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miR-155–5p predictive role to decelerate foam cell atherosclerosis through CD36, VAV3, and SOCS1 pathway

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ABSTRACT

MicroRNAs (miRNAs) are noncoding RNA molecules that play a significant role in atherosclerosis pathogenesis through post-transcriptional regulation. In the present work, a bioinformatic analysis using TargetScan and miRdB databases was performed to identify the miRNAs targeting three genes involved in foam cell atherosclerosis (CD36, Vav3, and SOCS1). A total number of three hundred and sixty-seven miRNAs were recognized and only miR-155–5p was selected for further evaluation based on Venn analysis. Another objective of this study was to evaluate the biological process and regulatory network of miR-155–5p associated with foam cell atherosclerosis using DIANA, DAVID, Cytoscape, and STRING tools. Additionally, the comprehensive literature review was performed to prove the miR-155–5p function in foam cell atherosclerosis. miR-155–5p might be related with ox-LDL uptake and endocytosis in macrophage cell by targeting CD36 and Vav3 genes which was showed from the KEGG pathways hsa04979, hsa04666, hsa04145 H, hsa04810, and GO:0099632, GO:0060100, GO:0010743, GO:001745. Furthermore, miR-155–5p was also predicted to increase the cholesterol efflux from macrophage by inhibit SOCS1 expression based on KEGG pathway hsa04120. Eleven original studies were included in the review and strongly suggest the role of miR-155–5p in foam cell atherosclerosis inhibition.

1. Introduction

Atherosclerosis is the etiology of coronary heart, cerebrovascular, and peripheral arterial diseases [1–4]. The foam cell, a lipid-loaded macrophage, determines early phase of atherosclerosis, induces a chronic inflammation state, and drives atherogenesis into an advanced phase. The continuous uptake of oxidized LDL (ox-LDL) by scavenger receptors and the decrease of cholesterol efflux by macrophages are two factors that contribute to foam cell formation [5,6].

Remarkable studies demonstrated that Cluster Differentiation 36 (CD36) accounts for a large proportion of foam cell formation by promoting the 50% uptake of ox-LDL. This receptor has two transmembrane domains located near the N and C termini, leaving only short cytoplasmic tails at each end. Despite having small intracellular domains, the involvement of CD36 through its related cognate ligand triggers a reaction that leads to the internalization of the resulting complex [7–12].

The precise mechanism of ox-LDL internalization after binding to CD36 is mediated by Vav kinase, which primarily act as guanine nucleotide exchange factors (GEF) for the Rho/Rac/Cdc42 family of small GTPases. A previous study reported that this protein is upregulated in the Src family kinase manner by activating the Fyn, Lyn, and subsequently Phospholipase C-γ1 (PLCγ1) expression. Furthermore, dynamin-2 is activated due to an increase of calcium influx. Hence, the endocytic vesicle size increase, membrane fission accelerates, and the ox-LDL endocytosis will increase. There are three structurally and functionally related members of the Vav kinase family. The Vav1 is expressed exclusively in hematopoietic cells, whereas Vav2 and Vav3 are found ubiquitously in many cells [13–15]. Moreover, cholesterol accumulation in macrophages stimulates not only ABCA1-PPARγ

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Table 1
Profile of miRNAs targeting CD36, Vav3, and SOCS1.

