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Diet and lifestyle factors associated with miRNA expression in colorectal tissue

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Martha L Slattery[1]
Jennifer S Herrick[1]
Lila E Mullany[1]
John R Stevens[2]
Roger K Wolff[1]

[1]Department of Internal Medicine, The University of Utah, Salt Lake City, [2]Department of Mathematics and Statistics, Utah State University, Logan, UT, USA

Abstract: MicroRNAs (miRNAs) are small non-protein-coding RNA molecules that regulate gene expression. Diet and lifestyle factors have been hypothesized to be involved in the regulation of miRNA expression. In this study it was hypothesized that diet and lifestyle factors are associated with miRNA expression. Data from 1,447 cases of colorectal cancer to evaluate 34 diet and lifestyle variables using miRNA expression in normal colorectal mucosa as well as for differential expression between paired carcinoma and normal tissue were used. miRNA data were obtained using an Agilent platform. Multiple comparisons were adjusted for using the false discovery rate *q*-value. There were 250 miRNAs differentially expressed between carcinoma and normal colonic tissue by level of carbohydrate intake and 198 miRNAs differentially expressed by the level of sucrose intake. Of these miRNAs, 166 miRNAs were differentially expressed for both carbohydrate intake and sucrose intake. Ninety-nine miRNAs were differentially expressed by the level of whole grain intake in normal colonic mucosa. Level of oxidative balance score was associated with 137 differentially expressed miRNAs between carcinoma and paired normal rectal mucosa. Additionally, 135 miRNAs were differentially expressed in colon tissue based on recent NSAID use. Other dietary factors, body mass index, waist and hip circumference, and long-term physical activity levels did not alter miRNA expression after adjustment for multiple comparisons. These results suggest that diet and lifestyle factors regulate miRNA level. They provide additional support for the influence of carbohydrate, sucrose, whole grains, NSAIDs, and oxidative balance score on colorectal cancer risk.

Keywords: colorectal cancer, carbohydrate, miRNA, NSAIDs, oxidative balance, sucrose

Introduction

MicroRNAs (miRNAs) are small non-protein-coding RNA molecules that regulate gene expression either by posttranscriptionally suppressing messenger RNA (mRNA) translation or by causing mRNA degradation.[1–6] We know that miRNAs play a critical role in regulation of proliferation, differentiation, apoptosis, and stress response and are involved in the majority of physiological processes.[7,8] While we are beginning to understand the role of miRNAs in various physiological functions, our understanding of what regulates miRNA expression is minimal. However, some studies have shown that some diet and other lifestyle factors such as specific dietary components, oxidative stress, and aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) alter miRNA expression.[9–13]

One of the factors most consistently inversely associated with colorectal cancer (CRC) risk is use of aspirin/NSAIDs. Likewise, dietary factors such as antioxidants have been associated with CRC risk.[14–17] We and others have shown that the oxida-

Correspondence: Martha L Slattery
Department of Internal Medicine, The University of Utah, 383 Colorow Drive, Salt Lake City, UT 84108, USA
Tel +1 801 585 6955
Email Marty.Slattery@hsc.utah.edu

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tive balance score (OBS) is associated with CRC,[18–20] with CRC risk lowest among those with a score that is higher in antioxidants and lower in prooxidant factors. The role that these diet and lifestyle factors have on miRNA expression is limited, especially as it applies to colorectal tissue.

In this study, we examine the linear association between diet and lifestyle factors and miRNA expression in colon and rectal tissues. Our hypothesis is that lifestyle factors commonly associated with CRC risk alter miRNA expression profiles. We test associations for colon and rectal cancers separately, since risk factors often differ by tumor location. We evaluate associations of diet and lifestyle factors with miRNA expression for normal colon and rectal mucosa as well as for the difference of miRNA expression between paired carcinoma and normal colorectal tissue to help determine if associations influence the disease process. If miRNA expression profiles are altered by diet and lifestyle factors, it would strengthen the biological support for these associations and provide avenues for cancer prevention.

Methods

Study participants

Study participants came from two population-based case–control studies that included all incident colon and rectal cancers between 30 and 79 years of age who resided along the Wasatch Front in Utah or were members of the Kaiser Permanente Medical Care Program (KPMCP) in Northern California. Participants were White, Hispanic, or Black for the colon cancer study; the rectal cancer study also included Asians and American Indians not living on reservations.[21,22] Cases had to have tumor registry verification of a first primary adenocarcinoma of the colon or rectum and were diagnosed between October 1991 and September 1994 for the colon cancer study and between June 1997 and May 2001 for the rectal cancer study. Detail study methods have been described earlier.[23] The study was approved by the Institutional Review Board of the University of Utah and at KPMCP and all participants provided written informed consent.

miRNA processing

RNA was extracted from formalin-fixed paraffin-embedded tissue. Both normal mucosa adjacent to the carcinoma tissue and matched carcinoma tissue were used. Normal mucosa tissue served as a control. The Agilent Human miRNA Microarray V19.0 (Agilent Technologies, Santa Clara, CA, USA) was used given the number of miRNAs, its high level of reliability (repeatability coefficient was 0.98 in our data), and the amount of RNA needed to run the platform. The microarray contains probes for 2,006 unique human miRNAs. About 100 ng total RNA was labeled with Cy3 and hybridized to the Agilent Microarray and were scanned on an Agilent SureScan microarray scanner model G2600D. Data were extracted from the scanned image using Agilent Feature Extract software v.11.5.1.1 (Agilent Technologies). Data were required to pass stringent quality control (QC) parameters established by Agilent that included tests for excessive background fluorescence, excessive variation among probe sequence replicates on the array, and measures of the total gene signal on the array to assess low signal. If samples failed to meet quality standards for any of these parameters, the sample was relabeled, hybridized to arrays, and scanned. If a sample failed QC assessment a second time, the sample was deemed to be of poor quality and was excluded from down-stream analysis.

Diet and lifestyle data

Data were collected by trained and certified interviewers using laptops. All interviews were audiotaped as previously described and reviewed for QC purposes.[24] The referent period for the study was 2 years prior to diagnosis. As part of the study questionnaire, information was collected on regular use and current use of aspirin and NSAIDs and on physical activity during the referent period and for 10 and 20 years prior to diagnosis. Physical activity was obtained for the physical intensity of activity performed as well as the frequency and duration of activity. Body size information, including height (measured at the time of interview), weight (recalled for referent period), and waist and hip circumference measurements, were also collected. Dietary information was obtained for the referent period using an extensive diet history questionnaire adapted from the validated CARDIA diet history.[25] Foods were converted to nutrients using the Nutrition Coding Center Nutrient Data System Version 19 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA) as well as being grouped into categories of similar foods. Foods units were standard servings per day, which was half cup of fruit, vegetable, or dairy product; meat servings were 2–3 oz of meat; grain products were half cup of rice-type grains or one slice of bread. Prudent and western dietary patterns were developed based on the principal component analysis.[26] Our prudent dietary pattern was heavily loaded toward diets high in fruits, vegetables, whole grains, fish, and chicken, whereas the western dietary pattern was highly loaded toward red meat, processed meats, and refined grains and high-sugar-high-fat foods. Additional questions were asked about meat consumption, doneness, and preparation methods that were combined and used to create a mutagen index score.[27]

Statistical methods

Of the 2,006 unique human miRNAs assessed, 1,278 were expressed in colorectal carcinoma tissue. To minimize differences in miRNA expression that could be attributed to the array, amount of RNA, location on array, or other factors that could erroneously influence expression, total gene signal was normalized by multiplying each sample by a scaling factor[28] (http://genespring-support.com/files/gs_12_6/GeneSpring-manual.pdf), which was the median of the 75th percentiles of all the samples divided by the individual 75th percentile of each sample. We limited our analysis to miRNAs that were expressed in at least 20% of the samples in the tissue(s) of interest. Data were assessed for colon and rectal cancer separately, and the number of miRNAs analyzed varied from 766 to 817 depending on tumor site and tissue type (ie, carcinoma or normal mucosa). Our sample consisted of 1,447 subjects with both miRNA expression data for carcinoma and paired normal mucosa as well as diet and lifestyle variables.

We assessed long-term vigorous physical activity, body mass index (kilogram per square meter) during referent year, waist circumference, and hip circumference. Assessment of aspirin/NSAID use included recent use (ie, using NSAIDs during the referent period or not) and ever regular use. We assessed 28 dietary variables including energy intake, western dietary pattern, prudent dietary pattern, mutagen index, total fat, total trans-fatty acid, total carbohydrates, sucrose, animal protein, vegetable protein, vitamins B12, C, D, and E, calcium, folic acid, dietary fiber, carotenoids, β-carotene, lutein + zeaxanthin, lycopene, and servings per day of dairy, fruit, vegetables, meat, processed meat, whole grains, and refined grains. All variables were analyzed as continuous variables unless they were collected as categorical (ie, NSAIDs variables). To summarize risk associated with multiple exposures, we developed an OBS that consisted of 13 diet and lifestyle factors that were prooxidants (dietary iron and polyunsaturated fat and cigarette smoking) and antioxidants (vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein + zeaxanthin, vitamin D, calcium, and folic acid and NSAID use).[18] To create the OBS, these diet and lifestyle factors were assigned values of 2 for low levels of exposure for each prooxidants or high exposure to antioxidants (low risk), 1 for intermediate levels of exposure, and 0 for high levels of exposure to prooxidants and low exposure to antioxidants (high risk). The individual scores for the 13 variables were then combined to obtain the OBS. Higher summary score corresponded to greater oxidative balance.

