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Identification of Key Genes in Atherosclerosis by Combined DNA Methylation and miRNA Expression Analyses

ORIGINAL INVESTIGATION

ABSTRACT

Background: Atherosclerosis is a significant cause of coronary heart disease, cerebral infarction, and peripheral vascular disease. The objective of this study was to identify the key genes aberrantly expressed in atherosclerosis, which were regulated by microRNAs and DNA methylation.

Methods: We acquired data on DNA methylation and microRNA and messenger RNA expression from Gene Expression Omnibus data sets (GSE46394, GSE53675, and GSE12288, respectively) and identified differentially methylated genes, differentially expressed genes, and differentially expressed microRNAs between atherosclerosis and control samples. The miRDB, miRTarBase, and TargetScan databases were used to predict differentially expressed microRNAs-targeted genes, which were then intersected with differentially methylated genes and differentially expressed genes to identify genes associated with aberrant DNA methylation and microRNA activity. The DAVID database was used to perform functional enrichment analysis of differentially methylated genes and the key genes involved in atherosclerosis. Potential therapeutic agents for atherosclerosis were predicted by Connectivity Map analysis.

Results: In total, we identified 47 upregulated hypomethylated and 90 downregulated hypermethylated genes in atherosclerosis. Among them, 24 differentially expressed genes were found to be modulated both through aberrant DNA methylation and microRNA expression, and 10 such differentially expressed genes were defined as the key genes in atherosclerosis. Fifteen chemicals were selected for their potential effect in atherosclerosis.

Conclusions: We identified 10 key genes significantly associated with aberrant DNA methylation and microRNA expression in atherosclerosis and suggested 15 chemicals with potential effects on these genes, which could be further investigated as candidate drugs for atherosclerosis.

Keywords: Atherosclerosis, DNA methylation, gene expression, microRNA

INTRODUCTION

Atherosclerosis, a chronic inflammatory cardiovascular disorder, is the main cause of cardiovascular diseases such as coronary heart disease, cerebral infarction, and peripheral vascular disease.[1] Atherosclerosis is characterized by thickening and hardening of arterial walls through deposition of fats and cholesterol, resulting in the loss of elasticity, narrowing of the lumen, and accumulation of macrophages.[2] Animal experiments and observations of human specimens indicate that atherogenesis is initially triggered by qualitative changes in the monolayer of endothelial cells lining the inner arterial surface.[3] Atherosclerosis mainly affects large- and medium-sized arteries, and its clinical manifestations occur after narrowing or occlusion of the affected vessels, which impede blood flow to different organs. The main consequences of aortic and coronary atherosclerosis are aortic aneurysm and coronary heart disease, respectively. In the early stages of atherosclerosis, atherogenic lipoproteins are cleared from the intima by macrophages, resulting in the formation of lipid-laden macrophage foam cells, which are accompanied by apoptosis or necrosis of endothelial and smooth muscle cells.[4]

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Emerging evidence suggests that apoptosis contributes to the instability of atherosclerotic lesions.[5] Atherosclerosis progresses with age through focal calcification of atherosclerotic plaques, and coronary artery calcification represents a more effective and reliable prognostic biomarker of coronary atherosclerosis than traditional risk factor scores.[6] Pathological angiogenesis of the vessel wall is a characteristic feature of high-risk atherosclerotic plaques and progression of the disease.[7] Plaque rupture, which frequently occurs during the evolution of coronary atherosclerotic lesions, is the most common cause of coronary thrombosis.[8]

MicroRNAs (miRNAs) are non-coding, single-stranded RNA with an average length of approximately 22 nucleotides, which play a regulatory role in gene expression through base-pairing with target messenger (m)RNAs. The formed silencing complex (RISC) is guided to the 3′-untranslated region of the target mRNA, resulting in its translational silencing or repression.[9] MicroRNAs can be expressed in tissue- and cell type-specific manner and affect various cell functions such as proliferation, apoptosis, migration, differentiation, and development. Recent studies have shown that specific miRNAs are closely involved in all stages of atherosclerosis, from endothelial dysfunction to plaque rupture.[10] Thus, miRNA-21, miRNA-210, and miRNA-14a have been identified and verified to be significantly upregulated in atherosclerosis-affected arteries, indicating their role in the formation of atherosclerotic plaques.[11]

Epigenetic modifications are reversible and heritable changes of gene function without altering its DNA sequence[12]; they comprise modifications in DNA (such as methylation), RNA, and histones, which affect gene expression. DNA methylation has been implicated in various pathological conditions, including cancer development. DNA methylation often occurs in the promoter region of tumor suppressor genes, decreasing their expression, which promotes carcinogenesis[13]; therefore, abnormal DNA methylation is often used as an important molecular marker of tumor prognosis, diagnosis, classification, and treatment. However, the regulatory effect of DNA methylation on gene expression in patients with atherosclerosis remains unclear.

In this study, we performed comprehensive analysis of genome-wide DNA methylation and mRNA and miRNA expression data sets to identify key genes regulated by both miRNAs and DNA methylation and differentially expressed in atherosclerosis. Integrative analysis of the roles of miRNAs and DNA methylation in atherosclerosis may provide new insights into the etiology of the disease at the genetic level and help develop novel diagnostic biomarkers.

HIGHLIGHTS

- Integrative multiomics analysis of atherosclerosis was performed to identify differentially expressed genes regulated by microRNAs (miRNAs) and DNA methylation in atherosclerosis.
- Ten key atherosclerosis-related differentially expressed genes regulated through both DNA methylation and miRNA activity were detected.
- Fifteen chemicals with potential effects on the key genes were revealed by Connectivity Map analysis; they could be investigated as possible candidate drugs to treat atherosclerosis in the future.