CD36			Vav3			SOCS1	
TargetScan 7.2		miRdB	TargetScan 7.2		miRdB	TargetScan 7.2	miRdB
miR-203a-3p.1	miR-330–3p.2	miR-203a-3p	miR-9-5p	miR-186–5p	miR-30–5p	miR-30–5p	miR-155–5p
miR-148–3p/152–3p	miR-670–3p	miR-148–3p	let-7-5p/98–5p	miR-374–5p	miR-19–3p	miR-19–3p	miR-142–5p
miR-146–5p	miR-376c-3p	miR-152–3p	miR-133a-3p.1	miR-154–5p	miR-142–5p	miR-142–5p	miR-193–3p
miR-21–5p/590–5p	miR-875–5p	miR-146–5p	miR-30–5p	miR-411–3p	let-7-5p/98–5p	miR-221–3p/222–3p	miR-23–3p
miR-375	miR-325–3p	miR-21–5p	miR-128–3p	miR-760	miR-221–3p/222–3p	miR-155–5p	miR-302–3p
miR-140–3p.2	miR-876–5p	miR-590–5p	miR-27–3p	miR-875–5p	miR-155–5p	miR-29–3p	miR-372–3p
miR-141–3p/200a-3p	miR-374–5p	miR-142–5p	miR-125–5p	miR-543	miR-29–3p	miR-324–5p	miR-373–3p
miR-128–3p	miR-382–3p	miR-375	miR-103–3p/107	miR-493–5p	miR-324–5p	miR-331–3p	miR-520–3p
miR-203a-3p.2	miR-224–5p	miR-140–3p.2	miR-208–3p	miR-873–5p.1	miR-331–3p	miR-665	miR-199–5p
miR-204–5p/211–5p	miR-339–5p	miR-140–3p.1	miR-499a-5p	miR-495–3p	miR-665	miR-149–5p	miR-34–5p
miR-205–5p	miR-377–3p	miR-200a-3p	miR-218–5p	miR-381–3p	miR-149–5p	miR-582–5p	miR-449–5p
miR-17–5p/20–5p/93–5p/106–5p/519–3p	miR-655–3p	miR-128–3p	miR-223–3p	miR-325–3p	miR-582–5p	miR-411–3p	miR-204–5p
miR-153–3p	miR-532–3p	miR-203a-3p	miR-129–3p	miR-5000–3p	miR-411–3p	miR-495–3p	miR-211–5p
miR-150–5p	miR-411–3p	miR-204–5p	miR-31–5p	miR-524–5p	miR-495–3p	miR-335–5p	miR-202–5p
miR-143–3p	miR-873–5p.1	miR-211–5p	miR-203a-3p.2	miR-766–5p	miR-335–5p	miR-556–3p	miR-140–3p.1
miR-194–5p	miR-382–5p	miR-205–5p	miR-143–3p	miR-1301–3p	miR-556–3p	miR-3179	miR-122–5p
miR-223–3p	miR-1193	miR-17–5p	miR-203a-3p.1	miR-4739	miR-3179	miR-3163	miR-217
miR-124–3p.1	miR-186–5p	miR-20–5p	miR-142–3p.2	miR-522–3p	miR-3163	miR-6720–5p	miR-216a-5p
miR-221–3p/222–3p	miR-411–5p.2	miR-93–5p	miR-338–3p	miR-3622b-5p	miR-6720–5p	miR-361–3p	miR-455–5p
miR-129–5p	miR-433–3p	miR-106–5p	miR-489–3p	miR-1908–5p	miR-361–3p	miR-380–3p	miR-214–5p
miR-122–5p	miR-486–5p	miR-143–3p	miR-155–5p	miR-3605–5p	miR-380–3p	miR-4640–3p	miR-147b
miR-217	miR-340–5p	miR-194–5p	miR-221–3p/222–3p	miR-624–5p	miR-4640–3p		
miR-216a-5p	miR-544a-5p	miR-223–3p	miR-145–5p	miR-514a-5p			
miR-455–3p.2	miR-335–5p	miR-519–3p	miR-182–5p	miR-450b-5p			
miR-455–5p	miR-410–3p	miR-153–3p	miR-142–5p	miR-5010–5p			
miR-155–5p	miR-495–3p	miR-150–5p	miR-193–3p	miR-4428			
miR-218–5p	miR-362–5p/500b-5p	miR-124–3p	miR-23–3p	miR-3173–5p			
miR-425–5p	miR-496.2	miR-221–3p	miR-302–3p/372–3p/373–3p/520–3p	miR-4766–3p			
miR-216b-5p	miR-299–5p	miR-222–3p	miR-199–5p	miR-374b-3p			
miR-302–3p/372–3p/373–3p/520–3p	miR-411–5p.1	miR-129–5p	miR-34–5p/449–5p	miR-3611			
miR-873–5p.2	miR-323–3p	miR-122–5p	miR-204–5p/211–5p	miR-3200–5p			
miR-493–3p	miR-154–3p/487–3p	miR-217	miR-202–5p	miR-889–3p			
miR-539–3p	miR-2115–3p	miR-216a-5p	miR-140–3p.1	miR-524–3p/525–3p			
miR-376–3p	miR-545–3p	miR-455–3p.2	miR-122–5p	miR-361–3p			
miR-329–3p/362–3p	miR-588	miR-455–5p	miR-217	miR-515–5p/519e-5p			
miR-219a-2-3p	miR-3605–5p	miR-155–5p	miR-216a-5p	miR-520g-3p			
miR-452–5p/892–3p	miR-3918	miR-218–5p	miR-455–5p	miR-2681–3p			
miR-494–3p	miR-3144–3p	miR-425–5p	miR-214–5p	miR-147a			
miR-448	miR-1301–3p	miR-216b-5p	miR-147b	miR-942–5p			
miR-379–5p	miR-3924	miR-302–3p	miR-7-5p	miR-1269			
miR-653–5p	miR-3146	miR-372–3p	miR-423–5p	miR-3194–5p			
miR-361–5p	miR-370–3p	miR-373–3p	miR-652–3p	miR-4766–5p			
miR-656–3p	miR-676–3p	miR-520–3p	miR-188–5p	miR-3612			
miR-1269	miR-450b-5p		miR-299–3p	miR-4731–5p			
miR-520g-3p	miR-2355–3p		miR-326	miR-1287–5p			
miR-599	miR-5579–3p		miR-1197	miR-5687			
miR-888–5p	miR-576–5p		miR-874–3p	miR-580–3p			
miR-526b-5p	miR-1286		miR-493–3p	miR-3140–3p			
miR-892–5p	miR-1179		miR-378–3p	miR-642a-5p			
miR-577	miR-525–5p		miR-744–5p	miR-6509–3p			
miR-144–5p	miR-642–3p		miR-369–3p	miR-3144–3p			
miR-3690	miR-5094		miR-655–3p	miR-345–3p			
miR-4428	miR-651–5p		miR-382–3p	miR-500a-5p			
miR-580–3p	miR-374a-3p		miR-330–3p.2	miR-3163			
miR-944	miR-1185–5p		miR-28–3p	miR-378g			
miR-3187–3p	miR-934		miR-758–3p	miR-770–5p			
miR-2355–5p	miR-552–3p		miR-340–5p	miR-3127–5p			
miR-3200–5p	miR-4761–3p		miR-496.2	miR-641/3617–5p			
miR-1287–5p	miR-873–3p		miR-382–5p	miR-105–5p			
miR-3121–3p	miR-514a-5p		miR-485–5p	miR-500a-3p			
miR-2278	miR-513b-5p		miR-3194–3p	miR-513b-5p			
miR-3163	miR-889–3p		miR-892–5p	miR-380–3p			
miR-1277–5p	miR-641/3617–5p		miR-670–5p	miR-2467–3p			
miR-1323	miR-512–3p		miR-576–5p	miR-323b-3p			
miR-4640–5p	miR-3622b-5p		miR-1180–5p	miR-525–5p			
miR-432–5p	miR-4766–5p		miR-671–5p	miR-498			
miR-3942–5p	miR-197–3p		miR-1277–5p	miR-561–5p			
miR-4731–5p			miR-579–3p	miR-628–5p			
				miR-1294			
				miR-324–3p/1913			

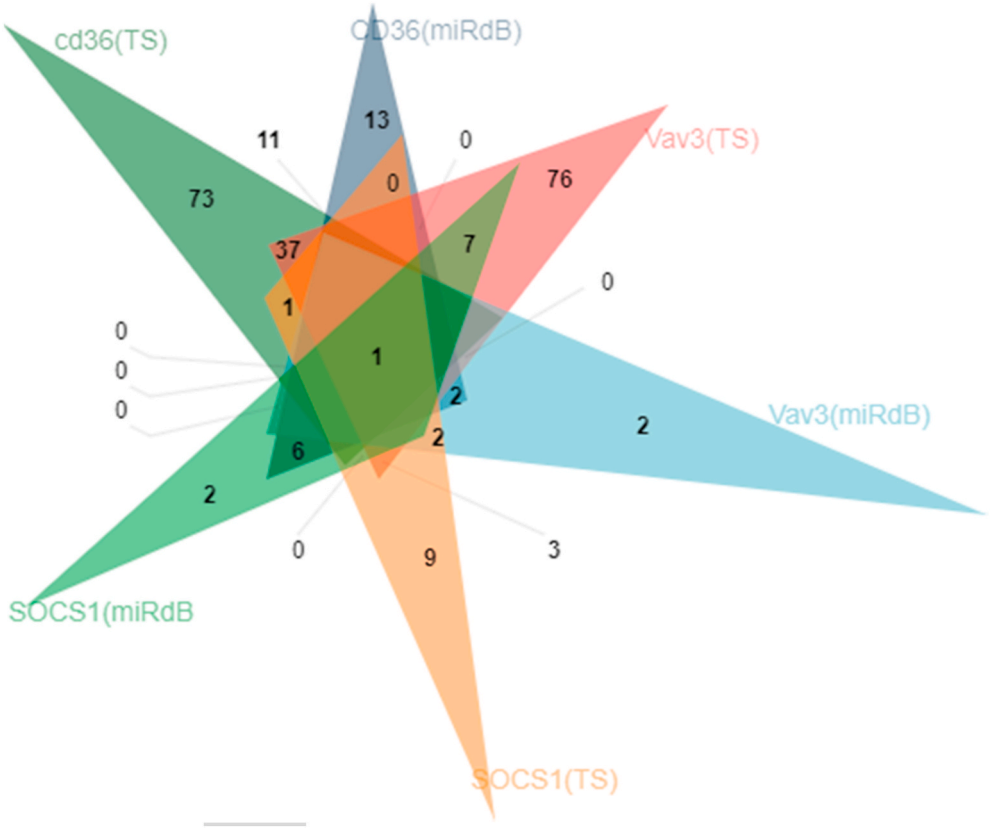

Fig. 1. The Venn analysis of miRNAs targeting 3′UTR of CD36, VAV3, and SOCS1 genes. Different shape and color represented the list of miRNAs targeting each gene. There was one miRNA shared by three genes which showed by shape overlaps.

dependent expression but also the reticulum-endoplasmic stress and inflammation process [16]. The Suppressor of Cytokine Signaling 1 (SOCS1), an inflammatory transcription factor, play as an E3 ligase that degrade ATP-binding cassette transporter 1 (ABCA1), which is the main receptor for the efflux of cholesterol [17–19].

