Published in final edited form as:

Inflamm Bowel Dis. 2011 January ; 17(1): 241–250. doi:10.1002/ibd.21450.

Peripheral blood microRNAs distinguish active ulcerative colitis and Crohn's disease

Feng Wu, Ph.D.¹, Natalie Jia Guo²[Undergraduate student], Hongying Tian¹, Michael Marohn, D.O.³, Susan Gearhart, M.D.³, Theodore M. Bayless, M.D.³, Steven R. Brant, M.D.^{4,5}, and John H. Kwon, M.D.-Ph.D.¹

¹Department of Medicine, Section of Gastroenterology, University of Chicago, Chicago, IL 60637

²Princeton University, Princeton, New Jersey

³Department of Surgery, Johns Hopkins University Medical Institutions, Baltimore, MD 21287

⁴The Harvey M. and Lyn P. Meyerhoff Inflammatory Bowel Disease Center, the Department of Medicine, Division of Gastroenterology, Johns Hopkins University Medical Institutions, Baltimore MD 21287

⁵Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD 21231

Abstract

Background—Crohn's disease (CD) and ulcerative colitis (UC) result from pathophysiologically distinct dysregulated immune responses, as evidenced by the preponderance of differing immune cell mediators and circulating cytokine expression profiles. MicroRNAs (miRNAs) are small, noncoding RNAs that act as negative regulators of gene expression and have an increasingly recognized role in immune regulation. We hypothesized that differences in circulating immune cells in CD and UC patients are reflected by altered miRNA expression and that miRNA expression patterns can distinguish CD and UC from normal healthy individuals.

Methods—Peripheral blood was obtained from patients with active CD, inactive CD, active UC, inactive UC and normal healthy adults. Total RNA was isolated and miRNA expression assessed using miRNA microarray and validated by mature miRNA quantitative RT-PCR.

Results—Five miRNAs were significantly increased and two miRNAs (149* and miRplus-F1065) were significantly decreased in the blood of active CD patients as compared to healthy controls. Twelve miRNAs were significantly increased and miRNA-505* was significantly decreased in the blood of active UC patients as compared to healthy controls. Ten miRNAs were significantly increased and one miRNA was significantly decreased in the blood of active UC patients as compared to active CD patients.

Conclusions—Peripheral blood miRNAs can be used to distinguish active CD and UC from healthy controls. The data support the evidence that CD and UC are associated with different circulating immune cells types and that the differential expression of peripheral blood miRNAs may form the basis of future diagnostic tests for IBD.

Keywords

microRNA; Crohn's disease; ulcerative colitis; peripheral blood

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are the two most common forms of chronic idiopathic inflammatory bowel diseases (IBD).¹ Both CD and UC are thought to arise as a consequence of an aberrant immune response to gut flora in genetically predisposed individuals and characterized by chronic relapsing and remitting inflammation of the gastrointestinal tract.¹ However, there are distinct clinical,² genetic,³ gene expression^{4–6} and immunologic characteristics¹ that indicate that CD and UC can be classified as two overlapping but distinct disease types. It is this overlap and our lack of comprehensive understanding of the underlying pathophysiology of CD and UC that have hampered the advancement in new diagnostic and treatment modalities for IBD.

MicroRNAs (miRNAs) are small (~22–24 nucleotide), noncoding RNAs that act as key regulators of gene expression.⁷ Briefly, they are initially transcribed as longer primary miRNA transcripts in the nucleus then subsequently processed by Drosha and DGCR8 into precursor miRNA (pre-miRNA). The pre-miRNA is exported to the cytoplasm by exportin 5 in a ras-related nuclear protein guanosine triphosphate-dependent manner. The cytoplasmic pre-miRNA is cleaved by Dicer and the functional miRNA strand incorporated into the RNA-inducing silencing complex (RISC). Once loaded, the miRNA binds to complementary sequences in the 3'-untranslated region (3'UTR) of target mRNAs, resulting in suppression of translation and/or degradation of mRNA.

