IRSTI 577.21 https://doi.org/10.26577/EJE.2022.v73.i4.07

O.Yu. Yurikova*, T.I. Abdullayeva, Sh.A. Atambayeva, A. Bekenkali

Al-Farabi Kazakh National University, Kazakhstan, Almaty
*e-mail: oksanayurikova@mail.ru

GENES AND MIRNAS INVOLVED IN THE DEVELOPMENT OF ATHEROSCLEROSIS

Atherosclerosis is a complex multifactorial disease. miRNAs, single-stranded RNA molecules may play an important role in the regulation genes expression which involved in atherosclerosis. The work studied binding of miRNAs with mRNA of genes responsible for development of human atherosclerosis. Based on the literature data of PubMed, a database of 14 genes sequences associated with development of atherosclerosis was created. The miRNA binding sites in mRNAs of the studied genes were found using the miRWalk, miRTarBase, TargetScan, miRDB, and MirTarget programs. 94 binding sites of 51 miRNAs were revealed in mRNA of genes responsible for the development of atherosclerosis. Of the studied binding sites, 15 sites are located in CDS, 17 in 5′UTR, and 62 in 3′UTR. It was defined that some mRNAs of genes have several binding sites with miRNAs. Thus, TNFRSF9 has 15, LDLR – 11 binding sites, TGFB1 – 14 binding sites. It was shown that miR-619-5p more frequently than others binds to mRNA of genes responsible for the development of atherosclerosis. miR-619-5p binds to CD36 with a ΔG/ΔGm ratio of 100%, miR-5096 with a ΔG/ΔGm equal to 100% binds to IL18. Among the studied genes, TGFB1 is the most preferred target of miRNA with high values of interaction, indicating the degree of miRNA binding to mRNA. Thus, using miRWalk, miRTarBase, TargetScan, and miRDB programs, it was shown that mRNA of TGFB1 is a target for many miRNAs in CDS, 5′UTR, and 3′UTR regions.

Key words: miRNA, mRNA, binding sites, atherosclerosis, CDS, 5′UTR, 3′UTR, and nucleotide sequences.

О.Ю. Юрикова*, Т.И. Абдуллаева, Ш.А. Атамбаева, А. Бекенқали

Әл-Фараби атындағы Қазақ ұлттық университеті, Қазақстан, Алматы қ.
*e-mail: oksanayurikova@mail.ru

Атеросклероздың дамуына жауап беретін гендер мен миРНК

Атеросклероз – көп факторлы ауру. miRNAs, бір тізбекті РНҚ молекулалары, атеросклерозға қатысатын гендердің экспрессиясын реттеуде маңызды рөл атқаруы мүмкін. Жұмыста миРНҚ-ның адамның атеросклерозының дамуына жауап беретін гендердің мРНҚ-мен байланысы зерттелді. PubMed әдебиеттерінің деректері негізінде атеросклероздың дамуына байланысты 14 геннің мәліметтер базасы құрылды. Таңдалған гендердің мРНҚ-дағы miRNA байланыстыратын сайттар miRWalk, miRTarBase, TargetScan, miRDB және MirTarget бағдарламаларының көмегімен табылды. Атеросклероздың дамуына жауап беретін гендердің мРНҚ-сында 51 миРНҚ-ны байланыстыратын 94 сайт табылды. Анықталған байланысу сайттардан 15 сайт CDS-де, 17-і 5′UTR-де және 62-і 3′UTR-де орналасқан. Атеросклероздың дамуына жауап беретін гендердің кейбір мРНҚ-сында миРНҚ-мен байланысатын бірнеше сайттар бар екендігі анықталды. Сонымен TNFRSF9 гендерінің 15 байланысу сайттары, LDLR – 11 байланысу сайттары, TGFB1 – 14 байланыс сайттары бар. Көрсетілгендей, атеросклероздың дамуына жауап беретін гендердің мРНҚ-мен миРНҚ miR-619-5p ең көп байланысады. miR-619-5p CD36 генімен ΔG/ΔGm қатынасы 100% болды, miR-5096 ΔG/ΔGm 100%-ке тең IL18 генімен байланысады. Барлық зерттелген гендердің ішінен TRFB1 гені миРНҚ-ның мРНҚ-мен байланыс дәрежесін сипаттайтын жоғары көрсеткіштерімен миРНҚ-ның ең қолайлы нысаны екендігі анықталды. Осылайша, miRWalk, miRTarBase, TargetScan және miRDB бағдарламаларын қолдана отырып, TGFB1 генінің мРНҚ-сы CDS, 5′UTR және 3′UTR аймақтарындағы көптеген миРНҚ-лардың нысаны екендігі көрсетілді.

