of this gene. Three miRNAs were associated with the mRNA of the *TGFBR1* gene from 16 nt to 61 nt. Not overlapping, they had a length of 66 nt, which would be 86% of the 5'UTR of this gene.

The average free energy of binding of miRNAs with mRNAs was -124.7 ± -10.4 kJ/mole. The number of associations of miRNAs with mRNAs having free interaction energy above -120 kJ/mole was 23. Of these, the highest free binding energy was associated with: mRNA of the *MBP* gene and miR-2-3313-3p.

Table 1 – Characteristics of miRNAs interaction in the 5 'UTR mRNA of myocardial infarction candidate gene

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ALDH2	miR-21-16482-3p	8	-123	92	21
ALMS1	miR-19-43069-3p	76	-119	90	23
ALOX5	miR-14-34410-3p	68	-127	91	24
AP3D1	miR-20-43118-5p	128	-121	92	21
	miR-7-17280-5p	152	-119	90	22
CD40	miR-1-3522-3p	10	-123	91	23
CHGA	miR-14-37896-3p	27	-113	91	21
	miR-16-14192-5p	155	-117	93	21
	miR-6-18590-3p	247	-113	91	21
DNASE1	miR-14-35161-5p	622	-117	89	24

Continuation of table 1

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
DPP4	miR-3-9273-3p	369	-108	93	20
DRD1	miR-14-35041-3p	50	-110	91	21
F2R	miR-6-16980-5p	56	-129	92	23
GATA2	miR-7-21068-3p	314	-138	94	24
GCLC	miR-17-38391-3p	453	-115	90	23
	miR-9-25099-3p	455	-119	93	22
GSTP1	miR-1-2336-3p	77	-115	93	21
HMOX1	miR-16-22443-3p	75	-113	95	20
HSPA12B	miR-19-43963-5p	36	-119	92	22
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
ILF3	miR-1-124-5p	46	-138	92	24
	miR-10-13751-3p	56	-121	92	21
	miR-5-14408-5p	167	-123	89	23
	miR-5-15733-3p	185	-134	90	24
KCNJ11	miR-2-4804-5p	82	-110	88	24
	miR-17-34996-5p	140	-115	93	23
KCNMA1	miR-8-22971-3p	36	-117	92	21
LAMA3	miR-9-20317-3p	13	-138	93	24
LRP8	miR-6-18255-5p	16	-127	95	22
MBP	miR-2-3313-3p	20	-142	89	25
NAMPT	miR-17-12804-3p	9	-117	96	20
NFKB	miR-10-13655-3p	126	-127	94	22
NFKB1	miR-9-13610-3p	231	-125	95	21
NFKBIL1	miR-5-14220-5p	32	-106	96	18
P2RY2	miR-6-16152-3p	49	-119	92	22
PDE4	miR-17-39416-3p	66	-121	92	22
SCAP	miR-12-30825-5p	22	-115	92	22
	miR-12-31721-3p	23	-108	91	21
	miR-8-23005-3p	49	-106	94	21
SEMA3F	miR-2-4005-5p	97	-132	89	24
	miR-2-4453-3p	97	-121	92	21
	miR-22-23987-3p	106	-121	92	21
	miR-5-8853-5p	109	-117	93	20
SH2B1	miR-11-23098-5p	225	-110	91	21
STAT3	miR-11-29089-3p	28	-115	92	22
TFAM	miR-11-29839-5p	221	-117	90	23
TGFBR1	miR-19-42639-3p	16	-117	92	22
	miR-5-15435-3p	31	-115	92	21
	miR-22-46282-5p	38	-123	89	23
THBS1	miR-19-43966-3p	113	-129	92	23

53 target genes mRNAs were associated with 94 miRNAs in CDS (Table 2).

There was a cluster of two binding sites in the mRNA of the *ADRA2B* gene from 455 nt to 486 nt equal to 31 nt, that without overlapping binding sites, would be 44 nt.

Two binding sites in the mRNA of the *APOE* gene formed a cluster from 766 nt to 788 nt with length of 22 nt. There is the cluster in the mRNA of the *CDKN1C* gene from 746 nt to 773 nt. Two binding sites formed a cluster with a length of 32 nt from 383 nt to 415 nt of mRNA of the *FGF2* gene. There was a cluster in the mRNA of the *HSPA12B* gene from 2072 nt to 2097 nt, with a length of 25 nt, that without superimposing lengths of miRNAs would be 46 nt. Two sites of binding miR miR-19-43132-3p and miR-2-6772-3p in the mRNA of the *SHH* gene formed a 39 nt cluster from 1341 nt to 1380 nt. There was a 41-nt cluster from 112 nt to 153 nt of mRNA of the *TGFBR1* gene, that would have a length of 138 nt, without overlapping binding sites.

The average free energy of binding of miRNAs with all mRNAs in the 5'UTR was -120.6 ± -8.4 kJ /mole. The number of associations of miRNAs with mRNAs having a free energy of binding of more than -120kJ/mole was equal to 48.

