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ASSOCIATIONS OF miRNA WITH mRNA OF ATHEROSCLEROSIS CANDIDATE GENES

Abstract. Atherosclerosis is a complex multifactorial disease where multiple genetic and environmental factors are involved. This research presents the characteristics of miRNA (mRNA-inhibiting RNA) interactions with mRNA of atherosclerosis candidate genes. 46, 59, and 33 target genes were identified with miRNA binding sites in the 5'-untranslated region (5'UTR), CDS (coding sequence), and 3′-untranslated region (3'UTR), respectively. Genes have been identified which are most susceptible to miRNA because of interaction with miRNA in more than one mRNA region: *ABCA1; ABCG8; ADCY9; ADRB3; APH1B; ALOX15; HMOX1; GAS6; GNB3; ID3; LRP6; PDE4D; PHACTR1; PROC RTN3; SERPINE1; TIMP3; TNFSF12* and *ZNF202*. Based on the criteria selected in our research, candidate genes were determined that have a free energy interaction with miRNAs equal to -120 kJ/mole and higher in the following associations: in 5'UTR - ID01336.3p-miR and *ADCY9*, ID02142.3p-miR and *ALDH2*, miR-4707-5p and *APH1B*, ID00216.3p-miR and *LGALS2*, ID02363.5p-miR and *NOS3*, ID00551.3p-miR and *NPC1*, ID01310.3p-miR and *PDE4D*, ID03397.3p-miR and *RTN4*, ID00561.3p-miR and *SERPINE1*, ID02903.3p-miR and *TIMP3,* ID01323.3p-miR and *TNFRSF11B*, ID01770 .3p-miR and *ZNF202*; in CDS region - ID03064.3p-miR and *ABCG8*; ID02771.3p-miR and *ADIPOR1*; ID00252.5p-miR and *ANGPTL2*; ID00457.3p-miR and *APOA1;* ID01641.3p-miR and *PDE4D*; ID02050.3p-miR and *TNF*; ID01804.3p-miR and *XBP1*; ID00182.5p-miR and *ZNF202*; in 3'UTR - ID00305.3p-miR and *ADRB3*; ID01213.5p-miR and *AGTR2*; ID02221.3p-miR and *CDK5*; ID03371.3p-miR and *ID3*; ID02229.3p-miR and *LCN2*; ID00704.5p-miR and *TLR9*. Identified associations could be used as biomarkers in diagnosis of atherosclerosis.

Key words: atherosclerosis, miRNA, mRNA, candidate genes, associations.

Introduction. The diagnosis, prevention and treatment of atherosclerosis are still the most important tasks of modern medicine. And accordingly, the success of treatment of diseases such as heart attack, stroke and other cardiovascular complications largely depends on the solution of such a problem like atherosclerosis [1]. According to statistical calculations, the share of deaths from cardiovascular diseases in the world, among which atherosclerosis is one of the first, continues to increase and in 2020 year could reach to 31.5% [2].

Despite the fact that modern clinical medicine is focused primarily on the use of drugs, non-pharmacological treatment methods attract specialists in the field of prevention and treatment. A growing number of studies have emphasized the importance of miRNAs (mRNA-inhibiting RNA) in the development of atherosclerosis. MiRNAs have been shown to be involved in the development of atherosclerosis by regulating the expression of atherosclerosis candidate genes [3].