Түйін сөздер: миРНҚ, мРНҚ, байланысатын сайттар, атеросклероз CDS, 5′UTR, 3′UTR, нуклеотидтік тізбектер.

© 2022 Al-Farabi Kazakh National University

О.Ю. Юрикова*, Т.И. Абдуллаева, Ш.А. Атамбаева, А. Бекенкали
Казахский национальный университет им. аль-Фараби, Казахстан, Казахстан, г. Алматы
*e-mail: oksanayurikova@mail.ru

Гены и миРНК, участвующие в развитии атеросклероза

Атеросклероз является многофакторным заболеванием. miRNA, одноцепочечные молекулы РНК, могут играть важную роль в регуляции экспрессии генов, участвующих в развитии атеросклероза. В работе было изучено связывание миРНК с мРНК генов, ответственных за развитие атеросклероза человека. На основе литературных данных публикаций PubMed была создана база данных из 14 генов, связанных с развитием атеросклероза. Сайты связывания миРНК в мРНК отобранных генов были найдены с использованием программ miRWalk, miRTarBase, TargetScan, miRDB и MirTarget. В mRNA генов, ответственных за развитие атеросклероза, найдено 94 сайта связывания для 51 миРНК. Из выявленных сайтов связывания миРНК, 15 расположены в CDS, 17 – в 5′UTR и 62 – в 3′UTR. Было выявлено, что некоторые мРНК генов, ответственных за развитие атеросклероза, имеют несколько сайтов связывания с миРНК. Так, гены TNFRSF9 имеют 15 сайтов связывания, LDLR – 11 сайтов связывания, TGFB1 – 14 сайтов связывания. Было показано, что больше всего с мРНК генов, ответственных за развитие атеросклероза, связывалась миРНК miR-619-5p. miR-619-5p связывается с геном CD36 сотношением ΔG/ΔGm, равным 100%, miR-5096 с ΔG/ΔGm, равным 100%, связываются с геном IL18. Было установлено, что из всех изученных генов ген TGFB1 является наиболее предпочитаемой мишенью миРНК с высокими показателями, характеризующими степень связывания миРНК с мРНК. Так, с помощью программ miRWalk, miRTarBase, TargetScan и miRDB было показано, что мРНК гена TGFB1 является мишенью для многих миРНК в CDS, 5′UTR и 3′UTR участках.

Ключевые слова: миРНК, мРНК, сайты связывания, атеросклероз, CDS, 5′UTR, 3′UTR, нуклеотидные последовательности.

Introduction

Atherosclerosis is a complex multifactorial disease of medium and large arteries influenced by many genetic and environmental factors. In recent years, the number of candidate genes for atherosclerosis has been growing rapidly. This, in turn, leads to a significant increase in interest in identifying additional genetic risk factors for atherosclerosis and initiating a large number of genetic studies to prove a genetic effect on atherosclerosis [1].

Atherosclerosis is a chronic inflammatory disease, in which lipid macrophages accumulate in the subendothelial layer of arteries [2, 3]. It is well known that adhesion of monocytes is stimulated by endothelial dysfunction of the artery wall, after which they divide into macrophages and absorb lipoprotein particles into foam cells [4, 5]. The accumulation of these lipids causes inflammation, which stimulates and intensifies the atherosclerotic process [6].

MicroRNAs (miRNAs) are short, single-stranded RNA molecules that interact with mRNA and may affect protein synthesis [7, 8]. They make up about 1-5% of the human genome and are formulated as evolutionary conserved components that control the post-transcriptional expression of certain genes [8- 10]. Due to this action, miRNA can suppress the expression of a particular protein and, therefore, bind to this gene and suppress it. miRNA suppresses the expression of a gene by dividing or disrupting its subsequent target mRNA, or by inhibiting the translation process. The method of action used to suppress a gene is determined by the degree of complementarity between the miRNA complex and its target mRNA [11].

