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Research Article

Evaluation of microRNA-29a expression and Dipeptidyl-peptidase 4 level in ulcerative colitis patients

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ABSTRACT

Objectives: Ulcerative colitis (UC) is a recurrent inflammatory bowel disease (IBD) that is increasing at an alarming rate worldwide. The microRNA-29 (miRNA-29) family has been implicated in the pathogenesis of UC. In recent years, alterations in dipeptidyl peptidase-4 (DPP4) levels have been reported in IBD patients. In the present study, we evaluated the relationship between miRNA-29a expression in intestinal tissue and serum DPP4 levels in UC patients and healthy subjects.

Methods: Blood samples and colonic punch biopsies were obtained from 35 UC patients and 29 healthy subjects. Serum levels of DPP4 were measured using the ELISA technique. The expression levels of miRNA-29a were assessed by qRT-PCR. Biochemical parameters and demographic information were collected based on clinical tests and questionnaires.

Results: The results showed a significant increase in miRNA-29a expression in the intestinal tissue of UC patients compared to controls. In addition, elevated miRNA-29a expression was accompanied by a decrease in serum DPP4 levels, although there was no significant difference between moderate and severe disease conditions. Furthermore, the levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and platelets were higher in UC patients than in healthy subjects.

Conclusion: These findings support the role of the miRNA-29a–DPP4 axis in the pathogenesis of UC and provide evidence for further evaluation of this axis as a potential biomarker for the disease.

Keywords: Inflammatory Bowl Diseases, Ulcerative colitis, Dipeptidyl-peptidase 4, miRNA-29a, Inflammation

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Introduction

Ulcerative colitis (UC) is one of the two major subtypes of inflammatory bowel disease (IBD) and is increasing at an alarming rate worldwide (1). UC is believed to result from a complex interaction of environmental, immune, genetic, and epigenetic factors. These factors lead to an inappropriate mucosal immune response, which may cause life-threatening complications such as toxic megacolon and colorectal cancer (CRC) (2). Therefore, to better understand the underlying mechanisms of UC, it is essential to investigate the molecular processes involved in the different stages of the disease.

Gene expression studies related to autophagy, inflammatory pathways, and immune responses have been conducted in UC patients (3). Gene expression is partly regulated by microRNAs (miRNAs), small molecules approximately 18–22 nucleotides in length that play important roles in gene splicing and post-transcriptional regulation (4). MicroRNAs have been linked to various human diseases, including IBD. Several studies have focused on miRNA profiling in UC patients, with results suggesting that miRNAs play a key role in the pathogenesis of UC. Wu and colleagues identified 11 miRNAs that were differentially expressed between active UC patients and controls, including eight upregulated miRNAs (miRNA-16, miRNA-21, miRNA-23a, miRNA-24, miRNA-29a, miRNA-126, miRNA-195, and let-7f) and three downregulated miRNAs (miRNA-192, miRNA-375, and miRNA-422b) (5).

There is a wealth of evidence showing that miRNA-21, miRNA-155, and miRNA-31 are frequently identified and among the most extensively studied miRNAs in relation to IBD (6–11). Recently, several observations have suggested that the miRNA-29 family is also involved in IBD pathogenesis. The miRNA-29 family consists of miRNA-29a, miRNA-29b, and miRNA-29c, which are known to regulate numerous biological processes (12). miRNA-29a has been implicated in the pathogenesis of UC through the regulation of intestinal epithelial apoptosis via Mcl-1 (13). Additionally, the influence of miRNA-29b overexpression on TGF-β has also been studied (14).

Dipeptidyl peptidase-4 (DPP4), also known as the T-cell antigen CD26, is a multifunctional enzyme that plays a key role in both metabolic and immune functions (15, 16). DPP4 has been reported to inhibit the production of incretin hormones, including glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). In addition to its metabolic regulatory effects, DPP4 cleaves and inactivates cytokines and chemokines involved in inflammation (16, 17). Recently, DPP4 activity has been implicated in various inflammatory diseases, such as rheumatoid arthritis, diabetes, and IBD (18). Notably, recent research has reported associations between DPP4 inhibitors and IBD risk, though findings have been conflicting (18). While DPP4 activity has been shown to be increased in the enterocytes of Crohn's disease (CD) patients (17), another study found that DPP4 levels were lower in IBD patients compared to control subjects (19).

