Original Article

Access this article online

Quick Response Code:

Website: http://www.braincirculation.org

DOI: 10.4103/bc.bc_63_23

Explore the role of long noncoding RNAs and mRNAs in intracranial atherosclerotic stenosis: From the perspective of neutrophils

Yilin Wang[1#], Tao Wang[2#], Ziping Han[1], Rongliang Wang[1], Yue Hu[1], Zhenhong Yang[1], Tong Shen[1], Yangmin Zheng[1], Jichang Luo[2,3], Yan Ma[2,3], Yumin Luo[1,3,4], Liqun Jiao[2,3]

[1]Institute of Cerebrovascular Disease Research, Xuanwu Hospital of Capital Medical University, [2]Department of Neurosurgery, Xuanwu Hospital of Capital Medical University, [3]Beijing Institute for Brain Disorders, Capital Medical University, [4]Beijing Geriatric Medical Research Center, Beijing Key Laboratory of Translational Medicine for Cerebrovascular Diseases, Beijing, China

[#]Yilin Wang and Tao Wang contribute equally to this article

Address for correspondence:
Dr. Yumin Luo, Institute of Cerebrovascular Diseases Research, Xuanwu Hospital of Capital Medical University, 45 Changchun Street, Beijing 100 053, China.
E-mail: yumin111@ccmu.edu.cn and Liqun Jiao: liqunjiao@sina.cn

Submission: 29-07-2023
Revised: 22-09-2023
Accepted: 26-09-2023
Published: 30-11-2023

Abstract:

CONTEXT: Circulating neutrophils and long noncoding RNAs (lncRNAs) play various roles in intracranial atherosclerotic stenosis (ICAS).

OBJECTIVE: Our study aimed to detect differentially expressed (DE) lncRNAs and mRNAs in circulating neutrophils and explore the pathogenesis of atherosclerosis from the perspective of neutrophils.

METHODS: Nineteen patients with ICAS and 15 healthy controls were enrolled. The peripheral blood of the participants was collected, and neutrophils were separated. The expression profiles of lncRNAs and mRNAs in neutrophils from five patients and five healthy controls were obtained, and DE lncRNAs and mRNAs were selected. Six lncRNAs were selected and validated using quantitative reverse transcription–polymerase chain reaction (qRT-PCR), and ceRNA and lncRNA-RNA binding protein (RBP)-mRNA networks were constructed. Correlation analysis between lncRNAs and mRNAs was performed. Functional enrichment annotations were also performed.

RESULTS: Volcano plots and heat maps displayed the expression profiles and DE lncRNAs and mRNAs, respectively. The qRT-PCR results revealed that the four lncRNAs showed a tendency consistent with the expression profile, with statistical significance. The ceRNA network revealed three pairs of regulatory networks: lncRNA RP3-406A7.3-NAGLU, lncRNA HOTAIRM1-MVK/IL-25/GBF1/CNOT4/ANKK1/PLEKHG6, and lncRNA RP11-701H16.4-ZNF416. The lncRNA-RBP-mRNA network showed five pairs of regulatory networks: lncRNA RP11-701H16.4-TEK, lncRNA RP11-701H16.4-MED17, lncRNA SNHG19-NADH-ubiquinone oxidoreductase core subunit V1, lncRNA RP3-406A7.3-Angel1, and lncRNA HOTAIRM1-CARD16.

CONCLUSIONS: Our study identified and verified four lncRNAs in neutrophils derived from peripheral blood, which may explain the transcriptional alteration of neutrophils during the pathophysiological process of ICAS. Our results provide insights for research related to the pathogenic mechanisms and drug design of ICAS.

Keywords:

Atherosclerosis, inflammation, stroke

Introduction

In recent years, stroke has become one of the main causes of death and disability in recent years.[1] It has been reported that 46.6% of stroke patients are diagnosed with intracranial occlusive diseases that can cause more severe stroke and increase the risk of recurrent stroke, especially intracranial atherosclerotic stenosis (ICAS).[2] Atherosclerosis is a lipid-driven inflammation of arteries.

How to cite this article: Wang Y, Wang T, Han Z, Wang R, Hu Y, Yang Z, *et al*. Explore the role of long noncoding RNAs and mRNAs in intracranial atherosclerotic stenosis: From the perspective of neutrophils. Brain Circ 2023;9:240-50.

Neutrophils heavily contribute to the initiation and progression of atherosclerosis, including promoting endothelial dysfunction, monocyte accumulation, and foam cell formation.[3,4] Neutrophils exacerbate endothelial dysfunction, recruit monocytes to atherosclerotic foci, and facilitate foam cells.[3] Neutrophils can form neutrophil extracellular traps (NETs) consisting of decondensed chromatin, cytoplasm, granular proteins, and nucleus.[3] NETs stimulate macrophages to release cytokines, activate Th17 cells, and promote immune cell accumulation around the atherosclerotic lesions.[5] Long noncoding RNAs (lncRNAs) are RNAs longer than 200 nucleotides that cannot be translated into proteins. More than 18,000 lncRNAs have been annotated in the human genome, which can interact with RNA, DNA, and proteins and participate in various cellular biology processes and the pathogenesis of human diseases.[6] Recently, several studies have reported that various lncRNAs participate in the pathological processes of atherosclerosis, plaque inflammation, and destabilization.[7] Despite the severe consequences of ICAS, the potential pathology and pathogenesis remain unclear. In our study, we collected peripheral blood from patients with ICAS and healthy controls, separated neutrophils, and detected the lncRNA expression profiles and mRNA expression profiles in neutrophils, aiming to explore the pathogenesis of atherosclerosis from the perspective of neutrophils.

Methods

Patient inclusion and clinical sample

This study conformed to the principles of the Declaration of Helsinki. The Ethics Committee of Xuanwu Hospital supported our study (No. [2021]083 in October 2021), and written informed consent was obtained from all enrolled participants. In this study, 19 patients with ICAS (diagnosed using high-resolution magnetic resonance imaging) and 15 healthy controls were enrolled. Regarding the patients, there were several exclusion criteria: previous history of other neurological diseases, other system diseases, and long-term drug intake. Neutrophils derived from the peripheral blood of all enrolled individuals were collected by density gradient centrifugation. LncRNA and mRNA microarray analyses were performed on five patients and five healthy controls. Further verification was done using quantitative reverse transcription–polymerase chain reaction (qRT-PCR) on the remaining 14 patients and 10 healthy controls.

Long noncoding RNA and mRNA microarrays and identification of differentially expressed long noncoding RNAs and mRNAs

Total RNAs were extracted, and quality and quantity were evaluated. Human lncRNA and mRNA microarray analyses were also performed. Sample labeling and array hybridization were performed according to the manufacturer's instructions. After cRNA was fragmented, it was heated. A gasket slide with a diluted hybridization solution was installed on the lncRNA and mRNA expression microarray slides. The slides were incubated, washed, fixed, and scanned using an Agilent DNA Microarray Scanner. Expression profiling of the ICAS group was performed using fold change (FC) against the average of the control subjects. Differentially expressed (DE) lncRNAs or mRNAs were defined as FC >2.00 and $P < 0.05$. Raw data were normalized, and DE lncRNAs and DE mRNAs were selected.

Quantitative reverse transcription–polymerase chain reaction verification of long noncoding RNAs

To verify the credibility of lncRNA microarray results, we selected several lncRNAs for validation using quantitative reverse transcriptionpolymerase chain reaction (qRTPCR). The selection criteria were as follows: (1) relatively novel, (2) higher FC, (3) lower *P* value, and (4) less intragroup heterogeneity. Finally, six DE lncRNAs were selected and validated using qRT-PCR in 14 patients with ICAS and 10 healthy controls. Total RNAs were extracted and reverse transcribed into cDNA using TRIzol reagent (Invitrogen) and SuperScript III reverse transcriptase (Invitrogen), respectively. LncRNA expression levels were estimated using qRT-PCR via the ViiA 7 Real-time PCR System (Applied Biosystems) with β-actin as internal parameters. The primers used are listed in Supplementary Table 1. All reactions were performed in triplicate. The expression level of lncRNAs was converted into FC by adopting the 2-ΔΔCt method.

