ARTICLE

Expression profiling of circular RNAs in sepsis-induced acute gastrointestinal injury: insights into potential biomarkers and mechanisms

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Abstract
This study aimed to investigate the role of circular RNAs (circRNAs) in sepsis-induced acute gastrointestinal injury (AGI), focusing on their potential as biomarkers and their involvement in disease progression. Peripheral blood samples from 14 patients with sepsis-induced AGI and healthy volunteers were collected. RNA sequencing was performed to profile circRNA and miRNA expression. Differential expression analysis identified key regulatory RNAs. Functional enrichment analysis was conducted to explore biological pathways, and circRNA-miRNA interaction networks were constructed. Significant differences in circRNA and miRNA expression profiles were observed between sepsis-induced AGI patients and healthy controls. Several circRNAs, including hsa_circ_0008381 and hsa_circ_0071375, exhibited stepwise expression increases correlating with AGI severity. Functional enrichment analysis indicated that the host genes of differentially expressed circRNAs are involved in key biological processes like protein ubiquitination, organelle maintenance, and cellular signaling pathways such as mitochondrial biogenesis and lipid metabolism. CircRNA-miRNA interaction networks suggested their role as miRNA sponges, regulating key downstream processes. This study demonstrated the potential of circRNAs as diagnostic biomarkers and therapeutic targets for sepsis-induced AGI. Further research is warranted to validate their clinical utility and unravel their mechanistic roles in AGI progression.

Keywords Sepsis · Acute gastrointestinal injury · circRNA · miRNA · Biomarker · TGF-β signaling · RNA sequencing

Introduction

Sepsis is a life-threatening condition characterized by a dysregulated host response to infection and remains a leading cause of mortality in intensive care unit (ICU) patients (Markwart et al. 2020). Every year, millions of people worldwide are affected by sepsis, leading to substantial healthcare costs and high mortality rates (Bauer et al. 2020; Fleischmann et al. 2021). Among the complications of sepsis, acute gastrointestinal injury (AGI) is particularly significant, as it serves both as a cause and a consequence of sepsis. AGI refers to gastrointestinal dysfunction in critically ill patients, which exacerbates systemic inflammation and promotes bacterial translocation, thereby aggravating multiple organ dysfunction syndrome (MODS) (Fink 1991; Wang 2021). Reports indicate that approximately 40% of patients in intensive care units exhibit

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varying degrees of AGI, and mortality rates significantly increase with the severity of AGI (Li et al. 2016; Zhang et al. 2018a, b). These findings highlight the critical role of gastrointestinal function in the onset and progression of sepsis. This bidirectional relationship creates a vicious cycle: Sepsis induces gastrointestinal dysfunction through hypoperfusion and inflammatory responses, while gastrointestinal dysfunction perpetuates systemic infection (Druzak et al. 2023).

Circular RNAs (circRNAs), a class of non-coding RNAs characterized by covalently closed loop structures, have emerged as crucial regulators in various biological processes. Unlike linear RNAs, circRNAs are resistant to exonuclease degradation and exhibit stable expression patterns, making them promising biomarkers and therapeutic targets (Kristensen et al. 2022; Verduci et al. 2021). Recent studies suggest that circRNAs modulate key pathways involved in inflammation, apoptosis, and cellular stress responses (Feng et al. 2020). It has been reported that circRNAs can mitigate intestinal ischemia–reperfusion injury by mediating signal transduction, serving as biomarkers for intestinal injury (Zhang et al. 2023). Furthermore, in sepsis, circRNAs have been shown to modulate inflammatory responses and immune cell function, impacting the progression of organ dysfunction and systemic inflammation (Qi et al. 2021). CircRNAs often act as molecular sponges for microRNAs (miRNAs), thereby influencing downstream gene expression networks (Jarlstad Olesen and Kristensen 2021). However, their specific roles in the progression of sepsis-induced AGI remain poorly understood, warranting further investigation.

This study aimed to elucidate the roles of circRNAs in sepsis-induced AGI, focusing on their potential as diagnostic biomarkers and therapeutic targets. By profiling circRNA expression in patients with varying AGI severities, this research seeked to identify specific circRNA-miRNA-mRNA interaction networks involved in disease progression. Understanding these regulatory mechanisms might offer novel insights into the molecular basis of sepsis-induced AGI and pave the way for innovative interventions to mitigate gastrointestinal and systemic complications in critically ill patients.

Methods

Study participants and diagnostic criteria

This study included patients with sepsis-induced AGI admitted to the intensive care unit (ICU) and healthy volunteers as controls. The diagnosis of sepsis is based on the third international consensus definitions for sepsis and septic shock (Sepsis-3) (Singer et al. 2016). Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Sepsis can be diagnosed if the Sequential Organ Failure Assessment (SOFA) score is increased by 2 points or more compared to the baseline (for patients with unknown baseline organ dysfunction, the baseline SOFA score is assumed to be zero), or if the quick SOFA (qSOFA) score is positive (a positive qSOFA score is defined as the presence of 2 or more qSOFA criteria at the onset of infection, including systolic blood pressure ≤ 100 mmHg, respiratory rate ≥ 22/min, and altered mental status). The inclusion criteria for patients were an age of ≥ 18 years, confirmed sepsis, and symptoms consistent with AGI. Patients were excluded if they had malignancies, hematologic disorders, autoimmune diseases, or neurological disorders. Health controls were age- and gender-matched to the patient group and had no history of significant illnesses, including gastrointestinal diseases, diabetes, or hypertension. Statistical adjustments were made for age and gender to control for these potential confounders, and no significant differences were observed between the groups in terms of these factors. The study included a sample of 14 AGI patients and 14 healthy volunteers. A post-hoc power analysis was performed to evaluate the statistical robustness of the sample size. The analysis revealed that the sample size provides adequate power (80%) to detect meaningful differences in the variables of interest, ensuring the reliability of the conclusions drawn from this study. Ethical approval was obtained from the Shenzhen People's Hospital Clinical Research Ethics Committee, and all participants provided written informed consent.

