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ORIGINAL ARTICLE

Correlation of Blood and Intestinal Mucosa miR-34a Expressions with Disease Severity in Ulcerative Colitis Patients

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SUMMARY

Background: **miR-34a has been implicated in many autoimmune diseases and gastrointestinal diseases. However, the expression of miR-34 in ulcerative colitis (UC) patients were not fully studied. This study was performed to investigate the association of blood and intestinal tissue miR-34a expression of patients with disease severity in UC patients.**
Methods: **Our study enrolled 82 patients with UC and 80 age- and gender- matched healthy individuals. Blood miR-34a expressions were detected using reverse transcription-polymerase chain reaction (RT-PCR). Local intestinal miR-34a, STAT3 mRNA and IL-23 mRNA expressions were also detected in the lesioned area and adjacent non-affected intestinal tissue in patients. Disease severity of UC was assessed by Mayo score. The diagnostic value of both blood and local miR-34a expression for UC patients was assessed by receiver operating characteristic (ROC) curve.**
Results: **Blood miR-34a was increased in UC patients in contrast with healthy individuals with statistical significance. In UC patients, local intestinal miR-34a expressions were markedly upregulated compared to adjacent non-affected intestinal tissue. Local intestinal miR-34a expressions were positively correlated with STAT3 mRNA and IL-23 mNRA. Both blood and local miR-34a expressions were significantly and positively related to Mayo scores. ROC curve analysis indicated that both blood and local miR-34a expressions may act as decent marker for Mayo grade.**
Conclusions: **Blood and intestinal tissue miR-34a expressions are correlated with disease severity in UC patients. Both blood and intestinal tissue miR-34a expressions may serve as potential diagnostic and prognostic makers for UC. Therapeutic methods targeting miR-34a may act as potential ways for UC treatment.**
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ulcerative colitis, miR-34a, inflammation, disease severity

INTRODUCTION

As an inflammatory bowel disease (IBD), ulcerative colitis (UC) is distinguished by mucosal inflammation that typically begins in the distal colon and can extend to the whole colon, manifesting as a relapsing and remitting condition [1]. The etiology of UC is believed to involve a complex interplay between environmental factors, immune system dysfunction, altered gut microbi-

ome composition, and genetic susceptibility to disease [2]. UC patients always suffer from bloody diarrhea, frequency, abdominal pain, fatigue, and fecal incontinence [3]. Traditionally, the diagnosis and assessment of UC has relied on clinical symptoms, laboratory tests, endoscopic findings, as well as pathological examinations [4]. Considering the limitations of the traditional diagnostic and evaluative methods, there is a pressing need for the development of a non-invasive and easily accessible biomarker for effective clinical management of UC.

MicroRNAs (miRNAs) are a collection of small endogenous ribonucleic acids (RNAs) with approximately 20 - 24 ribonucleotides in length [5]. miRNAs are produced by processing the single stranded RNA precursor with the size of 70 - 90 bases of hairpin structure by ribonuclease (mainly Dicer enzyme) [6]. The regulation of target genes by miRNA mainly depends on the degree of complementary pairing between miRNA and the transcriptome sequence of the target gene [7]. Bioinformatics analysis proved significant effects of miRNAs on diverse cellular processes, involving cell proliferation, differentiation, apoptosis, as well as immune response [8], indicating that miRNAs are involved in cell life activities. Many studies have shown that each miRNA can regulate the expression of hundreds of mRNA and regulate more than 30% of protein coding genes after transcription [9], and deregulation of miRNA may lead to many human diseases, such as tumors, autoimmune diseases, diabetes, cardiovascular diseases, and digestive diseases [10].

miR-34a together with two other members, miR-34b and miR-34c, comprise the extensively studied miR-34 family of microRNAs [11]. miR-34a is transcribed from a unique genomic locus on chromosome 1 (1p36.22). It displays ubiquitous expression across diverse tissue types, with the exception of lung tissue, and exhibits higher expression levels when compared to other members of the miR-34 family [12]. Besides, miR-34a also serves to regulate various target mRNAs that take part in the modulation of cell cycle, cell proliferation, senescence, migration, as well as cell invasion [13]. As previously reported, miR-34a is engaged in the development of cancers [14], osteoarthritis [15], cardiovascular diseases [16], neural diseases [17], and gut diseases [18], etc.

Moreover, miR-34a has been shown to exert a critical regulatory role in the modulation of inflammation pathways and immune response. It has been found that miR-34a serves to enhance the production of inflammatory chemokines and cytokines in keratinocytes. Furthermore, in studies involving wild-type mice with wounds, treatment with miR-34a-mimics has been shown to potentiate local inflammation and delay the healing process [19]. In addition, a prior investigation has revealed that miR-34a expression is under the influence of the pro-inflammatory cytokine IL-1β, and this regulatory interaction exerts a crucial effect on augmenting the inflammatory response in endometritis [20]. The miR-34a is also engaged in lipopolysaccharide mediated sepsis related renal function impairment via KLF4 [21]. Research has shown that miR-34a enhances the levels of inflammation/oxidative stress among patients with necrotizing enterocolitis by suppressing the expression of SIRT1 [18].

