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Identification of serum and tissue micro-RNA expression profiles in different stages of inflammatory bowel disease

M. Iborra,* F. Bernuzzi,† C. Correale,‡ S. Vetrano,‡ G. Fiorino,‡ B. Beltrán,* F. Marabita,** M. Locati,¶ A. Spinelli,§ P. Nos,* P. Invernizzi† and S. Danese‡

*Gastroenterology Unit, La Fe University and Politechnic Hospital, Health Research Institute, Valencia, Spain, †Center for Autoimmune Liver Diseases, ‡IBD Unit, Division of Gastroenterology and §Department and Chair of General Surgery, Humanitas Clinical and Research Center, ¶Department of Medical Biotechnologies and Translational Medicine, University of Milan School of Medicine, Humanitas Clinical and Research Center, Milan, Italy, and **Unit of Computational Medicine, Center for Molecular Medicine Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

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Correspondence: S. Danese, IBD Unit, Division of Gastroenterology, Istituto Clinico Humanitas, Via Manzoni 56, Rozzano, 20089 Milan, Italy.
E-mail: sdanese@hotmail.com

Summary

The altered expression of micro-RNA (miRNA) has been associated with Crohn's disease (CD) and ulcerative colitis (UC). The aim of this study was to establish specific miRNA expression patterns in the serum and mucosa of inflammatory bowel disease (IBD) patients (UC and CD with colonic involvement) at different stages of the disease. Serum and biopsies from nine active CD (aCD), nine inactive CD (iCD), nine active UC (aUC) and nine inactive UC (iUC) and serum from 33 healthy subjects were collected. Up to 700 miRNAs were evaluated by the *Taq*Man® human miRNA array. The ΔCt values were obtained using the mean expression values of all expressed miRNAs in a given sample as a normalization factor for miRNA real-time quantitative polymerase chain reaction data. The levels of serum miRNAs in CD and UC patients were different to healthy subjects. Thirteen serum miRNAs were expressed commonly in CD and UC patients. Two miRNAs were higher and four miRNAs were lower in the serum of aCD than iCD. No serum miRNA was regulated exclusively in aUC compared with iUC patients. Four miRNAs were higher and three miRNAs were lower in the mucosa of aCD than iCD. Two miRNAs were higher and three miRNAs were lower in the mucosa of aUC than iUC. No serum miRNAs coincided with tissue miRNAs in aCD and aUC patients. Our results suggest the existence of specific miRNA expression patterns associated with IBD and their different stages and support the utility of miRNA as possible biomarkers. This pilot study needs to be validated in a large prospective cohort.

Keywords: Crohn's disease, inflammation, inflammatory bowel disease, microRNA, ulcerative colitis

Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory gastrointestinal disorder, the pathophysiology of which remains unclear. The theory accepted most commonly is that IBD and the associated gastrointestinal inflammation are likely to be the result of the interaction between a defective immune response to a luminal factor (probably intestinal flora), epigenetic and environmental factors (e.g. smoking) and its influence in genetically predisposed subjects [1–3]. Genetic factors involved in inflammation and immune functions are known to play a very important role in IBD physiopathology.

Micro-RNAs (miRNAs) are a class of small non-coding RNAs, involved in the control of gene expression at the post-transcriptional level [4]. Following the discovery of miRNAs, the number of publications regarding their biogenesis and functions has been increasing exponentially and the miRNA sequence database, miRBase, is growing continuously [5,6]. MiRNAs are involved in the regulation of many biological processes such as the cell cycle, differentiation, proliferation, apoptosis, fibrosis and immune function [7]. Emerging evidence has demonstrated that miRNAs can also play an important role in the development of cancer as well as in the induction of chronic inflammatory and autoimmune diseases [8,9]. miRNAs have been found in tissues, serum, plasma and other body fluids. It has been demonstrated that the levels of miRNAs in serum are stable, reproducible and consistent among individuals of the same species [10]; for this reason, such levels are now being used as a non-invasive biomarker for different pathologies (i.e.

Table 1. Demographic characteristics and clinical features of inflammatory bowel disease (IBD) patients and control subjects.

