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Potential mechanisms of epigenetic regulation in diabetic retinopathy from the perspectives of multi-omics

Xin Luo[1] and Daxi Xue[1*]

Abstract

Purpose Diabetic retinopathy (DR) is a significant complication of diabetes, with complex pathogenesis involving epigenetic modifications. This study aimed to explore the epigenetic regulatory mechanisms contributing to DR.

Methods DR-related data, including DNA methylation, mRNA, and miRNA expression datasets, were obtained from the Gene Expression Omnibus database. Differential gene expression analysis was performed to identify differentially methylated genes and expressed mRNAs and miRNAs. Cross-analysis established the methylation-expression and miRNA-mRNA regulatory networks. A comprehensive DR-related epigenetic regulatory network was constructed, identifying hub genes. The expression characteristics of these hub genes in various immune cells were examined using single-cell RNA sequencing.

Results We identified 10,716 differentially methylated genes, 1,181 differentially expressed mRNAs, and 615 differentially expressed miRNAs in DR. The methylation-expression regulatory network was associated with pathways such as TGF-beta and ErbB signaling. The miRNA regulatory network was linked to pathways related to cellular senescence, adherents junctions, and endocytosis. Five hub genes were identified: TFRC, AP2M1, AP2A1, DAB2, and PPP1CB. Single-cell RNA sequencing revealed specific expression of these genes in particular immune cells, highlighting their potential roles in DR pathogenesis.

Conclusion This study constructed a comprehensive epigenetic regulatory network for DR and identified key regulatory genes, offering new insights into the molecular mechanisms underlying DR and potential therapeutic targets for diagnosis and treatment.

Keywords Diabetic retinopathy, DNA methylation, miRNA, Epigenetic regulation, Single-cell RNA sequencing

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Introduction

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes, and its prevalence continues to rise in parallel with the increasing incidence of diabetes [1]. The pathogenesis of DR is complex and involves multiple biological processes. Hyperglycemia induced by diabetes triggers a series of microvascular abnormalities [2] and neurodegenerative changes [3], along with chronic low-grade inflammation [4], which further exacerbates retinal vascular damage. Current treatments for DR, such as panretinal photocoagulation (PRP) and anti-vascular endothelial growth factor (anti-VEGF) therapies, can only slow down disease progression but do not cure it completely [5]. Therefore, elucidating the pathogenesis of DR is critical for advancing therapeutic development.

In recent years, a growing body of evidence suggests that epigenetic modifications play an important role in the onset and progression of DR [6]. Epigenetics, which refers to the regulation of gene function and expression without altering the DNA sequence, involves mechanisms such as DNA methylation, histone modification, and non-coding RNA (e.g., miRNA and lncRNA) regulation, thereby having a significant impact on the diseases [7, 8]. DNA methylation, an essential epigenetic modification, typically occurs in CpG islands within gene promoters and regulates gene expression by adding a methyl group to the fifth carbon of cytosine residues. This modification is pivotal in gene imprinting, X-chromosome inactivation, genome stability, and chromatin structure [9]. miRNAs, a class of small non-coding RNAs, regulate gene expression at the post-transcriptional level by binding to target mRNAs, inhibiting their translation or promoting their degradation. miRNAs are widely involved in diverse physiological processes such as cell cycle, cell growth, metabolism, and synaptic plasticity [10].

Epigenetic modifications, particularly DNA methylation and miRNA regulation, are closely linked to gene expression in DR. In a hyperglycemic environment, alterations in DNA methylation can affect the expression of retina-related genes. For instance, hyperglycemia-induced DNA methylation can enhance the transcription and expression of the matrix metalloproteinase-9 (MMP-9) gene, leading to mitochondrial damage and accelerated apoptosis of retinal vascular endothelial cells [11]. Additionally, miRNAs contribute to DR development by inhibiting the expression of target genes. Abnormal expressions of miRNAs, such as miR-21 and miR-93, are closely associated with DR progression, modulating key physiological parameters such as blood glucose levels and insulin resistance [12, 13]. Targeting these epigenetic modifications may offer effective interventions to slow or even reverse DR progression.

Despite extensive research on various epigenetic modifications in DR, a comprehensive characterization of the underlying epigenetic mechanisms remains lacking. This study addresses this knowledge gap by investigating the integrated epigenetic regulatory landscape of DR through comparative analysis of DNA methylation patterns, mRNA expression profiles, and miRNA signatures in clinical samples from DR patients. We constructed epigenetic regulatory networks to identify hub regulatory genes and performed single-cell RNA sequencing to further explore the potential functions and mechanisms of these hub genes. The results of this study are expected to provide new molecular targets for the early diagnosis and treatment of DR.

Materials and methods

Data collection

To investigate the epigenetic regulatory mechanisms in DR, the following data were integrated from several publicly available datasets. These included GSE121820, comprising DNA methylation data from 10 DR patients and 10 healthy controls; GSE189005, containing gene expression data from 10 DR cases and 9 controls; GSE189002, which encompassed miRNA expression profiles from 10 DR cases and 9 controls; and GSE204880, providing single cell sequencing data from DR patients and normal controls.

Data processing

Differentially methylated genes (DMGs) were identified from the GSE121820 dataset with a threshold of P-value < 1e-3. Differential expression analysis for mRNA and miRNA datasets was performed using the limma package in R software, with filtering criteria set at |log2 fold change| > 0.7 and P-value < 0.05.

Epigenetic network construction of methylation and miRNA

Cross-analysis between DMGs and differentially expressed genes (DEGs) was conducted to identify methylation-associated DEGs. Potential target genes of differentially expressed miRNAs were predicted using the Starbase database [14] (http://starbase.sysu.edu.cn/), and cross-analysis was performed between the predicted target genes and differentially expressed mRNAs to explore miRNA regulatory mechanisms [15].

Comprehensive epigenetic network construction and hub gene screening

Cross-analysis of DNA methylation, mRNA, and miRNA networks was conducted to identify potential regulatory genes. Protein-protein interaction (PPI) network among these genes was mapped using STRING [16], and hub

genes were identified via the MCODE clustering algorithm implemented in Cytoscapes (3.9.1) [17].