Since miRNAs play an important role in the silencing of target genes, they subsequently decrease protein synthesis and, therefore, affect cell function; hence, it has been hypothesized that miRNAs may indeed play a role in endothelial damage and cell fusion, growth, and inflammatory responses [12 – 15]. It has also been suggested that miRNAs may be significant regulators of smooth muscle proliferation and phenotypic changes [16 – 20] and may affect macrophage activity. Thus, understanding the effect of miRNAs on cells that provoke the development of atherosclerosis may be crucial for using their potential in clinical therapy in the process of vascular diseases. The aim of this study was to determine the genes and miRNAs involved in the development of atherosclerosis, as well as the identification of their miRNAs – candidate genes associations, effectively interacting to each other. As a result of the study, a database of genes responsible for the development of atherosclerosis was created, and the features of

the interaction of mRNA genes with miRNAs involved in the development of atherosclerosis were revealed.

Methods and materials

NCBI database (www.ncbi.nlm.nih.gov/) was used for the genes search; the name of the disease was used as a keyword (the choice of keywords was in different variations). For each request for atherosclerosis, there were several hundred candidate genes in the database, each of which was studied separately. The study was conducted by looking for the association of this gene with the corresponding disease in publications over the past decade (www.ncbi.nlm.nih.gov/PubMed). Thus, the relationship between the gene and the corresponding disease was identified, and a database of genes involved in the development of atherosclerosis was created. Further, a comparative analysis of the database of candidate genes was carried out.

All nucleotide sequences of selected genes mRNAs were obtained from GenBank (www.ncbi.nlm.nih.gov). The miRNA nucleotide sequences were obtained from miRBase (www.mirbase.org). The search for the target gene for miRNA was carried out using the MirTarget program [20]. The program was used to search for sites of interaction, free energy of interaction (ΔG) and schemes of their interaction. ΔG/ΔGm is a relative quantitative measure of the strength of interaction between miRNAs and mRNAs, where ΔGm is equal to the binding energy of the fully complementary miRNA nucleotide sequences. The location of the miRNA binding sites were determined at the 5'-UTR, protein Coding Sequence (CDS), or at the 3'UTR. The search for a target gene for miRNA was also carried out using miRWalk program [22 – 25].

Results and Discussion

14 genes involved in development of atherosclerosis were selected from NCBI database (Table 1).

Table 1 – Genes involved in the development of atherosclerosis

Gene	Full gene name	PMID
TNFRSF9	*TNF* receptor superfamily member 9	25032953
ABCA1	ATP binding cassette subfamily A member 1	25527331
ACE	angiotensin I converting enzyme	18298340
PDE4D	phosphodiesterase 4D	22045424
GCLM	glutamate-cysteine ligase modifier subunit	19126404
NQO1	NAD(P)H quinone dehydrogenase 1	31332605
IL18	interleukin 18	20350254
CD36	CD36 molecule	28691408
ICAM1	intercellular adhesion molecule 1	18420209
ITGB5	integrin subunit beta 5	30131040
IGFBP7	insulin like growth factor binding protein 7	30131040
LDLR	low density lipoprotein receptor	31834409
TGFB1	transforming growth factor beta 1	30942415
MTHFR	methylenetetrahydrofolate reductase	29501539

The binding of mRNA to miRNA and their characteristics are shown in Figure 1. Of the genes involved in the development of atherosclerosis: four genes interact with miRNAs in 5'UTR, six genes interact with miRNAs in CDS, and nine genes interact with miRNAs in 3'UTR. mRNAs of the genes presented in Table 1 are most often associated with the following miRNAs: miR-466, miR-619-5p, miR-1273g-3p, miR-1285-3p, miR-5095, and miR-5096.