The miRNAs are nanoscale RNAs which ranging in length from 18 to 27 nucleotides [4], capable of regulating the expression of more than 60% of all protein coding genes [5, 6, 7]. The miRNAs are able to regulate gene expression at the translation by binding to mRNA of the target gene. With complete complementarity of miRNA and mRNA, the latter either degrade or block the activity of the gene. However, incompleteness is most often observed, in which case miRNAs inhibit translation by binding to the 5'-untranslated region (5'UTR), 3′-untranslated region (3′UTR) or protein coding sequence (CDS) of mRNA [8,9,10]. The miRNAs are involved in many biological processes at the stages of development of atherosclerosis, from early endothelial dysfunction to rupture of an unstable atherosclerotic plaque [11]. The determination of miRNAs in the blood of patients could be a direction for the diagnosis of such clinical complications of atherosclerosis as ischemic stroke and myocardial infarction [8,11]. Several thousand publications describe changes in miRNAs concentrations in various diseases and changes in the expression of protein coding genes. In such experiments, correlations are usually established between changes in expression from one to tens of miRNAs and supposed target genes. In this situation, the question arises of the significance of such experiments in establishing the existing direct interactions of miRNAs and candidate genes. As a result, there are not methods for diagnosing diseases using miRNAs and are not therapeutic methods for treating diseases involving these molecules. There are many reasons for this outcome, and some of them are given below. Currently, more than seven thousand miRNAs are known that can regulate the expression of about 20 thousand human genes with varying efficiencies. When setting the task of studying the effect of miRNAs on genes using bioinformatics methods, it is possible to establish the most effective associations of miRNAs and target genes. However, existing programs for searching for miRNAs binding sites with mRNAs analyze only sequences of 6-8 nucleotides in length at the 5′UTR of miRNA. As a result, programs predict many false positive binding sites. This misconception is the main reason for the failure to identify miRNAs associations and target genes. An approach is needed to evaluate the comparative participation of all miRNAs with all genes and to determine the quantitative characteristics of miRNAs interactions with mRNAs. In addition, it is necessary to establish the estimated concentrations of miRNAs and mRNAs of target genes, because the result of miRNAs interactions with mRNAs will depend on their ratio. Further researches are needed to fully understanding the role of miRNAs in atherosclerosis pathogenesis and in the development of its complications, as well as in the development of targeted therapeutic approaches.

Materials and methods. The nucleotide sequences of mRNA candidate genes were obtained from GenBank (http://www.ncbi.nlm.nih.gov). The miRNA base consisted of 2565 miRNAs that were downloaded from miRBase (http://mirbase.org) and 3707 miRNAs were obtained from a report by Londin E. *et al.* [12]. A search for the target genes of miRNAs was performed using the MirTarget program [13]. This program defines the following binding characteristics: the start of the miRNA binding site of mRNA; the locations of miRNA binding sites in 5'UTR, CDS and 3'UTR regions; the interaction free energy (ΔG, kJ/mole). For each binding site, the ratio ΔG/ΔGm (%) was determined, where ΔGm is equal to the free energy binding of miRNA with its full complementary nucleotide sequence. The obtained miRNA-mRNA binding sites were selected with the ΔG/ΔGm ratio of 90% or more.

Results and discussion. The search for miRNA binding sites was carried out in the 5′UTR, CDS and 3′UTR mRNA of the candidate atherosclerosis genes in order to reveal interaction features of miRNAs in these regions. To option the most effective associations of miRNA and candidate genes, the following criteria and characteristics of the miRNAs interaction with mRNAs of target genes were selected: the value of the interaction free energy of miRNAs with mRNAs of the candidate target gene; the degree of complementarity of miRNA nucleotides and binding sites of mRNA candidate genes; the possibility of the candidate gene participation on the basis of its function in studied disease. There are others, depending on the characteristics of the disease in addition to these criteria and characteristics. Priority between criteria and characteristics is difficult to establish in advance, so there will always be uncertainty in the correct choice of their list. This difficulty is due to various causes of the disease at an early stage and deviations from the norm in subsequent stages. The most effective associations of miRNA and atherosclerosis candidate genes were selected which based on the above considerations.

Table 1 presents the characteristics of miRNAs interactions with mRNAs of 47 candidate atherosclerosis genes. Free energy interaction (ΔG) of miRNAs with mRNA is -120 kJ/mole or more could be recommended as associations: ID01336.3p-miR and *ADCY9*, ID02142.3p-miR and *ALDH2*,

miR-4707-5p and *APH1B*, ID00216.3p-miR and *CD40*, miR-6789-5p and *HIF1A*, ID01242.3p-miR and *LGALS2*, ID02363.5p-miR and *NOS3*, ID00551.3p-miR and *NPC1*, ID01310.3p-miR and *PDE4D*, ID03397.3p-miR and *PTGS2*, ID00561.3p-miR and *RTP4*, ID01098.3p-miR and *SERPINE1*, ID02903.3p-miR and *TIMP3*, ID01323.3p-miR and *TNFRSF11B*, ID01770.3p-miR and *ZNF202*. Three associations of miRNA and candidate genes (ID02813.3p-miR and *CAPN10*, ID01152.3p-miR and *HMOX1*, ID01840.5p-miR and *PHACTR1*) were characterized by the value of ΔG/ΔGm equal to 95%, which indicates an almost complete complementarity of the interaction of miRNA nucleotides and binding site nucleotides.