AGI grading and patient stratification

The severity of AGI was determined using the European Society of Intensive Care Medicine consensus criteria, which classify AGI into four grades (Reintam Blaser et al. 2012). AGI I indicates a risk of gastrointestinal dysfunction, AGI II represents gastrointestinal dysfunction impairing digestion and absorption, AGI III reflects gastrointestinal failure unresponsive to intervention, and AGI IV indicates severe gastrointestinal failure leading to MODS or shock. Upon admission to the ICU, physicians recorded APACHE II and SOFA scores to evaluate the overall severity of illness. Antibiotic therapy was initiated based on the patient's infection source, and fluid resuscitation was performed in cases of shock. Supportive care measures, such as mechanical ventilation for respiratory failure or continuous renal replacement therapy (CRRT) for renal dysfunction, were implemented according to the specific organ failures present in each patient.

Patients were stratified into two groups based on their AGI grade: The mild group (AGI I–II) and the severe group (AGI III–IV) (Ding et al. 2020). For patients with gastrointestinal failure, interventions included intravenous metoclopramide (10 mg three times daily) to enhance gastrointestinal motility, enema administration to facilitate bowel movements, and nutritional support tailored to their clinical condition. These treatments were combined with standard ICU care to manage the complications associated with AGI and underlying sepsis.

Clinical and laboratory data collection

Detailed clinical data were collected, including demographics, AGI grades, and ICU scoring systems (APACHE II and SOFA). Laboratory parameters recorded included inflammatory markers (hsCRP, PCT, IL-6), blood counts (WBC, neutrophil percentage, lymphocyte percentage), and coagulation indicators (PLT, AT-III). Bladder pressure was measured as a surrogate for intra-abdominal pressure. Peripheral blood (3 mL) was collected in RNA stabilization tubes (PAXgene, BD Biosciences) and stored at − 80 °C for subsequent analysis.

RNA extraction and quality control

Total RNA was extracted from stabilized blood samples using the TRIzol reagent (Invitrogen) following the manufacturer's protocol. RNA purity and concentration were assessed using a NanoDrop spectrophotometer, and integrity was confirmed using the Agilent 4200 TapeStation. Samples with a RIN ≥ 7.0 were used for downstream analysis. Ribosomal RNA was depleted to enrich for circRNA and miRNA.

Library construction and sequencing

CircRNA libraries were constructed by processing rRNA-depleted RNA samples. First, the RNA was fragmented, followed by reverse transcription into first-strand cDNA using random primers and M-MuLV reverse transcriptase. The RNA strand was subsequently degraded with RNase H, and second-strand cDNA synthesis was performed using DNA polymerase I. The resulting cDNA was purified and subjected to end-repair, A-tailing, and adapter ligation. After adapter ligation, libraries were amplified using PCR, and their quality was assessed with the Agilent TapeStation. Quantification was performed with the KAPA Library Quantification Kit to ensure uniformity before sequencing. For miRNA library preparation, total RNA was enriched for small RNAs using the Illumina TruSeq Small RNA Library Preparation Kit. During this process, specific adapters were ligated to the 5′ and 3′ ends of small RNA molecules. Reverse transcription was performed to create cDNA, which was then amplified by PCR to produce miRNA libraries. Following PCR, the libraries were size-selected to enrich for fragments corresponding to miRNA (~ 140–160 bp) using a gel-based purification method. The quality of the miRNA libraries was confirmed using the Agilent TapeStation, and quantification was performed with the KAPA Library Quantification Kit. Finally, all libraries, including both circRNA and miRNA libraries, were pooled in equimolar amounts

according to the desired sequencing depth and loaded onto the Illumina NovaSeq 6000 platform, with a sequencing depth of 30 million reads per sample. Pre-sequencing quality control included RNA integrity checks using the Agilent Bioanalyzer. Post-sequencing quality control was performed to evaluate read quality, including base quality score distribution and adapter trimming. For data normalization, the trimmed mean of M-values (TMM) method was applied to adjust for library size differences and compositional biases. Sequencing was performed using a paired-end 150 bp (PE150) strategy to ensure high coverage and accuracy for downstream analyses.

Identification and quantification of circRNA and miRNA

CircRNAs were identified using find_circ and CIRI2 software, and miRNAs were identified using miRDeep2. Expression levels were normalized as Transcripts Per Million (TPM) to account for library size differences. Raw reads in fastq format were processed with fastp for quality control, including adapter trimming and quality filtering. Only high-quality reads were used for downstream analyses.

Differential expression analysis

Differential expression analysis was performed using the EdgeR package in R, which models biological and technical variability using an overdispersed Poisson model. Adjusted P-values were calculated using the Benjamini–Hochberg method to control the false discovery rate (FDR). CircRNAs and miRNAs with $|\log2FoldChange| > 1$ and $FDR < 0.05$ were considered significantly differentially expressed. Volcano plots were generated to visualize the distribution of upregulated and downregulated RNAs.

Functional enrichment analysis

Functional enrichment of host genes of differentially expressed circRNAs was conducted using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The clusterProfiler R package was used, and adjusted P-values < 0.05 were considered significant.