	Control $n = 33$	aCD $n = 9$	iCD $n = 9$	aUC $n = 9$	iUC $n = 9$
Age (years)	47·3 ± 9·6	45·4 ± 14·6	45·9 ± 9·5	38·7 ± 9·1	42·3 ± 14·1
Sex (men/women)	18/15	6/3	3/6	6/3	6/3
Site of CD					
L1: ileal	–	0	0	–	–
L2: colonic	–	8	9	–	–
L3: ileocolonic	–	1	0	–	–
L4: upper disease	–	0	0		–
Site of UC					
E1: proctitis	–	–	–	0	0
E2: left-sided	–	–	–	6	3
E3: extensive	–	–	–	3	6
Duration of IBD (years)	–	13·5 ± 7·5	11·7 ± 5·8	11·3 ± 6·5	13·9 ± 7·5
Medications (*n*)					
Mesalamine	–	4	4	4	6
Antibiotics	–	0	1	1	3
Steroids	–	2	0	5	1
Inmunomodulators	–	1	2	3	2
Biologics	–	4	3	1	3

Continuous variables presented as mean ± standard error. CD: Crohn's disease; UC: ulcerative colitis.

cancer, autoimmune disease, inflammation) [10,11]. Previous studies, focused particularly on cancer, have discovered that circulating miRNA profiles can be correlated with tissue miRNA profiles [12,13]. In most cases, those changes in circulating miRNA profiles can precede the standard blood biomarkers and possess prognostic value [12,14,15]. These properties mean that miRNAs are attractive, blood-based, non-invasive biomarkers.

Recently, several papers have focused investigation on the altered expression of miRNAs in IBD and their important role as regulators and possible diagnostic biomarkers in IBD [8,16–18]. The majority of studies in IBD have been conducted in tissue and cellular cultures, and there are currently few reports on the quantitative assessment of circulating miRNA in IBD patients [19–21]. The first study where miRNAs were examined directly in the mucosa of UC patients was performed by Wu *et al.* [22] in 2008. Following publication of this study, other works have emerged aiming to identify all the miRNAs dysregulated in IBD; to elucidate the expression patterns in the diverse IBD subtypes; and to identify the targets of the miRNAs involved in IBD [23–25]. Finally, previous studies have identified peripheral blood miRNA expression profiles in IBD patients [19,21] and have demonstrated their potential utility as non-invasive biomarkers [20].

Our group has reviewed previously the importance of miRNA as an epigenetic mechanism in the development and induction of chronic inflammatory diseases and autoimmune diseases [8,26]. In this study, we proposed to identify the expression patterns of serum miRNAs associated with CD and UC and to compare them with healthy subjects, and explore whether miRNA expression patterns differ between patients with active and inactive disease. For the first time, we aimed to establish whether circulating miRNA profiles might correlate with tissue miRNA profiles in the same IBD patient. Finally, we attempted to develop an understanding of ways in which miRNAs can be regulated to promote the development of advanced therapies targeting several key molecules involved in IBD.

Materials and methods

Patients and controls

Blood samples and colonic punch biopsy samples were obtained from 36 IBD patients [nine active CD (aCD), nine inactive CD (iCD), nine active UC (aUC) and nine inactive UC (iUC)]. IBD patients were clustered in pools of three subjects according to sex, age and location or extent of disease. In the CD group, all patients had a colonic affectation (L2 or L3 in the Montreal Classification).

Blood samples were obtained from 33 healthy volunteers (control group) clustered in pools of three subjects according to sex and age for further analysis.

All participants were provided with complete information about the study. The clinical characteristics of the patients included are summarized in Table 1.

Serum and biopsy samples

Blood samples were drawn at the time of obtaining peripheral vein access for the endoscopic procedure. Serum samples were isolated by centrifugation (1500 ***g***) from 6 ml of total blood and stored at −80°C until use. In each subject, three punch biopsies were obtained from the left colon or sigma. In active IBD patients the colonoscopy punch

biopsies were collected from inflamed mucosa and in inactive IBD patients from healing mucosa. Tissue samples were preserved immediately in RNAlater®.