Table 1 - Characteristics of miRNA interactions
in the 5'UTR of the mRNAs of atherosclerosis candidate genes

Gene	miRNA	Start of site, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ABCA1	miR-4435	331	-110	91	22
ABCG8	ID00122.5p-miR	55	-110	90	22
ADCY9	ID01336.3p-miR	465	-136	89	24
ADRB3	ID01568.3p-miR	36	-115	90	22
AGT	miR-3126-5p	326	-108	91	22
ALDH2	ID02142.3p-miR	8	-123	92	21
APH1B	miR-4707-5p	5	-129	92	23
CAPN10	ID02813.3p-miR	105	-115	95	20
CD40	ID00216.3p-miR	10	-123	91	23
CX3CR1	ID01330.3p-miR	164	-119	89	23
DPP4	ID02385.3p-miR	369	-108	93	20
FGF23	miR-6878-3p	48	-102	91	21
GAS6	miR-4749-5p	320	-117	92	22
GNB3	ID00388.3p-miR	234	-110	87	22
HBEGF	ID03416.5p-miR	66	-117	92	20
HIF1A	miR-6789-5p	53	-132	90	24
HMOX1	ID01152.3p-miR	75	-113	95	20
ICAM1	ID00195.3p-miR	106	-117	89	23
ID3	ID00461.3p-miR	238	-113	90	22
IL15	ID01713.5p-miR	63	-115	92	20
IL18	miR-548au-3p	102	-100	90	21
IRS2	ID02344.3p-miR	66	-132	91	24
KDR	ID02534.5p-miR	143	-119	92	22
LGALS2	ID01242.3p-miR	75	-123	89	24
LPL	miR-4430	287	-96	94	18
LRP6	miR-6752-5p	68	-119	90	22
MMP2	ID02014.5p-miR	264	-117	89	23
NOS3	ID02363.5p-miR	200	-123	88	24
NPC1	ID00551.3p-miR	34	-121	88	24
NR4A1	ID01213.5p-miR	139	-119	89	23
PDE4D	ID01310.3p-miR	66	-121	92	22
PHACTR1	ID01840.5p-miR	96	-113	95	22
PLA2G7	miR-4722-5p	40	-119	90	23
PLTP	ID01382.3p-miR	108	-113	93	20
PON2	ID02200.3p-miR	8	-119	90	22
PTGS2	ID03397.3p-miR	108	-123	92	21
PTX3	miR-6866-5p	36	-106	91	23
RTN4	ID00561.3p-miR	247	-121	93	21
SCAP	ID00757.3p-miR	23	-108	91	21
SELP	ID03109.5p-miR	49	-106	94	21
SERPINE1	ID01098.3p-miR	30	-123	88	24
SOAT1	ID03036.3p-miR	46	-115	89	23
SPP1	miR-1913	60	-117	92	22
TIMP3	ID02903.3p-miR	1102	-121	90	22
TNFRSF11B	ID01323.3p-miR	115	-125	92	22
TNFSF12	ID01254.5p-miR	56	-110	91	21
ZNF202	ID01770.3p-miR	70	-123	94	22

The results of the analysis of miRNAs interactions in the CDS region of 59 candidate genes are given in table 2. Among them, associations can be distinguished with a free energy (ΔG) is -120 kJ/mole and higher: ID03064.3p-miR and *ABCG8*; ID02771.3p-miR and *ADIPOR1*; ID00252.5p-miR and *ANGPTL2*; ID00457.3p-miR and *APOA1*; ID01641.3p-miR and *PDE4D;* ID02050.3p-miR and *TNF*; ID01804.3p-miR and *XBP1;* ID00182.5p-miR and *ZNF202*. Four associations of miRNAs and candidate genes were also identified, which were characterized by a ΔG/ΔGm value of 95% or more: ID02129.5p-miR and *CD4*; ID01797.3p-miR and *PLA2G10*; miR-1281 and *PHACTR1*; ID00182.5p-miR and *ZNF202*.

