Association analysis of miRNA-related genetic polymorphisms in miR-143/145 and KRAS with colorectal cancer susceptibility and survival

Danyang Wang
Zhejiang University School of Medicine First Affiliated Hospital
Qingmin Liu
Hangzhou Center for Disease Control and Prevention
Yanjun Ren
Hangzhou Center for Disease Control and Prevention
Yan Zhang
Hangzhou Center for Disease Control and Prevention
Xin Wang
Ziyang community health service center
BING LIU (ELENALIU@163.COM)
Hangzhou Center for Disease Control and Prevention https://orcid.org/0000-0001-9759-1828

Research article

Keywords: MicroRNA, colorectal cancer, KRAS

Posted Date: December 19th, 2019

DOI: https://doi.org/10.21203/rs.2.19326/v1

Version of Record: A version of this preprint was published at Bioscience Reports on April 1st, 2021. See the published version at https://doi.org/10.1042/BSR20204136.

Abstract

Background MicroRNAs have important roles in tumorigenesis. There is accumulating evidence of aberrant expression of miR-143 and miR-145 and their target gene *KRAS* has been described in colorectal cancer (CRC). We hypothesize that single nucleotide polymorphisms (SNPs) within or near mRNA-miRNA binding sites may affect miRNA/target gene interaction, resulting in differential mRNA/protein expression and promoting the development and progression of CRC.

Methods We conducted a case-control study of 507 CRC cases recruited from a tertiary hospital and 497 population-based controls to assess the association of genetic polymorphisms in miR-143/145 and the *KRAS* 3' untranslated region (3' UTR) with CRC susceptibility and survival. Deaths and causes of death among the CRC cases were identified using the Hangzhou Cancer Registration System and Death Surveillance System. Genetic variations of genomic regions located from 500 bp upstream to 500 bp downstream of the miR-143/miR-145 gene and the 3' UTR of *KRAS* were selected using the Haploview and HaploReg software.

Results Using publicly available expression profiling data, we found that miR-143/145 and *KRAS* expression were all reduced in rectal cancer tissue compared with adjacent normal mucosa. The Rs74693964 C/T variant located 65 bp downstream of miR-145 genomic regions was observed to be associated with CRC susceptibility (adjusted odds ratio 2.414, 95% *CI*: 1.385–4.206). Among non-smokers, the miR-143 rs41291957 GA genotype and miR-145 rs74693964 CT genotype were borderline significantly associated with an increased risk of rectal cancer. However, there was no interaction effect between selected SNPs and smoking status. Cumulative effects of miR-143 and miR-145 on CRC risk were observed (P_{trend}=0.03). CRC cases carrying variant genotype TT of *KRAS* rs712 had poorer survival (log-rank *P*=0.044, adjusted hazard ratio 4.328, 95% *CI*: 1.236–15.147).

Conclusions Our results indicate that miRNA-related polymorphisms in miR-143/145 and *KRAS* are likely to be deleterious and represent potential biomarkers for CRC susceptibility and survival.

Introduction

Colorectal cancer (CRC) is one of the most commonly occurring malignancies worldwide. According to The Global Burden of Cancer 2013, colon and rectal cancer ranked third for cancer incidence and fourth for cancer deaths [1]. In China, CRC incidence and mortality statistics for 2014, published by the National Cancer Center, showed a similar trend, with CRC ranking in third and fifth place for cancer incidence and cancer deaths, respectively [2].

The development of CRC is a multifactorial and multistep process involving the gain and maintenance of specific genomic alterations [3]. Over the past few decades, many associations have been identified between the variation of protein-coding genes and CRC. In recent years, high-resolution maps of the human transcriptome have led to the discovery of a large number of non-protein-coding RNA genes and brought about a paradigm shift in our understanding of the function of variations in non-coding RNAs (ncRNAs) [4]. The ncRNAs include a class of short RNA molecules termed microRNAs (miRNAs), which are endogenous small ncRNAs that repress protein-coding genes by binding to target sites in the 3' untranslated region (3' UTR) of mRNAs. These miRNAs are involved in the regulation of almost all physiological and pathological processes, including cell proliferation, differentiation, and apoptosis [5].

MiR-143 and miR-145, which are located close to each other on 5q33, are co-transcribed from a single promoter and generate a primary transcript containing both miRNAs [6]. In 2003, miR-143 and miR-145 were reported to be downregulated in colorectal tissue for the first time [7]. Subsequently, a series of studies confirmed these results [8–10]. Decreased expression of these two miRNAs is involved in various cancer-related events, including proliferation, invasion, and migration, suggesting that they have anti-tumorigenic activity [11–13]. The KRAS oncogene is an important upstream mediator of the MAPK pathway, and its overexpression can lead to increased activation of the RAF/MEK/MAPK pathway, thereby promoting tumorigenesis [14]. KRAS is an important target of miR-143/145, which has been identified not only by computational predictions using software such as TargetScan, miRanda, and PicTar, but also by experimental validation [15, 16].

It is propsosed that mutations in either miRNAs or their coexpressed miRNA binding sites are often deleterious, which can affect miRNA/target gene interaction, resulting in differential mRNA or protein expression and increased susceptibility to common diseases [17]. This view was supported by some studies of miRNA-related genetic alterations with different types of cancer, including CRC[18–20]. However, published evidences for genetic variations of miR-143/145 and the 3'UTR of KRAS with CRC susceptibility are limited and not comprehensively investigated. Therefore, we conducted a case-control study to assess the association between these candidates biomarkers with CRC risk.