Construction of circRNA-miRNA-mRNA network

Interactions between circRNAs, miRNAs, and target mRNAs were predicted using the miRanda algorithm. Cytoscape software was used to construct and visualize the circRNA-miRNA-mRNA interaction network, highlighting potential regulatory axes involved in AGI pathophysiology.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) and analyzed using the Student's t-test for comparisons between two groups. Categorical variables were compared using the chi-square test, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to quantify associations. All statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY) and R (version 4.0.5). A P-value < 0.05 was considered statistically significant for all tests.

Results

Clinical characteristics of patients

Fourteen patients with sepsis-induced AGI were enrolled in this study, including eight patients classified as AGI III–IV (severe group) and six patients as AGI I–II (mild group). Patients in the severe group had higher

APACHE II (29.25 ± 7.27 vs. 23.17 ± 6.43) and SOFA scores (15.38 ± 4.41 vs. 9.83 ± 4.22), with significantly elevated bladder pressure (19.00 ± 3.42 mmHg vs. 10.83 ± 4.02 mmHg, OR = 8.17, 95% CI: 3.83–12.50). However, no significant difference was observed in mortality rate or ICU stay between the groups. The diagnoses of the patients included bowel perforation, septicemia, and pancreatitis, with higher frequencies of bowel perforation in the severe group. Laboratory parameters such as inflammatory markers (hsCRP, IL-6) and hepatic function indices (TBIL, DBIL) showed variability between groups but without statistical significance due to the limited sample size (Table 1).

Table 1 Clinical characteristics of patients with sepsis-induced acute gastrointestinal injury (AGI)

	AGI III ~ IV	AGI I ~ II	OR	95%CI
Cases	8	6		
Gerder (cases)			3.00	0.31 ~ 28.84
Male	6	3		
Female	2	3		
Age [years (SD)]	60.63 (22.87)	64.67 (17.08)	− 4.04	− 28.35 ~ 20.26
Diagnosis (cases)			–	–
Bowel perforation	3	0		
Septicemia	2	3		
Suppurative cholecystitis	1	1		
Severe pancreatitis	1	0		
Severe pneumonia	1	1		
Urinary tract infection	0	1		
Bladder pressure (mmHg)	19.00 (3.42)	10.83 (4.02)	8.17	3.83 ~ 12.50
APACHE II score	29.25 (7.27)	23.17 (6.43)	6.08	− 2.07 ~ 14.24
SOFA score	15.38 (4.41)	9.83 (4.22)	5.54	0.45 ~ 10.63
MV (cases)	1	2	3.50	0.24 ~ 51.90
CRRT (cases)	5	3	0.60	0.07 ~ 5.14
ECMO (cases)	1	1	1.40	0.07 ~ 28.12
Lab				
WBC ($\times 10^9$/L)	14.30 (9.37)	20.65 (14.81)	− 6.35	− 20.41 ~ 7.70
N (%)	85.74 (9.73)	93.71 (2.81)	− 7.97	− 16.98 ~ 1.03
L (%)	8.05 (7.02)	3.97 (2.05)	4.08	− 2.41 ~ 10.58
HLA-DR (%)	43.16 (21.19)	36.84 (20.05)	6.32	− 23.20 ~ 35.83
PCT (ng/ml)	39.67 (60.47)	64.16 (71.93)	− 24.49	− 101.55 ~ 52.57
hsCRP (mg/L)	173.30 (56.86)	104.13 (101.49)	69.17	− 23.32 ~ 161.66
IL-6 (mg/L)	2468.05 (2173.76)	1985.46 (2361.86)	482.59	− 2169.72 ~ 3134.91
TBIL (mmol/L)	51.46 (59.54)	30.87 (25.71)	20.60	− 36.36 ~ 77.56
DBIL (mmol/L)	40.17 (55.90)	16.50 (13.68)	23.67	− 27.63 ~ 74.97
GPT (U/L)	638.00 (1731.50)	579.20 (862.53)	58.80	− 1629.61 ~ 1747.21
GOT (U/L)	265.00 (587.40)	917.73 (1061.12)	− 652.73	− 1691.25 ~ 385.80
GGT (U/L)	67.25 (105.63)	79.50 (118.82)	− 12.25	− 143.23 ~ 118.73
ICU stay [mean days (SD)	17.00 (9.93)	30.00 (40.82)	− 13	− 45.26 ~ 19.26
Outcome				
Death (case)	1	2	3.50	0.24 ~ 51.90

SD Standard deviation, *OR* Odds ratio, *CI* Confidence interval, *APACHE II* Acute Physiology and Chronic Health Evaluation II score, *SOFA* Sequential Organ Failure Assessment score, *MV* Mechanical ventilation, *CRRT* Continuous renal replacement therapy, *ECMO* Extracorporeal membrane oxygenation, *WBC* White blood cell count, *N* Neutrophils, *L* Lymphocytes, *HLA-DR* Human leukocyte antigen-DR, *PCT* Procalcitonin, *hsCRP* High-sensitivity C-reactive protein, *IL-6* Interleukin-6

Expression profiles and characteristics of peripheral blood circRNAs and miRNAs

Peripheral blood samples from sepsis-induced AGI patients and healthy volunteers were analyzed to examine the expression profiles and characteristics of circRNAs and miRNAs. Figure 1A presented the workflow of the study, which includes patient sample collection, RNA sequencing, quality control, and downstream analyses for circRNAs and miRNAs. This comprehensive process ensured the reliability of the data used for comparative analyses. The genomic characteristics of circRNAs in peripheral blood were evaluated in both healthy volunteers and sepsis-induced AGI patients. Figure 1D illustrated the gene properties of circRNAs. Figure 1E showed the genomic distribution of circRNA reads in healthy volunteers, revealing that the majority of reads were localized to specific genomic regions. Similarly, Fig. 1F highlighted the distribution of circRNA reads in sepsis-induced AGI patients, showing notable changes in genomic localization compared to healthy controls. The length distribution of circRNAs across all samples was shown in Fig. 1G. Figure 1H presented the TPM density distribution of circRNA expression for each sample, indicating distinct expression patterns between the sepsis-induced AGI group and healthy volunteers. CircRNA chromosomal localization was visualized in Fig. 1I, showing that circRNA expression is distributed across all chromosomes, with similar patterns observed between healthy and patient samples.