Table 2 - Characteristics of miRNA interactions in the CDS of the mRNAs of atherosclerosis candidate genes

Gene	miRNA	Start of site, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
1	2	3	4	5	6
ABCA1	ID00653.3p-miR	6416	-110	90	23
ABCG5	ID03409.5p-miR	441	-115	93	20
ABCG8	ID03064.3p-miR	1727	-136	89	24
ADCY9	ID01569.3p-miR	2981	-115	92	20
ADIPOR1	ID02771.3p-miR	1092	-121	93	22
AGTR1	ID02795.5p-miR	102	-117	92	22
AHSG	miR-6794-5p	955	-108	93	20
ALOX15	ID01385.5p-miR	258	-110	91	21
ANGPTL2	ID00252.5p-miR	1145	-136	91	24
ANXA5	miR-3613-5p	475	-96	92	22
APOA1	ID00457.3p-miR	841	-123	91	22
APOL1	miR-146b-3p	1506	-113	93	22
BRAP	miR-1908-5p	233	-113	91	21
CD4	ID02129.5p-miR	1090	-113	95	20
CDKN1C	ID01313.3p-miR	360	-110	91	21
COMT	ID00215.3p-miR	199	-108	91	21
CPE	ID02448.5p-miR	355	-115	90	22
CX3CL1	ID02488.5p-miR	561	-113	93	21
CXCR3	ID00240.5p-miR	293	-106	91	21
CXCR4	miR-3119	736	-93	92	20
CYBA	ID01251.3p-miR	578	-119	92	22
CYP27A1	ID01201.5p-miR	557	-106	91	21
CYP2E1	miR-4445-3p	1366	-100	92	21
FADS2	ID01205.5p-miR	1447	-110	90	22
GAS6	ID01154.5p-miR	2163	-115	93	20
GNB3	miR-6736-3	1167	-104	91	21
GSTM1	ID01955.3p-miR	462	-108	89	23
HP	ID00253.5p-miR	1178	-98	92	20
LPCAT3	ID02232.3p-miR	740	-110	90	22
LRP6	ID03063.3p-miR	3421	-110	90	22
LTA	miR-6831-5p	581	-117	90	24
MMP3	ID00314.3p-miR	132	-119	93	23
NLRP3	ID00662.3p-miR	3638	-102	92	20
NPC1	miR-4459	1031	-119	93	22
PCSK9	ID01810.3p-miR	1052	-115	89	23
PDE4D	ID01641.3p-miR	335	-132	89	24
PHACTR1	miR-1281	1563	-93	96	17
PIN1	ID02643.3p-miR	627	-119	89	23
PLA2G10	ID01797.3p-miR	731	-110	95	20
PNPLA3	ID02224.3p-miR	918	-106	93	22
PON1	miR-5003-3p	330	-100	92	21
PROC	miR-185-3p	744	-110	90	22
RTN3	miR-718	254	-117	92	21
SELE	ID03022.3p-miR	829	-100	90	22
SERPINE1	miR-4758-3p	276	-119	90	23
SHBG	miR-6746-5p	821	-115	90	22
SOCS1	ID00171.3p-miR	461	-115	92	20
TGFB1	miR-6742-5p	2046	-110	90	22
THBS2	miR-598-3p	2941	-104	91	22

Continuation of table 2

1	2	3	4	5	6
TLR2	ID00935.5p-miR	295	-104	94	20
TNF	ID02050.3p-miR	230	-121	92	23
TNFSF12	miR-6739-3p	491	-100	92	21
TNNT2	ID02813.3p-miR	164	-113	93	20
TRIB3	miR-596	731	-113	91	21
TSPO	ID02332.3p-miR	134	-115	89	23
UCP2	miR-6878-3p	509	-102	91	21
XBP1	ID01804.3p-miR	110	-134	91	23
ZNF202	ID00182.5p-miR	935	-125	97	23

Data of the miRNAs interactions with mRNA of 33 candidate genes in 3'UTR region are shown in table 3. Based on these interactions, six associations can be distinguished, which were characterized by free energy equal to (ΔG) -120 kJ/mole and higher: ID00305.3p-miR and *ADRB3*; ID01213.5p-miR and *AGTR2*; ID02221.3p-miR and *CDK5*; ID03371.3p-miR and *ID3*; ID02229.3p-miR and *LCN2*; ID00704.5p-miR and *TLR9*. Of all the revealed interactions in the 3'UTRs, one maximum index of complementarity equal to 98% was determined between miR-1273g-3p and mRNA of *ALOX15* gene.

