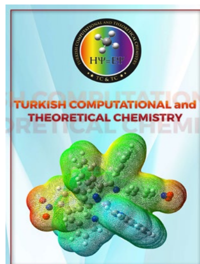

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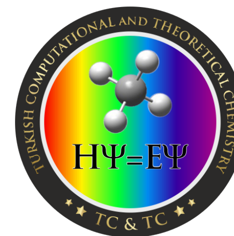

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Design of siRNAs Against Immune-Implicated Atherosclerosis Genes: Computational Study

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Abstract: Atherosclerosis is a chronic, immune-implicated, disease with high numbers of mortality globally. The aim of the current study is to target these genes by specific siRNA utilizing bioinformatics tools. Eight siRNAs were designed via RNAxs from C1QA and ITBG2 gene sequences retrieved from NCBI database. GC% and Tm of siRNAs were calculated through OligoCalc web interface. In addition, hybridization energy of siRNAs with the corresponding target sequences as well as docking to argonaute 2 protein were performed using DuplexFold and HDock. The designed siRNAs exhibited acceptable GC content and Tm values. Besides, the hybridization energy and docking scores were highly significant to block the expression of the mentioned genes. In conclusion, the designed siRNAs are superior candidates for silencing immune-mediated atherosclerotic genes which deserve further consideration.

Keywords: Atherosclerosis, siRNA, gene silencing, RNA interference, docking.

1. Introduction

siRNA (small interfering RNA) is a type of RNA molecule that acts as a gene silencing mechanism. It works by targeting specific mRNA molecules and cleaving them, preventing the production of the corresponding protein. siRNA is typically 20-25 nucleotides in length and is designed to be complementary to a specific target mRNA sequence [1]. siRNA is an important tool in molecular biology and is used in various applications, including therapeutic treatment for diseases, research, and biotechnology. The mechanism of siRNA-mediated gene silencing is a natural defense mechanism in cells against foreign genetic material and is also utilized as a powerful tool for regulating gene expression in a variety of experimental and therapeutic settings [2].

Several genes have been implicated in the pathogenesis of atherosclerosis, including those involved in lipid metabolism, inflammation, and immune response. By targeting these genes, siRNA molecules can regulate their expression and potentially modulate the development and progression of the disease. However, the efficacy of siRNA-based therapies for the treatment of atherosclerosis would depend on several factors, including the specific target gene, the design of the siRNA molecule, the delivery method, and the cellular and physiological context. In vitro and in vivo studies have demonstrated the potential of siRNA-based therapies for the treatment of immune-related diseases, including atherosclerosis [3].

C1qA is a subunit of the complement system C1 complex, which is part of the innate immune system. The complement system is involved in the recognition and removal of pathogens and damaged cells, and it is activated by a series of enzymatic reactions that generate a cascade of proteolytic cleavage products. C1qA is one of six subunits that make up the C1 complex, and it is thought to play a role in the recognition and binding of immune complexes and the initiation of the complement cascade [4]. The role of C1qA in the pathophysiology of disease is not fully understand. Thus, it is important to determine the potential of C1qA-targeted therapies for the treatment of various conditions, including atherosclerosis.

ITGB2 has been implicated in the pathogenesis of several immune-related diseases, including

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atherosclerosis, where it is involved in the recruitment of immune cells to the arterial wall and the regulation of inflammation [5]. Both C1qA and ITGB2 have been implicated in the regulation of immune cell function and the modulation of inflammation, and they are potential targets for the treatment of various immune-related diseases, including atherosclerosis. Therefore, the aim of the current study is to target these genes by specific siRNA utilizing bioinformatics tools.

2. Computational Method

2.1 Workflow methodology

The methodology and the employed webservers adopted in this study were summarized in Fig 1. The detailed methods employed were mentioned in detail below.