The expression profiles of miRNAs were also analyzed. Figure 1J depicted the TPM density distribution of miRNA expression across all samples, highlighting differences between the two groups. A heatmap of differentially expressed miRNAs (Fig. 1K) showed distinct miRNA expression patterns between healthy volunteers (HV) and sepsis-induced AGI cases. These findings suggested significant changes in miRNA expression profiles associated with sepsis-induced AGI. Volcano plots (Fig. 1L) were generated to identify differentially expressed miRNAs based on $|\log2FoldChange| > 1$ and $FDR < 0.05$. A total of 111 miRNAs were identified, Similarly, for circRNAs, a total of 479 circRNAs were found to be differentially expressed (Fig. 1B–C). These results indicated significant differences in the expression of both circRNAs and miRNAs between healthy controls and sepsis-induced AGI patients, providing potential biomarkers and therapeutic targets for further investigation.

circRNA-miRNA relationships and functional enrichment

Differentially expressed circRNAs and miRNAs were further analyzed for their roles in gene regulation. A total of 479 circRNAs (including the top 10 upregulated and downregulated circRNAs, Fig. 2A) and 111 miRNAs (top 10 upregulated and downregulated miRNAs, Fig. 2B) were identified. Interaction networks among circRNAs, miRNAs, and mRNAs were visualized (Fig. 2C), showing complex regulatory relationships. Gene Ontology (GO) enrichment analysis (Fig. 2D) revealed that circRNAs were predominantly associated with cellular components (CC) such as lysosomal membranes, lytic vacuole membranes, and vacuolar membranes. Additionally, they were involved in molecular functions (MF), including ubiquitin-like protein ligase activity and ubiquitin-protein transferase activity, indicating their potential roles in protein ubiquitination and cellular membrane processes. KEGG analysis (Fig. 2E) demonstrated significant enrichment in pathways related to organelle biogenesis and maintenance, G0 and early G1 regulation, and transcriptional activation of mitochondrial biogenesis, suggesting that these pathways may play critical roles in the cellular responses of sepsis-induced AGI. Additional enriched pathways include cholesterol biosynthesis regulation by SREBP, G2/M transition, and transcriptional processes mediated by YAP1 and RAF activation, highlighting the involvement of circRNAs in cellular signaling, lipid metabolism, and cell cycle progression. These results suggested that circRNAs participate in key biological processes and pathways, potentially contributing to the pathogenesis and progression of sepsis-induced AGI.

Correlation of circRNA expression with AGI grading

A total of 225 circRNAs were differentially expressed in the mild group (AGI I–II), while 332 circRNAs were differentially expressed in the severe group (AGI III–IV). Among these, 68 circRNAs were shared between the two