Isolation of serum RNA and miRNA

Three pools of three serum samples were analysed for each group (aCD, iCD, aUC, iUC and healthy subjects). Total RNA was isolated using 135 µl of each serum sample. We introduced a synthetic miRNA, *Caenorhabditis elegans* gene (cel-miR-39), as the exogenous housekeeping gene. The RNA fraction, highly enriched for small RNA species [≤ 200 base pairs (bp)], was isolated from pools of serum using the mirVana™Paris™ kit (Ambion/Applied Biosystems, Life Technologies, Foster City, CA, USA), in accordance with the manufacturer's protocol.

Isolation of tissue RNA and miRNA

To disrupt each sample of tissue we added 400 µl of cell disruption buffer and then homogenized the sample with a motorized rotorstator. Total RNA was isolated from tissue samples using the mirVana™Paris™ kit (Ambion/Applied Biosystems). The RNA obtained from each sample was then quantified by NanoDrop. Pools of three tissue samples in each were analysed using a final concentration of 50 ng/µl.

Reverse transcription and pre-amplification

A total of 3 µl of the small RNA fraction were reverse-transcribed using the miRNA Megaplex reverse transcription primers (for pools A and B) and the *Taq*Man® microRNA reverse transcription kit (both from Applied Biosystems). The cDNA obtained was amplified using *Taq*Man® PreAmp Master Mix and Megaplex PreAmp Primers (for pools A and B).

Reverse transcription of cel-miR39

For the reverse transcription of cel-miR-39, we prepared a reaction with RT master mix using the *Taq*Man® microRNA reverse transcription kit, cel-miR-39 RT primer (*Taq*Man MicroRNA assay) and total RNA. The reaction was incubated at 16°C for 30 min, followed by 42°C for 30 min and then 85°C for 5 min.

Initial assessment of sample quality

An initial reverse transcription–quantitative polymerase chain reaction (RT–qPCR) was performed to test the quality of cDNA before the definitive analysis. At this point, three types of quality control were used. Cel-miR-39 was used as a spiked-in control in serum samples. RNU48 was used to test the quality and integrity of the obtained cDNA tissue. Mammalian U6 (U6) was used in both types of samples (serum and tissue). Ct values of 16–19 in serum samples and 15–18 in tissue samples were considered as valid.

Each RT reaction was performed using *Taq*Man® 2× Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems).

RT–qPCR

Up to 700 miRNAs were evaluated by the *Taq*Man® human miRNA array. A *Taq*Man® human microRNA array card is a high-throughput PCR-based miRNA array that enables analysis of more than 700 miRNA assays on a microfluidic card. Simultaneous synthesis of cDNA for mature miRNAs was performed using Megaplex reverse transcription human pool A and B (Applied Biosystems). Each of these, A and B, is a set of predefined pools of 384 stem-looped reverse transcription primers. RT–qPCR was performed using the Applied Biosystems 7900HT fast real-time PCR system and default thermal-cycling conditions.

Data analysis

Data analysis was performed using Expression Suite software (Applied Biosystems) and the HTqPCR library in R [27]. The ΔCt values were obtained using the mean expression value of all expressed miRNAs in a given sample as a normalization factor for miRNA RT–qPCR data, according to the procedure described by Mestdagh *et al.* [28]. The results were expressed as $\log_2$ fold change from ΔCt values. We discarded fold change values between −2 and 2 in absolute terms, with mean values between −1 and 1 expressed as $\log_2$ fold change. Student's *t*-test was used to determine significance, and *P*-values < 0·05 were considered significant. To verify the quality and reproducibility of the results, pairwise correlation was performed.

The heatmap shows the expression on a *Z*-score scale obtained using ΔCt data. A large positive number means that the gene is less expressed, whereas a negative number means that the gene is more expressed.

We checked the list of genes expressed differentially using the TargetScan Human database (http://www.targetscan.org) for miRNA target identification.

Results

Figure 1 shows the comparison of expression levels of serum miRNAs in IBD patients and in the control group. The expression map of all serum miRNAs displayed a clear separation between patients and controls. Red indicates greater expression, blue indicates less expression.

