Published in final edited form as:

Exp Mol Pathol. 2014 June ; 96(3): 422–425. doi:10.1016/j.yexmp.2014.04.009.

Elevated Circulating miR-150 and miR-342-3p in Patients with Irritable Bowel Syndrome

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Abstract

Background and Aims—MicroRNAs (miRNAs) are small non-coding RNAs, which regulate gene expression and are thus of interest as diagnostic markers, and as clues to etiology and targets of intervention. This pilot study examined whether circulating miRNAs are differentially expressed in patients with IBS.

Methods—miRNA microarrays (Nanosttring) were run on the whole blood of 43 participants.

Results—hsa-miR-150 and hsa-miR-342-3p were found to be significantly elevated (FDR adjusted $p = 0.05$, 1.6 fold change) in IBS patients compared to healthy controls. Neither of these miRNAs showed any relationship to race or sex. hsa-miR-150 is associated with inflammatory

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Disclosure

None of the authors has potential conflicts of interest to disclose.

Author Contributions

Sarah K. Abey, LeeAnne B. Sherwin and Wendy A. Henderson performed the patient enrollment and administered the patient protocols, patient data collection and sample collection; they also curate and formatted clinical and patient data for analysis. Ralph M. Peace, Nicolaas H Fourie and Paul A. Smyser performed sample preparation and curation. Ralph M. Peace performed the RNA purification and prepared RNA for miRNA analysis. Nicolaas H. Fourie conducted the data analysis, interpreted the data, and was responsible for manuscript generation. Sarah K. Abey, John W. Wiley and Bridgett Rahim-Williams contributed to the data interpretation, functional network analyses. Wendy A. Henderson was responsible for protocol generation. All co-authors were required to contribute to the writing and editing of the manuscript.

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bowel disorders and pain, and interacts with a protein kinase (AKT2) through which it may affect inflammatory pathways. hsa-miR-342-3p is predicted to interact with mRNAs involved in pain signaling, colonic motility, and smooth muscle function.

Conclusions—This preliminary study reports the association of two miRNAs, detected in whole blood, with IBS. These miRNAs link to pain and inflammatory pathways both of which are thought to be dysregulated in IBS. Larger samples sizes are needed to confirm their importance and potential as biomarkers.

Keywords

irritable bowel syndrome; microRNA; hsa-miRNA-150; hsa-miR-342-3p

Introduction

Irritable Bowel Syndrome (IBS) is a common gastrointestinal (GI) disorder characterized by chronic abdominal pain (CAP) and changes in bowel habits, including the frequent occurrence of diarrhea (IBS-D), constipation (IBS-C) or a combination of both (IBS-M) (Drossman, 2007). The disorder affects an estimated 10–15% of the global population and is estimated to have a significant economic cost in terms of healthcare and lost productivity in the USA alone (Ashburn and Gupta, 2006). A better understanding of IBS pathogenesis and symptom etiology is important to improve patient outcomes.

The etiology and pathogenesis underlying IBS is currently unclear. Much of recent research, including ours, suggests dysregulated inflammatory and immune pathways and changes to epithelial barrier function in the etiology IBS (Henderson et al., 2012; Chadwick et al., 2002; Vivinus-Nebot et al., 2013). These results derive from studies based on the morphology (Demaude et al., 2006), functional performance (Piche et al., 2009), or protein (Miwa et al., 2001) and/or gene (Kerchoffs et al., 2012) expression of intestinal tissues. Recently, the expression of microRNAs (miRNAs), which are small RNAs capable of regulating protein levels, has piqued interests as diagnostic biomarkers, as indicators of etiology and as potential therapeutic targets. Although miRNAs are routinely examined in the study of a wide array of diseases relatively few studies of IBS have focused on miRNA expression.

Some notable studies have demonstrated the diagnostic and functional importance of miRNAs in IBS. Zhou et al. (2010) identified hsa-miR-29a as up-regulated in blood and colon tissue and described its role in modulating epithelial barrier function in patients with IBS-D and increased intestinal permeability. Kappelar et al. (2008) demonstrated the expression regulation of a serotonin receptor gene, associated with IBS-D, by hsa-miR-510. These discoveries have led to miRNA based therapeutic interventions in IBS being proposed (Zhou et al., 2011).