We examined lifestyle variables to determine if there was an association between each lifestyle variable and miRNA expression by fitting a linear model to the $\log_2$-transformed expression levels and adjusting for age at diagnosis, study center, and sex. We examined miRNA expression in both normal mucosa and the difference between miRNA expressions in carcinoma and in the normal colonic mucosa. *p*-values were generated using the bootstrap method by creating a distribution of 10,000 F statistics derived by resampling the residuals from the null hypothesis model of no association between the lifestyle variables and the miRNAs using the boot package in R. Associations were considered important if the false discovery rate (FDR) *q*-value was <0.11.[29] We standardized the β coefficient presented in order to compare the results across the miRNA and lifestyle factors by subtracting the mean of the mRNA and dividing the result by the standard deviation of the mRNA before calculating the slope. If the lifestyle variable in the regression model was continuous, then we applied the same technique to it as well.

Results

The study population consisted of 892 cases of colon cancer and 555 cases of rectal cancer (Table 1). The mean age for colon cancer cases was 64.7 years and for rectal cancer cases was 61.8 years. The majority of study participants were males and most reported never having used

Table 1 Description of study population

Lifestyle factor	Colon	Rectal
Sex[a]		
Male	485 (54.4)	316 (56.9)
Female	407 (45.6)	239 (43.1)
Center[a]		
Kaiser	626 (70.2)	340 (61.3)
Utah	266 (29.8)	215 (38.7)
OBS[a]		
Low	321 (36.0)	191 (34.4)
2	187 (21.0)	120 (21.6)
3	242 (27.1)	160 (28.8)
High	142 (15.9)	84 (15.1)
Ever used NSAIDs regularly[a]		
No	531 (59.5)	318 (57.3)
Yes	361 (40.5)	237 (42.7)
Recent NSAID use[a]		
No	583 (65.6)	350 (63.6)
Yes	306 (34.4)	200 (36.4)
Age at diagnosis[b]	64.7 (9.5)	61.8 (10.8)
Calories (Kcal)[b]	2,479 (1,223)	2,660 (1,261)
Total carbohydrates (gram per 1,000 Kcal)[b]	127.9 (22.6)	123.8 (21.8)
Sucrose (gram per 1,000 Kcal)[b]	20.6 (8.48)	21.8 (9.80)

Notes: [a]Values are given as N (%); [b]Values are given as mean (SD).
Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; OBS, oxidative balance score; SD, standard deviation.

aspirin/NSAIDs (subsequently referred to as NSAIDs) on a regular basis.

There were few diet and lifestyle factors associated with miRNA expression in either normal colonic mucosa or with differential miRNA expression between carcinoma and normal mucosa when applying an FDR *q*-value of <0.1. Body mass index, waist and hip circumference, and physical activity showed no associations with miRNA expression with the FDR *q*-value at <0.1 as did most dietary factors analyzed. Only three dietary factors, carbohydrate intake, servings per day of whole grains, and sucrose intake, were associated with miRNA expression. For carbohydrate intake and sucrose intake, there were 250 and 198 miRNAs significantly differentially expressed between colon carcinoma and normal mucosa tissue by level of dietary intake. Of these miRNAs, 166 were significantly differentially expressed for both carbohydrate intake and sucrose intake (Table 2 shows the top 85 miRNAs based on *q*-value and Table S1 shows all miRNAs significantly differentially expressed for both carbohydrate and sucrose intake). Although the adjusted *q*-values indicated more significant associations with carbohydrate intake, the β coefficients for the two variables were very similar. There were 32 miRNAs uniquely associated with sucrose intake (Table 3). The top 53 miRNAs associated uniquely with carbohydrate intake (Table 3) all had a *q*-value of <0.05. Evaluation of miRNA expression in whole grains showed 99 miRNAs differentially expressed by level of intake in normal colonic mucosa with an FDR of <0.1 (Table 4).

Associations with miRNAs were similar for all indicators of NSAIDs use and for both normal colonic mucosa and differential miRNA expression between carcinoma and normal colonic mucosa. Table 5 shows the top 85 of the 135 miRNAs differentially expressed in normal colonic mucosa by recent NSAID use versus no recent NSAID use. OBS was only associated with miRNA expression for rectal tissue

Table 2 Colon cancer differential miRNA expression (top 85) associated with both sucrose and carbohydrate intake

Sucrose	Tumor	Normal	Sucrose			Carbohydrate		
miRNA expression	**Mean**	**Mean**	**β**	***p*-value**	***q*-value**	**β**	***p*-value**	***q*-value**
hsa_miR_10b_3p	47.06	53.71	−0.0838	0.0128	0.0641	−0.0911	0.0074	0.0350
hsa_miR_1224_5p	910.91	1,009.30	−0.0891	0.0093	0.0641	−0.0988	0.0060	0.0350
hsa_miR_1225_5p	2,775.37	3,033.07	−0.0922	0.0084	0.0641	−0.1030	0.0037	0.0350
hsa_miR_1227_5p	966.19	1,082.86	−0.0991	0.0033	0.0641	−0.1071	0.0027	0.0350
hsa_miR_1307_3p	11.07	12.84	−0.0901	0.0100	0.0641	−0.1070	0.0028	0.0350
hsa_miR_135a_3p	146.18	161.98	−0.0770	0.0270	0.0743	−0.0925	0.0078	0.0350
hsa_miR_1471	237.51	257.92	−0.0801	0.0204	0.0680	−0.1097	0.0016	0.0350
hsa_miR_1587	1,337.63	1,396.92	−0.1067	0.0022	0.0641	−0.1108	0.0020	0.0350
hsa_miR_181b_5p	23.22	18.61	−0.0917	0.0067	0.0641	−0.0903	0.0079	0.0350
hsa_miR_188_5p	406.17	458.31	−0.0810	0.0186	0.0651	−0.1033	0.0036	0.0350
hsa_miR_1914_3p	120.96	130.99	−0.0821	0.0173	0.0641	−0.1031	0.0037	0.0350
hsa_miR_1915_3p	2,946.52	3,444.83	−0.0896	0.0101	0.0641	−0.0946	0.0068	0.0350
hsa_miR_195_3p	1.12	1.03	0.0838	0.0161	0.0641	0.0949	0.0061	0.0350
hsa_miR_197_5p	3,010.83	3,334.22	−0.1104	0.0010	0.0641	−0.1087	0.0018	0.0350
hsa_miR_2861	5,575.32	6,458.02	−0.0926	0.0073	0.0641	−0.0954	0.0054	0.0350
hsa_miR_3124_5p	1.67	2.22	0.1180	0.0009	0.0641	0.1103	0.0010	0.0350
hsa_miR_3137	235.66	244.41	−0.0776	0.0230	0.0714	−0.1062	0.0028	0.0350
hsa_miR_3138	78.43	80.84	−0.0730	0.0324	0.0826	−0.1020	0.0045	0.0350
hsa_miR_3156_5p	87.64	93.95	−0.0753	0.0313	0.0818	−0.1024	0.0026	0.0350
hsa_miR_3158_5p	29.98	32.45	−0.0823	0.0168	0.0641	−0.1053	0.0023	0.0350
hsa_miR_3162_5p	2,715.58	2,982.07	−0.1011	0.0040	0.0641	−0.1141	0.0016	0.0350
hsa_miR_3188	218.59	247.20	−0.0875	0.0112	0.0641	−0.0969	0.0056	0.0350
hsa_miR_3194_5p	153.17	167.90	−0.0693	0.0455	0.0976	−0.0965	0.0053	0.0350
hsa_miR_3196	1,228.77	1,388.20	−0.0786	0.0224	0.0712	−0.0933	0.0073	0.0350
hsa_miR_3197	29.50	31.14	−0.0801	0.0202	0.0678	−0.1000	0.0042	0.0350
hsa_miR_345_5p	58.98	65.81	−0.0753	0.0261	0.0728	−0.1094	0.0009	0.0350
hsa_miR_3610	109.25	128.54	−0.0870	0.0129	0.0641	−0.1080	0.0031	0.0350
hsa_miR_3621	36.22	38.31	−0.0787	0.0232	0.0715	−0.1010	0.0048	0.0350
hsa_miR_370	44.95	41.52	−0.1075	0.0017	0.0641	−0.1323	0.0004	0.0350
hsa_miR_378e	2.05	2.15	0.0811	0.0197	0.0667	0.0941	0.0060	0.0350
hsa_miR_3940_5p	754.26	842.23	−0.1024	0.0033	0.0641	−0.1072	0.0018	0.0350

(*Continued*)

Table 2 (*Continued*)