Functional enrichment analysis

The R package clusterProfiler(4.8.3) was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analyses of DEGs [18], with significance defined as adjusted P-value < 0.05 corrected by the Benjamini-Hochberg method [19].

Immunoinfiltration analysis

Immune cell infiltration levels were assessed using single-sample gene set enrichment analysis (ssGSEA) implemented in the R package GSVA (1.48.3) [20], leveraging immune-related gene sets including 782 genes and 28 cell types [21].

Single-cell analysis

Single-cell RNA sequencing data were processed using the Seurat package (5.2.1) [22]. Initially, quality control was applied to filter out low-quality cells, with removal of cells showing > 20% ribosomal gene expression and > 3% erythrocyte gene expression. Subsequently, the NormalizeData function was used to normalize the filtered data, the FindVariableFeatures function was employed to identify highly variable genes (HVGs), and the ScaleData function was utilized to normalize the HVGs. Principal component analysis (PCA) was conducted based on HVGs, and cell clustering was performed using the FindNeighbors and FindClusters functions. Finally, cell clusters were annotated according to typical marker genes provided by the reference gene set, followed by integration of kidney and retinal data using Harmony.

Statistical analysis

Data analysis and visualization were conducted using R software version 4.1.2. Differential expression analysis was performed using the limma package, with significance thresholds set at |logFC| > 0.7 and P-value < 0.05.

Results

Screening of differentially methylated genes

Analysis of the GSE121820 dataset identified 10,716 DMGs by comparing DNA methylation levels between DR patients and healthy controls with P-value less than 1e-3 (Fig. 1a and Supplementary Table 1). To further investigate the functions of these filtered genes and their involvement in signaling pathways, GO and KEGG functional enrichment analyses were performed. GO enrichment analysis revealed that these DMGs were significantly enriched in biological processes such as regulation of GTPase activity, positive regulation of GTPase activity, regulation of small GTPase mediated signal transduction, activation of GTPase activity, and peptidyl-lysine modification (Fig. 1b). KEGG analysis indicated significant enrichment in pathways including hippo signaling pathway, signaling pathways regulating pluripotency of stem cells, hepatocellular carcinoma, adherens junction, and cell cycle (Fig. 1c).

Screening of differentially expressed mRNA

Based on screening criteria of |log2FC| > 0.7 and $P < 0.05$, 1,181 differentially expressed mRNAs were identified from the GSE189005 dataset, comprising 885 upregulated genes and 296 downregulated genes (Fig. 2a and Supplementary Table 2). The top five significantly upregulated mRNAs were RNF182, SLC14A1, BBOF1, SELENBP1, and SLC4A1, while the top five significantly downregulated mRNAs were SNORA11, SCARNA5, SNORA38B, RNU12, and SNORA80E. A heatmap of these DEGs clearly distinguished patients with DR from healthy individuals (Fig. 2b). GO analysis indicated that the differentially expressed mRNAs were primarily enriched in biological processes related to myeloid cell development, erythrocyte differentiation, myeloid cell differentiation, mitotic cell cycle phase transition, and

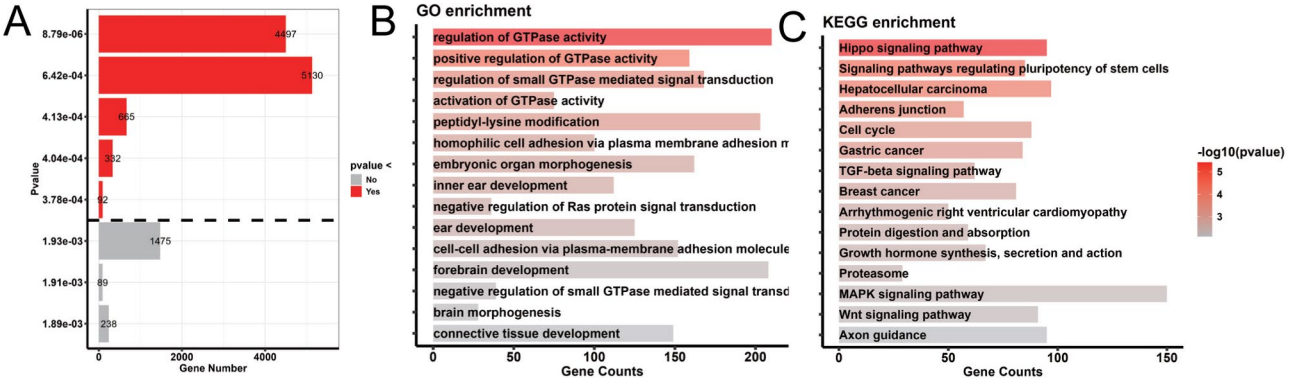

Fig. 1 Differentially methylated genes. (**a**) Number of DMGs identified based on different p values. (**b**) GO functional enrichment analysis. (**c**) KEGG enrichment analysis