These findings mentioned above indicate a pivotal role of miR-34a in immune homeostasis and immune regulation. However, to date, there has been no report regarding any alterations in miR-34a expression in the peripheral blood/intestinal mucosa among UC patients. To our knowledge, this is the first study to conduct miR-34a expression analysis in both peripheral blood and local mucosa of UC patients, aiming to explore whether it can be used as a potential diagnostic and prognostic biomarker of UC.

Patients and methods

Study patients

Eighty-two UC patients were selected from February 2022 to May 2023 in our hospital. The presence of UC in patients was confirmed based on a variety of investigative modalities, including endoscopic evaluation, radiological imaging, histological examination, and clinical parameters [22,23]. None of the patients received glucocorticoid, salicylic acid inhibitor, and immunosuppressive agents 30 days before enrollment. Exclusion criteria: 1) Patients with malignant tumors; 2) Patients with chronic heart, liver, and kidney diseases; 3) Accompanied by toxic colon dilation, colon stenosis, obstruction, perforation, etc.; 4) Patients with incomplete examination data. We collected baseline characteristics of patients, including age, gender, weight, smoking history, erythrocyte sedimentation rate (ESR), as well as C-reactive proteins (CRP). Meanwhile, eighty gender and age matched healthy individuals receiving regular body check were enrolled as healthy controls. The study was approved by the Ethical Committee of Changsha Hospital of Traditional Chinese Medicine (Ethical Number: 20220013). Prior to enrollment, all patients provided written consent for participation in this study.

Determination of disease severity

The severity of UC was determined using the Mayo score [24], which includes four items: frequency of bowel movements, blood in the stool, endoscopic examination, and overall comprehensive evaluation by the doctor. The degree of disease activity in UC patients was assessed via a grading system that comprised a 12-point scale. Each item was rated on a scale of 0 to 4 according to its severity, and the sum total score was indicative of the severity of disease activity, with a higher score indicating a more severe state. Patients with a Mayo total score of ≥ 3 were selected for this study, with disease severity stratified as mild (3 - 5 points), moderate (6 - 8 points), or severe (≥ 9 points). The evaluation of Mayo's scoring results was conducted by two gastroenterologists in our hospital, and the con-

sistency of the results was checked using Kappa values for endoscopic observation.

Blood collection and RT-PCR

A previous investigation has indicated that the most ideal approach for profiling miRNAs within a systemic context would entail the use of whole blood samples that have not undergone clotting, as opposed to blood or plasma samples [25]. For blood sample collection, non-fasting venous samples were obtained from all study participants and then placed in Vacuette EDTA K3E blood bottles (Grenier Bio-One International AG, Kremsmunster, Austria). The unprocessed whole blood samples were subsequently stored at 4℃ for further use [25].

Total RNA was isolated from both whole blood and intestinal mucosa using an adaptation of the TRI Reagent® BD technique (Molecular Research Center, Inc., Cincinnati, OH, USA). RNA concentration in the extracted samples was measured using a NanoDrop® spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). Typically, miRNA concentrations within a range of 30 - 300 ng/μL were obtained per sample. RNA integrity was evaluated using RNA 6000 Nano LabChip Series II Assays (for small RNA) on a 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). PCR reactions were conducted using an ABI 7900 HT Fast Real-Time PCR System (Applied Biosystems) in final volumes of 10 μL. Briefly, reactions consisted of 0.7 μL cDNA, 5 μL TaqMan® Universal PCR Fast Master Mix, and 0.2 μM TaqMan® primer-probe mix (Applied Biosystems). Amplification began with a 10-minute incubation step at 95℃, followed by 40 cycles of denaturation at 95℃ for 15 seconds and annealing/extension at 60℃ for 60 seconds.

RT-PCR quantification of miR-34a, IL-23 mRNA and STAT3 mRNA expression were performed using TaqMan MicroRNA® Assays (Applied Biosystems, Foster City, CA, USA) based on the protocol provided by the manufacturer. Total RNA was reverse transcribed using the MultiScribe™-based High-Capacity cDNA Archive kit (Applied Biosystems).

IL-23 forward:
5′-GTTCCCCATATCCAGTGTGG-3′,
reverse:
5′-GGATCCTTTGCAAGCAGAAC-3′;
STAT3 forward:
5′-GCCAGAGAGCCAGGAGCA-3′,
reverse:
5′-TGAAGCTGACCCAGGTAGCGCTGC-3′;
GAPDH forward:
5′-AACGTGTCAGTGGTGGACCTG-3′,
reverse:
5′-AGTGGGTGTCGCTGTTGAAGT-3′;
miR-34a: forward:
5′-ACACTCCAGCTGGGTGGCAGTGTCTTAGC,
reverse:
5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAG TTGAGACAACCA-3′;
U6 forward:
5′-CTCGCTTCGGCAGCACA-3′,
reverse:
5′-AACGCTTCACGAATTTGCGT-3′.

miR-16 and U6 were employed as an endogenous control for normalization of miRNA expression profiles in the blood samples and intestinal mucosa samples, respectively.