Table 2 – Characteristics of miRNAs interaction in the CDS of mRNA of myocardial infarction candidate gene

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ABCA1	miR-11-21109-3p	6416	-110	90	23
ABCC6	miR-19-38006-5p	967	-117	90	24
ADAM8	miR-17-39143-3p	1365	-123	89	24
	miR-10-25954-5p	2233	-119	89	24
ADAMTS7	miR-6-18378-3p	280	-117	92	22
	miR-17-39416-3p	877	-121	92	22
	miR-2-7425-5p	2570	-108	89	23
	miR-10-25694-5p	4745	-132	89	25
	miR-7-22066-3p	5030	-125	89	24
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
ADRB1	miR-9-27298-3p	130	-119	90	22
AGTR1	miR-6-3109-5p	102	-117	92	22
ALMS1	miR-9-26506-3p	169	-113	91	22
AP3D1	mir-1-2121-3p	2856	-140	89	25
	miR-11-29785-3p	3099	-108	91	21
APOA1	miR-10-13655-3p	841	-123	91	22
APOA1BP	miR-15-38746-3p	142	-108	93	21
APOB	miR-13-36375-5p	179	-119	90	23
APOE	miR-X-45440-5p	643	-121	95	22
	miR-9-23547-5p	766	-115	93	20
APOE	miR-9-24355-5p	768	-115	93	20
C4B	miR-2-6355-3p	2210	-106	93	20
	miR-X-45905-3p	5141	-123	91	24
CCL5	miR-19-42426-5p	181	-121	89	23

Continuation of table 2

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
CDKN1C	miR-22-21907-3p	360	-106	94	19
	miR-1-163-3p	746	-110	91	21
	miR-8-22944-3p	753	-119	97	20
	miR-5-16165-5p	831	-113	93	20
	miR-14-31624-3p	1026	-127	88	24
CDKN2B	miR-6-17811-3p	412	-132	89	24
CHGA	miR-7-17280-5p	704	-119	90	22
	miR-17-38391-3p (2)	769÷770	-115÷-119	90÷93	23
	miR-17-38391-3p	943	-115	90	23
	miR-9-25099-3p	1335	-117	92	22
CLEC16A	miR-19-34661-5p	3004	-119	90	23
DDAH2	miR-X-46104-3p	281	-110	91	21
DOT1L	miR-16-37746-3p	1113	-121	89	23
	miR-1-2781-5p	2711	-121	92	23
	miR-9-24407-3p	2874	-119	90	22
	miR-11-29723-5p	3924	-108	93	20
ENPP1	miR-X-44972-5p	32	-119	93	20
ESR1	miR-5-4100-5p	409	-106	91	22
	miR-4-5310-3p	1852	-115	90	23
FGF2	miR-8-22077-3p	344	-123	92	22
	miR-17-36033-3p	383	-129	87	25
	miR-6-18438-3p	392	-125	91	23
GATA2	miR-16-38537-3p	877	-125	89	24
GJA4	miR-20-44079-5p	401	-121	90	22
GP6	miR-19-28028-5p	964	-132	89	24
GSTM1	miR-2-6532-3p	462	-108	89	23
HSPA12B	miR-19-40174-5p	1102	-110	91	21
	miR-3-8603-3p	2072	-129	90	24
	miR-8-21932-3p	2073	-127	88	24
IL6R	miR-2-4533-3p	483	-125	89	23
ILF3	miR-10-26849-3p	2272	-113	90	23
KCNMA1	miR-17-39416-3p (4)	203÷215	-119÷-125	90÷95	22
LAMA3	miR-11-28567-3p	96	-125	91	23
LDLR	miR-10-26537-5p	2452	-108	96	20
LRP1	miR-19-38092-3p	486	-117	93	22
	miR-1-2791-5p	2848	-110	91	22
	miR-5-3136-5p	12102	-108	91	21
LRP8	miR-6-7754-5p	1317	-115	93	21
MEF2A	miR-22-45452-5p	1831	-104	94	20
MMP3	miR-10-24436-3p	132	-119	93	23
NFKB1	miR-11-29785-5p	2249	-108	91	21