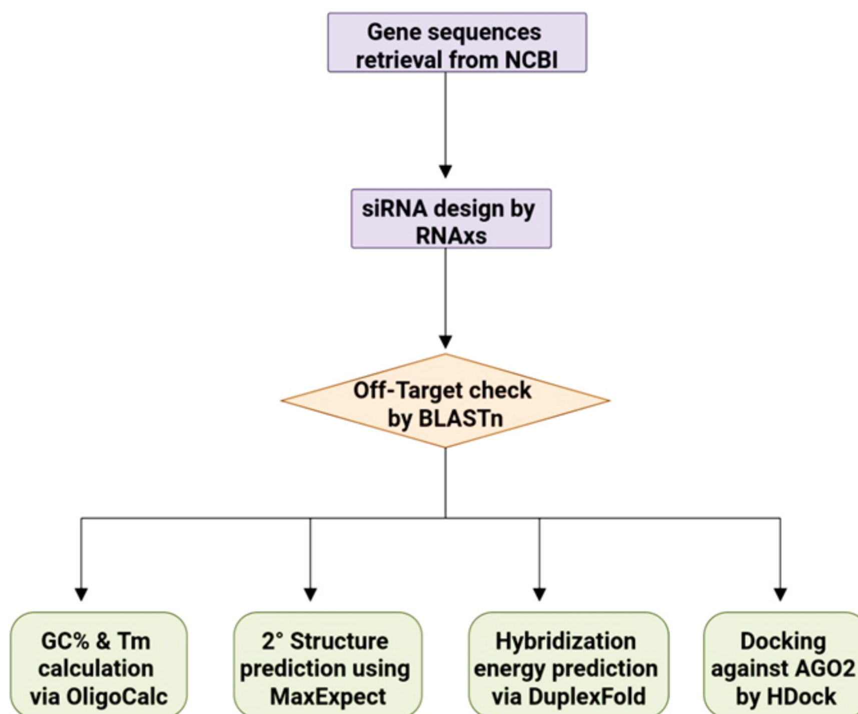

Figure 1 Flowchart of the adopted methodology used in the present study along with the utilized online servers

2.2 Retrieval of genes sequence

The genomic sequence of the two examined genes (ITBG2 and C1QA) was retrieved from national center for biotechnology information (NCBI) (https://www.ncbi.nlm.nih.gov/gene) [6]. The two genes have the accession numbers NM_015991 and NM_000211.4, respectively. Because ITBG2 is a large gene (~ 42 kb), only the first 1600 base was taken for further analysis.

2.3 Target selection and siRNAs design

The retrieved sequences were inputted to RNAxs server [7] for the selection and design of the most appropriate 19-mer siRNA candidates with least off-targets. 10 candidate siRNAs were produced to be assessed as efficient for silencing. The selection criteria of the server were kept as default.

2.4 Off-targets validation

Since 19-mer RNA is almost impossible not to complement other targets in the genomic or transcriptomic sequences, it became a regular routine to test the off-targets of any designed siRNA. The most popular tool is BLASTn [8] (https://blast.ncbi.nlm.nih.gov/Blast.cgi) which finds off-target similarity with human genomic as well as transcriptome excluding XM/XP models and setting E-value threshold at 0.01.

2.5 Calculation of GC content and Tm

The designed siRNAs successfully passed the off-target check, their GC% content and Tm were calculated via the online program OligoCalc [9].

2.6 Prediction of RNA secondary structures

The siRNA secondary structure was predicted using MaxExpect webserver (https://rna.urmc.rochester.edu/RNAstructureWeb/Servers/MaxExpect/MaxExpect.html) [10] keeping default parameters. This tool predicts the best RNA secondary structure with the lowest free energy of folding from 1000 structures.

2.7 Hybridization energy

The binding energy between the target and guide siRNA strand was predicted via DuplexFold server [10], (https://rna.urmc.rochester.edu/RNAstructureWeb/Servers/DuplexFold/DuplexFold.html). This server upon folding 2 sequences together, forms intermolecular bonding giving the confirmation with the least hybrid free energy.

2.8 Molecular docking

The siRNAs with lowest (stronger) complementarity strength of the two genes were subjected to docking against the argonaute 2 protein (AGO2). The receptor (AGO2) 3D-structure was downloaded from PDB (PDB ID#5JS1). The x-ray crystallography had a resolution of 2.50 Å and R-value of 0.247. The PDB file as well as the guide siRNA sequence were co-inputted to HDock platform [11] (http://hdock.phys.hust.edu.cn/) for performing molecular docking using default settings.

3. Results and discussion

3.1 Selection of siRNAs and their GC% and Tm

According the data from table 1, the GC% of the designed siRNAs range from 53 to 58 with a corresponding Tm range 51.1 to 53.2. as GC content increases, Tm follows in increase due to the fact that G is 3 H-bonded with C which necessitates the higher required Tm to break apart those bonds [12]. Conflicting evidences detect a desired range for GC content. Some 31 – 58 [13], others prefer 36 – 52 interval [14]. It is an important criteria as low GC content gives rise to unspecific or weak binding, whilst strands are harder to unwind if GC content was high [15]. Our results fall within the desired range. Notably, as GC% increases, the strength of complementarity increases also (as shown in hybridization energies below).

