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Association of Cigarette Smoking and microRNA Expression in Rectal Cancer: Insight into Tumor Phenotype

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Abstract

Smoking is known to influence messenger RNA (mRNA) expression in colorectal cancer (CRC) cases. As microRNAs (miRNAs) are known repressors of mRNAs, we hypothesize that smoking may influence miRNA expression, thus altering mRNA expression. Our sample consisted of 1447 CRC cases that had normal colorectal mucosa and carcinoma miRNA data and lifestyle data. We examined current smoking, current versus never and former versus never (C/F/N) smoking[1], and pack-years smoked with miRNA expression in normal mucosa as well as differential miRNA expression between paired normal and carcinoma tissue for colon and rectal tissue to determine associations between smoking and miRNA expression. We adjusted for multiple comparisons using the Benjamini Hochberg false discovery rate (FDR).

Significant associations were seen for rectal differential miRNA expression only. We analyzed miRNAs significantly associated with smoking with CIMP and MSI status, using a polytomous logistic regression. Two hundred and thirty-one miRNAs were differentially expressed with current smoking, 172 with C/F/N, and 206 with pack-years smoked; 111 were associated with all three. Forty-three miRNAs were unique to current smoking, 14 were unique to C/F/N and 57 were

[1]C/F/N smoking: Differential miRNA expression in subjects who were current smokers at time of diagnosis compared to subjects who never smoked, and differential miRNA expression in former smokers compared to those who had never smoked.

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Ethics and Consent to Participate

All participants signed an informed consent and this study was approved by the Institutional Review Board at the University of Utah; the committee numbers for this paper are IRB_00055877 and IRB_00002335.

Conflicts of Interest

Conflicts of interest: none.

Lila Mullany wrote the manuscript. Martha Slattery obtained funding and the data used in the manuscript and assisted in writing the manuscript. Roger Wolff oversaw microRNA assays. Jennifer Herrick analyzed the data. John Stevens assisted in determining appropriate statistical methodologies. All authors reviewed and provided input to the manuscript. All authors have read and approved the final version of the manuscript.

unique to pack years smoked. Of the 306 unique miRNAs associated with cigarette smoking, 41 were inversely associated and 200 were directly associated with CIMP high or MSI tumor molecular phenotype for either colon or rectal cancer. Our results suggest that cigarette smoking can alter miRNA expression and, given associations with CIMP high and MSI tumor molecular phenotype, it is possible that smoking influences tumor phenotype through altered miRNA expression.

Keywords

microRNAs; smoking; rectal; neoplasms; life style; CIMP; MSI

1. Introduction

Long-term and current cigarette smoking has been shown to increase risk of developing and dying from many cancers, including CRC (1–3). We have previously shown that certain mRNAs are differentially expressed in current smokers as compared to people who have never smoked (4). MiRNAs are small (~22 nucleotides), endogenously expressed, non-coding RNA molecules that bind to the 3' untranslated region of the mRNAs to alter mRNA translation into protein (5). Typically, miRNAs cause mRNA degradation or block translation (6), however there is some evidence that in certain environments miRNAs may facilitate mRNA translation (7). MiRNAs have been shown to be differentially expressed in carcinoma tissue as compared to normal tissue (8–10), and, as miRNAs regulate many different target genes (6, 11), they have been implicated as key effector molecules in the carcinogenesis process (12). MiRNAs influence the translation of mRNAs, which we have seen are impacted by current cigarette use; it is possible, then, that smoking alters miRNA expression and contributes to the previously observed altered mRNA levels.

In this study we evaluate whether current smoking and pack-years of cigarettes smoked influence miRNA expression in CRC in both normal colon and rectal mucosa and differential (paired carcinoma tissue compared to normal colon and rectal mucosa) expression. We hypothesize that smoking will influence miRNA levels, with greater or temporal exposure having a larger influence on differential expression.

2. Materials and methods

2.1 Study population

Data come from participants in the population-based Diet, Activity, and Lifestyle study that were recruited from Utah or the Kaiser Permanente Medical Care Program of Northern California (KPMCP). Colon cancer cases were identified as having a primary adenocarcinoma diagnosed between 1 October 1991 and 30 September 1994, while rectal cancer cases were diagnosed between May 1997 and May 2001. Eligible cases were between 30 and 79 years of age at diagnosis, currently living in the study area, spoke English and were able to complete an interview, and had no prior history of CRC, Crohn's disease, ulcerative colitis, or known familial adenomatous polyposis. This study was

approved by the Institutional Review Board at the University of Utah; all participants signed an informed consent form.

2.2 Smoking Data

Data were collected by trained and certified interviewers using laptop computers. All interviews were audio-taped as previously described and reviewed for quality control purposes (13). The referent period for the study was two years prior to diagnosis for cases. As part of the study questionnaire, information was collected on cigarette smoking history. Anyone who reported having smoked at least 100 cigarettes in their lifetime was considered to have been a cigarette smoker. Regular smokers were then defined as anyone having smoked at least one cigarette a day for six months or longer. Data also were collected for start and stop dates for smoking in order to calculate pack-years smoked. We collected data on number of cigarettes smoked per day, and used amount smoked along with years smoked to calculate the pack-years. The pack-years variable was defined as the number of years a subject smoked a pack or 20 cigarettes a day. For rectal cancer subjects we also analyzed miRNA expression with exposure to smoke of others in and outside of the house in non-smokers only.

2.3 MiRNA Processing

RNA was extracted from formalin-fixed paraffin embedded tissues and processed as previously described (14). 100 ng total RNA was labeled with Cy3 and hybridized to Agilent Human miRNA Microarrays V19.0 and were scanned on an Agilent SureScan microarray scanner model G2600D using Agilent Feature Extract software v.11.5.1.1. Data were required to pass stringent 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. Samples that failed to meet QC standards were repeated, and 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. The Agilent platform was found to be highly reliable (r=0.98), and to have reasonable agreement with NanoString (15) as well as excellent agreement with qRT-PCR (8). For unpaired samples due to missing normal scans, we imputed values whenever possible for normal mucosa as previously described (16). In order to minimize differences 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 which was the median of the 75^{th} percentiles of all the samples divided by the 75^{th} percentile of each individual sample (17). This scaling factor was implemented using SAS 9.4.

2.4 Statistical Analysis

Our sample consisted of 1447 carcinoma tissue and paired normal colorectal mucosa from subjects for whom smoking data were available. In this analysis, we included only the miRNAs in which at least 20% of the samples had some level of detectable expression in the tissue(s) of interest. We stratified the data by tumor site, i.e. colon vs. rectal cancer. The number of miRNAs analyzed varied from 766 to 817 depending on tissue and tumor site. We examined four smoking variables (ever smoked, smoking status (current/former/never),

average number of cigarettes smoked per day, and pack-years smoked for rectal cancer only, since years starting and stopped was not collected for colon cancer cases) to determine if there was an association between smoking cigarettes and miRNA expression. We examined associations in both normal colon and rectal mucosa and the difference in expression between paired carcinoma tissue and normal colon and rectal mucosa. This was done by fitting a linear model to the log base 2 transformed miRNA expression levels and adjusting for age at diagnosis, study center and sex. 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 (18) using the boot package in R. Associations were considered significant if the Benjamini Hochberg (FDR) adjusted p-values were less than 0.05 (19). We standardized the slopes by transforming the miRNAs and continuous smoking variables to standard normal in order to better compare the results across the miRNA and cigarette smoking variables.