Continuation of table 2

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
NFKBIL1	miR-11-27076-3p	815	-123	89	24
	miR-19-42140-3p	923	-110	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
NOX5	miR-11-29675-5p	410	-100	92	20
PDE4D	miR-19-33623-3p	335	-132	89	24
	miR-19-21199-3p (2)	337÷344	-140	89	25
	miR-1-155-3p	344	-129	94	22
PLAUR	miR-17-40348-5p	233	-123	91	23
PTX3	miR-11-30283-5p	573	-117	93	21
S100A1	miR-11-32270-3p	221	-110	93	21
SCAP	miR-12-17092-3p	2486	-125	91	22
SELE	miR-7-21003-3p	829	-100	90	22
SEMA3F	miR-7-19239-3p	2519	-125	89	23
SERPINE1	miR-2-3962-5p	542	-125	88	24
SH2B1	miR-4-13219-5p	2834	-106	91	22
SHH	miR-17-41315-3p	1000	-119	93	22
	miR-14-31624-3p	1112	-127	88	24
	miR-19-43132-3p	1341	-119	90	22
	miR-2-6772-3p	1356	-129	88	24
SIRT1	miR-5-13181-3p	232	-123	89	24
	miR-19-43644-3p	264	-123	89	23
SMTN	miR-X-46721-5p	1920	-104	92	21
	miR-11-18690-5p	2371	-113	91	22
	miR-14-35670-5p	2990	-121	90	23
SOD3	miR-17-41183-5p	259	-123	89	23
	miR-2-6532-3p	502	-108	89	23
TGFBR1	miR-7-18119-3p	112	-123	92	22
	miR-9-20317-3p (3)	127÷133	-134÷-136	90÷91	24
	miR-17-39416-3p (2)	128÷131	-121	92	22
TNF	miR-20-42898-3p	230	-121	92	23
TNNI3	miR-17-39011-3p	322	-119	90	23
VEGFA	miR-9-26506-3p	775	-113	91	22

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

Two binding sites in the mRNA of the *ANGPT2* gene formed a 29 nt long cluster from 3064 nt to 3093 nt, that without overlapping miRNAs lengths would be equal to 46 nt.

miR-11-27078-5p and miR-3-5147-5p bound to the *CDKN2B* and *OLR1* genes in the same sites, indicating that they had matched sequences. From 1746 nt to 1775 nt of mRNA of the *CDKN2B* gene, a 29 nt long cluster would have a length of 92 nt. The cluster, formed from 1504 nt to 1529 nt in the *OLR1*

mRNA, had a length of 25 nt, without overlapping the binding sites would be 45 nt. Such cluster arrangement of binding sites in these genes indicated the need to reduce the proportion of binding sites in the site, as well as the enhanced control of the expression of these genes.

Two binding sites in the mRNA of the *FAIM2* gene from 3392 nt to 3414 nt were combined into a cluster of 22. From 2845 nt to 2870 nt in the mRNA of the *FGF2* cluster was 25 nt long, without overlapping the length of the miRNAs would be 44 nt. mRNA of the *SP1* gene contained eight and seven binding sites for miR-10-29282-3p and miR-15-36862-3p, respectively, from 4147 nt to 4184 nt, forming a 37 nt cluster which, without overlapping the miRNAs lengths, would be 184 nt. Therefore, the compaction of binding sites was necessary to reduce the proportion of binding sites.

The average free energy of binding of miRNA with mRNA in the 5'UTR was -111.1 ± -8.7 kJ/mole. The number of associations of miRNA with mRNA having a free energy of binding of more than -120 kJ/mole was equal to 9, *STAT3* and miR-19-30988-5p had the highest energy.

Table 3 – Characteristics of miRNAs interaction in the 3'UTR of mRNA of myocardial infarction candidate gene.

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ABCC9	miR-5-12351-3p	6665	-104	89	23
ABO	miR-19-38604-5p	1094	-117	92	22
ANGPT2	miR-7-21133-5p	3064	-121	89	24
	miR-5-18072-3p	3071	-102	91	22
AP3D1	miR-2-7340-3p	4735	-110	91	22
CASR	miR-17-39495-3p	4776	-113	90	23
CCL5	miR-X-45975-5p	616	-96	92	22
	miR-2-4826-5p (2)	738÷739	-113	90	23
	miR-10-11641-3p	816	-121	90	23
CD40LG	miR-5-14114-5p	1365	-123	89	23
CDKN2B	miR-11-27078-5p (4)	1746÷1752	-108	89	23
	miR-3-5147-5p (4)	1746÷1752	-100	90	22
CXCR2	miR-17-40078-3p	1850	-113	88	24
	miR-22-45902-3p	1972	-113	93	22
	miR-10-26483-5p	2008	-113	91	22
	miR-19-43804-3p	2547	-110	91	21
	miR-17-34996-5p	2722	-110	90	23
	miR-6-19858-3p	2925	-108	91	22
CYP4A11	miR-3-10329-5p	1708	-121	89	24
1 DKK	miR-10-29282-3p	1580	-106	91	23
DOT1L	miR-16-39493-5p	6992	-115	90	23
ENPP1	miR-2-4804-5p (2)	6751÷6752	-113÷117	90÷93	24
FAIM2	miR-4-11676-5p	1817	-110	91	21
	miR-19-43146-3p	2464	-113	91	21
	miR-19-41434-3p	3392	-113	93	21
	miR-2-5276-5p	3394	-102	92	20
	miR-15-36006-5p	3952	-119	90	23
	miR-22-46603-5p	4522	-121	89	24