METHODS

Data Source

Microarray data on gene methylation profiling data set (GSE46394) and miRNA (GSE53675) and mRNA (GSE12288) expression were retrieved from Gene Expression Omnibus (GEO) data sets (https://www.ncbi.nlm.nih.gov/geo/). The GSE46394 genome-wide DNA methylation data set comprises 49 samples (34 atherosclerosis and 15 control samples). The GSE53675 miRNA data set includes 18 samples (13 for cardiovascular disease and 5 for control), and the GSE12288 mRNA data set contains 222 peripheral blood samples (110 for coronary artery disease and 112 for control). Additionally, the GSE20686 data set containing 337 whole blood gene expression profiles (186 of coronary artery disease and 151 of control) was used for verification.

Methylation Analysis

R package ChAMP (Version 2.20.1) was used to process gene methylation data from the GSE46394 data set. The champ function was used to load methylation data files; probes with the detection level of $P > .01$ and probes with <3 beads in at least 5% of samples were excluded. We also removed all non-CpG, single-nucleotide polymorphism-related, multi-hit probes, and probes those on the X and Y chromosomes. After probe filtering, the beta value of patients with atherosclerosis was normalized using the Beta MIxture Quantile dilation method for type I and II probe correction with the thresholds of $|\Delta\beta| > 0.2$ and P-value $< .05$.

Differential Expression Analysis

After data pre-processing, differentially methylated probes (DMPs), differentially expressed genes (DEGs), and differentially expressed miRNAs (DEMs) in atherosclerosis and control samples were identified using the limma package (Version 3.42.2) in R. In the GSE46394 data set, DMPs located in the gene region were assigned to the corresponding genes, which were regarded as differentially methylated genes (DMGs). Differentially expressed genes from GSE12288 and DMGs from GSE46394 were intersected using Venn analysis to obtain methylated DEGs (MDEGs) in atherosclerosis. The cutoff criteria for DMPs were $|\Delta\beta| > 0.2$ and $P < .05$. We used $P < .05$ to screen out DEGs and DEMs.

Enrichment of Genomic Characterization Annotations

The distribution of DMPs in functional regions was explored by the ChAMP package (Version 2.20.1) in R, including the gene regions of the body, transcriptional start site 200 (TSS 200), TSS1500, 5′ untranslated region (5′ UTR), 3′ UTR, intergenic region, first exon (1st Exon), and CpG islands (island, shore, and shelf).

Establishment of a Regulatory Network

The targets of the selected DEMs mentioned above were predicted by the online bioinformatics tools miRDB