Table 3 - Characteristics of miRNA interactions
in the 3'UTR of the mRNAs of atherosclerosis candidate genes

Gene	miRNA	Start of site, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ADCY9	miR-2392	5360	-102	92	20
ADIPOR2	ID01700.3p-miR	1398	-119	90	23
ADRB3	ID00305.3p-miR	2347	-121	88	24
AGTR2	ID01213.5p-miR	2307	-121	90	23
ALOX15	miR-1273g-3p	2393	-115	98	21
APLN	miR-3661	1260	-110	90	22
APH1B	ID01642.3p-miR	2230	-119	90	24
CDK5	ID02221.3p-miR	1096	-125	89	24
CHI3L1	ID01707.5p-miR	1497	-110	93	22
CXCL12	ID00483.3p-miR	932	-119	90	23
CXCL13	miR-4326	830	-100	92	20
CXCL5	miR-567	1817	-108	91	23
CYP27A1	ID00964.5p-miR	2078	-119	90	24
DAP	miR-6762-5p	525	-119	90	23
DKK1	ID00436.3p-miR	1580	-106	91	23
FASLG	ID00790.3p-miR	1595	-104	89	23
HMOX1	miR-3155a	1227	-106	91	21
ID3	ID03371.3p-miR	974	-123	91	23
IL10	ID01332.3p-miR	1200	-110	90	22
LCN2	ID02229.3p-miR	682	-123	94	21
LRP6	miR-4693-3p	9159	-108	94	23
NCEH1	miR-6728-3p	3101	-106	91	21
PCSK9	miR-6877-3p	2468	-110	91	21
PDE4D	ID02141.5p-miR	7731	-100	90	22
PROC	miR-6736-3p	1672	-106	93	21
PSMA6	ID02529.5p-miR	954	-106	93	20
ROCK1	miR-5010-3p	5650	-106	91	22
RTN3	miR-6785-5p	4652	-110	90	22
S100A9	ID02629.5p-miR	436	-108	93	21
SELPLG	ID02248.5p-miR	1818	-106	94	20
TIMP3	miR-1224-5p	3267	-104	96	19
TLR9	ID00704.5p-miR	3779	-123	88	24
TNFSF12	miR-3151-5p	907	-108	93	21

From the above Tables it follows that in the protein coding region, more than all single interactions of different miRNAs with mRNAs were determined in comparison with the 3'UTR and 5'UTR regions. In the course of studying the miRNAs interactions with mRNAs various regions of the atherosclerosis candidate genes, there were identified that single associations not only in one mRNA region. Candidate genes which had interactions with miRNAs in two regions: *ABCA1; ABCG8; ADRB3; APH1B; ALOX15; HMOX1; GAS6; GNB3; ID3; PHACTR1; PROC RTN3; SERPINE1; TIMP3* and *ZNF202*, and in three regions: *ADCY9; LRP6; PDE4D* and *TNFSF12*. Accordingly, these genes are most affected by different miRNAs.

Genes selected in associations, including which have significant interactions with miRNAs with high free energy, are directly involved in the development of atherosclerosis. For example, disorders of lipid metabolism often accompany the development of cardiovascular pathology. Polymorphisms of genes involved in the control of lipid metabolism have been identified. These are the genes of apolipoproteins A (LPA), B (APOB), C (APOC1-3), E (APOE), low density lipoprotein receptor (LPLR), paraoxonase (PON1), etc. [14]. An inverse relationship has been established between high density lipoproteins (HDL) and atherosclerosis. Lecithin-cholesterol-acyltransferase (LCAT) is a key enzyme in cholesterol reverse transport and HDL metabolism. Mutations in *LCAT* gene are associated with low HDL and an increased risk of dyslipidemia and atherosclerosis [15]. Also, the *LRP* gene is a multifunctional receptor that is involved in several biological processes associated with the development of atherosclerosis [16]. The absorption of exogenous cholesterol occurs in enterocytes using a special NPCL transport system. Mutations in the *NPC1* gene contribute to the accumulation of huge amounts of cholesterol due to impaired intracellular transport of lipids, which leads to the development of atherosclerosis [17]. It was found that the level and activity of the *CETP* gene is associated with the level of HDL in plasma, which affects the risk of developing atherosclerosis [18]. Endothelial dysfunction plays a key role in the development and progression of this disease. The reduced bioavailability of nitric oxide (NO) obtained by endothelial NO synthase (eNOS) leads to deterioration in endothelial relaxation of the arteries. eNOS is encoded by the *NOS3* gene, the polymorphisms of which are associated with atherosclerosis [19]. Genes, which connected with inflammation, are also associated with the development of this disease. It has been suggested that toll-like receptors (TLRs) may be a key link between the development of cardiovascular disease and the immune system. TLR expression is regulated in endothelial cells and macrophages of atherosclerotic lesions. A299G polymorphism of *TLR* gene is associated with a risk of carotid arteriosclerosis, acute coronary syndrome [20]. Polymorphisms of *LGALS2* gene of galectin-2 protein were also identified as a genetic risk factor for myocardial infarction and coronary atherosclerosis [21].