Table 1 Designed siRNAs from C1QA and ITBG2 genes along with the corresponding GC content and Tm.

Position	Target sequences	Guide siRNA Sequence	GC%	Tm
C1QA				
340	CAGGGAGGGAGGGAAAUAA	UUAUUUCCCUCCCUCCCUG	53	51.1
339	GCAGGGAGGGAGGGAAAUA	UAUUUCCCUCCCUCCCUGC	58	53.2
518	CCUGGCCCCAUAACACAUA	UAUGUGUUAUGGGGCCAGG	53	51.1
2937	GGCUUCCUCAUCUUCCCAU	AUGGGAAGAUGAGGAAGCC	53	51.1
ITBG2				
80	UGGGUGGAGGAAUGAGGUU	AACCUCAUUCCUCCACCCA	53	51.1
278	CCCUCCCUCCUUCCUACAC	AAGAGUCAGGUCAGUGAGG	53	51.1
1496	CCUCACUGACCUGACUCUU	AAGAGUCAGGUCAGUGAGG	53	51.1
1592	CCCUCUCCAUCCUUAGCAU	AUGCUAAGGAUGGAGAGGG	53	51.1

It is important to emphasize that the designed siRNAs exhibit a high degree of specificity towards the targeted messenger RNAs (mRNAs), as demonstrated by the absence of significant off-target effects. This specificity is reflected by the fact that the E-value of the off-targets is higher than the predetermined threshold, indicating a low likelihood of binding to unintended targets. This high level of specificity enhances the reliability and accuracy of the siRNA-mediated gene silencing process and reduces the likelihood of unintended consequences.

3.2 Secondary structure prediction

The predicted secondary structure of the designed siRNAs varied considerably between the two groups (Fig 2) albeit no such big structural difference was found within the same group. The folding energy of the two groups ranged from 1.6 to 1.7. The secondary structure of the guide siRNA strand influences target mRNA and AGO2 binding,

i.e. circular or semi-circular structures tend to have more free energy interfering with siRNA silencing activity. On the other hand, hairpin-like structures are more likely to strongly bind targets [16]. Moreover, siRNA with hairpin-like structure (siRNA 3 and siRNA 4 of ITBG2) correlates with higher docking score as well.