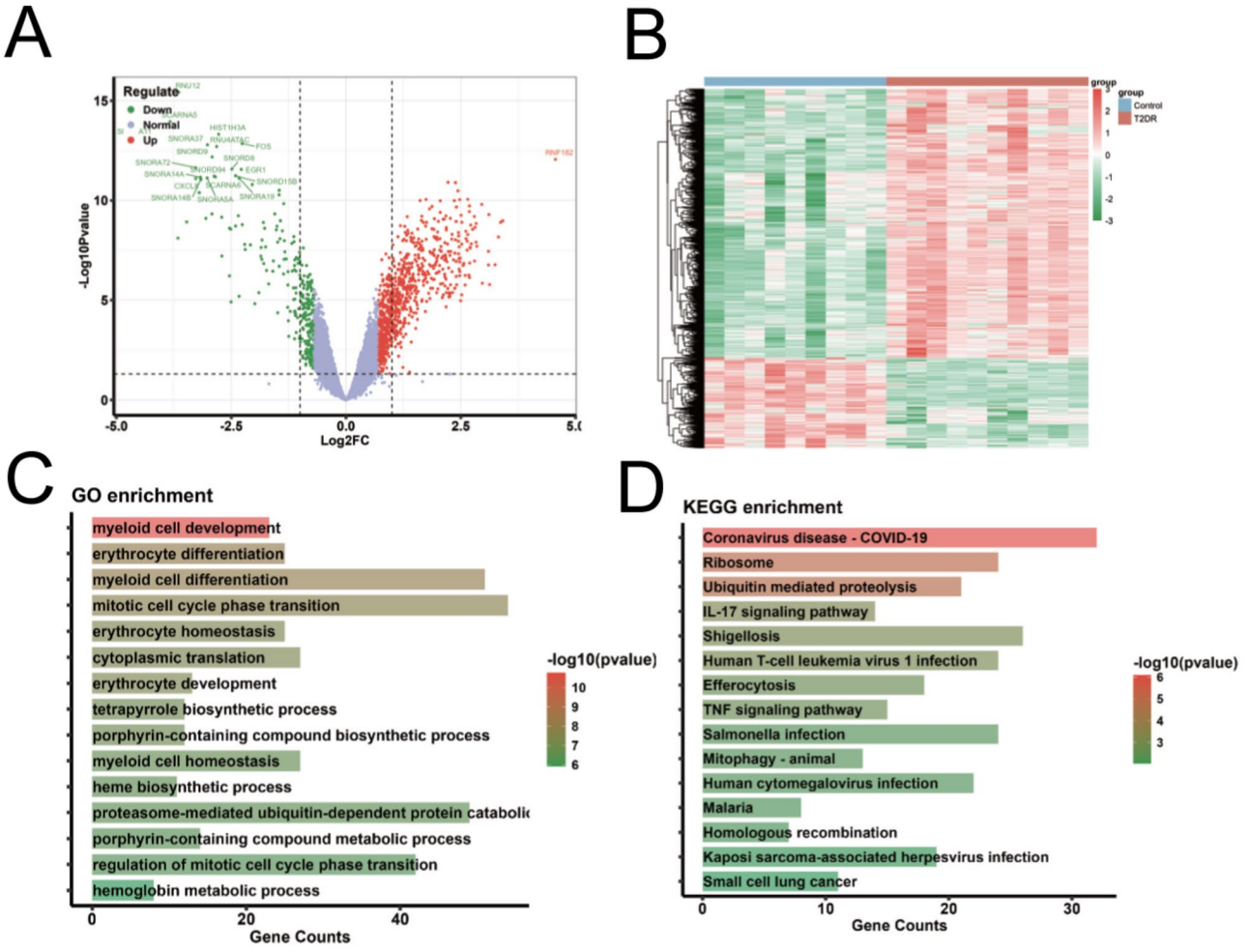

Fig. 2 Differentially expressed mRNA. (**a**) A Volcano plot visualizing differential mRNA in DR patients compared to healthy controls. (**b**) Heatmap of the differential mRNA distinguishing DR patients from healthy controls. (**c**) Histogram showing the top 15 GO terms ranked by the GO functional enrichment analysis. (**d**) Histogram showing the top 15 significant KEGG pathways

erythrocyte homeostasis (Fig. 2c). KEGG analysis showed significant differences in 34 pathways ($P<0.05$), including ribosome, ubiquitin mediated proteolysis, IL-17 signaling pathway, and (Fig. 2d).

Analysis of differential miRNA expression

In the GSE189002 dataset, 615 differentially expressed miRNAs were identified with the selection criteria (|log2FC| > 0.7 and $P<0.05$), including 368 upregulated and 247 downregulated DEGs (Fig. 3a and Supplementary Table 3). The top five significantly upregulated miRNAs were hsa-miR-5189-3p, hsa-miR-1226-3p, hsa-miR-4685-3p, hsa-miR-584-3p, and hsa-miR-6747-3p, while hsa-miR-29a-3p, hsa-miR-143-3p, hsa-miR-27a-3p, hsa-miR-30e-3p, and hsa-miR-31-5p were the top five significantly downregulated miRNAs. A heatmap demonstrated the differential expression of miRNAs between DR patients and healthy individuals (Fig. 3b). GO analysis showed enrichment in biological processes such as regulation of protein catabolic process, cell growth, peptidyl-serine modification, protein localization to nucleus, and post-embryonic development (Fig. 3c). KEGG results implicated several disease-associated pathways, including protein processing in endoplasmic reticulum, adherens junction, proteoglycans in cancer, cellular senescence, and regulation of actin cytoskeleton (Fig. 3d).

Construction of differential mRNA methylation regulatory network

A regulatory network for mRNA methylation was constructed to explore the relationship between DMGs and DEGs in DR. 537 differentially methylated mRNA were obtained through cross-analysis (Fig. 4a and Supplementary Table 4). GO analysis demonstrated that these genes were primarily involved in mitotic cell cycle phase transition, proteasome-mediated ubiquitin-dependent protein catabolic process, regulation of ubiquitin protein ligase activity, regulation of mitotic cell cycle phase transition,