Some mRNAs involved in the development of atherosclerosis have been associated with two or more miRNAs. *CD36*, *IL18*, and *ICAM1* genes interact with four miRNAs, *GCLM* – with six miRNAs, *LDLR* and *MTHFR* – with seven miRNAs, and *TNFRSF9* gene – with eight miRNAs. In 5'UTR, mRNA of *ITGB5* gene is associated with five miRNAs. The greatest number of miRNAs-mRNA interactions (14 binding sites) were identified in mRNA of *TGFB1* gene (Figure 1-2).

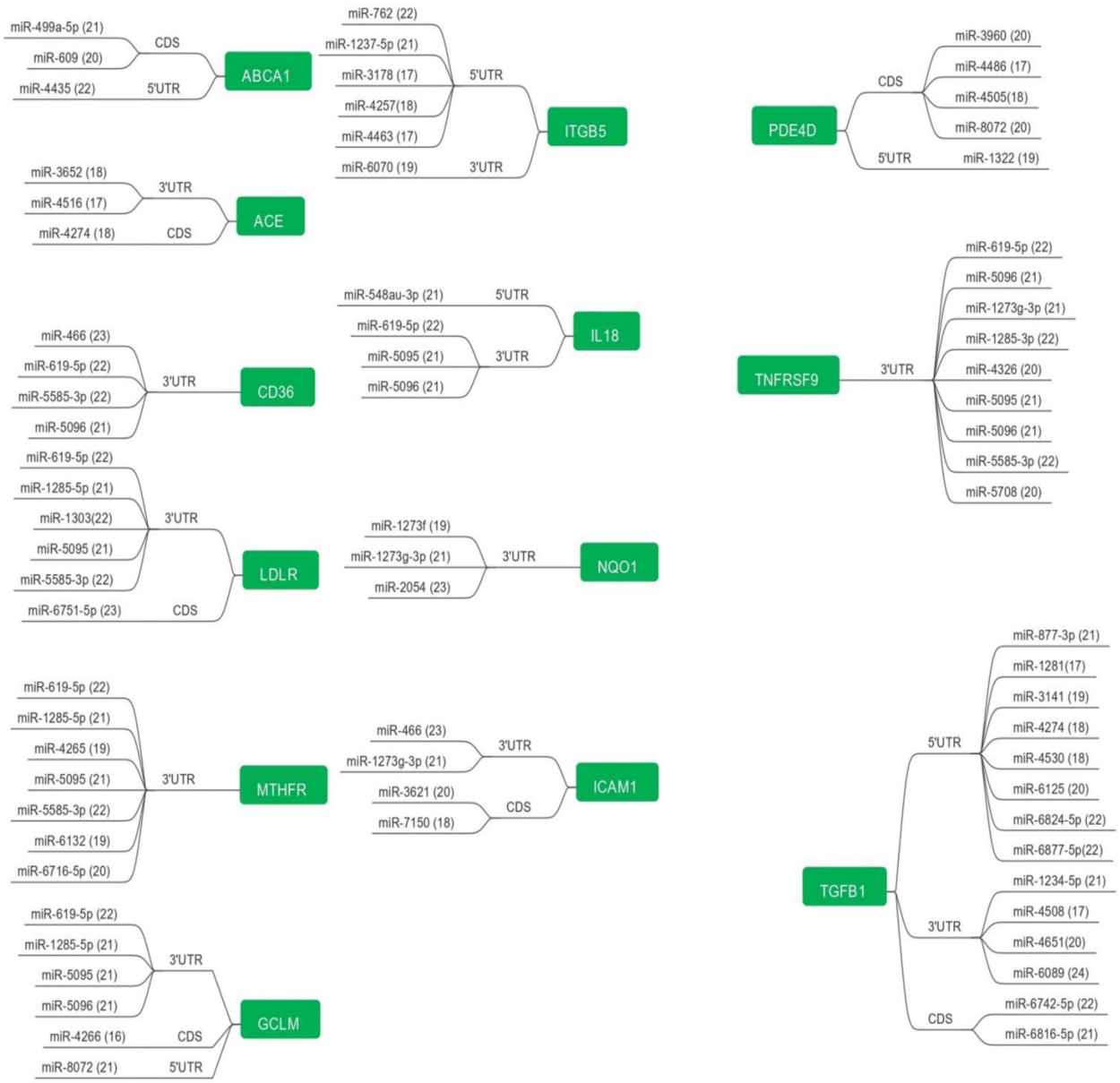

Figure 1 – Interactions of miRNAs and mRNAs of the genes involved in development of atherosclerosis