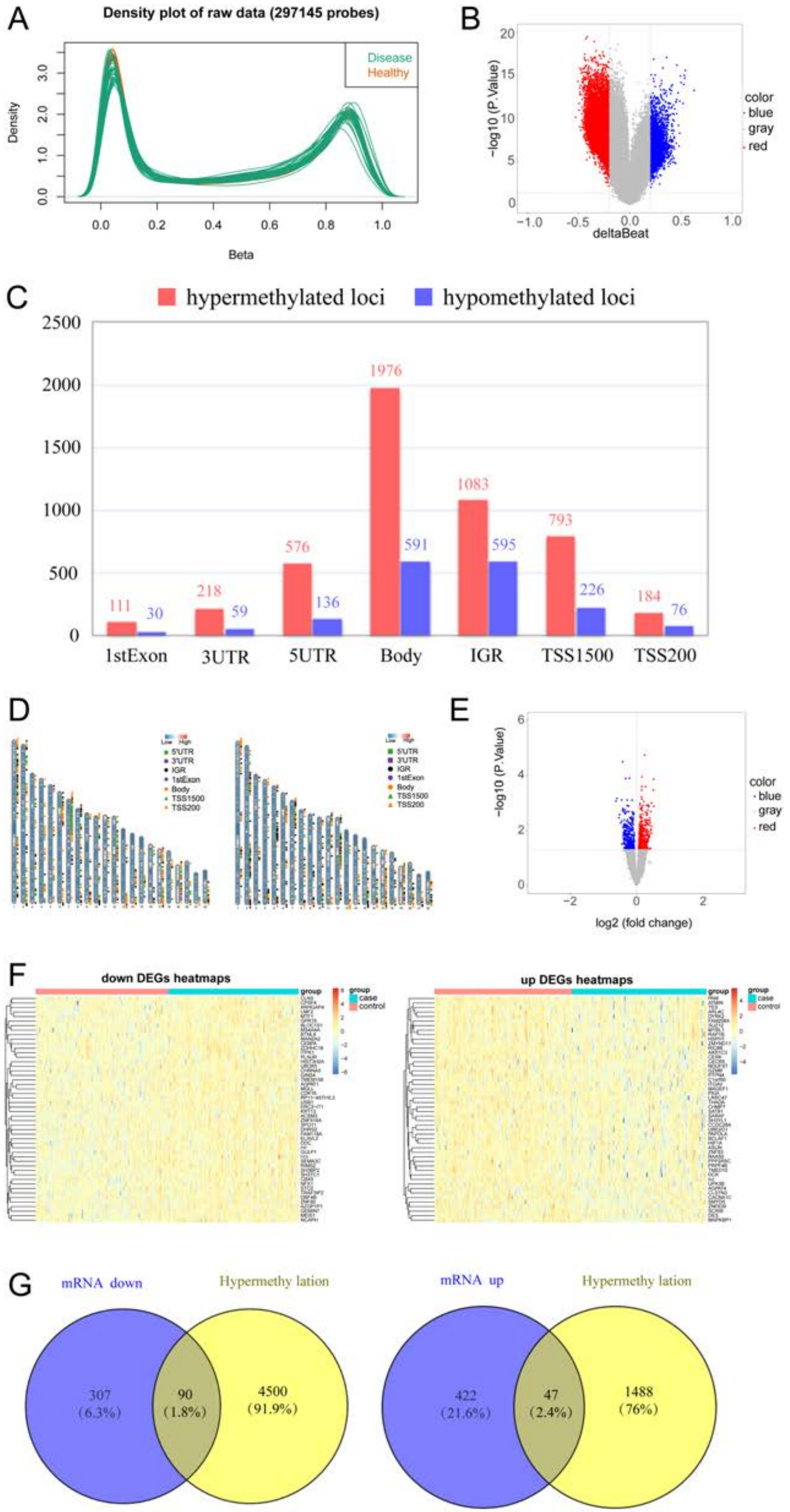

Figure 1. Identification of MDEGs in atherosclerosis. (a) Beta score density curve of methylation distribution. (b) Volcano plot of sites differentially methylated in atherosclerosis and control samples. (c) Average DNA methylation levels in the gene (left) and CpG island (right) regions. (d) Distribution of hypermethylated (left) and hypomethylated (right) chromosomes. (e) Volcano plot of DEGs in atherosclerosis and control samples. (f) Heat map of the expression levels of the top 50 downregulated (left) and upregulated (right) DEGs. (g) Venn diagrams showing overlapping between downregulated DEGs and hypomethylated genes (left) and between upregulated DEGs and hypermethylated genes (right). Methylated differentially expressed genes, MDEGs; DEGs, differentially expressed genes.

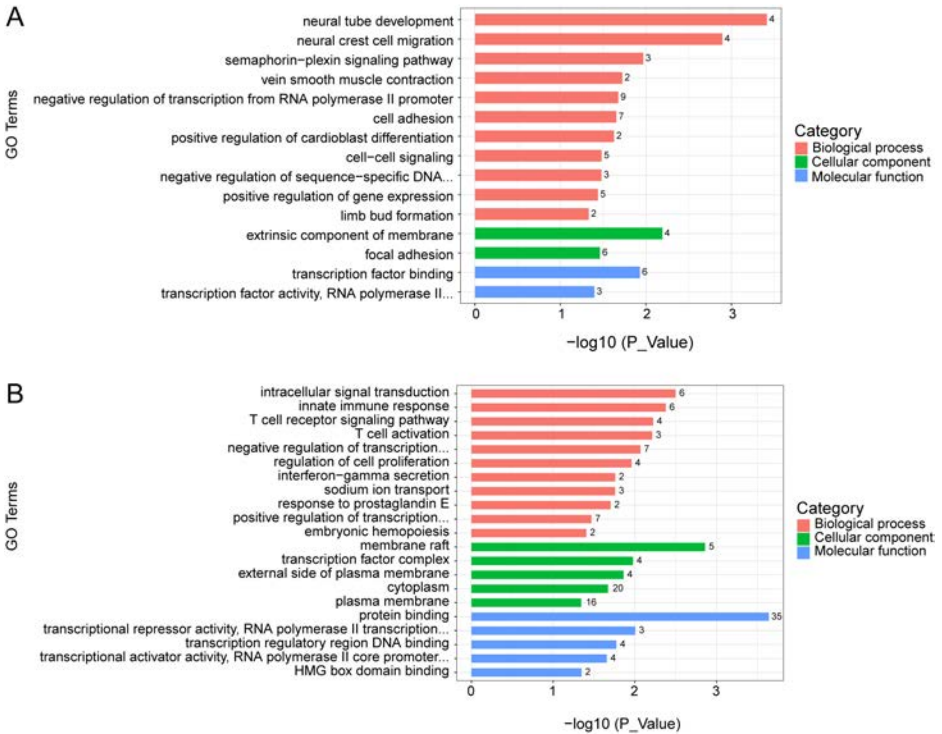

Figure 2. Functional enrichment analysis of methylated differentially expressed genes. GO term enrichment of 90 hypermethylated downregulated genes (a) and 47 hypomethylated upregulated genes (b). GO, gene ontology.

(https://www.mirdb.org/miRDB/), miRTarBase (https://mirtarbase.mbc.nctu.edu.tw/), and TargetScan (http://www.targetscan.org/). Target genes from each database were overlapped to improve the reliability of prediction. Differentially expressed microRNA targets were integrated with DEGs to construct an mRNA–miRNA regulatory network using Cytoscape software (https://www.cytoscape.org/). The miRWalk (http://mirwalk.umm.uni-heidelberg) and miRDB databases were used to predict miRNAs targeting the key genes, and a miRNA–mRNA regulatory network was constructed.

Functionally Enrichment Analysis

To explore biological functions of the identified MDEGs and key genes, gene ontology (GO) annotation analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis were performed using the DAVID database. Statistical significance was set at $P < .05$.

Drugs Screening with Connectivity Map

Connectivity Map (CMap) build 02 is an open database (https://www.broadinstitute.org) designed to explore the functional association between small-molecule drugs, gene expression, and disease.[14] In this study, CMap analysis was performed to screen potential small-molecule drugs targeting the identified key genes in atherosclerosis based on the following criteria: $|\text{enrichment score}| \geq 0.7$ and $P < .01$.

Statistical Analysis

Student's *t*-test was used to reveal candidate genes differentially expressed in atherosclerosis and control samples. The Rideogram package (Version 0.2.2) in R was used to visualize the distribution of DMPs in chromosomes. Differences with P-value $< .05$ were considered statistically significant.

RESULTS

Identification of Aberrantly Differentially Methylated Genes in Atherosclerosis

In the GSE46394 data set, the methylation distribution density diagram demonstrated that the beta-values of DMPs were <0.2 and >0.8, suggesting that no outlier samples were observed. Differential expression analysis was conducted across all samples (Figure 1a), and 17 751 DMPs were identified between atherosclerosis and control groups, including 13 984 hypermethylated and 3767 hypomethylated sites (Figure 1b). The annotation of these DMPs revealed that the majority of them were located in non-promoter areas, mostly in gene body and intergenic regions. A small portion of DMPs were located in CpG islands; however, most were identified in CpG island shores and shelves (Figure 1c). Analysis of DMP global distribution in chromosomes indicated that hypomethylated and hypermethylated DMPs had very similar proportions in the genome (Figure 1d).