Overall, miRNAs are thought to contribute to the regulation of over 30% of all protein coding genes,⁸ including those involved in development, metabolism, cell cycle control and fibrosis.^{7, 9} Recently growing evidence indicates that miRNAs play a significant role in immune function.¹⁰ The differential expression of miRNAs has been noted in T cell development and T cell subtypes.^{11, 12} They have demonstrable roles in intestinal epithelial chemokine expression,¹³ toll-like receptor signaling and cytokine signaling.¹⁴

It has been hypothesized that the differential expression of miRNAs may distinguish disease states.¹⁵ Indeed, altered miRNA profiles have been noted a vast array of diseases including multiple cancer subtypes,¹⁶ cardiovascular diseases,¹⁷ diabetes,¹⁸ and several inflammatory and autoimmune diseases,^{19–22} including CD and UC.^{13, 23–25} Recently, several studies indicate that the differential expression of miRNAs in the peripheral blood or plasma may be useful tools for the diagnosis or differentiation of disease.^{26–31}

In this study, we hypothesized that the differences in circulating immune cells associated with CD and UC are reflected by altered peripheral blood miRNA expression and that these expression patterns can distinguish CD and UC from normal healthy individuals. We performed miRNA microarray on peripheral blood samples isolated from CD patients, UC patients and healthy controls. We compared miRNA expression patterns between these three groups and validated their expression using mature miRNA qRT-PCR. Our findings suggest that peripheral blood miRNAs can be useful in the differentiation between active IBD subtypes and may be a useful tool for future determination of IBD diagnosis and disease activity.

MATERIALS AND METHODS

Patient recruitment and human peripheral blood collection

Healthy individuals undergoing colonoscopies for colorectal cancer screening and patients with CD or UC were recruited for blood collection following a protocol approved by the John Hopkins University Institutional Review Board. Upon obtaining informed consent, approximately 2 ml of peripheral blood were collected into a PAXgeneTM tube containing

6.9 ml RNA stabilizing solution (Fisher Scientific, Waltham, MA). Samples were drawn at the time of obtaining peripheral vein access for the planned endoscopic procedure.

The CD and UC diagnoses were established by clinical, endoscopic and histological criteria. CD and UC disease activity was determined by the presence or absence of acute and chronic inflammation assessed on histopathology of endoscopic pinch biopsies of the ileum and colon taken on the day of the blood collection. The healthy controls were defined as having no intestinal inflammation on the day of the blood collection. Clinical characteristics of patients utilized in the study are summarized in Table 1.

Total RNA isolation from blood stored in PAXgene™ tube

The PAXgene blood tube was incubated at room temperature for 2 h to ensure complete lysis of blood cells then centrifuged for 10 min at 3000g using a swing-out rotor. The supernatant was discarded and RNase-free water (4 ml) was added to the pellet then vortexed until the pellet was visibly dissolved. After centrifuging again for 10 min at 3000g, the miRNeasy Mini Kit protocol (QIAGEN, Valencia, CA) was used to isolate total RNA from the pellet following the manufacturer's protocol. The quality of the total RNA was verified by an Agilent 2100 Bioanalyzer profile (Agilent, Oswego, IL). The RNA samples were stored at -80°C .

microRNA microarray

Total RNA (1 μg per sample) was mixed with 10 different synthetic unlabeled miRNA spike-in controls and labeled with Hy3™ fluorescent using the miRCURY™ LNA Array Power Labeling kit (Exiqon, Vedbaek, Denmark) following the manufacturer's protocol. The Hy3™-labeled samples were hybridized to the miRCURY™ LNA Array v. 11.0 (Exiqon), which contains capture probes targeting all miRNAs for human, mouse or rat registered in the miRBase version 11.0 at the Sanger Institute. The hybridization and slide washing were performed according to the miRCURY™ LNA array manual. The microarray slides were scanned using the Microarray Scanner System (Agilent) and the image analysis was carried out using the Agilent Feature Extraction software v.9.5.3.