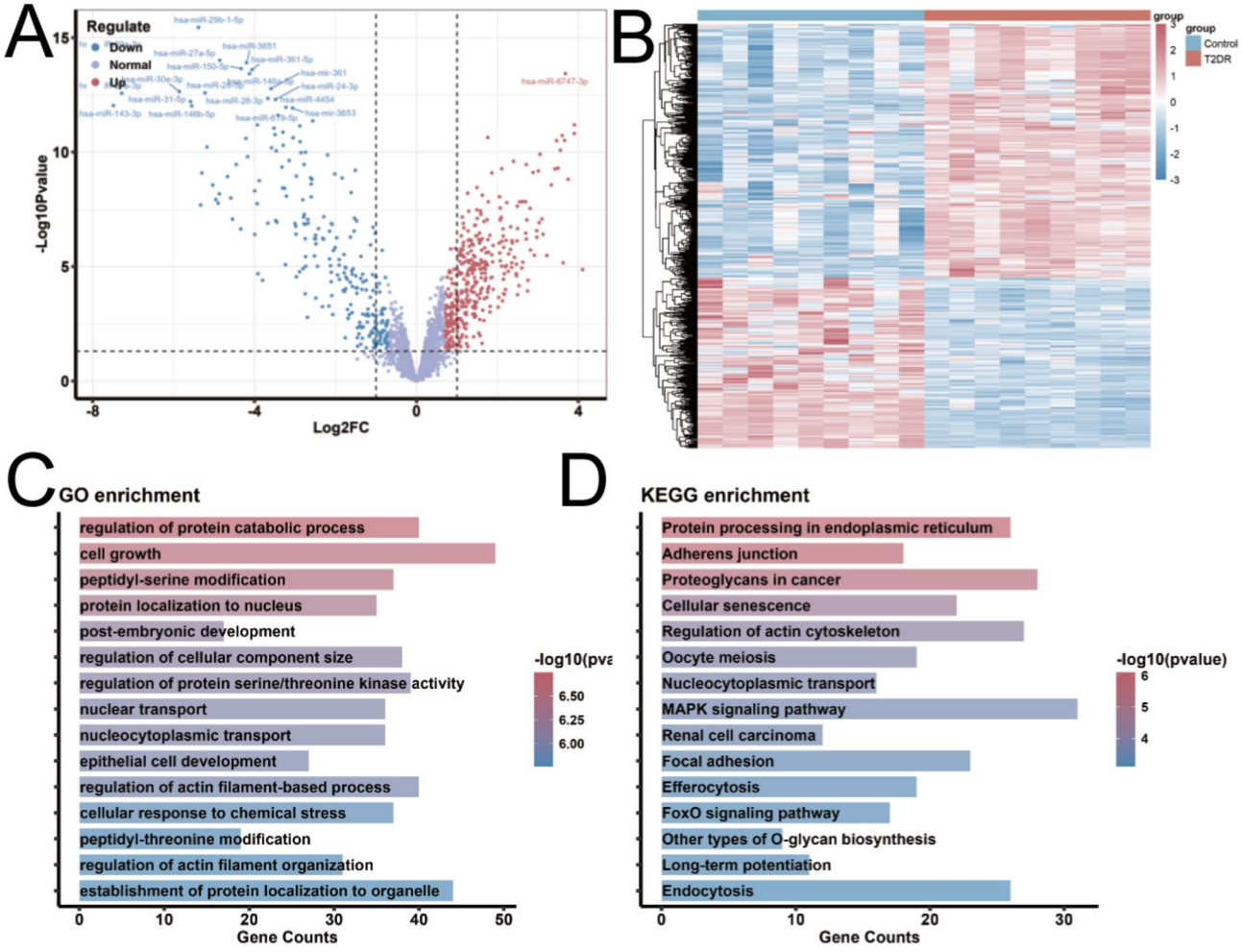

Fig. 3 Differential miRNA expression profile. (**a**) A Volcano plot visualizing differential miRNA expression between DR patients and healthy controls. (**b**) Heatmap of the differential miRNA expression between DR patients and healthy controls. (**c**) Histogram showing the top 15 GO terms ranked by the GO functional enrichment analysis. (**d**) Histogram showing the top 15 significant KEGG pathways

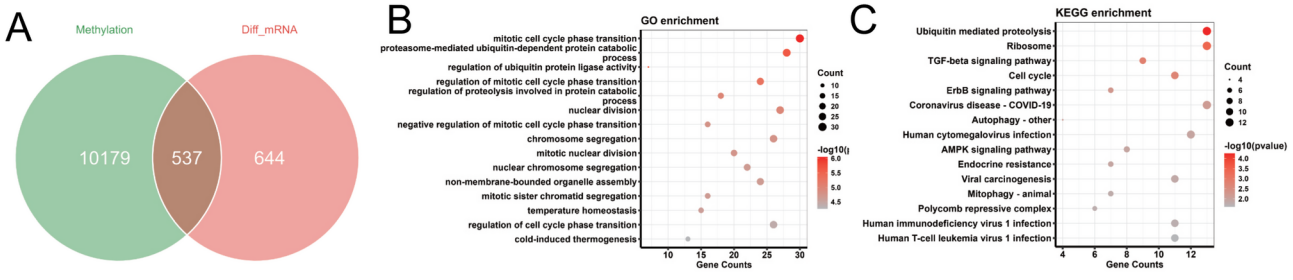

Fig. 4 The mRNA methylation regulatory network in DR. (**a**) Venn diagram of the differentially methylated mRNA. (**b**) GO functional enrichment analysis of the differentially methylated mRNA. (**c**) KEGG pathway enrichment analysis of the differentially methylated mRNA

and regulation of proteolysis involved in protein catabolic process (Fig. 4b). The KEGG pathways mainly included ubiquitin mediated proteolysis, ribosome, TGF-beta signaling pathway, cell cycle, and ErbB signaling pathway (Fig. 4c).

Construction of miRNA-mRNA regulatory network

The miRNA-mRNA regulatory network for DR was constructed by cross-analysis between differentially expressed miRNAs of potential target genes and differentially expressed mRNAs, resulting in 220 miRNA-mRNA pairs (Fig. 5a and Supplementary Table 5). GO analysis revealed that 73 DEGs were significantly enriched in

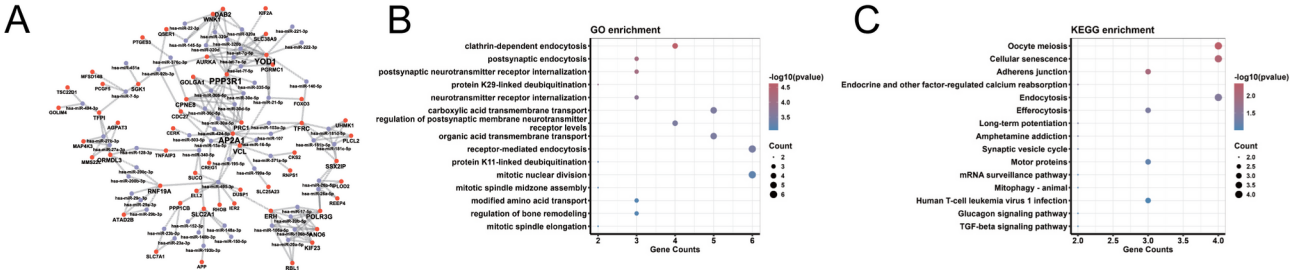

Fig. 5 The miRNA regulatory network in DR. (**a**) A miRNA-mRNA regulatory network. (**b**) GO functional enrichment analysis of DEGs. (**c**) KEGG pathway enrichment analysis of DEGs