Overall, 866 DEGs were identified in atherosclerosis compared to control samples; among them, 469 were upregulated and 397 downregulated (Figure 1e). The heatmap plot shows the top 50 downregulated and upregulated DEGs (Figure 1f); specific genes and their expression levels are indicated in Tables S1 and S2, respectively. Then, 47 upregulated hypomethylated and 90 downregulated hypermethylated genes were selected through intersection of DMGs and DEGs (Figure 1g) and considered MDEGs.

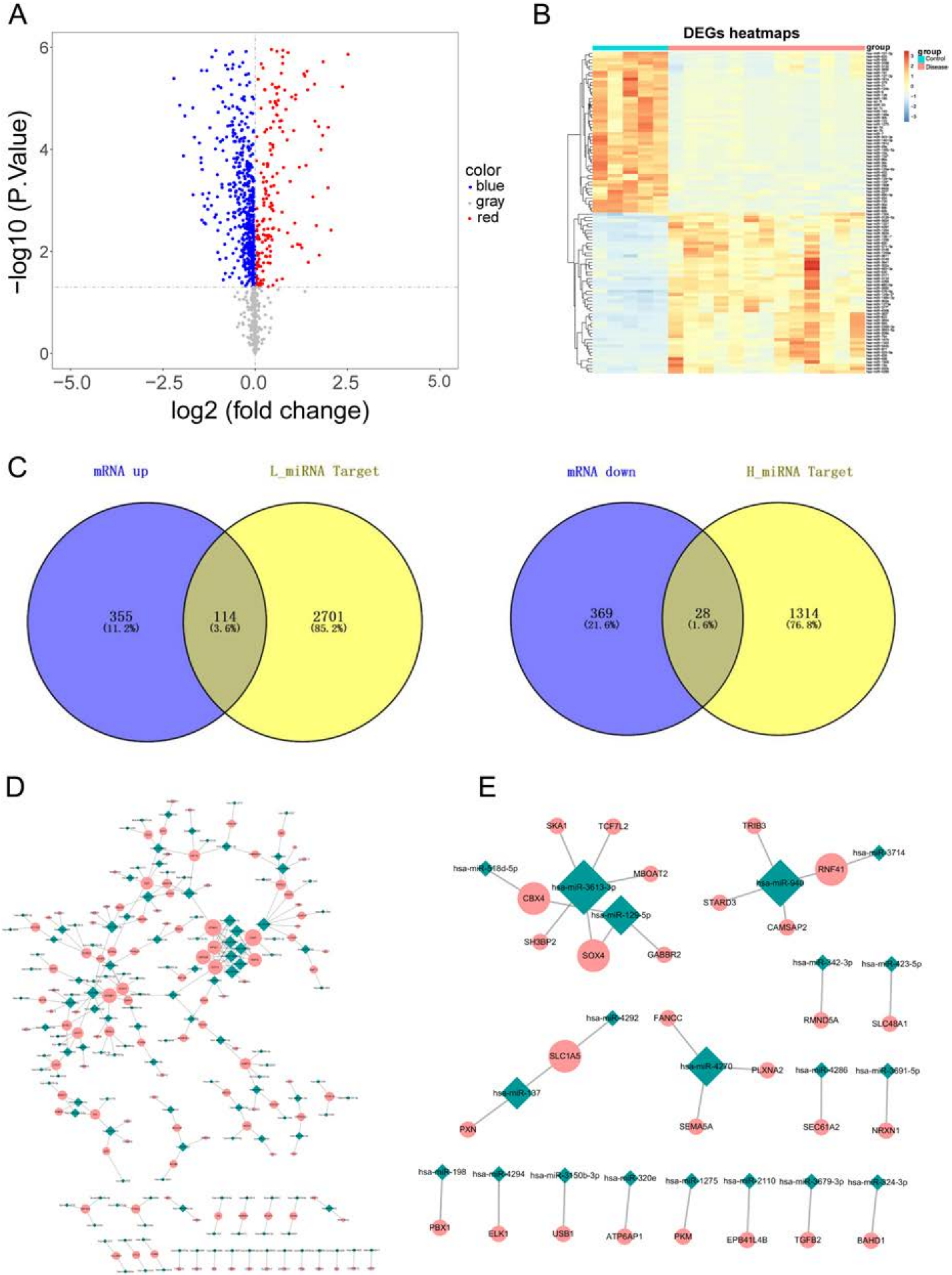

Figure 3. MiRNA–mRNA regulatory networks in atherosclerosis. (a) Volcano plot of miRNAs differentially expressed in atherosclerosis and control samples. (b) Heatmap of the expression levels of the top 50 upregulated and downregulated miRNAs. (c) Venn diagrams showing overlapping between upregulated DEGs and the predicted targets of downregulated miRNAs (left) and between downregulated DEGs and the predicted targets of upregulated miRNAs (right). (d, e) MiRNA–mRNA regulatory networks composed of upregulated miRNAs and downregulated DEGs (d) and downregulated miRNAs and upregulated DEGs (e). MiRNA, microRNA, mRNA, messengerRNA; DEGs, differentially expressed genes.