We analyzed any miRNAs that were significantly differentially expressed between carcinoma and normal mucosa for any smoking variable with CIMP and MSI tumor molecular phenotype in rectal and colon cancer subjects. Using a polytomous logistic regression we assessed associations for CIMP High/MSS, CIMP Low/MSI, and CIMP High/MSI relative to CIMP Low/MSS. For this analysis we report odds ratios and 95 percent confidence intervals.

3. Results

The study population consisted of 892 colon cancer cases and 555 rectal cancer cases; approximately 54% of cases were male and 46% were female for colon cases, and 57% of cases were male and 43% were female for rectal cases (Table 1). Approximately 11% of rectal cases and 14% of colon cases had a family history of CRC. About 55% of rectal cases and 58% of colon cases had ever smoked, with approximately 16% of rectal cases and 14% of colon cases smoking at the time of diagnosis. The average age at diagnosis was approximately 65 years old for colon subjects and 62 years old for rectal subjects. The average years smoked was 17.5 for colon cases and 14.8 for rectal cases; the average pack-years smoked for rectal cases was 16.1 pack-years.

We saw significant findings for differential miRNA expression, between carcinoma and normal colonic mucosa tissue, for rectal cancer only. We saw no significant findings for exposure to smoke of others in non-smoking rectal cancer cases. After adjustment for multiple comparisons, 231 miRNAs had significantly different differential expression (carcinoma tissue compared to normal colon and rectal mucosa) in current smokers as compared to cases that never smoked or were a former smoker. Thirty-six of these miRNAs were downregulated with current smoking and 195 were upregulated. One hundred and seventy-two miRNAs were associated significantly with C/F/N smoking; four of these miRNAs changed direction from never to former and from never to current, while 168 had differential expression increase or decrease in the same direction progressively from never to former to current. Of the 168 that had progressive changes in expression, 138 were upregulated and 30 were downregulated in current versus former or never smokers. Two hundred and six miRNAs were associated with pack-years smoked; of these, 37 were

downregulated and 169 were upregulated with more pack-years smoked. Forty-three miRNAs were unique to current smokers (Table 2), 14 were unique to C/F/N smoking (Table 3), and 57 were unique to pack-years smoked (Table 4).

One hundred and eleven miRNAs were associated with all three smoking variables (Table 5). Of these, 110 the same direction of association for every variable; one miRNA, hsa-miR-432-5p, had a positive change from never to former but negative changes in current smoking, never to current smoking, and pack-years smoked. Of the 110 with the same direction of association, 11 were downregulated with current smoking, never to former as well as never to current smoking, and with pack-years smoked; 99 miRNAs were upregulated with these variables.

Associations between the miRNAs associated with cigarette smoking the tumor molecular phenotype are summarized in Table 6 (Supplemental Table 1 has complete data for rectal cancer and Supplemental Table 2 has complete data for colon cancer). Differential expression of 39 miRNAs was directly associated, and of five miRNAs was inversely associated, with CIMP Low/MSI tumors in both colon and rectal cancer subjects (Table 6), however the magnitude of the associations, although less precise for rectal cancer, were much stronger. Nine miRNAs in rectal subjects and 11 in colon had differential expression that was inversely associated with CIMP Low/MSI, while 28 miRNAs in rectal and 79 in colon cancer subjects were differentially expressed in a direct relationship to CIMP Low/MSI. Few miRNAs associated with smoking were significantly associated with a CIMP High/MSS tumor. The strongest associations with the most miRNAs associated with cigarette smoking were observed when the tumor phenotype was CIMP High/MSI. Current smoking versus never smoked was significantly associated with CIMP High/MSI, OR = 2.42, 95% CI 1.16 – 5.03. Twelve miRNAs were differentially expressed in an inverse manner with CIMP High/MSI in both colon and rectal cancer cases, and 135 were differentially expressed in a direct manner for both tumor sites. Additionally, five miRNAs were inversely associated with CIMP High/MSI rectal tumors and of 54 miRNAs were directly associated with CIMP High/MSI rectal tumors

4. Discussion

In this study, we investigated whether cigarette smoking influences miRNA expression in CRC subjects. Two hundred and thirty-one miRNAs were associated with current smoking, 172 with C/F/N smoking, and 206 with pack-years smoked. One hundred and ninety-two miRNAs were associated with two or more smoking criteria, while 111 were associated with all three variables and 114 were unique to only one. Together, these results suggest that a lifestyle factor, such as cigarette smoking, is indeed able to alter endogenous levels of miRNAs in rectal cancer subjects. Additionally, while some miRNAs are altered with smoking in general, different amounts and type of exposure also results in different dysregulated miRNAs.

Previously, we identified miRNAs whose differential expression between carcinoma tissue and normal colorectal mucosa were associated significantly with survival after being diagnosed with rectal cancer (20). In that study, hazard ratios and 95% confidence intervals

were adjusted for age, sex, AJCC stage, and MSI tumor status. Of the 43 miRNAs associated with only current smoking, differential expression of 11 miRNAs had previously been identified with altered survival after diagnosis with rectal cancer. One of these miRNAs (hsa-miR-196b-5p) had improved survival in rectal cases when this miRNA was upregulated in rectal carcinoma tissue relative to normal mucosa, and this miRNA had decreased differential (carcinoma minus normal mucosa) expression in current smokers. Ten miRNAs (hsa-miR-1914-3p, hsa-miR-4327, hsa-miR-4470, hsa-miR-4665-3p, hsa-miR-4673, hsa-miR-548q, hsa-miR-550b-2-5p, hsa-miR-6074, hsa-miR-6165, and hsa-miR-939-5p) were upregulated in tumors of rectal cases of current smokers and the differential expression of these miRNAs was previously shown to be associated with worse survival when expression in carcinoma tissue increased. Five of the 14 miRNAs associated significantly with only C/F/N smoking were previously identified as being differentially expressed between carcinoma and normal mucosa and this change in expression was also shown to alter survival of rectal cancer cases. Three of these miRNAs (hsa-miR-17-5p, hsa-miR-29a-3p, and hsa-miR-425-5p) improved survival, and were downregulated in tumors of current and former smokers compared to those who never smoked. Two of the miRNAs (hsa-miR-3150b-5p and hsa-miR-6084) increased risk of death from colorectal cancer in cases diagnosed with rectal cancer when expression increased in carcinoma tissue; both miRNAs had higher expression in carcinoma tissue in both former and current smokers compared to carcinoma tissue of cases who never smoked. Twelve of the fifty-seven of the miRNAs associated with only pack-years smoked were associated with differential expression between carcinoma and normal mucosa and with colorectal cancer survival in cases diagnosed with rectal cancer. Four of these miRNAs (hsa-miR-196a-5p, hsa-miR-429, hsa-miR-508-5p, and hsa-miR-93-5p) reduced the risk of death from colorectal cancer after being diagnosed with rectal cancer when expression increased in carcinoma tissue, and all but one of these (hsa-miR-508-5p) was downregulated in tumors in cases who smoked more pack-years. Eight of the miRNAs (hsa-miR-139-3p, hsa-miR-3667-5p, hsa-miR-425-3p, hsa-miR-4298, hsa-miR-4429, hsa-miR-4481, hsa-miR-4685-5p, and hsa-miR-4783-3p) increased risk of colorectal cancer death among rectal cancer cases with expression increases in carcinoma tissue, and these miRNAs had higher expression in carcinoma tissue with subjects who smoked more pack-years. Of the 111 miRNAs associated with all three smoking variables, 69 were previously identified as being associated with rectal survival. Four miRNAs (hsa-miR-106b-5p, hsa-miR-19b-3p, hsa-miR-20b-5p, and hsa-miR-432-5p) were seen to improve survival and these were generally downregulated in all smoking variables. Of the 69 previously associated with CRC survival in cases diagnosed with rectal cancer, 65 were seen to worsen survival and these were upregulated with every smoking variable. These data suggest that smoking alters miRNA levels in a manner conducive to poorer outcomes.