Materials And Methods

Study population

This study was conducted in Hangzhou City, Zhejiang Province, China. A total of 507 CRC patients and 497 cancer-free controls were enrolled in the study from May 2014 to May 2015. CRC patients were recruited from a tertiary hospital in Hangzhou, Zhejiang Province, China. Eligible cases were newly diagnosed and histologically confirmed CRC without radiotherapy or chemotherapy. The control population was recruited from among the individuals who came to the community health service center for medical examinations. The controls had no cancer history or intestinal diseases. All participants were Han Chinese and had lived in Zhejiang Province for more than 20 years.

The study was approved by the Medical Ethical Committee of Hangzhou Center for Disease Control and Prevention. All participants supplied informed written consent. Face-to-face interviews were conducted by trained interviewers who administered a structured questionnaire asking about demographic characteristics, family history of cancer, previous medical history, and lifestyle-related factors. Smoking history was defined as having smoked at least one

cigarette per day for more than 1 year. Alcohol drinking or tea drinking was defined as having consumed an alcoholic drink or tea at least once per day for more than 3 months.

Polymorphism selection and genotyping

First, single nucleotide polymorphisms (SNPs) of genomic regions located from 500 bp upstream to 500 bp downstream of the miR-143/miR-145 gene and the 3' UTR of *KRAS* were downloaded from 1000 Genomes (http://www.internationalgenome.org/) if they had minor allele frequency> 0.05 within the Southern Han Chinese population. Then, tagSNPs representing SNPs with pairwise correlation of r^2>0.8 were further selected using the tagger algorithm implemented in the Haploview software. The function of the tagSNPs was predicted using RegulomeDB and HaploReg. Finally five polymorphisms were selected for study: *KRAS* rs712, rs1137196, miR-143 rs41291957, miR-145 rs74693964, and rs80026971. Detailed information regarding the selected SNPs are listed in Supplementary table.

Genomic DNA was extracted from peripheral blood samples using a magnetic bead method with KingFisher Flex (Thermo Scientific, USA). The concentration and purity of the DNA samples were determined using a NanoDrop2000 spectrophotometer (Thermo Scientific, USA). Genotyping was performed using the Agena MassArray Genotyping Platform (Agena Inc. San Diego, CA, USA). Five percent blinded samples were repetitively genotyped and a negative control was interspersed throughout the genotyping assays. The detection rates of all SNP genotyping assays were ≥96%. The concordance rates for duplicated samples were 100%.

Gene expression analysis

The miR-143/145 microarrays were downloaded from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo), accession no. GSE38389. From this dataset, 66 paired samples from rectal tumor tissue and normal samples were collected, and microRNA expression profiles were detected using the GPL11039 platform (Exiqon miRCURY LNA microRNA array v.9.2 Extended Version).

We used Oncomine (www.oncomine.org last accessed on Dec 5, 2019) to conduct a meta-analysis for *KRAS* gene expression. We extracted the qualified datasets by key words as follows: Gene "*KRAS*" ; Cancer type: "Colorectal cancer" ; Analysis type: "Cancer vs. Normal Analysis"; Data Type: "mRNA" from the the Oncomine database. There are 8 arrays (Kaiser Colon, Sabates-Bellver Colon, Skrzypczak Colorectal 2, TCGA Colorectal, Skrzypczak Colorectal, Gaspar Colon, Hong Colorectal, Gaedcke Colorectal) including 639 colorectal cancer cases and 202 controls involved in the meta-analysis.

CRC death surveillance

A follow-up survey of CRC cases with Hangzhou household registration was conducted using the Cancer Registration System and Death Surveillance System of Hangzhou Center for Disease Control and Prevention. The date of censorship was Jan 1 2019. We applied the identification card(ID) number and name of CRC cases to match and acquire the survival outcome from the Surveillance Systems. Date and cause of death were recorded for the survival analysis.

Statistical analysis

A two-sided student's t-test was used to compare the differences in the quantitative data, and a chi-square test was used to compare categorical data between the two groups. Departures from Hardy–Weinberg equilibrium were tested using goodness-of-fit chi-square test. Multivariate logistic regression analysis was performed to explore the association between the selected SNPs and CRC risk with adjustment for age, sex, and family history of cancer. A likelihood ratio test was used to assess the interaction effects between the SNPs and smoking with respect to CRC. The Cochran-Armitage test was used for trend analysis. Kaplan–Meier survival analysis and log rank test were used to assess survival outcome, that is, overall survival in relation to the genotypes. Multivariate Cox regression analysis was performed to calculate relative risk [hazard ratio (HR)] and 95% confidence interval (CI) associated with genetic polymorphisms from cancer diagnosis until the end of the study or death. Statistical analyses were performed using the SPSS V.17.0 and SAS V.9.2 software. A *P* value < 0.05 was considered statistically significant.