Construction of ceRNA network and long noncoding RNA-RNA binding protein-mRNA network

We constructed a ceRNA network and lncRNA-RNA binding protein (RBP)-mRNA network for the validated lncRNAs. The detailed processes are shown in Figure 1. In the preliminary ceRNA network, lncRNA–miRNA and miRNA–mRNA interactions were predicted using TargetScan and miRanda, respectively. To simplify the ceRNA network, the predicted mRNAs intersected with DE mRNAs. mRNAs intersecting with their corresponding miRNAs were extracted from the preliminary network and placed into a simplified ceRNA network. In the lncRNA-RBP-mRNA network, lncRNA-RBP–RBP-mRNA interactions were predicted using the RBPDB and Encori databases. Predicted mRNAs intersected with DE mRNAs. Intersecting mRNAs and their corresponding RBPs were combined to form a preliminary lncRNA-RBP-mRNA network.

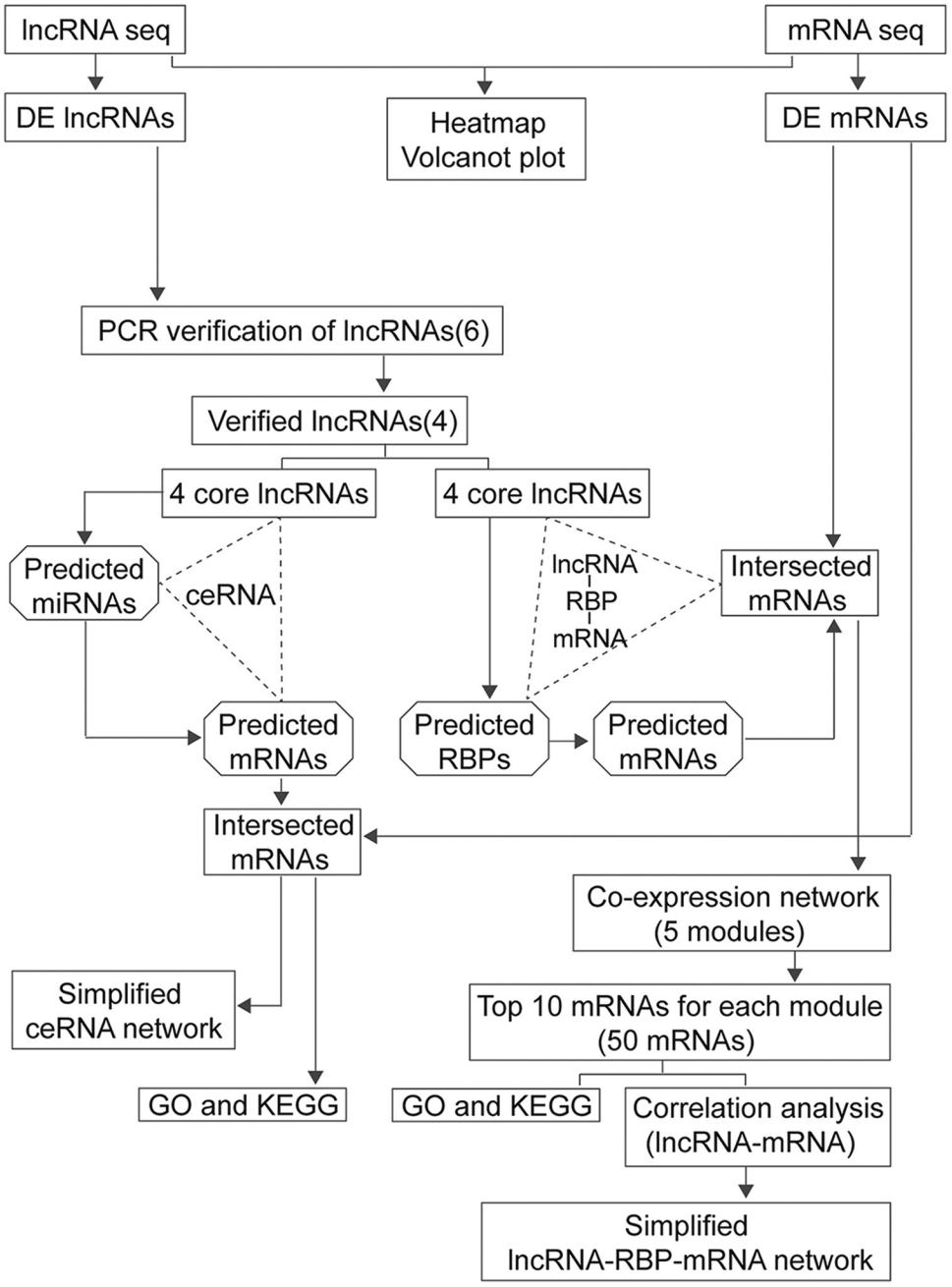

Figure 1: Flowchart and quantitative reverse transcription–polymerase chain reaction (qRT-PCR) verification of long noncoding RNAs (lncRNAs). (a) Flowchart displaying the analysis procedure. DE: Differentially expressed, RBP: RNA binding protein, GO: Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes. (b) qRT-PCR verification of lncRNAs. Red, upregulated; blue, downregulated; ICAS: intracranial atherosclerotic stenosis; ,: P <0.05; ,,: P <0.01; ,,,: P <0.001

Co-expression network construction and functional enrichment annotation

A co-expression network was established for all mRNAs in the preliminary lncRNA-RBP-mRNA network, and the top 10 hub genes for each module were selected via Integrated Differential Expression and Pathway analyses (IDEP. 96). Correlation analysis was performed for all hub genes and lncRNAs using the OECloud tool (https://cloud.oebiotech.com). The hub genes with the highest correlation coefficient in each module were placed in the simplified lncRNA-RBP-mRNA network, combined with their corresponding RBPs. Meanwhile, gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of all the hub genes in the co-expression network and all the mRNAs in the simplified ceRNA network were performed via the "clusterProfiler" package in R and WEB-based Gene SeT AnaLysis Toolkit (WebGestalt), respectively. The GO terms included biological processes (BP), cellular components (CC), and molecular functions (MF).

Statistical analysis and graph plotting

Statistical analysis was executed via SPSS Statistics 23.0 (Armonk, NY: IBM Corp.), and a $P < 0.05$ was considered statistically significant. Graphs were drawn using GraphPad Prism 8.0 (SanDiego, CA, USA), R software (version 4.4.1, the R foundation, New Zealand), Cytoscape 3.9.1, Adobe Illustrator 2020, IDEP. 96, WebGestalt, and two online platforms (https://www.bioinformatics.com.cn and http://www.genome.ad.jp/kegg/).

Results

Identification of differentially expressed long noncoding RNAs and mRNAs and quantitative reverse transcription–polymerase chain reaction verification of long noncoding RNAs

The expression profiles of lncRNAs and mRNAs were visualized using volcano plots, and DE lncRNAs and mRNAs (FC >2.00 and $P < 0.05$) were visualized using heat maps [Figure 2a]. Subsequently, six DE lncRNAs were selected and validated using qRT-PCR. LncRNA-RP3-406A7.3, lncRNA-RP11-70C1.3, and lncRNA-SNHG19 were upregulated, and lncRNA-HOTAIRM1, lncRNA-RP1-244F24.1, and lncRNA-RP11-701H16.4 were downregulated [Figure 2b]. The results of qRT-PCR showed that there were four lncRNAs (lncRNA-RP3-406A7.3, lncRNA-SNHG19, lncRNA-HOTAIRM1, and lncRNA-RP11-701H16.4) that showed expression tendencies consistent with the results of lncRNA microarray with statistical significance, which were considered the core lncRNAs in this study.

ceRNA network and functional enrichment annotation

A preliminary ceRNA regulatory network was constructed for the four core lncRNAs [Figure 3a]. The predicted mRNAs were then intersected with the DE mRNAs to obtain eight mRNAs, forming a simplified ceRNA network with their corresponding miRNAs [Figure 3b]. The simplified ceRNA network showed three pairs of lncRNA-mRNA regulatory networks: lncRNA RP3-406A7.3-NAGLU, lncRNA HOTAIRM1-MVK/interleukin (IL)-25/GBF1/CNOT4/ANKK1/PLEKHG6, and lncRNA RP11-701H16.4-ZNF416. GO and KEGG analyses were performed on these eight mRNAs [Figure 3c]. Enriched BP mainly consists of metabolic processes, biological regulation, response to stimuli, and cell communication. The enriched CC mainly consisted of the cytosol, extracellular space, membrane-enclosed lumen, membrane, microbody, and nucleus. The enriched MF mainly consisted of ion binding, protein binding, and transferase activities. The enriched KEGG primarily consisted of terpenoid