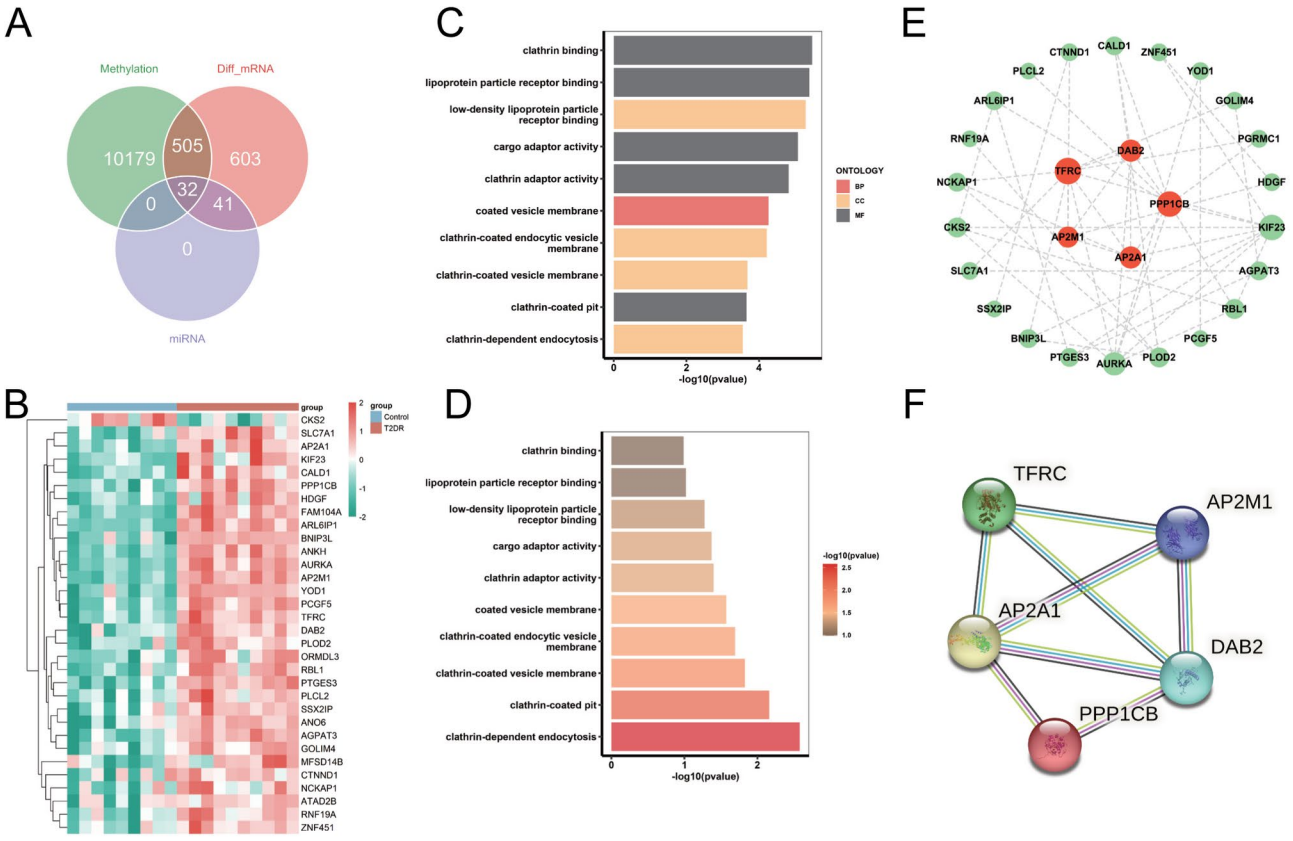

Fig. 6 The epigenetic regulatory network in DR. (**a**) Venn diagram containing three lists of epigenetic regulation-related genes. (**b**) Heatmap of the intersection genes distinguishing DR patients from healthy controls. (**c**) GO functional enrichment analysis of the intersection genes. (**d**) KEGG pathway enrichment analysis of the intersection genes. (**e**) PPI network of the intersection genes. (**f**) The significant modules of hub genes

biological processes such as clathrin-dependent endocytosis, postsynaptic endocytosis, postsynaptic neurotransmitter receptor internalization, neurotransmitter receptor internalization, and carboxylic acid transmembrane transport (Fig. 5b). These genes were associated with 124 signaling pathways, highlighting the involvement of oocyte meiosis, cellular senescence, adherens junction, endocrine and other factor-regulated calcium reabsorption, and endocytosis (Fig. 5c).

Construction of DR-related epigenetic regulatory network and identification of hub genes

Cross-analysis of the mRNA methylation network and miRNA-mRNA network identified 32 genes co-regulated by both epigenetic regulatory networks (Fig. 6a and Supplementary Table 6). The heatmap illustrated that these intersection genes distinctly distinguished DR patients from healthy controls (Fig. 6b). GO analysis was performed to describe the biological processes (BP), cellular components (CC), and molecular functions (MF) correlated with these genes. In terms of BP, these genes showed significant enrichment in coated vesicle

membrane. Genes in the CC category were primarily related to low-density lipoprotein particle receptor binding, clathrin-coated endocytic vesicle membrane, clathrin-coated vesicle membrane, and clathrin-dependent endocytosis. In the category of MF, these genes were mainly enriched in clathrin binding, lipoprotein particle receptor binding, cargo adaptor activity, clathrin adaptor activity, and clathrin-coated pit (Fig. 6c). KEGG analysis demonstrated significant associations with signaling pathways such as clathrin-dependent endocytosis, clathrin-coated pit, clathrin-coated vesicle membrane, clathrin-coated endocytic vesicle membrane, and coated vesicle membrane (Fig. 6d). PPI network analysis showed 38 nodes and 50 edges in total (Fig. 6e). The hub gene modules were defined using the MCODE plugin in Cytoscape, with five hub genes identified, including TFRC, AP2M1, AP2A1, DAB2, and PPP1CB (Fig. 6f).