Complex genomic interaction in macrophages regulate foam cell formation in a positive or negative manner and act in several stages either transcriptionally, post-transcriptionally, or post-translationally. The microRNAs (miRNAs) are small non-coding transcript RNA with a length of 21–25 nucleotides and functions in post-transcriptional

Table 2
Profile of miR-155–5p seed sequences binding with 3′UTR target genes.

Gene	Position	Sequences	Site type	Context++ Score	Context++ Score percentile	Weighted context ++ score	Conserved branch length	PCT
CD36	480–487 miR-155–5p	5’ ... UCAGAAUGCUUUUCUAGCAUUAA ||||||| 3′ UGGGGAUAGUGCUAAUCGUAAUU	8mer	−0.30	95	−0.30	0.313	<0.1
	928–934 miR-155–5p	5’…UUCACUUAUUCUGAGAGCAUUAG ||||||| 3′ UGGGGAUAGUGCUAAUCGUAAUU	7mer-m8	−0.02	26	0.00	0.237	<0.1
	1103–1109 miR-155–5p	5’…CCAGAGUAAAUGUUGAGCAUUAC ||||||| 3′ UGGGGAUAGUGCUAAUCGUAAUU	7mer-m8	−0.02	26	0.00	0.062	<0.1
	2707–2713 miR-155–5p	5’…CCUGCAUAUACCAAUAGCAUUAC ||||||| 3′ UGGGGAUAGUGCUAAUCGUAAUU	7mer-m8	−0.09	68	0.00	0.134	<0.1
VAV3	1357–1364 miR-155–5p	5’…UUGGGAAAAAAAGAAAGCAUUAA ||||||| 3′ UGGGGAUAGUGCUAAUCGUAAUU	8mer	−0.39	98	−0.39	2.442	<0.1
	1385–1392 miR-155–5p	5’…UAGAACUGAACCAGGAGCAUUAA ||||||| 3′ UGGGGAUAGUGCUAAUCGUAAUU	8mer	−0.25	93	−0.25	0.052	<0.1
SOCS1	24–31 miR-155–5p	5’… GCCCCGCCGUGCACGCAGCAUUAA ||||||| 3′ UGGGGAUAGUGCUAAUCGUAAUU	8mer	−0.33	97	0.33	3.65	<0.1

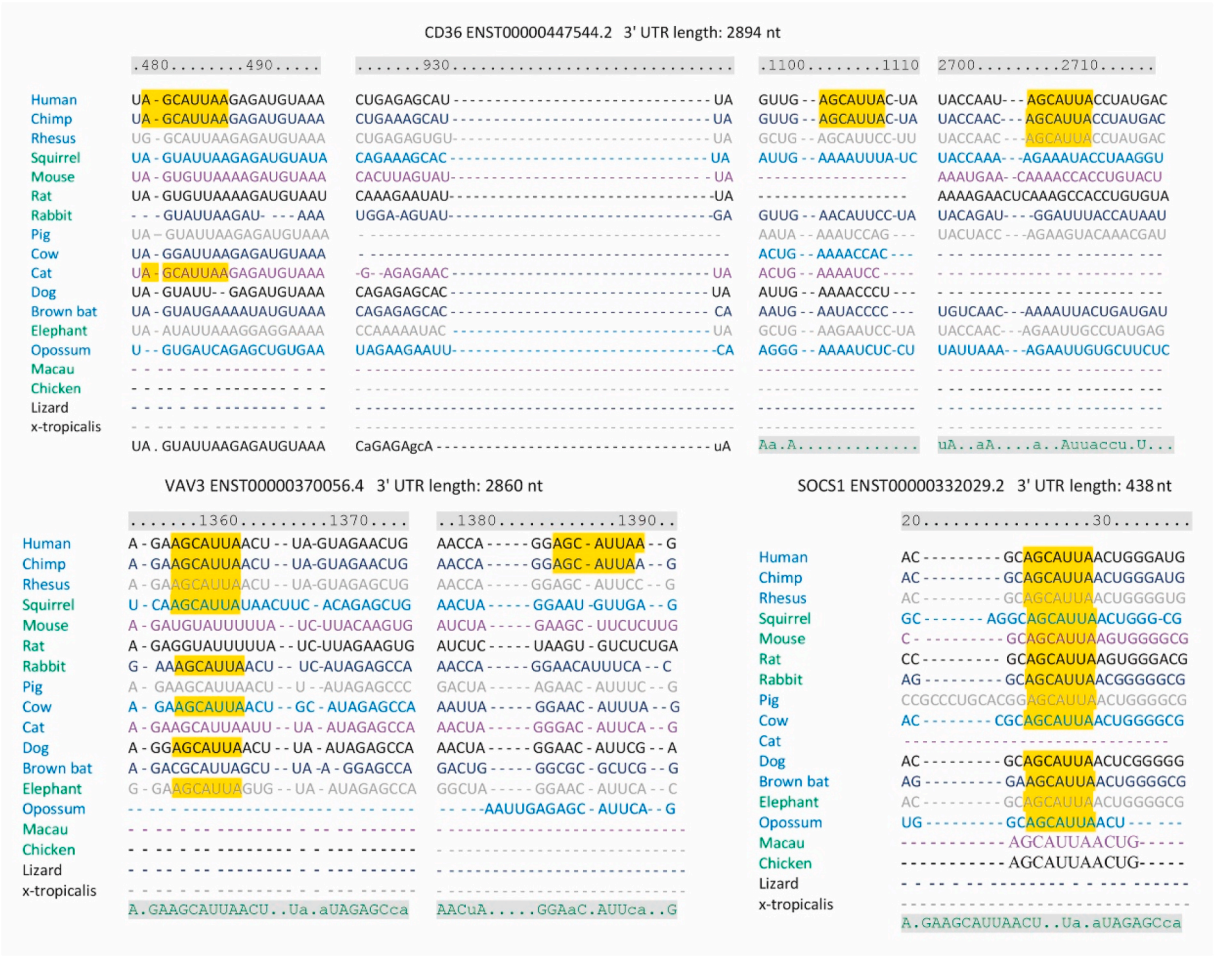

Fig. 2. The conserved sites for miR-155–5p binding in 3′UTR of CD36, Vav3, and SOCS1 in different species. The yellow color indicated the similar 3′UTR sequences among species.

regulation of gene expression by translational repressor or by promoting the degradation of mRNA. Many miRNAs identified in human diseases include atherosclerosis-based disease. Nevertheless, there are lack of studies has proven the role of miRNAs in foam cell formation. Therefore, exploring specific miRNAs targeting the important molecule as the key player of atherogenesis has a potential role for the invention of new atherosclerosis' therapeutic agents [20].

The aim of this study was to identify the miRNAs targeting 3′Untranslated Region (UTR) of CD36, Vav3, and SOCS1 using open database tools. Furthermore, the evaluation of mature sequence, physiological binding, and also conserved sites among different species were also presented. The other goal of this study was to investigate the biological process or molecular function of selected miRNA and its target genes which might be related to foam cell atherosclerosis. Additionally, the current literatures regarding the underlying function of miR-155–5p in foam cell formation were reviewed.