Sucrose	Tumor	Normal	Sucrose			Carbohydrate		
miRNA expression	**Mean**	**Mean**	**β**	***p*-value**	***q*-value**	**β**	***p*-value**	***q*-value**
hsa_miR_4257	332.86	361.83	−0.0765	0.0267	0.0740	−0.0989	0.0060	0.0350
hsa_miR_4270	482.93	483.89	−0.0925	0.0097	0.0641	−0.0910	0.0077	0.0350
hsa_miR_4271	277.51	288.76	−0.0976	0.0043	0.0641	−0.1020	0.0045	0.0350
hsa_miR_4296	1.91	1.37	0.0860	0.0137	0.0641	0.0964	0.0055	0.0350
hsa_miR_4428	1,303.07	1,361.56	−0.0730	0.0354	0.0873	−0.1105	0.0025	0.0350
hsa_miR_4442	288.05	293.52	−0.0822	0.0176	0.0641	−0.0959	0.0065	0.0350
hsa_miR_4478	292.78	326.72	−0.0929	0.0070	0.0641	−0.1151	0.0012	0.0350
hsa_miR_4481	79.73	85.64	−0.1077	0.0020	0.0641	−0.1187	0.0008	0.0350
hsa_miR_4486	178.26	186.85	−0.0962	0.0061	0.0641	−0.0926	0.0080	0.0350
hsa_miR_4487	92.03	100.77	−0.0755	0.0301	0.0797	−0.0932	0.0067	0.0350
hsa_miR_4507	1,821.93	2,056.47	−0.0926	0.0048	0.0641	−0.0933	0.0084	0.0350
hsa_miR_4515	305.06	344.83	−0.0746	0.0289	0.0780	−0.0926	0.0083	0.0350
hsa_miR_4516	15,260.03	17,545.65	−0.0850	0.0136	0.0641	−0.1005	0.0039	0.0350
hsa_miR_4651	216.67	229.48	−0.0985	0.0048	0.0641	−0.0970	0.0053	0.0350
hsa_miR_4695_5p	356.79	385.84	−0.0913	0.0079	0.0641	−0.1046	0.0024	0.0350
hsa_miR_4707_5p	95.29	98.20	−0.0880	0.0102	0.0641	−0.0947	0.0071	0.0350
hsa_miR_4721	1,590.35	1,739.00	−0.0951	0.0058	0.0641	−0.0993	0.0039	0.0350
hsa_miR_4725_3p	15.13	16.09	−0.0952	0.0070	0.0641	−0.1045	0.0034	0.0350
hsa_miR_4734	189.46	206.40	−0.0872	0.0097	0.0641	−0.0938	0.0073	0.0350
hsa_miR_4758_5p	139.37	134.74	−0.0871	0.0112	0.0641	−0.0974	0.0063	0.0350
hsa_miR_4763_3p	1,338.05	1,402.42	−0.1138	0.0013	0.0641	−0.1065	0.0023	0.0350
hsa_miR_4776_5p	36.75	37.60	−0.0679	0.0476	0.0993	−0.0929	0.0073	0.0350
hsa_miR_4788	327.29	342.72	−0.0962	0.0051	0.0641	−0.0988	0.0056	0.0350
hsa_miR_5006_5p	653.96	737.60	−0.0729	0.0330	0.0836	−0.1011	0.0044	0.0350
hsa_miR_5196_5p	76.16	66.21	−0.1040	0.0032	0.0641	−0.0999	0.0039	0.0350
hsa_miR_520b	16.21	20.36	−0.0825	0.0177	0.0641	−0.0990	0.0040	0.0350
hsa_miR_525_5p	1.95	2.41	0.0833	0.0149	0.0641	0.0959	0.0048	0.0350
hsa_miR_548q	71.52	91.25	−0.0952	0.0066	0.0641	−0.1037	0.0021	0.0350
hsa_miR_557	75.73	75.51	−0.1129	0.0016	0.0641	−0.1068	0.0026	0.0350
hsa_miR_572	441.84	510.90	−0.0898	0.0092	0.0641	−0.0940	0.0073	0.0350
hsa_miR_6068	2,305.93	2,676.49	−0.0963	0.0055	0.0641	−0.1001	0.0046	0.0350
hsa_miR_6127	1,378.02	1,494.38	−0.0894	0.0097	0.0641	−0.1036	0.0035	0.0350
hsa_miR_623	45.79	48.87	−0.0815	0.0220	0.0710	−0.1015	0.0035	0.0350
hsa_miR_638	3,686.15	4,168.08	−0.1014	0.0031	0.0641	−0.1064	0.0017	0.0350
hsa_miR_642a_3p	3,880.94	4,192.71	−0.0802	0.0191	0.0657	−0.1002	0.0039	0.0350
hsa_miR_642b_3p	659.58	707.40	−0.0896	0.0096	0.0641	−0.1017	0.0033	0.0350
hsa_miR_6511b_5p	70.13	67.42	−0.0966	0.0051	0.0641	−0.1016	0.0031	0.0350
hsa_miR_6722_3p	98.64	103.55	−0.0867	0.0128	0.0641	−0.0912	0.0084	0.0350
hsa_miR_6724_5p	842.08	891.10	−0.0958	0.0051	0.0641	−0.0995	0.0044	0.0350
hsa_miR_718	103.79	115.55	−0.0897	0.0091	0.0641	−0.0960	0.0060	0.0350
hsa_miR_877_5p	39.26	39.14	−0.0839	0.0156	0.0641	−0.0973	0.0051	0.0350
hsa_miR_937_5p	658.60	673.28	−0.1139	0.0011	0.0641	−0.1061	0.0017	0.0350
hsa_miR_1249	34.36	34.94	−0.0915	0.0100	0.0641	−0.0912	0.0094	0.0357
hsa_miR_4632_5p	212.75	224.93	−0.0775	0.0254	0.0728	−0.0912	0.0095	0.0357
hsa_miR_4787_5p	1,109.55	1,394.63	−0.0815	0.0166	0.0641	−0.0904	0.0092	0.0357
hsa_miR_5787	1,928.96	2,138.52	−0.0709	0.0401	0.0919	−0.0926	0.0094	0.0357
hsa_miR_601	42.38	46.88	−0.0803	0.0184	0.0651	−0.0903	0.0091	0.0357
hsa_miR_1233_1_5p	178.73	198.48	−0.0727	0.0372	0.0888	−0.0902	0.0105	0.0367
hsa_miR_3656	2,793.88	2,941.93	−0.0991	0.0042	0.0641	−0.0871	0.0106	0.0367
hsa_miR_371b_5p	1,139.60	1,438.06	−0.0871	0.0107	0.0641	−0.0909	0.0102	0.0367
hsa_miR_4497	3,447.19	3,736.33	−0.0824	0.0179	0.0643	−0.0895	0.0100	0.0367
hsa_miR_4633_5p	7.36	7.84	0.1123	0.0011	0.0641	0.0897	0.0101	0.0367
hsa_miR_4687_3p	1,902.37	2,193.37	−0.0937	0.0063	0.0641	−0.0912	0.0105	0.0367
hsa_miR_5001_5p	968.46	1,138.75	−0.0955	0.0057	0.0641	−0.0871	0.0103	0.0367

Abbreviation: miRNA, micro RNA.

Table 3 Differential miRNA expression between colon carcinoma and normal colonic mucosa uniquely associated with either sucrose or carbohydrate intake

miRNA	Tumor	Normal	β	*p*-value	*q*-value
	Mean	Mean			
Sucrose					
hsa_miR_1229_3p	15.00	17.87	−0.0680	0.0454	0.0976
hsa_miR_1247_3p	27.62	26.20	−0.0738	0.0300	0.0797
hsa_miR_1273g_5p	9.21	10.05	0.0943	0.0064	0.0641
hsa_miR_155_5p	44.51	46.29	−0.0699	0.0422	0.0947
hsa_miR_181a_5p	34.21	24.91	−0.0778	0.0245	0.0726
hsa_miR_200a_5p	6.09	5.12	0.0795	0.0238	0.0723
hsa_miR_210	23.66	14.90	−0.0815	0.0165	0.0641
hsa_miR_2277_3p	3.86	5.01	0.0857	0.0125	0.0641
hsa_miR_302c_5p	2.70	2.40	0.0688	0.0473	0.0993
hsa_miR_3170	1.86	2.44	0.0888	0.0108	0.0641
hsa_miR_320b	82.25	80.23	−0.0765	0.0258	0.0728
hsa_miR_3614_5p	5.13	6.55	0.0715	0.0381	0.0894
hsa_miR_3648	331.00	274.75	−0.0767	0.0246	0.0726
hsa_miR_365b_5p	13.34	14.46	0.0704	0.0424	0.0947
hsa_miR_373_5p	17.58	18.43	−0.0718	0.0348	0.0871
hsa_miR_3976	2.15	1.11	0.0817	0.0177	0.0641
hsa_miR_4419a	69.63	66.94	−0.0728	0.0374	0.0888
hsa_miR_4448	9.30	9.04	0.0828	0.0174	0.0641
hsa_miR_4484	64.10	71.60	−0.0754	0.0261	0.0728
hsa_miR_4488	109.70	113.97	−0.0846	0.0110	0.0641
hsa_miR_4532	316.22	368.80	−0.0776	0.0256	0.0728
hsa_miR_4700_5p	1.34	0.96	0.0693	0.0459	0.0977
hsa_miR_4715_5p	4.40	5.06	0.0802	0.0208	0.0682
hsa_miR_4717_3p	3.67	3.39	0.0912	0.0082	0.0641
hsa_miR_4733_5p	67.07	69.46	−0.0753	0.0287	0.0780
hsa_miR_4793_3p	5.89	6.42	0.0815	0.0195	0.0666
hsa_miR_5003_3p	19.76	20.35	−0.0704	0.0408	0.0921
hsa_miR_602	44.22	47.11	−0.0707	0.0378	0.0892
hsa_miR_6069	18.95	19.15	−0.0674	0.0467	0.0989
hsa_miR_6075	209.46	246.60	−0.0713	0.0355	0.0873
hsa_miR_6134	3.05	3.18	0.0752	0.0293	0.0786
hsa_miR_659_5p	2.28	3.28	0.0862	0.0134	0.0641
Carbohydrate					
hsa_miR_1291	5.62	3.31	0.0966	0.0053	0.0350
hsa_miR_129_5p	9.26	9.98	−0.0973	0.0048	0.0350
hsa_miR_1305	112.80	129.42	−0.0920	0.0081	0.0350
hsa_miR_30c_1_3p	10.17	11.17	−0.0911	0.0082	0.0350
hsa_miR_3198	165.30	171.97	−0.0927	0.0076	0.0350
hsa_miR_3945	43.34	47.78	−0.0938	0.0063	0.0350
hsa_miR_4459	20,580.45	24,456.49	−0.0951	0.0075	0.0350
hsa_miR_4470	34.38	40.65	−0.1108	0.0016	0.0350
hsa_miR_4499	376.55	430.33	−0.1030	0.0031	0.0350
hsa_miR_4656	182.68	185.98	−0.0909	0.0084	0.0350
hsa_miR_575	561.79	607.76	−0.1092	0.0014	0.0350
hsa_miR_6131	129.70	148.03	−0.0925	0.0071	0.0350
hsa_miR_1236_5p	186.86	188.18	−0.0908	0.0092	0.0357
hsa_miR_221_3p	11.21	2.53	0.0896	0.0093	0.0357
hsa_miR_3652	150.82	160.30	−0.0909	0.0093	0.0357
hsa_miR_769_3p	21.60	24.95	−0.0900	0.0091	0.0357
hsa_miR_4740_5p	31.44	33.08	−0.0898	0.0103	0.0367
hsa_miR_1288	50.64	56.68	−0.0896	0.0111	0.0374
hsa_miR_4730	6.40	5.81	0.0882	0.0119	0.0386
hsa_miR_4314	60.89	63.69	−0.0866	0.0135	0.0388