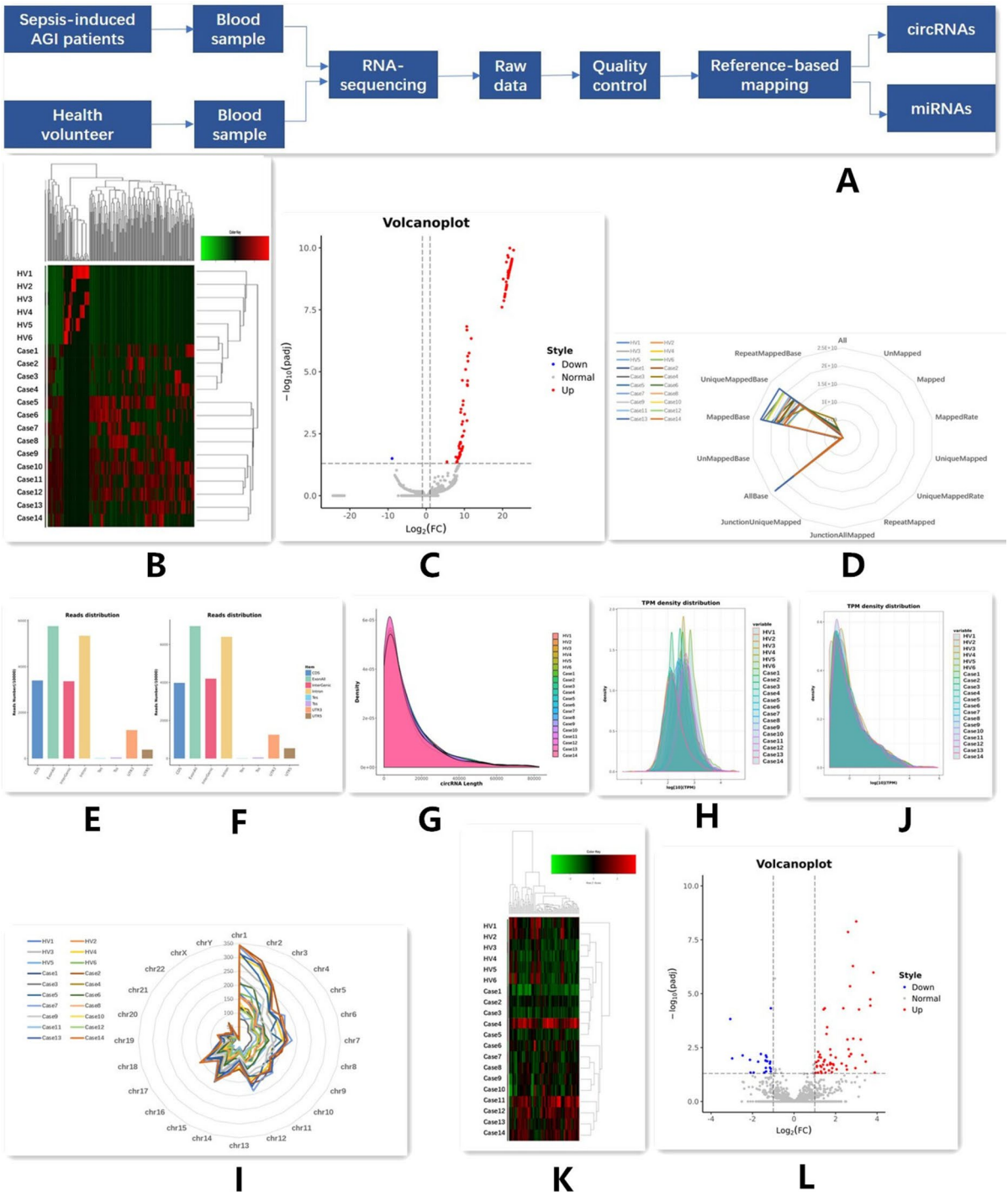

Fig. 1 Peripheral blood circRNA and miRNA profiling in patients with sepsis-induced acute gastrointestinal injury (AGI). **A** Workflow of peripheral blood circRNA and miRNA detection in sepsis-induced AGI patients and healthy volunteers. **B** Heatmap of differentially expressed circRNAs between sepsis-induced AGI patients and healthy volunteers. **C** Volcano plot of differentially expressed circRNAs. **D** Characteristics of circRNAs in terms of mapping and alignment metrics. **E** Genomic distribution of circRNA reads in healthy volunteers. **F** Genomic distribution of circRNA reads in sepsis-induced AGI patients. **G** Distribution of circRNA lengths across samples. **H** TPM density distribution of circRNA expression in sepsis-induced AGI patients and healthy volunteers. **I** Chromosomal distribution of circRNAs. **J** TPM density distribution of miRNA expression. **K** Heatmap of differentially expressed miRNAs. **L** Volcano plot of differentially expressed miRNAs. HV indicates healthy volunteers, and Case refers to sepsis-induced AGI patients

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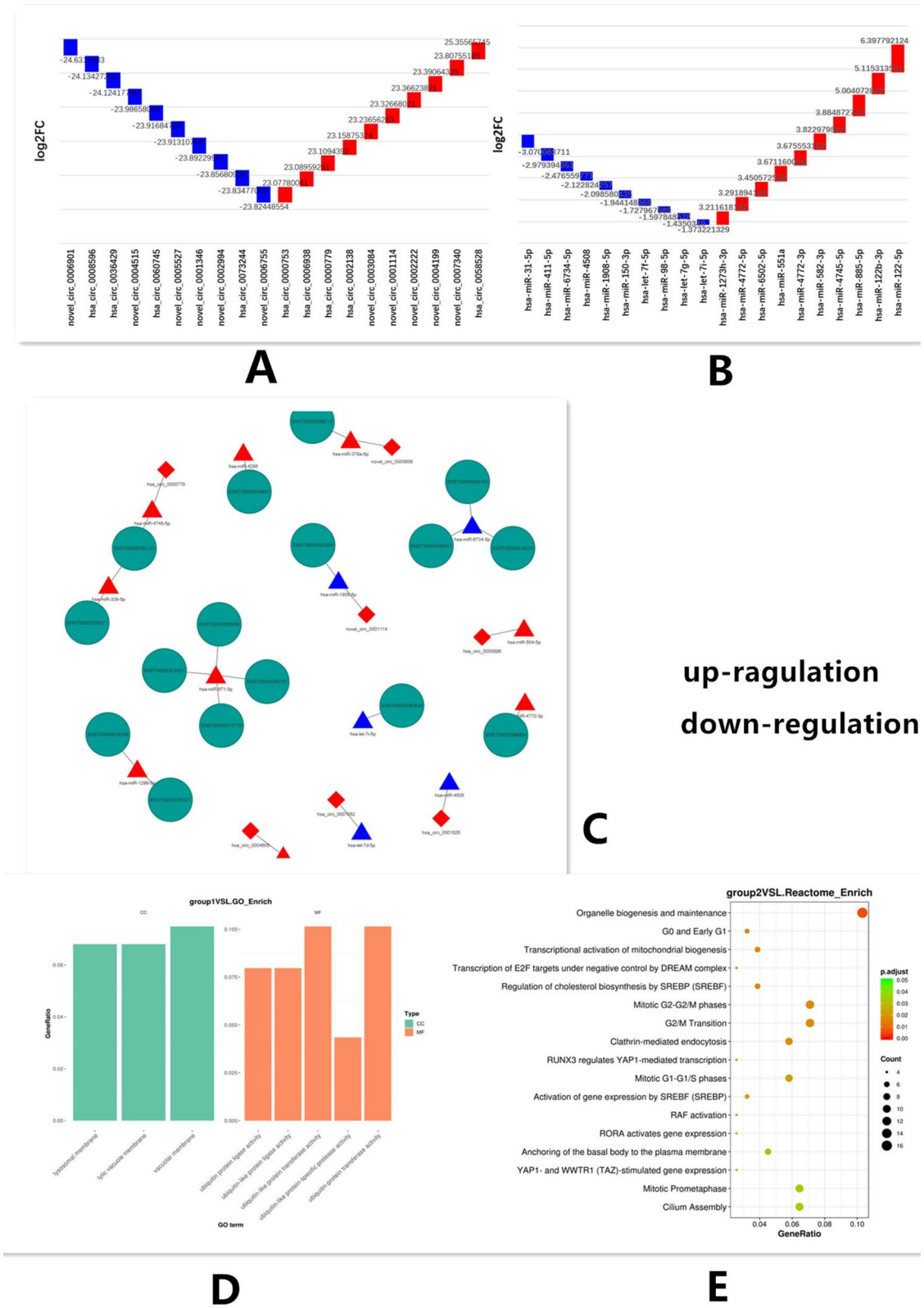