Single-cell expression of hub genes

Single-cell RNA sequencing was employed to examine the expression characteristics of DR-related hub genes across various immune cell types, including B cells, retinal endothelial cells (EC-R), glomerular capillary endothelial cells (GC-EC), macrophages, neutrophils, podocytes, and retinal pericytes (RPC) (Fig. 7a). Notably, PPP1CB exhibited high expression in B cells, while macrophages demonstrated elevated AP2A1 expression. AP2M1 and PPP1CB were highly expressed in RPC. Hub genes such as TFRC, AP2M1, AP2A1, and DAB2 were specifically expressed in EC-R and GC-EC (Fig. 7b and c). Figure 7d illustrated the expression state of the hub genes in DR tissue compared to normal tissue. The Uniform Manifold Approximation and Projection(UMAP) of single-cell gene expression distribution revealed distinct clustering patterns (Fig. 7e).

To further elucidate the molecular mechanisms of diabetic complications, the analysis of the aforementioned hub genes was extended to diabetic kidney disease (DKD). In DKD, EC-R and GC-EC showed specific expression of AP2A1 and DAB2. RPC expressed high levels of AP2A1 and PPP1CB, while podocytes exhibited high expression of AP2M1. Other cells displayed enrichment of DAB2 and TFRC (Figs. 8a-d). In order to gain further insights into the transcriptomic features of immune cells in diabetic complications, the DR and DKD data were integrated. Batch effect correction was performed on the merged dataset using Harmony [23], thereby achieving an integrated single-cell transcriptome.

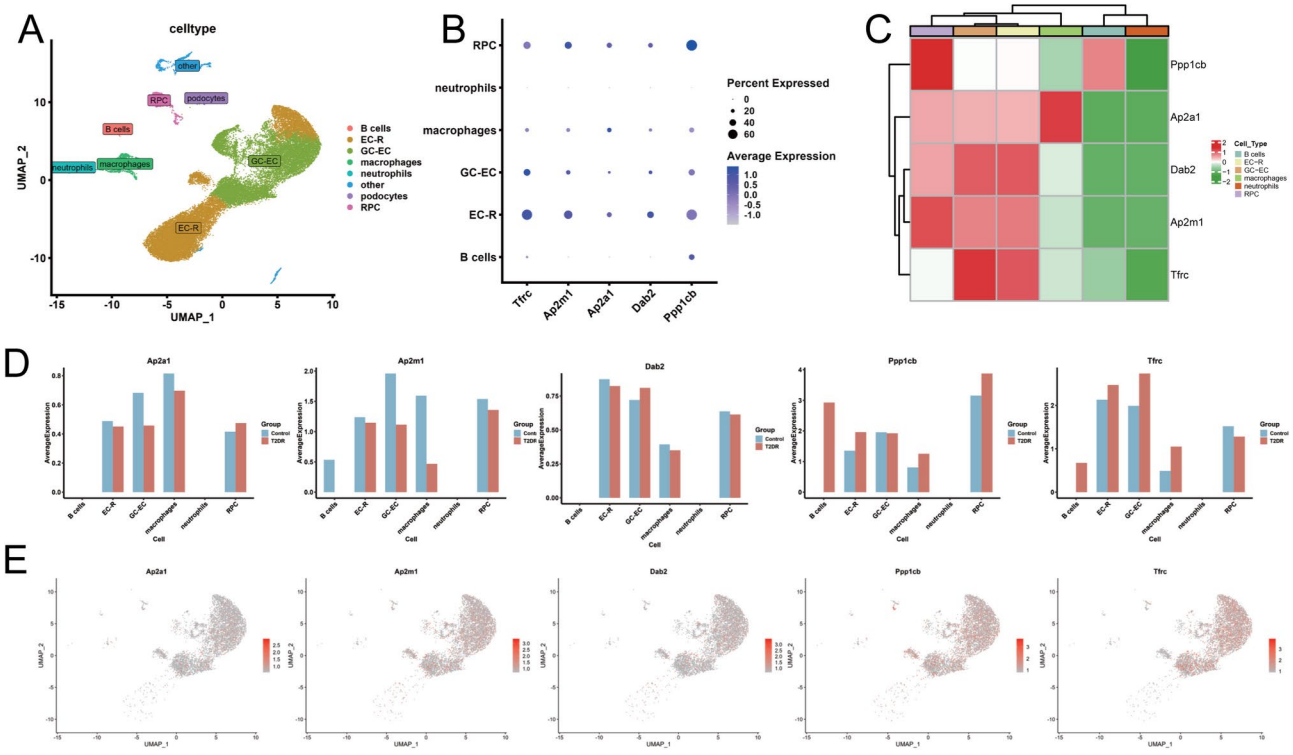

Fig. 7 Single-cell expression of hub genes in DR. (**a**) Major cell types. (**b**) Bubble plot showing the average expression levels of hub genes and fractions of expressed cells. (**c**) Heatmap of hub gene expression in the cell types. (**d**) Comparison of hub gene expression in the cell types between DR patients and healthy controls. (**e**) UMAP plots with the expression of hub genes in the cell types