Identification of serum miRNA expression in CD patients

We compared serum samples from CD patients (nine aCD patients and nine iCD patients) and healthy control

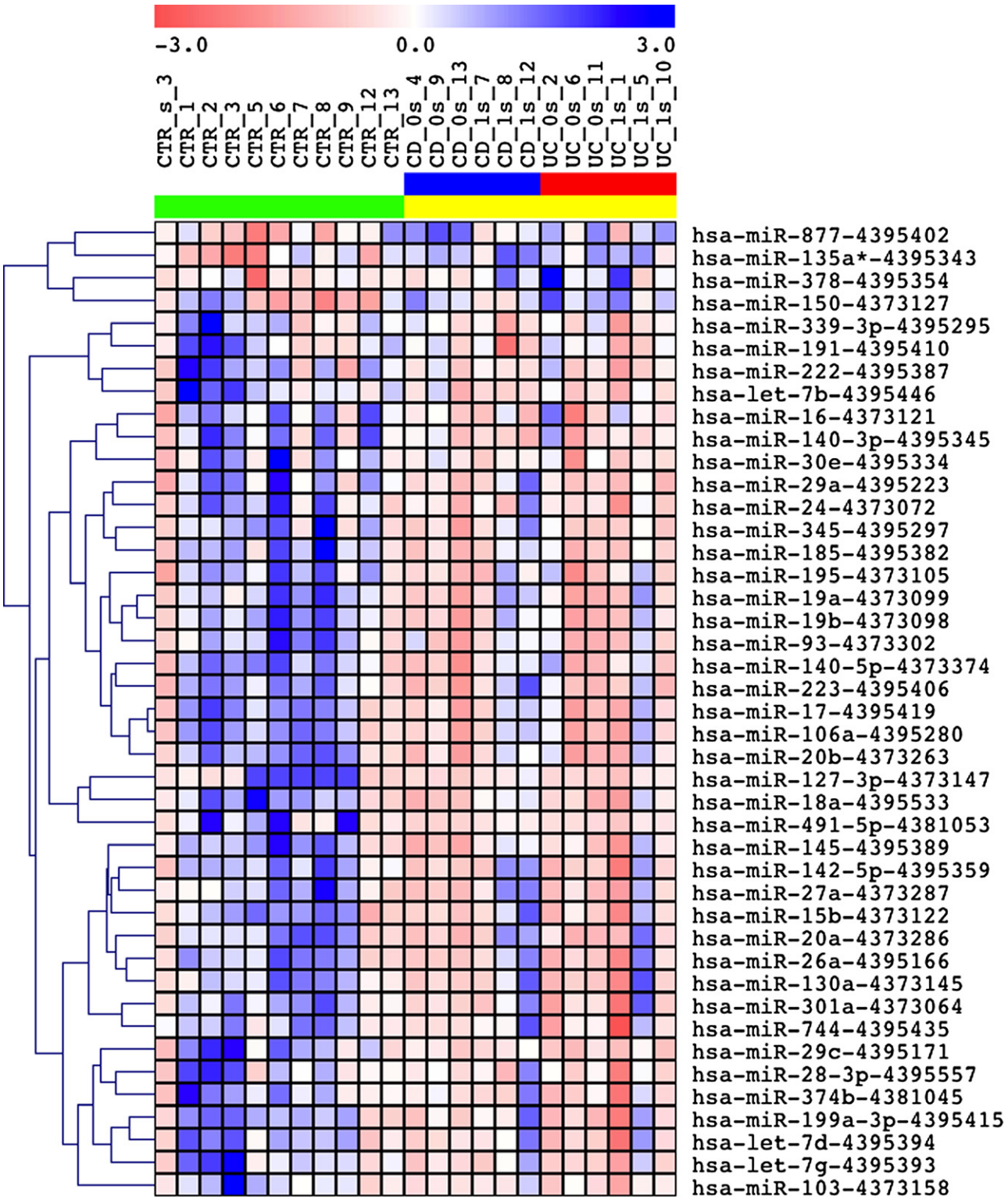

Fig. 1. Heatmap of inflammatory bowel disease-associated peripheral blood micro-RNAs (miRNAs). The heatmap shows the expression on a *Z*-score scale obtained using ΔCt data. The lightly shaded area indicates higher than mean intensity across all samples and the ares that are shaded darker indicates lower than mean intensity across all samples. CTR: control; CD-1: Crohn's disease active; CD-0: Crohn's disease inactive; UC-1: ulcerative colitis active; UC-0: ulcerative colitis inactive.