Conclusion. The associations of a large number of genes with atherosclerosis reflect the enormous complexity of this disease. Therefore, it is important to establish associations of these genes with miRNAs. The data obtained in this research significantly expand the understanding of the dependence of atherosclerosis candidate genes expression from miRNAs. These data allow us to consider miRNAs with target genes as perspective diagnostic and therapeutic molecular markers of atherosclerosis.

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АТЕРОСКЛЕРОЗ КАНДИДАТТЫҚ ГЕННІҢ мРНК-МЕН миРНК-НЫҢ АССОЦИАЦИЯЛАРЫ

Аннотация. Атеросклероз – бұл көптеген генетикалық және экологиялық факторлар қатысатын күрделі, мультифакторлы ауру болып табылады. Соңғы жылдары атеросклероздың генетикалық қауіп факторларын анықтауға деген қызығушылық өте тез өсуде. Атеросклероздың патогенезіне липидтердің алмасу ақаулары, эндотелиалды дисфункциясы, тотығу стрессі, қабыну және иммундық реакциялар, жасуша пролиферациясы және гемостатикалық ақаулар сияқты бірнеше факторлар қатысады. Тиісінше, атеросклерозға кандидаттық

гендер анықталды, олар осы процестерге тікелей әсер етеді. Алайда, осы аурудың дамуына әсер ететін, әртүрлі биологиялық процестерге қатысатын гендерден басқа, miRNA (mRNA-inhibiting RNA) кандидаттық атеросклероз генінің экспрессиясын реттеу арқылы, атеросклероздың дамуына қатысатындығы анықталды. miRNA-тың гендерге әсерін зерттеу міндетін қою кезінде, биоинформатикалық әдістерді қолдана отырып, miRNA пен мақсатты гендердің ең тиімді бірлестіктерін құруға болады. Бұл жұмыста miRNA-ның (mRNA-inhibiting RNA) атеросклероз үшін кандидаттық гендер mRNA-ға өзара әрекеттесу сипаттамалары келтірілген. miRNA-тың mRNA-ға кандидаттық гендерімен байланыстыратын сайттар MirTarget бағдарламасын пайдаланып анықталды. Бұл бағдарлама mRNA -ның әртүрлі аймақтарында байланыстыратын сайттардың орналасуымен қатар, miRNA-ның mRNA-ға байланысатын сайттардың басталуын, miRNA-ның mRNA-ға еркін энергетикалық әсерлесу көрсеткішін (ΔGm, kJ/mole) және miRNA нуклеотидтерінің mRNA-ның байланыстыратын сайттарының (ΔG/ΔGm) толықтау дәрежесін анықтайды. Осы аймақтардағы miRNA -ның өзара әрекеттесу ерекшеліктерін анықтау үшін miRNA -ны байланыстыратын сайттарды іздеу 5'-трансляцияланбайтын аймақта (5´UTR), ақуызды кодтау аймағында (CDS) және mRNA атеросклероздың кандидаттық гендерінің 3′- трансляцияланбайтын аймағында (3´UTR) жүргізілді. CDS аймағында әр түрлі miRNA-ның mRNA -мен өзара жеке әрекеттесу көбінесе 3'UTR және 5'UTR аймақтарына қарағанда, көбірек болатындығы анықталды. miRNA-мен атеросклерозаның кандидатты гендері mRNA-ның әртүрлі аймақтарымен өзара әрекеттесуін зерттеу кезінде, жеке ассоциациялары mRNA-ның бір ғана емес аймағында бар, гендер анықталды. Кандидат гендерінің miRNA-мен өзара әрекеттесуі екі бағытта болды: *ABCA1; ABCG8; ADRB3; APH1B; ALOX15; HMOX1; ГА36; GNB3; ID3; PHACTR1; PROC*; *RTN3; SERPINE1; TIMP3* және *ZNF202*, және үш бағытта: *ADCY9; LRP6; PDE4D* және *TNFSF12*. Тиісінше, бұл гендерге көбінесе миRNA әсер етеді. Біздің зерттеуімізде таңдалған критерийлер негізінде, келесі кандидаттық гендер анықталды. Олар miRNA-мен еркін энергетикалық әрекеттесіп, -120 kJ/mole-ға тең және келесі ассоциацияларда жоғарырақ болады: 5'UTR-да - ID01336.3p-miR және *ADCY9*, ID02142.3p-miR және *ALDH2*, miR-4707-5p және *APH1B*, ID00216.3p-miR және *CD40*, miR-6789-5p және *HIF1A*, ID01242.3p-miR және *LGALS2*, ID02363.5p-miR және *NOS3*, ID00551.3p-miR және *NPC1*, ID01310.3p-miR және *PDE4D*, ID03397.3p-miR және *PTGS2*, ID00561.3p-miR және *RTN4*, ID01098.3p-miR және *SERPINE1*, ID02903.3p-miR және *TIMP3*, ID01323.3p-miR және *TNFRSF11B*, ID01770.3p-miR және *ZNF202*; в CDS - ID03064.3p-miR және *ABCG8*; ID02771.3p-miR және *ADIPOR1*; ID00252.5p-miR және *ANGPTL2*; ID00457.3p-miR және *APOA1*; ID01641.3p-miR және *PDE4D*; ID02050.3p-miR және *TNF*; ID01804.3p-miR және *XBP1*; ID00182.5p-miR және *ZNF202*; 3'UTR-да - ID00305.3p-miR және *ADRB3*; ID01213.5p-miR және *AGTR2*; ID02221.3p-miR және *CDK5*; ID03371.3p-miR және *ID3*; ID02229.3p-miR және *LCN2*; ID00704.5p-miR және *TLR9*. MiRNA мен кандидаттардың гендерінің ассоциациясы анықталды, олар 95% G/ΔGm мәнімен сипатталды, бұл miRNA нуклеотидтері мен байланыстырушы жердің нуклеотидтерінің өзара әрекеттесуінің толықтай үйлесімділігін көрсетеді: 5'UTR - ID02813.3p-miR және *CAPN10*, ID01152.3p- miR және *HMOX1*, ID01840.5p-miR және *PHACTR1*; CDS - ID02129.5p-miR және *CD4* бойынша; ID01797.3p-miR және *PLA2G10*; miR-1281 және *PHACTR1*; ID00182.5p-miR және *ZNF202*; 3'UTR - miR-1273g-3p және *ALOX15*. Анықталған қауымдастықтарды биомаркер ретінде атеросклероздың диагностикасында қолдануға болады.

Түйін сөздер: атеросклероз, miRNA, mRNA, кандидаттық гендер, ассоциациялар.

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АССОЦИАЦИИ miRNA С mRNA КАНДИДАТНЫХ ГЕНОВ АТЕРОСКЛЕРОЗА

Аннотоация. Атеросклероз представляет собой комплексное мультифакторное заболевание, в котором задействованы множественные генетические и экологические факторы. В последние годы очень быстро возрастает интерес к идентификации факторов генетического риска для атеросклероза. Множественные факторы, такие как дефекты в липидном метаболизме, эндотелиальная дисфункция, окислительный стресс, воспаление и иммунные ответы, клеточная пролиферация и гемостатические дефекты участвуют в патогенезе атеросклероза. Соответственно, выявились гены-кандидаты атеросклероза, которые непосредственно влияют на данные процессы. Однако помимо генов, вовлеченных в различные биологические процессы, которые влияют на развитие данного заболевания, было определено, что miRNA (mRNA-inhibiting RNA) также участвуют в развитии атеросклероза посредством регуляции экспрессии кандидатных генов атеросклероза. При постановке задачи изучения влияния miRNA на гены с помощью