Results

Characteristics of the study population

A total of 507 CRC patients (209 colon cancer cases and 298 rectal cancer cases) and 497 cancer-free controls were involved in our study. The baseline characteristics and lifestyle factors are shown in Table 1. There was no significant difference in age between the cases and controls, but the proportion of males was higher in the case group (64.89% vs. 57.95%). CRC patients were more likely to have a lower education level and lower body mass index (P<0.05). CRC patients also reported higher percentages of family history of cancer and history of appendicitis in comparison with controls (P=0.034, P<0.001 respectively). However, no significant differences were found between the case and control groups with respect to tobacco smoking, alcohol drinking, or tea drinking.

mRNA expression analysis of miR-143, miR-145, and *KRAS*

We extracted published microarray data from GEO datasets GSE38389 and compared the mRNA expression of miR-143, miR-145 between rectal cancer tissue and adjacent normal mucosa. As shown in Figure 1, miR-143 expression was under-expressed (log2-fold difference<-1) 26 out of 66 matched pairs of rectal tumor samples and normal samples (*P* value for paired *t* test <0.001). MiR-145 showed the same trend, with decreased expression(log2-fold difference<-1) in 28 out of 66 pairs of samples (*P* value for paired *t* test <0.001). (Figure1, 2)

We performed a statistical comparison of *KRAS* expression from multiple colorectal cancer studies published in Oncomine database. Eight independent microarray studies comprising a total of 639 colorectal cancer and 202 normal colorectal mucosa samples were evaluated from meta-analysis data by Oncomine. Meta-analysis identified that *KRAS* mRNA was under-expressed no matter in colon cancer or rectal cancer(P<0.001, P=0.016 respectively).

Polymorphisms of miR-143, miR-145, and *KRAS* 3' UTR and CRC risk

KRAS rs712 and rs1137196, miR-143 rs41291957, and miR-145 rs74693964 and rs80026971 were genotyped in this study. The genotype distribution of the five SNPs in the control group all conformed to Hardy–Weinberg equilibrium; their associations with the risk of CRC are presented in Table 2. As shown in the table, subjects with the heterozygous genotype CT of rs74693964 were more than twice as likely to have CRC as subjects with the wild genotype CC [adjusted odds ratio (*OR*)=2.414, 95% *CI*:1.385–4.206]. MiR-145 rs74693964 was associated with a significantly increased risk of CRC. However, *KRAS* rs712 and rs1137196, miR-143 rs41291957, and miR-145 rs80026971 showed no association with the risk of CRC. In the subgroup analysis, rs41291957 and rs74693964 were found to be associated with an increased risk of rectal cancer but not colon cancer (rs41291957 GA vs. AA: adjusted *OR*=1.367, 95% *CI*: 1.005–1.860; rs74693964 CT vs. CC: adjusted *OR*=2.820, 95% *CI*: 1.547–5.140) (Tables 2, 3).

When stratified by smoking status, we found that the genotype distributions of miR-143 rs41291957 among non-smokers differed significantly between cases and controls. Compared with the GG genotype, those carrying heterozygous genotype GA had a nearly 40% increased risk for developing CRC (adjusted *OR*=1.397, 95% *CI*: 1.007–1.936). In non-smokers, miR-145 rs74693964 remained a significant risk factor for CRC among subjects carrying the CT genotype (adjusted *OR* =3.086, 95% *CI*: 1.468–6.484). Interaction analyses of the two SNPs and tobacco smoking were conducted using a multiplicative model; neither interaction effect showed statistical significance (Table 4).

Although miR-143 and miR-145 located close to each other on 5q33, our analysis showed no linkage disequilibrium between them. To evaluate the potential cumulative effects of miR-143 and miR-145, we defined at-risk genotypes as those with *OR* values greater than 1 under a dominant model of rs41291957 and rs74693964. We compared the distributions of the number of at-risk genotypes between cases and controls. CRC risk increased with the number of at-risk genotypes (P_{trend}=0.003). When split by cancer type, individuals harboring two at-risk genotypes had an increased risk of rectal cancer relative to those with none (*OR*=3.738, 95% *CI*: 1.725–8.101) (Table 5).

Polymorphisms of miR-143, miR-145, and *KRAS* 3' UTR and CRC survival

We collected and evaluated the overall survival time of 222 CRC cases with Hangzhou household registration from the Cancer Registration System and Death Surveillance System of Hangzhou Center for Disease Control and Prevention. Of the 222 cases recruited between May 2014 and May 2015, a total of 34 had died of CRC by Jan 1 2019. Associations between polymorphisms of miR-143, miR-145, and the *KRAS* 3' UTR and CRC survival were explored. First, we used the Kaplan–Meier method to compare overall survival among different genotypes of selected SNPs. Then, adjusted HRs were obtained by Cox regression analysis for further confirmation of the relationships between the genotypes and CRC survival. The results showed that the mutant homozygote TT of rs712 was associated with decreased survival time in CRC (log-rank P=0.044). Compared with the reference genotype GG of rs712, the CRC cases with TT genotype had a significant increase in number of deaths (adjusted *HR*=4.328, 95% *CI*: 1.236–15.147). The polymorphisms of miR-143 and miR-145 did not show any statistical association with prognosis of CRC cases (Table 6).

Discussion

MiR-143 and miR-145 which are located on 5q23 may originate from the same primary miRNA. Michael et al. showed that miR-143 and miR-145 displayed consistently decreased expression levels of mature miRNA at the colorectal neoplasm stages, in comparison with healthy colon mucosa. Several other studies have confirmed this finding [9, 21]. Our study found that both miR-143/145 showed reduced expression in rectal cancer compared with adjacent normal mucosa based on microarray gene expression datasets from GEO, which is consistent with previous studies. KRAS is one of the most frequently mutated genes associated with CRC risk. A number of recent studies have demonstrated the significance of KRAS mutation in CRC carcinogenesis [15, 22]; however, KRAS gene expression status in CRC has been less reported. We conducted a meta-analysis for KRAS gene expression from multiple colorectal cancer studies published in Oncomine database. We found that KRAS expression was downregulated in CRC tissues, especially in rectal cancers. Mazza et al. evaluated of the miRNAome and transcriptome of matched pairs of tumour and adjacent non-tumorous mucosa samples of CRC. He found concurrent downregulation of KRAS and the miR-143/145 cluster in CRC tissue [16]. This result was interpreted in terms of a feed-forward mechanism in which the miR-143/145 polycistronic cluster targets the RAS-responsive element-binding protein RREB1 and KRAS, which, in turn, induce downregulation of the cluster [14].