Table 2. Serum micro-RNAs (miRNAs) expressed differentially in inflammatory bowel disease (IBD) patients compared with healthy subjects.

CD *versus* control			UC *versus* control		
miRNA	$\log_2$ FC	*P*-value	miRNA	$\log_2$ FC	*P*-value
miR-27a*	2·66	0·035	miR-760	5·07	0·048
miR-140-3p	1·89	0·012	miR-423-5p	4·34	0·044
miR-140-5p	1·44	0·017	miR-128	4·25	0·036
miR-16	1·43	0·020	miR-196b	4·17	0·034
miR-195	1·33	0·035	miR-103	4·01	0·029
miR-877	−1·13	0·029	miR-221	3·68	0·047
			miR-532-5p	2·45	0·030
			miR-15b	2·27	0·031
			miR-27a	2·04	0·026
			let-7g	1·94	0·012
			miR-93	1·89	0·013
			let-7d	1·76	0·040
			miR-598	1·74	0·006
			miR-142-5p	1·71	0·024
			let-7e	1·60	0·037
			miR-223	1·45	0·023
			miR-374b	1·40	0·021
			miR-19a	1·40	0·038
			miR-345	1·39	0·015
			miR-199a-3p	1·38	0·027
			miR-24	1·15	0·013
			miR-30e	1·09	0·044
			miR-29a	1·07	0·006
			miR-28-3p	1·01	0·039
			miR-150	−1·54	0·011

The table shows the serum miRNAs expressed specifically in Crohn's disease (CD) patients and ulcerative colitis (UC) patients when compared with control subjects. The miRNAs exposed showed significant differences (*t*-test, *P*-value < 0·05). The data are expressed as $\log_2$ fold change (FC) from ΔCt values. Under-expressed miRNAs are shown in italic type.

subjects. Only 21 of these 768 miRNAs showed expression levels that differed significantly ($P < 0{\cdot}05$) between CD (both active and inactive) and healthy subjects (Table 2). Fourteen of the 21 identified miRNAs were expressed commonly in the peripheral blood of CD and UC patients, with the remaining six miRNAs expressed specifically in CD patients.

We identified six miRNAs expressed differentially in the serum of aCD patients compared with iCD patients (Table 3).

Identification of serum miRNA expression in UC patients

Thirty-nine differentially expressed miRNAs were identified in UC patients ($P < 0{\cdot}05$ UC *versus* healthy). However, only 25 miRNAs were expressed specifically in UC (Table 2).

We subsequently attempted to determine whether serum miRNAs would allow us to distinguish aUC from iUC.

Table 3. Serum micro-RNAs (miRNAs) expressed in active Crohn's disease (aCD) *versus* inactive CD (iCD).

aCD *versus* iCD		
miRNA	$\log_2$ FC	*P*-value
miR-188-5p	2·00	0·008
miR-877	1·50	0·005
miR-140-5p	−1·47	0·044
miR-145	−2·53	0·008
miR-18a	−2·67	0·001
miR-128	−3·00	0·004

The miRNAs exposed showed significant differences (*t*-test, *P* value < 0·05). The data are expressed as $\log_2$ fold change (FC) from ΔCt values. Under-expressed miRNAs are shown in italic type.

Fifteen miRNAs demonstrated expression levels in aUC, but the expression levels of these did not differ significantly from those in iUC patients (data not shown).

Identification of serum miRNAs expressed commonly in UC and CD patients

We compared peripheral blood miRNA expression in UC and CD patients and found that 13 miRNAs shared common altered expression in both groups, of which 12 (all but miR-135a*) were over-expressed (Table 4). Most of the commonly altered miRNAs showed a similar increase in expression in CD and UC.