2. Results

2.1. Identification of miRNAs targeting CD36, Vav3, and SOCS1

CD36, VAV3, and SOCS1 are proatherogenic proteins due to their action in macrophage cell. Therefore, identifying molecules that degrade these mRNAs is one important strategy to reduce the foam cell formation. An analysis using TargetScan 7.2 (http://www.targetscan.org/) showed that one hundred and thirty-five, one hundred and thirty-eight, and twenty-two miRNAs targeting 3′UTR of CD36, Vav3, and SOCS1, respectively (21). Based on miRdB analysis (http://mirdb.org/), forty-two, nine, and twenty-one miRNAs targeting 3′UTR of CD36, Vav3, and SOCS1, respectively (Table 1) [22,23]. Interestingly, Venn diagram analysis (http://jvenn.toulouse.inra.fr/app/example.html) demonstrated only one miRNA targeting three genes simultaneously (Fig. 1) [24].

2.2. Profile of common predicted miRNA

From the previous step, miR-155–5p was selected for further analysis. The gene transcribed miR-155–5p was MIR155 (NCBI Gene ID 406947) and located in chr21:25573980–25574044 (+). miR-155–5p mature sequences was 5′-UUAAUGCUAAUCGUGAUAGGGGUU-3' (length = 24). Understanding the interaction between miR-155–5p seed sequences with 3′UTR of CD36, Vav3, and SOCS1 was crucial to make a prediction of miRNA stability and function. The interaction of this miRNA with 3′UTR of the genes were predicted by bioinformatic analysis in TargetScan 7.2 presented with context ++ score, conserved branch lengths, and PCT value (Table 2). From the data, it was concluded that seed sequences of miR-155–5p was UCGUAAU.

The conservation analysis may provide evidence that a predicted miRNA target is functional and indicates that the sequences have been maintained by natural selection. The conserved sequences sites among eighteen species were depicted in Fig. 2. There was one conserved site for miR-155–5p interaction with Vav3 and SOCS1, four poorly conserved site for CD36, and one poorly conserved site for Vav3 among vertebrates.

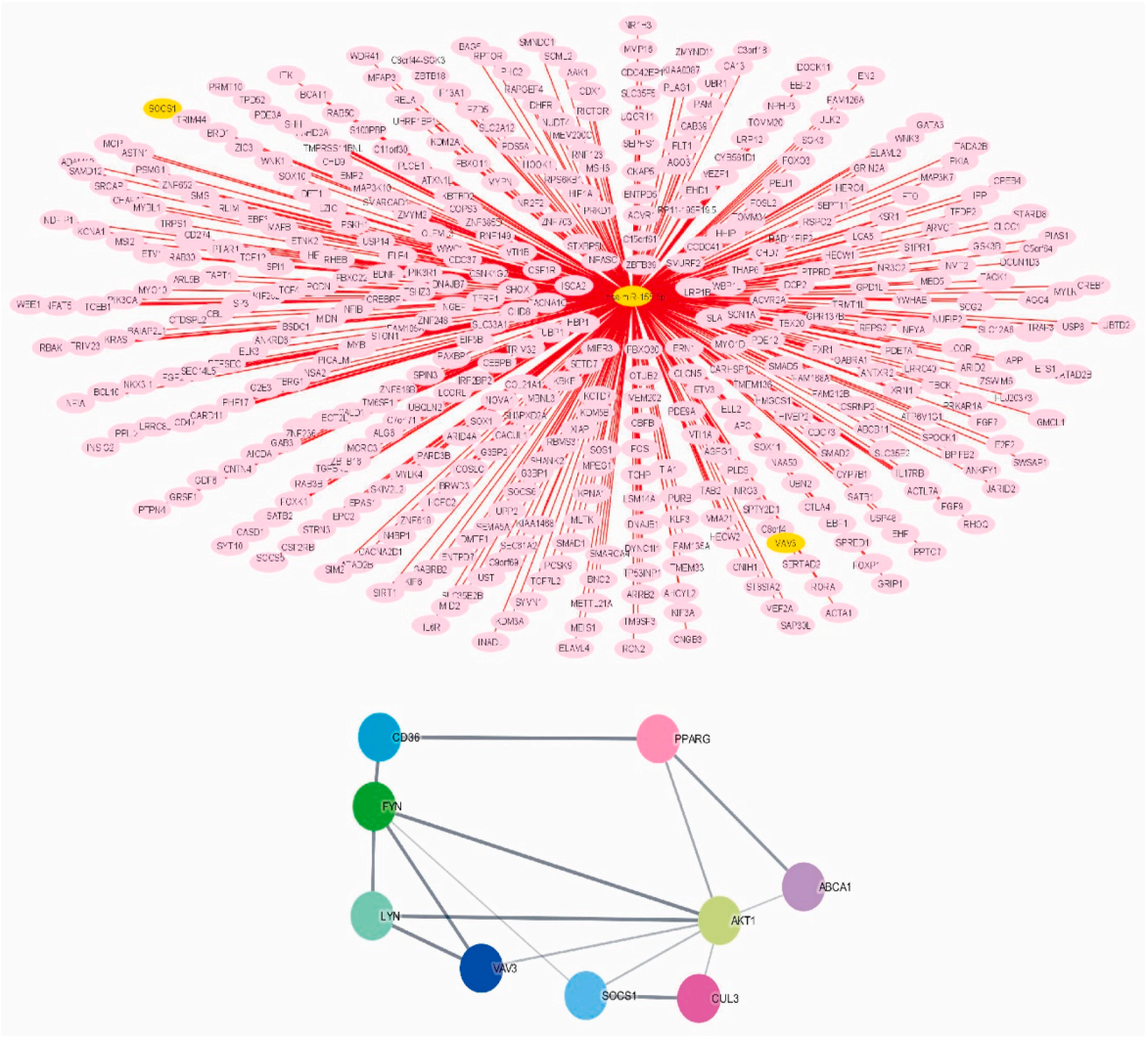

Fig. 3. Network analysis for miR-155–5p and target genes. The above network analysis, miRNA-gene targets networks, was performed using Cytoscape 3.8.3. The below network analysis, protein-protein of miRNAs candidate target interaction was conducted using String. The bold line indicated strong correlation, whereas the thin line showed the opposite.

2.3. Functional analysis of miR-155–5p and target genes

The relatedness of miR-155–5p with CD36, Vav3, and SOCS1 in cellular network was investigated by Cytoscape 3.8.3, followed by determination pathway enrichment analysis with DIANA TOOLS - miRPath v.3 (http://snf-515788.vm.okeanos.grnet.gr/), Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (https://david.ncifcrf.gov/), and STRING 11.0 (https://string-db.org/) [25–27]. From Fig. 3 it could be seen that Vav3 and SOCS1 involved in miR-155–5p networking, but not with CD36. It might be caused by the poorly conserved interaction between miR-155–5p and CD36. The below part of Fig. 3 showed that there was strong interaction between CD36, Fyn, Lyn, and Vav3. This finding supported the previous studies that reported the role of these proteins in ox-LDL endocytosis [14,15]. The activation of PPAR-γ cholesterol derivatives induce ABCA1 expression [28,29]. However, the accumulation of this lipid activates the SOCS1 which act with Cullin and NEDD4 to degrade ABCA1 so the cholesterol efflux will be suppressed [30]. The role of miR-155–5p in this pathway still needs to be validated by experimental studies.