Due to conflicting findings in previous studies and the limited data regarding the association between DPP4 concentration and the risk of developing IBD, we aimed to compare serum DPP4 levels between UC patients and healthy subjects. Additionally, TargetScan analysis predicted a binding site for miRNA-29a in the 3′ untranslated region (3′UTR) of the DPP4 gene. It has been demonstrated that this miRNA-29a binding site negatively regulates DPP4 gene expression (20). Based on previous findings and bioinformatics predictions, we selected miRNA-29a for further investigation. Specifically, we assessed the expression of miRNA-29a in intestinal tissue from UC patients and examined its relationship with serum DPP4 levels.

Material and Methods

Patients

Blood samples and colonic punch biopsies were obtained from 35 patients with ulcerative colitis (UC) and 29 healthy control subjects. The diagnosis of UC was established based on clinical and histopathological criteria. Informed consent was obtained from all participants prior to sample collection. Blood samples were centrifuged at 2,500 rpm for 15 minutes, and the resulting serum was transferred to microtubes and stored at −80 °C until further analysis. For each subject, three punch biopsies were collected from the descending colon or sigmoid. In UC patients, biopsies were taken specifically from inflamed mucosal areas. All tissue samples were immediately snap-frozen and stored at −80 °C. The expression levels of miRNA-29a were evaluated in colonic biopsies from both UC patients and healthy controls.

Laboratory Measurements

Fasting blood samples were collected after a minimum 8-hour fast to assess fasting blood glucose (FBS), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complete blood count (CBC), and DPP4 levels. Serum DPP4 concentrations were measured using a quantitative enzyme-linked immunosorbent assay (ELISA) kit (ZellBio), following the manufacturer's instructions. FBS, CRP, ESR, and CBC were measured using standard laboratory techniques.

RNA extraction and qRT-PCR from tissue samples

Total RNA was extracted from colonic tissue samples

using a column-based method (AnaCell, Iran), as per the manufacturer's protocol. RNA concentration and purity were assessed using a NanoDrop spectrophotometer by determining the absorbance ratio at 260/280 nm. Complementary DNA (cDNA) synthesis was performed using the AnaCell microRNA Detection Kit. For each sample, 500 ng of total RNA was used in the reverse transcription (RT) reaction, along with specific stem-loop primers for miRNA and housekeeping controls, RT buffer, dNTPs, and RT enzyme. The RT reaction was carried out at 37 °C for 60 minutes, followed by enzyme inactivation at 70 °C for 5 minutes.

Quantitative real-time PCR (qRT-PCR) was conducted using RealQ Plus Master Mix Green (Amplicon) on a Rotor-Gene 6000 qPCR system (Qiagen, Germany). Each reaction was performed in duplicate under the following thermal cycling conditions: 95 °C for 15 minutes, followed by 40 cycles of 95 °C for 15–30 seconds, 55–60 °C for 60 seconds, and a melt curve analysis from 55 to 95 °C. The relative expression levels of miRNA-29a were calculated using the comparative 2^–ΔΔCT method, with normalization to U6 small nuclear RNA.

Statistical analysis

Data are presented as the mean ± standard error (S.E.) of three independent experiments. Group comparisons were performed using Student's t-test. Pearson correlation analysis was used to assess the relationship between miRNA-29a levels and other variables. Statistical analyses and figure generation were performed using IBM SPSS Statistics software version 26.0 and GraphPad Prism software version 9. A p-value of < 0.05 was considered statistically significant.

Clinical characteristics of the subjects

As summarized in Table 1, the study included 29 healthy controls and 35 individuals diagnosed with ulcerative colitis (UC). There were no significant differences in age, sex distribution, or body mass index (BMI) between

Table 1. Demographic characteristics and clinical features of UC patients and control subjects