(*Continued*)

Table 3 (*Continued*)

miRNA	Tumor	Normal	β	*p*-value	*q*-value
	Mean	Mean			
hsa_miR_4682	2.04	1.82	0.0873	0.0133	0.0388
hsa_miR_196b_5p	15.41	6.06	0.0833	0.0156	0.0410
hsa_miR_4462	328.28	365.23	−0.0829	0.0157	0.0410
hsa_miR_4716_3p	103.29	116.31	−0.0848	0.0153	0.0410
hsa_miR_3972	3.95	2.61	0.0841	0.0170	0.0420
hsa_miR_4739	913.21	1,025.15	−0.0858	0.0171	0.0420
hsa_miR_3925_5p	42.61	46.66	−0.0825	0.0184	0.0435
hsa_miR_4665_5p	59.37	61.84	−0.0836	0.0185	0.0435
hsa_miR_1207_5p	1,881.80	2,044.90	−0.0825	0.0191	0.0438
hsa_miR_378a_3p	112.01	146.82	−0.0806	0.0196	0.0438
hsa_miR_4701_3p	78.10	75.38	−0.0798	0.0201	0.0438
hsa_miR_4793_5p	225.94	220.22	−0.0787	0.0192	0.0438
hsa_miR_497_5p	1.21	5.89	0.0800	0.0196	0.0438
hsa_miR_5700	2.39	2.49	0.0806	0.0201	0.0438
hsa_miR_5581_5p	106.65	121.34	−0.0816	0.0206	0.0441
hsa_miR_4496	56.08	60.47	−0.0793	0.0213	0.0448
hsa_miR_3130_5p	11.48	11.83	0.0815	0.0219	0.0455
hsa_miR_4737	1.10	0.94	0.0797	0.0220	0.0455
hsa_miR_5194	54.57	60.38	−0.0799	0.0227	0.0466
hsa_miR_6129	78.07	86.34	−0.0788	0.0231	0.0469
hsa_miR_6717_5p	118.74	134.59	−0.0798	0.0236	0.0474
hsa_miR_4294	21.43	22.45	−0.0784	0.0239	0.0475
hsa_miR_4713_3p	229.14	258.22	−0.0785	0.0238	0.0475
hsa_miR_1307_5p	51.08	67.75	−0.0772	0.0241	0.0476
hsa_miR_3185	18.39	17.80	−0.0776	0.0253	0.0478
hsa_miR_4304	16.80	18.22	−0.0784	0.0247	0.0478
hsa_miR_4673	79.93	94.86	−0.0783	0.0252	0.0478
hsa_miR_494	15,260.32	15,700.29	−0.0771	0.0247	0.0478
hsa_miR_5088	47.41	55.74	−0.0795	0.0250	0.0478
hsa_miR_6515_3p	8.54	8.71	0.0793	0.0251	0.0478
hsa_miR_518c_5p	1.84	2.58	0.0791	0.0256	0.0479
hsa_miR_4646_5p	110.21	112.97	−0.0780	0.0263	0.0483
hsa_miR_378i	56.62	73.03	−0.0757	0.0276	0.0490
hsa_miR_887	53.78	63.17	−0.0765	0.0287	0.0500
hsa_miR_3679_5p	672.88	742.50	−0.0760	0.0291	0.0504
hsa_miR_345_3p	62.14	60.39	−0.0749	0.0295	0.0509
hsa_miR_3125	56.40	60.80	−0.0747	0.0305	0.0523
hsa_miR_191_3p	8.84	9.03	0.0749	0.0327	0.0542
hsa_miR_4508	48.75	57.69	−0.0752	0.0324	0.0542
hsa_miR_622	89.80	88.61	−0.0747	0.0323	0.0542
hsa_miR_4690_5p	164.97	209.18	−0.0736	0.0342	0.0560
hsa_miR_4419b	25.39	26.54	−0.0735	0.0354	0.0568
hsa_miR_4534	224.12	255.34	−0.0735	0.0355	0.0568
hsa_miR_4659b_3p	4.15	5.06	0.0750	0.0367	0.0583
hsa_miR_520e	11.01	14.21	−0.0736	0.0371	0.0585
hsa_miR_3150b_5p	14.06	19.14	−0.0731	0.0374	0.0587
hsa_miR_590_5p	1.93	2.43	0.0743	0.0380	0.0594
hsa_miR_4538	140.02	191.59	−0.0741	0.0393	0.0609
hsa_miR_1303	3.89	4.35	0.0708	0.0395	0.0610
hsa_miR_513c_5p	91.98	100.86	−0.0726	0.0398	0.0612
hsa_miR_339_3p	3.81	3.95	0.0725	0.0401	0.0613
hsa_miR_1470	4.48	5.86	0.0715	0.0409	0.0620
hsa_miR_550b_2_5p	37.51	39.99	−0.0712	0.0409	0.0620
hsa_miR_3195	892.68	1,067.06	−0.0697	0.0424	0.0635
hsa_miR_1208	29.73	32.93	−0.0704	0.0430	0.0639

(*Continued*)

Table 3 (*Continued*)

miRNA	Tumor	Normal	β	*p*-value	*q*-value
	Mean	Mean			
hsa_miR_2276	101.73	96.46	−0.0715	0.0429	0.0639
hsa_miR_3200_5p	25.29	28.98	−0.0702	0.0441	0.0648
hsa_miR_4710	43.12	40.75	−0.0693	0.0446	0.0651
hsa_miR_500a_5p	20.68	23.85	−0.0694	0.0454	0.0658
hsa_miR_4535	67.37	72.01	−0.0690	0.0478	0.0687
hsa_miR_630	345.63	402.87	−0.0678	0.0486	0.0695
hsa_miR_4253	45.58	48.10	−0.0685	0.0494	0.0700
hsa_miR_452_5p	8.81	10.34	0.0683	0.0496	0.0700
hsa_miR_510	1.10	0.97	0.0674	0.0498	0.0700

Abbreviation: miRNA, micro RNA.

Table 4 miRNA expression in normal colonic mucosa associated with whole grain intake

miRNA	Tumor	Normal	β	*p*-value	*q*-value
	Mean	Mean			
hsa_let_7b_5p	304.74	250.81	0.0844	0.0080	0.0722
hsa_miR_103a_3p	58.12	40.90	0.0771	0.0166	0.0919
hsa_miR_107	37.67	26.20	0.0746	0.0222	0.0988
hsa_miR_10a_3p	3.98	4.44	−0.0860	0.0096	0.0753
hsa_miR_1234_3p	40.56	47.47	0.0790	0.0162	0.0919
hsa_miR_1237_5p	6.52	7.50	−0.0905	0.0091	0.0742
hsa_miR_1254	19.24	21.91	−0.1183	0.0012	0.0536
hsa_miR_125a_5p	11.66	9.00	0.0713	0.0213	0.0981
hsa_miR_1260a	317.07	341.64	0.0973	0.0020	0.0536
hsa_miR_1260b	125.83	98.87	0.0858	0.0089	0.0742
hsa_miR_1261	3.20	4.23	−0.1051	0.0021	0.0536
hsa_miR_1273d	27.61	28.00	0.0847	0.0108	0.0790
hsa_miR_1273f	261.20	268.96	0.0752	0.0196	0.0943
hsa_miR_1273g_3p	2,181.39	2,647.15	0.1032	0.0027	0.0536
hsa_miR_1291	5.62	3.31	−0.0791	0.0160	0.0919
hsa_miR_1295a	9.30	10.75	−0.1146	0.0018	0.0536
hsa_miR_1303	3.89	4.35	−0.0951	0.0031	0.0536
hsa_miR_1323	6.54	7.09	−0.0755	0.0194	0.0943
hsa_miR_141_3p	38.27	28.27	0.0869	0.0078	0.0722
hsa_miR_145_5p	116.51	184.46	0.0760	0.0143	0.0903
hsa_miR_192_5p	81.69	123.55	0.0895	0.0079	0.0722
hsa_miR_194_3p	5.56	6.87	0.1202	0.0005	0.0536
hsa_miR_194_5p	79.56	107.89	0.0996	0.0031	0.0536
hsa_miR_1972	113.21	113.89	0.0873	0.0090	0.0742
hsa_miR_197_3p	13.39	14.16	0.0799	0.0170	0.0919
hsa_miR_200b_3p	141.48	120.08	0.0819	0.0142	0.0903
hsa_miR_200c_3p	128.38	103.59	0.0988	0.0038	0.0581
hsa_miR_215	38.97	64.59	0.0799	0.0155	0.0919
hsa_miR_23b_3p	59.36	57.72	0.0754	0.0209	0.0973
hsa_miR_24_3p	89.32	52.20	0.0800	0.0136	0.0903
hsa_miR_28_5p	1.36	1.91	0.0741	0.0145	0.0903
hsa_miR_30b_3p	5.46	4.85	−0.1061	0.0023	0.0536
hsa_miR_30d_5p	30.09	27.11	0.0851	0.0063	0.0722
hsa_miR_3163	9.39	9.74	−0.1131	0.0010	0.0536
hsa_miR_3173_3p	8.76	8.51	−0.1031	0.0026	0.0536
hsa_miR_320d	42.91	39.97	0.0772	0.0174	0.0919
hsa_miR_320e	37.87	36.13	0.0765	0.0209	0.0973
hsa_miR_339_3p	3.81	3.95	−0.0858	0.0087	0.0742
hsa_miR_3605_5p	14.36	14.33	−0.0985	0.0063	0.0722
hsa_miR_3614_5p	5.13	6.55	−0.0918	0.0069	0.0722
hsa_miR_3617_5p	4.71	5.20	−0.0777	0.0180	0.0919
hsa_miR_3651	50.76	21.67	0.0881	0.0094	0.0752