The biological pathways of genes under regulation of the miR-155–5p were investigated using DIANA TOOLS-miRPath v.3 confirmed by DAVID. These applications are related to kyoto encyclopedia of genes and genomes (KEGG) Pathway and Gene Ontology (GO). The result showed sixteen and thirteen pathways were correlated with miR-155–5p (Table 3).

Several signaling pathways related to foam cell formation were discovered. The role of miR-155–5p in ox-LDL uptake by targeting 3′UTR of CD36 was shown in cholesterol metabolism pathway (hsa04979) and phagosome (hsa04145). The endocytosis of ox-LDL was also predicted to be regulated by miR-155–5p by translational repression of Vav3 from the functional annotations' plasma membrane (GO:0099632: protein transport within plasma membrane) and regulation of actin cytoskeleton (hsa04810). Furthermore, suggestive role of miR-155–5p in increasing ABCA1 expression by inhibit the expression of SOCS1 could be seen from the signaling pathway related with Ubiquitin-mediated proteolysis (hsa04120).

The ox-LDL uptake and endocytosis are two parts of phagocytosis function from macrophage that determine the foam cell formation. Several biological processes and molecular functions from STRING database were matched from DIANA and DAVID analysis (Table 4). The biological processes GO:0010887, GO:0010885, GO:0060100GO: 0071404, GO:0038096, hsa04666 were linked with cholesterol metabolism, phagosome pathways and associate with foam cell formation (GO:0010745, GO:0010743). The similar pathway was found for Ubiquitin mediated Proteolysis. Therefore, the regulation of ABCA1 degradation-SOCS1 dependent by miR-155–5p was important to be

Table 3
List of biological pathways related to miR-155–5p.

	DIANA TOOLS - miRPath v.3	DAVID	
hsa04350	TGF-beta signaling pathway	GO:0007155	Cell adhesion
hsa04010	MAPK signaling pathway	GO:0045121	Membrane raft
hsa04722	Neurotrophin signaling pathway	GO:0009986	Cell surface
hsa00601	Glycosphingolipid biosynthesis - lacto and neolacto series	GO:0005886	Plasma membrane
hsa04014	Ras signaling pathway	GO:0005829	Cytosol
hsa00601	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	GO:0001954	Positive regulation of cell-matrix adhesion
hsa05161	Hepatitis B	hsa04660	T cell receptor signaling pathway
hsa04390	Hippo signaling pathway	hsa04810	Regulation of actin cytoskeleton
hsa04917	Prolactin signaling pathway	hsa04662	B cell receptor signaling pathway
hsa00510	N-Glycan biosynthesis	GO:0061630	Ubiquitin protein ligase activity
hsa04668	TNF signaling pathway	GO:0016567	Protein ubiquitination
hsa04550	Signaling pathways regulating pluripotency of stem cells)	hsa04979	Cholesterol metabolism
hsa04662	B cell receptor signaling pathway	hsa4145	Phagosome
hsa05212	Pancreatic cancer		
hsa04660	T cell receptor signaling pathway		
hsa05142	Chagas disease (American trypanosomiasis)		

validated in wet laboratory studies.

The integrated pathways simulation for CD36, SOCS1, and Vav3 regulated by miR-155–5p in foam cell context was presented with Biorender application as seen in Fig. 4.

2.4. Literature studies of miR-155–5p function in atherosclerosis

The roles of miRNAs in the atherosclerosis provides new perspectives on disease mechanisms and have revealed potential diagnostic and therapeutic targets. Bioinformatic tools determined the common miRNA and predicted the function associate with foam cell atherosclerosis. It is overestimate to conclude the role of miR-155–5p in foam cell atherosclerosis only by bioinformatic analysis. Therefore, the review from available studies was performed to give brief overview about the role of miR-155–5p in foam cell atherosclerosis.

A total of forty and two hundred and sixth articles was retrieved in the first search using Pubmed and ProQuest database. The inclusion criteria in this study were [1]: one key word must be involved miR-155 or miR-155–5p [2]; the sample was macrophage cell [2], must have one parameter that assess the foam cell number, and [3] the cell must be treated with ox-LDL. The exclusion were as follows [1]: the review articles [2], the original articles that was not relevant for key terms [3], the in vivo, human, and pharmacological studies. By reading the title and abstract, a total of eighteen articles were eligible for further review (Fig. 5.). Subsequently, after reading the text comprehensively, eleven articles were included in the review (Table 5).

Out of the eleven selected original articles, ten papers used cell line RAW 264.7 or THP1, while only one study worked with macrophage primary culture. Furthermore, in all experiments, ox-LDL was used to create foam cell model with the range dose from 20 to 80 μg/ml for different time. Several studies measured foam cell as the outcome parameter, while others combined the balance between lipid uptake and efflux (Table 5).

Table 4
Biological pathways related to the protein-protein interaction.

Biological process (GO)		KEGG Pathway	
Index	Description	Index	Description
GO:0010887	Negative regulation of cholesterol storage	hsa04664	Fc epsilon RI signaling pathway
GO 0050702	Interleukin-1 beta secretion	hsa04662	B cell receptor signaling pathway
GO:0010885	Regulation of cholesterol storage	hsa04975	Fat digestion and absorption
GO:0060100	Positive regulation of phagocytosis, engulfment	hsa04666	Fc gamma R-mediated phagocytosis
GO:0071404	Cellular response to low density lipoprotein particle stimulus	hsa04979	Cholesterol metabolism
GO:0010745	Negative regulation of macrophage derived foam cell differentiation	hsa04660	T cell receptor signaling pathway
GO:0042953	Lipoprotein transport	hsa04650	Natural killer cell mediated cytotoxicity
GO:0010743	Regulation of macrophage derived foam cell differentiation	hsa03320	PPAR signaling pathway
GO:0043552	Positive regulation of phosphatidylinositol 3-kinase activity	hsa04670	Leukocyte *trans*-endothelial migration
GO:0060334	Regulation of interferon gamma mediated signaling pathway	hsa04380	Osteoclast differentiation
GO:0038096	Fc-gamma receptor signaling pathway involved in phagocytosis	hsa04120	Ubiquitin mediated proteolysis
GO:1904645	Response to amyloid beta	hsa04062	Chemokine signaling pathway
GO:0050663	Cytokine secretion	hsa04510	Focal adhesion
GO:0038095	Fc-epsilon receptor signaling pathway	hsa04152	AMPK signaling pathway
GO:0030032	Lamellipodium assembly	hsa04611	Platelet activation
GO:0048010	Vascular endothelium growth factor receptor signaling pathway	hsa04024	cAMP signaling pathway
GO:0050853	B cell receptor signaling pathway	hsa04810	Regulation of actin cytoskeleton
GO:0031295	T cell co-stimulation		

3. Discussion

Numerous reports clearly indicates the important role of miRNAs in atherosclerosis. Foam cell is a key factor that not only play as the early marker of atherosclerosis, but also drive the inflammation process in atherogenesis. Our findings demonstrated that 3′UTR of CD36, Vav3, and SOCS1, the molecules that involved in foam cell formation, was targeted by miR-155–5p. BIC gene (MIR155) is encoded miR-155. This gene which consists of 3 exons has many starts and stop codons but lack Open Reading Frame (ORF). The transcription of the MIR155 produces pri-miR-155. Exportin-5 translocate the pri-miR-155 from the nucleus to cytoplasm. Dicer enzyme cleaves the terminal loop of this molecule resulting in RNA duplexes of ~22 nucleotides or pre-miR-155. Following Dicer cleavage, an Argonaute (AGO) protein binds to the short RNA duplexes, forming the core of a multi-subunit complex called the RNA-induced silencing complex (RISC). The passenger miRNA is released and degraded, while the other strand, the guide strand, is retained within the RISC. Recent data suggest that both arms of the pre-miRNA hairpin (-5p and -3p) can give rise to mature miRNAs [20,42].