Most studies of gene expression in IBS focus on intestinal gene expression; these require invasive sampling of the intestinal mucosa. The purpose of this preliminary investigation was to examine circulating miRNA expression as a less invasive measure of molecular dysregulation in patients with IBS.

Materials and Methods

Sample

Patients who met Rome II criteria (i.e., chronic or recurrent abdominal pain, and altered stooling habits) for IBS (IBS-D = 5, IBS-C = 5, or IBS-M = 2) and healthy controls (i.e., no known organic disease or GI symptoms, n = 31) were recruited (clinicaltrials.gov #NCT00824921; see Table 1 for demographic and clinical indicators), at the National Institutes of Health (NIH) Hatfield Clinical Research Center. Participants signed a written consent to have their samples stored and used for research purposes. Whole blood samples were collected via venipuncture in PAXGene tubes (PreAnalytiX, Qiagen, Valencia, CA, USA) as per the manufacturer's instructions, and stored at -80°C until RNA purification.

microRNA Expression

Total RNA, including miRNA, was extracted using the PAXGene Blood miRNA extraction kit (PreAnalytiX, Qiagen, Valencia, CA, USA). The nCounter Human miRNA Expression Assay Kit V1.4 (NanoString Technologies, Seattle, WA, USA) was used to anneal miRNAs to target specific barcode probes. No amplification was required. The sample was then immobilized on an nCounter Cartridge using the nCounter Prep Station. Barcode probes were counted and recorded for each miRNA target (735 human miRNAs) using the nCounter Digital Analyzer. The data were normalized to the top 100 expressed miRNAs, and the background was corrected using the nSolver software package. A 25 count threshold was set. miRNA species with more than 75% of cases at this threshold were excluded as such low counts cannot be accurately reproduced. Using these criteria 138 miRNAs were included in subsequent analyses. Repeated samples and internal controls show a high degree of consistency between assays.

Statistical Analysis

Statistical analysis of the demographic and clinical data was performed using SPSS v15.0 with *a priori* statistical significance set at $p = 0.05$. Comparisons of each parameter were conducted using the parametric independent sample Student's t-test.

Statistical analysis of the genetic data was performed using BRB-Array Tools (Biometric Research Branch, National Cancer Institute) with the *a priori* statistical significance set at $p = 0.05$. A priori p-values were adjusted for False Discovery Rate (FDR). miRNA data from IBS patients and healthy controls were compared while assessing the presence of race and gender effects. Integrated Pathway Analysis (IPA, Ingenuity Systems, Inc.) was used to explore the functional associations of miRNA species of interest.

Results

Demographic and Clinical Data

The IBS group was not significantly different in terms of age, BMI, hematocrit, CRP, ESR, ALT, AST, or amylase compared to healthy controls (Table 1). Lipase was significantly higher in IBS patients compared to healthy controls (Table 1). Although a difference in

lipase was observed among groups, none of the values fell outside of the normal clinical range for these indices.

miRNA Expression

Both hsa-miR-150 and hsa-miR-342-3p were found to be elevated (FDR adjusted $p = 0.05$) in patients with IBS compared to healthy controls (Figures 1a and b). The expression of hsa-miR-150 and hsa-miR-342-3p were 1.6 and 1.7 fold higher, respectively, in IBS patients (mean $\pm$ 1 standard deviation: hsa-miR-150 = 3988 ± 1195 ; hsa-miR-342-3p = 491 ± 152) than in healthy controls (hsa-miR-150 = 2509 ± 1135 ; hsa-miR-342-3p = 292 ± 87). The IBS-constipation (hsa-miR-342-3p = 560 ± 225) cohort but not the IBS-diarrhea (hsa-miR-342-3p = 444 ± 44) cohort had elevated ($p < 0.005$) hsa-miR-342-3p expression compared to healthy controls (Figure 1b). Expression of these miRNAs was not found to have any relationship to race or gender.

Integrated Pathway Analysis of IBS-Associated miRNAs

To explore possible interactions of these miRNAs with other genes and proteins, and their role in different molecular pathways, they were entered into an Ingenuity Pathway Analysis (IPA; Ingenuity Systems Inc., Redwood City, CA). The analysis merged networks associated with the following diseases: cancer, hematological disease, organismal injury and abnormalities, endocrine system disorders, and reproductive system disease. In addition, functional networks related to cellular development, growth, proliferation, movement, death, survival, and cell-to-cell signaling were merged. Several genes with both direct and indirect bindings to the two miRNA species were revealed, including the calcium channel voltage-dependent L type alpha 1C subunit (CACNA 1C), caspase-3, and the serine-threonine protein kinase AKT2 (Figure 2).