Identification of tissue miRNAs that distinguish active *versus* inactive IBD patients

We found seven miRNAs expressed differentially in the mucosa of aCD *versus* iCD (Table 5). None of the tissue

Table 4. Serum micro-RNAs (miRNAs) expressed commonly in Crohn's disease (CD) and ulcerative colitis (UC).

	CD		UC	
miRNA	$\log_2$ FC	*P*-value	$\log_2$ FC	*P*-value
miR-127-3p	6·92	0·027	8·02	0·012
miR-491-5p	5·46	0·019	5·71	0·015
miR-18a	2·57	0·016	2·97	0·010
miR-145	2·55	0·009	2·33	0·021
let-7b	1·75	0·012	1·70	0·013
miR-185	1·52	0·022	1·72	0·008
miR-29c	1·50	0·011	1·97	0·002
miR-19b	1·34	0·027	1·45	0·021
miR-20b	1·20	0·012	1·23	0·024
miR-106a	1·05	0·043	1·33	0·024
miR-17	1·03	0·038	1·24	0·027
miR-222	0·91	0·041	1·07	0·043
*miR-135a**	−2·23	0·005	−1·82	0·011

The miRNAs exposed showed significant differences (*t*-test, *P* value < 0·05). The data are expressed as $\log_2$ fold change (FC) from ΔCt values. Under-expressed miRNAs are shown in italic type.

Table 5. Tissue miRNAs expressed in inflammatory bowel disease (IBD) patients.

aCD *versus* iCD			aUC *versus* iUC		
miRNA	$\log_2$ FC	*P*-value	miRNA	$\log_2$FC	*P*-value
miR-18a*	2·10	0·028	miR-650	1·95	0·0009
miR-629*	1·06	0·008	miR-548a-3p	1·12	0·001
let-7b	0·81	0·041			
miR-140-3p	0·78	0·007			
miR-422a	−1·21	0·006	*miR-630*	−1·45	0·037
miR-885-5p	−1·35	0·048	*miR-489*	−1·88	0·049
miR-328	−1·76	0·031	*miR-196b*	−2·18	0·029

The miRNAs exposed showed significant differences between active and inactive patients (*t*-test, *P* value < 0·05). The data are expressed as $\log_2$ fold change (FC) from ΔCt values. Under-expressed miRNAs are shown in italic type. CD: Crohn's disease; UC: ulcerative colitis.

miRNAs obtained in aCD coincided with serum miRNAs in aCD. However, miR-140-3p was expressed exclusively in the blood of CD patients.

We identified five tissue miRNAs able to distinguish aUC and iUC (Table 5). Of the five miRNAs, only miR-196b was expressed exclusively in the blood of UC patients, but serum expression was increased. None of the mucosa miRNAs found exclusively in aUC coincided with mucosa miRNAs in aCD.

Discussion

The regulatory role of different miRNAs in many cellular processes, as well as their role in the process of inflammation in IBD patients, deserves exploration. In this study, we have identified different miRNA expression patterns in the serum of CD patients with participation of the colon, UC patients and healthy controls. These patterns are modified in patients with active and inactive disease. Our results provide insight into the potential biological function of these genes in disease pathogenesis.

There is a lack of studies in the literature evaluating the differential expression of circulating miRNAs and their role in IBD [19–21,29]. In the current study, six serum miRNAs were expressed specifically in CD patients (aCD and iCD *versus* control). In previous reports, increased expression of miR-16 and miR-195 was identified in peripheral blood of CD patients compared with healthy controls, a finding supported by our results [20,21]. In addition, miR-16 was found in the mucosa of the terminal ileum of aCD patients [25]. Pauley *et al*. reported that miR-16 was elevated in the peripheral blood cells of patients with rheumatoid arthritis (another autoimmune disease), and that its expression was correlated with disease activity, demonstrating the potential role of this miRNA as a biomarker for disease activity [30]. The main function of miR-16 is to regulate the production of inflammatory mediators and immunity through co-operation with other miRNAs; its target is tumour necrosis factor (TNF)-α [9,31]. MiR-16 expression is increased in T cell subtypes and is able to modulate several aspects of innate and adaptive immunity [17,22,32]. MiR-16 has been shown to be involved in the induction of apoptosis by targeting bcl-2 and the modulation of the nuclear factor kappa B (NF-κB)-regulated transactivation of the IL-8 gene [14,32,33]. The potential regulatory role of miR-16 on cellular processes in patients with CD warrants further exploration.