Most interactions were found predominantly in 3'UTR of mRNAs. The largest number of miRNA bindings are in 3'-untranslated region of the studied mRNAs. *TGFB1* gene was the only that has miRNA binding sites in 5'UTR, CDS, and 3'UTR. The length of miRNAs associated with genes associated with the development of atherosclerosis varied within 17-23 nucleotides. Some miRNA-mRNA interactions was more effective than others as the free energy of binding was high: mRNA of *CD36* and miR-619-5p interacted with the free energy value equal to -121 kJ/mol, *ITGB5* and miR-762 (free energy of interaction -123 kJ/mol), *TGFB1* and miR-6089 (-136 kJ/mol). The average free energy of binding in 5'UTR, CDS and 3'UTR was calculated by dividing the sum of free energy value of all binding sites by the number of these sites and it was around -104 kJ/mol for 5'UTR located binding sites, -101 kJ/mol for CDS located binding sites, and -108 kJ/mol for 3'UTR located binding sites. Some mRNAs have multiple binding sites of miRNAs. The largest number of interactions was found

between miR-619-5p, miR-1273g-3p, miR-1285-5p and *TNFRSF9*, *LDLR*, and *TGFB1 mRNAs*. Notably, these several miRNAs – mRNAs interactions were with full complementarity and therefore with ΔG/ΔGm value equal to 100%. There were such pairs miR-619-5p and *CD36* mRNA, miR-5096 and *IL18* mRNA. The high ΔG/ΔGm ratio that is around 98% was revealed between miR-1273g-3p, miR-619-5p, miR-5096 and mRNAs of *ICAM1*, *IL18*, *NQO1*, *LDLR*, *TNFRSF9*. In addition, high ΔG/ΔGm values were found in genes *GCLM* and *ACE*.

In result of the conducted analysis 14 miRNA binding sites were revealed in mRNA of *TGFB1* gene. As it was established that mRNA of gene *TGFB1* interacted with different miRNAs, it might be associated with the development of atherosclerosis. In addition the prediction of miRNAs interacting with *TGFB1* was performed using miRWalk open-source platform [22-25]. Table 2 shows the identified interactions of mRNA of *TGFB1* with various miRNAs at Score 1,000 in its, 5'UTR, CDS, and 3'UTR.