Discussion

In this preliminary study of circulating miRNA expression in IBS, hsa-miR-150 and hsa-miR-342-3p were identified as up-regulated in IBS patients compared to healthy controls. Despite the small sample size, a consistent differential expression of hsa-miR-342-3p between the different IBS-subtypes (diarrhea, constipation, or mixed) compared to healthy controls is evident. Neither race nor gender was found to explain variation in these miRNAs. We have previously identified hsa-miR-342-3p as part of a dysregulated miRNA profile in a weight- and sex-matched Caucasian cohort of IBS patients and healthy controls (Peace et al., in review). This dysregulation of miR-342-3p in independent cohorts provides additional evidence for the consistency of an association between this miRNA species and IBS.

The hsa-miR-342-3p has been found to be up-regulated in Bladder Pain Syndrome (BPS) (Gheinani et al., 2013). This finding is intriguing, because similar to the symptom of abdominal pain with unknown cause presenting in our IBS patients, BPS patients present with pelvic pain in the absence of a specific cause. The IPA provides a potential molecular link between this miRNA and pain signaling pathways by revealing an association with the voltage-dependent L type calcium channel alpha 1C protein (CACNA 1C). Regulated processing of the CACNA1 mRNA has been functionally associated with nociceptive pain

transmission (Lipscombe et al., 2013). Dysregulated expression of L-type calcium channels is also associated with altered colonic motility and smooth muscle contraction in a rat model of IBS (Zhang et al., 2010). In addition to chronic pain, inflammation also underlies the pathogenesis of BPS. We have previously observed that inflammatory markers are disproportionately represented in colonic tissues from IBS patients, which suggests a sub-clinical inflammatory component in IBS (Henderson et al., 2012). Taken together, it is likely that the functional role of the hsa-miR-342-3p involves inflammatory, pain signaling, smooth muscle contractility, and GI tract motility pathways.

The hsa-miR-150 also has a postulated role in inflammation and has been previously associated with Crohn's disease (CD), ulcerative colitis (UC), and colorectal cancer (Pekow et al., 2012; Ma et al., 2012). This miRNA was found to be over expressed in the colon epithelium of patients with CD and UC (Pekow et al., 2012) and has also been associated with pain (Orlova et al., 2011). Targets of the hsa-miR-150 include the telomerase-related proteins Dyskerin (dyskeratosis congenita 1, DKC1), as well as AKT2, a pro-survival protein kinase activated via the PI3K pathway, and is expressed in many human malignancies. AKT, which is an important factor in inflammatory pathways, has also been linked to inflammatory bowel diseases (Anderson and Wong, 2010).

In summary, our findings identify two miRNA species, hsa-miR-150 and hsa-miR-342-3p, that are differentially expressed in the peripheral circulation of patients suffering from IBS. Both miRNAs are implicated in inflammation and have previously been associated with pain. Further research will reveal the specific roles of hsa-miR-150 and hsa-miR-342-3p in these pathways and whether they may be developed as biomarkers and/or therapeutic targets in IBS.

Acknowledgments

Funding

The authors acknowledge funding from the US Department of Health and Human Services, National Institutes of Health, National Institute of Nursing Research, Division of Intramural Research (to Wendy A. Henderson, 1Z1ANR000018-01-04; Intramural Research Training Awards to Nicolaas H. Fourie, Ralph M. Peace, Sarah K. Abey, Leeanne B. Sherwin, and Paul A. Smyser; Howard Hughes-NIH Research Scholar RMP; Special support from the Burroughs Wellcome Fund to Ralph M. Peace.