Emerging evidence has shown that miRNA-related SNPs may alter an individual's susceptibility to CRC by disrupting miRNAs' procession, expression, or interaction with target mRNA [23]. However, no SNP of the miR-143 and miR-145 genes could be identified by the HapMap and dbSNP database retrieval. Thus we selected the SNPs within the miRNA regulatory region/transcription factor-binding sites for further study. MiR-145 Rs74693964 is located 65 bp downstream of miR-145. According to functional predictions based on HaploReg annotations [24] and the RegulomeDB database [25], this SNP has been identified as a promoter histone modification or enhancer histone modification region in more than 20 tissues, including colonic mucosa and rectal mucosa. We observed that individuals with the CT genotype of rs74693964 in the Chinese population had a two-fold increased risk for developing CRC compared with those carrying the CC genotype. After stratification by smoking status, miR-145 rs74693964 was found to be significantly associated with an increased risk of CRC among non-smokers. To date, only two studies have reported an association of miR-145 rs74693964 with risk of cancer; one was a study of cervical cancer and the other of non-small-cell lung cancer [26, 27]. No similar study involving CRC has yet been reported. To our knowledge, the present work is the first investigation of the link between miR-145 rs74693964 and CRC risk in the Chinese population.

In previous studies, Li et al. [28] reported a significant effect of mutant genotypes or alleles of rs41291957 on CRC risk, although Ying et al. [29] failed to find any association between rs41291957 and CRC susceptibility. In our study, rectal cancer risk was shown to be associated with the rs41291957 heterozygous

genotype. Rs41291957 is located 91 bp upstream of miR-143. Saini et al. demonstrated that up to 60% of miRNAs have transcription factor binding sites within 1 kb of the start of the pre-miRNA [30], indicating that rs41291957 in the promoter region may be involved in the transcriptional activation of miR-143. Furthermore, bioinformatic predictions using HaploReg and RegulomeDB indicated that rs41291957 is probably involved in epigenetic modifications that promote colorectal tumorigenesis.

The cumulative effects of significant polymorphisms of miR-143 and miR-145 were evaluated. CRC risk increased with the number of at-risk genotypes, especially in rectal cancer. The average SNP density of clustered miRNAs was significantly lower than that of the individual miRNAs, which may to some degree reflect the critical biological functions regulated by clustered miRNAs [31]. The miR-143/145 cluster coordinately plays an important part in the carcinogenesis of CRC [32]. It is thus a reasonable assumption that the more mutations occur in the miR-143/145 cluster, the greater the risk of CRC.

KRAS is a direct target of miR-143/145. In this study, no SNP was identified within the binding region of miR-143/145, nor was there any association with CRC risk. However, our results indicated that the rs712 G > T polymorphism in the 3' UTR of the KRAS gene may modulate survival outcome in CRC. Multiple miRNAs, including miR-200b, miR-200c, and miR-429, target rs712. The miR-200 family (miR-200b, miR-200c, and miR-429) has been widely investigated with regard to its role in tumor metastasis [33]. Pichler et al. found that miR-200 family expression was associated with poor prognosis in CRC patients and with cancer stem cell properties in CRC [34]. Therefore, the rs712 G > T change might attenuate its binding capacity with the miR-200 family. Although the association between the KRAS rs712 polymorphism and cancer risk has been widely studied [35–37], the effects of this polymorphism on CRC survival are still unclear. Schneiderova et al. [38] indicated that individuals with colon cancer carrying the heterozygous GT genotype had longer overall survival. The survival impact of rs712 on CRC survival was not significant in a study by Dai[39]. Our study suggests that a poor prognosis in CRC is associated with the homozygous TT genotype. The limited and conflicting results on the prognostic value of KRAS rs712 as a predictor for CRC survival indicate that larger studies are required.

There were some limitations to this study. First, owing to the lack of RNA samples for the study population, we were unable to carry out functional validation tests. The biological functions of the selected SNPs in CRC were inferred and predicted using the available online tools. Second, the participants in the case group and control group were collected from a hospital and from the community, respectively; thus, selection bias cannot be ignored. Finally, the relatively small sample size, especially for the survival analysis, may have hindered the ability of the study to detect weak gene-disease associations and gene-environment interactions.

Conclusions

In conclusion, our results suggest that rs74693964 C/T and rs41291957 G/A in the miR-143/145 cluster might have cumulative effects on rectal cancer risk. Rs712 G/T in KRAS might be associated with poorer survival in CRC. Further large population-based prospective studies as well as functional validation are warranted to advance our understanding of the role of these factors in CRC.