Continuation of table 3

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
FGB	miR-20-43646-5p	2081	-121	89	24
	miR-2-4826-5p	2291	-113	90	23
FGF2	miR-17-39466-3p	2845	-113	91	22
	miR-8-11096-5p	2848	-115	92	22
FTO	miR-17-34996-5p	3710	-110	90	23
GATA2	miR-2-6824-3p	2340	-119	97	22
GP6	miR-X-45975-5p	1686	-96	92	22
	miR-22-45335-5p	2206	-113	90	23
GSTCD	miR-8-23744-5p	3096	-119	90	23
HNRNPUL1	miR-17-40012-5p	2918	-113	91	21
ICAM1	miR-15-36862-3p	2987	-108	89	23
IL23R	miR-2-4826-5p (2)	2767÷2768	-115÷117	92÷93	23
IL6R	miR-14-35161-5p	3063	-119	90	24
	miR-X-45975-5p	4004	-96	92	22
	miR-2-4826-5p (2)	4607÷4608	-113÷115	90÷92	23
	miR-7-20771-3p	4974	-89	91	21
LPL	miR-10-28550-3p (2)	300÷301	-119÷121	90÷92	23
LTA	miR-16-9117-3p	1258	-98	92	21
MPO	miR-10-17453-5p	2743	-100	92	21
	miR-22-46211-3p	3001	-104	91	22
MTAP	miR-2-4826-5p (2)	2530÷2531	-115÷117	92÷93	23
MTHFR	miR-9-24450-5p	3350	-117	89	24
	miR-X-44909-3p	6342	-108	91	22
	miR-2-4684-5p	6844	-117	93	22
	miR-22-45902-3p	7051	-113	93	22
OLR1	miR-11-27078-5p (2)	1504÷1506	-108	89	23
	miR-3-5147-5p (2)	1504÷1506	-100	90	22
PSMA6	miR-4-11714-5p	954	-106	93	20
SEMA3F	miR-17-38738-5p	3293	-119	92	22
SMTN	miR-17-39570-5p	3166	-125	92	22
SP1	miR-10-29282-3p (8)	4147÷4161	-104	89	23
	miR-15-36862-3p (7)	4147÷4159	-108	89	23
STAT3	miR-2-4826-5p	3359	-117	93	23
	miR-19-30988-5p	3782	-129	90	23
TFAM	miR-10-29282-3p (3)	3823÷3837	-104	89	23
	miR-19-42814-5p	3824	-106	91	23
TNFSF4	miR-19-42814-5p (2)	2489÷2493	-106	91	23

Of the 183 genes involved in the development of MI, 35 genes were associated with 51 miRNAs in the 5'UTR, 53 genes with 94 miRNA in the CDS, and 37genes with 50 miRNAs in the 3'UTR.

Of these, 24 genes that bind to only one miRNA were arranges in the 5'UTR region, 36 genes in the CDS, and 24 genes in the 3'UTR. Thus, these genes, weakly interacting with miRNA,

did not need an enhanced control of the express. The remaining mRNAs of the genes involved in the development of MI were associated with two or more miRNAs. Thus, 9 genes under the control of miRNAs were bound in the 5'UTR, 21 genes in the CDS, and 15 in the 3'UTR. Most of the interactions occurred at the CDS site. The largest number of binding sites had the mRNA of the genes *CXCR2* and *FAIM2* in the 3'UTR (6 miRNAs).

The binding sites located in the CDS had the strongest interaction. Only three genes had binding sites in all regions: *AP3D1, GATA2, SEMA3F*.

Characteristics of miRNA interaction with 5'UTR, CDS and 3'UTR mRNA candidate genes of ischemic heart disease.

miRNA bound to the mRNA of IHD genes with high complementarity. From 34 target genes, their mRNAs were associated with 43 miRNAs at 5'UTR (table 4).

A cluster from 3 nt to 28 nt, located in the mRNA of the *DOCK7* gene, occupied most part of the gene 5'UTR equal to 34 nt. Starting from 49 nt to 70 nt of the *SELP* gene mRNA contained a cluster of 21 nt long, occupying a third of 5'UTR equal to 65 nt.

The average free energy of binding of miRNAs with mRNAs for 5'UTR of all genes mRNAs was -120.4 ± -15.4 kJ / mol. The number of associations with free energy of binding above -120 kJ / mol was equal to 20. Of these, the mRNA of the *DOCK7* gene and miR-19-21199-3p had the largest free energy of binding.