In situ hybridization (ISH)

Intestinal mucosa specimens from the UC patients were first fixed with 4% paraformaldehyde and subsequently embedded in paraffin. miR-34a ISH was conducted on Tissue Microarrays (TMAs) using the MiRCURY LNA miRNA ISH Optimization kit (Exiqon, Vedbaek, Denmark). Microscopic evaluation of staining intensity was conducted utilizing a 4-point scale, wherein the observed results were graded as follows: 0 (negative), 1 (+), 2 (++), and 3 (+++), in accordance with predefined criteria [26].

Statistical analysis

Data analysis was performed by Graphpad Prism 8.0. Normal distribution was evaluated by the Kolmogorov-Smirnov test. Continuous data with normal distribution were presented as mean ± standard deviation (SD); in contrast, those with skewed distribution were reported as median (interquartile [IQR]). Besides, a categorical variable was described by frequency. The general data comparison between UC patients and healthy control group was conducted using *t*-tests, Mann-Whitney U-tests, or chi squared tests, where appropriate. The miR-34a expressions among different UC patients were compared using one way ANOVA or Kruskal-Wallis tests, followed by and Tukey or Tamhan tests. miR-34a expressions for multiple comparisons. Correlations of miR-34a expressions with Mayo grade and other measurement indicators were tested using Spearman's or Pearson's correlation. The diagnostic value of both blood and local miR-34a expression in UC was determined by ROC curve. p-value less than 0.05 was indicative for statistical significance.

RESULTS

Demographic data

The clinical characteristics of the patients and controls are summarized in Table 1. There were no significant differences of age, gender distribution, BMI, and smoking habits between UC patients and healthy controls. The localization of UC was 10 cases in E1 (ulcerative proctitis), 25 cases in E2 (left-sided ulcerative colitis, distal UC) and 47 cases in E3 (extensive ulcerative colitis, pancolitis). The mean Mayo scores were 6.70 ± 2.28

Table 1. Demographic statistics of enrolled participants.

	UC patients (n = 82)	Healthy controls (n = 80)	p-value
Age (year)	53.1 ± 11.8	52.8 ± 12.0	0.511
Gender (female/male)	40/42	37/43	0.899
BMI (kg/m^2)	23.8 ± 3.3	23.6 ± 2.9	0.431
Smoking (yes/no)	35/47	32/48	0.913
Localization (E1/E2/E3)	10/25/47	/	
Mayo Score	6.79 ± 2.28	/	
hsCRP levels (mg/L)	1.3 - 18.0	/	

The statistical method for age and BMI was Student's *t*-test, and gender distribution and smoking were evaluated by chi squared test. UC - ulcerative colitis, BMI - body mass index, CRP - C-reactive protein.