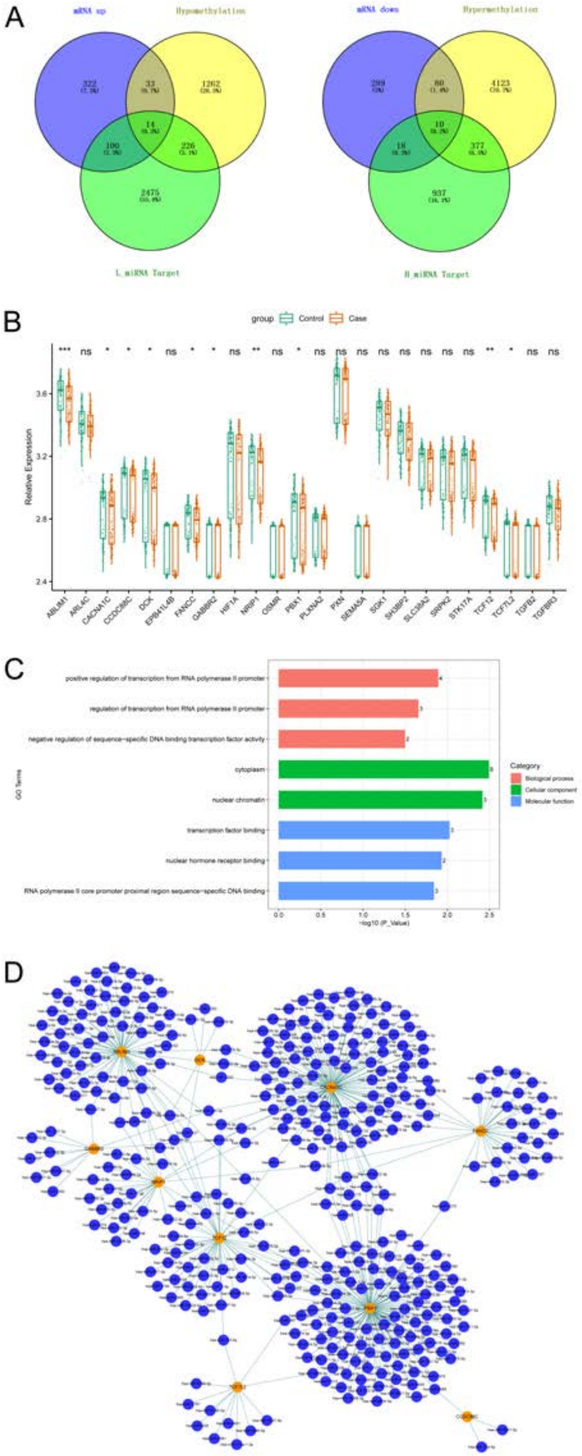

Figure 4. Identification and functional analysis of the key genes regulated through both DNA methylation and miRNAs in atherosclerosis. (a) Venn diagrams showing overlapping between hypomethylation-upregulated DEGs and targets of downregulated miRNAs (left) and hypermethylation-downregulated DEGs and targets of upregulated miRNAs (right). (b) Expression levels of 24 genes in atherosclerosis and control samples from the GSE20686 data set. (c) Functional enrichment analysis of the key genes. (d) The miRNA–mRNA regulatory network of the key genes. MiRNA, microRNA, mRNA, messengerRNA; DEGs, differentially expressed genes.

Functional Enrichment of Methylated Differentially Expressed Genes

To understand the biological functions of the identified MDEGs, we performed GO annotation analysis. The results indicated that the 90 hypermethylation-downregulated genes were significantly enriched in the processes of negative regulation of transcription from RNA polymerase II promoter, cell adhesion, cell–cell signaling, positive regulation of gene expression, focal adhesion, transcription factor binding, and transcription factor binding (Figure 2a). In terms of the 47 hypomethylation-upregulated genes, the main enriched GO terms were positive regulation of transcription from RNA polymerase II promoter, innate immune response, T cell receptor signaling pathway, T cell activation, interferon-gamma secretion, intracellular signal transduction, negative regulation of transcription from RNA polymerase II promoter, cytoplasm, protein binding, and transcription regulatory region DNA binding (Figure 2b).

Differentially Expressed Genes Targeted by Differentially Expressed miRNAs

A total of 874 DEMs (240 upregulated and 634 downregulated) were identified in patient and control samples (Figure 3a). According to the miRDB, miRTarBase, and TargetScan databases, 1342 and 2815 genes were predicted as targets of upregulated and downregulated DEMs, respectively (Figure 3b). Intersection of these genes with DEGs revealed that the expression of 114 genes targeted by downregulated DEMs was increased, whereas that of 28 genes targeted by upregulated DEMs was decreased (Figure 3c). Detailed miRNA–mRNA networks are shown in Figure 3d and 3e.

Key Genes with Aberrant DNA Methylation Targeted by Differentially Expressed Genes

Next, we identified candidate genes affected by changes both in DNA methylation and miRNA expression, which may provide valuable information on the mechanisms underlying atherosclerosis progression. A total of 24 such genes were selected: 14 targeted by underexpressed DEMs and upregulated by hypomethylation and 10 targeted by overexpressed DEMs and downregulated by hypermethylation (Figure 4a). Among the 24 genes, 10 genes, namely *TCF7L2*, *CACNA1C*, *NRP1*, *GABBR2*, *FANCC*, *DCK*, *CCDC88C*, *TCF12*, *ABLIM1*, *and PBX1* were differentially expressed in the atherosclerosis group compared to the control group in the GSE20686 data set; therefore, we considered them as the key genes in atherosclerosis development (Figure 4b). To understand the biological functions of the key genes, we applied GO annotation analysis. The results suggested that the 10 key genes were significantly enriched in the processes of positive regulation of transcription from RNA polymerase II promoter, negative regulation of sequence-specific DNA binding transcription factor activity, transcription factor binding, transcription factor binding, and nuclear hormone receptor binding (Figure 4c). The miRNA–mRNA regulatory network for the key genes is shown in Figure 4d.

Connectivity Map Analysis of the Key Genes

Furthermore, we conducted CMap analysis, which revealed 15 small-molecule drugs that might potentially target the

Table 1. Predicted Chemical Drugs Targeting Key Genes in Atherosclerosis

CMap Name	Mean	n	Enrichment	*P*	Specificity	Percent Non-null
Rottlerin	−0.724	3	−0.931	<.001	0.0252	100
Picotamide	−0.516	5	−0.757	.002	0	100
Cephaeline	−0.588	5	−0.747	.002	0.1386	100
Dacarbazine	−0.423	4	−0.738	.009	0.0052	75
Mycophenolic acid	0.606	3	0.886	.003	0.0503	100
Etynodiol	0.54	4	0.875	<.001	0	100
Meteneprost	0.591	4	0.86	<.001	0	100
Ifosfamide	0.554	3	0.844	.007	0	100
Iopamidol	0.485	4	0.799	.003	0.0189	100
Furosemide	0.407	4	0.782	.004	0.0116	100
Enalapril	0.495	4	0.759	.006	0.007	75
Benzathine benzylpenicillin	0.36	4	0.747	.008	0.0286	75
Dipivefrine	0.422	4	0.737	.009	0.0063	75
Ambroxol	0.39	4	0.735	.010	0.0654	75

key genes, and by ranking the enrichment score in descending order, the top 4 chemicals were identified as being potential treatment options for atherosclerosis, including rottlerin, picotamidethe, mycophenolic acid, and ethynodiol (Table 1). Therefore, these drugs could be used for drug development research in atherosclerosis.