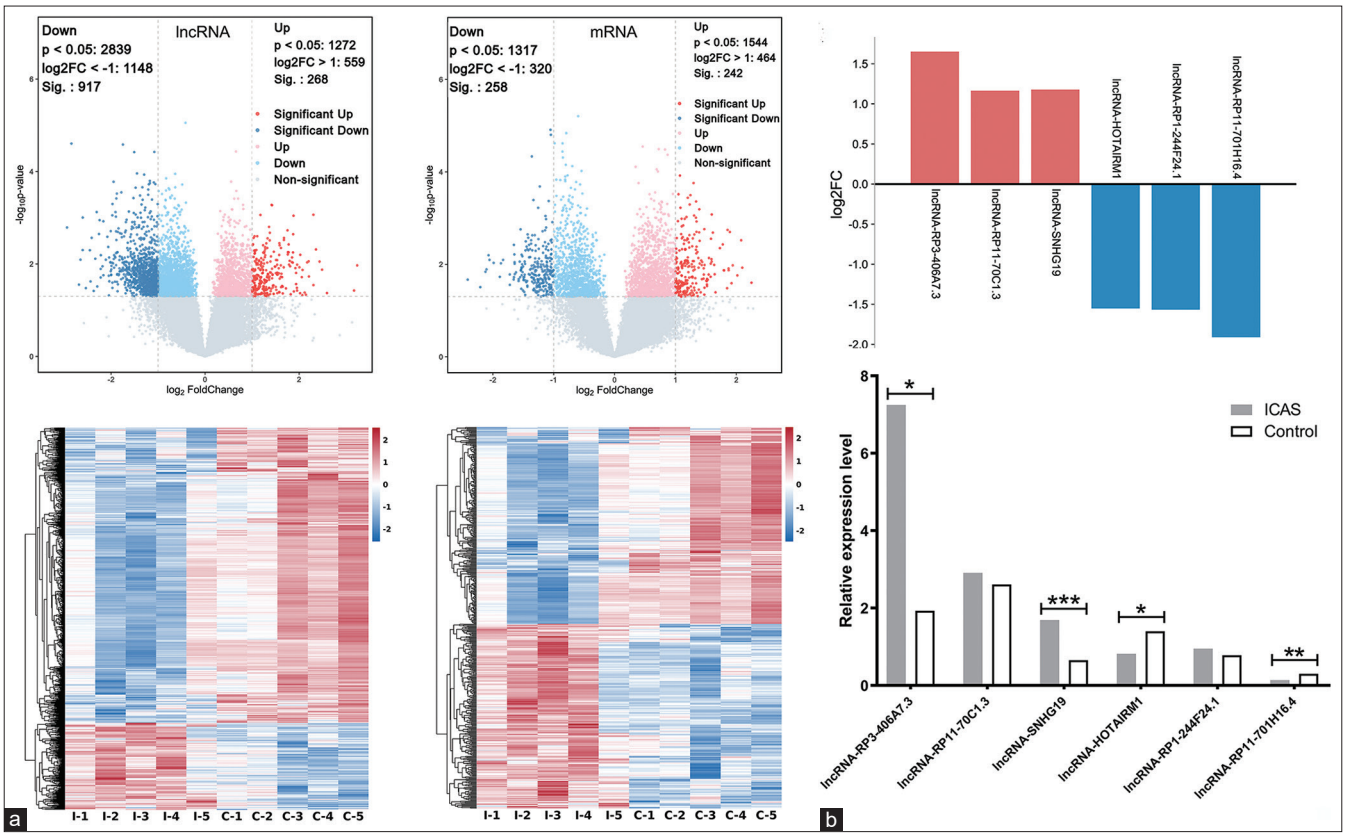

Figure 2: LncRNAs and mRNAs microarray profiles and qRT-PCR verification results. (a)Volcano plots and heatmaps of lncRNAs and mRNAs. Red: upregulated; blue: downregulated. (b) The expression level of six lncRNAs verified by qRT-PCR. Red: upregulated; blue: downregulated. FC: fold changes; ICAS: intracranial atherosclerotic stenosis; qRT-PCR: quantitative reverse transcription polymerase chain reaction. *P<0.05, **P<0.01, ***P<0.001.

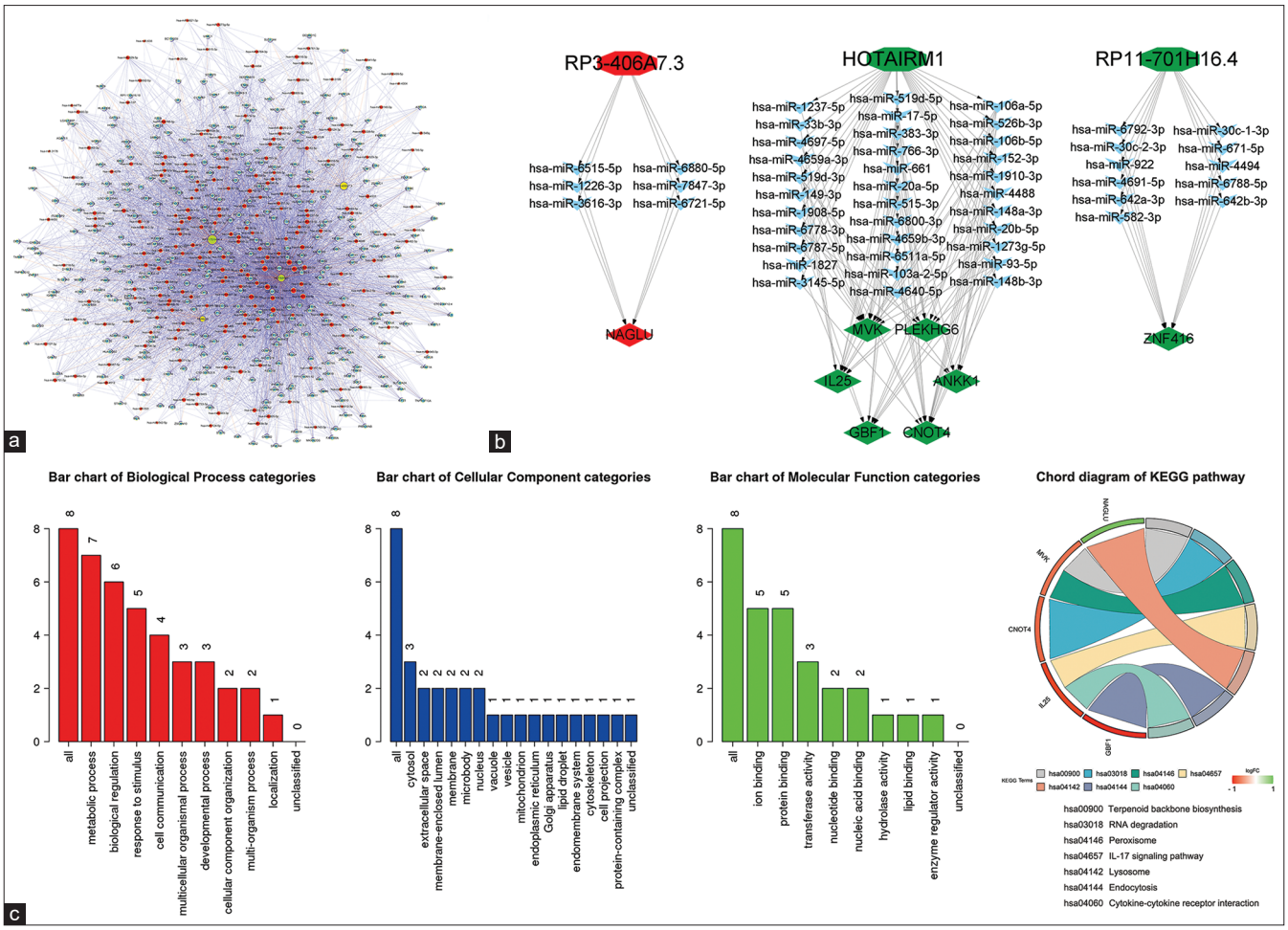

Figure 3: ceRNA network and functional enrichment annotation. (a) Preliminary ceRNA regulatory network. (b) Simplified ceRNA network. Octagons indicate long noncoding RNAs (lncRNAs); V-shapes indicate miRNAs; diamonds indicate mRNAs; red means upregulated; green means downregulated. (c) Functional enrichment annotation of the mRNAs in the simplified ceRNA network. KEGG: Kyoto Encyclopedia of Genes and Genomes

backbone biosynthesis, RNA degradation, peroxisomes, IL-17 signaling pathway, lysosomes, endocytosis, and cytokine–cytokine receptor interactions.

