Heliyon 10 (2024) e36988

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Unveiling circRNA-mediated ceRNA networks in ischemic stroke by integrative analysis of multi-source gene expression profiling

Ya Zhang, Xiao-Ou Zhang *

Shanghai Key Laboratory of Maternal and Fetal Medicine, Clinical and Translational Research Center of Shanghai First Maternity and Infant Hospital, Frontier Science Center for Stem Cell Research, School of Life Sciences and Technology, Tongji University, Shanghai, China

A B S T R A C T

Background: Circular RNAs (circRNAs) are emerging as potential therapeutic targets for ischemic stroke (IS) due to their regulatory roles in inflammation and apoptosis. This study aimed to develop a comprehensive and robust IS-specific competing endogenous RNA (ceRNA) network to facilitate the identification of novel diagnostic and therapeutic targets.

Methods: We integrated expression data from 15 IS studies using the Rank-In algorithm to minimize batch effects. Differentially expressed circRNAs, miRNAs, and mRNAs were identified by comparing IS and control samples. Functional enrichment analysis of differentially expressed circRNA host genes revealed significantly enriched pathways and Gene Ontology (GO) terms relevant to IS pathogenesis. We further predicted miRNA-circRNA and mRNA-miRNA interactions, enabling the construction of a comprehensive ceRNA network to identify circRNA-related genes with diagnostic potential for IS.

Results: Integrated analysis revealed 199 differentially expressed circRNAs, 103 miRNAs, and 1736 mRNAs in IS patients. Functional enrichment analysis implicated these molecules in relevant pathways like the neurotrophin signaling pathway and p53 signaling pathway. The constructed circRNA-miRNA-mRNA regulatory network provided insights into potential mechanisms underlying IS. Three circRNA-related genes (RGS2, CDK5R1, and NSF) displayed promising diagnostic potential for IS when combined.

Conclusions: We successfully constructed a robust and informative IS-specific ceRNA network by integrating data from diverse sources. This network identified differentially expressed RNAs and revealed enriched pathways potentially involved in IS pathogenesis. Notably, our analysis identified CDK5R1, RGS2, and NSF as potential diagnostic biomarkers for IS. This study sheds light on a circRNA-mediated regulatory network with potential diagnostic and therapeutic implications for ischemic stroke.

1. Introduction

Stroke, a leading global health concern, encompasses hemorrhagic and ischemic subtypes. Ischemic stroke (IS), accounting for roughly 85 % of all cases, is the second leading cause of death and the third leading contributor to combined mortality and morbidity worldwide [1]. In China, IS has become the primary cause of death and disability, imposing significant burdens on individuals, families, and healthcare systems. The financial impact of managing IS in China is substantial, consuming about 4.13 % of the total national medical expenditure [2]. Therefore, effective strategies for prevention, early detection, and innovative therapeutic interventions are critical to address this growing public health challenge.

Circular RNAs (circRNAs), a novel class of endogenous non-coding RNAs characterized by a covalently closed-loop structure formed through back-splicing [3,4], have emerged as potential players in IS development [5]. Dysregulated circRNA expression

* Corresponding author. Shanghai Key Laboratory of Maternal and Fetal Medicine, Clinical and Translational Research Center of Shanghai First Maternity and Infant Hospital, Frontier Science Center for Stem Cell Research, School of Life Sciences and Technology, Tongji University, Shanghai, 200092, China.

E-mail address: zhangxiaoou@tongji.edu.cn (X.-O. Zhang).

https://doi.org/10.1016/j.heliyon.2024.e36988

Received 30 January 2024; Received in revised form 24 August 2024; Accepted 26 August 2024

Available online 27 August 2024

profiles have been observed in both animal models and clinical samples from IS patients [6–8]. These circRNAs are implicated in key biological processes relevant to IS, including neuroinflammation, apoptosis, autophagy, oxidative stress, angiogenesis, blood-brain barrier integrity, neural plasticity modulation, and neuronal regeneration facilitation [9,10]. Unraveling the complex roles of circRNAs in these processes can enhance our understanding of IS pathophysiology and pave the way for novel treatment strategies.

Non-coding RNAs (ncRNAs) such as microRNAs (miRNAs) and circRNAs are increasingly recognized as potent regulators of complex protein-coding gene networks [11]. The competitive endogenous RNA (ceRNA) hypothesis proposes that specific RNAs compete for binding to miRNAs through shared miRNA binding motifs [12]. Within this intricate regulatory network, circRNAs can act as miRNA sponges, competing for miRNA interactions via complementary base pairing [13]. This mechanism reduces the suppressive effect of miRNAs on target mRNA expression, allowing circRNAs to indirectly regulate gene expression through networks. By forming the circRNA-miRNA-mRNA regulatory axis, circRNAs play a crucial role in the miRNA-mediated gene expression regulatory network. Growing evidence suggests that circRNAs may also function in IS through the ceRNA mechanism [6,14], although the exact functional mechanisms require further elucidation.

Despite promising findings on circRNA involvement in IS, challenges persist. Previous studies have identified a remarkably diverse set of circRNAs potentially associated with IS, primarily from peripheral blood samples [6,14]. This reliance on a single source raises concerns about sampling bias due to inherent heterogeneity within study populations. Additionally, circRNAs offer greater stability than other RNA species, but their abundance in peripheral blood remains relatively low [15]. Variability in circRNA capture efficiency during sequencing procedures may also contribute to biases in final detection outcomes.