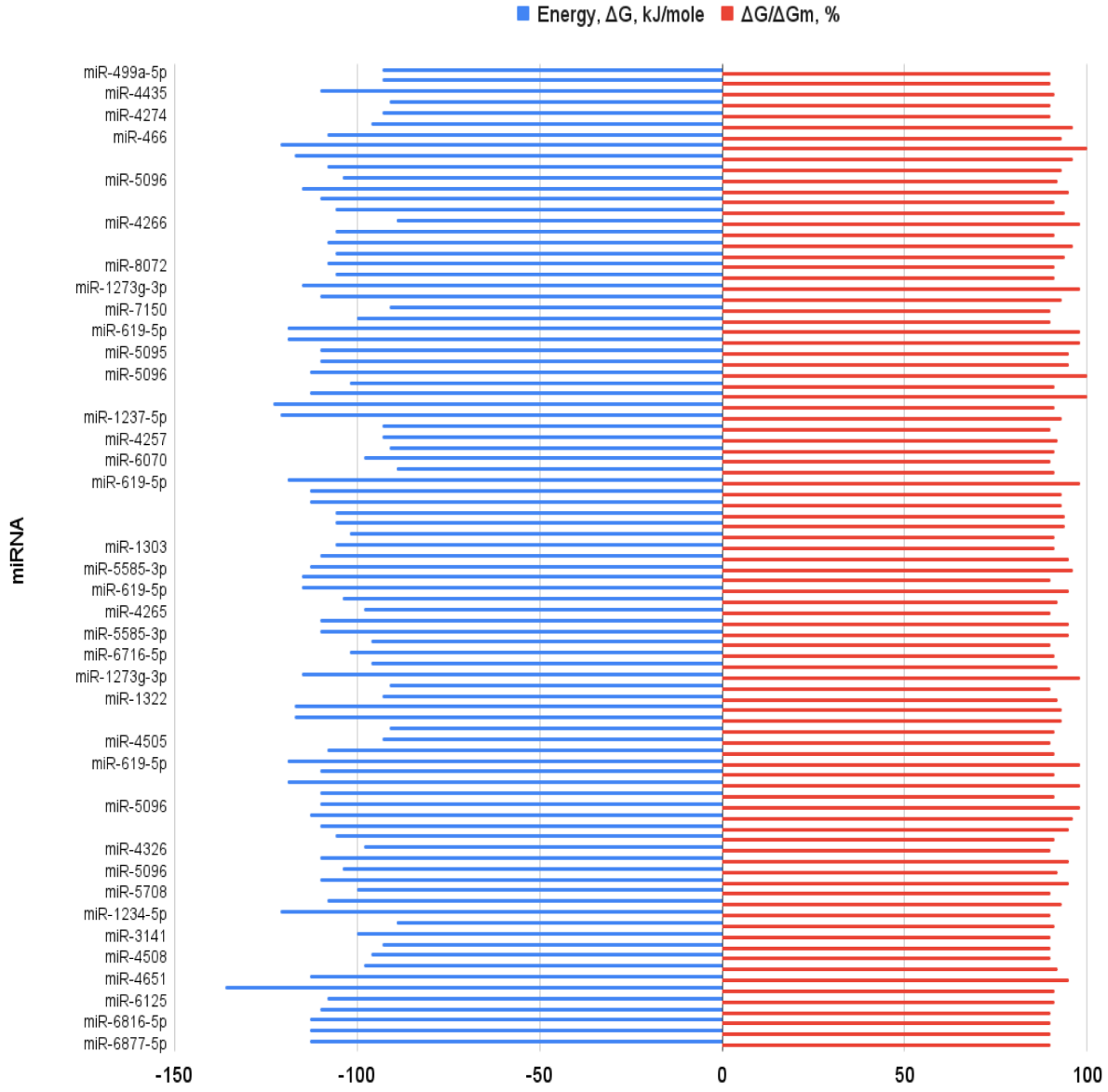

Figure 2 – Characteristics of binding sites of miRNAs and mRNAs of the genes involved in development of atherosclerosis

Table 2 – Interactions of mRNAs with miRNAs, involved in the development of atherosclerosis, obtained using miRWalk

miRNA	Target gene	Site location	Score	Localization	miRTarBase	Target Scan	miRDB
hsa-miR-17-5p	*TGFB1*	2279	1,000	3UTR	MIRT437865		
hsa-miR-106a-5p	*TGFB1*	2279	1,000	3UTR	MIRT734164		
hsa-miR-124-3p	*TGFB1*	1177	0,981	CDS			+
hsa-miR-361-3p	*TGFB1*	2739	1,000	3UTR			+
hsa-miR-609	*TGFB1*	2616	1,000	3UTR			+
hsa-miR-609	*TGFB1*	2465	1,000	3UTR			+
hsa-miR-654-5p	*TGFB1*	1025	1,000	CDS			+
hsa-miR-744-5p	*TGFB1*	1891	1,000	CDS	MIRT037719	+	+
hsa-miR-1827	*TGFB1*	1834	1,000	CDS			+
hsa-miR-3907	*TGFB1*	2648	1,000	3UTR			+
hsa-miR-4472	*TGFB1*	519	1,000	5UTR			+
hsa-miR-4510	*TGFB1*	726	1,000	5UTR			+
hsa-miR-6129	*TGFB1*	2247	1,000	3UTR			+
hsa-miR-6130	*TGFB1*	2220	1,000	3UTR			+
hsa-miR-6856-3p	*TGFB1*	2742	1,000	3UTR			+

Note: miRTarBase is experimentally confirmed miRNA-database of interactions with targets;

TargetScan is a web server that predicts miRNA targets;

miRDB is an online database for miRNA targets prediction;

The score is calculated by running the TarPmiR algorithm to predict miRNA targets based on the random-forest method.