Long noncoding RNA-RNA binding protein-mRNA network, co-expression network, and functional enrichment annotation

Four core lncRNA-targeted RBPs were identified. The RBP-targeted mRNAs were then predicted and intersected with the DE mRNAs to obtain 447 DE-targeted mRNAs. Subsequently, combining the 447 mRNAs and their corresponding RBPs, a preliminary lncRNA-RBP-mRNA network was constructed for the four core lncRNAs [Figure 4]. For the mRNAs in the network, a co-expression network was established to obtain five modules [Figure 5]. Figure 6 shows the top 10 hub genes and correlation analysis between the four core lncRNAs and the top 10 hub genes in each module. The lncRNA-mRNA pair with the highest correlation coefficient is highlighted in the green box for each module: RP11-701H16.4-TEK (Module 1), lncRNA RP11-701H16.4-MED17 (Module 2), lncRNA SNHG19-NADH-ubiquinone oxidoreductase core subunit V1 (NDUFV1) (Module 3), lncRNA RP3-406A7.3-Angel1 (Module 4), and lncRNA HOTAIRM1-CARD16 (Module 5). For these four core lncRNAs and five mRNAs, the corresponding RBPs were extracted from the preliminary network and inserted into a simplified lncRNA-RBP-mRNA network [Figure 7]. GO and KEGG analyses were performed for the top 10 hub genes of each module [Figure 8]. The GO and KEGG terms related to the five mRNAs are highlighted in red boxes. TEK may function as a tyrosine kinase. TEK was enriched in the following BP: cell-substrate junction organization and assembly and focal adhesion assembly. TEK was also enriched in the HIF-1 signaling pathway. MED17 may enrich in the mediator complex. MED17 may function as a transcriptional coactivator, nuclear receptor-binding protein, and coactivator. In addition, MED17 was enriched in the thyroid hormone signaling pathway. NDUFV1 mainly enrich in the BP, CC, and MF that are involved in oxidative phosphorylation and mitochondrial function. In addition, NDUFV1 was enriched in retrograde endocannabinoid signaling.

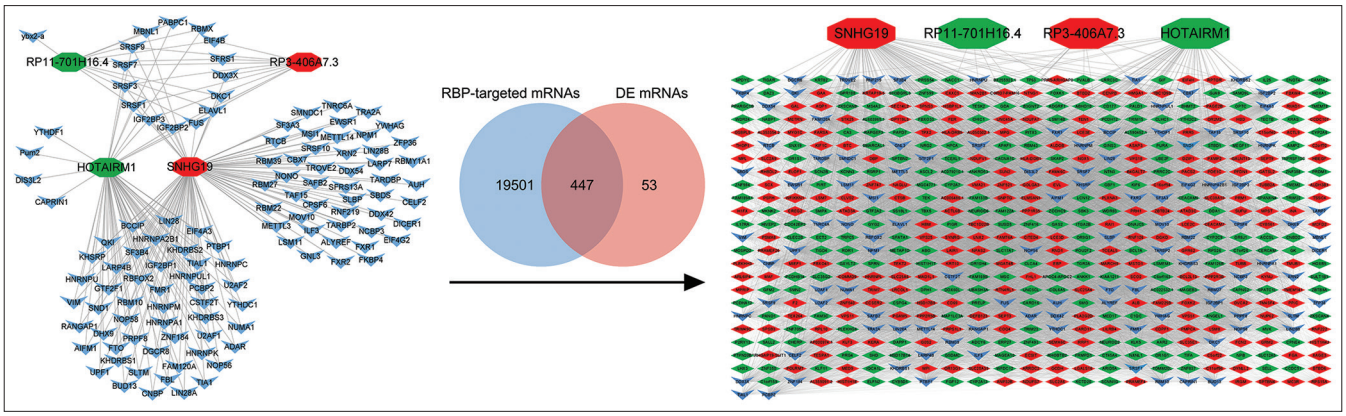

Figure 4: Construction of preliminary long noncoding RNA-RNA binding protein (lncRNA-RBP)-mRNA network. Gain of lncRNA-targeted RBPs; gain of RBP-targeted mRNAs; targeted mRNAs intersected with differentially expressed mRNAs; 447 intersected mRNAs and corresponding RBPs and lncRNAs constitute preliminary lncRNA-RBP-mRNA network. Octagons indicate lncRNAs; V-shapes indicate RBPs; diamonds indicate mRNAs; red, upregulated; green, downregulated

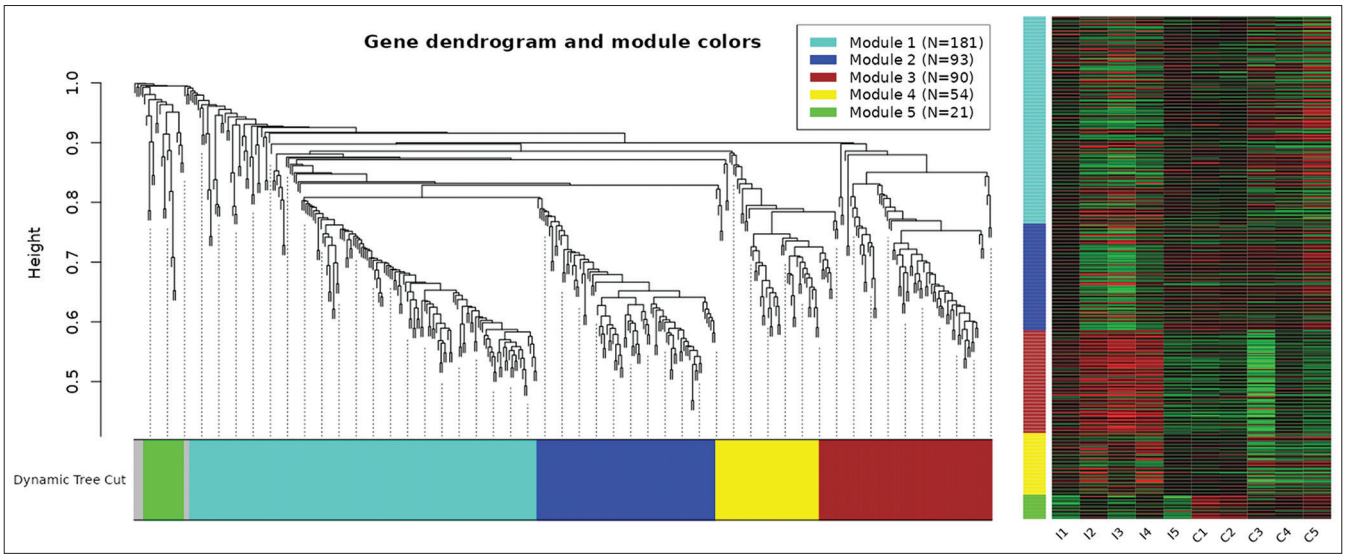

Figure 5: Construction of co-expression network. Co-expression network of mRNAs in the preliminary long noncoding RNA-RNA binding protein-mRNA network (5 modules)

Discussion

Recently, neutrophils have been considered regulators of ICAS, and previous studies have concentrated more on the effect of neutrophils on other cells or components of ICAS. Accordingly, the alteration of neutrophils *per se* has been ignored, and our study fills this knowledge gap. The present study uncovers four lncRNAs in neutrophils as potential therapeutic targets for ICAS. The ceRNA and lncRNA-RBP-mRNA networks may elucidate how neutrophils are responsible for ICAS development.

Long noncoding RNA RP3-406A7.3

In our study, lncRNA RP3-406A7.3 was upregulated in the ICAS group. To date, no literature is available regarding lncRNA RP3-406A7.3. Our study suggests that lncRNA RP3-406A7.3 may regulate NAGLU via miRNAs and Angel1 via RBPs, participating in the pathophysiological process of ICAS.

N-acetyl-alpha-glucosaminidase (NAGLU) can degrade heparan sulfate.[8] A previous study identified abnormally expressed NAGLU in endothelial cells as a biomarker of atherosclerosis.[9] However, the relationship between NAGLU and neutrophils has not yet been reported. In the present study, NAGLU expression was found to be upregulated. Further studies are required to explore the role of NAGLU in neutrophils in ICAS.