To gain deeper insights into the role of circRNAs in IS, we employed a meticulous data integration strategy. This comprehensive approach combined data from diverse sources, mitigating potential biases arising from batch effects and inter-individual variability. Additionally, we carefully considered the limitations of various circRNA detection methodologies to ensure robust analysis. As a result, we have constructed a reliable and informative IS-specific ceRNA network encompassing 7 circRNAs, 44 miRNAs, and 51 mRNAs. This network provides a valuable framework for exploring the intricate interplay between circRNAs, miRNAs, and mRNAs in IS pathogenesis. We focused on two key signaling pathways, the neurotrophin, and p53 signaling pathways, to elucidate potential molecular mechanisms underlying IS. Furthermore, our analysis identified three circRNA-associated genes (RGS2, CDK5R1, and NSF) with potential as diagnostic biomarkers for IS. This comprehensive investigation paves the way for the development of targeted therapeutic strategies and novel diagnostic tools for IS.

2. Methods

2.1. Data acquisition and differential expression screening

We conducted a systematic literature search to identify relevant research articles encompassing circRNA, miRNA, or mRNA expression profiling data specifically in human IS samples. Studies were included if they: (1) directly compared samples from IS patients and healthy controls, (2) employed microarray or high-throughput sequencing methods to report relative expression of circRNAs, miRNAs, or mRNAs, and (3) provided details on disease classification, sample preparation, and population size. Studies not meeting these criteria were excluded (detailed in Table 1) [6,16–23].

Expression profiles of circRNAs, miRNAs, and mRNAs were retrieved from the Gene Expression Omnibus (GEO) database. Due to platform variations, distinct normalization procedures were applied. Microarray data underwent base-2 logarithmic transformation. Probe IDs were mapped to gene IDs using the latest annotation files. When multiple probes corresponded to a single gene, their values were averaged. For RNA-seq data, normalized expression metrics (FPKM, TPM, or TMM) were downloaded. Genes with zero counts across all profiles were excluded for quality control.

Following data acquisition and filtering, expression data was normalized and standardized using a universal log-transformation with pseudocount addition. The Rank-In algorithm [24] was employed to mitigate batch effects arising from diverse data sources within the circRNA, miRNA, and mRNA expression profiles. This algorithm was further used to identify differentially expressed circRNAs (DEcircRNAs), miRNAs (DEmiRNAs), and mRNAs (DEmRNAs) between IS and control samples, with a significance threshold of FDR <0.05.

2.2. Functional enrichment analysis

To elucidate the biological functions potentially regulated by the identified DEcircRNAs, we performed functional enrichment analysis on their host genes. We retrieved the host genes from the circBase database and subjected them to enrichment analysis using the DAVID [25]. We investigated enrichments for both Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Pathways and GO terms with a p-value threshold of less than 0.05 were considered significantly enriched.

2.3. Prediction of miRNA–circRNA and mRNA–miRNA interactions

To identify potential regulatory interactions relevant to IS, we constructed a ceRNA network using only DEcircRNAs, DEmiRNAs, and DEmRNAs identified by comparing IS patient samples with controls. We utilized the circMine database [26] to predict circRNA-miRNA interactions, retaining only those with a prediction score exceeding 300 and an energy score lower than -7. For mRNA-miRNA interaction prediction, target mRNAs potentially regulated by the identified DEmiRNAs were retrieved from the TargetScan database [27]. We prioritized high-confidence interactions by setting a weighted context++ score threshold below -0.5 in

Table 1
Basic information of datasets.

Profiles	Title	Journal	Dataset	Tissue	N. of case	N. of control	Sex (M/F) in case	Sex (M/F) in control	Age in case	Age in control	Assay
CircRNAs	Circular RNA hsa_circ_0003574 as a biomarker for prediction and diagnosis of ischemic stroke caused by intracranial atherosclerotic stenosis	Front Pharmacol	–	Blood	5	5	4/1	5/0	59.4 ± 8.1	59.8 ± 10.2	Microarray
CircRNAs	Profile and Functional Prediction of Plasma Exosome-Derived CircRNAs From Acute Ischemic Stroke Patients	Front Genet	GSE195442	Blood	10	10	5/5	5/5	75.8 ± 7.8	68.2 ± 10.9	Microarray
CircRNAs	Identification of Circular RNA hsa_circ_0001599 as a Novel Biomarker for Large-Artery Atherosclerotic Stroke	DNA Cell Biol	GSE161913	Blood	5	4	3/2	2/2	60.3 ± 9.5	58.6 ± 8.6	Sequencing
CircRNAs	Circulating Circular RNAs as Biomarkers for the Diagnosis and Prediction of Outcomes in Acute Ischemic Stroke	Stroke	GSE133768	Blood	3	3	1/2	1/2	63.0 ± 1.5	59.3 ± 2.6	Microarray
CircRNAs	Comprehensive Analysis of Peripheral Exosomal circRNAs in Large Artery Atherosclerotic Stroke	Front Cell Dev Biol	GSE173719	Blood	5	5	–	–	–	–	Sequencing
CircRNAs	Expression profile and bioinformatics analysis of circular RNAs in acute ischemic stroke in a South Chinese Han population	Sci Rep	GSE140275	Blood	3	3	–	–	–	–	Sequencing
CircRNAs	Screening for differentially expressed circRNAs in ischemic stroke by RNA sequencing	BMC Neurol	GSE178764	Blood	3	3	–	–	61.3 ± 8.4	63.7 ± 4.5	Sequencing
mRNAs	Profile and Functional Prediction of Plasma Exosome-Derived CircRNAs from Acute Ischemic Stroke Patients	Front Genet	GSE195442	Blood	10	10	5/5	5/5	75.8 ± 7.84	68.2 ± 10.9	Microarray
mRNAs	Expression Profile and Potential Functions of Circulating Long Noncoding RNAs in Acute Ischemic Stroke in the Southern Chinese Han Population	Front Mol Neurosci	GSE140275	Blood	3	3	–	–	–	–	Sequencing
mRNAs	Dysregulated lncRNA and mRNA may promote the progression of ischemic stroke via immune and inflammatory pathways: results from RNA sequencing and bioinformatics analysis	Genes Genom	GSE180470	Blood	3	3	3/0	3/0	51.0 ± 1.0	51.3 ± 0.6	Sequencing
mRNAs	TTC7B emerges as a novel risk factor for ischemic stroke through the convergence of several genome-wide approaches	J Cerebr Blood F Met	GSE22255	Blood	20	20	10/10	10/10	60.2 ± 10.6	58.7 ± 11.0	Microarray
miRNAs	–	–	GSE169353	Blood	3	3	–	–	–	–	Sequencing
miRNAs	–	–	GSE199942	Blood	5	5	–	–	–	–	Sequencing
miRNAs	–	–	GSE231431	Blood	21	14	–	–	–	–	Sequencing
miRNAs	RNA-Seq identifies circulating miR-125a-5p, miR-125b-5p, and miR-	Circ Res	GSE110993	Blood	20	20	8/12	10/10	74.7 ± 9.7	72.7 ± 10.1	Sequencing