Declarations

Acknowledgements

Not applicable.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 81302506), Zhejiang Provincial Administration of Traditional Chinese Medicine(No. 2018ZA068) and Education of Zhejiang Province(No. Y201738284).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Danyang Wang and Bing Liu designed the study and Bing Liu applied for Research Ethics Board approval. Danyang Wang, Bing Liu, and Xin Wang recruited the participants and collected the data. Bing Liu and Yan Zhang conducted the experiments. Qingmin Liu and Yanjun Ren analyzed the data and prepared the tables. Danyang Wang and Bing Liu drafted and completed the manuscript. All authors approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Medical Ethical Committee of Hangzhou Center for Disease Control and Prevention. All participants supplied informed written consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

3'-UTR 3'-untranslated region;

CRC colorectal cancer;

TagSNPs tag single nucleotide polymorphisms;

GEO Gene Expression Omnibus;

ncRNAs non-coding RNAs;

miRNAs microRNAs;

FDR false discovery rate;

OS overall survival;

OR odds ratio

HR hazard ratio;

CHS Southern Han Chinese

TFBS transcription factor binding site

ID identification card

References

1. Global Burden of Disease Cancer C, Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, Allen C, Hansen G, Woodbrook R *et al*: **The Global Burden of Cancer 2013**. *JAMA oncology* 2015, **1**(4):505-527.
2. Chen W, Sun K, Zheng R, Zeng H, Zhang S, Xia C, Yang Z, Li H, Zou X, He J: **Cancer incidence and mortality in China, 2014**. *Chinese journal of cancer research = Chung-kuo yen cheng yen chiu* 2018, **30**(1):1-12.
3. Yin Y, Song M, Gu B, Qi X, Hu Y, Feng Y, Liu H, Zhou L, Bian Z, Zhang J *et al*: **Systematic analysis of key miRNAs and related signaling pathways in colorectal tumorigenesis**. *Gene* 2016, **578**(2):177-184.
4. Bhartiya D, Scaria V: **Genomic variations in non-coding RNAs: Structure, function and regulation**. *Genomics* 2016, **107**(2-3):59-68.
5. Rao CV, Yamada HY: **Genomic instability and colon carcinogenesis: from the perspective of genes**. *Frontiers in oncology* 2013, **3**:130.
6. Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, Lee TH, Miano JM, Ivey KN, Srivastava D: **miR-145 and miR-143 regulate smooth muscle cell fate and plasticity**. *Nature* 2009, **460**(7256):705-710.
7. Michael MZ, SM OC, van Holst Pellekaan NG, Young GP, James RJ: **Reduced accumulation of specific microRNAs in colorectal neoplasia**. *Molecular cancer research : MCR* 2003, **1**(12):882-891.
8. Akao Y, Nakagawa Y, Naoe T: **MicroRNA-143 and -145 in colon cancer**. *DNA and cell biology* 2007, **26**(5):311-320.
9. Slattery ML, Herrick JS, Pellatt DF, Stevens JR, Mullany LE, Wolff E, Hoffman MD, Samowitz WS, Wolff RK: **MicroRNA profiles in colorectal carcinomas, adenomas and normal colonic mucosa: variations in miRNA expression and disease progression**. *Carcinogenesis* 2016, **37**(3):245-261.
10. Xu P, Wang J, Sun B, Xiao Z: **Integrated analysis of miRNA and mRNA expression data identifies multiple miRNAs regulatory networks for the tumorigenesis of colorectal cancer**. *Gene* 2018, **659**:44-51.
11. Gomes SE, Pereira DM, Roma-Rodrigues C, Fernandes AR, Borralho PM, Rodrigues CMP: **Convergence of miR-143 overexpression, oxidative stress and cell death in HCT116 human colon cancer cells**. *PloS one* 2018, **13**(1):e0191607.
12. Sheng N, Tan G, You W, Chen H, Gong J, Chen D, Zhang H, Wang Z: **MiR-145 inhibits human colorectal cancer cell migration and invasion via PAK4-dependent pathway**. *Cancer medicine* 2017, **6**(6):1331-1340.
13. Slattery ML, Mullany LE, Sakoda LC, Wolff RK, Samowitz WS, Herrick JS: **Dysregulated genes and miRNAs in the apoptosis pathway in colorectal cancer patients**. *Apoptosis : an international journal on programmed cell death* 2018, **23**(3-4):237-250.
14. Kent OA, Chivukula RR, Mullendore M, Wentzel EA, Feldmann G, Lee KH, Liu S, Leach SD, Maitra A, Mendell JT: **Repression of the miR-143/145 cluster by oncogenic Ras initiates a tumor-promoting feed-forward pathway**. *Genes & development* 2010, **24**(24):2754-2759.
15. Chen X, Guo X, Zhang H, Xiang Y, Chen J, Yin Y, Cai X, Wang K, Wang G, Ba Y *et al*: **Role of miR-143 targeting KRAS in colorectal tumorigenesis**. *Oncogene* 2009, **28**(10):1385-1392.
16. Mazza T, Mazzoccoli G, Fusilli C, Capocefalo D, Panza A, Biagini T, Castellana S, Gentile A, De Cata A, Palumbo O *et al*: **Multifaceted enrichment analysis of RNA-RNA crosstalk reveals cooperating micro-societies in human colorectal cancer**. *Nucleic acids research* 2016, **44**(9):4025-4036.
17. Chen K, Rajewsky N: **Natural selection on human microRNA binding sites inferred from SNP data**. *Nature genetics* 2006, **38**(12):1452-1456.
18. Gholami M, Larijani B, Sharifi F, Hasani-Ranjbar S, Taslimi R, Bastami M, Atlasi R, Amoli MM: **MicroRNA-binding site polymorphisms and risk of colorectal cancer: A systematic review and meta-analysis**. *Cancer medicine* 2019.