(*Continued*)

Table 4 (*Continued*)

miRNA	Tumor	Normal	β	*p*-value	*q*-value
	Mean	Mean			
hsa_miR_3659	12.37	12.99	–0.0919	0.0075	0.0722
hsa_miR_3666	18.85	20.61	–0.1040	0.0031	0.0536
hsa_miR_3680_3p	7.44	6.56	–0.0879	0.0089	0.0742
hsa_miR_3713	6.29	6.87	–0.0787	0.0172	0.0919
hsa_miR_375	15.73	42.48	0.1002	0.0029	0.0536
hsa_miR_378a_3p	112.01	146.82	0.0830	0.0135	0.0903
hsa_miR_378c	6.82	7.25	–0.1000	0.0033	0.0536
hsa_miR_378i	56.62	73.03	0.0746	0.0207	0.0973
hsa_miR_3926	34.69	33.62	0.0777	0.0219	0.0987
hsa_miR_4260	8.28	7.84	–0.1340	0.0011	0.0536
hsa_miR_4261	17.42	16.92	0.0762	0.0231	0.0988
hsa_miR_4284	1,257.36	1,195.39	0.0895	0.0061	0.0722
hsa_miR_4286	1,057.76	1,096.92	0.1091	0.0009	0.0536
hsa_miR_4316	5.73	7.66	–0.0921	0.0054	0.0717
hsa_miR_4418	5.37	5.37	–0.0919	0.0057	0.0717
hsa_miR_4425	9.73	10.92	–0.1217	0.0006	0.0536
hsa_miR_4436a	4.75	5.23	–0.0806	0.0145	0.0903
hsa_miR_4436b_3p	7.66	7.03	–0.0794	0.0171	0.0919
hsa_miR_4436b_5p	19.16	23.14	0.0753	0.0226	0.0988
hsa_miR_4444	9.63	8.77	–0.0969	0.0043	0.0626
hsa_miR_4446_3p	12.28	15.26	–0.0830	0.0144	0.0903
hsa_miR_4454	41,752.17	43,243.72	0.1086	0.0009	0.0536
hsa_miR_4455	72.03	70.07	0.0783	0.0171	0.0919
hsa_miR_4485	1,034.22	1,284.96	0.0910	0.0075	0.0722
hsa_miR_4489	11.85	12.56	–0.1249	0.0006	0.0536
hsa_miR_4502	17.43	19.16	–0.0867	0.0110	0.0790
hsa_miR_4510	6.03	6.45	–0.0832	0.0113	0.0798
hsa_miR_4519	8.59	8.71	–0.1052	0.0020	0.0536
hsa_miR_4526	3.59	4.36	–0.0912	0.0051	0.0708
hsa_miR_452_5p	8.81	10.34	–0.0901	0.0078	0.0722
hsa_miR_4660	5.17	5.04	–0.0830	0.0118	0.0819
hsa_miR_4684_3p	2.88	3.29	–0.0751	0.0231	0.0988
hsa_miR_4691_5p	11.56	12.49	–0.1010	0.0032	0.0536
hsa_miR_4748	5.83	6.27	–0.0765	0.0179	0.0919
hsa_miR_4750_5p	8.69	6.66	–0.0790	0.0183	0.0923
hsa_miR_4755_3p	6.86	7.81	–0.1098	0.0011	0.0536
hsa_miR_4773	8.16	9.29	–0.0771	0.0217	0.0987
hsa_miR_486_5p	16.64	21.50	0.0792	0.0186	0.0927
hsa_miR_500a_3p	2.93	2.96	–0.0797	0.0162	0.0919
hsa_miR_5093	3.64	3.86	–0.0750	0.0227	0.0988
hsa_miR_5096	52.50	51.07	0.1018	0.0028	0.0536
hsa_miR_509_5p	7.11	8.08	–0.1400	0.0002	0.0536
hsa_miR_5100	3,645.51	4,386.60	0.1074	0.0012	0.0536
hsa_miR_516a_5p	27.00	29.72	–0.1258	0.0011	0.0536
hsa_miR_518a_5p	4.60	5.94	–0.0780	0.0190	0.0936
hsa_miR_5195_5p	8.30	8.56	–0.0842	0.0110	0.0790
hsa_miR_550a_5p	7.64	8.54	–0.0866	0.0109	0.0790
hsa_miR_5572	7.65	8.28	–0.1117	0.0011	0.0536
hsa_miR_5685	1.93	2.93	–0.0762	0.0172	0.0919
hsa_miR_6083	30.20	37.23	0.0839	0.0102	0.0786
hsa_miR_659_3p	11.22	11.27	–0.0969	0.0057	0.0717
hsa_miR_6716_5p	5.41	5.25	–0.1045	0.0015	0.0536
hsa_miR_6718_5p	7.76	7.68	–0.0924	0.0072	0.0722
hsa_miR_708_5p	9.33	10.69	–0.0954	0.0067	0.0722
hsa_miR_711	20.37	18.07	–0.0806	0.0177	0.0919
hsa_miR_770_5p	5.58	6.29	–0.0910	0.0079	0.0722
hsa_miR_92a_3p	94.06	35.74	0.0772	0.0171	0.0919

Abbreviation: miRNA, micro RNA.

Table 5 miRNA expression in normal colonic mucosa associated with recent NSAIDs use