Table 4 – Characteristics of miRNAs interaction in the 5'UTR of mRNA of ischemic heart disease candidate gene

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ABCA1	miR-11-21109-3p	6416	-110	90	23
ABCC6	miR-19-38006-5p	967	-117	90	24
ADAM8	miR-17-39143-3p	1365	-123	89	24
	miR-10-25954-5p	2233	-119	89	24
ADAMTS7	miR-6-18378-3p	280	-117	92	22
	miR-17-39416-3p	877	-121	92	22
	miR-2-7425-5p	2570	-108	89	23
	miR-10-25694-5p	4745	-132	89	25
	miR-7-22066-3p	5030	-125	89	24
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
ADRB1	miR-9-27298-3p	130	-119	90	22
AGTR1	miR-6-3109-5p	102	-117	92	22
ALMS1	miR-9-26506-3p	169	-113	91	22
AP3D1	mir-1-2121-3p	2856	-140	89	25
	miR-11-29785-3p	3099	-108	91	21
APOA1	miR-10-13655-3p	841	-123	91	22
APOA1BP	miR-15-38746-3p	142	-108	93	21
APOB	miR-13-36375-5p	179	-119	90	23
APOE	miR-X-45440-5p	643	-121	95	22
	miR-9-23547-5p	766	-115	93	20
APOE	miR-9-24355-5p	768	-115	93	20
C4B	miR-2-6355-3p	2210	-106	93	20
	miR-X-45905-3p	5141	-123	91	24
CCL5	miR-19-42426-5p	181	-121	89	23

Continuation of table 4

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
CDKN1C	miR-22-21907-3p	360	-106	94	19
	miR-1-163-3p	746	-110	91	21
	miR-8-22944-3p	753	-119	97	20
	miR-5-16165-5p	831	-113	93	20
	miR-14-31624-3p	1026	-127	88	24
CDKN2B	miR-6-17811-3p	412	-132	89	24
CHGA	miR-7-17280-5p	704	-119	90	22
	miR-17-38391-3p (2)	769÷770	-115÷-119	90÷93	23
	miR-17-38391-3p	943	-115	90	23
	miR-9-25099-3p	1335	-117	92	22
CLEC16A	miR-19-34661-5p	3004	-119	90	23
DDAH2	miR-X-46104-3p	281	-110	91	21
DOT1L	miR-16-37746-3p	1113	-121	89	23
	miR-1-2781-5p	2711	-121	92	23
	miR-9-24407-3p	2874	-119	90	22
	miR-11-29723-5p	3924	-108	93	20
ENPP1	miR-X-44972-5p	32	-119	93	20
ESR1	miR-5-4100-5p	409	-106	91	22
	miR-4-5310-3p	1852	-115	90	23
FGF2	miR-8-22077-3p	344	-123	92	22
	miR-17-36033-3p	383	-129	87	25
	miR-6-18438-3p	392	-125	91	23
GATA2	miR-16-38537-3p	877	-125	89	24
GJA4	miR-20-44079-5p	401	-121	90	22
GP6	miR-19-28028-5p	964	-132	89	24
GSTM1	miR-2-6532-3p	462	-108	89	23
HSPA12B	miR-19-40174-5p	1102	-110	91	21
	miR-3-8603-3p	2072	-129	90	24
	miR-8-21932-3p	2073	-127	88	24
IL6R	miR-2-4533-3p	483	-125	89	23
ILF3	miR-10-26849-3p	2272	-113	90	23
KCNMA1	miR-17-39416-3p (4)	203÷215	-119÷-125	90÷95	22
LAMA3	miR-11-28567-3p	96	-125	91	23
LDLR	miR-10-26537-5p	2452	-108	96	20
LRP1	miR-19-38092-3p	486	-117	93	22
	miR-1-2791-5p	2848	-110	91	22
	miR-5-3136-5p	12102	-108	91	21
LRP8	miR-6-7754-5p	1317	-115	93	21
MEF2A	miR-22-45452-5p	1831	-104	94	20
MMP3	miR-10-24436-3p	132	-119	93	23
NFKB1	miR-11-29785-5p	2249	-108	91	21
NFKBIL1	miR-11-27076-3p	815	-123	89	24
	miR-19-42140-3p	923	-110	91	21

Continuation of table 4

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
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
NOX5	miR-11-29675-5p	410	-100	92	20
PDE4D	miR-19-33623-3p	335	-132	89	24
	miR-19-21199-3p (2)	337÷344	-140	89	25
	miR-1-155-3p	344	-129	94	22
PLAUR	miR-17-40348-5p	233	-123	91	23
PTX3	miR-11-30283-5p	573	-117	93	21
S100A1	miR-11-32270-3p	221	-110	93	21
SCAP	miR-12-17092-3p	2486	-125	91	22
SELE	miR-7-21003-3p	829	-100	90	22
SEMA3F	miR-7-19239-3p	2519	-125	89	23
SERPINE1	miR-2-3962-5p	542	-125	88	24
SH2B1	miR-4-13219-5p	2834	-106	91	22
SHH	miR-17-41315-3p	1000	-119	93	22
	miR-14-31624-3p	1112	-127	88	24
	miR-19-43132-3p	1341	-119	90	22
	miR-2-6772-3p	1356	-129	88	24
SIRT1	miR-5-13181-3p	232	-123	89	24
	miR-19-43644-3p	264	-123	89	23
SMTN	miR-X-46721-5p	1920	-104	92	21
	miR-11-18690-5p	2371	-113	91	22
	miR-14-35670-5p	2990	-121	90	23
SOD3	miR-17-41183-5p	259	-123	89	23
	miR-2-6532-3p	502	-108	89	23
TGFBR1	miR-7-18119-3p	112	-123	92	22
	miR-9-20317-3p (3)	127÷133	-134÷-136	90÷91	24
	miR-17-39416-3p (2)	128÷131	-121	92	22
TNF	miR-20-42898-3p	230	-121	92	23
TNNI3	miR-17-39011-3p	322	-119	90	23
VEGFA	miR-9-26506-3p	775	-113	91	22

Of the 55 target genes, their mRNAs in the CDS were bound to 90 miRNAs (table 5).