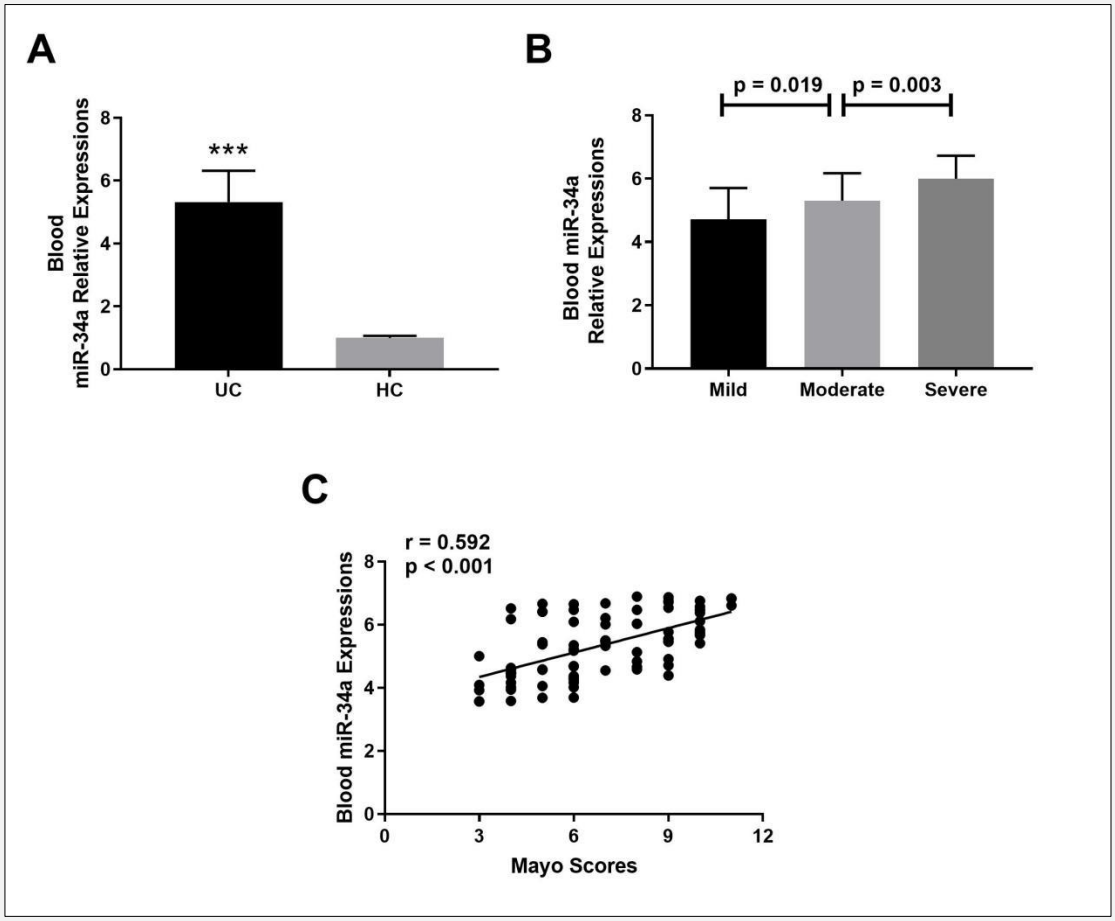

Figure 1. A - Comparison of blood miR-34a expressions between UC and HC patients, B - Comparison of blood miR-34a expressions among different degrees of UC, C - Correlation of blood miR-34a expressions with Mayo Scores (* p < 0.001 vs. HC). Statistical methods: A - Student's *t*-test, B - One way ANOVA followed by Tukey's test, C - r value for Spearman's correlation test.**