Angel1 belongs to the CCR4 deadenylase family and has a eukaryotic initiation factor 4E (eIF4E)-binding motif. Generally, Angel1 is located in the Golgi apparatus or endoplasmic reticulum rather than being widely distributed throughout the cell.[10] eIF4E plays various roles in the mRNA life cycle.[10] In eukaryotes, eIF4E binds with mRNA 5′ cap and eIF4G and then eIF3, and bridges

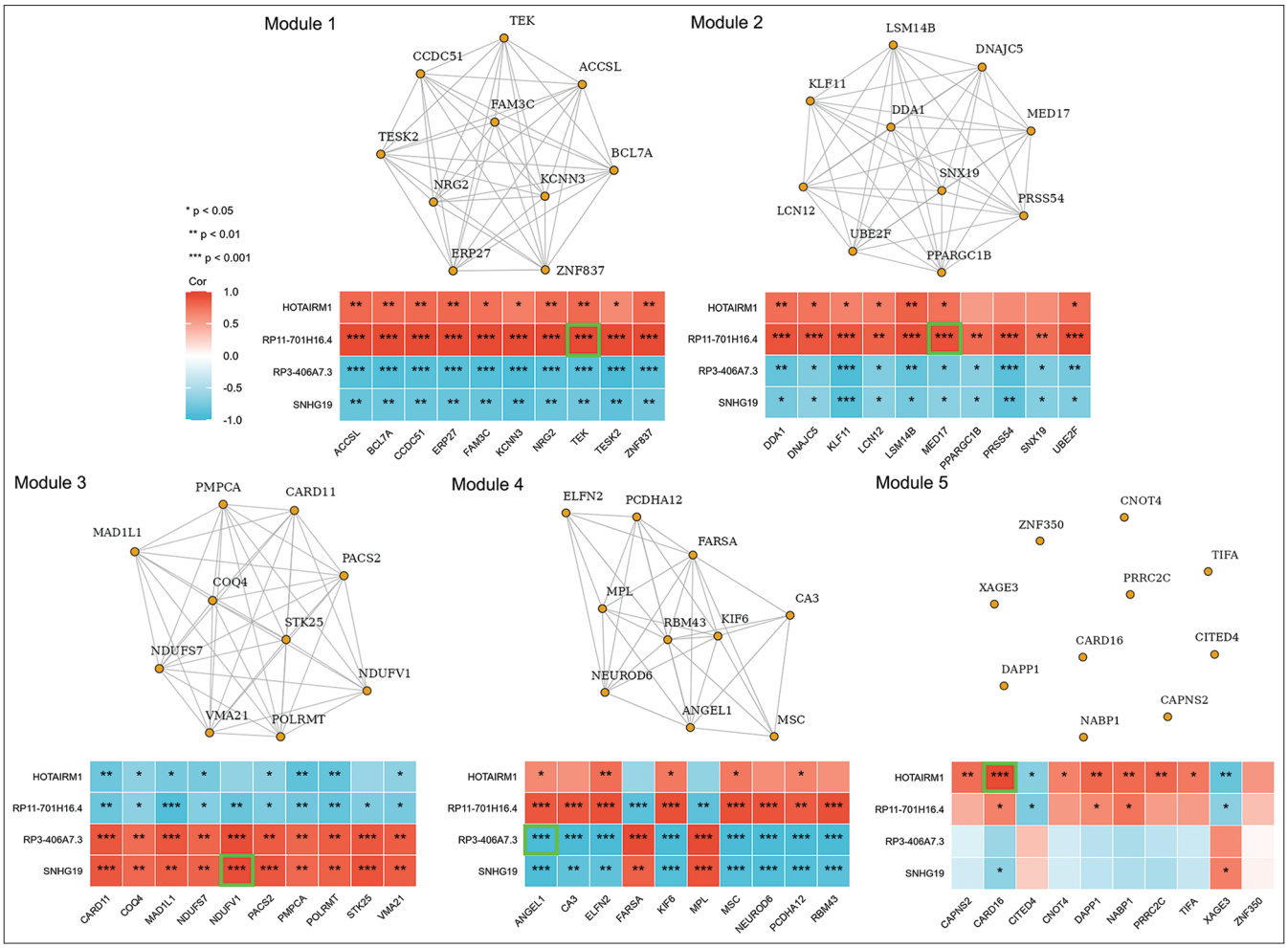

Figure 6: Correlation analysis of long noncoding RNAs (lncRNAs) and top 10 hub genes of each module. Red rectangle means positive correlation; blue rectangle means negative correlation; the darker the color is, the more significant the correlation is. Green box indicates the pair of lncRNA-mRNA with the highest correlation coefficient in each module. Protein–protein interaction network presents the interaction between the top 10 hub genes in each module. In the network, the gene and the interaction are defined as the node and the edge, respectively

mRNAs to ribosomes. eIF4E is a rate-limiting factor in protein synthesis.[11] Angel1 was identified as a partner of eIF4E that can compete with eIF4G for binding to eIF4E.[11] According to the literature, an original peptide derived from Angel1 can interact with eIF4E, inhibiting translation and causing massive cell death in cancer cell lines.[11] In the tumor microenvironment, eIF4E phosphorylation facilitates neutrophil survival and promotes metastasis. Inhibition of eIF4E phosphorylation can inhibit pathological neutrophil accumulation in a mouse model.[12] Neutrophil infiltration is vital in atherosclerosis.[13] In the present study, Angel1 expression was downregulated in the ICAS group. We deduced that decreased Angel1 expression might increase translation and neutrophil survival, contributing to the development of ICAS.

Long noncoding RNA HOX antisense intergenic RNA myeloid 1

HOX antisense intergenic RNA myeloid 1 (HOTAIRM1) is located between HOXA1 and HOXA2 and exhibits myeloid-specific expression.[14] The lncRNA HOTAIRM1 was initially identified in myeloid lineage cells and was shown to participate in cell differentiation. Recently, lncRNA HOTAIRM1 has been observed in various cells, such as neurons. LncRNA HOTAIRM1 facilitates autophagy and proliferation.[15] In our study, lncRNA HOTAIRM1 was downregulated in the ICAS group. Our study suggests that lncRNA HOTAIRM1 may regulate MVK, IL-25, GBF1, CNOT4, ANKK1, and PLEKHG6 via miRNAs and regulate CARD16 via RBPs, contributing to the pathophysiological process of ICAS.

MVK is located on chromosome 12q24 and consists of ten coding exons and one noncoding exon.[16] Previous studies have reported that MVK participates in cholesterol synthesis, a risk factor for atherosclerosis.[17] However, regulatory interactions between MVK and neutrophils have not yet been reported. CCR4-NOT transcription complex subunit 4 (CNOT4), an E3 ubiquitin ligase, interacts with ubiquitin-conjugating enzymes and

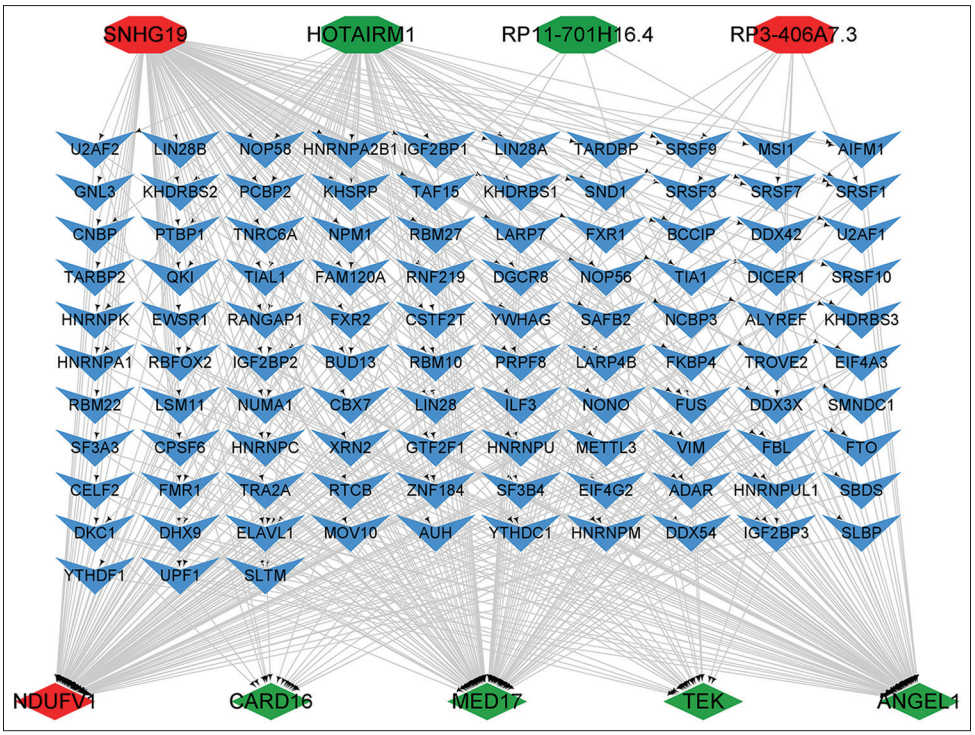

Figure 7: Simplified long noncoding RNA-RNA binding protein (lncRNA-RBP)-mRNA network. Pair of lncRNA-mRNA with the highest correlation coefficient in each module were extracted from the preliminary network, and put into the simplified network, combined with their corresponding RBPs. Octagons indicate lncRNAs; V-shapes indicate. RBPs; diamonds indicate mRNAs; red means upregulated; green means downregulated