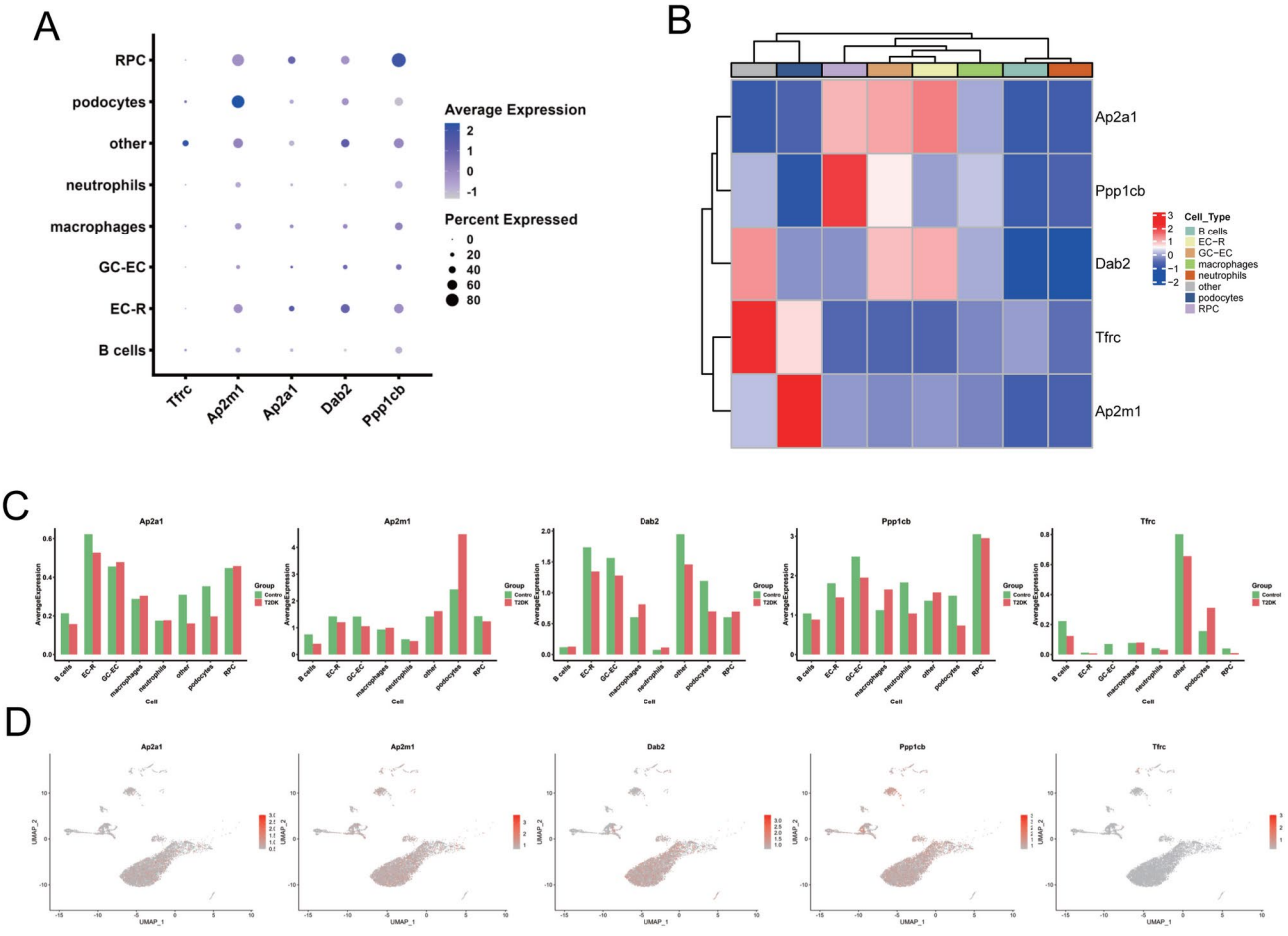

Fig. 8 Single-cell expression of hub genes in DKD. (**a**) Bubble plot showing the average expression levels of hub genes and fractions of expressed cells. (**b**) Heatmap of hub gene expression in the cell types. (**c**) Comparison of hub gene expression in the cell types between T2DK patients and healthy controls. (**d**) UMAP plots with the expression of hub genes in the cell types

Discussion

This study provided a comprehensive multi-omics analysis of the epigenetic regulatory mechanisms in DR, highlighting the critical roles of DNA methylation, mRNA, and miRNA in modulating disease progression. The findings revealed hub genes and their regulatory networks in DR, offering new insights into the pathophysiology of DR and potential molecular targets for clinical interventions.

Our study further confirmed significant alterations in DNA methylation in DR, which could potentially modulate the expression and function of specific genes involved [24]. Several DMGs identified in DR were found to be closely associated with signaling pathways such as hippo signaling pathway and hepatocellular carcinoma. Previous research has indicated that the hippo signaling pathway involved in angiogenesis is implicated in abnormal vascular responses in DR [25]. Moreover, Azuma et al.'s investigation has revealed that DR stands as an independent risk factor for the development of hepatocellular carcinoma (HCC) in patients with nonalcoholic fatty liver disease (NAFLD) [26].

At the mRNA level, our study identified a series of differentially expressed mRNAs in DR, primarily associated with pathways like ribosome, ubiquitin mediated proteolysis, and IL-17 signaling. Ribosomal protein networks have been observed to change significantly following retinal ischemia and reperfusion (I/R) injury [27], suggesting that ribosomal damage may lead to retinal cell dysfunction [28]. Studies have shown that changes in mRNA do not significantly alter basic functions of retinal pigment epithelium (RPE) cells, but may regulate cellular responses to diabetes and hypoxic stress through the ubiquitin mediated proteolysis pathway [29]. The IL-17 signaling pathway is particularly relevant in the activation of retinal microglial cells, which secrete pro-inflammatory cytokines and chemokines, exacerbating retinal inflammation and vascular damage, thus contributing to the progression of DR [30, 31].

Our study also identified a series of miRNAs specifically expressed in DR, which may exert key regulatory roles in DR development through pathways such as adherens junction and cellular senescence. Genes involved

in the adherens junction pathway show abnormal methylation and differential expression, potentially altering intercellular adhesion and signal transmission, thereby affecting vascular stability and extracellular matrix organization [32]. In DR, activation of the Notch1 signaling pathway leads to damage in the adherens junction pathway between vascular endothelial cells, and particularly results in the dissociation of VE-cadherin from β-catenin, disrupting vascular barrier function and increasing vascular permeability [33]. Under hyperglycemic conditions, retinal cells undergo significant senescence changes, characterized by the secretion of various inflammatory factors and growth factors that constitute the senescence-associated secretory phenotype (SASP), promoting the spread of cellular senescence and pathological neovascularization, thereby exacerbating the pathological conditions of DR [34–36].