(*continued on next page*)

Table 1 (*continued*)

Profiles	Title	Journal	Dataset	Tissue	N. of case	N. of control	Sex (M/F) in case	Sex (M/F) in control	Age in case	Age in control	Assay
	143–3p as potential biomarkers for acute ischemic stroke										

TargetScan and further filtered by overlapping the predicted target genes with our set of DEmRNAs.

2.4. Construction of circRNA–miRNA–mRNA network

Through the integration of high-confidence circRNA-miRNA pairs and miRNA-mRNA pairs, we constructed a comprehensive circRNA-miRNA-mRNA regulatory network specific to IS. Only nodes that formed complete circRNA-miRNA-mRNA axes were retained in the final network. Cytoscape software (version 3.10.0) [28] was employed to create a visually informative representation of the network.

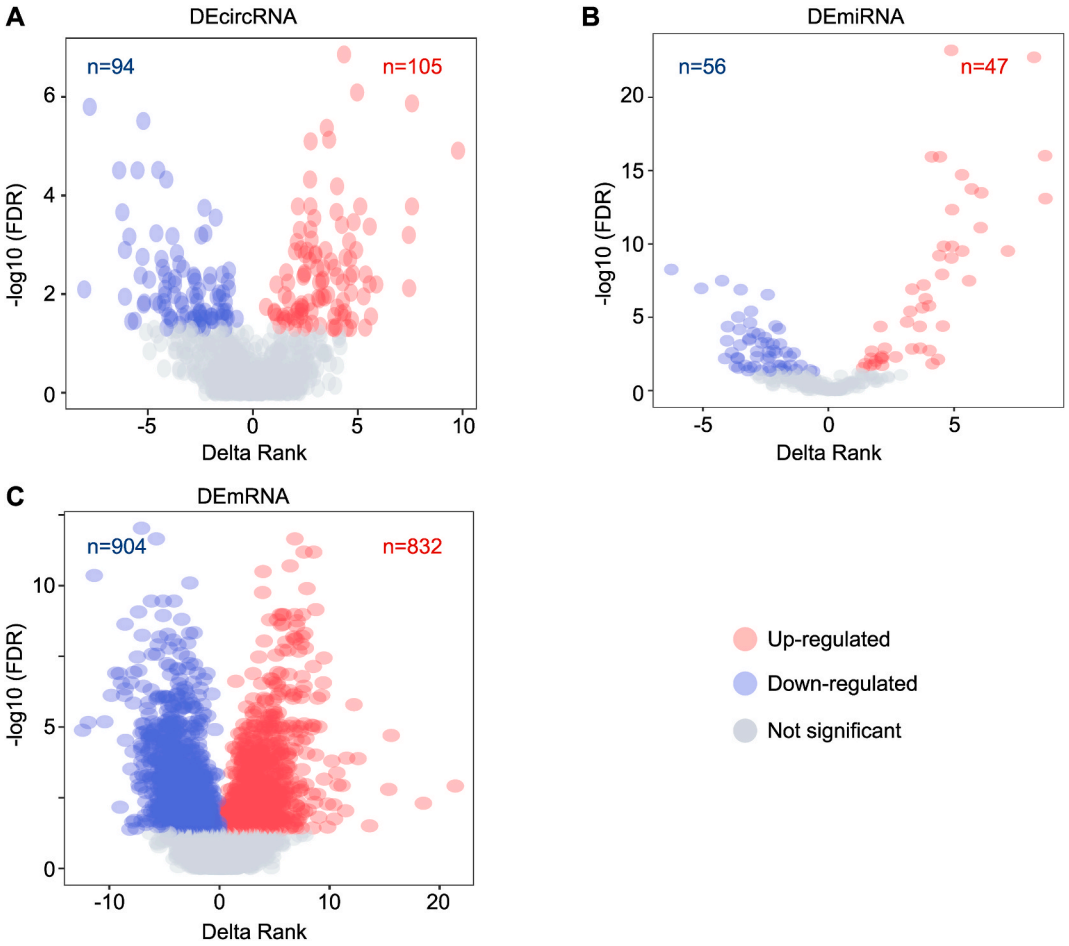

Fig. 1. Volcano plots depicting the differential expression of circRNAs (A), miRNAs (B), and mRNAs (C) between IS patients and control samples. These volcano plots were generated using delta rank values and the false discovery rate (FDR) calculated using the Rank-In method. Red points represent significantly (FGR <0.05) up-regulated circRNAs (A), miRNAs (B), and mRNAs (C), while blue points represent down-regulated ones, all of which exhibit statistical significance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Results