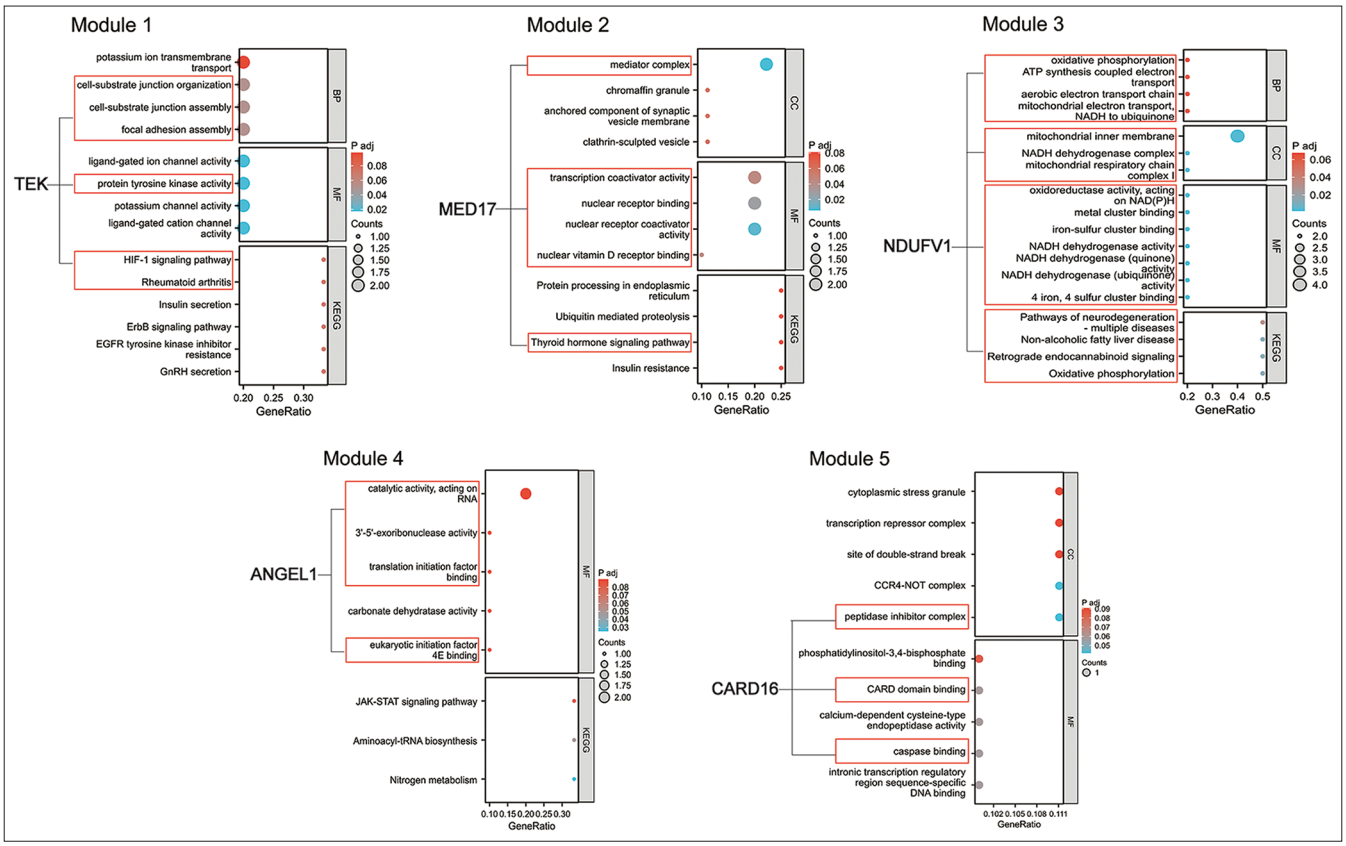

Figure 8: Functional enrichment annotation of the top 10 hub genes of each module and simplified long noncoding RNA-RNA binding protein (lncRNA-RBP)-mRNA network. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses for the top 10 hub genes of each module. GO terms consist of BP, MF, and CC. BP: biological process; MF: molecular function; CC: cellular component

participates in ubiquitination. CNOT4 is involved in transcriptional regulation.[18] There is no available data regarding CNOT4, neutrophils, and atherosclerosis. Ankyrin repeat and kinase domain containing 1 (ANKK1) is a receptor-interacting protein (serine/threonine kinase) that participates in various BP, such as cell survival and tissue differentiation.[19,20] To the best of our knowledge, no data is available on ANKK1, neutrophils, and atherosclerosis. We deduced that ANKK1 might regulate the survival of neutrophils that participate in atherosclerosis. Pleckstrin homology domain-containing family G with RhoGef domain member 6 (PLEKHG6) belongs to a guanine nucleotide exchange factor.[21] PLEKHG6 rapidly activates RhoG under the epidermal growth factor stimulation, which is involved in cell migration.[22] To the best of our knowledge, no literature is available regarding PLEKHG6, neutrophils, and atherosclerosis. Thus, we deduced that PLEKHG6 regulates neutrophil migration and participates in atherosclerosis. GBF1 indirectly regulates neutrophil chemotaxis.[23] In this study, GBF1 expression was downregulated. We deduced that GBF1 may modulate neutrophil chemotaxis and participate in ICAS. Studies have demonstrated that IL-25 has an anti-atherosclerosis role.[24] Consistent with this, in our study, IL-25 was downregulated, resulting in decreased anti-atherosclerotic effects. However, the relationship between the remaining four mRNAs in the ceRNA network and neutrophils/atherosclerosis has not been reported.

The caspase recruitment domain (CARD) regulates signaling pathways related to innate immune responses, caspase activation, and inflammasome assembly.[25] CARD16 (also known as pseudo-ICE) comprises 97 amino acids containing a solitary CARD motif.[25,26] CARD16 can bind with the CARD of caspase-1 (CASP1) and negatively regulate CASP1 activity.[27] According to the literature, the loss of CASP1 can decrease the transcription levels of inflammatory factors and reduce the recruitment of pulmonary neutrophils.[28] Additionally, IL-1beta (IL-1β) is known as a pro-inflammatory cytokine.[29] CARD16 can bind with CASP1, reducing IL-1β levels.[26] CARD16 interacts with Bcl-10, possibly by participating in other inflammatory processes.[27] Consistent with this finding, CARD16 expression was downregulated in the ICAS group in our study. We deduced that decreased CARD16 may carry increased CASP1 activity, increasing the recruitment of neutrophils and causing the development of ICAS.

Long noncoding RNA RP11-701H16.4

In our study, lncRNA RP11-701H16.4 was downregulated in the ICAS group. To the best of our knowledge, to date, no literature is available regarding lncRNA RP11-701H16.4. To the best of our knowledge, this is the first study to suggest that lncRNA RP11-701H16.4 can regulate ZNF416 via miRNAs and regulate MED17 and TEK via RBPs, thereby contributing to the pathophysiological process of ICAS.

ZNF416 is a histone acetyltransferase that has been identified as a novel transcriptional activator and repressor. ZNF416 regulates fibroblast differentiation and proliferation.[30] However, the relationship between ZNF416, neutrophils, and atherosclerosis remains unclear.

Mediator (MED) is a coactivator that connects transcriptional regulators and RNA polymerase (Pol) II and initiates gene transcription.[31,32] MED is a multiprotein complex consisting of more than 30 subunits and four modules: kinase, head, middle, and tail.[33] Mediator 17 (MED17), a subunit of the head module of MED, interacts with other subunits and is a key scaffold component. Generally, MED17 promotes transcription initiation and DNA repair.[34] Increasing evidence suggests that the transcriptional activity of neutrophils is vital in chronic inflammatory diseases. In early atherosclerosis, neutrophils present a nonresolving inflammatory state with the inhibition of homeostatic transcription factors in endotoxemia.[35] To the best of our knowledge, no previous study has investigated the relationship between MED17 and neutrophils. We deduced that MED17 may regulate the transcriptional activity of neutrophils, participating in inflammation and ICAS development.