Abbreviations

AKT2	serine-threonine protein kinase
Alk. Phos	alkaline phosphatase
ALT	alanine transaminase
AST	aspartate aminotransferase
BMI	body mass index
BPS	bladder pain syndrome
CACNA 1C	calcium channel voltage-dependent L type alpha 1C subunit

CAP	chronic abdominal pain
CD	Crohn's Disease
CRP	C-reactive protein
DKC1	dyskerin
ESR	erythrocyte sedimentation rate
FDR	false discovery rate
GGTP	gamma glutamyl transpeptidase
GI	gastrointestinal
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IBS-C	irritable bowel syndrome constipation
IBS-D	irritable bowel syndrome diarrhea
IBS-M	irritable bowel syndrome mixed
IPA	Ingenuity Pathway Analysis
miRNA	microRNA
NIH	National Institutes of Health
UC	Ulcerative Colitis

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Highlights

- Potential circulating miRNA biomarkers of IBS suggests non-invasive methods to understand, diagnose, and monitor the condition.
- The primary aim of this study was to examine circulating miRNA expressions in patients with ROME II IBS and compare them to healthy controls.
- Whole genome miRNA profiling was used to characterize peripheral circulating miRNA expression in patients with IBS and healthy controls.
- miR-150 and miR-342-3p were significantly elevated in patients with IBS compared to healthy controls.

Figure 1.

Figure 1a and 1b: Box plots showing the differential expression of (a) hsa-miR-150 and (b) hsa-miR-342-3p in healthy controls and IBS patients.

Figure 2.

Integrated Pathway Analysis (IPA) network generated outlining probable relationships between hsa-miR-342-3p and hsa-miR-150 and related mRNAs and proteins. Direct and indirect relationships are shown by solid and dashed lines, respectively. The arrow indicates specific directionality of interactions.

Table 1

Sample characteristics and clinical results

Variable	Healthy Controls (n=31)	IBS ^a patients (n=12)	P value
Gender (n)			
Male	15	3	-
Female	16	9	-
Race (n)			
Asian	7	2	-
African American/Black	13	8	-
Caucasian	7	1	-
Other	4	1	-
Age	27.9±8.5	29.2±7	p>0.05
Range (years)	13–45	16–45	
IBS-Diarrhea	-	5	
IBS-Constipation	-	5	
IBS-Mixed	-	2	
BMI^b	24.5±4.7	26.6±5.8	p>0.05
Range	28.5–48.2	20.5–41.3	
Hematocrit	40.4±5.0	39.4±4.6	p>0.05
Range (%)	28.5–48.2	30.2–47.9	
CRP^c	1.8±1.8	3.1±4.8	p>0.05
Range (mg L ⁻¹)	0.2–7.3	0.2–17.5	
ESR^d	9.6±8.4	12.5±7.7	p>0.05
Range (mm/hr)	2.0–30.0	3.0–25.0	
Alk. Phos.^e	75.2±56.6	66.0±19.6	p>0.05
Range (U L ⁻¹)	36.0–367.0	40.0–110.0	
ALT^f	27.5±10.6	28.6±13.7	p>0.05
Range (U L ⁻¹)	15.0–66.0	17.0–55.0	
AST^g	17.8±8.9	20.5±15.0	p>0.05
Range (U L ⁻¹)	6.0–53.0	6.0–61.0	
GGTP^h	25.0±12.6	34.2±28.3	p>0.05
Range (U L ⁻¹)	9.0–57.0	11.0–113.0	
Amylase	64.5±20.8	71.4±20.9	p>0.05
Range (U L ⁻¹)	29.0–117.0	46.0–109.0	
Lipase	127.6±46.5	168.5±72.3	p=0.034
Range (U L ⁻¹)	63.0–255.0	85.0–357.0	

All values expressed as mean ±standard deviation.

^aIBS, Irritable Bowel Syndrome.

^b BMI, Body Mass Index.

^c CRP, C-reactive protein.

^d ESR, Erythrocyte sedimentation rate.

^e Alk. Phos., Alkaline phosphatase.

^f ALT, Alanine transaminase.

^g AST, Aspartate Aminotransferase.

^h GGTP, Gamma Glutamyl Transpeptidase. Normal ranges – Hematocrit: 34.1–44.9%, CRP: <3.0mg L⁻¹, ESR: <42mm hr⁻¹, Alk Phos.: 37–116 U L⁻¹, ALT: 6–41 U L⁻¹, AST: 9–34 U L⁻¹, GGTP: 5–85 U L⁻¹, Amylase: 25–115 U L⁻¹, Lipase: 76–491 U L⁻¹.