miRNA	No recent use		Recent use		β	*p*-value	*q*-value
	Tumor	Normal	Tumor	Normal			
	Mean	Mean	Mean	Mean			
hsa_miR_1185_1_3p	396.08	442.93	414.35	413.20	−0.16	0.0114	0.0845
hsa_miR_1185_2_3p	136.55	150.38	147.62	139.56	−0.15	0.0182	0.0845
hsa_miR_1225_3p	17.01	19.03	16.84	17.73	−0.17	0.0112	0.0845
hsa_miR_1227_5p	976.85	1,110.48	949.54	1,030.83	−0.16	0.0121	0.0845
hsa_miR_1228_3p	34.12	35.62	33.55	33.29	−0.16	0.0163	0.0845
hsa_miR_1229_5p	659.51	722.70	692.39	665.34	−0.15	0.0179	0.0845
hsa_miR_1233_1_5p	180.18	202.49	176.16	190.91	−0.18	0.0081	0.0845
hsa_miR_1268b	1,303.23	1,289.54	1,295.27	1,189.93	−0.17	0.0077	0.0845
hsa_miR_129_5p	9.32	10.23	9.18	9.50	−0.18	0.0065	0.0845
hsa_miR_150_3p	256.88	283.63	266.29	266.67	−0.16	0.0140	0.0845
hsa_miR_1587	1,352.23	1,428.35	1,312.10	1,337.13	−0.16	0.0140	0.0845
hsa_miR_195_3p	1.05	0.97	1.23	1.12	0.17	0.0106	0.0845
hsa_miR_196a_5p	6.01	3.56	6.23	3.86	0.17	0.0115	0.0845
hsa_miR_197_5p	3,005.55	3,430.32	3,033.62	3,151.71	−0.17	0.0097	0.0845
hsa_miR_2392	228.38	235.42	229.30	214.90	−0.16	0.0170	0.0845
hsa_miR_2861	5,626.17	6,628.80	5,494.23	6,135.84	−0.17	0.0096	0.0845
hsa_miR_28_3p	1.36	1.47	1.36	1.63	0.16	0.0178	0.0845
hsa_miR_3141	217.64	209.30	230.06	194.84	−0.18	0.0089	0.0845
hsa_miR_3147	36.50	35.54	37.03	33.73	−0.17	0.0126	0.0845
hsa_miR_3185	18.46	18.12	18.28	17.20	−0.16	0.0169	0.0845
hsa_miR_3188	217.86	253.49	220.57	235.46	−0.16	0.0167	0.0845
hsa_miR_3197	29.37	31.66	29.82	30.16	−0.17	0.0155	0.0845
hsa_miR_3591_3p	1.34	1.29	1.40	1.40	0.18	0.0080	0.0845
hsa_miR_3648	326.34	281.62	339.96	261.57	−0.18	0.0080	0.0845
hsa_miR_3656	2,807.67	3,026.73	2,776.46	2,782.58	−0.16	0.0133	0.0845
hsa_miR_3665	3,199.26	3,688.47	3,103.18	3,381.05	−0.18	0.0053	0.0845
hsa_miR_3679_5p	673.97	761.63	671.02	706.29	−0.17	0.0101	0.0845
hsa_miR_371b_5p	1,170.13	1,490.03	1,085.20	1,339.67	−0.22	0.0010	0.0845
hsa_miR_3911	96.51	89.05	99.12	84.30	−0.18	0.0075	0.0845
hsa_miR_3917	97.60	99.91	97.40	94.78	−0.17	0.0087	0.0845
hsa_miR_3923	0.58	1.32	0.80	1.65	0.18	0.0072	0.0845
hsa_miR_3940_5p	760.89	864.96	743.30	799.19	−0.17	0.0106	0.0845
hsa_miR_423_5p	53.16	58.48	54.22	54.85	−0.22	0.0012	0.0845
hsa_miR_4257	337.07	372.94	325.74	340.76	−0.19	0.0056	0.0845
hsa_miR_4271	276.36	295.46	280.32	276.17	−0.16	0.0140	0.0845
hsa_miR_4281	3,828.07	4,117.96	3,928.63	3,793.36	−0.16	0.0172	0.0845
hsa_miR_4298	183.19	168.33	191.21	157.16	−0.19	0.0052	0.0845
hsa_miR_4306	24.83	23.12	25.54	22.04	−0.18	0.0103	0.0845
hsa_miR_4322	108.18	102.21	108.10	95.91	−0.18	0.0088	0.0845
hsa_miR_4327	248.12	251.53	256.93	233.14	−0.18	0.0061	0.0845
hsa_miR_4417	211.11	191.02	221.11	179.36	−0.15	0.0182	0.0845
hsa_miR_4419a	68.39	68.52	72.01	63.97	−0.17	0.0091	0.0845
hsa_miR_4433_3p	294.45	316.29	302.33	289.43	−0.16	0.0103	0.0845
hsa_miR_4433_5p	19.68	20.35	19.09	18.90	−0.17	0.0162	0.0845
hsa_miR_4447	14.11	15.79	14.69	14.63	−0.18	0.0057	0.0845
hsa_miR_4466	1,784.94	1,996.87	1,780.90	1,849.17	−0.16	0.0182	0.0845
hsa_miR_4479	1.92	2.27	2.19	2.54	0.17	0.0167	0.0845
hsa_miR_4486	180.46	191.15	174.64	178.73	−0.18	0.0073	0.0845
hsa_miR_4488	111.67	117.23	106.27	107.89	−0.17	0.0095	0.0845
hsa_miR_4505	3,082.72	3,378.63	2,949.31	3,173.69	−0.17	0.0143	0.0845
hsa_miR_4507	1,847.97	2,105.80	1,776.12	1,963.15	−0.17	0.0118	0.0845
hsa_miR_4532	319.46	378.39	310.60	350.83	−0.16	0.0167	0.0845
hsa_miR_4669	349.43	374.17	355.47	348.03	−0.17	0.0155	0.0845
hsa_miR_4690_5p	166.05	213.24	163.22	201.57	−0.16	0.0165	0.0845
hsa_miR_4695_5p	359.45	392.74	352.89	372.91	−0.17	0.0112	0.0845

(*Continued*)

Table 5 (*Continued*)

miRNA	No recent use		Recent use		β	*p*-value	*q*-value
	Tumor	Normal	Tumor	Normal			
	Mean	Mean	Mean	Mean			
hsa_miR_4700_3p	1.29	1.53	2.24	2.85	0.25	<0.0001	0.0845
hsa_miR_4734	192.44	211.44	184.33	196.97	−0.17	0.0120	0.0845
hsa_miR_4763_3p	1,335.39	1,438.92	1,346.77	1,333.66	−0.16	0.0165	0.0845
hsa_miR_4787_5p	1,138.11	1,440.10	1,058.08	1,308.81	−0.21	0.0018	0.0845
hsa_miR_4800_5p	186.25	167.82	201.33	158.36	−0.17	0.0131	0.0845
hsa_miR_498	25.41	28.61	25.97	26.90	−0.17	0.0131	0.0845
hsa_miR_5001_5p	990.75	1,177.94	928.90	1,065.39	−0.22	0.0013	0.0845
hsa_miR_514b_5p	32.51	32.24	33.75	30.55	−0.17	0.0102	0.0845
hsa_miR_5189	33.44	34.64	33.18	33.00	−0.16	0.0144	0.0845
hsa_miR_5196_5p	75.47	67.46	77.74	63.88	−0.17	0.0107	0.0845
hsa_miR_572	448.42	524.02	430.84	486.13	−0.16	0.0176	0.0845
hsa_miR_590_5p	1.89	2.25	1.92	2.74	0.20	0.0043	0.0845
hsa_miR_6068	2,337.20	2,750.48	2,254.49	2,536.41	−0.18	0.0064	0.0845
hsa_miR_6075	2,14.34	254.50	200.64	231.62	−0.23	0.0007	0.0845
hsa_miR_6086	346.89	377.05	359.93	345.16	−0.19	0.0061	0.0845
hsa_miR_6088	3,169.09	3,495.65	3,177.39	3,219.68	−0.17	0.0105	0.0845
hsa_miR_6089	28,714.26	33,589.79	28,275.38	31,010.21	−0.16	0.0157	0.0845
hsa_miR_6125	9,092.99	11,058.09	8,810.33	10,118.16	−0.20	0.0024	0.0845
hsa_miR_6126	603.41	713.44	599.82	671.34	−0.16	0.0170	0.0845
hsa_miR_6127	1,375.55	1,531.09	1,385.68	1,425.00	−0.18	0.0062	0.0845
hsa_miR_6165	359.86	332.79	380.59	311.21	−0.17	0.0131	0.0845
hsa_miR_623	45.99	49.99	45.50	46.77	−0.18	0.0072	0.0845
hsa_miR_6511a_5p	47.59	44.90	46.82	42.60	−0.16	0.0171	0.0845
hsa_miR_670	1.22	1.53	1.76	1.77	0.16	0.0175	0.0845
hsa_miR_671_5p	259.87	299.18	266.28	278.64	−0.16	0.0150	0.0845
hsa_miR_6724_5p	843.41	914.95	839.90	845.93	−0.18	0.0083	0.0845
hsa_miR_765	42.12	41.45	43.68	39.09	−0.18	0.0060	0.0845
hsa_miR_874	204.36	226.29	202.36	212.98	−0.19	0.0043	0.0845
hsa_miR_937_5p	660.48	689.07	657.22	643.64	−0.16	0.0116	0.0845
hsa_miR_939_5p	642.45	703.34	628.40	648.33	−0.18	0.0046	0.0845
hsa_miR_320a	99.91	102.52	101.43	97.48	−0.16	0.0189	0.0867
hsa_miR_1183	63.53	65.28	63.11	62.42	−0.16	0.0201	0.0872
hsa_miR_1207_5p	1,878.63	2,100.91	1,890.05	1,940.24	−0.15	0.0209	0.0872
hsa_miR_1226_5p	52.30	56.08	53.73	52.71	−0.15	0.0210	0.0872
hsa_miR_1915_3p	2,957.00	3,531.48	2,930.60	3,282.81	−0.15	0.0210	0.0872
hsa_miR_3621	36.35	39.02	36.09	36.99	−0.16	0.0210	0.0872
hsa_miR_4314	60.70	64.77	61.42	61.71	−0.15	0.0198	0.0872
hsa_miR_4707_5p	95.66	100.51	94.84	93.87	−0.15	0.0199	0.0872
hsa_miR_6069	18.92	19.58	19.01	18.32	−0.16	0.0202	0.0872
hsa_miR_6511b_5p	70.44	68.60	69.78	65.25	−0.15	0.0202	0.0872
hsa_miR_1181	218.07	244.37	226.56	231.00	−0.16	0.0221	0.0888
hsa_miR_1234_5p	4,635.89	5,342.62	4,700.32	4,968.38	−0.15	0.0232	0.0888
hsa_miR_3170	1.81	2.40	1.94	2.51	0.16	0.0242	0.0888
hsa_miR_365a_5p	25.42	26.06	25.19	24.84	−0.15	0.0239	0.0888
hsa_miR_4315	0.07	2.10	0.25	2.42	0.16	0.0243	0.0888
hsa_miR_4442	283.26	299.26	297.28	282.82	−0.15	0.0220	0.0888
hsa_miR_4672	827.58	883.29	794.89	831.14	−0.15	0.0238	0.0888
hsa_miR_4706	17.89	17.95	17.83	17.30	−0.15	0.0229	0.0888
hsa_miR_4745_5p	260.36	297.48	264.24	279.82	−0.15	0.0241	0.0888
hsa_miR_4767	26.01	28.99	26.52	27.00	−0.15	0.0221	0.0888
hsa_miR_6076	249.40	277.34	250.34	261.52	−0.16	0.0224	0.0888
hsa_miR_663a	409.92	278.30	433.93	261.51	−0.15	0.0240	0.0888
hsa_miR_6722_3p	98.16	106.16	99.81	98.69	−0.15	0.0228	0.0888
hsa_miR_1208	29.68	33.39	29.87	32.06	−0.15	0.0250	0.0897
hsa_miR_638	3,709.77	4,267.16	3,653.32	3,982.11	−0.15	0.0250	0.0897

Abbreviations: miRNA, micro RNA; NSAIDs, nonsteroidal anti-inflammatory drugs.