3.1. Differential expression of circRNAs, miRNAs, and mRNAs in IS

To investigate the expression profiles of circRNAs, miRNAs, and mRNAs in IS, we performed a systematic analysis of RNA-seq and microarray datasets derived from peripheral blood samples. The datasets included samples from IS patients and age- and sex-matched healthy controls. We integrated data from 7 circRNA profiling studies (34 IS cases, 33 controls), 4 miRNA profiling studies (36 IS cases, 36 controls), and 4 mRNA profiling studies (49 IS cases, 42 controls) (Table 1). Utilizing the Rank-In integrative algorithm (Supplementary Table S1), we identified a total of 199 DEcircRNAs (Fig. 1A), 103 DEmiRNAs (Fig. 1B), and 1736 DEmRNAs (Fig. 1C). Notably, the Rank-In algorithm facilitated the identification of circRNAs consistently dysregulated across datasets and platforms (Supplementary Fig. S1), potentially mitigating the effects of individual variability and technical variations in sequencing.

3.2. Functional enrichment analysis of DEcircRNA target genes

To elucidate the potential biological roles of DEcircRNAs in IS, we performed comprehensive Gene Ontology and KEGG pathway enrichment analyses using the parent genes of the identified DEcircRNAs (Fig. 2). GO enrichment analysis (Fig. 2A and B) revealed that the parent genes were significantly enriched for biological processes encompassing microtubule nucleation, endodermal cell differentiation, positive regulation of phosphorylation, and membrane fission. Notably, KEGG pathway enrichment analysis (Fig. 2C) consistently highlighted a significant enrichment of DEcircRNA parent genes within pathways crucial for IS pathophysiology, including the neurotrophin signaling pathway, focal adhesion, and p53 signaling pathway. Interestingly, enrichments were also observed for pathways like Human papillomavirus infection and DNA replication, warranting further investigation. To further explore the functional roles of the identified mRNAs, we conducted GO and KEGG pathway enrichment analyses on DEmRNA. Interestingly, the DEmRNAs exhibited distinct GO and pathway enrichment patterns compared to DEcircRNAs (Supplementary Table S2). DEmRNAs were primarily enriched in biological processes related to cellular organization, protein transport and processing, and responses to stress. These findings suggest that circRNAs and mRNAs may contribute to IS pathogenesis through different pathways.

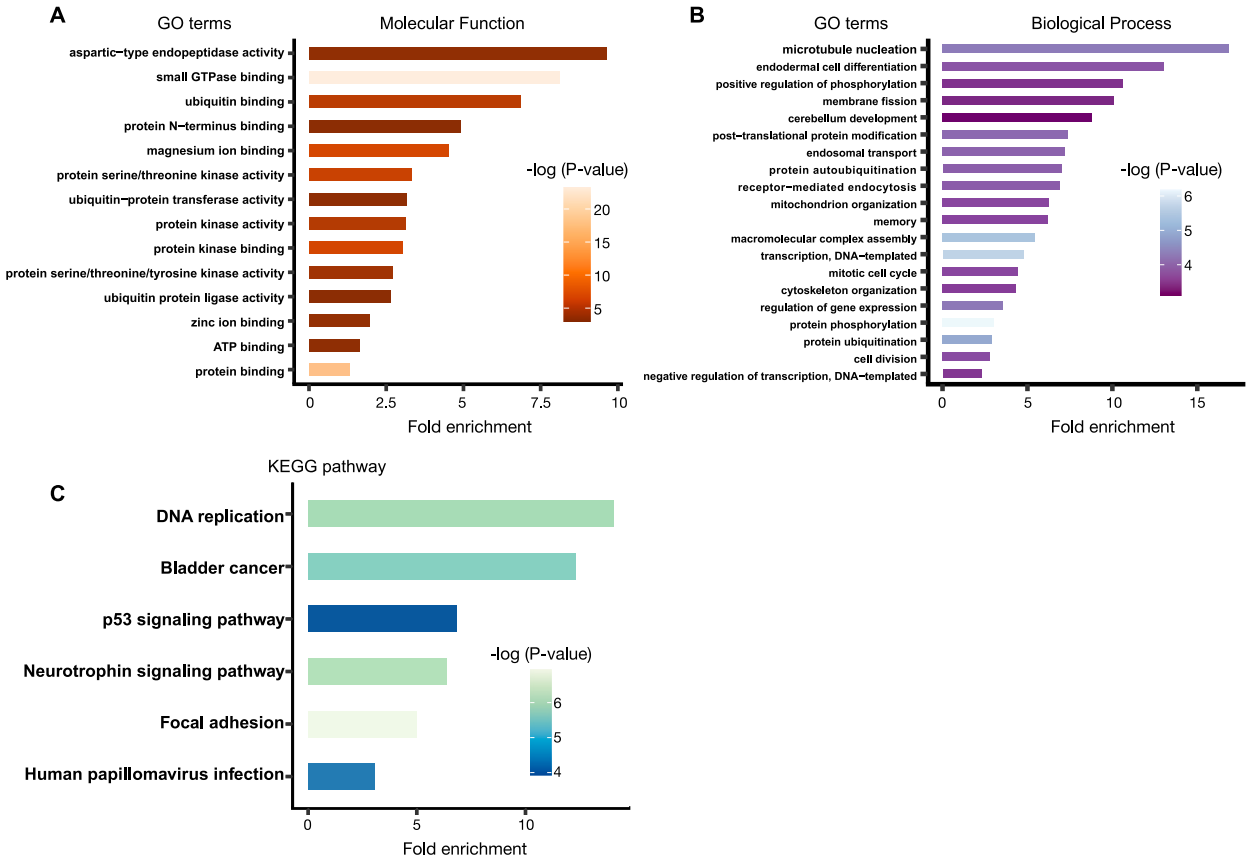

Fig. 2. GO enrichment and pathway analysis of parent genes associated with differentially expressed circRNAs. Bar plots illustrating the most significantly enriched GO terms, categorized by molecular functions (A) and biological processes (B). (C) The top enrichment scores of significantly enriched KEGG pathways.