19. Wu S, Sun H, Wang Y, Yang X, Meng Q, Yang H, Zhu H, Tang W, Li X, Aschner M *et al*: **MALAT1 rs664589 Polymorphism Inhibits Binding to miR-194-5p, Contributing to Colorectal Cancer Risk, Growth, and Metastasis**. *Cancer research* 2019, **79**(20):5432-5441.
20. Schmit SL, Gollub J, Shapero MH, Huang SC, Rennert HS, Finn A, Rennert G, Gruber SB: **MicroRNA polymorphisms and risk of colorectal cancer**. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2015, **24**(1):65-72.
21. Luo X, Burwinkel B, Tao S, Brenner H: **MicroRNA signatures: novel biomarker for colorectal cancer?** *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2011, **20**(7):1272-1286.
22. Wan XB, Wang AQ, Cao J, Dong ZC, Li N, Yang S, Sun MM, Li Z, Luo SX: **Relationships among KRAS mutation status, expression of RAS pathway signaling molecules, and clinicopathological features and prognosis of patients with colorectal cancer**. *World journal of gastroenterology* 2019, **25**(7):808-823.
23. Ryan BM, Robles AI, Harris CC: **Genetic variation in microRNA networks: the implications for cancer research**. *Nature reviews Cancer* 2010, **10**(6):389-402.
24. Ward LD, Kellis M: **HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease**. *Nucleic acids research* 2016, **44**(D1):D877-881.
25. Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Park J, Hitz BC, Weng S *et al*: **Annotation of functional variation in personal genomes using RegulomeDB**. *Genome research* 2012, **22**(9):1790-1797.
26. Chuanyin L, Xiaona W, Zhiling Y, Yu Z, Shuyuan L, Jie Y, Chao H, Li S, Hongying Y, Yufeng Y: **The association between polymorphisms in microRNA genes and cervical cancer in a Chinese Han population**. *Oncotarget* 2017, **8**(50):87914-87927.
27. Li C, Zhang Y, Li Y, Ma Q, Liu S, Yao Y, Tan F, Shi L, Yao Y: **The association of polymorphisms in miRNAs with nonsmall cell lung cancer in a Han Chinese population**. *Cancer management and research* 2018, **10**:697-704.
28. Li L, Pan X, Li Z, Bai P, Jin H, Wang T, Song C, Zhang L, Gao L: **Association between polymorphisms in the promoter region of miR-143/145 and risk of colorectal cancer**. *Human immunology* 2013, **74**(8):993-997.
29. Ying HQ, Peng HX, He BS, Pan YQ, Wang F, Sun HL, Liu X, Chen J, Lin K, Wang SK: **MiR-608, pre-miR-124-1 and pre-miR26a-1 polymorphisms modify susceptibility and recurrence-free survival in surgically resected CRC individuals**. *Oncotarget* 2016, **7**(46):75865-75873.
30. Saini HK, Griffiths-Jones S, Enright AJ: **Genomic analysis of human microRNA transcripts**. *Proceedings of the National Academy of Sciences of the United States of America* 2007, **104**(45):17719-17724.
31. Han M, Zheng Y: **Comprehensive analysis of single nucleotide polymorphisms in human microRNAs**. *PloS one* 2013, **8**(11):e78028.
32. Chivukula RR, Shi G, Acharya A, Mills EW, Zeitels LR, Anandam JL, Abdelnaby AA, Balch GC, Mansour JC, Yopp AC *et al*: **An essential mesenchymal function for miR-143/145 in intestinal epithelial regeneration**. *Cell* 2014, **157**(5):1104-1116.
33. O'Brien SJ, Carter JV, Burton JF, Oxford BG, Schmidt MN, Hallion JC, Galandiuk S: **The role of the miR-200 family in epithelial-mesenchymal transition in colorectal cancer: a systematic review**. *International journal of cancer* 2018, **142**(12):2501-2511.
34. Pichler M, Ress AL, Winter E, Stiegelbauer V, Karbiener M, Schwarzenbacher D, Scheideler M, Ivan C, Jahn SW, Kiesslich T *et al*: **MiR-200a regulates epithelial to mesenchymal transition-related gene expression and determines prognosis in colorectal cancer patients**. *British journal of cancer* 2014, **110**(6):1614-1621.
35. Pan XM, Sun RF, Li ZH, Guo XM, Zhang Z, Qin HJ, Xu GH, Gao LB: **A let-7 KRAS rs712 polymorphism increases colorectal cancer risk**. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2014, **35**(1):831-835.
36. Jin H, Liang Y, Wang X, Zhu J, Sun R, Chen P, Nie X, Gao L, Zhang L: **Association between a functional polymorphism rs712 within let-7-binding site and risk of papillary thyroid cancer**. *Medical oncology* 2014, **31**(10):221.
37. Du XY, Hu YY, Xie C, Deng CY, Liu CY, Luo ZG, Niu YM, Shen M: **Significant association between Let-7-KRAS rs712 G > T polymorphism and cancer risk in the Chinese population: a meta-analysis**. *Oncotarget* 2017, **8**(8):13863-13871.
38. Schneiderova M, Naccarati A, Pardini B, Rosa F, Gaetano CD, Jiraskova K, Opattova A, Levy M, Veskrna K, Veskrnova V *et al*: **MicroRNA-binding site polymorphisms in genes involved in colorectal cancer etiopathogenesis and their impact on disease prognosis**. *Mutagenesis* 2017, **32**(5):533-542.
39. Dai Q, Wei HL, Huang J, Zhou TJ, Chai L, Yang ZH: **KRAS polymorphisms are associated with survival of CRC in Chinese population**. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2016, **37**(4):4727-4734.