DISCUSSION

Atherosclerosis is one of the leading causes of mortality worldwide, and the absolute number of deaths is increasing each year.[15] Previous studies have shown that both DNA methylation and miRNA expression are implicated in the pathogenesis of cardiovascular diseases;[16] however, their role in the regulation of gene expression in atherosclerosis remains unclear. In this study, we performed comparative analysis of DNA methylation (GSE46394) and mRNA (GSE12288) and miRNA (GSE53675) expression data from the GEO database and identified key atherosclerosis-rela ted genes whose expression could be regulated both through DNA methylation and miRNA activity.

By overlapping DEGs and DMGs, we identified 90 hypermethylated and 47 hypomethylated genes downregulated and upregulated in atherosclerosis, respectively. Because there are few methylation and gene expression data sets from the same tissue, we selected the methylation and corresponding gene expression data sets from aortas and blood, respectively, to identify MDEGs. On the other hand, it has been revealed that brain tissue of patients with Alzheimer's disease and blood of patients with atherosclerosis share some similar methylation profiles.[17] Therefore, our study may help to better understand the regulatory mechanisms of methylation in atherosclerosis. However, further validation is necessary. Moreover, the functional enrichment analysis results indicated that these genes were involved in the processes relevant to atherosclerosis, such as immune response and cell adhesion. Atherosclerosis is a chronic inflammatory disease that affects the arterial wall,[18] and both innate and adaptive immune responses contribute to the development of atherosclerotic lesions by influencing lipoprotein deposition and oxidation in arterial walls.[19] Interferon-gamma (IFN-γ), a key cytokine implicated in both innate and adaptive immunity, is an important factor in atherogenesis, affecting vascular inflammation, oxidative stress, vascular smooth muscle cell (VSMC) proliferation and migration, and plaque formation and rupture.[20]

Our integrative analysis of miRNA and mRNA expression data revealed 142 miRNA-targeted DEGs in atherosclerosis; among them, 24 DEGs from the GSE53675 data set were regulated by both miRNAs and DNA methylation. The expression patterns of *DCK*, *CACNA1C*, *NRP1*, *CCDC88C*, *TCF12*, *ABLIM1*, *TCF7L2*, *GABBR2*, *FANCC*, and *PBX1* were validated in another atherosclerosis data set, indicating that these genes may be key players in the etiology of the disease. Functional enrichment analysis showed that the 10 key genes were mainly involved in transcriptional regulation, including negative regulation of sequence-specific DNA-binding transcription factor activity. Increasing amounts of evidence have suggested that DNA methylation may play an important role in plaque progression and vulnerability,[21] which indicates that DNA methylation may affect the binding of transcription factors to the corresponding targets, thus leading to alterations of gene expression in atherosclerosis. Despite some preliminary studies, some of the key genes have not been linked to atherosclerosis.

TCF7L2 is a transcription factor closely involved in the maintenance of vascular integrity. It has been shown that changes in TCF7L2 function can induce VSMC plasticity and initiate vessel wall reconstruction,[22] confirming that TCF7L2 is a regulator of vessel wall integrity and suggesting it as a potential therapeutic target in atherosclerosis. Neuropilin 1 (NRP1) is a receptor for class 3 semaphorins and a member of the vascular endothelial growth factor family, which plays a key role in embryonic angiogenesis.[23] In the mature vascular system, NRP1 exerts pleiotropic effects, acting as a proangiogenic factor and attenuating pathological tissue ischemia in a gene dose-dependent manner.[24,25] Previous reports have illustrated that *Npr1*-deficient mice have significantly reduced ventricular dilation and functional shortening, increased levels of inflammatory cytokines, and elevated nuclear factor-kappa B and activating protein-1-binding activity. The *CACNA1C* gene encodes an α-subunit of a voltage-dependent calcium channel, which is expressed in cardiac and smooth muscles, and is essential for the regulation of the plateau phase of cardiac action potential, cellular excitability, excitation-contraction coupling, and gene expression. CACNA1C serves as a target for Ca^{2+} channel blockers, playing a role in blood pressure control, and has been implicated in the pathogenesis of cardiovascular diseases such as atherosclerosis, essential hypertension, inherited arrhythmias, and sudden cardiac death.[26-29] A previous study suggests that the upregulation of *CACNA1C* expression may be used as a strategy to treat atherosclerosis-related diseases through inhibition of inflammatory response.[30] Cumulatively, these findings and our results suggest that the aberrant expression of the identified key genes due to dysregulation of DNA methylation and miRNA synthesis may disturb their functional activity in the vascular system, thus contributing to the development of atherosclerosis.

Conventional therapies for atherosclerosis are non-specific, and effective drugs are insufficient. Therefore, we searched the CMap database to identify potential drugs, which may be related to the identified key genes, and guide the development of novel atherosclerosis treatment strategies. Rottlerin is a natural product that is composed of chalcone and flavonoid scaffolds with multiple target molecules and biological effects. Rottlerin may be valuable in the development of therapeutic agents against angiogenesis through its anti-angiogenic and anti-proliferative therapies by blocking the NFjB—cyclin D-1 cascade and causing a decrease in ET-1 levels.[31,32] Picotamide, a derivative of methoxy-isophtalic acid, acts as an antiplatelet agent and exerts dual pharmacological effects *in vivo* through inhibition of the thromboxane A2 receptor and thromboxane A2 synthase.[33] Large randomized studies have shown that picotamide could slow the evolution of early carotid atherosclerotic lesions by inhibiting plaque growth and preventing their formation, suggesting that the drug may be effective in treating individuals at risk for atherosclerotic thrombosis.[34] Mycophenolic acid is an immunosuppressant used in clinics to prevent graft rejection, which also shows anti-cancer and anti-viral properties.[35] However, the mechanism of these prospective medicines is unclear, and additional experiments are required to validate the therapeutic effects of these prospective medicines on atherosclerosis.