Microarray data analysis

This miRCURY™ LNA Array chip has four replicate probes for each of 834 human miRNAs, 19 small nucleolar RNAs, 10 positive controls and 8 negative controls. In addition, this array contains capture probes for 429 new proprietary miRPlus™ human miRNAs which are not reported in miRBase. The hybridization signal of 5SrRNA was one of the highest signals in the arrays. Therefore, each background-subtracted median fluorescence intensity was Log2 transformed and normalized to the corresponding 5SrRNA signal of each array. The normalized data was analyzed using dChip software (<http://www.dchip.org/>) to identify differential miRNA expression between analysis groups. The criteria for significance included (1) a miRNA signal higher than mean + 2SD of the negative controls on an array, (2) a T-test on any given two groups, $p < 0.05$.

Quantitative reverse transcription and PCR (qRT-PCR)

We used the NCode SYBR green miRNA qRT-PCR Kit (Invitrogen, La Jolla, CA) to validate the expression of miRNAs identified by microarray. Briefly, total RNA (200 ng) was used to add a poly-A tail and then converted to first strand cDNA according to manufacturer's protocol. For miRNA qPCR, the reverse primer was the NCode miRNA universal qPCR primer (Invitrogen). Forward primers were DNA form of the mature miRNA sequences, which were obtained from Operon (Huntsville, Alabama) and listed in Table 2. The qPCR was performed in an ABI7900 cyclor (Applied Biosystem, Carlsbad,

CA) and the cycle threshold (Ct) passed was recorded. The expression of each target miRNA in sample was calculated relative to U6B, a ubiquitously expressed small nuclear RNA that has been widely used as an internal control. Data is presented as target miRNA expression = $2^{\Delta Ct}$, with $\Delta Ct = (U6B\ Ct - \text{target miRNA}\ Ct)$.

Statistical analysis

Experimental results are expressed as mean values $\pm$ standard error. Statistical analyses for qRT-PCR were performed using one-way ANOVA for multiple groups (GraphPad Prism 5). $P < 0.05$ was considered significant.

RESULTS

Identification of CD-associated peripheral blood miRNAs

We sought to test the hypothesis that peripheral blood miRNAs can distinguish IBD subtypes. Total RNA was extracted from the peripheral blood of patients with endoscopically and histologically confirmed active CD, inactive CD, active UC, inactive UC and healthy control subjects. Blood samples from 55 patients were utilized for this study. The clinical characteristics of each patient group are listed in Table 1.

A miRNA microarray capable of measuring the expression of 834 known and 429 putative human microRNA genes was used to compare miRNA expression among all collected samples. The 55 miRNA microarrays were performed and analyzed using relatively low stringency criteria to maximize the identification of candidate miRNAs. A comparison of blood samples from healthy controls to active CD samples identified 18 miRNAs with differential expression (Figure 1). Of these miRNAs, 12 were increased and 6 were decreased in blood samples collected from patients with active CD as compared to healthy controls. When the active CD patients were subgrouped into Crohn's ileitis and Crohn's colitis patients, there was no significant difference in the expression of these 18 miRNAs between the two subgroups (**data not shown**). In addition, while the expression of these 18 miRNAs in the blood of active CD patients appeared relatively consistent, there appeared to be increased heterogeneity of the expression of these 18 miRNAs in the blood of patients with inactive CD.

We next performed a higher stringency qRT-PCR on 11 of the 18 identified miRNAs in an attempt to validate the miRNA microarray results. Seven of the 11 CD-associated peripheral blood miRNAs were confirmed, including miRs-199a-5p, -362-3p, -340*, -149* and -532-3p (Figure 2). Of these miRNAs, miR-362-3p demonstrated the most significant difference in expression with a 4.7 fold increase seen in the peripheral blood of active CD patients. Two additional putative miRNAs, miRplus-E1271 and miR-plus-F1065, were differentially expressed and highly expressed in the peripheral blood of patients with active CD.

Overall, miRs-199a-5p, -362-3p and -532-3p and miRplus-E1271 were increased in the peripheral blood of patients with active CD but not in the blood of patients with inactive CD as compared to healthy controls. In contrast, the peripheral blood of both active and inactive CD patients exhibited increased expression of miR-340*. Similarly, miRplus-F1065 was decreased only in the blood of active CD patients while miR-149* was decreased in the blood of both active and inactive CD patients.