When we compared active and inactive CD, we discovered six serum miRNAs expressed differentially. No serum miRNAs in aCD patients were found to coincide with tissue miRNAs in aCD (see below). None of our six miRNAs regulated exclusively in the serum of aCD patients has been described previously in the same conditions. However, miR-188-5p has been found previously to be up-regulated in the peripheral blood of UC patients [21], down-regulated in the mucosa of UC patients [23] and up-regulated in the mucosa of rectal cancer [34]. Similarly, miR-145 was lower in the UC colonic mucosa than normal mucosa, and this suppression could predispose to IBD-associated neoplasic transformation in long-standing UC [35].

Although some groups have described miRNA expression patterns in the peripheral blood of aCD patients [19–21], none of these produced results similar to those of the current study. Potential reasons for these differences may be: (i) the small and heterogenic population in the studies, particularly the lack of clustering according to medications, behaviour, disease duration and previous surgery; (ii) differences in type of sample used (platelets, serum, total blood); and (iii) differences in the methodology employed (sample collection and approach method) in each study. Larger studies are required to elucidate fully the clinical utility of these profiles.

We found that 25 miRNAs were expressed specifically in the serum of UC patients; 24 miRNAs were up-regulated and one was down-regulated (miR-150). None of the serum miRNAs found specifically in UC patients has been described previously in the peripheral blood of these patients. In the peripheral blood of UC patients we found a significant increase in miR-29a, which regulates innate and adaptive immune responses by targeting interferon (IFN)-γ [36]. Moreover, serum miR-29a has strong potential as a novel non-invasive biomarker for early detection of color-

ectal cancer [37,38]. In accordance with our results, two studies have demonstrated an increase of miR-29a expression in the colon of active and inactive UC patients [22,23]. This finding suggests that circulating miRNAs profiles may correlate with tissue miRNA profiles, indicating a potential role of miRNAs as non-invasive biomarkers, and also demonstrates that the inflammation in IBD has an impact beyond the mucosa, generating a systemic reaction. In addition, colorectal cancer is known to represent a well-defined complication of long-standing UC. It has been demonstrated that miR-29a is associated with active and inactive UC [22,23] and is a good biomarker for the early detection of colorectal cancer [37,38]. For this reason, we hypothesized that the altered expression of miR-29a could be involved in UC-associated neoplasic transformation.

In the literature, there are no previous studies comparing miRNA expression patterns in the peripheral blood of aUC and iUC patients. In our study, no serum miRNAs were regulated specifically in aUC patients compared with iUC patients. Although colonoscopy is the gold standard technique for the activity evaluation in UC, this invasive technique is complex and is not considered safe. Thus, there is a pressing need for new non-invasive biomarkers to improve the detection of disease activity in UC in order to determine prognosis and to monitor response to therapy.

Although the exact pathogenesis of CD and UC remains unknown, it is well established that both arise as a consequence of a genetic predisposition and immune gut flora dysregulation. Both diseases share similarities, such as a chronic relapsing–remission course, the involvement of the intestinal mucosa as well as a number of common extra-intestinal manifestations. However, CD and UC do not share localization, endoscopic findings or histology. In this study, we have demonstrated that UC and CD have miRNAs in common as well as some differences, which is in concordance with other studies [19,21]. We found an overlap of 13 miRNAs in the blood of CD and UC patients. Only Wu *et al.* have published previously that the blood expression of five miRNAs (miR-199a-5p, -363-3p, -340*, -532-3p and miRplus-1271) were elevated in both aCD and aUC compared with healthy controls. None of these miRNAs are the same as the miRNAs found by our group. However, a number of these miRNAs in common between CD and UC and their targets have been associated with other immune-mediated diseases such as systemic lupus erythematosus, rheumatoid arthritis, primary biliary cirrhosis, psoriasis and multiple sclerosis [9,39–41]. The overlap of these miRNAs in the blood of UC and CD patients suggests a generalized inflammatory status common to both diseases as well as other autoimmune diseases.