Current smoking was associated with the largest number of dysregulated miRNAs, with 231 miRNAs whose differential expression was significantly associated with current versus not current smoking; pack-years smoked was associated significantly with 206 miRNAs, and had the highest number of unique miRNAs that were differentially expressed (N=57). These data show that, not only are many miRNAs generally dysregulated with any smoking, there are specific miRNAs that represent current as well as amount and duration (embodied by

pack-years smoked) of smoking. Additionally, differential expression of these unique miRNAs has been shown to be associated significantly with altered survival in rectal cancer subjects. These data support the hypothesis that current smoking does indeed influence a larger number of miRNAs, however it also supports the notion that cigarette smoking in a large amount, regardless of contemporariness to diagnosis, is able to influence miRNA expression in a unique manner and possibly alter survival of rectal cancer in a negative manner.

Previously, we identified mRNAs that were differentially expressed in colon tissue of current smokers versus people who never smoked (4). In order to see if miRNAs associated with smoking could be influencing mRNA expression, we found the target genes for the five miRNAs that were unique to C/F/N smoking whose differential expression was previously seen to be associated with rectal cancer survival, as these miRNAs are more likely to be influencing mRNA expression. Three mRNAs from this list were previously shown to be dysregulated in current smokers: *FGA* (fold change = 28.01), *RBM20* (fold change = 2.99), and *YY2* (fold change = 3.34) were all upregulated in current smokers (4). Hsa-miR-17-5p is known to regulate *RBM20* and hsa-miR-29a-3p is known to regulate *FGA* and *YY2*; both of these miRNAs have decreasing expression in carcinoma tissue and increasing expression in normal colonic mucosa in current smokers compared to people who never smoked ($Beta_{C\ vs.\ N} = -0.3429$ for hsa-miR-17-5p; $Beta_{C\ vs.\ N} = -0.3469$ for hsa-miR-29a-3p). This could explain the increased expression of these target genes. Our past study on mRNA used differential expression in colon tissue. However, the miRNAs that are differentially expressed in regards to smoking are differentially expressed in rectal tissue. This may account for incomplete overlapping between target genes of miRNA and our previous report of smoking and mRNA expression. It is likely that multiple miRNAs regulate these target genes, and while the observed miRNA-mRNA expression direction is in concordance with the hypothesis that the altered miRNA expression contributes to the mRNA differential expression, it is not the only possible interaction.

We analyzed all miRNAs significantly differentially expressed across any of the smoking variables for associations with CIMP and MSI tumor phenotype in both colon and rectal tissues. We have previously shown that cigarette smoking is more likely to be associated with MSI and CIMP High tumors in colon cancer cases (21–23). Overall, of the 306 miRNAs associated with cigarette smoking, 41 were inversely associated with either CIMP High or MSI tumor phenotype and 200 were directly associated with CIMP High or MSI tumor molecular phenotype for either colon or rectal cancer, with directionality consistent across tissue types. Associations with these miRNAs were generally stronger for rectal tumors, however given the few MSI rectal cases, the estimates were much less precise than those observed for colon cancer. These results suggest that smoking may alter miRNAs, whose differential expression may influence CIMP and MSI tumor phenotype.

Ten miRNAs were differentially expressed in rectal tissue across current smokers versus those who had never smoked as well as with CIMP High/MSI tumor phenotype. Five of these, hsa-miR-29a-3p, hsa-miR-29b-3p, hsa-miR-30b-5p, hsa-miR-3150b-5p, and hsa-miR-425-5p, were associated with both current versus never smoking as well as CIMP High/MSI phenotype for both rectal and colon differential tissue, while hsa-miR-17-5p, hsa-

miR-200a-3p, hsa-miR-4485 were associated only in colon tissue, and hsa-miR-141-3p and hsa-miR-6084 were associated only in rectal tissue. Seven of ten miRNAs, hsa-miR-29a-3p, hsa-miR-29b-3p, hsa-miR-30b-5p, hsa-miR-425-5p, hsa-miR-17-5p, hsa-miR-200a-3p, and hsa-miR-141-3p had decreased differential expression in current smokers as compared to never smokers, and this differential expression was indirectly associated with CIMP High/MSI status. Two miRNAs, hsa-miR-3150b-5p and hsa-miR-6084, had increased differential expression in current smokers compared to never smokers, and this increase in expression was directly associated with CIMP High/MSI. One miRNA, hsa-miR-4485, had increased differential expressed in current smokers with colon cancer, and this was indirectly associated with CIMP High/MSI. Current smoking compared to never smoked showed a significant association with CIMP High/MSI tumor phenotype in colon cancer cases, OR = 2.42, 95% CI 1.16 – 5.03. As nine of the 10 miRNAs associated with both current versus never smoking and CIMP High/MSI tumor phenotype had agreement in the direction of their associations, i.e. increased differential expression with current versus never smoking as well as a direct relationship with CIMP High/MSI or decreased differential expression and an inverse relationship, our data suggests that current versus never smoking alters miRNA expression, and this alteration influences the development of a CIMP High/MSI tumor.

The study has several strengths including our large sample size. This enabled us to look at colon and rectal samples separately. Since we only found miRNAs significantly differentially expressed with smoking among rectal cancer cases, we may not have found significant results if we had combined colon and rectal cancers or examined only colon cancer cases. We also consider our platform as an asset in this study. As previously stated, our platform has very high reliability and good concordance with other methods (8, 15). The large microarray platform enables a discovery approach, which is needed since previous studies have not examined miRNA expression and cigarette smoking in colorectal tissue. Another strength is the range of data available for these analyses. These data enabled us to investigate a variety of smoking variables, which was essential to this study, as we have shown that smoking temporality, duration, and amount have different influences on miRNA expression. Additionally, we have been able to examine not only how smoking is associated with miRNA expression but also how miRNAs associated with cigarette smoking are also associated with tumor molecular phenotypes that have been associated with cigarette smoking.

A limitation in this study is that, given the large numbers of miRNAs, and the overlap in genes targeted by the different miRNAs, it is very difficult to discern distinct biological pathways altered by specific miRNAs associated with smoking. This limitation is not unique to our study, but a limitation of the field in general. Since each miRNA can regulate thousands of genes, it is unknown which of the many pathways and genes targeted are most important for colorectal cancer. Additionally, most miRNA-functionality tools available are not tissue specific, and this is likely a detriment to performing functional analysis based on miRNAs only identified as being associated with cigarette smoking in rectal tissue. However, while we cannot identify the exact functionality these miRNAs have, we are able to see that these miRNAs have been associated with CRC prognosis, and this supports the assertion that smoking can have a functional impact in rectal cancer, possibly through regulation of miRNA expression. Our rectal cancer sample size was not large enough to have

sufficient power to detect significant associations between smoking and CIMP/MSI tumor phenotype given the rare nature of MSI rectal tumors. We encourage others to replicate this analysis with larger samples.