Identification of UC-associated peripheral blood miRNAs

A comparison of peripheral blood samples from active UC patients to healthy control subjects was performed using the miRNA microarray (Figure 3). Seventeen differentially

expressed miRNAs were identified with 12 miRNAs increased and 5 miRNAs decreased in the blood of active UC patients. When the active UC patients were subgrouped into pan-colitis and distal colitis subgroups, there was no significant difference in the expression of these 17 miRNAs between the two subgroups (**data not shown**). Similar to the CD-associated miRNAs, there appeared to be an increased heterogeneity of the expression of these 17 miRNAs in the blood of patients with inactive UC.

A higher stringency qRT-PCR was performed on 10 of the 17 identified miRNAs in an attempt to validate the miRNA microarray results. Nine of the 10 UC-associated peripheral blood miRNAs were confirmed, including miRs-28-5p, -151-5p, -103-2*, -199a-5p, -340*, -362-3p, -532-3p, -505* and miRplus-E1271 (Figure 4). A 7.2 fold decreased expression of miR-505* was noted in active UC patients' blood. Conversely, miR-103-2* and miR-362-3p demonstrated the greatest increase in blood expression in active UC patients with a 3.1 and 5.2-fold increase, respectively.

Overall, miRs-28-5p, -151-5p, -199a-5p, -340* and miRplus-E1271 were increased in the peripheral blood of patients with active UC but not in inactive UC. In contrast, miRs-103-2*, -362-3p and -532-3p were increased in the blood of both inactive and active UC patients. One miRNA, miR-505*, was decreased in the blood of both active and inactive UC patients.

Comparison of peripheral blood miRNA expression in active CD and UC

We next sought to determine whether peripheral blood miRNAs could distinguish active CD from active UC. Of the 21 miRNAs assessed for validation qRT-PCR to identify CD associated (Figure 2) and UC-associated (Figure 4) miRNAs, five miRNAs were common to both sets of analyses, including miRs-199a-5p, -362-3p, -340*, -532-3p and miRplus-E1271. To determine whether any of these 21 miRNAs were further able to distinguish active CD from active UC, we proceeded to perform qRT-PCR comparisons of the active CD group, the active UC group and the healthy control group (Figure 5). We confirmed that 8 miRNAs, including miRs-28-5p, -103-2*, 149*, -151-5p, -340*, -505*, -532-3p, and miR-plus-E1153, were able to distinguish active CD from active UC. Of these miRNAs, seven were significantly increased in active UC compared to active CD while miR-505* was significantly decreased by 7.2-fold in active UC compared to active CD. Of note, in this validation set, miRplus-E1153 was additionally found to not only distinguish active UC from active CD but active UC from healthy controls as well.

To determine whether we could identify additional miRNAs capable of distinguishing active CD and active UC, we directly compared the miRNA microarray profiles of the two patient groups (Figure 6A). Seventeen differentially expressed miRNAs were identified with 10 miRNAs increased and 7 decreased in the peripheral blood of active UC patients as compared to active CD patients. Of note, miR-149* and miR-505* were among the 17 miRNAs identified by directly comparing the active CD and active UC groups. Further validation qRT-PCR was performed on 5 these miRNAs and three miRNAs were confirmed to be differentially expressed (Figure 6B). The peripheral blood expression of miRs-3180-3p, miRplus-E1035 and miRplus-F1159 were significantly increased in the active UC patients as compared to active CD patients. The expression of these three miRNAs was not only differentially expressed when comparing active UC to active CD but also when comparing active UC to healthy controls. This differential expression of these three miRNAs in active UC in comparison to healthy controls was not noted in the initial miRNA microarray comparison of peripheral blood miRNAs from active UC patients with healthy controls.

DISCUSSION

We present the first evidence that peripheral blood miRNAs can distinguish active IBD subtypes from each other and healthy controls. Overall, we identified 10 miRNAs significantly increased and one miRNA significantly decreased in the peripheral blood of active UC patients as compared to CD. We identified 12 miRNAs significantly increased and one miRNA significantly decreased in the blood of active UC patients compared to healthy controls. We identified 5 miRNAs significantly increased and 2 miRNAs significantly decreased in the blood of active CD patients compared to healthy controls.