3.3. Construction of a putative circRNA–miRNA–mRNA regulatory network

Given the established role of circRNAs as competing endogenous RNAs (ceRNAs) that sequester miRNAs and influence mRNA regulation, we constructed a putative circRNA-miRNA-mRNA regulatory network based on our identified differentially expressed genes (Fig. 3). In this network, circRNAs potentially act as miRNA sponges, sequestering miRNAs and consequently affecting their ability to target specific mRNAs.

Our analysis identified a network comprising 7 DEcircRNAs, 44 DEmiRNAs, and 51 DEmRNAs. The network revealed two sub-networks: one centered on hsa_circ_0026958 and hsa_circ_0002110, and another involving hsa_circ_0102489, hsa_circ_0027768, hsa_circ_0030855, hsa_circ_0000397, and hsa_circ_0033809. Interestingly, the network highlights the versatility of circRNA-miRNA interactions. We observed instances where a single circRNA interacts with multiple miRNA families, while conversely, a single miRNA family can be targeted by multiple circRNAs. For example, hsa_circ_0000397 interacts with diverse miRNA families, while the let-7 miRNA family is targeted by multiple circRNAs within the network.

3.4. Diagnostic potential of circRNA-associated mRNAs in IS

To assess the potential diagnostic utility of mRNAs within the constructed circRNA-ceRNA network, we performed a receiver operating characteristic (ROC) curve analysis using an additional independent case-control study encompassing 39 IS cases and 24 controls with mRNA microarray data [29]. This analysis (Fig. 4) identified three genes, RGS2, CDK5R1, and NSF, as promising diagnostic markers for IS based on their area under the curve (AUC) values (RGS2: 0.888, CDK5R1: 0.634, NSF: 0.630). Notably, combining these three mRNAs yielded a significantly enhanced cumulative AUC (AUC = 0.92) compared to individual markers, indicating a superior ability to distinguish IS cases from controls. This combined marker panel demonstrated a sensitivity of 90 % and a specificity of 79 % for IS diagnosis.

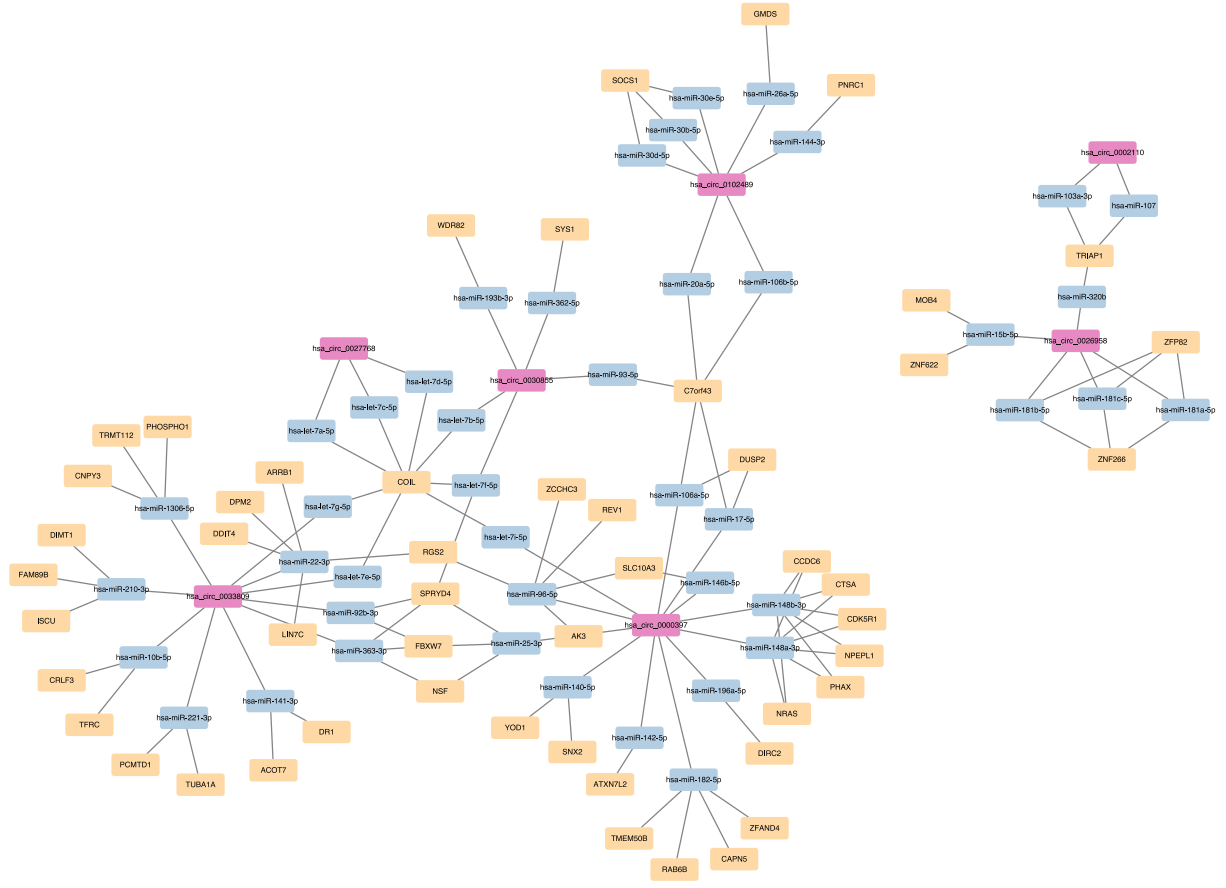

Fig. 3. CircRNA-ceRNA network analysis in IS. The ceRNA networks were based on circRNA-miRNA and miRNA-mRNA interactions. Pink nodes represent circRNAs, blue nodes represent miRNAs, and yellow nodes represent mRNAs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