Fig. 2 circRNA-miRNA relationships and functional enrichment analysis. **A** Top 10 upregulated and downregulated circRNAs in sepsis-induced AGI patients. **B** Top 10 upregulated and downregulated miRNAs in sepsis-induced AGI patients. **C** Interaction network of circRNAs and miRNAs. **D** Gene Ontology (GO) enrichment analysis of host genes of differentially expressed circRNAs. **E** KEGG pathway enrichment analysis of differentially expressed circRNAs

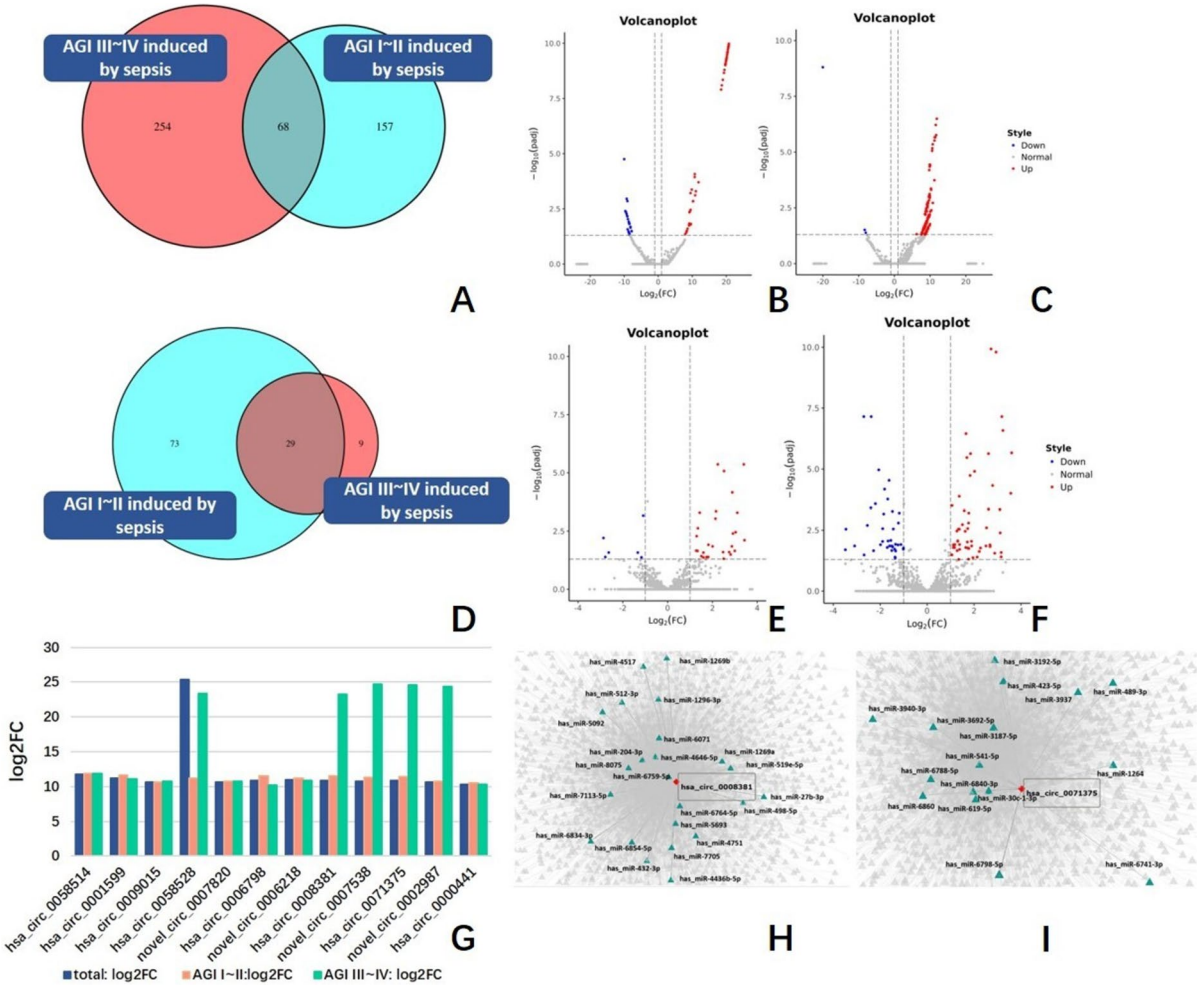

Fig. 3 Differential expression of circRNAs in sepsis-induced AGI with different severities. **A** Venn diagram of differentially expressed circRNAs between AGI I–II and AGI III–IV groups. **B** Volcano plot of differentially expressed circRNAs in the AGI I–II group. **C** Volcano plot of differentially expressed circRNAs in the AGI III–IV group. **D** Venn diagram of differentially expressed miRNAs between AGI I–II and AGI III–IV groups. **E** Volcano plot of differentially expressed miRNAs in the AGI I–II group. **F** Volcano plot of differentially expressed miRNAs in the AGI III–IV group. **G** Expression profiles of key circRNAs (e.g., hsa_circ_0008381 and hsa_circ_0071375) across AGI severity groups. **H** Interaction network of hsa_circ_0008381 with associated miRNAs. **I** Interaction network of hsa_circ_0071375 with associated miRNAs