Of these miRNAs, several appear to have similar patterns of expression in the blood of both active CD and UC. Specifically, the blood expression of miRs-199a-5p, -362-3p, -340*, -532-3p and miRplus-1271 were elevated in both CD and UC as compared to healthy controls. The blood expression levels of three of these miRNAs were similar in both the CD and UC groups. The overlap of these miRs in the blood of active CD and UC patients indicates that these miRNAs may reflect a generalized inflammatory state common to both CD and UC and other autoimmune diseases. This is supported by the finding that miR-199a-5p was also previously found to be elevated in peripheral blood mononuclear cells (PBMCs) of African American patients with systemic lupus erythematosus (SLE).²⁹

The identification of peripheral blood miRNAs distinct to CD and UC supports recent studies utilizing blood based miRNAs to distinguish disease. Voellenkle *et al*³⁰ identified miRNAs differentially expressed in PBMCs of patients with ischemic cardiomyopathy (CM) and non-ischemic CM as compared to healthy controls. Furthermore, similar studies using serum and plasma have reported utility in distinguishing patients with sepsis³¹ and malignancies, including prostate,²⁶ ovarian,³² colorectal cancer²⁸ and non-small cell lung cancer.²⁷ In the context of autoimmune diseases, Te *et al*²⁹ reported the differential expression of 21 miRNAs in PBMCs isolated from African American patients with SLE compared to healthy controls. As stated above, only miR-199a-5p was found to be expressed in our CD and UC samples, while the other 20 SLE-associated PBMC miRNAs were not differentially expressed in either CD or UC. This supports the possibility that peripheral blood miRNAs may not only distinguish IBD subtypes but that specific autoimmune diseases may be associated with distinct miRNA expression patterns.

In this study, we chose to study whole peripheral blood miRNA expression using a blood collection technique with proven miRNA stability. The PaxGene™ tube, for the collection of RNA, has demonstrable stability through 48hrs at room temperature,³³ which would be relevant in the clinical setting. At the time of the initiation of this study, a commercially available technique to extract miRNA from a PaxGene™ tube was not yet available, however, we favored the establishment of our own miRNA extraction technique in favor of the utilization of a stable miRNA collection technique.

Of note, while the expression of miRNAs in plasma and serum are thought to reflect the extrusion of miRNAs from relevant remote tissues or organs or disease processes,²⁶ it is likely that peripheral blood miRNAs do not reflect miRNAs expressed in remote tissues. This is consistent with our results demonstrating that the peripheral blood miRNAs associated with active CD and UC were not similar to our previous studies identifying differentially expressed miRNAs from colonoscopic pinch biopsies of active CD and UC tissues.^{13, 23} Specifically, the 11 active UC-associated miRNAs,¹³ 5 active Crohn's colitis miRNAs²³ and 4 Crohn's ileitis miRNAs²³ previously identified as differentially expressed in biopsy tissues were not differentially expressed in the peripheral blood of IBD patients in this study. It is more likely, that the peripheral blood miRNAs identified in our study reflect expression in circulating white blood cells.

CD and UC have been associated with distinct immune responses with CD and UC differing in associated circulating and intestinal immune cell types and Th1, Th2 and Th17 cytokine profiles.³⁴ Interestingly, altered miRNA expression profiles have been reported in both T and B cell subtypes.¹⁰ The identification of differing peripheral blood miRNA associated with CD and UC is consistent with the hypothesis that each IBD subtype is associated with differing immune cell types. In addition, the finding that peripheral blood miRNAs differ in active CD and UC indicates that the miRNAs expressed in each subtype do not reflect a simple, systemic inflammatory response. This specificity of miRNAs in peripheral blood is supported by the lack of significant overlap between IBD-associated miRNAs found in our study with miRNAs found in the PBMCs of SLE patients.

Overall, the identification of differentially expressed miRNAs in the tissues and peripheral blood of patients with active CD and UC supports the hypothesis that CD and UC involve distinct pathophysiologic mechanisms. While the miRNAs that we validated do not represent a comprehensive list of all differentially expressed miRNAs in the peripheral blood of IBD patients and future results may vary based upon newer miRNA extraction protocols and arrays, the results also support the potential that blood-based miRNAs may be useful diagnostic tools for IBD. Further studies are necessary to confirm the ability of blood-based miRNAs to distinguish IBD subtypes and to determine the correlation of these and other miRNAs with disease severity and other variables, such as CD location and UC extent.