However, it is overestimate to prove the role of miR-155–5p in the foam cell atherosclerosis only by identify the miRNAs targeting three genes. The reason was because CD36, Vav3, and SOCS1 could be expressed in many cell types, disease, and can be induced by different stimulus. Thus, the next strategy in this study was exploring the possible role of miR-155–5p in foam cell atherosclerosis by performing

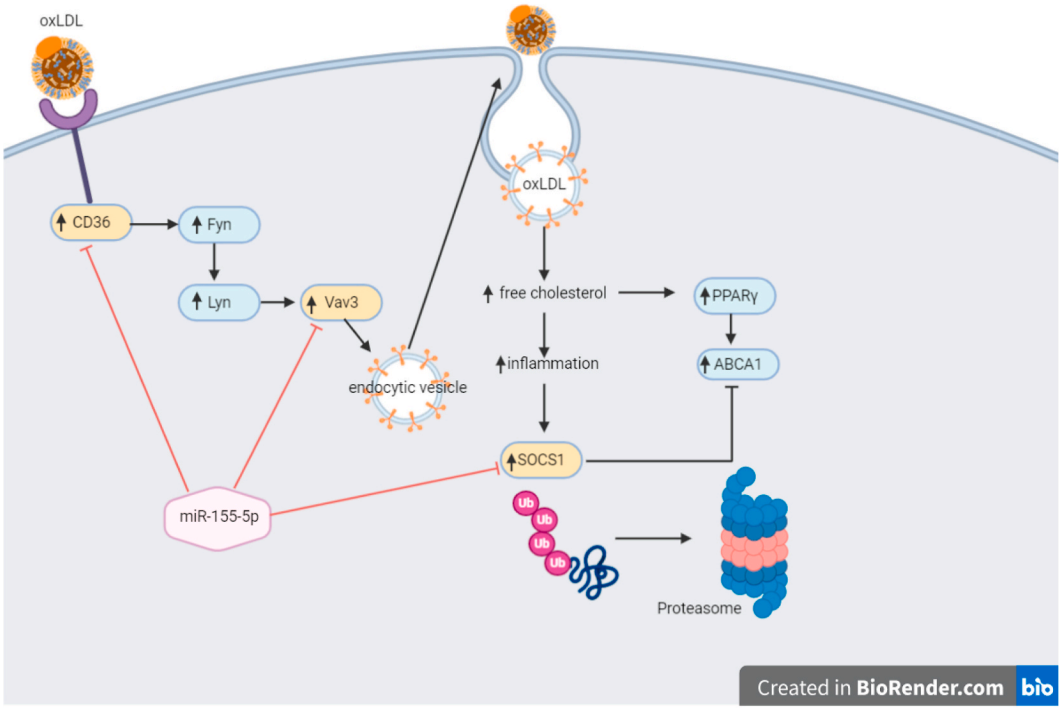

Fig. 4. The proposed role of miR-155–5p in foam cell atherosclerosis inhibition through CD36, VAV3, and SOCS1. The ox-LDL uptake is mediated by CD36. The binding of ox-LDL with CD36 activates the Lyn which subsequently activates the Vav3. Vav3 increases the expression of Rac/Rho kinase, which is an important molecule in the up-regulation of dynamin that build the endocytic vesicle structure. The vesicles fuse with the membrane cell and endocytose the ox-LDL. Inside the cell, the content of ox-LDL is hydrolyzed and the free cholesterols are released into the cytoplasm. High lipid induces the inflammation process that stimulates the SOCS1. This transcription factor acts as the E3 ligase that leads the ABCA1 to proteasome for degradation.

functional enrichment analysis. Interestingly, several pathways such as cholesterol metabolism (hsa04979), phagosome (hsa4145), positive regulation of phagocytosis (GO:0060100), Fc gamma receptor signaling pathway involved in phagocytosis (GO:0038096), regulation of actin cytoskeleton (hsa04810), regulation of macrophage derived foam cell differentiation (GO:0010743), PPAR signaling pathway (hsa03320), ubiquitin mediated proteolysis (hsa04120) were related to foam cell atherosclerosis.

Recent studies showed the miR-155–5p was high not only in *in vitro* atherosclerosis models, but also in both circulating and atherosclerotic lesions in both mice and humans. According to the researches conducted by Du et al., in 2014 and by Nazari-Jahantigh et al., in 2012, C57BL/6, ApoE -/-, LDR -/- mice aged 3–7 months administered with High Fat Diet (HFD) or partially ligated in their carotid arteries, then stained with Oil Red O (ORO) and Monocyte + Macrophage antibody (MOMA), showed the increase number of foam cell compared to control [43](38). Significant differences in circulating miR-155–5p and atherosclerotic lesion in individuals with coronary artery disease were higher compared with healthy individuals [44].

Interestingly, the role of miR-155–5p in the foam cell formation remains controversial. Several works showed an anti-atherogenic profile, while others demonstrated pro-atherogenic properties. The papers that concluded miR-155–5p as pro-atherogenic did not specifically measure the outcome of atherosclerosis. Most of the publications used the inflammatory cytokine production to conclude the effect of this miRNA, but not specifically measure the number of foam cell, the lipid uptake and the cholesterol efflux. Moreover, the pro-atherogenic studies used macrophage cells that treated with LPS, which not naturally express the preference type of macrophage to produce foam cell. M2 macrophage phenotype with high endocytic capacity is the origin of foam cell since the foam cell formation is a physiological process to phagocytose ox-LDL. Nevertheless, in the chronic state, the foam cell function change into pro-inflammatory due to cholesterol metabolism dysregulation. The uptake of ox-LDL predominantly occurs through CD36 which expressed higher in M2 macrophages compared to M1 [45]. Therefore, the examination of new perspectives for the development of the foam model was considered. We suggest to use M-CSF, IL-4 as inducer for macrophages differentiation than LPS, which hopefully could answer the contradiction role of miR-155–5p in atherosclerosis [46,47].

Another possibility of this conflicting result was answered by Bruen analysis which concluded that the function of miR-155–5p depend on the phase of atherosclerosis. The miR-155–5p suppress atherosclerosis in the early phase, while it shows the opposite effect in the advanced phase [48]. Several studies using ApoE−/− mice as a model for advance phase of atherosclerosis demonstrated that the injection of antagomir-155 attenuated atherosclerosis development and progression in ApoE$^{-/-}$ mice [35]. In contrast, LDLr$^{-/-}$ mice transplanted with miR-155-deficient bone marrow as model for early atherosclerosis had increased atherosclerotic plaques, elevated levels of pro-inflammatory monocytes, and decreased IL-10 production from peritoneal macrophages [49].