Tables

Table 1 Baseline characteristics of study population

Characteristics	Cases（n=507）	Controls（n=497）	Statistics	*P value*
Age (years), (mean±SD)	62.55±11.88	62.75±11.99	0.264	0.792
Gender, N (%)				
Males	329（64.89%）	288（57.95%）	5.109	0.024
Females	178（35.11%）	209（42.05%）		
Former BMI (kg/m2), N (%)[]				
<18.5	18（3.56%）	15（3.27%）	13.038	0.001
18.5~23.9	298（58.89%）	219（47.71%）		
>=23.9	190（37.55%）	225（49.02%）		
Education level, N (%)				
Illiterate	63（12.45%）	25（5.20%）	63.428	<0.0001
Primary school	182（35.97%）	91（18.92%）		
Middle school and above	261（51.58%）	365（75.88%）		
Marital status, N (%)				
Married	504（99.41%）	412（84.08%）	78.418	<0.0001
Unmarried	3（0.59%）	78（15.92%）		
Family history of cancer, N (%)				
No	408（80.47%）	425（85.51%）	4.511	0.034
Yes	99（19.53%）	72（14.49%）		
History of appendicitis				
No	477（94.08%）	489（98.39%）	12.787	<0.0001
Yes	30（5.92%）	8（1.61%）		
Smoking, N (%)*				
No	324（63.91%）	338（69.12%）	0.353	0.552
Yes	183（36.09%）	151（30.88%）		
Alcohol consumption, N (%)*				
No	359（70.81%）	353（71.03%）	0.772	0.380
Yes	148（29.19%）	144（28.97%）		
Tea consumption, N (%)*				
No	230（45.36%）	239（48.09%）	0.046	0.830
Yes	277（54.64%）	258（51.91%）		

*Cochran-Mantel-Haenszel test, adjusted by sex

[] BMI of five years before investigation

Table 2 Genetic association analyses of selected SNPs with colorectal cancer risk

Gene symbol	Genotype	Cases (N%)	Controls (N%)	*OR*(95%*CI*) *	*P value*
KRAS	rs712				
	GG	301 (60.4%)	313 (64.1%)	1.0	
	GT	179 (35.9%)	159 (32.6%)	1.190(0.911-1.555)	0.201
	TT	18 (3.6%)	16 (3.3%)	1.184(0.591-2.372)	0.634
	G allele	781(78.4%)	785(80.4%)		
	T allele	215(21.6%)	191(19.6%)		
KRAS	rs1137196				
	CC	295 (59.7%)	299 (62.2%)	1.0	
	CA	180 (36.4%)	167 (34.7%)	1.108 (0.849-1.446)	0.452
	AA	19 (3.8%)	15 (3.1%)	1.303 (0.648-2.621)	0.458
	C allele	770 (77.9%)	765 (79.5%)		
	A allele	218 (22.1%)	197 (20.5%)		
miR-143	rs41291957				
	GG	208 (41.4%)	226 (46.1%)	1.0	
	GA	246 (48.9%)	210 (42.9%)	1.275(0.979-1.662)	0.072
	AA	49 (9.7%)	54 (11%)	0.977(0.634-1.505)	0.915
	G allele	662 (65.8%)	662 (67.5)		
	A allele	344 (34.2%)	318 (32.5)		
miR-145	rs74693964				
	CC	462 (91.1%)	470 (96.1%)	1.0	
	CT	45 (8.9%)	19 (3.9%)	2.414(1.385-4.206)	0.002
	C allele	969 (95.6%)	959 (98.1%)		
	T allele	45 (4.4%)	19 (1.9%)		
miR-145	rs80026971				
	GG	497 (98%)	487 (98.6%)	1.0	
	GC	10 (2%)	7 (1.4%)	1.220(0.457-3.258)	0.692
	C allele	1004 (99.0%)	981 (99.3%)		
	T allele	10 (1.0%)	7 (0.7%)		

* *OR*(95%*CI*) adjusted by age, sex and family history of cancer .

Table 3 Genetic association analyses of selected SNPs with colon cancer and rectal cancer risk

Gene symbol	Genotype	Colon Cancer（N=209）				Rectal Cancer（N=298）			
		Cases(N%)	Controls(N%)	*OR*(95%*CI*)*	*P value*	Cases(N%)	Controls(N%)	*OR*(95%*CI*) *	*P value*
KRAS	rs712								
	GG	118(57.8)	315(64.3)	1.0		183(62.2)	315(64.3)	1.0	
	GT	74(36.3)	159(32.4)	1.260(0.889-1.787)	0.194	105(35.7)	159(32.4)	1.147(0.842-1.562)	0.385
	TT	12(5.9)	16(3.3)	1.998(0.916-4.362)	0.082	6(2.0)	16(3.3)	0.654(0.250-1.712)	0.387
KRAS	rs1137196								
	CC	113(55.9)	301(62.3)	1.0		182(62.3)	301(62.3)	1.0	
	CA	78(38.6)	167(34.6)	1.269(0.897-1.796)	0.179	102(34.9)	167(34.6)	1.010(0.741-1.377)	0.949
	AA	11(5.4)	15(3.1)	1.943(0.864-4.372)	0.108	8(2.7)	15(3.1)	0.913(0.377-2.211)	0.840
miR-143	rs41291957								
	GG	88(42.3)	226(45.9)	1.0		120(40.7)	226(45.9)	1.0	
	GA	97(46.6)	212(43.1)	1.177(0.833-1.663)	0.355	149(50.5)	212(43.1)	1.367(1.005-1.860)	0.046

Table 4 Association of selected SNPs with colorectal cancer risk after stratification by smoking status