Acknowledgments

We thank the Cancer Center Microarray Core Facility at Johns Hopkins University, Baltimore, MD, USA.

Sources of support: This work was supported by the Broad Medical Research Program grant IBD-0212 (F.W. and J.H.K.). This work was also supported by National Institutes of Health grant K08DK078046 (J.H.K.). J.H.K. was also supported by the Sherlock Hibbs IBD Research Fund, the M. Alan Guerrieri Family Fund and the Harvey M. and Lyn P. Meyerhoff Inflammatory Bowel Disease Center at Johns Hopkins University.

REFERENCES

1. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol.* 2010; 28:573–621. [PubMed: 20192811]
2. Podolsky DK. Inflammatory bowel disease. *N Engl J Med.* 2002; 347:417–429. [PubMed: 12167685]
3. Cooney R, Jewell D. The genetic basis of inflammatory bowel disease. *Dig Dis.* 2009; 27:428–442. [PubMed: 19897957]
4. Costello CM, Mah N, Hasler R, et al. Dissection of the inflammatory bowel disease transcriptome using genome-wide cDNA microarrays. *PLoS Med.* 2005; 2:e199. [PubMed: 16107186]
5. Lawrance IC, Fiocchi C, Chakravarti S. Ulcerative colitis and Crohn's disease: distinctive gene expression profiles and novel susceptibility candidate genes. *Hum Mol Gen.* 2001; 10:445–456. [PubMed: 11181568]
6. Wu F, Dassopoulos T, Cope L, et al. Genome-wide expression differences between Crohn's and ulcerative colitis from endoscopic pinch biopsies: insights into distinctive pathogenesis. *Inflamm Bowel Dis.* 2007; 13:807–821. [PubMed: 17262812]
7. Guarnieri DJ, DiLeone RJ. MicroRNAs: a new class of gene regulators. *Ann Med.* 2008; 40:197–208. [PubMed: 18382885]
8. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell.* 2005; 120:15–20. [PubMed: 15652477]
9. Jiang X, Tsitsiou E, Herrick SE, et al. MicroRNAs and the regulation of fibrosis. *FEBS J.* 2010; 277:2015–2021. [PubMed: 20412055]
10. Davidson-Moncada J, Papavasiliou FN, Tam W. MicroRNAs of the immune system: roles in inflammation and cancer. *Ann N Y Acad Sci.* 2010; 1183:183–194. [PubMed: 20146715]