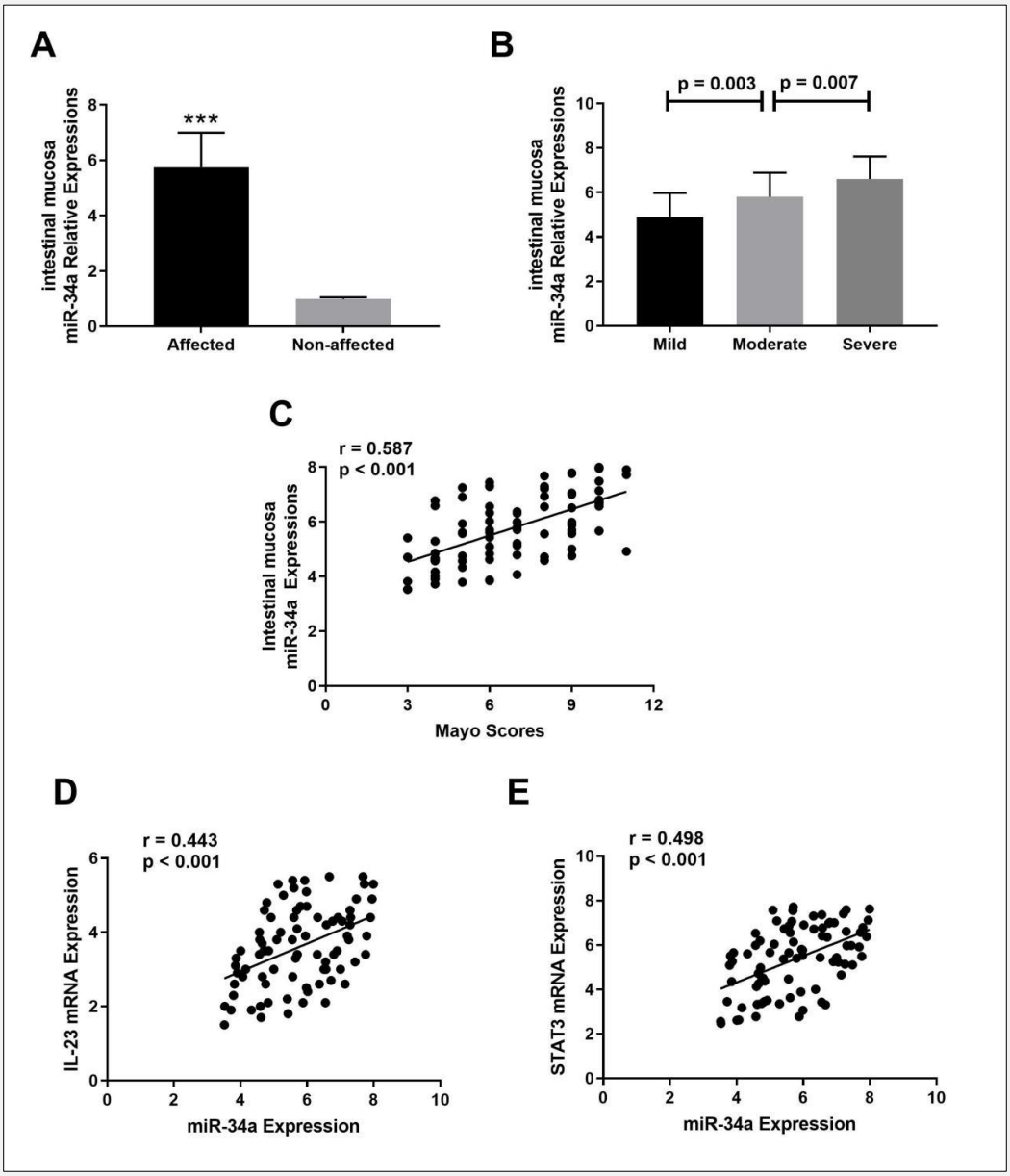

Figure 2. A - Comparison of intestinal mucosa miR-34a expressions between affected and non-affected tissues, B - Comparison of intestinal mucosa miR-34a expressions among different degree of UC, C - Correlation of intestinal mucosa miR-34a expressions with Mayo Scores, D - Correlation of intestinal mucosa miR-34a expressions with IL-23 mRNA expressions, E - Correlation of intestinal mucosa miR-34a expressions with STAT3 mRNA expressions.
Statistical methods: A - Student's *t*-test, B - One way ANOVA followed by Tukey's test, C, D, E - r value for Spearman's correlation test.

with hsCRP levels ranging from 1.3 - 18.0 mg/L.

Peripheral blood miR-34a was enhanced among UC patients

As shown in Figure 1A, the miR-34a expression was elevated in the peripheral blood of UC patients with statistical significance in contrast to the levels observed in normal control group (5.32 ± 1.00 vs. 1.00 ± 0.06, $p < 0.001$). Besides, patients with severe UC exhibited significantly higher miR-34a expressions in serum compared to moderate UC (6.00 ± 0.73 vs. 5.31 ± 0.86, $p < 0.001$) (Figure 1B). Patients with moderate UC showed

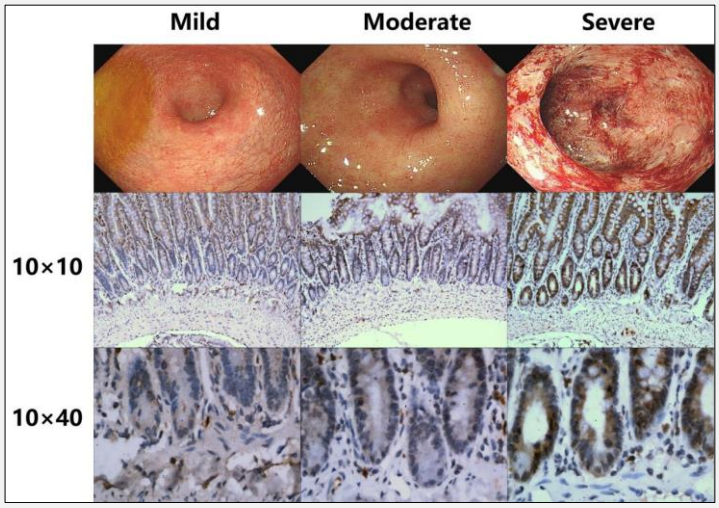

Figure 3. Intestinal mucosa specimen miR-34a expressions among mild, moderate, and severe UC patients.