Overall, our findings give predictions which need to be validated by laboratory experiments to conclude the role of miR-155–5p in foam cell atherosclerosis inhibition through CD36, Vav3, and SOCS1. Another limitation of this study is our review did not specify the method to show that miR-155–5p regulate directly the target genes. Many papers used miR-155 mimic or inhibitor to study the role of this miRNA in foam cell formation. Recent publications by Ye et al. 2016, Chang et al. 2016, Zhang et al., 2020 demonstrated that SOCS1 was the direct target of miR-155–5p by performing a luciferase reporter assay using HEK293 cells. The cells were co-transfected with the wild-type (WT) or mutated (Mut) SOCS1 luciferase reporter vector, together with miR-155 mimic and the control for 24 and 48 h. The result showed that luciferase activity was significantly inhibited in cells transfected with WT SOCS1 and miR-155 mimic, but not in cells transfected with mutation SOCS1 and miR-155 mimic [33,50,51]. Therefore, our results provide justification

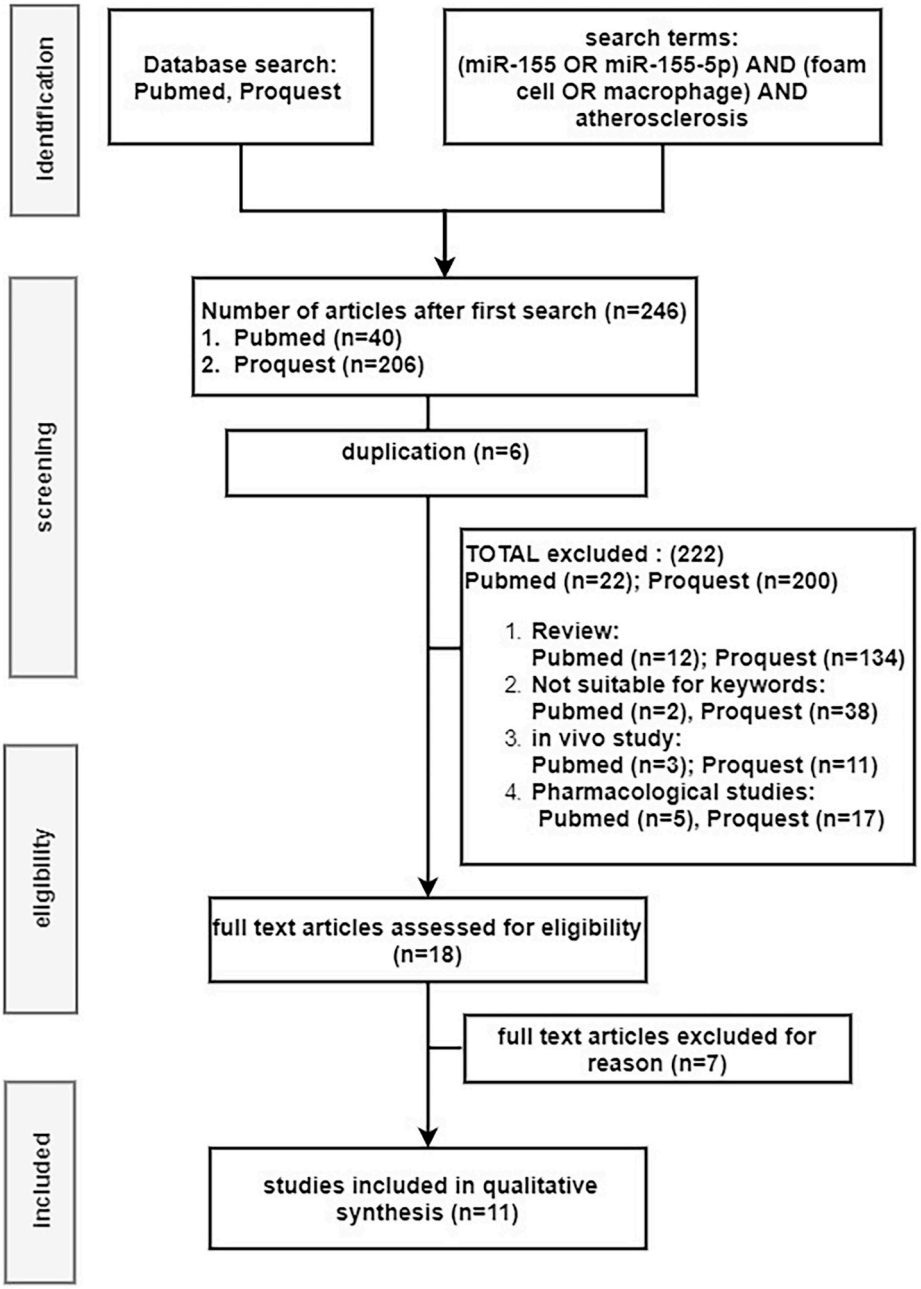

Fig. 5. PRISMA flowchart for systematic literature review to evaluate the role of miR-155–5p in foam cell atherosclerosis.

for further evaluation about the role of miR-155–5p in foam cell atherosclerosis by doing luciferase assay to get transcriptional activity profile of miRNA with 3′UTR of gene targets.

Available studies did not clearly mention the type of miR-155 use, either -5p or -3p. miRdB database informs that the previous name of miR-155–5p is miR-155. Moreover, miR-155–5p is the predominant functional miR-155 and also expressed 20-fold to 200-higher than miR-155–3p [52]. Further studies need to specify which type of miR-155 is used. Furthermore, the comparative studies about the role of miR-155–5p and -3p in foam cell atherosclerosis should be performed.

4. Conclusion

Foam cell atherosclerosis is not only determined the beginning of atherosclerosis, but also plays a key role in its progression. The miR-155–5p is upregulated in macrophages treated with ox-LDL. Our findings revealed the predictive role of miR-155–5p to inhibit foam cell atherosclerosis through CD36, Vav3, and SOCS1 pathway. However, given some conflicting results, further studies are required to investigate the stage-specific effects of miR-155–5p inhibition during atherosclerosis progression using M2 phenotype macrophage. In addition, the direct binding of miR-155–5p to the gene targets should be studied. Moreover, the miR-155–5p function in several other key aspects of foam cell formation such as autophagy process, the preference needs of metabolic supply is needed to be elucidated in further investigation.

5. Material and method

5.1. Identification of microRNA targeting CD36, VAV3, and SOCS1

The identification of miRNAs targeting 3′UTR of CD36, VAV3, and SOCS1 were performed using TargetScan 7.2 (http://www.targetscan.

Table 5
Summary of recent papers studying the role of miR-155–5p in foam cell atherosclerosis.