(Table 6 for top dysregulated miRNAs); 137 miRNAs were differentially expressed between rectal carcinoma and paired normal rectal mucosa based on OBS level.

Discussion

We assessed 34 diet and lifestyle variables with miRNA expression levels in colorectal tissue and observed that only

Table 6 Rectal cancer differential miRNA expression between carcinoma and normal rectal mucosa associated with OBS

miRNA	Low		Q2		Q3		High		β	*p*-value	*q*-value
	Tumor	Normal	Tumor	Normal	Tumor	Normal	Tumor	Normal			
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean			
hsa_miR_106b_5p	12.71	3.43	11.09	3.00	13.24	3.13	13.87	3.24	0.10	0.015	0.0748
hsa_miR_1183	57.93	56.90	55.82	59.18	55.29	57.24	55.26	58.99	−0.09	0.0124	0.0748
hsa_miR_1202	1,148.16	1,137.83	1,094.67	1,255.86	1,059.69	1,180.33	1,101.84	1,276.40	−0.11	0.0049	0.0748
hsa_miR_1207_5p	1,539.78	1,681.81	1,473.82	1,870.36	1,399.16	1,714.49	1,455.64	1,792.49	−0.09	0.0213	0.0748
hsa_miR_1234_5p	3,735.98	4,230.54	3,564.89	4,536.04	3,362.81	4,236.96	3,549.80	4,499.46	−0.09	0.0203	0.0748
hsa_miR_126_3p	13.82	13.46	14.19	13.43	15.97	13.18	17.38	13.39	0.11	0.004	0.0748
hsa_miR_1275	586.07	640.38	561.24	722.57	526.32	673.33	539.49	711.07	−0.10	0.0084	0.0748
hsa_miR_134	216.89	238.07	209.35	259.08	200.11	240.91	206.40	251.41	−0.09	0.0225	0.0748
hsa_miR_135a_3p	135.39	147.31	126.90	149.81	124.85	150.89	125.52	153.09	−0.10	0.0125	0.0748
hsa_miR_138_2_3p	5.24	6.19	5.63	5.52	5.71	5.82	6.18	5.95	0.11	0.0053	0.0748
hsa_miR_1471	165.81	183.08	159.25	195.46	150.04	182.99	161.60	219.81	−0.10	0.0086	0.0748
hsa_miR_200a_5p	6.04	4.90	6.45	4.31	6.38	4.45	6.81	4.38	0.11	0.0056	0.0748
hsa_miR_215	43.09	62.38	38.57	55.61	44.92	58.67	46.40	55.97	0.10	0.0099	0.0748
hsa_miR_2276	93.32	84.53	88.94	86.76	88.26	85.53	90.51	89.73	−0.09	0.0156	0.0748
hsa_miR_3135b	430.17	460.54	417.24	483.00	408.83	467.76	404.70	472.46	−0.09	0.0212	0.0748
hsa_miR_3138	62.94	66.48	61.35	69.57	58.39	66.75	61.50	72.66	−0.10	0.0106	0.0748
hsa_miR_3141	193.39	169.59	183.86	180.15	177.63	172.04	185.06	178.89	−0.09	0.0196	0.0748
hsa_miR_3150b_3p	1.77	1.72	2.50	1.58	2.38	1.49	2.63	1.09	0.12	0.0027	0.0748
hsa_miR_3195	759.99	899.09	722.37	914.86	681.46	900.11	708.87	942.07	−0.10	0.0088	0.0748
hsa_miR_320c	151.24	155.10	139.99	183.66	134.47	173.06	142.97	203.22	−0.12	0.0026	0.0748
hsa_miR_345_3p	51.06	47.48	48.74	51.42	46.23	47.27	49.63	51.55	−0.09	0.0175	0.0748
hsa_miR_3622b_3p	2.07	1.15	1.60	0.89	2.52	0.71	1.92	0.46	0.11	0.0057	0.0748
hsa_miR_3660	3.65	3.85	3.92	3.64	3.88	3.45	3.90	3.42	0.10	0.0111	0.0748
hsa_miR_3663_3p	127.73	136.18	122.73	144.56	118.88	142.37	120.45	149.26	−0.11	0.0038	0.0748
hsa_miR_3667_5p	20.38	20.81	19.63	23.34	18.59	22.91	19.58	25.88	−0.12	0.0023	0.0748
hsa_miR_3676_5p	3,259.89	3,130.99	2,810.34	2,948.81	2,963.05	3,084.49	2,760.10	2,921.68	−0.09	0.024	0.0748
hsa_miR_3945	37.95	42.59	37.48	45.93	35.96	44.61	37.22	48.01	−0.10	0.0117	0.0748
hsa_miR_3960	8,167.42	8,562.89	7,843.76	9,715.45	7,369.85	8,884.25	7,907.71	9,999.93	−0.09	0.0185	0.0748
hsa_miR_4270	394.51	389.95	378.71	417.22	361.86	397.35	377.73	425.26	−0.11	0.0057	0.0748
hsa_miR_4317	1.80	1.05	2.07	0.76	2.21	0.62	2.00	0.84	0.10	0.016	0.0748
hsa_miR_4425	10.32	11.70	10.92	11.70	10.77	11.82	11.06	11.43	0.09	0.0183	0.0748
hsa_miR_4429	23.18	22.00	21.56	23.20	20.28	22.05	22.23	23.42	−0.10	0.0099	0.0748
hsa_miR_4459	18,407.67	21,996.85	17,732.08	23,874.45	16,907.97	23,689.13	17,443.91	24,811.06	−0.10	0.0115	0.0748
hsa_miR_4476	61.75	64.72	59.36	67.13	57.54	66.43	60.05	69.97	−0.10	0.0102	0.0748
hsa_miR_4484	60.87	63.18	56.16	64.10	55.79	63.11	57.17	66.04	−0.09	0.0235	0.0748
hsa_miR_4508	45.27	54.18	42.90	54.98	40.93	53.04	42.33	55.39	−0.09	0.0235	0.0748
hsa_miR_4516	12,859.39	14,765.57	12,208.17	16,233.84	11,758.21	15,345.88	12,308.14	16,542.65	−0.11	0.0044	0.0748
hsa_miR_4534	186.77	207.00	179.24	219.34	169.13	209.22	182.00	231.13	−0.09	0.0213	0.0748
hsa_miR_4634	261.48	284.84	251.85	313.20	239.29	300.46	247.34	336.48	−0.12	0.0029	0.0748
hsa_miR_4655_5p	28.98	28.13	27.79	30.28	26.73	28.36	28.74	30.61	−0.09	0.0221	0.0748
hsa_miR_4689	109.63	101.83	104.67	110.46	100.56	103.75	106.31	112.06	−0.10	0.0107	0.0748
hsa_miR_4709_3p	1.12	1.28	1.57	1.01	1.40	1.10	1.50	1.05	0.09	0.0242	0.0748
hsa_miR_4739	756.23	852.13	735.58	934.44	703.38	876.68	725.94	916.12	−0.09	0.024	0.0748
hsa_miR_4767	25.36	27.24	24.33	27.67	23.66	27.15	23.14	27.98	−0.09	0.0197	0.0748
hsa_miR_4768_3p	2.87	3.64	3.25	3.37	3.43	3.31	3.47	3.56	0.10	0.014	0.0748
hsa_miR_4783_3p	11.69	13.14	11.56	13.93	10.87	13.27	11.44	14.63	−0.09	0.0246	0.0748
hsa_miR_4787_3p	87.24	100.63	84.74	101.87	77.10	96.98	81.83	105.01	−0.10	0.0158	0.0748

(*Continued*)

Table 6 (*Continued*)