Two binding sites in the mRNA of the *APOE* gene from 766 nt to 788 nt formed a 22 nt cluster for binding miR-9-23547-5p and miR-9-24355-5p. Without the overlapping lengths of the miRNAs, the length of this cluster would be 136 nt instead of 31 nt, hence the sites binding arrangement is necessary to decrease the length of the sites. miR-11-18690-5p and miR-1-1351-3p had binding sites in the mRNA of the *CNDP1* gene from 285 nt and 290 nt respectively, forming a 28 nt cluster. A cluster of 32 nt was located from 383 nt to 415 nt in the *FGF2* mRNA, it would be equal to 48 nt without overlapping miR-17-36033-3p and miR-6-18438-5p. In the mRNA of the *GP1BA* miR-1-654-3p and miR-5-8853-5p had paired sites from 1325 nt, 1364 nt, 1403 nt, therefore had particularly overlapping sequences. There was a cluster of 28 nt long from 5080 nt to 5108 nt of the mRNA of the *SMARCA4* gene, that without miRNAs overlapping would have a length of 46 nt. There was a cluster in the

mRNA of the *ZNF259* gene from 84 nt to 114 nt for binding two miRNAs: miR-7-21068-3p and miR-2-4736-5p.

The average free energy of binding of miRNA with mRNA for CDS of all mRNAs was -117.5 ± -9.8 kJ/mol. The largest free energy had such associations as: mRNA of the *DAB2I* gene and mir-1-2121-3p, *CELSR2* and mir-1-2121-3p, *SMARCA4* and miR-5-15733-3p, which can be used as diagnostic markers of CVD.

Table 5 – Characteristics of miRNAs interaction in the CDS of mRNA of ischemic heart disease candidate gene

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ABCA1	miR-11-21109-3p	6416	-110	90	23
AGTR1	miR-6-3109-5p	102	-117	92	22
ALDH2	miR-1-3575-5p	1559	-119	89	23
ANGPTL2	miR-1-1510-5p	1145	-136	91	24
APOA1	miR-10-13655-3p	841	-123	91	22
APOB	miR-13-36375-5p	179	-119	90	23
	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
C3	miR-5-14995-5p	102	-106	91	21
CELSR2	mir-1-2121-3p	86	-142	91	25
	miR-8-22507-5p(2)	4856÷4857	-115	92	22
	miR-3-8028-5p	5803	-115	89	23
	miR-19-41434-3p	7753	-110	91	21
	miR-1-3181-5p(2)	8113÷8114	-123÷-129	95÷100	23
	miR-6-12155-5p	8691	-125	91	22
CNDP1	miR-11-18690-5p	285	-110	90	22
	miR-1-1351-3p	290	-117	89	23
	miR-16-20362-3p	526	-102	91	22
CTCF	miR-19-41018-5p	2532	-123	91	24
DAB2I	miR-14-36753-5p	1049	-110	90	22
	miR-8-23953-5p	2371	-129	88	24
	miR-19-33623-3p(3)	2743÷2750	-132÷-136	89÷91	24
	mir-1-2121-3p	2750	-146	93	25
DDAH2	miR-X-46104-3p	281	-110	91	21
DNAH11	miR-9-26506-3p	137	-113	91	22
ENPP1	miR-X-44972-5p	32	-119	93	20
ESR1	miR-5-4100-5p	409	-106	91	22
	miR-4-5310-3p	1852	-115	90	23
F2	miR-11-30672-3p	532	-119	100	21
F2RL3	miR-9-24119-5p	824	-102	91	21
F7	miR-1-4241-5p	215	-119	89	23
FADS2	miR-16-33426-5p	1447	-110	90	22