11. Wu H, Neilson JR, Kumar P, et al. miRNA Profiling of Naive, Effector and Memory CD8 T Cells. *PLoS ONE*. 2007; 2:e1020. [PubMed: 17925868]
12. Malumbres R, Sarosiek KA, Cubedo E, et al. Differentiation stage-specific expression of microRNAs in B lymphocytes and diffuse large B-cell lymphomas. *Blood*. 2009; 113:3754–3764. [PubMed: 19047678]
13. Wu F, Zikusoka M, Trindade A, et al. MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2 alpha. *Gastroenterology*. 2008; 135:1624–1635. [PubMed: 18835392]
14. Taganov KD, Boldin MP, Chang KJ, et al. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A*. 2006; 103:12481–12486. [PubMed: 16885212]
15. Perron MP, Boissonneault V, Gobeil LA, et al. Regulatory RNAs: Future Perspectives in Diagnosis, Prognosis, and Individualized Therapy. *Methods Mol Biol*. 2006; 361:311–326. [PubMed: 17172720]
16. Cho WC. MicroRNAs in cancer - from research to therapy. *Biochim Biophys Acta*. 2010; 1805:209–217. [PubMed: 19931352]
17. Catalucci D, Gallo P, Condorelli G. MicroRNAs in cardiovascular biology and heart disease. *Circ Cardiovasc Genet*. 2009; 2:402–408. [PubMed: 20031613]
18. Tang X, Tang G, Ozcan S. Role of microRNAs in diabetes. *Biochim Biophys Acta*. 2008; 1779:697–701. [PubMed: 18655850]
19. Sonkoly E, Wei T, Janson PC, et al. MicroRNAs: novel regulators involved in the pathogenesis of Psoriasis? *PLoS One*. 2007; 2:e610. [PubMed: 17622355]
20. Stanczyk J, Pedrioli DM, Brentano F, et al. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. *Arthritis Rheum*. 2008; 58:1001–1009. [PubMed: 18383392]
21. Nakasa T, Miyaki S, Okubo A, et al. Expression of microRNA-146 in rheumatoid arthritis synovial tissue. *Arthritis Rheum*. 2008; 58:1284–1292. [PubMed: 18438844]
22. Tan Z, Randall G, Fan J, et al. Allele-specific targeting of microRNAs to HLA-G and risk of asthma. *Am J Hum Genet*. 2007; 81:829–834. [PubMed: 17847008]
23. Wu F, Zhang S, Dassopoulos T, et al. Identification of microRNAs associated with ileal and colonic Crohn's disease. *Inflamm Bowel Dis*. 2010 In Press.
24. Olaru AV, Selaru FM, Mori Y, et al. Dynamic changes in the expression of microRNA-31 during inflammatory bowel disease-associated neoplastic transformation. *Inflamm Bowel Dis*. 2010
25. Ahmed FE, Jeffries CD, Vos PW, et al. Diagnostic microRNA markers for screening sporadic human colon cancer and active ulcerative colitis in stool and tissue. *Cancer Genomics Proteomics*. 2009; 6:281–295. [PubMed: 19996134]
26. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A*. 2008; 105:10513–10518. [PubMed: 18663219]
27. Hu Z, Chen X, Zhao Y, et al. Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. *J Clin Oncol*. 2010; 28:1721–1726. [PubMed: 20194856]
28. Huang Z, Huang D, Ni S, et al. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *Int J Cancer*. 2010; 127:118–126. [PubMed: 19876917]
29. Te JL, Dozmorov IM, Guthridge JM, et al. Identification of unique microRNA signature associated with lupus nephritis. *PLoS One*. 2010; 5:e10344. [PubMed: 20485490]
30. Voellenkle C, van Rooij J, Cappuzzello C, et al. microRNA signatures in peripheral blood mononuclear cells of chronic heart failure patients. *Physiol Genomics*. 2010
31. Wang JF, Yu ML, Yu G, et al. Serum miR-146a and miR-223 as potential new biomarkers for sepsis. *Biochem Biophys Res Commun*. 2010; 394:184–188. [PubMed: 20188071]
32. Resnick KE, Alder H, Hagan JP, et al. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. *Gynecol Oncol*. 2009; 112:55–59. [PubMed: 18954897]

33. Rainen L, Oelmueller U, Jurgensen S, et al. Stabilization of mRNA expression in whole blood samples. Clin Chem. 2002; 48:1883–1890. [PubMed: 12406972]
34. Blumberg RS. Inflammation in the intestinal tract: pathogenesis and treatment. Dig Dis. 2009; 27:455–464. [PubMed: 19897960]

Figure 1.

Heatmap of CD-associated peripheral blood miRNAs. Eighteen miRNAs were identified in the miRNA microarray profiling of peripheral blood miRNAs isolated from active and inactive CD patients and normal, healthy controls. Red color indicates higher than mean intensity (white) across all samples. Blue color indicates lower than mean intensity (white) across all samples.

Figure 2.

miRNA expression in the peripheral blood of active CD patients, inactive CD patients and healthy controls. The expression of active CD-associated peripheral blood miRNAs was assessed by qRT-PCR. Seven miRNAs were identified as significantly different. Data are presented as box-whisker plots (box, 25%–75%; whisker, 10%–90%; line, median). *P < 0.05; **P < 0.01; ***P < 0.001 as compared to healthy controls.

Figure 3. Heatmap of UC-associated peripheral blood miRNAs. Seventeen miRNAs were identified in the miRNA microarray profiling of peripheral blood miRNAs isolated from active and inactive UC patients and normal, healthy controls. Red color indicates higher than mean intensity (white) across all samples. Blue color indicates lower than mean intensity (white) across all samples.