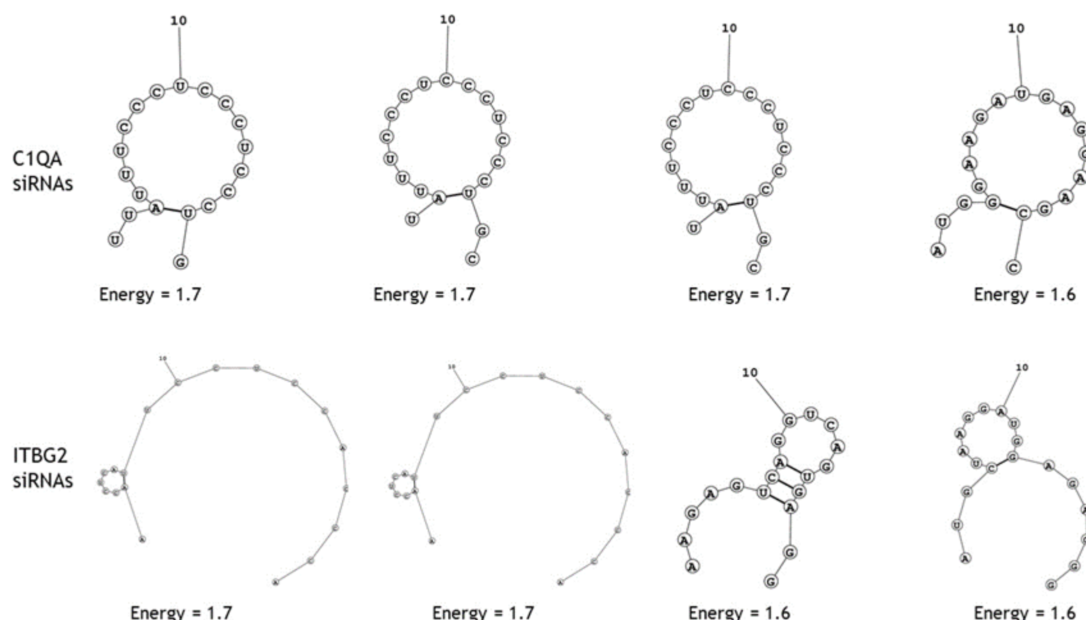

Figure 1 Secondary structures of designed siRNAs plus their folding energy

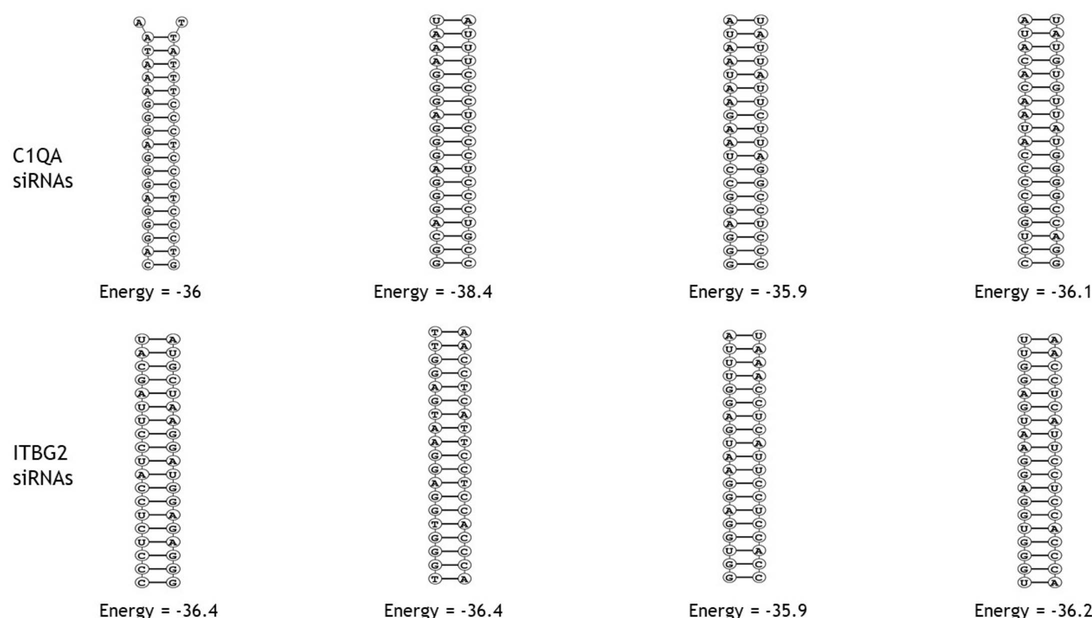

Figure 3 Obtained hybridization energy of designed siRNAs and their target sequences.

3.3 Hybridization energy

The hybridization energy that determines the strength of complementarity between target sequence and guide siRNA strand revealed high values (Fig 1). This confirms the suitability of siRNAs selection (based on the range of hybridization energy) and, at the same time, suggests the high possibility of being superior candidates for silencing C1QA and ITBG2 genes. Best of which was siRNA 2 of C1QA gene and siRNA 2 in ITBG2 gene with a hybridization energy in the range -36 to -38 kcal/mol.

3.4 Molecular docking

The docking profile of the designed siRNAs were summarized in Table 2. The docking score indicates high binding energy between designed siRNAs and AGO2 protein. Consequently, this gives them superiority regarding the likelihood of being potent siRNAs as this binding is the first stage of gene silencing prior to hybridization to the corresponding mRNA of the target gene. siRNA 1 of C1QA gave the highest free energy of binding while siRNA 3 of ITBG2 was the best with docking scores -352.88 and -334.5 respectively.

The 3D illustration of the interaction between siRNAs and AGO2 protein is depicted in Fig 3. It is obvious the typical fitness of the designed siRNAs toward the binding pocket of AGO2 protein except for siRNA 4 of C1QA. The RNA-binding motif lies at the interface of protein subunits. siRNA 1 of C1QA exhibited the highest binding energy (-352.88 kcal/mole) compared to the rest of others. This is evidenced by the typical form of binding, i.e. most of the siRNA got bound to the active pocket of AGO2 (wide-range binding) shown in Fig 4.

It should be mentioned that as GC content increases, hybridization energy increases and docking score gets higher as well. Taking siRNA 2 as an example, GC content, hybridization energy and docking score were 58%, -38.4 and -324.3 kcal/mole.