Parameter	Control (n = 29)	UC (n = 35)	*P*-value
IBD Grade (Severe/Moderate)	-	17/18	
Duration of Disease (Years) (Severe/Moderate)	-	11.92 ± 2.5 / 10.0 ± 2.0	0.56
Age (Years)	43.4 ± 12.9	40.7 ± 1.9	0.378
Sex (Men/Women)	15/14	16/19	0.63
BMI	24.5 ± 1.0	24.7 ± 1.0	0.94
CRP (mg/L)	3.4 ± 0.2	48.8 ± 4.6	0.000***
ESR	18.0 ± 9.8	52.0 ± 8.2	0.03*
PLT (10^3/μL)	$240.1\times10^3 \pm 13.4\times10^3$	$354.3\times10^3 \pm 43.6\times10^3$	0.02*
UC Location (Severe/Moderate) (%)			
E1: Proctitis	NA	6	-
E2: Left-sided	NA	10	-
E3: Extensive	NA	19	-
Medication (Severe/Moderate) (%)			
Infliximab (Remicade)	NA	0/1 (2.9)	-
Rhofanib (Tofacitinib)	NA	1/1 (5.7)	-
Sulfasalazine	NA	2/1 (8.6)	-
Prednisolone	NA	3/0 (8.6)	-
Azathioprine	NA	1/6 (20.0)	-
6-MP	NA	0/1 (2.9)	-
Adalimumab	NA	1/1 (5.7)	-
Mesalazine	NA	8/9 (48.6)	-
Pentasa	NA	1/0 (2.9)	-
Asacol	NA	2/3 (14.3)	-

UC: Ulcerative colitis, IBD: Inflammatory bowel disease, BMI: Body mass index, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, PLT: Platelet

the two groups. The median disease duration for UC patients was 11.92 ± 2.5 years for those with severe UC and 10.0 ± 2.0 years for those with moderate UC. Clinical parameters such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and platelet (PLT) count were significantly different between UC patients and healthy controls. Mesalazine was the most commonly prescribed medication, with 48.6% of severe and moderate UC patients reporting its use.

The level of miRNA-29a is significantly increased in colonic biopsies of UC patient

As shown in Figure 1, miRNA-29a expression was significantly higher in the colonic biopsies of UC patients compared to healthy controls ($p < 0.001$). In the UC group, CRP and PLT levels were positively correlated with miRNA-29a expression ($p < 0.001$). However, miRNA-29a expression did not correlate significantly with disease duration, age, BMI, sex, or ESR in UC patients (Table 2). Additionally, no significant correlation was observed between miRNA-29a expression and DPP4 levels in either UC or control groups.

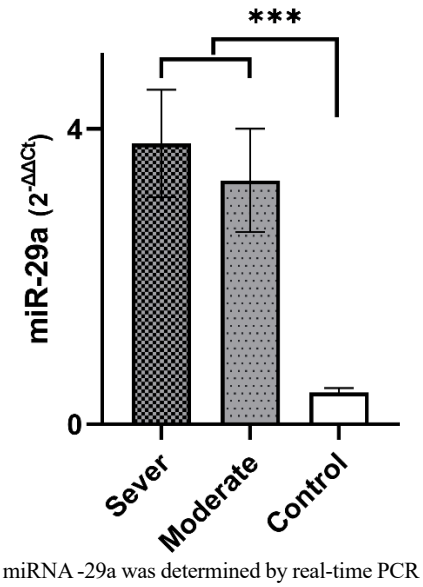

Figure 1. miRNA -29a was determined by real-time PCR in colonic punch biopsies. $2^{-\Delta\Delta Ct}$ method was used to determine significant differences for UC patients (severe and moderate) relative to controls. Quantified data were shown as mean ± S.E. ***$P < 0.001$

Table 2. Correlation between the expression of miR-29a and parameters in UC subjects

Parameter	UC (n = 35)
Age (Years)	r= -0.09
BMI (kg/m^2)	r= -0.09
Duration of the disease (Years)	r= -0.08
CRP (mg/L)	r= 0.3*
ESR (mm/h)	r= 0.26
PLT (10^3/µL)	r= 0.46**
DPP4 (ng/ml)	r= -0.08

The data in the table are Pearson correlation coefficient (r) between miR-29a and other parameters. *$P < 0.05$, **$P < 0.001$. BMI: Body mass index, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, PLT: Platelet

DPP4 enzyme is significantly decreased in serum of UC patients

DPP4 levels were assessed in the serum of UC patients and healthy controls using ELISA. The results indicated that serum DPP4 levels were significantly lower in UC patients (mean = 2.52 ± 0.09 ng/ml) compared to healthy controls (mean = 2.93 ± 0.16 ng/ml, p = 0.032) (Figure 2). No significant differences in DPP4 levels were observed between UC patients with moderate and severe disease. Furthermore, DPP4 levels were not significantly correlated with disease duration, age, BMI, ESR, or PLT levels in UC patients.