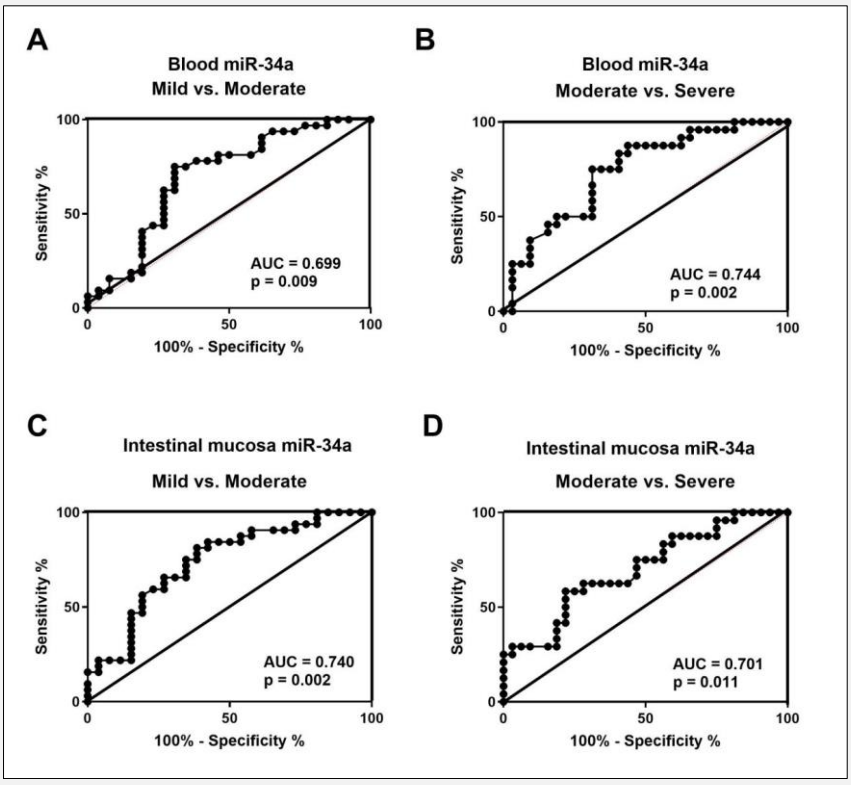

Figure 4. A - ROC curve of blood miR-34a expression between mild and moderate UC, B - ROC curve of blood miR-34a expression between moderate and severe UC, C - ROC curve of miR-34a expression in intestinal mucosa between mild and moderate UC, D - ROC curve of miR-34a expression in intestinal mucosa between moderate and severe UC.
UC - ulcerative colitis, ROC - receiver operating characteristic, AUC - Area Under Curve, all p-value for prediction accuracy.

significantly increased expressions of miR-34a in compared to those with mild UC (5.31 ± 0.86 vs. 4.72 ± 0.98, $p < 0.001$) (Figure 1B). As shown in Figure 1C, a positive association was established between the serum miR-34a expression and the Mayo scores observed in UC patients ($r = 0.592$, $p < 0.001$).

Intestinal mucosa miR-34a expression was elevated in affected tissues

The expression level of miR-34a in intestinal mucosa affected tissue was much higher in contrast to that of the adjacent non-affected tissue with statistical significance (5.75 ± 1.24 vs. 1.05 ± 1.19, $p < 0.001$) (Figure 2A). Besides, patients with severe UC showed significantly higher miR-34a expressions in intestinal mucosa compared to moderate UC (6.60 ± 1.02 vs. 5.81 ± 1.09, $p < 0.001$) (Figure 2B). Patients with moderate UC showed significantly increased expressions of miR-34a in compared with those with mild UC (5.81 ± 1.08 vs. 4.90 ± 1.08, $p < 0.001$) (Figure 2B). Similar to the observations within serum, a positive correlation was also noted between miR-34a expression levels within the intestinal mucosa and Mayo scores observed in UC patients ($r = 0.587$, $p < 0.001$) (Figure 2C). Next, ISH with intestinal mucosa tissue microarrays showed that miR-34a overexpression was more prominent in UC patients with severe than in moderate and mild patients (Figure 3), indicating that dysregulation of miR-34a may contribute to the severity of UC.

We further detected the correlation between miR-34a expressions with IL-23 and STAT3 mRNA. As shown in Figure 2D and 2E, we found local miR-34a expressions were significantly and positively related to IL-23 mRNA ($r = 0.443$, $p < 0.001$) as well as STAT3 mRNA ($r = 0.498$, $p < 0.001$) expressions.

Diagnostic value of blood and intestinal mucosa miR-34a in UC patients

Finally, a ROC curve analysis was conducted to evaluate the diagnostic potential of miR-34a levels in both blood and intestinal mucosa as biomarkers for UC progression. As shown in Figure 4 for mild UC vs. moderate UC and moderate UC vs. severe UC, increased blood miR-34a expression exhibited significant AUC for both mild UC vs. moderate UC (AUC = 0.699, $p = 0.009$) (Figure 4A) and moderate UC vs. severe UC (AUC = 0.744, $p = 0.002$) (Figure 4B). In addition, increased intestinal mucosa miR-34a expression also demonstrated significant AUC for both mild UC vs. moderate UC (AUC = 0.740, $p = 0.002$) (Figure 4C) and moderate UC vs. severe UC (AUC = 0.701, $p = 0.011$) (Figure 4D). These findings implicated that both blood and intestinal mucosa miR-34a expressions might serve as good diagnostic markers to evaluate disease development of UC.

DISCUSSION

It is reported that many people suffer from various diseases that are tightly associated with dysregulation of the intestinal immune system, including UC [27]. Currently, some studies have indicated abnormal miRNA alternations in patients suffering from UC [28,29]. However, much information remains unknown regarding the miRNA expression of local peripheral blood as well as their important role in UC. In the current study, we mainly focused on miR-34a, which is proven to be involved in the pathogenesis of many autoimmune diseases [30,31]. However, the expression of miR-34a in peripheral blood and local intestinal mucosa of UC patients has not been reported. Here, the findings revealed that the relative miR-34a expressions in peripheral blood and the local intestinal mucosa of UC patients were higher than control. Both local and blood miR-34a expressions were correlated with disease severity, suggesting a potential influence of miR-34a on the progression of UC.

Evidence has implicated the role of miR-34a across colon diseases. For example, high levels of circulating miR-34a are associated with colorectal cancer progression [32]. Furthermore, elevated levels of miR-34a have been found to predict an increased risk of disease recurrence and overall poorer survival rates in patients with colorectal adenocarcinoma [33]. Research has shown that resveratrol may alleviate colitis, likely due in part to its ability to significantly decrease the expression levels of miR-34a and pro-inflammatory cytokines [34].