miRNA	Low		Q2		Q3		High		β	*p*-value	*q*-value
	Tumor	Normal	Tumor	Normal	Tumor	Normal	Tumor	Normal			
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean			
hsa_miR_4792	21.93	22.99	21.38	22.56	20.25	22.61	21.27	23.81	–0.09	0.0193	0.0748
hsa_miR_4800_5p	189.78	151.55	177.22	156.88	177.34	154.11	177.96	158.70	–0.10	0.0125	0.0748
hsa_miR_483_5p	106.80	89.08	99.14	94.34	98.44	91.77	102.22	96.52	–0.09	0.0226	0.0748
hsa_miR_512_3p	9.99	11.50	9.59	10.68	9.67	10.89	10.15	10.60	0.10	0.0079	0.0748
hsa_miR_518c_5p	1.36	2.04	1.73	1.87	1.78	1.55	2.10	1.99	0.09	0.0177	0.0748
hsa_miR_5195_3p	138.45	142.76	131.18	149.95	125.68	143.20	131.49	150.95	–0.09	0.0178	0.0748
hsa_miR_525_5p	1.38	1.41	1.94	1.80	1.65	1.36	2.36	1.49	0.10	0.0102	0.0748
hsa_miR_5580_3p	10.61	11.28	10.80	10.84	10.79	11.08	10.84	10.85	0.11	0.0043	0.0748
hsa_miR_5585_3p	315.49	306.63	310.14	309.20	305.59	305.96	294.30	311.32	–0.09	0.0221	0.0748
hsa_miR_5703	207.72	265.79	207.86	312.71	185.76	305.53	200.05	363.43	–0.09	0.0243	0.0748
hsa_miR_5787	1,616.14	1,778.11	1,552.86	1,982.27	1,448.39	1,915.68	1,489.32	2,116.47	–0.12	0.0018	0.0748
hsa_miR_601	37.10	39.13	34.70	41.09	34.18	40.12	35.64	41.07	–0.09	0.0232	0.0748
hsa_miR_6085	580.90	584.83	559.94	631.04	546.52	603.35	563.01	638.08	–0.10	0.0088	0.0748
hsa_miR_6087	13,089.85	14,902.63	12,300.31	15,684.35	11,770.32	15,080.69	12,590.52	15,866.09	–0.09	0.0188	0.0748
hsa_miR_6088	2,478.37	2,649.04	2,371.03	2,878.27	2,188.64	2,626.39	2,373.45	2,823.12	–0.09	0.0244	0.0748
hsa_miR_6090	4,618.18	5,049.31	4,401.44	5,582.73	4,096.36	5,193.18	4,462.14	5,805.83	–0.11	0.0046	0.0748
hsa_miR_6124	448.21	420.35	424.87	454.58	406.85	435.30	438.91	474.68	–0.10	0.0138	0.0748
hsa_miR_630	291.06	381.14	289.74	458.60	261.19	438.12	275.15	510.63	–0.09	0.0175	0.0748
hsa_miR_642a_3p	3,285.09	3,486.19	3,239.54	3,762.13	3,040.61	3,625.40	3,190.24	3,892.74	–0.09	0.021	0.0748
hsa_miR_6500_5p	15.46	17.28	14.61	16.89	14.28	17.34	14.60	17.85	–0.10	0.0097	0.0748
hsa_miR_652_3p	1.45	0.78	1.45	0.88	2.24	0.67	1.72	0.62	0.09	0.0204	0.0748
hsa_miR_671_3p	0.71	1.07	0.77	0.86	0.92	0.98	0.99	0.99	0.09	0.0197	0.0748
hsa_miR_6722_3p	82.02	85.64	78.46	92.24	73.78	85.08	79.56	91.22	–0.09	0.0239	0.0748
hsa_miR_6723_5p	108.28	110.46	104.38	117.18	102.39	113.55	105.10	120.56	–0.11	0.0032	0.0748
hsa_miR_718	87.83	97.01	84.47	101.62	80.61	99.43	84.62	106.95	–0.11	0.0053	0.0748
hsa_miR_769_3p	15.91	18.80	15.66	19.78	14.77	18.84	15.64	20.19	–0.11	0.0064	0.0748
hsa_miR_940	611.90	774.01	603.08	766.65	592.77	773.30	559.65	809.99	–0.09	0.0204	0.0748

Abbreviations: miRNA, micro RNA; OBS, oxidative balance score; Q, quartile.

five of these factors altered miRNA expression level after adjusting for multiple comparisons (FDR q-value <0.1). These variables fell into two categories: 1) dietary carbohydrate, sucrose, and whole grains that could be operating through an insulin-related pathway and 2) NSAIDs and OBS that could be influencing miRNA expression level because of their role in inflammation and oxidative stress. Although others have suggested that diet and lifestyle factors could alter disease risk through their impact on miRNA expression,[13,30] we have been able to test this hypothesis broadly within a large population-based study with detailed diet and lifestyle data along with miRNA expression data.

In evaluating these results, there are several considerations, such as miRNA expression in this study is from colorectal carcinoma and normal colorectal mucosa, and miRNA expression could be different in other tissue types. Perhaps the largest limitation of the study relates to the interpretation of results. While we can show that factors such as carbohydrate intake are associated with miRNA expression after adjustment for multiple comparisons, it is much more difficult to determine the specific biological mechanism associated with alteration in miRNA expression. For instance, the 250 miRNAs differentially expressed by level of carbohydrate intake are associated with 7,152 unique validated target genes. It is difficult to determine the relative importance of the multitude of pathways associated with these genes that relate specifically with carbohydrate intake. However, we can say that some lifestyle factors do influence miRNA expression levels, giving credence to reports of these factors in disease processes. We have also compared our current findings to our previous findings from these data for miRNAs that were differentially expressed between carcinoma and normal colorectal mucosa.[23,31] We observed that 223 of the 250 miRNAs associated with carbohydrate intake, 175 of the 198 miRNAs associated with sucrose intake, 75 of the 99 miRNAs associated with whole grain intake, 121 of the 135 miRNAs associated with recent NSAID use, and 116 of the 137 miRNAs associated with OBS were also differentially expressed between carcinoma and normal colorectal mucosa, suggesting a role in tumor development.

Dietary factors have been cited as being important regulators of miRNAs.[9,30] Studies have primarily been done in mice and have focused on targeted miRNAs. Reported associations have been found between dietary folate and let-7a, miR-21, miR-23, miR-130, miR-190, miR-17-92, and miR-122 in liver samples and between retinoic acid and let-7a, miR-15a/miR-16-1, and miR-23 in acute promyelocytic leukemia.[9] Others have reported associations between miRNAs and polyphenols such as the antioxidant resveratrol with miR-663, miR-155, miR-21, miR-181b, and miR-30c2 in breast tissue cells.[32] We did not replicate these findings. In a review by Garcia-Segura et al,[30] carbohydrates were cited as being associated with miR-29c and miR-21. In our study, miR-21-3p was associated with carbohydrates. One controlled study using colorectal cells showed that starch consumption upregulated expression of the miR-17-92 cluster.[33] We did not see associations within this miR cluster. Sucrose was associated with dysregulated miRNAs in a similar manner that carbohydrate intake was associated with miRNA expression. It has been proposed that sucrose metabolism downregulates expression of miR-156[34] and that miR-398 and miR-408 are responsive to sucrose levels.[35] Again, we did not see associations between these miRNAs and dietary sucrose level in our data. Although there was overlap of nine miRNAs that differentially expressed by level of carbohydrate intake and by level of whole grain intake, the direction of the associations was different.

NSAIDs have been examined with miRNAs in a few studies. Celecoxib has been associated with miR-222 levels in breast tissue in mice,[36] and miR-271 has been associated with an NSAID and reactive oxygen species pathway.[11] Other studies have focused on COX-2 expression and miRNAs and have shown that miR-101a and miR-199a are associated with higher COX-2 expression and that miR-10b and miR-21 had a high influence on Cox-2 expression. None of these miRNAs were associated with recent NSAID use in our study population, although miR-199a was associated with ever using NSAIDs.

Inflammation and oxidative stress are key elements in the CRC carcinogenic process. We developed an OBS to account for dietary and lifestyle factors that could act together to influence CRC risk.[18] Oxidative stress also has been examined with miRNA expression in a limited number of studies.[10] miR-200a has been associated with oxidative stress in breast cells; miR-155 has been linked to inflammatory and oxidative stress pathways; miR-21, miR-125b, miR-196, and miR-210 have been linked to inflammatory cytokines and signaling pathways.[10] Others have cited miR-181a, miR-205, miR-1, miR-21, miR-24, miR-25, miR-185, miR-214, miR-133, miR-145, and miR-495 as being modulated by reactive oxygen species.[37] Of these, only miR-200a-5p was associated with OBS in our data.

While we have found associations between a limited number of diet and lifestyle factors and miRNA expression levels, we have failed to replicate other findings that have been cited in the literature. These differences could stem from several sources, the primary reason being our study is the only study conducted in humans, while others have relied on mouse models and cell lines and were usually conducted in noncolorectal tissue. Additionally, while others have targeted a few miRNAs, we have incorporated a platform of over 2,000 miRNAs. This methodological difference has resulted in our level of adjustment being considerable, while other studies have no or minimal adjustment for multiple comparisons. Our data are based on recall of diet and lifestyle factors from cases, mainly for a referent period of 2 years to diagnosis. While other referent periods may be important, more distant referent periods would represent a time less temporal to the time of the miRNA expression. We do however believe that our data are excellent, in that results obtained from this study in terms of risk are similar to several other large cohort studies. However, if there is bias toward the null in our recalled data, it could influence our ability to detect associations. Other factors such as potentially different effects by age of participant are possible. Although we adjusted for age to control for confounding, we did not conduct separate age-stratified analysis. Our sample size, although large, would be too small for detailed subgroup analysis. Likewise, we have used an Agilent platform and have previously compared platform results to those obtained from quantitative polymerase chain reaction. Our results were in 100% agreement in direction of association and fold changes calculated from data on the Agilent platform and that obtained from quantitative polymerase chain reaction.[31]

Conclusion

In summary, we have shown that carbohydrate intake, sucrose intake, NSAID use, and OBS are associated with miRNA expression level. Additionally, most of these miRNAs were differentially expressed between colorectal carcinoma and normal mucosa, suggesting a role in CRC. We believe that these findings lend support to the hypothesis that miRNAs are regulated by diet and lifestyle factors. It is possible that other diet and lifestyle factors could be important in control settings, which we were unable to detect at the population level. We urge other researchers to replicate these findings utilizing laboratory-based studies to better understand the

functional significance of these findings. These findings, if replicated, could provide further support for these diet and lifestyle factors in cancer prevention.

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Author contributions

MLS designed research; RW and MLS conducted research; JSH, LEM, and JRS analyzed data and performed statistical analysis; MLS wrote paper and had primary responsibility for the final content; all authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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