TEK, a specific receptor for angiopoietin-1 (Ang-1), also known as TIE-2, with a molecular weight of 140kD.[36] Tie2 activates several pathways and triggers multiple downstream effects.[36] The Ang-Tie2 system is vital for the modulation of vascular integrity and quiescence. Ang-Tie2 is an important regulator of inflammation.[37] The Tie2 receptor is expressed on human neutrophils[38] and on neutrophils via active site ligation by Ang1 or Ang2.[39] According to the literature, Ang1-transfected mesenchymal stem cells alleviate granulocyte infiltration, pro-inflammatory cytokine expression, and alveolar inflammation.[40] Ang-1 inhibits granulocyte infiltration, chemokines, and pro-inflammatory cytokine IL-1β in the lungs of HV_T-ventilated mice model.[41] In our study, TIE-2 was downregulated in the ICAS group, which may have alleviated the anti-inflammatory effect of Ang-1. In other words, decreased TIE-2 may carry increased inflammation, resulting in the development of ICAS. However, other studies have suggested that Ang-1, a pro-inflammatory mediator, mediates neutrophil migration in Tie2 and CD18.[42] Notably, higher concentrations of Ang-1 decreased migratory response. Additionally, Tie2 inhibition did not completely block the chemotactic response to Ang-1, indicating that other factors may assist Ang-1 in mediating neutrophil migration.[42] Further studies are required to explore the role of TIE-2 in ICAS.

Long noncoding RNA SNHG19

SNHG19 has recently been identified as a novel lncRNA involved in several diseases.[43] lncRNA SNHG19 has been observed in the brains of patients with Alzheimer's disease[44] and breast cancer tissues.[45] To date, no studies have reported the relationship between lncRNA SNHG19 and neutrophils or atherosclerosis. To the best of our knowledge, this is the first study to suggest that lncRNA SNHG19 regulates NDUFV1 via RBPs, contributing to the pathophysiology of ICAS.

NDUFV1 is located on the mitochondrial complex I.[46,47] Clinically, mitochondrial complex I dysfunction is common in genetic diseases, especially human mitochondrial diseases.[48] Mitochondrial dysfunction is related to inflammation, oxidative stress, and cell apoptosis, and participates in the progression of atherosclerosis. The inhibition of mitochondrial activity can significantly alleviate atherosclerosis.[49] Activated neutrophils can produce reactive oxygen species (ROS), which are beneficial for host defense and are conducive to the elimination of bacteria and pathogens. However, the overproduction of ROS by neutrophils may cause organ system dysfunction during inflammation. Inhibition of mitochondrial complex I in neutrophils can diminish proinflammatory cytokine production and nuclear factor kappa B (NF-κB) activation.[50] Previous studies reported that NDUFV1 increases the activity of mitochondrial complex I.[47] In this study, NDUFV1 expression was upregulated in the ICAS group. Combined with the studies mentioned above, the increased NDUFV1 in neutrophils may promote the activity of mitochondrial complex I, increasing pro-inflammatory cytokine production and NF-κB activation and promoting the development of ICAS.

However, this study had some limitations. First, our conclusions may be compromised by the small sample size. Second, the ceRNA and lncRNA-RBP-miRNA networks were constructed via bioinformatics analysis, which requires identification and validation using molecular biology experiments.

Conclusions

We suggest that lncRNA RP11-701H16.4, lncRNA SNHG19, lncRNA RP3-406A7.3, and lncRNA HOTAIRM1 of neutrophils may participate in the development of ICAS and construct a regulatory network to explore the potential downstream mechanism. We elaborated on the transcriptional alterations of neutrophils in the pathophysiological process of ICAS, and provided insights for research related to the pathogenesis mechanism and drug design in ICAS.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

We would like to thank all the participants in this study.

Financial support and sponsorship

This work was supported by grants from the Beijing Natural Science Foundation (7222083) and the National Natural Science Foundation of China (82171303, 82171301).

Conflicts of interest

Dr. Yumin Luo is an Editorial Board member of Brain Circulation. The article was subject to the journal's standard procedures, with peer review handled independently of them and their research groups.

References

1. Wolf VL, Ergul A. Progress and challenges in preclinical stroke recovery research. Brain Circ 2021;7:230-40.
2. Wang Y, Zhao X, Liu L, Soo YO, Pu Y, Pan Y, *et al*. Prevalence and outcomes of symptomatic intracranial large artery stenoses and occlusions in China: The Chinese intracranial atherosclerosis (CICAS) study. Stroke 2014;45:663-9.
3. Döring Y, Soehnlein O, Weber C. Neutrophil extracellular traps in atherosclerosis and atherothrombosis. Circ Res 2017;120:736-43.
4. Bir SC, Kelley RE. Carotid atherosclerotic disease: A systematic review of pathogenesis and management. Brain Circ 2022;8:127-36.
5. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. Science 2015;349:316-20.
6. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol 2021;22:96-118.
7. Zhang Z, Salisbury D, Sallam T. Long noncoding RNAs in atherosclerosis: JACC review topic of the week. J Am Coll Cardiol 2018;72:2380-90.
8. Lopergolo D, Salvatore S, Sorrentino V, Malandrini A, Santorelli FM, Battisti C. Early-onset motor polyneuropathy associated with a novel dominant NAGLU mutation. Neurol Sci 2023;44:1415-8.
9. Xing C, Jiang Z, Wang Y. Downregulation of NAGLU in VEC increases abnormal accumulation of lysosomes and represents a predictive biomarker in early atherosclerosis. Front Cell Dev Biol 2021;9:797047.
10. Gosselin P, Martineau Y, Morales J, Czjzek M, Glippa V, Gauffeny I, *et al*. Tracking a refined eIF4E-binding motif reveals angel1 as a new partner of eIF4E. Nucleic Acids Res 2013;41:7783-92.
11. Masse M, Glippa V, Saad H, Le Bloas R, Gauffeny I, Berthou C, *et al*. An eIF4E-interacting peptide induces cell death in cancer cell lines. Cell Death Dis 2014;5:e1500.
12. Robichaud N, Hsu BE, Istomine R, Alvarez F, Blagih J, Ma EH, *et al*. Translational control in the tumor microenvironment promotes lung metastasis: Phosphorylation of eIF4E in neutrophils. Proc Natl Acad Sci U S A 2018;115:E2202-9.
13. Balta S, Celik T, Mikhailidis DP, Ozturk C, Demirkol S, Aparci M, *et al*. The relation between atherosclerosis and the neutrophil-lymphocyte ratio. Clin Appl Thromb Hemost