We also found that miR-34a levels in intestinal mucosa were positively associated with STAT3 mRNA and IL-23 mRNA expressions. As a member of the transcription factor family, signal transducer and activator of transcription (STAT) 3 mediates transmembrane signaling between cell surface-bound receptors and the intracellular nuclear machinery in response to diverse stimuli, including peptide hormones, cytokines, and growth factors. Numerous cytokines act as ligands for cell surface receptors that initiate downstream signaling pathways leading to the activation of STAT3 [35, 36]. Studies have demonstrated that STAT3 activation is evident in inflamed colonic tissue obtained from patients suffering from IBD [37]. In addition, upregulation of STAT3 expression in T-cells, macrophages, as well as epithelial cells has been found to be directly associated with the degree of histological inflammation in colonic tissue [38,39].

Genetic investigations indicate that certain IBD patients possess specific single nucleotide polymorphisms in the gene of IL-23 receptor (IL-23R), thereby suggesting the impacts of IL-23R signaling on disease susceptibility [40]. An increase in the production of IL-23 has been observed in macrophages, dendritic cells, and granulocytes in UC patients [41].

Several limitations existed in this study. First, as a cross-sectional study, it was conducted exclusively

among Chinese participants, thereby potentially limiting its generalizability to other populations or ethnicities. Further multicenter studies with larger samples among different areas and countries are needed. Moreover, the causal relationship between miR-34a and UC remains unclear. Second, only miR-34a is studied in this study, and investigation of other miRNAs may provide much more valuable information. Finally, further investigation is warranted to determine whether miR-34a expression is dysregulated in other forms of IBD disease such as Crohn's disease.

Collectively, our study revealed an obvious increase in blood miR-34a expression levels among patients with ulcerative colitis (UC) when compared to healthy control subjects. In UC patients, local intestinal miR-34a expressions were markedly upregulated in comparison with adjacent non-affected intestinal tissue. Both blood and local miR-34a expressions may act as potential diagnostic and prognostic markers for UC.

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Declaration of Interest:

The authors declare that they have no competing interests.

References:

1. Segal JP, LeBlanc JF, Hart AL. Ulcerative colitis: an update. Clin Med (Lond) 2021;21(2):135-9. (PMID: 33762374)
2. Du L, Ha C. Epidemiology and Pathogenesis of Ulcerative Colitis. Gastroenterol Clin North Am 2020;49(4):643-54. (PMID: 33121686)
3. Eisenstein M. Ulcerative colitis: towards remission. Nature 2018; 563(7730):S33. (PMID: 30405234)
4. Kaenkumchorn T, Wahbeh G. Ulcerative Colitis: Making the Diagnosis. Gastroenterol Clin North Am 2020;49(4):655-69. (PMID: 33121687)
5. Mohr AM, Mott JL. Overview of microRNA biology. Semin Liver Dis 2015;35(1):3-11. (PMID: 25632930)
6. Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol 2018;141(4):1202-7. (PMID: 29074454)
7. Ho PTB, Clark IM, Le LTT. MicroRNA-Based Diagnosis and Therapy. Int J Mol Sci 2022;23(13):7167. (PMID: 35806173)
8. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH. An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. J Cell Physiol 2019;234(5):5451-65. (PMID: 30471116)
9. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010; 11(9):597-610. (PMID: 20661255)
10. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 2017;16(3):203-22. (PMID: 28209991)
11. Zhang L, Liao Y, Tang L. MicroRNA-34 family: a potential tumor suppressor and therapeutic candidate in cancer. J Exp Clin Cancer Res 2019;38(1):53. (PMID: 30717802)
12. Ghandadi M, Sahebkar A. MicroRNA-34a and its target genes: Key factors in cancer multidrug resistance. Curr Pharm Des 2016; 22(7):933-9. (PMID: 26648462)
13. Chen F, Hu SJ. Effect of microRNA-34a in cell cycle, differentiation, and apoptosis: a review. J Biochem Mol Toxicol 2012;26(2): 79-86. (PMID: 22162084)
14. Li S, Wei X, He J, et al. The comprehensive landscape of miR-34a in cancer research. Cancer Metastasis Rev 2021;40(3):925-48. (PMID: 33959850)
15. Luo C, Liang JS, Gong J, et al. The function of microRNA-34a in osteoarthritis. Bratisl Lek Listy 2019;120(5):386-91. (PMID: 31113203)
16. Chen X, Zhou JY, Zhou JY. MicroRNA-34a: role in cancer and cardiovascular disease. Curr Drug Targets 2014;15(4):361-73. (PMID: 24438466)
17. Chua CEL, Tang BL. miR-34a in Neurophysiology and Neuropathology. J Mol Neurosci 2019;67(2):235-46. (PMID: 30488149)
18. Zhu H, Lin Y, Liu Y. miR-34a increases inflammation and oxidetive stress levels in patients with necrotizing enterocolitis by downregulating SIRT1 expression. Mol Med Rep 2021;24(3): 664. (PMID: 34296298)
19. Wu J, Li X, Li D, et al. MicroRNA-34 Family Enhances Wound Inflammation by Targeting LGR4. J Invest Dermatol 2020; 140(2):465-476.e11. (PMID: 31376385)
20. Ma X, Yin B, Guo S, et al. Enhanced Expression of miR-34a Enhances Escherichia coli Lipopolysaccharide-Mediated Endometritis by Targeting LGR4 to Activate the NF-κB Pathway. Oxid Med Cell Longev 2021;2021:1744754. (PMID: 34504639)
21. Khan MJ, Singh P, Dohare R, et al. Inhibition of miRNA-34a Promotes M2 Macrophage Polarization and Improves LPS-Induced Lung Injury by Targeting Klf4. Genes (Basel) 2020;11(9):966. (PMID: 32825525)
22. Pabla BS, Schwartz DA. Assessing Severity of Disease in Patients with Ulcerative Colitis .Gastroenterol Clin North Am 2020 49(4):671-688. (PMID: 33121688)
23. Podolsky DK. Inflammatory bowel disease (2). N Engl J Med 1991;325:1008-16. (PMID: 1886623)
24. Sandborn WJ, Sands BE, Vermeire S, et al. Modified Mayo score versus Mayo score for evaluation of treatment efficacy in patients with ulcerative colitis: data from the tofacitinib OCTAVE program. Therap Adv Gastroenterol 2022;15:17562848221136331. (PMID: 36506749)
25. Heneghan HM, Miller N, Sweeney KJ, Newell J, Kerin MJ. Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg 2010;251:499-505. (PMID: 20134314)
26. Yoshii A, Koji T, Ohsawa N, Nakane PK. In situ localization of ribosomal RNAs is a reliable reference for hybridizable RNA in tissue sections. J Histochem Cytochem 1995;43(3):321-7. (PMID: 7532657)