The first papers published on miRNA expression patterns in IBD patients were performed in tissue samples [22–25]. We have found seven miRNAs expressed specifically in the mucosa of aCD. None of these miRNAs have been described previously in the mucosa of aCD patients. One tissue miRNA of aCD, miR-140-3p, coincided with one of the miRNAs expressed exclusively in the blood of CD patients (aCD and iCD together). Previous studies have demonstrated that miR-140-3p was down-regulated in tumour samples of colorectal cancer [42] and could regulate the expression of a membrane protein (CD38) through the activation of TNF-α and NF-κB [43]. We believe that miR-140-3p should be explored specifically in the blood of aCD to gain an understanding of its role in the pathogenesis of CD and to confirm the mucosa and serum correlation. We hypothesized that miR-140-3p could be used as a biomarker of active disease.

In contrast to the serum findings, we found five tissue miRNAs that were able to distinguish aUC from iUC. None of these tissue miRNAs have been described previously for aUC patients. In contrast, Fasseu *et al.* described a decreased expression of miR-196b in the mucosa of iUC patients [23]. None of the mucosa miRNAs found exclusively in aUC coincided with mucosa miRNAs in aCD, which suggests the possibility of using tissue miRNAs expression patterns to distinguish both pathologies.

The available evidence indicates that miRNA expression in plasma and serum appears to reflect the extrusion of miRNAs from distant tissues or organs or disease pathways [11–13,20]. In this regard, the results of Wu *et al.* did not identify the same expression patterns in mucosa and peripheral blood. They hypothesized that the peripheral blood miRNAs of their study possibly identified the expression in circulating white blood cells [19]. Our results do not show an exact correlation between the miRNA expression profiles of the serum and mucosa of the same patients. We believe that this dissimilarity may be because of the small number of patients, who were extremely heterogeneous, and the treatments employed during the disease could cause epigenetic changes with an impact on the miRNA expression profiles. Nevertheless, we have shown throughout the discussion that some of our serum miRNAs have been found previously in the mucosa under the same conditions. The most surprising finding was that miR-127-3p was shown to be the miRNA with increased expression in both UC and CD patients. Similar to our findings, Fasseu *et al.* [23] have also described increased expression of miR-127-3p in the mucosa of active and inactive CD, as well as in active and inactive UC patients compared with healthy subjects. This finding suggests that miR-127-3p could be a potential IBD biomarker.

In conclusion, our results suggest that there are specific miRNA expression patterns associated with different stages of IBD. These findings demonstrate that miRNAs may play a certain role in the development of the flare and relapse of inflammation in IBD patients. miRNAs may be useful for distinguishing IBD from healthy controls and the different expression in CD patients (with colonic involvement); UC and control patients support the utility of miRNA as possible biomarkers. The small population, the dissimilar

samples and methodology employed in the published studies may explain the different miRNA expression patterns identified by our group. The overlap miRNAs among CD, UC and other autoimmune diseases suggests that the mechanisms involved in the development of these disease are similar. Indirectly, our results suggest the use of some miRNAs as non-invasive biomarkers, as we have demonstrated that circulating miRNA profiles are correlated with tissue miRNA profiles. To date, current evidence, including the findings from this study, suggests that miRNAs play an important role in oncogenesis and that they are involved in the regulation of cellular processes and inflammatory pathways. However, it is necessary to confirm the results of our pilot study in larger samples, with subtypes of patients according to treatment, disease duration, behaviour or localization and previous surgery, for example, in order to clarify the role of certain miRNAs as biomarkers and as therapeutic targets.

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Disclosure

The authors declare no conflicts of interest.

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