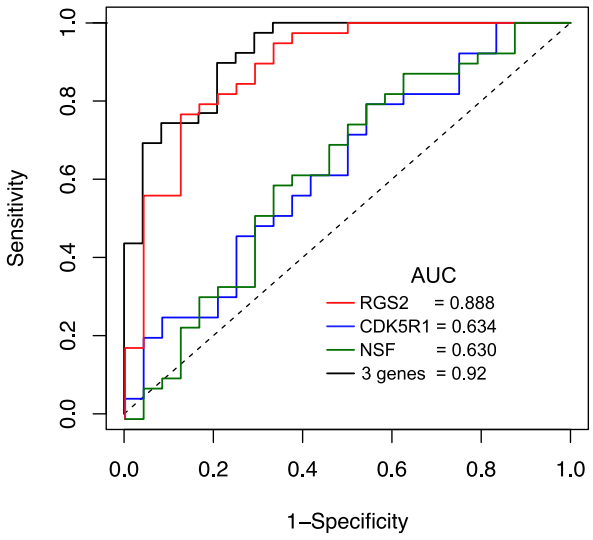

Fig. 4. ROC curve analysis of the representative genes in the circRNA ceRNA network.

4. Discussion

IS poses a significant global health burden due to its high prevalence, associated disability, elevated mortality rates, frequent recurrence, and substantial economic implications. Consequently, there is an urgent need for reliable biomarkers to improve IS diagnosis, risk stratification, and treatment. However, the current landscape of circRNA research in IS faces challenges due to individual variability, heterogeneity of blood samples used for circRNA detection, and batch effects in analysis methods. These factors contribute to inconsistencies in identified circRNA biomarkers across different IS studies. To address these challenges, we integrated diverse datasets from multiple sources to systematically analyze differentially expressed circRNAs, mRNAs, and miRNAs in individuals with and without IS. This comprehensive approach enabled us to unravel the circRNA-related transcriptome landscape of IS and identify potential biomarkers for diagnosis and treatment.

Our analysis identified 199 differentially expressed circRNAs associated with IS. To gain deeper insights into the biological functions of these circRNAs, we performed GO and KEGG pathway enrichment analyses. The results implicated circRNAs in regulating diverse signaling pathways involved in IS pathogenesis, including neurotrophin signaling, p53 signaling, and human papillomavirus (HPV) infection. The neurotrophin signaling pathway, comprising neurotrophins such as NGF, BDNF, NT-3, and NT-4, plays a critical role in promoting neuronal survival, synaptic plasticity, and neurogenesis [30]. One pathway significantly enriched in our analysis was neurotrophin signaling, which plays a critical role in neuronal survival and function. Interestingly, studies suggest that a combination of Panax Ginseng and Ginkgo biloba extracts might contribute to IS treatment by activating calmodulin and cyclic AMP-responsive element-binding protein through the neurotrophin signaling pathway [31]. Additionally, the importance of this pathway in preventing neuronal apoptosis following injury has been emphasized in previous research [32]. Furthermore, a high-throughput sequencing study in a rat model of ischemia-reperfusion provided further evidence supporting the involvement of the neurotrophin signaling pathway in IS [33]. These findings, together with the enrichment of the neurotrophin signaling pathway in our analysis, strongly suggest its involvement in IS pathogenesis. The p53 signaling pathway, identified as significantly enriched in our analysis, plays a complex and paradoxical role in IS. While it functions as a quintessential tumor suppressor, orchestrating cell cycle arrest and apoptosis in response to DNA damage, p53 activation can also exacerbate neuronal injury and contribute to ischemic stroke pathology. An exploration into p53-responsive microRNAs – including miR-192, miR-194, and miR-34a – revealing their involvement in ischemic heart failure following myocardial infarction, primarily via the p53 pathway [34]. These microRNAs regulate crucial cellular processes like proliferation, apoptosis, and angiogenesis [34]. Notably, modulating p53 levels through the CHIP protein has shown promise in mitigating infarct-related damage. Additionally, p53's influence on angiogenesis suggests a potential role in the progression from cardiac hypertrophy to heart failure [35,36]. Our findings highlight the potential involvement of circRNAs in modulating p53 signaling, opening avenues for further investigation into therapeutic strategies that balance p53's protective and detrimental effects in IS. Intriguingly, our analysis identified the HPV infection pathway as one of the differentially enriched pathways. While HPV is primarily associated with cervical cancer, recent studies have suggested its potential involvement in cardiovascular diseases [37]. Our findings raise the possibility that HPV infection might contribute to IS pathogenesis through circRNA-mediated mechanisms. Further research is needed to elucidate the underlying molecular mechanisms linking HPV infection to IS. In summary, our findings support the notion that circRNA-associated mechanisms intricately intersect with neurotrophin signaling, p53 signaling pathways, and HPV infection.