groups, as shown in the Venn diagram (Fig. 3A). Volcano plots depict the distribution of differentially expressed circRNAs in AGI I–II (Fig. 3B) and AGI III–IV (Fig. 3C) groups compared to healthy controls, identifying circRNAs with significant expression changes based on |log2FoldChange|> 1 and FDR < 0.05. Additionally, a total of 102 miRNAs were differentially expressed in the mild group (AGI I–II), while 38miRNAs were differentially expressed in the severe group (AGI III–IV), with 29 miRNAs shared between the two groups, as illustrated in the Venn diagram (Fig. 3D). Volcano plots highlight the distribution of differentially expressed miRNAs in AGI I–II (Fig. 3E) and AGI III–IV (Fig. 3F) groups, showing miRNAs with statistically significant expression changes. Several circRNAs demonstrated stepwise increases in expression correlated with AGI severity. Notable examples include hsa_circ_0008381, novel_circ_0007538, hsa_circ_0071375, and novel_circ_0002987, which exhibited progressively higher expression levels from AGI I–II to AGI III–IV groups (Fig. 3G). These circRNAs may serve as potential biomarkers for AGI progression. To investigate the regulatory mechanisms, circRNA-miRNA interaction networks were analyzed. hsa_circ_0008381 was found to interact with multiple miRNAs, forming a complex regulatory network as shown in Fig. 3H. Similarly, hsa_circ_0071375 exhibited associations with several

miRNAs (Fig. 3I), suggesting its role in regulating downstream mRNA expression. These networks underscored the potential of circRNAs as sponges for miRNAs in the context of sepsis-induced AGI.

Discussion

Sepsis-induced AGI has a complex pathogenesis, posing significant challenges in critically ill patients. AGI occurs in approximately 40–50% of sepsis patients and is directly associated with increased disease severity and ICU mortality (Zhang et al. 2018a, b). This highlights the urgent need to identify novel biomarkers and therapeutic targets for the early diagnosis and treatment of AGI in sepsis. This study investigated the role of circRNAs in sepsis-induced AGI, revealing their potential as biomarkers and therapeutic targets. Fourteen patients with AGI caused by severe infections were enrolled, and circRNA and miRNA expression in peripheral blood were analyzed. The results showed significant differences in circRNA and miRNA expression between sepsis-induced AGI patients and healthy volunteers. The stepwise differences observed suggested that these circRNAs may serve both as biomarkers for sepsis-induced AGI and as contributors to its progression.

Previous studies have shown that circRNAs are primarily derived from exons of protein-coding genes, forming covalently closed circular structures by exon circularization or intron back-splicing, which joins the 3′ and 5′ ends together (Jeck et al. 2013). Many circRNAs have been found to act as molecular sponges for miRNAs, thereby regulating gene expression (Hansen et al. 2013). During sepsis, the differential expression of miRNAs may result from changes in the expression of specific circRNAs associated with these miRNAs. Sepsis often leads to organ dysfunction, and sepsis-induced myocardial injury significantly increases patient mortality. Our previous research revealed morphological changes in myocardial cells of rats injected with lipopolysaccharide (LPS), along with abnormalities in calcium-regulating enzymes in the sarcoplasmic reticulum and mitochondria (Dong et al. 2005). Another study using a hypodynamic infection-induced septic shock model in adolescent rats found 851 circRNAs in myocardial tissues, with 11 showing differential expression (Zhang et al. 2019). In a cecal ligation and puncture (CLP)-induced acute lung injury (ALI) mouse model, macrophages isolated from lung homogenates revealed 11 upregulated and 126 downregulated circRNAs. GO analysis indicated that the upregulated circRNAs were involved in various biological functions, such as mitochondrial distribution regulation and Notch binding, while the downregulated circRNAs were associated with processes like histone H3K27 methylation. KEGG pathway analysis suggested a correlation between upregulated circRNAs and the TGF-β signaling pathway (Bao et al. 2019). In patients with urosepsis and LPS-treated HK2 cells, circPRKCI was significantly downregulated. Overexpression of circPRKCI reversed LPS-induced reductions in cell viability, increases in apoptosis, and elevated levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8. CircPRKCI negatively regulated miR-545 expression, and upregulation of miR-545 counteracted the protective effects of circPRKCI overexpression against LPS-induced HK2 cell injury (Shi et al. 2020). These findings indicated that circRNAs can serve as biomarkers in sepsis-induced organ failure and help elucidate the biological mechanisms of disease onset.