Overall, the tested siRNAs suggested their significant potential as silencers of C1QA and ITBG2 immune genes that are involved in the initiation as well as progression of atherosclerosis. The silencing of two specific genes through the use of designed small interfering RNAs (siRNAs) presents a novel approach to combating the involvement of the immune system in the development of atherosclerosis. This innovative technique effectively blocks chronic inflammation, the root cause of the disease, and ultimately leads to the reversal of its progression. The targeted suppression of these genes through siRNAs holds great promise for providing a more effective means of treating this debilitating disease [17,18].

Table 2. Molecular docking scores of selected siRNAs in addition to AGO2 interacting residues at the interface.

siRNA	Docking score	Receptor interface residues*
C1QA		
1	-352.88	Ser 672, Gln 228, Pro 120, Gln 160, Arg 280, Arg 69
2	-314.3	Cys 352, Gln 633, Gln 708, His 681
3	-297.4	Arg 635, Phe 156, Gln 160, Gln 675, Gly 674, Arg 761, Ile 353, Ser 672, Glu 673, Asn 99, Arg 351
4	-291.5	Gln 588, Arg 663, Arg 773, Thr 662, Gln 589
ITBG2		
1	-315.7	Gln 473, Val 598, Arg 710, Glu 637, Lys 726, His 600, Tyr 790, Lys 476, Arg 792
2	-315.72	Gln 473, Val 598, Arg 710, Glu 637, Lys 726, His 600, Tyr 790, Lys 476, Arg 792
3	-334.5	Asp 358, Thr 559, His 807, Tyr 804, Lys 525, Arg 710, Tyr 815
4	-333.9	Arg 710, Ser 672, Lys 263, Gln 678, Arg 351, Thr 222, Glu 333, His 600, Glu 637

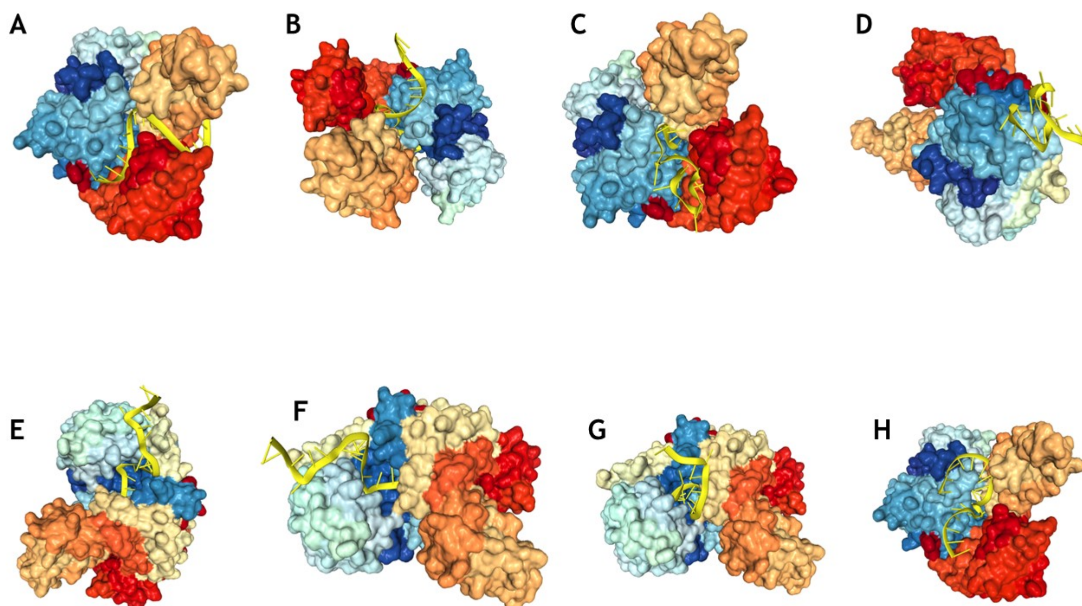

Figure 4. 3D visualization of interaction between selected C1QA siRNAs (A through D), ITBG2 siRNAs (E thgrough H) and AGO2. AGO2 protein is colored in rainbow while siRNAs are shown in yellow.