5. Conclusions

Overall, our findings suggest that smoking is able to alter miRNA expression in rectal carcinoma tissue, and they lend support to the hypothesis that this alteration is responsible for worsening survival for rectal cancer cases and may influence CIMP and MSI tumor phenotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- As both smoking and miRNA expression have been associated with cancer risk, we propose that current smoking and pack-years smoked alter microRNA expression in colorectal cancer.
- We found that differential expression of numerous unique miRNAs are associated with current smoking or pack-years smoked, and various others were associated with all smoking variables, in rectal cancer cases.
- We found that cigarette smoking was associated significantly with CIMP High/MSI tumor phenotype, and that this phenotype was associated with the differential expression of many miRNAs in both colon and rectal cancer cases.
- Our findings support the hypothesis that smoking, as a lifestyle variable, is able to alter microRNA expression in rectal cancer and that this may in turn contribute to tumor molecular phenotype.

Table 1

Descriptive Table.

		Colon		Rectal	
		N/Mean	**(%/SD)**	**N/Mean**	**(%/SD)**
Age at diagnosis		64.7	(9.5)	61.8	(10.8)
Sex	*Male*	485	(54.4)	316	(56.9)
	Female	407	(45.6)	239	(43.1)
Center	*Kaiser*	626	(70.2)	340	(61.3)
	Utah	266	(29.8)	215	(38.7)
Family history of colorectal cancer	*No*	770	(86.3)	493	(89.0)
	Yes	122	(13.7)	61	(11.0)
Ever Smoked	*No*	373	(41.9)	252	(45.4)
	Yes	517	(58.1)	303	(54.6)
Smoking Status	*Never*	373	(41.9)	264	(47.6)
	Former	390	(43.8)	200	(36.0)
	Current	127	(14.3)	91	(16.4)
Average # of cigarettes/day	*None*	373	(42.0)	264	(47.7)
	1–10	142	(16.0)	74	(13.4)
	11–20	215	(24.2)	146	(26.4)
	>20	159	(17.9)	70	(12.6)
Years smoked		17.5	(18.3)	14.8	(17.8)
Pack-years smoked				16.1	(23.4)

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Table 2

MiRNAs differentially expressed between carcinoma tissue and normal rectal mucosa and associated with only current smoking.

	Never & Former		Current				
	Mean miRNA Expression		Mean miRNA Expression			P-values	
miRNA	Carcinoma Tissue	Normal Mucosa	Carcinoma Tissue	Normal Mucosa	Beta[1]	Raw	FDR
hsa-miR-1208	26.48	30.79	28.12	30.02	0.3125	0.0077	0.0328
hsa-miR-1224-5p	692.89	820.71	756.13	800.96	0.3061	0.0076	0.0328
hsa-miR-1236-5p	127.80	136.98	140.37	134.74	0.2980	0.0084	0.0344
hsa-miR-1276	2.32	2.00	1.71	2.20	−0.3268	0.0057	0.0280
hsa-miR-1914-3p[2]	106.17	121.11	117.74	121.75	0.3159	0.0066	0.0301
hsa-miR-196b-5p[2]	14.93	2.31	13.55	2.43	−0.2829	0.0135	0.0456
hsa-miR-197-5p	2553.58	2891.42	2837.27	2837.46	0.2992	0.0075	0.0328
hsa-miR-199b-5p	3.83	0.96	2.70	1.30	−0.3156	0.0053	0.0277
hsa-miR-2276	89.25	86.67	96.83	83.14	0.3298	0.0035	0.0230
hsa-miR-3174	12.41	10.63	11.76	11.19	−0.2838	0.0149	0.0495
hsa-miR-3187-5p	3.84	2.27	3.10	2.52	−0.3043	0.0072	0.0321
hsa-miR-3189-5p	5.19	5.12	4.40	5.45	−0.3166	0.0066	0.0301
hsa-miR-320e	34.84	36.31	40.31	37.27	0.2885	0.0126	0.0441
hsa-miR-3676-5p	2912.88	3019.95	3453.19	3181.99	0.3046	0.0076	0.0328
hsa-miR-423-3p	15.79	19.47	17.74	20.02	0.2829	0.0146	0.0489
hsa-miR-4257	267.62	316.63	302.40	318.08	0.3193	0.0053	0.0277
hsa-miR-4311	2.99	2.06	2.22	2.21	−0.3104	0.0067	0.0304
hsa-miR-4313	22.87	27.84	26.76	28.83	0.2910	0.0113	0.0403
hsa-miR-4322	88.99	88.90	97.53	88.46	0.2852	0.0130	0.0451
hsa-miR-4327[2]	201.55	202.77	223.68	198.38	0.3095	0.0057	0.0280
hsa-miR-4450	4.15	3.17	3.43	3.49	−0.3095	0.0065	0.0301
hsa-miR-4470[2]	31.28	37.96	34.27	37.99	0.2977	0.0098	0.0370
hsa-miR-4487	94.43	106.26	101.39	103.66	0.3258	0.0049	0.0263
hsa-miR-4515	280.18	336.05	308.11	333.67	0.3322	0.0041	0.0240

	Never & Former		Current				
	Mean miRNA Expression		Mean miRNA Expression			P-values	
miRNA	**Carcinoma Tissue**	**Normal Mucosa**	**Carcinoma Tissue**	**Normal Mucosa**	**Beta[1]**	**Raw**	**FDR**
hsa-miR-4665-3p[2]	87.69	110.91	102.16	113.04	0.3050	0.0094	0.0364
hsa-miR-4673[2]	66.94	81.77	74.74	81.75	0.3028	0.0098	0.0370
hsa-miR-4676-5p	2.51	1.80	1.60	2.05	−0.3245	0.0045	0.0247
hsa-miR-4695-5p	299.23	347.39	324.06	341.22	0.2873	0.0131	0.0452
hsa-miR-4746-3p	370.75	429.01	409.40	428.00	0.3224	0.0055	0.0280
hsa-miR-5006-5p	544.48	647.79	581.01	634.52	0.2835	0.0132	0.0452
hsa-miR-5189	26.37	28.82	28.72	28.62	0.2977	0.0091	0.0354
hsa-miR-548q[2]	50.06	69.60	57.11	67.71	0.3043	0.0071	0.0320
hsa-miR-550b-2-5p[2]	26.33	30.43	28.98	30.15	0.2897	0.0119	0.0419
hsa-miR-6074[2]	14.42	14.68	15.16	14.07	0.3116	0.0085	0.0344
hsa-miR-6134	3.12	2.73	2.89	3.19	−0.3222	0.0056	0.0280
hsa-miR-6165[2]	299.51	275.50	331.99	272.61	0.3083	0.0079	0.0331
hsa-miR-642a-3p	3129.76	3671.40	3499.61	3525.75	0.2994	0.0087	0.0344
hsa-miR-6510-5p	237.90	255.86	271.61	250.33	0.2993	0.0099	0.0370
hsa-miR-6511b-5p	50.18	53.48	55.37	53.09	0.2901	0.0141	0.0474
hsa-miR-652-5p	64.67	72.15	69.38	69.04	0.3105	0.0059	0.0285
hsa-miR-934	5.21	0.70	4.43	0.86	−0.2899	0.0111	0.0398
hsa-miR-936	44.86	48.55	48.41	48.26	0.3020	0.0075	0.0328
hsa-miR-939-5p[2]	467.20	552.48	529.55	547.50	0.3040	0.0079	0.0331

[1]Adjusted for age at diagnosis, study center, and sex.

[2]These miRNAs have been previously associated with differential expression and survival

Table 3

MiRNAs differentially expressed between carcinoma tissue and normal rectal mucosa and associated with only C/F/N smoking.