By delving into the complex interplay between coding and noncoding RNAs, our study harnessed miRNA binding sites to establish intricate regulatory networks across the transcriptome [38]. This comprehensive approach enabled us to identify novel circRNA-miRNA-mRNA interactions that may contribute to IS pathogenesis. Intriguingly, our ceRNA network underscored

hsa_circ_0000397's capacity to modulate miR-148b-3p and miR-148a-3p in IS patients, leading to upregulated CDK5R1 expression. This observation aligns with murine models where circHIPK3-induced miR-148b-3p reduction correlated with elevated CDK5R1 and diminished SIRT1, culminating in apoptotic events and mitochondrial dysfunction [39]. CDK5R1, a neuronal cyclin-dependent kinase, has been implicated in IS pathogenesis, with its activation contributing to neuronal damage and excitotoxicity. Our findings suggest that the hsa_circ_0000397-miR-148b-3p-miR-148a-3p-CDK5R1 axis may play a role in IS progression by modulating CDK5R1 expression. Building upon prior research [40], our findings corroborated the biomarker potential of RGS2 in IS. RGS2, a regulator of G protein signaling, has been shown to play a protective role in IS, with its reduced expression associated with poor outcomes [40]. Our identification of RGS2 as a potential biomarker further highlights its potential utility in IS diagnosis and risk stratification. Furthermore, our exploration into NSF's biomarker candidacy aligns with murine research involving middle cerebral artery occlusion [41]. NSF, an N-ethylmaleimide-sensitive factor, is involved in vesicular trafficking and has been linked to neuronal injury and apoptosis in IS models [41]. Our findings suggest that NSF expression levels might serve as a biomarker for IS diagnosis and prognosis. However, further studies are necessary to validate this possibility in larger patient cohorts. Overall, our study highlights the potential of circRNA-miRNA-mRNA regulatory networks in uncovering novel biomarkers for IS. The identified genes, CDK5R1, RGS2, and NSF, merit further investigation to fully elucidate their roles in IS and explore their potential as diagnostic markers or therapeutic targets.

Integrative analysis approaches offer substantial advantages for identifying differentially expressed genes across diverse datasets. However, this approach also presents certain limitations. Batch effects arising from different experimental protocols, sample heterogeneity due to variations in patient demographics and clinical characteristics, and platform variability associated with different microarray or RNA-seq technologies can potentially introduce biases into the analysis. In our study, we employed the Rank-In pipelines to minimize these potential biases. Rank-In facilitated the identification of differentially expressed circRNAs that showed highly consistent expression patterns across different platforms and samples (Supplemental Fig. S1). While these approaches enhance the generalizability of our findings, it is important to acknowledge that some residual biases might remain. For instance, even though Rank-In minimizes technical variations across platforms, subtle differences in patient demographics or clinical characteristics could still introduce some heterogeneity into the analysis. Future studies with larger and more diverse patient cohorts could help mitigate these limitations. Additionally, performing stratified analysis based on relevant clinical factors could provide more specific insights into the role of circRNAs in different patient subgroups. Future studies with experimental validation are crucial for further strengthening our findings. Functional validation of the identified circRNAs, including their interactions with miRNAs and mRNAs, is essential to elucidate their mechanistic roles in IS pathogenesis. Additionally, prospective clinical studies with larger cohorts are warranted to validate the diagnostic potential of the proposed circRNA-associated mRNA markers.

5. Conclusion

In conclusion, this study sheds light on the role of circRNAs in IS by integrating multi-source patient data and identifying a novel circRNA-mediated ceRNA network. This network reveals potential interactions between circRNAs, miRNAs, and mRNAs that may contribute to IS pathophysiology. Notably, our findings suggest circRNA-mediated ceRNA networks as potential targets for future IS therapies, warranting further investigation. Additionally, the identified circRNA associations with CDK5R1, RGS2, and NSF hold promise as diagnostic biomarkers for IS. This comprehensive analysis provides valuable insights and data to advance our understanding of IS and pave the way for the development of novel therapeutic strategies.

Data availability statement

No new data were generated or analyzed in support of this research. The public data used in this article are available in the Gene Expression Omnibus (GEO) database and summarized in Table 1.

CRediT authorship contribution statement

Ya Zhang: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xiao-Ou Zhang:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China [32170553 to X.-O.Z.] and Fundamental Research Funds for the Central Universities [22120240435 to X.-O.Z.].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36988.