Type	Title	Reference	Sample and Treatment	Results
antiatherogenic	miR-155 acts as an anti-inflammatory factor in atherosclerosis-associated foam cell formation by repressing calcium-regulated heat stable protein 1	[31]	the monocyte THP-1 cell line. After stimulated with 100 nM PMA, the monocyte differentiated to macrophage. The macrophage were transfected with 100 nM miR-155 mimic or miR-155 inhibitor for 0, 6, 12, 24, or 48 h, followed by treatment with 50 μg/ml oxLDL for 24 h.	↓ TNFα ↓ foam cell
	MiR-155 inhibits transformation of macrophages into foam cells via regulating CEH expression	[32]	Human THP-1 cells were differentiated into macrophages by adding 100 PMA for 72 h. The macrophages were transformed into foam cells by co-incubating in 50 μg/ml ox-LDL, 0.3% bovine serum albumin (BSA) in serum-free RPMI 1640 medium for 48 h foam cells were transfected with miR-155 mimics (40 nM) for 24 h at 37 °C, then grown for 24 h in 10% fetal bovine serum and without antibiotics.	↑CEH ↑cholesterol efflux ↓contents of CE, FC, TC and CE/TC ratio ↓ TNF ↓ SRA ↑ ABCA1 ↑ IL10
	miR-155 Regulated Inflammation Response by the SOCS1-STAT3-PDCD4 Axis in Atherogenesis.	[33]	macrophage Raw264.7 cell line were exposed with 20 μg/ml ox-LDL for 24 h and transfected with anti miR-155	↑PDCD4 ↓IL6 ↓TNF ↑IL10 ↓ foam cell
	MicroRNA-155 silencing enhances inflammatory response and lipid uptake in oxidized low-density lipoprotein-stimulated human THP-1 macrophages.	[34]	THP-1 cell line was differentiated into macrophage by adding PMA 100 nm for 24 h. Silencing of endogenous miR-155 in THP-1 cells using locked nucleic acid-modified antisense oligonucleotides. The cells were incubated for 24 h posttransfection and then exposed to oxLDL (50 kg/ml) for another 24 h	↑oxLDL-induced lipid uptake, ↑LOX-1, CD36, and CD68 ↑ IL-6, -8, and TNFα
	miR-155 inhibits oxidized low-density lipoprotein-induced apoptosis of RAW264.7	[35]	RAW 264.7 cells were transfected with synthetic miR-155 mimics (M, 80 nM)	↑FADD ↑Apoptosis
	Regulation of microRNA-155 in atherosclerotic inflammatory responses by targeting MAP3K10.	[36]	The human monocytic cell line THP-1 were cultured with 100 nM PMA for 24 h	↓TNFα ↓IL6 ↓MAP3K10
	miR-155 inhibits oxidized low-density lipoprotein-induced apoptosis in different cell models by targeting the p85α/AKT pathway.	[37]	Raw264.7 cells were transfected with miR-155 mimics or inhibitor for 24 h. Following transfection, the cells were stimulated with OxLDL 80 μg/ml for 12 h	Prevent cytotoxicity ↓apoptosis
Proatherogenic	MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages.	[38]	BM cells were harvested from the femurs of Mir155+/+/Apoe−/− and Mir155−/−/Apoe−/− mice, cultured for 7 days to allow differentiation into primary macrophages treated with siRNA or siRNA against Socs1, Sfpi1, and Bcl6 on Day 4 stimulated with moxLDL and IFNγ on Day 7.	Loss of Mir155 reduced the expression of CCL2
	MicroRNA-155 Promotes Atherosclerosis Inflammation via Targeting SOCS1.	[39]	THP-1 cells were exposed to PMA 100 nm. Then the cells were stimulation with oxLDL 50 μg/ml for 24 h. Thus the cells transfected with miR-155 mimic or miR-155 inhibitor.	↓SOCS1 ↓TNFα ↓ IL1 ↓CCL2 ↓ CCL4 ↓CCL7
	microRNA-155 promotes the ox-LDL-induced activation of NLRP3 inflammasomes via the ERK1/2 pathway in THP-1 macrophages and aggravates atherosclerosis in ApoE−/− mice.	[40]	THP-1 monocytes were stimulated with. PMA 100 ng/ml to induce the differentiation of THP-1 monocytes into macrophages for 48 h. The differentiated macrophages were then treated with 50 μg/ml ox-LDL for 24 h. The cells transfected with miR-155 mimic and miR-155 inhibitor 50 nm for 24 h	↑ ERK1/2 ↑ phospho–NF–κB ↑ NLRP3 ↑ caspase-1 ↑ IL-1β ↑ IL-18
	Elevated microRNA-155 promotes foam cell formation by targeting HBP1 in atherogenesis.	[41]	RAW264.7 were transfected with miR-155 mimic or inhibitor for 48 h than stimulated with oxLDL for 24 h	↑ lipid uptake ↑ ROS ↓ HBP1

org/vert_72/) and MicroRNA Target Prediction Database (miRdB) (http://www.mirdb.org/) for cross validation. The CD36, Vav3, and SOCS1 gene reference sequences were retrieved from the https://www.ensembl.org/with reference numbers ENSG00000135218, ENSG00000134215, and ENSG00000185338, respectively. The validation prediction of miRNAs targeting CD36, Vav3 and SOCS1 genes by TargetScan 7.2 was showed in context ++ score, conserved branch lengths, and PCT [21,53]. The prediction of miRNAs using miRdB was demonstrated with the cut off value of 80 [22,23].

5.2. Profile of common miRNA targeting CD36, VAV3, and SOCS1

The similar miRNA targeting CD36, SOCS1, and Vav3 based on miRdB and TargetScan 7.2 was selected with Venn diagram analysis. This miRNA was predicted to be a strong candidate for studying its role in foam cell atherosclerosis. The profile of common miRNA and the interaction with the genes was evaluated using miRdB and TargetScan 7.2 database. The data includes the mature miRNA sequences, the interaction position between miRNA's seed sequences and 3′UTR of target genes. The conserved physiological binding sites of 3′ UTR in three genes across among different species was also demonstrated using TargetScan 7.2.

5.3. Functional enrichment analysis of miRNA targets

The Cytoscape 3.8.3 was used to construct the miRNAs-mRNAs network followed by determination pathway enrichment analysis with DIANA TOOLS-miRPath v.3 and Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 [25,54,55]. These tools provide information on the functional notations of miRNA that are experimentally supported using Gene Ontology (GO) or GO Slim terms, combined with statistically-enriched pathways, such as Kyoto Encyclopedia of Genes and Genomes (KEGG) molecular pathways, and was based on target genes that query miRNAs targets. Moreover, to ensure the validity

of the results, STRING 11.0 (https://string-db.org/) database were also performed to provide a critical assessment and integration of protein–-protein interactions, including direct (physical) as well as indirect (functional) associations. All data available in STRING were provided with a probabilistic confidence score. Targets with a confidence score greater than 0.4 were selected to construct the network (26). The proposed mechanism of miR-155–5p role in foam cell inhibition through CD36, Vav3, and SOCS1 pathway was demonstrated using Biorender application (16).

5.4. Review of miR-155–5p function from available studies

The articles from Pubmed and Proquest database, written in English language, and published for the past 10 years (2010–2020) was carried out. A retrieval strategy was created with the input from an expert librarian, and the search strategy was performed encompassing terms such as (miR-155 OR miR-155–5p) AND ((foam cell) OR (macrophage)) AND atherosclerosis. The expression level of miR-155–5p was high in monocyte-macrophage cells whereas miR-155–3p expression was very low. Furthermore, miRdB showed that miR-155–5p previous name was miR-155. Therefore, we used miR-155 for inclusion criteria to performed the review.

The data excluded following criteria [1]: Article review, editorial, comment, or interview [2]; in vivo or human studies [3]; Studies that does not include the macrophages cell [4]; Pharmacological studies; and duplication.

CRediT authorship contribution statement

Ermin Rachmawati: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Djanggan Sargowo:** Project administration, Supervision. **M. Saifur Rohman:** Project administration, Supervision, Funding acquisition. **Nashi Widodo:** Visualization, Supervision, Writing – review & editing. **Umi Kalsum:** Visualization, Supervision.

Declaration of competing interest

The authors claim that the research was conducted without any business or financial relationships that may be construed as a potential conflict of interest.

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