It was also found that the interaction between hsa-miR-6856-3p and *TGFB1* is conservative, which is demonstrated by the Diana Tools platform (Figure 3) [26].

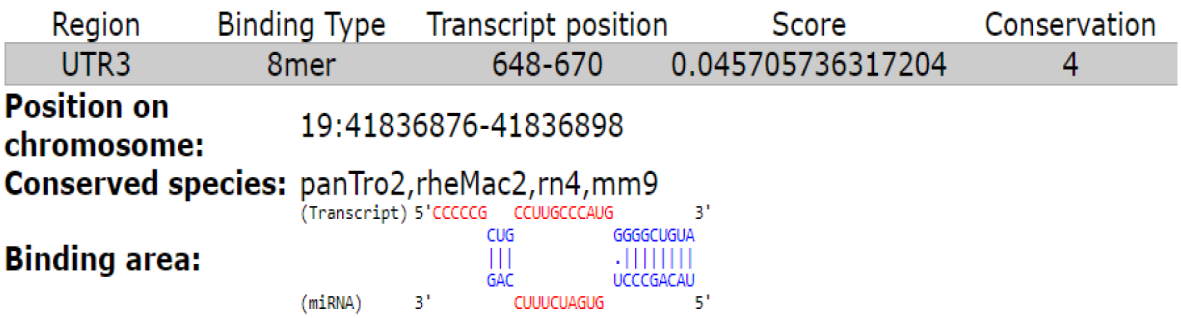

Figure 3 – Characteristics of binding sites of hsa-miR-6856-3p and TGFB1 obtained using program Diana Tools

It should be noted that interactions between *TGFB1* gene mRNA and hsa-miR-17-5p, hsa-miR-106A-5p, hsa-miR-744-5p have been experimentally confirmed. The relationship between *TGFB1* mRNA and hsa-miR-744-5p was demonstrated by miRWalk program, and also confirmed by TargetScan and miRDB programs. Most interactions between *TGFB1* mRNA and miRNAs shown in Table 2 are also determined by miRDB miRNA target prediction database.

Conclusion

The analysis revealed that *TGFB1* gene, which is responsible for the development of atherosclerosis, may become the most effective biomarker, and it was shown that its expression is regulated by various miRNAs. The efficiency of using mRNA of *TGFB1* gene as a regulator of the development of atherosclerosis was also shown using miRWalk, miRTarBase, TargetScan, miRDB and Diana Tools programs.