Continuation of table 5

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
FGF2	miR-8-22077-3p	344	-123	92	22
	miR-17-36033-3p	383	-129	87	25
	miR-6-18438-5p	392	-125	91	23
GCKR	miR-10-26109-5p	820	-119	92	22
GP1BA	miR-1-654-3p	1325	-115	92	20
	miR-5-8853-5p	1325	-115	92	20
	miR-1-654-3p	1364	-115	92	20
	miR-5-8853-5p	1364	-115	92	20
	miR-1-654-3p	1403	-115	92	20
	miR-5-8853-5p	1403	-115	92	20
	miR-17-20448-3p	1532	-93	92	20
GSTM1	miR-2-6532-3p	462	-108	89	23
HNF1A	miR-4-12346-5p	1463	-108	89	23
	miR-8-24124-3p	1825	-113	90	22
HP-v-1	miR-1-3943-5p	1178	-98	92	20
IL6R	miR-2-4533-3p	483	-125	89	23
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
IRF8	miR-5-14452-5p	786	-121	88	24
ITGB3	miR-5-15104-5p	52	-115	89	23
ITIH4	miR-10-24312-3p	1682	-100	92	20
KALRN	miR-1-2704-3p	1631	-110	91	21
LDLR	miR-20-43477-3p	223	-110	85	22
	miR-10-26537-5p	2452	-108	96	20
MADD	miR-6-11241-3p(2)	516÷517	-113÷-115	93÷95	21
MEF2A	miR-22-45452-5p	1831	-104	94	20
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
MMP3	miR-10-24436-3p	132	-119	93	23
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
NCAN	miR-1-2228-3p	1071	-123	88	24
	miR-5-8853-5p	3447	-115	92	20
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

Continuation of table 5

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
NPC1L1	miR-15-36925-3p	1793	-136	93	24
	miR-15-37642-3p	2221	-123	88	24
	miR-15-33456-5p	2937	-117	92	22
PCSK9	miR-2-4035-3p	1052	-115	89	23
PPARD	miR-12-30825-5p	431	-113	90	22
	miR-2-5142-5p	855	-102	91	21
SELE	miR-7-21003-3p	829	-100	90	22
SELPLG	miR-1-1788-3p	334	-115	90	22
SERPINE1	miR-2-3962-5p	542	-125	88	24
SMARCA4	miR-16-39450-3p	656	-123	94	23
	miR-4-12861-5p	681	-117	90	22
	miR-17-38067-3p	972	-127	90	23
	miR-9-25099-3p	5080	-119	93	22
	miR-9-26506-3p	5086	-110	90	22
	miR-5-15733-3p	5191	-140	94	24
	miR-4-12154-5p	5194	-127	88	24
TFR2	miR-4-12266-3p	2054	-121	89	23
THRA	miR-19-43985-3p	1931	-108	91	22
TNF	miR-20-42898-5p	230	-121	92	23
TRIB1	miR-8-24549-5p	756	-127	90	24
VEGFA	miR-9-26506-3p	775	-113	91	22
	miR-8-21883-3p	887	-123	88	24
VWF	miR-17-40267-5p	5029	-127	90	24
ZNF259	miR-7-21068-3p	84	-129	88	24
	miR-2-4736-5p	93	-123	94	21

35 target genes contacted in their 3'UTR with 53 mRNAs (table 6).

Two miRNAs – miR-7-21133-5p and miR-5-18072-3p, bound to the mRNA of the *ANGPT2* gene, formed a 29-nt cluster from 3064 nt to 3093 nt. There was a cluster in the mRNA of the *F7* gene from 1561 nt to 1584 nt with length of 23 nt, which without lapping binding sites would be 45 nt. In the mRNA of the *FGF* gene, from 2845 nt at 2870 nt, a cluster of binding sites of 25 nt in length was formed. The binding sites of miR-17-39935-3p and miR-10-26483-5p formed a cluster in the mRNA of the *ICAM1* gene with a length of 25 nt from 3022 to 3047 nt. There was a cluster of binding sites for two miRNAs of 25 nt long, from 3887 nt to 3912 nt in the mRNA of the *LDLR* gene which would have been 44 nt without overlapping.

The average free energy of binding of miRNAs with mRNAs for 3'UTR of all mRNAs was -111.6 ± -97.8 kJ/mole. The number of associations having a free energy of binding above -120 kJ/mole was 8. The largest free energy of binding was possessed by the association of the mRNA of the *TGFB1* gene and mir-1-2121-3p, which can be used as a diagnostic marker for IHD.

Table 6 – Characteristics of miRNAs interaction in the 3'UTR of mRNA of ischemic heart disease candidate gene

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ABO	miR-19-38604-5p	1094	-117	92	22
	miR-17-12804-3p	1125	-113	93	20
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
AGTR2	miR-16-40163-5p	2307	-121	90	23
ANGPT2	miR-7-21133-5p	3064	-121	89	24
	miR-5-18072-3p	3071	-102	91	22
AS3MT	miR-5-18072-3p	1375	-102	91	22
	miR-14-35161-5p	1403	-117	89	24
	miR-2-5355-3p	2038	-115	90	22
CSMD1	miR-16-16298-5p	13931	-119	90	24
CTCF	miR-3-10087-5p	3865	-108	91	22
CXCL12	miR-11-25459-3p	932	-119	90	23
DNAH11	miR-2-4826-5p(2)	13853÷13854	-115	92	23
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
F2RL3	miR-2-4826-5p(2)	2091÷2092	-115÷-117	92÷93	23
	miR-2-4826-5p(2)	2426÷2427	-113÷-115	90÷92	23
F7	miR-9-25099-3p	1561	-115	90	22
	miR-17-38391-3p	1561	-115	90	23
FADS	miR-1-41-3p	1515	-108	91	21
	miR-19-43386-3p	2407	-117	89	23
	miR-1-1412-5p	2712	-117	90	22
	miR-17-39583-3p	2803	-119	89	23
FGB	miR-20-43646-5p	2081	-121	89	24
	miR-2-4826-5p	2291	-113	90	23
FGF	miR-10-24600-3p	2289	-102	89	23
	miR-17-39466-3p	2845	-113	91	22
	miR-8-11096-5p	2848	-115	92	22
GCKR	miR-6-18764-3p	1956	-121	88	24
GHR	miR-6-19858-3p	4139	-108	91	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
IGFBP3	miR-19-43165-3p	1753	-108	91	21
IL10	miR-17-39466-3p	1200	-110	90	22