	Never		Former			Current				
	Mean miRNA Expression		Mean miRNA Expression			Mean miRNA Expression			P-values	
miRNA	Carcinoma Tissue	Normal Mucosa	Carcinoma Tissue	Normal Mucosa	Beta[1]	Carcinoma Tissue	Normal Mucosa	Beta[1]	Raw	FDR
hsa-miR-141-3p	33.88	22.13	29.52	23.59	−0.2704	32.73	24.52	−0.2624	0.0084	0.0438
hsa-miR-16-5p	77.93	58.03	67.77	55.89	−0.2250	65.75	58.32	−0.3325	0.0079	0.0427
hsa-miR-17-5p[2]	58.74	12.85	49.85	13.12	−0.1944	47.11	13.85	−0.3429	0.0098	0.0461
hsa-miR-200a-3p	25.70	17.69	21.28	18.25	−0.3084	23.06	19.24	−0.3464	0.0017	0.0271
hsa-miR-21-5p	404.36	117.53	360.56	120.50	−0.2770	374.43	128.14	−0.3106	0.0049	0.0383
hsa-miR-29a-3p[2]	103.95	39.54	87.04	39.29	−0.2676	90.14	40.54	−0.3469	0.0019	0.0271
hsa-miR-29b-3p	20.28	6.72	17.14	7.26	−0.3570	17.01	7.09	−0.3591	<.0001	0.0256
hsa-miR-30b-5p	21.97	20.00	18.53	19.26	−0.1801	21.11	20.73	−0.3485	0.0107	0.0484
hsa-miR-3150b-5p[2]	11.39	16.30	12.30	15.89	0.2478	14.18	15.96	0.3330	0.0069	0.0411
hsa-miR-3620-3p	7.04	8.66	7.35	8.06	0.3440	7.52	7.90	0.2400	0.0016	0.0271
hsa-miR-425-5p[2]	11.85	5.52	9.95	5.80	−0.3016	10.45	5.92	−0.2500	0.0054	0.0394
hsa-miR-4485	1074.42	1298.17	1012.29	1335.83	−0.2928	1127.54	1347.92	0.0495	0.0043	0.0362
hsa-miR-509-3-5p	5.58	6.67	5.96	6.40	0.2902	6.00	6.36	0.2117	0.0099	0.0463
hsa-miR-6084[2]	13.00	14.82	14.26	15.10	0.2296	15.58	14.63	0.3358	0.0069	0.0411

[1] Adjusted for age at diagnosis, study center, and sex.

[2] These miRNAs have been previously associated with differential expression and survival.

Table 4

MiRNAs differentially expressed between carcinoma tissue and normal rectal mucosa and associated with only pack-years smoked.

	Mean miRNA Expression			P-values	
miRNA	**Carcinoma Tissue**	**Normal Mucosa**	**Beta[1]**	**Raw**	**FDR**
hsa-miR-1273g-5p	8.35	8.60	−0.0418	0.0130	0.0491
hsa-miR-127-3p	2.27	0.85	−0.0744	0.0080	0.0386
hsa-miR-1290	147.04	117.28	0.0578	0.0029	0.0269
hsa-miR-139-3p[2]	7.94	10.64	0.1110	0.0124	0.0475
hsa-miR-146b-5p	3.35	1.27	−0.1280	0.0041	0.0299
hsa-miR-192-3p	1.98	2.54	−0.1243	0.0054	0.0351
hsa-miR-196a-5p[2]	6.29	2.68	−0.1548	0.0004	0.0240
hsa-miR-203a	12.40	3.42	−0.1715	0.0002	0.0240
hsa-miR-2277-3p	3.99	4.62	0.0277	0.0004	0.0240
hsa-miR-28-5p	1.36	1.69	−0.0737	0.0061	0.0363
hsa-miR-3147	31.02	29.77	0.1183	0.0082	0.0388
hsa-miR-320b	76.00	75.48	0.0998	0.0087	0.0397
hsa-miR-320d	41.27	41.01	0.0944	0.0090	0.0406
hsa-miR-330-3p	3.27	5.68	−0.1205	0.0060	0.0359
hsa-miR-339-3p	3.41	3.16	−0.1126	0.0103	0.0429
hsa-miR-3648	302.77	265.50	0.1188	0.0073	0.0381
hsa-miR-3667-5p[2]	19.58	22.73	0.1104	0.0133	0.0495
hsa-miR-3677-3p	4.49	2.39	−0.1125	0.0116	0.0463
hsa-miR-3911	84.61	75.53	0.1290	0.0034	0.0269
hsa-miR-3934-5p	41.73	42.63	0.1307	0.0033	0.0269
hsa-miR-425-3p[2]	12.37	15.09	0.0846	0.0090	0.0406
hsa-miR-4259	17.67	15.88	0.0795	0.0036	0.0273
hsa-miR-429[2]	11.43	5.11	−0.1131	0.0098	0.0425
hsa-miR-4298[2]	164.14	149.05	0.1330	0.0036	0.0273

	Mean miRNA Expression			P-values	
miRNA	Carcinoma Tissue	Normal Mucosa	Beta[1]	Raw	FDR
hsa-miR-4419a	62.35	59.78	0.1238	0.0049	0.0342
hsa-miR-4419b	23.99	25.29	0.1165	0.0074	0.0381
hsa-miR-4421	1.62	1.90	−0.1106	0.0122	0.0475
hsa-miR-4429[2]	21.85	22.49	0.1257	0.0048	0.0341
hsa-miR-4436b-3p	7.98	6.42	−0.1067	0.0126	0.0478
hsa-miR-4481[2]	64.90	69.70	0.1377	0.0022	0.0266
hsa-miR-4513	25.75	25.91	0.1300	0.0034	0.0269
hsa-miR-4640-5p	27.75	25.32	0.1151	0.0097	0.0425
hsa-miR-4667-5p	56.42	56.33	0.1110	0.0106	0.0437
hsa-miR-4685-5p[2]	19.37	20.14	0.1089	0.0124	0.0475
hsa-miR-4698	33.40	34.05	0.1324	0.0031	0.0269
hsa-miR-4710	36.12	34.94	0.1188	0.0074	0.0381
hsa-miR-4743-5p	54.09	53.32	0.1098	0.0126	0.0478
hsa-miR-4758-5p	106.67	105.94	0.1197	0.0075	0.0381
hsa-miR-4778-5p	68.39	52.84	0.1307	0.0034	0.0269
hsa-miR-4783-3p[2]	11.39	13.57	0.1374	0.0024	0.0266
hsa-miR-4800-5p	181.69	154.52	0.1448	0.0014	0.0240
hsa-miR-483-5p	102.04	92.12	0.1398	0.0017	0.0246
hsa-miR-487b	1.78	2.05	−0.1290	0.0031	0.0269
hsa-miR-508-5p[2]	2.99	3.42	0.0278	0.0062	0.0363
hsa-miR-514b-5p	29.50	28.13	0.1212	0.0060	0.0359
hsa-miR-5187-5p	1.95	2.19	−0.1196	0.0074	0.0381
hsa-miR-5190	26.14	26.81	0.1147	0.0087	0.0397
hsa-miR-532-3p	2.56	1.34	−0.1185	0.0079	0.0386
hsa-miR-5585-3p	308.27	307.70	0.1290	0.0046	0.0333
hsa-miR-5585-5p	5.18	5.83	−0.1261	0.0051	0.0343
hsa-miR-616-3p	1.22	0.77	−0.0628	0.0021	0.0266

	Mean miRNA Expression			P-values	
miRNA	**Carcinoma Tissue**	**Normal Mucosa**	**Beta[1]**	**Raw**	**FDR**
hsa-miR-659-5p	1.73	2.59	−0.1311	0.0031	0.0269
hsa-miR-6716-3p	4.90	4.75	−0.0409	0.0053	0.0350
hsa-miR-6716-5p	3.80	3.73	0.0838	0.0009	0.0240
hsa-miR-758-5p	27.84	29.46	−0.0508	0.0103	0.0429
hsa-miR-766-3p	22.63	27.90	0.0713	0.0070	0.0381
hsa-miR-93-5p[2]	35.97	12.16	−0.0681	0.0076	0.0383

[1] Adjusted for age at diagnosis, study center, and sex.