4. Conclusions

The current study focuses on the development and assessment of the effectiveness of small interfering RNAs (siRNAs) directed against two genes, C1QA and ITBG2, that play a role in immune-mediated atherosclerosis. The researchers designed 8 different siRNAs, each of which showed a strong affinity to its respective target sequence, indicated by high hybridization energy. Furthermore, these siRNAs had a high docking score to the AGO2 protein, which suggests that they could effectively silence the targeted genes.The results of the evaluation suggest that the selected siRNAs could potentially be used to suppress the role of the immune system in the progression of atherosclerosis. However, to fully establish their efficacy, further validation is necessary through experiments in both in vitro (test tube) and in vivo (live organism) settings.

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References

[1] Dorsett Y, Tuschl T. siRNAs: applications in functional genomics and potential as therapeutics. Nat Rev Drug Discov 2004;3:318–29. https://doi.org/10.1038/nrd1345.

[2] Friedrich M, Aigner A. Therapeutic siRNA: State-of-the-Art and Future Perspectives. BioDrugs 2022;36:549–71. https://doi.org/10.1007/s40259-022-00549-3.

[3] Stephan MT. Empowering patients from within: Emerging nanomedicines for in vivo immune cell reprogramming. Seminars in Immunology 2021;56:101537. https://doi.org/10.1016/j.smim.2021.101537.

[4] Yang H, Che D, Gu Y, Cao D. Prognostic and immune-related value of complement C1Q (C1QA, C1QB, and C1QC) in skin cutaneous melanoma. Front Genet 2022;13:940306. https://doi.org/10.3389/fgene.2022.940306.

[5] Zu L, He J, Zhou N, Zeng J, Zhu Y, Tang Q, et al. The Profile and Clinical Significance of ITGB2 Expression in Non-Small-Cell Lung Cancer. Journal of Clinical Medicine 2022;11:6421. https://doi.org/10.3390/jcm11216421.

[6] Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt KD, et al. GenBank. Nucleic Acids Research 2018;46:D41–7.

[7] Tafer H, Ameres SL, Obernosterer G, Gebeshuber CA, Schroeder R, Martinez J, et al. The impact of target site accessibility on

the design of effective siRNAs. Nature Biotechnology 2008;26:578–83.

[8] Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. BMC Bioinformatics 2009;10:1–9.

[9] Kibbe WA. OligoCalc: an online oligonucleotide properties calculator. Nucleic Acids Research 2007;35:W43–6.

[10] Bellaousov S, Reuter JS, Seetin MG, Mathews DH. RNAstructure: web servers for RNA secondary structure prediction and analysis. Nucleic Acids Research 2013;41:W471–4.

[11] Yan Y, Zhang D, Zhou P, Li B, Huang S-Y. HDOCK: a web server for protein–protein and protein–DNA/RNA docking based on a hybrid strategy. Nucleic Acids Research 2017;45:W365–73.

[12] Liu F, Tøstesen E, Sundet JK, Jenssen T-K, Bock C, Jerstad GI, et al. The Human Genomic Melting Map. PLoS Comput Biol 2007;3:e93. https://doi.org/10.1371/journal.pcbi.0030093.

[13] Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A. Rational siRNA design for RNA interference. Nature Biotechnology 2004;22:326–30.

[14] Amarzguioui M, Prydz H. An algorithm for selection of functional siRNA sequences. Biochemical and Biophysical Research Communications 2004;316:1050–8.

[15] Ishizuka A, Siomi MC, Siomi H. A Drosophila fragile X protein interacts with components of RNAi and ribosomal proteins. Genes & Development 2002;16:2497–508.

[16] Sun Y, Gui J, Chen Z. The Influence of RNA Secondary Structure on Efficiency of siRNA Silencing. In: Long M, editor. World Congress on Medical Physics and Biomedical Engineering May 26-31, 2012, Beijing, China, Berlin, Heidelberg: Springer; 2013, p. 398–401. https://doi.org/10.1007/978-3-642-29305-4_106.

[17] Xu J, Chen C, Yang Y. Identification and Validation of Candidate Gene Module Along With Immune Cells Infiltration Patterns in Atherosclerosis Progression to Plaque Rupture via Transcriptome Analysis. Frontiers in Cardiovascular Medicine 2022;9. https://doi.org/10.3389/fcvm.2022.894879.

[18] Soehnlein O, Libby P. Targeting inflammation in atherosclerosis — from experimental insights to the clinic. Nat Rev Drug Discov 2021;20:589–610. https://doi.org/10.1038/s41573-021-00198-1.