References

[1] V.L. Feigin, et al., Global burden of stroke and risk factors in 188 countries, during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013, Lancet Neurol. 15 (9) (2016) 913–924.
[2] L.P. Liu, et al., Current status of endovascular procedures in management of ischemic stroke in China, CNS Neurosci. Ther. 20 (6) (2014) 483–484.
[3] P. Zhang, et al., Comprehensive identification of alternative back-splicing in human tissue transcriptomes, Nucleic Acids Res. 48 (4) (2020) 1779–1789.
[4] X.O. Zhang, et al., Diverse alternative back-splicing and alternative splicing landscape of circular RNAs, Genome Res. 26 (9) (2016) 1277–1287.
[5] Z. Liu, Y. Zhou, J. Xia, CircRNAs: key molecules in the prevention and treatment of ischemic stroke, Biomed. Pharmacother. 156 (2022) 113845.
[6] L. Zuo, et al., Circulating circular RNAs as biomarkers for the diagnosis and prediction of outcomes in acute ischemic stroke, Stroke 51 (1) (2020) 319–323.
[7] S.L. Mehta, G. Pandi, R. Vemuganti, Circular RNA expression profiles alter significantly in mouse brain after transient focal ischemia, Stroke 48 (9) (2017) 2541–2548.
[8] D. Lu, et al., Identification of blood circular RNAs as potential biomarkers for acute ischemic stroke, Front. Neurosci. 14 (2020) 81.
[9] Y. Gao, et al., Circ_0093887 upregulates CCND2 and SUCNR1 to inhibit the ox-LDL-induced endothelial dysfunction in atherosclerosis by functioning as a miR-876-3p sponge, Clin. Exp. Pharmacol. Physiol. 48 (8) (2021) 1137–1149.
[10] R. Yang, et al., Non-coding RNAs: the extensive and interactive regulators of the blood-brain barrier permeability, RNA Biol. 18 (sup1) (2021) 108–116.
[11] X.O. Zhang, et al., Species-specific alternative splicing leads to unique expression of sno-lncRNAs, BMC Genom. 15 (2014) 287.
[12] T.B. Hansen, et al., Natural RNA circles function as efficient microRNA sponges, Nature 495 (7441) (2013) 384–388.
[13] L.S. Kristensen, et al., The biogenesis, biology and characterization of circular RNAs, Nat. Rev. Genet. 20 (11) (2019) 675–691.
[14] X.Z. Wang, et al., Construction of circRNA-mediated immune-related ceRNA network and identification of circulating circRNAs as diagnostic biomarkers in acute ischemic stroke, J. Inflamm. Res. 15 (2022) 4087–4104.
[15] X.O. Zhang, et al., Complementary sequence-mediated exon circularization, Cell 159 (1) (2014) 134–147.
[16] L. Li, et al., Circular RNA hsa_circ_0003574 as a biomarker for prediction and diagnosis of ischemic stroke caused by intracranial atherosclerotic stenosis, Front. Pharmacol. 13 (2022) 961866.
[17] J. Yang, et al., Profile and functional prediction of plasma exosome-derived CircRNAs from acute ischemic stroke patients, Front. Genet. 13 (2022) 810974.
[18] S. Li, et al., Identification of circular RNA hsa_circ_0001599 as a novel biomarker for large-artery atherosclerotic stroke, DNA Cell Biol. 40 (3) (2021) 457–468.
[19] Q. Xiao, et al., Comprehensive analysis of peripheral exosomal circRNAs in large artery atherosclerotic stroke, Front. Cell Dev. Biol. 9 (2021) 685741.
[20] S. Li, et al., Expression profile and bioinformatics analysis of circular RNAs in acute ischemic stroke in a South Chinese Han population, Sci. Rep. 10 (1) (2020) 10138.
[21] D. Wei, et al., Screening for differentially expressed circRNAs in ischemic stroke by RNA sequencing, BMC Neurol. 21 (1) (2021) 370.
[22] Y. Wang, et al., Dysregulated lncRNA and mRNA may promote the progression of ischemic stroke via immune and inflammatory pathways: results from RNA sequencing and bioinformatics analysis, Genes Genomics 44 (1) (2022) 97–108.
[23] T. Krug, et al., TTC7B emerges as a novel risk factor for ischemic stroke through the convergence of several genome-wide approaches, J. Cerebr. Blood Flow Metabol. 32 (6) (2012) 1061–1072.
[24] K. Tang, et al., Rank-in: enabling integrative analysis across microarray and RNA-seq for cancer, Nucleic Acids Res. 49 (17) (2021) e99.
[25] B.T. Sherman, et al., DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update), Nucleic Acids Res. 50 (W1) (2022) W216–W221.
[26] W. Zhang, et al., circMine: a comprehensive database to integrate, analyze and visualize human disease-related circRNA transcriptome, Nucleic Acids Res. 50 (D1) (2022) D83–D92.
[27] S.E. McGeary, et al., The biochemical basis of microRNA targeting efficacy, Science 366 (6472) (2019).
[28] P. Shannon, et al., Cytoscape: a software environment for integrated models of biomolecular interaction networks, Genome Res. 13 (11) (2003) 2498–2504.
[29] G.C. O'Connell, et al., Machine-learning approach identifies a pattern of gene expression in peripheral blood that can accurately detect ischaemic stroke, NPJ Genom Med 1 (2016) 16038.
[30] T. Numakawa, H. Odaka, N. Adachi, Actions of brain-derived neurotrophin factor in the neurogenesis and neuronal function, and its involvement in the pathophysiology of brain diseases, Int. J. Mol. Sci. 19 (11) (2018).
[31] A. Zhao, et al., Combination of panax ginseng and ginkgo biloba extracts attenuate cerebral ischemia injury with modulation of NLRP3 inflammasome and CAMK4/CREB pathway, Front. Pharmacol. 13 (2022) 980449.
[32] D.H. Kim, et al., Prevention of apoptotic but not necrotic cell death following neuronal injury by neurotrophins signaling through the tyrosine kinase receptor, J. Neurosurg. 100 (1) (2004) 79–87.
[33] C. Wang, et al., Identification of conserved and novel microRNAs in cerebral ischemia-reperfusion injury of rat using deep sequencing, J. Mol. Neurosci. 54 (4) (2014) 671–683.
[34] S. Matsumoto, et al., Circulating p53-responsive microRNAs are predictive indicators of heart failure after acute myocardial infarction, Circ. Res. 113 (3) (2013) 322–326.
[35] A.T. Naito, et al., Promotion of CHIP-mediated p53 degradation protects the heart from ischemic injury, Circ. Res. 106 (11) (2010) 1692–1702.
[36] M. Sano, et al., p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload, Nature 446 (7134) (2007) 444–448.
[37] X. Liang, O.H.I. Chou, B.M.Y. Cheung, The effects of human papillomavirus infection and vaccination on cardiovascular diseases, NHANES 2003-2016, Am. J. Med. 136 (3) (2023) 294–301 e2.
[38] L. Salmena, et al., A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 146 (3) (2011) 353–358.
[39] G. Chen, et al., circHIPK3 regulates apoptosis and mitochondrial dysfunction induced by ischemic stroke in mice by sponging miR-148b-3p via CDK5R1/SIRT1, Exp. Neurol. 355 (2022) 114115.
[40] A. Simats, et al., A mouse brain-based multi-omics integrative approach reveals potential blood biomarkers for ischemic stroke, Mol. Cell. Proteomics 19 (12) (2020) 1921–1936.
[41] K. Hu, et al., Cathepsin B knockout confers significant brain protection in the mouse model of stroke, Exp. Neurol. 368 (2023) 114499.