Study Limitations

First, the sample size was small. Second, because of the lack of clinical information for patients with atherosclerosis in the public data sets we used, the relationship between the key genes and clinicopathological features could not be evaluated. Finally, the CMap 02 database is a bioinformatics tool to identify novel applications for established drugs; however, this target protein-based approach to drug discovery involves many different pathways. Therefore, additional basic and clinical research is required to evaluate the role of the identified key genes in atherosclerosis and the regulatory influence of DNA methylation and miRNAs on their expression.

CONCLUSION

We identified *TCF7L2*, *CACNA1C*, *NRP1*, *GABBR2*, *FANCC*, *DCK*, *CCDC88C*, *TCF12*, *ABLIM1*, and *PBX1* as the key genes regulated through DNA methylation and miRNA activity in atherosclerosis. Fifteen chemicals with potential effects on the key genes were suggested as possible therapeutic agents for atherosclerosis worth further investigation. Our findings lay a foundation for future research on specific genes with a role in atherosclerosis, which would be beneficial in developing novel diagnostic and treatment approaches.

Ethics Committee Approval: The current research follows the GEO data access policies and publication guidelines. Thus, the present study was exempted from the approval of local ethics committees.

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.C., F.Y.; Design – Y.C., Y.H., Z.Z.; Supervision – Z.Z., Y.L., Y.C., F.Y; Fundings – F.Y.; Materials – Y.L., Y.H., C.L.; Data collection &/or processing – Y.H., L.W., B.W., C.L.; Analysis &/or interpretation – Y.C., L.W., Y.L., B.W.; Literature search – C.L., B.W., L.W., Y.C.; Writing – Y.C.; Critical review – Z.Z., F.Y.

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Declaration of Interests: The authors declare that they have no competing interest.

Funding: GEO belongs to public databases. The patients involved in the databases have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest. In addition, the code for this study can be found at https://github.com/YankunCuiLabs/Atherosclerosis.

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Table S1. Top 50 Downregulated Differentially Expressed Genes and Expression Levels ($P < .05$)

Gene	LogFC	AveExpr	*t*	*P*	adj.*P*.Val	B
NCAPH	−0.42420	4.36604	−4.24336	.00003	.20262	1.18864
CEBPA	−0.21650	8.48234	−3.89966	.00013	.29192	0.22175
ACSM3	−0.33151	5.24816	−3.89145	.00013	.29192	0.19947
BTNL8	−0.59549	6.43693	−3.45462	.00066	.56048	−0.92729
AZGP1P1	−0.45334	4.83423	−3.41707	.00075	.56048	−1.01873
ITPK1	−0.13419	8.39107	−3.41103	.00077	.56048	−1.03336
ZNF518A	−0.28367	5.26105	−3.41063	.00077	.56048	−1.03432
TMEM158	−0.62621	6.10389	−3.38641	.00084	.56048	−1.09274
RIMS2	−0.28365	5.06438	−3.27722	.00122	.56048	−1.35157
UBOX5	−0.23991	6.72277	−3.26572	.00126	.56048	−1.37837
GPR15	−0.40961	4.77313	−3.20278	.00156	.56048	−1.52371
GINS4	−0.41712	5.06484	−3.16961	.00174	.56048	−1.59928
MS4A4A	−0.35216	5.46324	−3.16506	.00177	.56048	−1.60960
DBF4B	−0.41520	5.40963	−3.13769	.00193	.56048	−1.67135
DHRS2	−0.29505	4.72509	−3.12552	.00201	.56048	−1.69865
TRAF3IP2	−0.23782	6.14006	−3.12026	.00205	.56048	−1.71043
LMF2	−0.13520	8.10620	−3.11599	.00207	.56048	−1.71997
CHRNA5	−0.36838	5.37261	−3.11024	.00211	.56048	−1.73279
CLN3	−0.09600	8.55706	−3.09711	.00220	.56048	−1.76202
RP11-457I16.2	−0.33091	5.67464	−3.08396	.00230	.56048	−1.79119
MEIS1	−0.35010	5.57833	−3.06973	.00241	.56048	−1.82261
SH3BP2	−0.08729	7.58459	−3.06163	.00247	.56048	−1.84044
MAN2A2	−0.11816	8.02322	−3.05729	.00251	.56048	−1.84996
ELAVL2	−0.51443	3.64348	−3.04639	.00260	.56048	−1.87386
AGPAT1	−0.09885	8.71547	−3.04156	.00264	.56048	−1.88442
SEMA3C	−0.17196	5.60622	−2.97836	.00322	.61736	−2.02126
CBX5	−0.17351	7.06001	−2.97528	.00325	.61736	−2.02785
NFX1	−0.10961	6.68208	−2.97362	.00327	.61736	−2.03142
ZNF80	−0.53684	3.21149	−2.96068	.00340	.61736	−2.05906
FAM118A	−0.20646	6.96513	−2.95756	.00343	.61736	−2.06573
PLAUR	−0.11682	8.47853	−2.93966	.00363	.63288	−2.10376
ARHGAP4	−0.16113	8.10204	−2.92976	.00374	.64190	−2.12471
GULP1	−0.27422	4.29483	−2.90953	.00399	.64190	−2.16733
PIP	−0.37139	5.67257	−2.90277	.00407	.64190	−2.18150
MTF1	−0.10478	7.75893	−2.89953	.00411	.64190	−2.18828
MGLL	−0.14377	8.07346	−2.89398	.00418	.64190	−2.19989
KRT13	−0.44882	4.16985	−2.87121	.00448	.64190	−2.24730
DDC	−0.29891	5.07996	−2.86142	.00462	0.64190	−2.26757
SH3TC1	−0.13177	7.61029	−2.85577	.00470	.64190	−2.27924
FEV	−0.23702	4.14836	−2.84238	.00489	.64190	−2.30683
HIST3H2A	−0.24503	7.79990	−2.84086	.00492	.64190	−2.30996
CDK16	−0.12661	6.88073	−2.84050	.00492	.64190	−2.31070
CPSF4	−0.12119	7.97814	−2.82983	.00508	.64190	−2.33258
SPO11	−0.48025	4.54656	−2.82092	.00522	.64190	−2.35078
ZDHHC18	−0.14369	9.36060	−2.81533	.00531	.64190	−2.36218
USB1	−0.18880	8.27328	−2.81082	.00538	.64190	−2.37138
STC2	−0.22912	5.57037	−2.81043	.00539	.64190	−2.37216
ERC2-IT1	−0.37091	5.03421	−2.79647	.00562	.64190	−2.40049
BLOC1S1	−0.09496	9.08088	−2.77594	.00597	.64190	−2.44194
GEMIN7	−0.33456	6.05866	−2.77050	.00607	.64190	−2.45287