2016;22:405-11.
14. Zhang X, Lian Z, Padden C, Gerstein MB, Rozowsky J, Snyder M, *et al.* A myelopoiesis-associated regulatory intergenic noncoding RNA transcript within the human HOXA cluster. Blood 2009;113:2526-34.
15. Jing Y, Jiang X, Lei L, Peng M, Ren J, Xiao Q, *et al.* Mutant NPM1-regulated lncRNA HOTAIRM1 promotes leukemia cell autophagy and proliferation by targeting EGR1 and ULK3. J Exp Clin Cancer Res 2021;40:312.
16. Zeng K, Zhang QG, Li L, Duan Y, Liang YH. Splicing mutation in MVK is a cause of porokeratosis of Mibelli. Arch Dermatol Res 2014;306:749-55.
17. Chen W, Xu J, Wu Y, Liang B, Yan M, Sun C, *et al.* The potential role and mechanism of circRNA/miRNA axis in cholesterol synthesis. Int J Biol Sci 2023;19:2879-96.
18. Zhang B, Zhao B, Han S, Chen S. CNOT4 suppresses nonsmall cell lung cancer progression by promoting the degradation of PAF1. Mol Carcinog 2023;62:1563-71.
19. Leggieri A, García-González J, Torres-Perez JV, Havelange W, Hosseinian S, Mech AM, *et al.* Ankk1 loss of function disrupts dopaminergic pathways in Zebrafish. Front Neurosci 2022;16:794653.
20. Rubio-Solsona E, Martí S, Vílchez JJ, Palau F, Hoenicka J. Correction: ANKK1 is found in myogenic precursors and muscle fibers subtypes with glycolytic metabolism. PLoS One 2018;13:e0198880.
21. D'Angelo R, Aresta S, Blangy A, Del Maestro L, Louvard D, Arpin M. Interaction of ezrin with the novel guanine nucleotide exchange factor PLEKHG6 promotes RhoG-dependent apical cytoskeleton rearrangements in epithelial cells. Mol Biol Cell 2007;18:4780-93.
22. Samson T, Welch C, Monaghan-Benson E, Hahn KM, Burridge K. Endogenous RhoG is rapidly activated after epidermal growth factor stimulation through multiple guanine-nucleotide exchange factors. Mol Biol Cell 2010;21:1629-42.
23. Mazaki Y, Nishimura Y, Sabe H. GBF1 bears a novel phosphatidylinositol-phosphate binding module, BP3K, to link PI3Kγ activity with Arf1 activation involved in GPCR-mediated neutrophil chemotaxis and superoxide production. Mol Biol Cell 2012;23:2457-67.
24. Mantani PT, Dunér P, Bengtsson E, Ljungcrantz I, Sundius L, To F, *et al.* Interleukin-25 (IL-25) has a protective role in atherosclerosis development in the aortic arch in mice. J Biol Chem 2018;293:6791-801.
25. Chen Z, Zhong Y, Chen J, Sun S, Liu W, Han Y, *et al.* Disruption of β-catenin-mediated negative feedback reinforces cAMP-induced neuronal differentiation in glioma stem cells. Cell Death Dis 2022;13:493.
26. Aral K, Milward MR, Cooper PR. Inflammasome dysregulation in human gingival fibroblasts in response to periodontal pathogens. Oral Dis 2022;28:216-24.
27. Karasawa T, Kawashima A, Usui F, Kimura H, Shirasuna K, Inoue Y, *et al*. Oligomerized CARD16 promotes caspase-1 assembly and IL-1β processing. FEBS Open Bio 2015;5:348-56.
28. Lin Z, Xia Y, Guo J, Xu G, Liu Y, Yang Y, *et al.* Caspase-1 deficiency impairs neutrophils recruitment and bacterial clearance in *Streptococcus equi* ssp. *Zooepidemicus* infected mice. Vet Microbiol 2022;268:109411.
29. Lee SH, Stehlik C, Reed JC. Cop, a caspase recruitment domain-containing protein and inhibitor of caspase-1 activation processing. J Biol Chem 2001;276:34495-500.
30. Cheng D, Li Z, Wang Y, Xiong H, Sun W, Zhou S, *et al.* Targeted delivery of ZNF416 siRNA-loaded liposomes attenuates experimental pulmonary fibrosis. J Transl Med 2022;20:523.
31. Jean-Jacques H, Poh SL, Kuras L. Mediator, known as a coactivator, can act in transcription initiation in an activator-independent manner *in vivo*. Biochim Biophys Acta Gene Regul Mech 2018;1861:687-96.
32. Larivière L, Plaschka C, Seizl M, Wenzeck L, Kurth F, Cramer P. Structure of the mediator head module. Nature 2012;492:448-51.
33. Terabayashi T, Hashimoto S. Correction to: Increased unfolded protein responses caused by MED17 mutations. Neurogenetics 2022;23:75-6.
34. Giustozzi M, Freytes SN, Jaskolowski A, Lichy M, Mateos J, Falcone Ferreyra ML, *et al.* Arabidopsis mediator subunit17 connects transcription with DNA repair after UV-B exposure. Plant J 2022;110:1047-67.
35. Garratt LW. Current understanding of the neutrophil transcriptome in health and disease. Cells 2021;10:2406.
36. Makinde T, Agrawal DK. Intra and extravascular transmembrane signalling of angiopoietin-1-Tie2 receptor in health and disease. J Cell Mol Med 2008;12:810-28.
37. Fiedler U, Augustin HG. Angiopoietins: A link between angiogenesis and inflammation. Trends Immunol 2006;27:552-8.
38. Neagoe PE, Dumas E, Hajjar F, Sirois MG. Angiopoietin-1 but not angiopoietin-2 induces IL-8 synthesis and release by human neutrophils. J Cell Physiol 2012;227:3099-110.
39. Sturn DH, Feistritzer C, Mosheimer BA, Djanani A, Bijuklic K, Patsch JR, *et al.* Angiopoietin affects neutrophil migration. Microcirculation 2005;12:393-403.
40. Mei SH, McCarter SD, Deng Y, Parker CH, Liles WC, Stewart DJ. Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin 1. PLoS Med 2007;4:e269.
41. Hegeman MA, Hennus MP, van Meurs M, Cobelens PM, Kavelaars A, Jansen NJ, *et al.* Angiopoietin-1 treatment reduces inflammation but does not prevent ventilator-induced lung injury. PLoS One 2010;5:e15653.
42. Burnett A, Gomez I, De Leon DD, Ariaans M, Progias P, Kammerer RA, *et al.* Angiopoietin-1 enhances neutrophil chemotaxis *in vitro* and migration *in vivo* through interaction with CD18 and release of CCL4. Sci Rep 2017;7:2332.
43. Li Y, Jin L, Wang F, Ren L, Pen R, Bo G, *et al.* Epigenetic axis of SNHG19/miR-137/TNFAIP1 modulates amyloid beta peptide 25-35-induced SH-SY5Y cytotoxicity. Epigenomics 2022;14:187-98.
44. Cao M, Li H, Zhao J, Cui J, Hu G. Identification of age- and gender-associated long noncoding RNAs in the human brain with Alzheimer's disease. Neurobiol Aging 2019;81:116-26.
45. Li XX, Wang LJ, Hou J, Liu HY, Wang R, Wang C, *et al.* Identification of long noncoding rnas as predictors of survival in triple-negative breast cancer based on network analysis. Biomed Res Int 2020;2020:8970340.
46. Xue W, Li X, Li W, Wang Y, Jiang C, Zhou L, *et al.* Intracellular CYTL1, a novel tumor suppressor, stabilizes NDUFV1 to inhibit metabolic reprogramming in breast cancer. Signal Transduct Target Ther 2022;7:35.
47. Wang R, Kairen C, Li L, Zhang L, Gong H, Huang X. Overexpression of NDUFV1 alleviates renal damage by improving mitochondrial function in unilateral ureteral obstruction model mice. Cell Biol Int 2022;46:381-90.
48. Varghese F, Atcheson E, Bridges HR, Hirst J. Characterization of clinically identified mutations in NDUFV1, the flavin-binding subunit of respiratory complex I, using a yeast model system. Hum Mol Genet 2015;24:6350-60.
49. Xia W, Li Y, Wu M, Yin J, Zhang Y, Chen H, *et al.* Inhibition of mitochondrial activity ameliorates atherosclerosis in ApoE(-/-) mice via suppressing vascular smooth cell activation and macrophage foam cell formation. J Cell Biochem 2019;120:17767-78.
50. Zmijewski JW, Lorne E, Zhao X, Tsuruta Y, Sha Y, Liu G, *et al.* Mitochondrial respiratory complex I regulates neutrophil activation and severity of lung injury. Am J Respir Crit Care Med 2008;178:168-79.

Supplementary Table 1: Sequence of primers used for quantitative reverse transcription–polymerase chain reaction

Gene name	Primer sequence	Temperature (C)	Product length (bp)
β-actin	F:5'GTGGCCGAGGACTTTGATTG3'	60	73
	R:5'CCTGTAACAACGCATCTCATATT3'		
RP3-406A7.3	F:5'TTGAACCTTTTTGGACTGGCT3'	60	137
	R:5'CTCAGCTGGAACAGCATTGG3'		
RP11-70C1.3	F:5'CAACGCCTCACTGTCATTCT3'	60	58
	R:5'GGCACCAGTTTCTCCAATGT3'		
RP11-701H16.4	F:5'GCTTGGTGTGTGTTCCTACC3'	60	126
	R:5'GCAAGGTGAGTGGTCTGTC 3'		
RP1-244F24.1	F:5'CCACCTCTTTGCATCCACTC3'	60	78
	R:5'CTTACCTCTTGGCACCTTGA3'		
HOTAIRM1	F:5'ATGAACTGGCGAGAGGTCTGT 3'	60	210
	R:5' CCAGGAATGAGTAACACGGAGT 3'		
SNHG19	F:5'GCTACGATCTTGGGACGAAC3'	60	119
	R:5'CGCTAAGGGACAAAGTGGTG3'		