Continuation of table 6

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
IL6R	miR-14-35161-5p	3063	-119	90	24
KIF6	miR-12-31649-3p	3234	-113	91	22
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	90	23
	miR-7-20771-3p	4974	-89	91	21
LTA	miR-16-9117-3p	1258	-98	92	21
MLXIPL	miR-6-17487-3p	3189	-115	92	23
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
NOS1	miR-17-40078-3p	9425	-113	88	24
	miR-12-31701-3p	11355	-117	92	22
	miR-2-7331-5p	11355	-123	89	23
	miR-22-45902-3p	11608	-110	91	22
NQO1	miR-17-34996-5p	1719	-110	90	23
PPARA	miR-18-41332-3p	5939	-123	89	23
	miR-19-29188-3p	5948	-113	90	22
	miR-16-38689-3p	9118	-102	91	22
PPP1R3B	miR-14-35161-5p	2150	-117	89	24
SELPLG	miR-22-43699-5p	1818	-106	94	20
TGFB1	mir-1-2121-3p	2093	-140	89	25
TNFSF4	miR-19-42814-5p(2)	2489÷2493	-106	91	23

Earlier it has been shown that genes of the sarkomerny proteins connected with a myocardial infarction and also some genes of coronary heart disease, are targets for miRNAs (Pinskij, 2017: 54-69). In this research with the purpose to reveal features of target genes at them interaction in 5'UTR, CDS and 3'UTR of mRNAs also other genes participating in these types cardiovascular diseases have been studied. It has been established that the 174 genes participating in the development of IHD, 34 genes were associated with 43 miRNAs in the 5'UTR, 55 genes with 90 miRNAs in the CDS, and 35 genes with 53 miRNAs in the 3'UTR. Of them, 23 genes that bound to only one miRNA were located in the 5'UTR region, 34 genes in the CDS, and 19 genes in the 3'UTR. Consequently, these target genes, weakly interacting with miRNAs, did not need an enhanced control of the expression. The remaining mRNAs of genes involved in the development of IHD were associated with two or more miRNAs. Thus, 8 genes under the control of miRNAs were bound in the 5'UTR, 20 genes in the CDS, and 15 in the 3'UTR. Consequently, most of the interactions occurred in the CDS site. The mRNA of the *THRA* gene (three miRNAs) in 5'UTR, the *SMARCA4* gene in the CDS (seven miRNAs), and the *LDLR* gene in the 3'UTR (six miRNAs) had the largest number of interactions. The binding sites located at 5'UTR had the strongest interaction. Only two genes had binding sites in all sites: *CTCF* and *F2RL3*.

Conclusion

It was important to find out what functions genes hold, which expression was most often regulated by miRNAs. Conducted analysis of the function of the studied target genes for miRNA showed that a significant part of them encoded: a)myocardial infarction candidate genes: cell signaling genes *PDED4* was regulated by 13 miRNA with free energy of binding miRNAs with mRNAs -144 kJ/mole; transcription factor genes *ILF3, GATA2* were regulated by 5 and 3 miRNAs respectively with the highest free energy of binding miRNAs with mRNAs -138 kJ/mole; cell proliferation genes *TGFBR1* and *AP3D1* were regulated by 6, 5 miRNAs with highest free energy of binding miRNAs with mRNAs -136 kJ/mole and -140 kJ/mole respectively; b)ischemic heart disease candidate genes: transcription regulators gene *SMARCA4* and cell proliferation regulators genes *TGFB1* and *DOCK7* were regulated by 7, 5 and 2 miRNAs with highest free energy of binding miRNAs with mRNAs -140 kJ/mole, -140 kJ/mole and -138 kJ/mole respectively.

Despite the fact that some of the genes were common for several cardiovascular diseases subtypes, there were genes characteristic for only one subtype: myocardial infarction – *ADRB1, ILF3, GATA2, TGFBR1, AP3D1;* ischemic heart disease – *SMARCA4, DOCK7, CELSR2, TRIB1*. All of the above mentioned interactions can be used as the promising markers of myocardial infarction and ischemic heart disease.

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