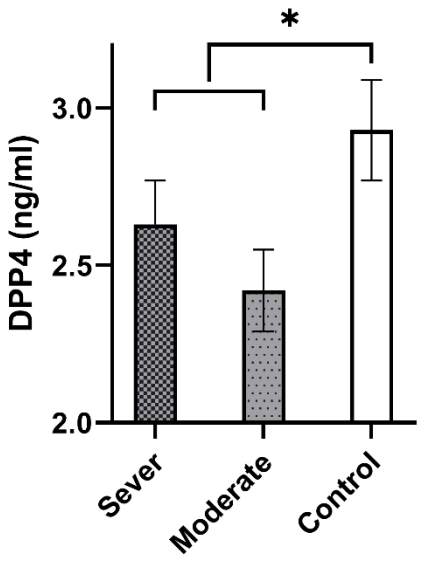

Figure 2. DPP4 was determined by ELISA in serum of UC patients and control subjects. Quantified data were shown as mean ± S.E. *$P < 0.05$

Table 3. Correlation between the level of DPP4 and parameters in UC subjects

Parameter	UC (n = 35)
Age (Years)	r= 0.086
Duration of the disease (Years)	r= 0.05
BMI (kg/m^2)	r= -0.08
CRP (mg/L)	r= 0.17
ESR (mm/h)	r= -0.18
PLT (10^3/µL)	r= -0.1

The data in the table are Pearson correlation coefficient (r) between DPP4 and other parameters. BMI: Body mass index, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, PLT: Platelet

Discussion

There is growing evidence suggesting that various subgroups of inflammatory bowel disease (IBD) exhibit distinct gene expression profiles (21). While much attention has been given to protein-coding genes, microRNAs (miRNAs)—a class of well-known non-coding transcripts—have not been extensively explored in the context of IBD and its subtypes (22). The involvement of miRNAs in the pathogenesis of ulcerative colitis (UC) was first suggested by recent studies, which identified a unique miRNA expression signature in UC. These findings have been corroborated by several subsequent studies, indicating the potential role of aberrant miRNA expression in the inflammatory processes underlying UC (23, 24).

Therefore, these documents proposed the possible involvement of aberrant expression of miRNAs in the development of inflammatory responses of UC. On the other hand, a great number of studies have elucidated that DPP4/CD26 and its substrates play a crucial role in inflammation and immunity, and they are involved in the pathogenesis of UC disease (25, 26). As far as the authors are aware, there was no available data on miRNA-29a expression and its possible correlation with DPP4 in UC patients. Hence, we evaluated miRNA-29a gene expression in tissue samples of UC patients and the probable correlation of this miRNA with serum status of DPP4.

Our findings indicate that miRNA-29a expression was significantly elevated in the colonic tissues of UC patients compared to healthy controls. This aligns with previous reports, such as one study that found a mean serum expression level of miRNA-29a of 1.23 in UC patients, compared to 1.07 in healthy controls (27). In line with these results, other studies have demonstrated the upregulation of miRNA-29a alongside other miRNAs, including miRNA-16, -21, -23a, -24, -126, -195, and Let-7f, in active UC tissues, while miRNAs such as -192, -375, and -422b were found to be down-regulated (5).

Supporting our findings, Lv et al. reported an elevated expression of miRNA-29a in the colonic tissues of both UC patients and mice with dextran sodium sulfate (DSS)-induced experimental colitis (13). Additionally, miRNA-29 expression has been shown to increase in the intestinal tissues of IBS patients compared to non-IBS controls. Experimental models of colitis also exhibited elevated miRNA-29a and -29b expression along with increased intestinal permeability (28). Furthermore, Fasseu et al. reported upregulation of miRNA-29a in the quiescent mucosa of UC patients (29).

The precise mechanisms by which miRNAs contribute to the pathogenesis of ulcerative colitis (UC) remain unclear. However, several studies have proposed potential targets of miRNA-29 that could shed light on its role. For example, miRNA-29 has been shown to target and downregulate Claudin-1 expression, as well as NF-kB-repressing factors, thereby promoting intestinal permeability in both knockout mice and the intestinal tissue of patients with irritable bowel syndrome (IBS) (28). Additionally, miRNA-29a inhibits the expression of Myeloid cell leukemia 1 (Mcl-1), a member of the Bcl-2 family, by targeting its 3' UTR in colon samples from UC patients and in DSS-induced colitis mice (13).