References

1. Arnett, D. K., Baird, A. E., Barkley, R. A., Basson, C. T., Boerwinkle, E., Ganesh, S. K., Herrington, D. M., Hong, Y., Jaquish, C., McDermott, D. A., O'Donnell, C. J. Relevance of genetics and genomics for prevention and treatment of cardiovascular disease: a scientific statement from the American Heart Association Council on Epidemiology and Prevention, the Stroke Council, and the Functional Genomics and Translational Biology Interdisciplinary Working Group. // Circulation. – 2007. – Vol. 115, No. 22. – Pp. 2878-2901.
2. Hansson G.K., Libby P., Tabas I. Inflammation and plaque vulnerability // Journal of Internal Medicine. – 2015. – Vol. 278. – Pp. 483-493.
3. Libby P, Hansson G.K. Inflammation and immunity in diseases of the arterial tree: players and layers // Circulation Research. – 2015. – Vol. 116. – Pp. 307-311.
4. Vozenilek A.E., Navratil A.R., Green J.M., et al. Macrophage-Associated Lipin-1 Enzymatic Activity Contributes to Modified Low-Density Lipoprotein-Induced Proinflammatory Signaling and Atherosclerosis // Arteriosclerosis, Thrombosis, and Vascular Biology. – 2018. – Vol. 38. – Pp. 324-334.
5. Violi F., Carnevale R., Loffredo L., et al. NADPH Oxidase-2 and Atherothrombosis: Insight from Chronic Granulomatous Disease // Arteriosclerosis, Thrombosis, and Vascular Biology. – 2017. – Vol. 37. – Pp. 218-225.
6. Tabas I. Russell Ross Memorial Lecture in Vascular Biology: Molecular-Cellular Mechanisms in the Progression of Atherosclerosis // Arteriosclerosis, Thrombosis, and Vascular Biology. – 2017. – Vol. 37. – Pp. 183-189.
7. Thum T, Mayr M. Review focus on the role of microRNA in cardiovascular biology and disease // Cardiovascular Research. – 2012. – Vol. 93. – Pp. 543-544.
8. Winter J., Jung S., Keller S., et al. Many roads to maturity: microRNA biogenesis pathways and their regulation // Nature Cell Biology. – 2009. – Vol. 11. – Pp. 228-234.
9. Maitrias P., Metzinger-Le Meuth V., Nader J., et al. The Involvement of miRNA in Carotid-Related Stroke // Arteriosclerosis, Thrombosis, and Vascular Biology. – 2017. – Vol. 37. – Pp. 1608-1617.
10. Zampetaki A., Mayr M. MicroRNAs in vascular and metabolic disease // Circulation Research. – 2012. – Vol. 110. – Pp. 508-522.
11. Yates L., Norbury C., Gilbert R. The long and short of microRNA // Cell. – 2013. – Vol. 153. – Pp. 516-519.
12. Sunderland N., Skroblin P., Barwari T., et al. MicroRNA Biomarkers and Platelet Reactivity: The Clot Thickens // Circulation Research. – 2017. – Vol. 120. – Pp. 418-435.
13. Lima J., Batty J., Sinclair H., Kunadian V. MicroRNAs in Ischemic Heart Disease: From Pathophysiology to Potential Clinical Applications // Cardiology in Review. – 2017. – Vol. 25. – Pp. 117-125.
14. Alexandru N., Badila E., Weiss E., et al. Vascular complications in diabetes: Microparticles and microparticle associated microRNAs as active players // Biochemical and Biophysical Research Communications. – 2016. – Vol. 472. – Pp. 1-10.
15. Reddy M., Das S., Zhuo C., et al. Regulation of Vascular Smooth Muscle Cell Dysfunction under Diabetic Conditions by miR-504 // Arteriosclerosis, Thrombosis, and Vascular Biology. – 2016. – Vol. 36. – Pp. 864-873.
16. Albinsson S., Sward K. Targeting smooth muscle microRNAs for therapeutic benefit in vascular disease // Pharmacological Research. – 2013. – Vol. 75. – Pp. 28-36.
17. Robinson H., Baker A. How do microRNAs affect vascular smooth muscle cell biology? // Current Opinion in Lipidology. – 2012. – Vol. 23. – Pp. 405-411.
18. Hergenreider E., Heydt S., Treguer K., et al. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNA // Nature Cell Biology. – 2012. – Vol. 14. – Pp. 249–256.
19. Ivashchenko A., Berillo O., Pyrkova A., et al. The properties of binding sites of miR-619-5p, miR-5095, miR-5096 and miR-5585-3p in the mRNAs of human genes // BioMed Research International. – 2014. – Vol. 2014. – P. 720715.
20. Sticht C., De La Torre C., Parveen A., Gretz N. miRWalk: An online resource for prediction of microRNA binding sites // PLOS One. – 2018. – Vol. 13, №10.
21. Joshi SR, Comer BS, McLendon JM, Gerthoffer WT. MicroRNA Regulation of Smooth Muscle Phenotype // Mol Cell Pharmacol. – 2012. – №4 (1). – Pp. 1-16.
22. Sticht C, De La Torre C, Parveen A, Gretz N. miRWalk: An online resource for prediction of microRNA binding sites // PLoS One. – 2018. – №3 (10):e0206239.
23. Dweep H, Gretz N, Sticht C. miRWalk database for miRNA-target interactions // Methods Mol Biol. – 2014. – №1182. – Pp. 289-305.
24. Griffiths-Jones, Sam miRBase: microRNA sequences and annotation // Current protocols in bioinformatics. – 2010. – Vol. Chapter 12. – Unit 12.9.1-10.
25. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data // Nucleic Acids Res. – 2011. – №39. – D152-D157.
26. Paraskevopoulou MD, Georgakilas G, Kostoulas N, et al. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows // Nucleic Acids Res. – 2013. – №41 (Web Server issue). – W169-W173.