[2] These miRNAs have been previously associated with differential expression and survival.

Table 5

MiRNAs associated with current smoking, C/F/N smoking and pack-years smoked.

	Beta[1]			
miRNA	**Current Smoking**	**Never vs. Former**	**Never vs. Current**	**Pack-Years**
hsa-miR-106b-5p[2]	−0.3240	−0.2585	−0.4337	−0.1597
hsa-miR-1185-1-3p	0.3492	0.0624	0.3756	0.1237
hsa-miR-1185-2-3p	0.3925	0.0841	0.4282	0.1450
hsa-miR-12021	0.4067	0.0485	0.4273	0.1481
hsa-miR-1207-5p[2]	0.3785	0.1107	0.4255	0.1361
hsa-miR-1225-5p[2]	0.3685	0.0829	0.4037	0.1424
hsa-miR-1226-5p[2]	0.3854	0.1882	0.4652	0.1525
hsa-miR-1227-5p[2]	0.3413	0.1035	0.3852	0.1160
hsa-miR-1229-3p	0.4794	0.1478	0.5421	0.1095
hsa-miR-1229-5p[2]	0.3419	0.1298	0.3970	0.1410
hsa-miR-1234-5p[2]	0.3967	0.0929	0.4361	0.1395
hsa-miR-125a-3p	0.3628	0.1325	0.4191	0.1470
hsa-miR-130b-3p	−0.3470	−0.1079	−0.3928	−0.1344
hsa-miR-134[2]	0.3889	0.1307	0.4444	0.1423
hsa-miR-138-2-3p	−0.3258	−0.1233	−0.3782	−0.1187
hsa-miR-1469	0.3908	0.1560	0.4570	0.1196
hsa-miR-149-3p[2]	0.3351	0.1743	0.4090	0.1181
hsa-miR-150-3p[2]	0.3640	0.0670	0.3924	0.1352
hsa-miR-1587	0.3449	0.0882	0.3823	0.1173
hsa-miR-188-5p	0.3828	0.2098	0.4718	0.1620
hsa-miR-19b-3p[2]	−0.2856	−0.2561	−0.3942	−0.1222
hsa-miR-20b-5p[2]	−0.3292	−0.2362	−0.4294	−0.1305
hsa-miR-3121-3p	−0.3705	−0.0328	−0.3844	−0.1219

	Beta[1]			
miRNA	**Current Smoking**	**Never vs. Former**	**Never vs. Current**	**Pack-Years**
hsa-miR-3138[2]	0.2932	0.1901	0.3738	0.1380
hsa-miR-3162-5p	0.3507	0.1443	0.4119	0.1340
hsa-miR-3185[2]	0.3578	0.1743	0.4318	0.1343
hsa-miR-3187-3p[2]	0.3029	0.1543	0.3683	0.1225
hsa-miR-3188[2]	0.3751	0.1140	0.4235	0.1320
hsa-miR-33b-3p[2]	0.3843	0.1037	0.4283	0.1408
hsa-miR-345-3p[2]	0.3234	0.1109	0.3705	0.1488
hsa-miR-3621[2]	0.2991	0.1617	0.3678	0.1051
hsa-miR-3651	−0.2860	−0.2557	−0.3945	−0.1114
hsa-miR-3656	0.3801	0.1196	0.4309	0.1216
hsa-miR-3663-3p[2]	0.4170	0.1177	0.4670	0.1433
hsa-miR-3665[2]	0.3961	0.1056	0.4409	0.1316
hsa-miR-3679-5p[2]	0.3396	0.0987	0.3815	0.1300
hsa-miR-373-5p	0.3683	0.0888	0.4060	0.1120
hsa-miR-3937[2]	0.3675	0.1251	0.4205	0.1290
hsa-miR-3940-5p[2]	0.3637	0.0924	0.4029	0.1223
hsa-miR-3945[2]	0.3190	0.1568	0.3855	0.1159
hsa-miR-3960[2]	0.3710	0.1280	0.4254	0.1444
hsa-miR-4270[2]	0.4566	0.1074	0.5022	0.1746
hsa-miR-4271	0.3615	0.1231	0.4138	0.1388
hsa-miR-4281[2]	0.4036	0.0565	0.4275	0.1452
hsa-miR-4304	0.3229	0.1970	0.4065	0.1571
hsa-miR-432-5p[2]	−0.3634	0.1652	−0.2933	−0.0580
hsa-miR-4433-3p[2]	0.3621	0.1150	0.4109	0.1420
hsa-miR-4442	0.3462	0.0744	0.3777	0.1404

	Beta[1]			
miRNA	**Current Smoking**	**Never vs. Former**	**Never vs. Current**	**Pack-Years**
hsa-miR-4443[2]	0.3592	0.0282	0.3711	0.1311
hsa-miR-4459[2]	0.3270	0.1111	0.3741	0.1133
hsa-miR-4463[2]	0.3475	0.0852	0.3836	0.1497
hsa-miR-4466[2]	0.4036	0.1375	0.4619	0.1422
hsa-miR-4476	0.3621	0.1176	0.4121	0.1313
hsa-miR-4484	0.3822	0.0646	0.4096	0.1357
hsa-miR-4496	0.3775	0.1499	0.4411	0.1602
hsa-miR-4499[2]	0.3372	0.1257	0.3905	0.1393
hsa-miR-4508[2]	0.3430	0.1441	0.4041	0.1302
hsa-miR-4516[2]	0.4308	0.1052	0.4754	0.1434
hsa-miR-4530	0.3611	0.0590	0.3861	0.1180
hsa-miR-4534[2]	0.3288	0.1076	0.3744	0.1416
hsa-miR-4535	0.3563	0.1111	0.4035	0.1234
hsa-miR-4538	0.3610	0.0744	0.3926	0.1160
hsa-miR-4539	0.5240	0.2030	0.6101	0.1816
hsa-miR-4634[2]	0.3583	0.1556	0.4243	0.1526
hsa-miR-4646-5p	0.3163	0.1306	0.3717	0.1245
hsa-miR-4651	0.3795	0.1688	0.4511	0.1427
hsa-miR-4655-5p[2]	0.3497	0.0956	0.3902	0.1173
hsa-miR-4664-3p[2]	0.3634	0.1665	0.4340	0.1137
hsa-miR-4665-5p[2]	0.3253	0.2652	0.4378	0.1404
hsa-miR-4669	0.3472	0.1225	0.3992	0.1116
hsa-miR-4687-3p[2]	0.3893	0.0693	0.4187	0.1363
hsa-miR-4690-5p[2]	0.3449	0.0919	0.3839	0.1307
hsa-miR-4700-5p	−0.3031	−0.1716	−0.3759	−0.1163
hsa-miR-4701-3p	0.2990	0.1822	0.3763	0.1418