The integration of DNA methylation and miRNA expression data allowed us to identify methylated DEGs and construct a mRNA methylation regulatory network in DR. In this network, pathways such as the TGF-beta signaling pathway and ErbB signaling pathway were found to be associated with DR. Elevated TGF-β expression in DR tissue suggests activation of TGF-beta signaling pathway, which may affect the retinal fibrotic process and vascular barrier function [37, 38]. High glucose levels in diabetes can lead to aberrant activation of the ErbB signaling pathway, promoting apoptosis and inhibiting cell proliferation, which may be potentially linked to impaired corneal epithelial healing in diabetes [39]. Abnormal activation of the ErbB signaling pathway is also associated with inflammation and neovascularization in DR, increasing risks of hemorrhage and vision loss [40].

A miRNA-mRNA regulatory network was also constructed and revealed critical pathways for specific miRNAs in DR. The network including important hub miRNAs such as hsa-miR-27a-3p and hsa-miR-31-5p, were found to be closely associated with signaling pathways such as cellular senescence, adherens junction, and endocytosis. Mastropasqua et al. have reported downregulation of hsa-miR-27a-3p in DR, which may be related to the role of hsa-miR-27a-3p in insulin signaling and glucose metabolism [41]. Additionally, hsa-miR-31-5p has been identified to be involved in the pathological process of DR [42]. It has been demonstrated that the activation of soluble epoxide hydrolase (sEH) in the retina in DR promotes the endocytosis of cell adhesion molecules like VE-cadherin and N-cadherin, disrupting vascular endothelial cell junctions, leading to vascular leakage and retinal edema [43].

Through comprehensive DR-related epigenetic regulatory network construction, five hub genes were identified, including TFRC, AP2M1, AP2A1, DAB2, and PPP1CB. Studies have shown that regulation of transferrin receptor (TFRC) and related iron metabolism pathways may provide novel strategies for the prevention and treatment of DR [44, 45]. Disabled-2 (DAB2), a protein associated with the endocytosis of VEGFR2 (vascular endothelial growth factor receptor 2), may become impaired with consequent disruption of VEGFR2 signaling and inhibited angiogenesis, highlighting its potential as a therapeutic target for DR [46]. Evidence suggests that Arf-directed GTPase-activating protein 1 (ARAP1) may regulate the phagocytic function of retinal pigment epithelial (RPE) cells through interactions with proteins like AP-2 complex subunit alpha-1 (AP2A1) [47]. Following retinal ischemia/reperfusion (I/R) injury, the AP2M1 level is markedly upregulated, aiding in the regulation of retinal neurotransmitter release and improving the survival of retinal ganglion cells (RGCs) [48]. Changes in the activity of protein phosphatase 1 (PP1) also have a significant impact on RGC survival and function in DR [49], and PPP1CB, a subtype of PP1, is likely to influence retinal function under diabetic conditions.

Furthermore, single-cell RNA sequencing elucidated the distinct expression of DR-related hub genes in different immune cells. The five identified hub genes were found to be associated with immune cells implicated in DR pathogenesis and their abnormal expression in specific cell types might drive DR onset and progression [8]. The high PPP1CB expression in B cells may be linked to metabolic regulation [50], while the elevated AP2A1 expression in macrophages suggests its potential role in the inflammatory response of DR [51]. The pronounced expression of AP2M1 and PPP1CB in retinal pericytes (RPCs) may affect retinal vascular stability and inflammation [52]. The high expression of TFRC, AP2M1, AP2A1, and DAB2 in EC-R and GC-EC further indicates their role in diabetes-induced vascular abnormalities and retinal damage [53, 54]. The single-cell analysis also revealed key roles for these hub genes in DKD. The specific expression of AP2A1 and DAB2 in EC-R and GC-EC may influence microvascular permeability [55], and the high levels of AP2A1 and PPP1CB in RPCs may be related to kidney filtration function and vascular damage [56]. These findings are similar to those observed in DR and could suggest shared epigenetic regulatory mechanisms between DR and DKD, particularly in endothelial cells [57]. The differential expression of these hub genes in immune cells reveals their potential role in diabetic complications through immune cell activation and endothelial cell function, highlighting their potential as biomarkers and therapeutic targets in diabetic complications.

Although this study has revealed the epigenetic regulatory mechanisms of DR through multi-omics integration, several limitations remain. First, the analysis utilizes a small sample size of high throughput sequencing data, making it difficult for the analysis to filter further, which

may limit the generality and statistical robustness of the findings. Second, while the study focused on DNA methylation and miRNAs in DR, other epigenetic mechanisms such as histone modifications and long non-coding RNAs (lncRNAs) require further exploration. Lastly, the influence of epigenetic modifications on gene expression in DR needs further functional validation to clarify the biological roles of key genes and pathways.

Conclusion

This study systematically investigated the epigenetic regulatory mechanisms of DR. Through an integrative multi-omics analysis, we identified significant alterations in DNA methylation, and mRNA and miRNA expression associated with DR, and constructed a comprehensive epigenetic regulatory network, highlighting five hub genes. Single-cell RNA sequencing analysis further revealed the specific expression of these hub genes in various immune cell, underscoring their crucial roles in the onset and progression of DR. The findings not only offer new insights into the pathophysiology of DR but also provide potential molecular targets for its diagnosis and treatment.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13098-025-01723-7.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Acknowledgements
NA.

Author contributions
Xin Luo contributed to the conceptualization and design of the study, performed the data analysis, and drafted the manuscript. Additionally, Xin was involved in the interpretation of the results and provided critical revisions to the manuscript for important intellectual content.Daxi Xue served as the principal investigator, overseeing the project and coordinating the research activities. Daxi contributed to the study's design, supervised the data collection and analysis processes, and played a significant role in writing and revising the manuscript. Daxi also provided guidance on the overall direction of the research and ensured the integrity of the work. Both authors have read and approved the final manuscript and agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
not applicable.

Consent for publication
All authors agree to the publication of this article.

Competing interests
The authors declare no competing interests.

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