биоинформатических методов можно установить наиболее эффективные ассоциации miRNA и генов-мишеней. В данной работе представлены характеристики взаимодействий miRNA с mRNA кандидантных генов атеросклероза. Сайты связывания miRNA с mRNA кандидатных генов определяли с помощью программы MirTarget. Данная программа, помимо расположения сайтов связывания в различных регионах mRNA, определяет начало сайтов связывания miRNA с mRNA, показатель свободной энергии взаимодействия miRNA с mRNA (ΔGm, kJ/mole), а также степень комплементарности нуклеотидов miRNA с сайтом связывания mRNA (ΔG/ΔGm). Поиск сайтов связывания miRNA проводили в 5'-нетранслируемой области (5´UTR), белок-кодирующей области (CDS) и 3'-нетранслируемой области (3´UTR) mRNA кандидатных генов атеросклероза с целью выявления особенностей взаимодействия miRNA в этих регионах. Выявлены 46, 59 и 33 генов-мишеней, имеющие сайты связывания miRNA в 5'UTR, CDS и 3'UTR, соответственно. В CDS области установлено больше одиночных взаимодействий различных miRNA с mRNA в сравнении с областями 3'UTR и 5'UTR. В ходе изучения взаимодействий miRNA с различными областями mRNA кандидатных генов атеросклероза были выявлены гены, которые имели одиночные ассоциации не только в одной области mRNA. Гены-кандидаты имели взаимодействия с miRNA в двух областях: *ABCA1; ABCG8; ADRB3*; *APH1B*; *ALOX15; HMOX1*; *GAS6*; *GNB3*; *ID3*; *PHACTR1*; *PROC*; *RTN3*; *SERPINE1*; *TIMP3* и *ZNF202*, и в трех областях: *ADCY9*; *LRP6*; *PDE4D* и *TNFSF12*. Соответственно, данные гены наиболее подвержены влиянию со стороны miRNA. Основываясь на критериях, выбранных в нашем исследовании, были определены кандидатные гены, имеющие свободную энергию взаимодействия с miRNA равной 120 kJ/mole и выше в следующих ассоциациях: в 5'UTR - ID01336.3p-miR and *ADCY9,* ID02142.3p-miR and *ALDH2,* miR-4707-5p and *APH1B,* ID00216.3p-miR and *CD40,* miR-6789-5p and *HIF1A*, ID01242.3p-miR and *LGALS2*, ID02363.5p-miR and *NOS3,* ID00551.3p-miR and *NPC1*, ID01310.3p-miR and *PDE4D,* ID03397.3p-miR and *PTGS2*, ID00561.3p-miR and *RTN4,* ID01098.3p-miR and *SERPINE1*, ID02903.3p-miR and *TIMP3,* ID01323.3p-miR and *TNFRSF11B*, ID01770.3p-miR and *ZNF202*; в CDS - ID03064.3p-miR *и ABCG8*; ID02771.3p-miR и *ADIPOR1;* ID00252.5p-miR и *ANGPTL2*; ID00457.3p-miR и *APOA1;* ID01641.3p-miR и *PDE4D*; ID02050.3p-miR и *TNF;* ID01804.3p-miR и *XBP1*; ID00182.5p-miR и *ZNF202;* в 3'UTR - ID00305.3p-miR и *ADRB3*; ID01213.5p-miR и *AGTR2*; ID02221.3p-miR и *CDK5*; ID03371.3p-miR и *ID3*; ID02229.3p-miR и *LCN2*; ID00704.5p-miR и *TLR9*. Выявлены ассоциации miRNA и кандидатных генов, которые характеризовались величиной ΔG/ΔGm, равной 95%, что свидетельствует о почти полной комплементарности взаимодействия нуклеотидов miRNA и нуклеотидов сайта связывания: в 5'UTR - ID02813.3p-miR и *CAPN10*, ID01152.3p-miR и *HMOX1*, ID01840.5p-miR и *PHACTR1*; в CDS - ID02129.5p-miR и *CD4*; ID01797.3p-miR и *PLA2G10*; miR-1281 и *PHACTR1*; ID00182.5p-miR и *ZNF202;* в 3'UTR- miR-1273g-3p и *ALOX15*. Выявленные ассоциации можно использовать в качестве биомаркеров при диагностике атеросклероза.

Ключевые слова: атеросклероз, miRNA, mRNA, кандидатные гены, ассоциации.

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