	Beta[1]			
miRNA	**Current Smoking**	**Never vs. Former**	**Never vs. Current**	**Pack-Years**
hsa-miR-4734[2]	0.3823	0.1301	0.4376	0.1230
hsa-miR-4739[2]	0.3876	0.0814	0.4221	0.1334
hsa-miR-4740-5p	0.3805	0.1893	0.4608	0.1324
hsa-miR-4741[2]	0.3299	0.1453	0.3916	0.1415
hsa-miR-4745-5p	0.3852	0.1719	0.4582	0.1319
hsa-miR-4763-3p	0.3866	0.0937	0.4263	0.1292
hsa-miR-4767	0.3502	0.1117	0.3975	0.1141
hsa-miR-4787-3p	0.3618	0.1469	0.4241	0.1119
hsa-miR-4787-5p[2]	0.3331	0.1020	0.3764	0.1146
hsa-miR-484	−0.3872	−0.0332	−0.4013	−0.1189
hsa-miR-498[2]	0.3321	0.1042	0.3763	0.1183
hsa-miR-5001-5p[2]	0.3668	0.0957	0.4074	0.1214
hsa-miR-500a-3p	−0.3082	−0.1597	−0.3760	−0.1226
hsa-miR-5195-3p[2]	0.3657	0.0876	0.4029	0.1438
hsa-miR-550a-3-5p[2]	0.3391	0.0485	0.3597	0.1250
hsa-miR-557	0.3773	0.1589	0.4447	0.1435
hsa-miR-572[2]	0.3640	0.0900	0.4022	0.1293
hsa-miR-5787	0.2933	0.1829	0.3709	0.1195
hsa-miR-601[2]	0.3915	0.1433	0.4523	0.1451
hsa-miR-6068[2]	0.3695	0.1019	0.4128	0.1225
hsa-miR-6076	0.3567	0.0598	0.3821	0.1212
hsa-miR-6086[2]	0.3344	0.1146	0.3831	0.1256
hsa-miR-6087[2]	0.4023	0.0505	0.4238	0.1354
hsa-miR-6088[2]	0.3818	0.0590	0.4068	0.1370
hsa-miR-6089[2]	0.3912	0.1205	0.4423	0.1331

	Beta[1]			
miRNA	**Current Smoking**	**Never vs. Former**	**Never vs. Current**	**Pack-Years**
hsa-miR-6090[2]	0.4363	0.1023	0.4797	0.1529
hsa-miR-6124[2]	0.3394	0.0816	0.3740	0.1457
hsa-miR-6125[2]	0.3672	0.1027	0.4107	0.1214
hsa-miR-6126[2]	0.3887	0.1204	0.4398	0.1268
hsa-miR-617	0.3833	0.1717	0.4562	0.1249
hsa-miR-638[2]	0.3835	0.1030	0.4272	0.1300
hsa-miR-6722-3p[2]	0.3570	0.1116	0.4044	0.1481
hsa-miR-6723-5p	0.3597	0.0792	0.3933	0.1418
hsa-miR-6724-5p[2]	0.3419	0.0944	0.3820	0.1313
hsa-miR-770-5p	−0.3457	−0.0423	−0.3637	−0.1213
hsa-miR-877-5p[2]	0.3441	0.1394	0.4032	0.1504
hsa-miR-887[2]	0.3314	0.1118	0.3788	0.1162

[1] Adjusted for age at diagnosis, study center, and sex.

[2] These miRNAs have been previously associated with differential expression and survival.

Table 6

MicroRNAs that are significantly differentially expressed with cigarette smoking and are associated with CIMP and/or MSI status in colon and rectal cancer subjects with an FDR < 0.05.

	CIMP Low / MSI	CIMP High / MSS	CIMP High / MSI
Colon and Rectal Tissue			
Directly Associated miRNAs	hsa-miR-320c, hsa-miR-1207-5p, hsa-miR-1225-5p, hsa-miR-1229-3p, hsa-miR-1234-5p, hsa-miR-125a-3p, hsa-miR-134, hsa-miR-135a-3p, hsa-miR-188-5p, hsa-miR-1914-3p, hsa-miR-3610, hsa-miR-3917, hsa-miR-3945, hsa-miR-3960, hsa-miR-4270, hsa-miR-4459, hsa-miR-4470, hsa-miR-4499, hsa-miR-4508, hsa-miR-4516, hsa-miR-4530, hsa-miR-4532, hsa-miR-4673, hsa-miR-4739, hsa-miR-4745-5p, hsa-miR-5006-5p, hsa-miR-5088, hsa-miR-520b, hsa-miR-575, hsa-miR-5787, hsa-miR-6086, hsa-miR-6090, hsa-miR-6126, hsa-miR-617, hsa-miR-718, hsa-miR-762, hsa-miR-1469, hsa-miR-3676-5p, hsa-miR-877-5p	hsa-miR-3622a-5p	hsa-miR-1202, hsa-miR-1226-5p, hsa-miR-1307-5p, hsa-miR-149-3p, hsa-miR-150-3p, hsa-miR-3150b-5p, hsa-miR-3646, hsa-miR-3652, hsa-miR-3666, hsa-miR-3667-5p, hsa-miR-371a-5p, hsa-miR-423-3p, hsa-miR-4253, hsa-miR-4463, hsa-miR-4476, hsa-miR-4515, hsa-miR-4655-5p, hsa-miR-4664-3p, hsa-miR-4665-3p, hsa-miR-4665-5p, hsa-miR-4783-3p, hsa-miR-4787-3p, hsa-miR-550b-2-5p, hsa-miR-601, hsa-miR-623, hsa-miR-6723-5p, hsa-miR-6724-5p, hsa-miR-887, hsa-miR-940, hsa-miR-99b-3p, hsa-miR-5189, hsa-miR-1185-1-3p, hsa-miR-1185-2-3p, hsa-miR-1207-5p, hsa-miR-1225-5p, hsa-miR-1227-5p, hsa-miR-1229-3p, hsa-miR-1229-5p, hsa-miR-1233-1-5p, hsa-miR-1234-5p, hsa-miR-125a-3p, hsa-miR-1275, hsa-miR-129-5p, hsa-miR-134, hsa-miR-135a-3p, hsa-miR-1587, hsa-miR-188-5p, hsa-miR-1914-3p, hsa-miR-1915-3p, hsa-miR-197-5p, hsa-miR-2861, hsa-miR-3162-5p, hsa-miR-3187-3p, hsa-miR-3188, hsa-miR-3610, hsa-miR-3620-5p, hsa-miR-3665, hsa-miR-3679-5p, hsa-miR-3917, hsa-miR-3937, hsa-miR-3940-5p, hsa-miR-3945, hsa-miR-4257, hsa-miR-4270, hsa-miR-4271, hsa-miR-4281, hsa-miR-4419b, hsa-miR-4429, hsa-miR-4459, hsa-miR-4466, hsa-miR-4470, hsa-miR-4499, hsa-miR-4505, hsa-miR-4507, hsa-miR-4508, hsa-miR-4516, hsa-miR-4530, hsa-miR-4532, hsa-miR-4535, hsa-miR-4538, hsa-miR-4651, hsa-miR-4669, hsa-miR-4672, hsa-miR-4673, hsa-miR-4687-3p, hsa-miR-4690-5p, hsa-miR-4721, hsa-miR-4739, hsa-miR-4740-5p, hsa-miR-4745-5p, hsa-miR-4746-3p, hsa-miR-4763-3p, hsa-miR-4787-5p, hsa-miR-5001-5p, hsa-miR-5006-5p, hsa-miR-5088, hsa-miR-5195-3p, hsa-miR-520b, hsa-miR-548q, hsa-miR-557, hsa-miR-572, hsa-miR-575, hsa-miR-5787, hsa-miR-6068, hsa-miR-6076, hsa-miR-6084, hsa-miR-6086, hsa-miR-6087, hsa-miR-6088, hsa-miR-6089, hsa-miR-6090, hsa-miR-6125, hsa-miR-6126, hsa-miR-6127, hsa-miR-617, hsa-miR-638, hsa-miR-671-5p, hsa-miR-6722-3p, hsa-miR-718, hsa-miR-762, hsa-miR-874, hsa-miR-937-5p, hsa-miR-939-5p, hsa-miR-1208, hsa-miR-1224-5p, hsa-miR-1469,