It is important to note that many UC-associated miRNAs are involved in regulating adaptive immune responses and inflammation. One study demonstrated that miRNA-29 is upregulated by nucleotide oligomerization domains-2 (NOD2), an intracellular sensor that plays a role in inflammation and immune defense. NOD2 binds to IL-12p40 directly and IL-23p19 indirectly, which suppresses IL-23 production in dendritic cells (DCs) and consequently inhibits Th17 differentiation (31). Furthermore, miRNA-29 has been shown to inhibit the production of IFN-γ by targeting T-bet (Tbx21) and eomesodermin (Eomes), two key transcription factors involved in Th1 differentiation. This suggests that miRNA-29 also plays a role in modulating Th2 cell activity, which is implicated in UC pathogenesis (32).

Dipeptidyl-peptidase 4 (DPP4), a near-ubiquitous membrane-bound protease, cleaves N-terminal dipeptides from a variety of endogenous and exogenous peptides and is known to modulate immune responses (18). Emerging evidence suggests that DPP4 plays a crucial role in the pathogenesis of inflammatory bowel disease (IBD) subtypes (33). Hildebrandt et al. demonstrated that DPP4 activity was significantly reduced in patients with ulcerative colitis (UC) and Crohn's disease (CD) compared to healthy controls. They observed negative correlations between DPP4 activity and clinical disease activity scores, as well as inflammatory indices like orosomucoid and C-reactive protein (CRP) in both UC and CD patients (34).

Similarly, Moran and colleagues reported decreased DPP4 protein and gene expression in intestinal tissue biopsies and plasma samples from CD patients when compared to non-CD individuals. They also found weak negative correlations between plasma DPP4 protein levels and CRP, although no significant association was observed with clinical disease activity index. This suggests that DPP4 may be part of a broader network of inflammatory disease markers (19). Furthermore, other studies have shown that both serum DPP4 activity and CD26 levels were reduced in CD patients compared to healthy controls, while CRP levels were elevated in CD patients (35). In line with these findings, serum DPP4 activity was notably reduced in both UC and CD patients, and for both diseases, DPP4 levels were negatively correlated with disease severity (36).

The literature supports our findings, as we observed a significant reduction in serum DPP4 levels in UC patients compared to non-UC subjects. Additionally, our data indicated that there was no significant difference

in DPP4 serum levels between patients with severe and moderate UC. In contrast, a statistically significant difference was reported between Truelove and Witts'-mild and Truelove and Witts'-severe patients (36). The discrepancies between studies could be attributed to variations in sample size, inclusion and exclusion criteria, and patient selection methods. Therefore, more clinical studies are needed to further investigate this issue.

CRP and ESR, both well-known indicators of inflammation, are frequently monitored during regular follow-ups of UC patients to assess disease activity and guide treatment decisions (37). Our data revealed that serum levels of both CRP and ESR were elevated in UC patients compared to healthy controls, with CRP levels showing a more significant increase. However, conflicting reports exist regarding the ability of ESR and CRP to predict disease activity in UC. Evidence suggests that the site of the disease plays a significant role, with inflammation confined to the rectum or sigmoid colon often showing lower ESR and CRP levels, even in severe disease (38).

According to the results of the reporter luciferase assay, miRNA-29a has been proposed as a direct modulator of DPP4 (39). Furthermore, the miRNA-29 family clusters inhibit elevated DPP4 protein levels by targeting the 3′UTR of its mRNA. This miRNA has been linked to various human diseases, including cancer (40) and diabetes (41), although its precise role in UC pathogenesis remains unclear. In the present study, we observed elevated expression of miRNA-29a and decreased DPP4 levels in UC patients. To the best of our knowledge, this is the first study to report the involvement of miRNA-29a in the pathogenesis of UC.

In summary, our findings demonstrate that miRNA-29a expression is significantly higher in UC patients compared to healthy individuals. Additionally, we observed reduced serum levels of DPP4 in UC patients compared to non-UC controls. These results provide concurrent evidence for the potential involvement of miRNA-29 and DPP4 in the pathogenesis of UC.

Declarations

Ethics approval

This study was approved by the ethics committee of the Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1399.349).

Competing interests

The authors declare no conflict of interest.

Author contributions

Golnaz Goodarzi, Sadra Samavarchi Tehrani, and Saeed Ebrahimi Fana: Performed real-time PCR and ELISA, analyzed data. prepared the writing and drafting.
Nafiseh Saleknezhad: Collected the data.
Ghodratollah Panahi: Completed the review and editing
Maryam Rayatpisheh: Collected sample
Amir Anushiravani: Supervised the data and approved the final manuscript

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