The mechanisms of sepsis-induced intestinal failure are intricate. Bacteria and their products (PAMPs), including endotoxins (LPS) and bacterial DNA, are recognized by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) on macrophages, neutrophils, dendritic cells, and even intraepithelial lymphocytes (IELs). This recognition triggers a "cytokine storm" of pro-inflammatory mediators, driving local intestinal and systemic inflammation (Møller et al. 2005). Increased recruitment of neutrophils and monocytes elevates pro-inflammatory cytokine and reactive oxygen species (ROS) levels, exacerbating vascular dilation and causing high capillary leakage as interstitial edema progresses (Dubniks and Grände 2008; Omonijo et al. 2019). Localized disseminated intravascular coagulation (DIC), often observed in sepsis, reduces oxygen and nutrient supply while increasing carbon dioxide concentrations, leading to heightened apoptosis and necrosis of intestinal epithelial cells (IECs). The regeneration of IECs is inhibited during sepsis, which further contributes to systemic inflammatory responses. Activation of intestinal immune cells also increases intestinal permeability by altering tight junctions. Experimental sepsis models have shown redistribution of

tight junction proteins such as occludin and claudins (claudin-1, -3, -4, -5, and -8). Similarly, endotoxemia in mice disrupts the ultrastructure of occludin and zonulin-1 (ZO-1) in intestinal epithelial cells (Yoseph et al. 2016). Pathological changes in tight junction proteins and associated enzymes, including myosin light chain kinase (MLCK), β-catenin, and zonulin, compromise IEC integrity, thereby promoting bacterial translocation. Additionally, pancreatic proteases can induce self-digestion, further promoting MODS. This self-digestion exacerbates the release of damage-associated molecular patterns (DAMPs) (Fitzal et al. 2003). The transcription and expression of mRNAs encoding these proteins have been associated with miRNAs and circRNAs. In our study, by stratifying patients based on disease severity, we identified circRNAs with stepwise increases in expression. These findings provided a foundation for further research into the functional roles of circRNAs in sepsis-induced AGI.

In recent years, increasing evidence has highlighted the potential of circular RNAs (circRNAs) as important regulators in various pathological processes, including intestinal injury and sepsis. For example, a recent study found that circEZH2_005 plays a crucial role in protecting the intestinal mucosa by promoting the proliferation of Lgr5 + stem cells through its interaction with hnRNPA1, thereby alleviating I/R-induced damage (Zhang et al. 2023). Similarly, another study focusing on abdominal sepsis revealed that circFLNA is significantly upregulated in the intestinal epithelium after sepsis induced by intestinal perforation, circFLNA/miR-766-3p/Fas axis offers a promising avenue for therapeutic intervention in sepsis-induced intestinal injury, underscoring the potential of circRNAs as both biomarkers and therapeutic targets in sepsis-related gastrointestinal complications (Ye et al. 2023). These studies support the idea that circRNAs are not only involved in intestinal injury but also show promise as diagnostic biomarkers and therapeutic targets in various contexts, including sepsis. Our findings align with these observations, suggesting that circRNAs could have similar roles in the pathophysiology of sepsis-induced AGI. Additionally, targeting circRNAs presents a promising therapeutic strategy for sepsis-induced AGI. Several approaches could be explored to modulate circRNA expression for therapeutic purposes. CircRNA mimics could be used to restore the function of beneficial circRNAs, while antisense oligonucleotides or small molecules could inhibit harmful circRNAs. Additionally, exosomal delivery systems could facilitate the targeted delivery of circRNAs or their inhibitors to specific tissues, minimizing off-target effects. However, several challenges must be addressed for these strategies to be effective. The delivery of circRNA-based therapeutics to the appropriate tissues remains a significant hurdle. Furthermore, off-target effects may occur if circRNAs or their inhibitors interact with unintended RNAs or proteins, which could lead to adverse outcomes. Therefore, refining delivery methods to ensure selectivity and safety is crucial. Future research will need to focus on optimizing these strategies and conducting experimental validations to fully explore the therapeutic potential of circRNAs in sepsis-induced AGI.

This study has several limitations that should be addressed in future research. First, the relatively small sample size of 14 patients with sepsis-induced AGI and 14 healthy volunteers limits the generalizability of the findings. While the sample size was determined based on preliminary analyses and clinical constraints, we acknowledge that statistical robustness could be further improved. To assess the adequacy of the sample size, we have now performed a post-hoc power analysis, which confirms that the current sample size provides sufficient statistical power (80%) to draw meaningful conclusions. However, larger, multicenter studies are needed to further validate the observed correlations between circRNA expression patterns and AGI severity. Second, while the bioinformatics analyses provided valuable insights into potential circRNA-miRNA-mRNA regulatory networks, we did not conduct functional validation of the identified circRNAs, including hsa_circ_0008381 and hsa_circ_0071375. Experimental studies are required to elucidate the specific biological roles of these circRNAs in sepsis-induced AGI. Additionally, while the circRNA-miRNA network construction and functional enrichment were based on computational approaches, no further validation using external datasets or independent cohort validation was performed. These aspects will be addressed in future studies to provide experimental confirmation and external validation of our findings. Third, the cross-sectional design of this study restricts the ability to infer causal relationships. Longitudinal studies examining circRNA dynamics over the course of sepsis and AGI progression would provide more comprehensive insights into the temporal changes associated with AGI severity. Lastly, the study focused solely on circRNA and miRNA interactions, and exploring additional non-coding RNA species or

proteomics could further enhance the understanding of the complex molecular mechanisms underlying sepsis-induced AGI.

Conclusions

In summary, this study highlighted the potential role of circRNAs as biomarkers and therapeutic targets in sepsis-induced acute gastrointestinal injury. By profiling peripheral blood circRNA and miRNA expression, significant differences were identified between patients with AGI and healthy controls, with stepwise expression changes correlating with AGI severity. These findings provided novel insights into the molecular mechanisms of sepsis-induced AGI, emphasizing the regulatory role of circRNAs in the progression of this condition.

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Author contributions CHS and LXJ conceived and designed the study. LXJ, LCX, HCY, CYT, NCC and LSL performed the experiments; analyzed the data. LXJ, LCX and HCY wrote the manuscript. CHS supervised the study. All authors reviewed and approved the final version of the manuscript.

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Data availability The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interests The authors declare no competing interests.

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Consent to participate Written informed consent was obtained from all participants.

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