27. Geremia A, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. Autoimmun Rev 2014;13(1):3-10. (PMID: 23774107)

28. Zhou J, Liu J, Gao Y, Shen L, Li S, Chen S. miRNA-Based Potential Biomarkers and New Molecular Insights in Ulcerative Colitis. Front Pharmacol 2021;12:707776. (PMID: 34305614)

29. Yarani R, Shojaeian A, Palasca O, et al. Differentially Expressed miRNAs in Ulcerative Colitis and Crohn's Disease. Front Immunol 2022;13:865777. (PMID: 35734163)

30. Xie M, Wang J, Gong W, et al. NF-κB-driven miR-34a impairs Treg/Th17 balance via targeting Foxp3. J Autoimmun 2019;102: 96-113. (PMID: 31130368)

31. Taheri F, Ebrahimi SO, Shareef S, Reiisi S. Regulatory and immunomodulatory role of miR-34a in T cell immunity. Life Sci 2020;262:118209. (PMID: 32763292)

32. Aherne ST, Madden SF, Hughes DJ, et al. Circulating miRNAs miR-34a and miR-150 associated with colorectal cancer progression. BMC Cancer 2015;15:329. (PMID: 25924769)

33. Rapti SM, Kontos CK, Christodoulou S, Papadopoulos IN, Scorilas A. miR-34a overexpression predicts poor prognostic outcome in colorectal adenocarcinoma, independently of clinicopathological factors with established prognostic value. Clin Biochem 2017;50:918-24. (PMID: 28624481)

34. Zhou X, Zhang Y, Hu M, Ge Z, Zhou G. Resveratrol enhances MUC2 synthesis via the ANRIL-miR-34a axis to mitigate IBD. Am J Transl Res 2023;15:363-72. (PMID: 36777862)

35. Levy DE, Darnell JE Jr. Stats: Transcriptional control and biological impact. Nat Rev Mol Cell Biol 2002;3:651-62. (PMID: 12209125)

36. Rawlings JS, Rosler KM, Harrison DA. The JAK/STAT signaling pathway. J Cell Sci 2004;117:1281-3. (PMID: 15020666)

37. Lovato P, Brender C, Agnholt J, et al. Constitutive STAT3 activation in intestinal T cells from patients with Crohn's disease. J Biol Chem 2003;278:16777-81. (PMID: 12615922)

38. Mudter J, Weigmann B, Bartsch B, et al. Activation pattern of signal transducers and activators of transcription (STAT) factors in inflammatory bowel diseases. Am J Gastroenterol 2005;100: 64-72. (PMID: 15654782)

39. Musso A, Dentelli P, Carlino A, et al. Signal transducers and activators of transcription 3 signaling pathway: An essential mediator of inflammatory bowel disease and other forms of intestinal inflammation. Inflamm Bowel Dis 2005;11:91-8. (PMID: 15677901)

40. Neurath MF. IL-23 in inflammatory bowel diseases and colon cancer. Cytokine Growth Factor Rev 2019;45:1-8. (PMID: 30563755)

41. Bauche D, Joyce-Shaikh B, Jain R, et al. LAG3+ Regulatory T Cells Restrain Interleukin-23-Producing CX3CR1+ Gut-Resident Macrophages during Group 3 Innate Lymphoid Cell-Driven Colitis. Immunity 2018;49:342-352.e5. (PMID: 30097293)