Table S2. The top 50 Upregulated Differentially Expressed Genes and Expression Levels ($P < .05$)

Gene	LogFC	AveExpr	*t*	*P*.Value	adj.*P*.Val	B
PTPN4	0.24502	7.95240	4.37944	.00002	.20262	1.58983
ATMIN	0.15971	6.88938	3.92778	.00011	.29192	0.29830
KIZ	0.51418	4.77742	3.87615	.00014	.29192	0.15808
ZMYND11	0.15893	7.74797	3.72580	.00025	.38852	−0.24128
PAM	0.26714	6.31302	3.72476	.00025	.38852	−0.24399
ZNF639	0.50583	4.63412	3.64945	.00033	.41184	−0.43894
SUZ12	0.15684	7.84603	3.64871	.00033	.41184	−0.44083
TMED10	0.15021	8.65123	3.48591	.00059	.56048	−0.85041
BCLAF1	0.15646	7.18669	3.43258	.00071	.56048	−0.98107
NAA50	0.21904	7.11358	3.37507	.00087	.56048	−1.11997
MAPKBP1	0.44910	5.56383	3.34306	.00097	.56048	−1.19639
PAPOLA	0.10131	7.61747	3.33271	.00101	.56048	−1.22097
PKIA	0.34683	5.99390	3.29420	.00115	.56048	−1.31181
CCDC28A	0.10400	8.66035	3.28556	.00118	.56048	−1.33207
SMYD5	0.29589	4.50978	3.28190	.00120	.56048	−1.34061
HIF1A	0.18443	8.73137	3.28002	.00120	.56048	−1.34502
ASUN	0.37387	5.41993	3.26229	.00128	.56048	−1.38638
AKR1C3	0.51856	5.04867	3.25866	.00129	.56048	−1.39480
RIC8B	0.35473	6.07938	3.24680	.00135	.56048	−1.42233
DYRK2	0.15684	6.75151	3.23386	.00141	.56048	−1.45227
LRRC47	0.16261	7.58968	3.21846	.00148	.56048	−1.48774
FAM208A	0.13743	7.27653	3.21355	.00151	.56048	−1.49901
TES	0.11509	8.26281	3.20906	.00153	.56048	−1.50933
CHMP7	0.12180	8.63754	3.18846	.00164	.56048	−1.55642
SCRIB	0.34049	6.16392	3.16815	.00175	.56048	−1.60258
SARAF	0.16012	10.10779	3.15726	.00181	.56048	−1.62724
SATB1	0.16816	9.34139	3.14840	.00187	.56048	−1.64725
ZNF83	0.42936	5.26587	3.12431	.00202	.56048	−1.70135
DCK	0.26040	6.02135	3.11564	.00208	.56048	−1.72074
MYBL1	0.31415	6.78184	3.09819	.00220	.56048	−1.75962
MAGEF1	0.30696	6.14272	3.06497	.00245	.56048	−1.83309
PRPF4B	0.18728	6.55797	3.05409	.00253	.56048	−1.85699
UPK3B	0.31561	5.84882	3.05169	.00255	.56048	−1.86226
UBE2G1	0.11653	7.92087	3.04735	.00259	.56048	−1.87176
DES	0.30145	5.09683	3.03083	.00273	.57030	−1.90785
CECR5	0.11043	7.59183	2.99704	.00303	.61736	−1.98109
NDUFS7	0.12770	8.30733	2.99202	.00308	.61736	−1.99190
PPP2R5C	0.11450	7.78984	2.97626	.00324	.61736	−2.02576
THADA	0.17096	6.36384	2.96766	.00333	.61736	−2.04418
C1orf50	0.39750	5.74435	2.95669	.00344	.61736	−2.06757
ARL4C	0.12577	8.70878	2.94134	.00361	.63288	−2.10020
GZMB	0.25464	9.12107	2.91362	.00394	.64190	−2.15873
CACNA1C	0.29952	4.53222	2.91261	.00395	.64190	−2.16086
AGPAT4	0.37032	5.48568	2.89529	.00416	.64190	−2.19715
CLSTN3	0.39282	6.12442	2.88668	.00428	.64190	−2.21512
ITGAV	0.33998	5.11404	2.88399	.00431	.64190	−2.22072
RAP1B	0.13669	9.95654	2.87896	.00438	.64190	−2.23121
HSPH1	0.17842	6.50064	2.87863	.00438	.64190	−2.23189
SH3YL1	0.25741	7.21868	2.87779	.00439	.64190	−2.23364
CERK	0.12678	8.67370	2.86467	.00457	.64190	−2.26085