		CIMP Low / MSI	CIMP High / MSS	CIMP High / MSI
				hsa-miR-3621, hsa-miR-3622a-5p, hsa-miR-373-5p, hsa-miR-4486, hsa-miR-4497, hsa-miR-4695-5p, hsa-miR-4734, hsa-miR-769-3p, hsa-miR-877-5p
	Inversely Associated miRNAs	hsa-miR-196a-5p, hsa-miR-196b-5p, hsa-miR-20b-5p, hsa-miR-215, hsa-miR-30b-5p	hsa-miR-196b-5p	hsa-miR-196b-5p, hsa-miR-127-3p, hsa-miR-19b-3p, hsa-miR-20a-5p, hsa-miR-20b-5p, hsa-miR-215, hsa-miR-29a-3p, hsa-miR-29b-3p, hsa-miR-30b-5p, hsa-miR-425-5p, hsa-miR-501-3p, hsa-miR-93-5p
Rectal Tissue Only				
	Directly Associated miRNAs	hsa-miR-3663-3p, hsa-miR-4534, hsa-miR-4634, hsa-miR-1202, hsa-miR-1225-3p, hsa-miR-1226-5p, hsa-miR-1307-5p, hsa-miR-139-3p, hsa-miR-3150b-5p, hsa-miR-345-5p, hsa-miR-3646, hsa-miR-3652, hsa-miR-3667-5p, hsa-miR-4253, hsa-miR-4476, hsa-miR-4664-3p, hsa-miR-4665-3p, hsa-miR-4689, hsa-miR-4767, hsa-miR-4783-3p, hsa-miR-5190, hsa-miR-601, hsa-miR-6085, hsa-miR-6510-5p, hsa-miR-6723-5p, hsa-miR-877-3p, hsa-miR-887, hsa-miR-940	hsa-miR-4721, hsa-miR-4470	hsa-miR-1183, hsa-miR-1236-5p, hsa-miR-1249, hsa-miR-2392, hsa-miR-3138, hsa-miR-3156-5p, hsa-miR-3158-5p, hsa-miR-3185, hsa-miR-3194-5p, hsa-miR-320a, hsa-miR-320b, hsa-miR-33b-3p, hsa-miR-345-3p, hsa-miR-3648, hsa-miR-3656, hsa-miR-3934-5p, hsa-miR-423-5p, hsa-miR-4259, hsa-miR-4304, hsa-miR-4322, hsa-miR-4327, hsa-miR-4417, hsa-miR-4433-3p, hsa-miR-4433-5p, hsa-miR-4442, hsa-miR-4484, hsa-miR-4487, hsa-miR-4488, hsa-miR-4646-5p, hsa-miR-4685-5p, hsa-miR-4707-5p, hsa-miR-4726-5p, hsa-miR-4741, hsa-miR-4743-5p, hsa-miR-4758-5p, hsa-miR-4788, hsa-miR-4793-5p, hsa-miR-498, hsa-miR-5196-5p, hsa-miR-550a-3-5p, hsa-miR-602, hsa-miR-642b-3p, hsa-miR-6511b-5p, hsa-miR-652-5p, hsa-miR-936, hsa-miR-1225-3p, hsa-miR-139-3p, hsa-miR-345-5p, hsa-miR-4689, hsa-miR-4767, hsa-miR-5190, hsa-miR-6085, hsa-miR-6510-5p, hsa-miR-877-3p
	Inversely Associated miRNAs	hsa-miR-106b-5p, hsa-miR-140-3p, hsa-miR-425-5p, hsa-miR-501-3p, hsa-miR-126-3p, hsa-miR-200a-3p, hsa-miR-203a, hsa-miR-429, hsa-miR-4709-3p	hsa-miR-1273g-5p, hsa-miR-130b-3p	hsa-miR-141-3p, hsa-miR-106b-5p, hsa-miR-140-3p, hsa-miR-15b-5p, hsa-miR-3174
Colon Tissue Only				
	Directly Associated miRNAs	hsa-miR-5001-5p, hsa-miR-5195-3p, hsa-miR-548q, hsa-miR-557, hsa-miR-572, hsa-miR-6068, hsa-miR-6076, hsa-miR-6084, hsa-miR-6087, hsa-miR-6088, hsa-miR-6089, hsa-miR-6125, hsa-miR-6127, hsa-miR-638, hsa-miR-671-5p, hsa-miR-6722-3p, hsa-miR-874, hsa-miR-937-5p, hsa-miR-939-5p, hsa-miR-1208, hsa-miR-1224-5p, hsa-miR-330-3p, hsa-miR-3621, hsa-miR-3622a-5p, hsa-miR-373-5p, hsa-miR-4486, hsa-miR-4497, hsa-miR-4695-5p, hsa-miR-4734, hsa-miR-769-3p	hsa-miR-509-3-5p, hsa-miR-5189, hsa-miR-1208, hsa-miR-1224-5p, hsa-miR-1469, hsa-miR-3621, hsa-miR-3676-5p, hsa-miR-373-5p, hsa-miR-4486, hsa-miR-4497, hsa-miR-4695-5p, hsa-miR-4734, hsa-miR-769-3p, hsa-miR-877-5p	hsa-miR-3663-3p, hsa-miR-4481, hsa-miR-4534, hsa-miR-4634, hsa-miR-3960, hsa-miR-330-3p, hsa-miR-3676-5p

	CIMP Low / MSI	CIMP High / MSS	CIMP High / MSI
Inversely Associated miRNAs	hsa-miR-17-5p, hsa-miR-19b-3p, hsa-miR-20a-5p, hsa-miR-29b-3p, hsa-miR-3651, hsa-miR-4485, hsa-miR-199b-5p, hsa-miR-29a-3p, hsa-miR-758-5p, hsa-miR-3174, hsa-miR-6716-3p	hsa-miR-17-5p, hsa-miR-196a-5p, hsa-miR-19b-3p, hsa-miR-20a-5p, hsa-miR-20b-5p, hsa-miR-215, hsa-miR-29b-3p, hsa-miR-3651, hsa-miR-4485, hsa-miR-200a-3p, hsa-miR-425-5p, hsa-miR-429, hsa-miR-532-3p, hsa-miR-192-3p, hsa-miR-28-5p, hsa-miR-484	hsa-miR-17-5p, hsa-miR-4485, hsa-miR-200a-3p, hsa-miR-196a-5p, hsa-miR-3651, hsa-miR-199b-5p, hsa-miR-758-5p, hsa-miR-429, hsa-miR-532-3p, hsa-miR-107, hsa-miR-3121-3p, hsa-miR-3135b, hsa-miR-4313, hsa-miR-4436b-3p, hsa-miR-4800-5p