Figure 4. miRNA expression in the peripheral blood of active UC patients, inactive UC patients and healthy controls. The expression of active UC-associated peripheral blood miRNAs was assessed by qRT-PCR. Nine miRNAs were identified as significantly different. Data are presented as box-whisker plots (box, 25%–75%; whisker, 10%–90%; line, median). *P < 0.05; **P < 0.01; ***P < 0.001 as compared to healthy controls.

Figure 5. qRT-PCR identification of 8 miRNAs differentiating active CD patients from active UC patients using miRNAs initially identified via peripheral blood miRNA microarray profiling of active CD with healthy controls and active UC with healthy controls. Data are presented as box-whisker plots (box, 25%–75%; whisker, 10%–90%; line, median). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 6.

A. Heatmap comparing peripheral blood miRNAs from active UC and CD. Twenty-one miRNAs were identified when comparing the miRNA microarray profiling of peripheral blood miRNAs isolated from active UC and CD. Red color indicates higher than mean intensity (white) across all samples. Blue color indicates lower than mean intensity (white) across all samples. **B.** miRNA validation of 3 miRNAs identified in the heatmap comparison of peripheral blood miRNAs from active CD and UC. Data are presented as box-whisker plots (box, 25%–75%; whisker, 10%–90%; line, median). *P < 0.05; **P < 0.01; ***P < 0.001.

Table 1

Clinical characteristics of patients.

	Control	Active CD	Inactive CD	Active UC	Inactive UC
No. of patients	13	14	5	13	10
Male, n (%)	6 (46.2)	9 (64.3)	3 (60)	4 (30.8)	4 (40)
Age (y)					
Mean	56.3	40.3	58.6	48.5	46.5
Range	43–75	21–67	34–73	32–81	29–64
Duration of IBD (y)					
Mean	NA	15.9	20.6	11.1	14.4
Range		0–45	7–37	3–33	5–29
Medications, n (%)					
Mesalamine	0	6 (42.9)	4 (80)	11 (84.6)	8 (80)
Antibiotics	0	2 (14.3)	1 (20)	0	0
Steroids	0	2 (14.3)	2 (40)	3 (23.1)	0
Immunomodulators	0	2 (14.3)	1 (20)	3 (38.5)	3 (30)
Biologics	0	6 (42.9)	1 (20)	2 (15.4)	2 (20)

Table 2

Primers used for quantitative real-time PCR of miRNA

Name	Direction	Primer (5'-3')
universal qPCR primer	reverse	NCode miRNA Fiest-strand cDNA Synthesis kit (Invitrogen)
miR-28-5p	forward	AAGGAGCTCACAGTCTATTGAG
miR-149*	forward	AGGGACGGGGGCTGTGCA
miR-151-5p	forward	TCGAGGAGCTCACAGTCTAGT
miR-199a-5p	forward	CCCAGTGTTCAGACTACCTGTTC
miR-340*	forward	TCCGTCTCAGTTACTTTATAGC
miR-362-3p	forward	AACACACCTATTCAAGGATTCA
miR-1908	forward	CGGCGGGACGGCGATTGGTC
miR-505*	forward	GGGAGCCAGGAAGTATTGATGT
miR-532-3p	forward	CCTCCACACCCAAGGCTTGCA
Let-7b	forward	TGAGGTAGTAGGTTGTGTGGTT
miRplus-A1056/hsa-miR-3180-3p	forward	TGGGGCGGAGCTTCCGGAGGCC
miRplus-C1040/hsa-miR-103-2*	forward	GGCTTCTTTACAGTGCTGCCTT
miRplus-E1035	forward	AGGCGGCGGAGGGGCG
miRplus-E1045	forward	AACTAGGCGGCTATGGTAT
miRplus-E1153	forward	ATGAGGTGGCAAGAAATGGGCT
miRplus-E1271	forward	GAACGCGGTCTGAGTGGT
miRplus-F1029	forward	TCGGAAGAGGAGCGGAAAGGAAA
miRplus-F1065	forward	CTCGGGCGGCGGGAGGAG
miRplus-F1159	forward	AGCGGCGGCGGCGGAAGG
U6B	forward	CGCAAGGATGACACGCAAATTCG