Gene symbol	Genotype	SMOKER				NON-SMOKER			
		Cases (N%)	Controls (N%)	*OR* (95%*CI*) *	*P value*	Cases (N%)	Controls (N%)	*OR* (95%*CI*) *	*P value*
KRAS	rs712								
	GG	108(60.3%)	100(66.7%)	1.0		193(60.5%)	209(63.0)	1.0	
	GT	66(36.9%)	48(32.0%)	1.361(0.848-2.184)	0.202	113(35.4%)	109(32.8)	1.112(0.799-1.546)	0.529
	TT	5(2.8%)	2(1.3%)	2.358(0.444-12.514)	0.314	13(4.1%)	14(4.2)	0.960(0.438-2.106)	0.920
	GT+TT	71(39.7%)	50(33.3%)	1.402(0.880-2.232)	0.155	126(39.5%)	123(37.0)	1.094(0.796-1.504)	0.579
Multiplicative Interaction								1.263(0.725-2.203)	0.410
KRAS	rs1137196								
	CC	105(59.7)	98(66.7)	1.0		190(59.7)	198(60.2)	1.0	
	CA	65(36.9)	47(32.0)	1.354(0.841-2.180)	0.212	115(36.2)	118(35.9)	1.003(0.723-1.391)	0.986
	AA	6(3.4)	2(1.4)	2.835(0.555-14.479)	0.210	13(4.1)	13(4.0)	1.001(0.449-2.229)	0.999
	CA+AA	71(40.3)	49(33.4)	1.415(0.887-2.259)	0.145	128(40.3)	131(39.9)	1.003(0.730-1.377)	0.987
Multiplicative Interaction								1.423(0.814-2.487)	0.215
miR-143	rs41291957								
	GG	78(43.1%)	66(44.0%)	1.0		130(40.4%)	157(46.9%)	1.0	
	GA	83(45.9%)	67(44.7%)	1.079(0.677-1.721)	0.748	163(50.6%)	141(42.1%)	1.397(1.007-1.936)	0.045
	AA	20(11.0%)	17(11.3%)	0.956(0.460-1.986)	0.905	29(9.0%)	37(11.0%)	0.918(0.533-1.580)	0.756
	GA+AA			1.053(0.677-1.640)	0.818			1.296(0.949-1.770)	0.103
Multiplicative Interaction								0.805(0.470-1.378)	0.429
miR-143	rs74693964								
	CC	167(91.3%)	140(94.0%)	1.0		295(91.0%)	324(97.0%)	1.0	
	CT	16(8.7%)	9(6.0%)	1.672(0.687-4.070)	0.257	29(9.0%)	10(3.0%)	3.086(1.468-6.484)	0.003
Multiplicative Interaction								0.473(0.153-1.462)	0.194
miR-145	rs80026971								
	GG	181(98.9%)	150(99.3)	1.0		316(97.5%)	331(98.2%)	1.0	
	GC	2(1.1%)	1(0.7)	1.290(0.113-14.738)	0.838	8(2.5%)	6(1.8%)	1.199(0.406-3.539)	0.742
Multiplicative Interaction								1.141(0.080-16.168)	0.922

* *OR*(95%*CI*) adjusted by age, sex and family history of cancer

Table 5 Genetic association analyses of number of at-risk genotypes within rs41291957 and rs74693964 with colon cancer and rectal cancer risk

Number of at-risk genotypes	Colon Cancer				Rectal Cancer				Colorectal	
	Cases(N%)	Controls(N%)	*OR*(95%*CI*)*	*P value*	Cases(N%)	Controls(N%)	*OR*(95%*CI*)*	*P value*	Cases(N%)	Controls(N%
0	106 (51.0)	270 (55.4)	1.0		136 (46.1)	270 (55.4)	1.0		242 (48.1)	270 (55.4)
1	92 (44.2)	206 (42.3)	1.151(0.824-1.607)	0.411	139 (47.1)	206 (42.3)	1.378(1.026-1.874)	0.033	231 (45.9)	206 (42.3)
2	10 (4.8)	11 (2.3)	2.242(0.918-5.475)	0.076	20 (6.8)	11 (2.3)	3.738(1.725-8.101)	0.001	30 (6.0)	11 (2.3)
P_{trend}				0.128				0.001		

* *OR*(95%*CI*) adjusted by age, sex and family history of cancer

Table 6 Kaplan–Meier survival estimation of mean survival and hazard ratios (HRs) of selected SNPs

Gene symbol	Genotypes	N%	Mean Survival (in months)	Log rank, *P value*	*HR*(95%*CI*)*, *P value*
KRAS	rs712				
	GG	137(62.5)	51.94	0.044	1.0
	GT	74(33.8)	48.66		1.672(0.815-3.432), 0.161
	TT	8(3.7)	37.00		4.328(1.236-15.147), 0.022
KRAS	rs1137196				
	CC	131(61.5)	52.28	0.098	1.0
	CA	75(35.2)	47.49		1.860(0.913-3.788),0.088
	AA	7(3.3)	41.29		3.030(0.676-13.578),0.147
miR-143	rs41291957				
	GG	93(42.5)	47.49	0.123	1.0
	GA	105(47.9)	52.07		0.573(0.280-1.174),0.128
	AA	21(9.6)	48.82		0.375(0.078-1.613),0.188
miR-145	rs74693964				
	CC	201(90.5)	51.14	0.441	1.0
	CT	21(9.5)	49.43		0.491(0.117-2.056),0.330
miR-145	rs80026971				
	GG	218(98.2)	51.34	0.557	1.0
	GC	4(1.8)	41.25		1.481(0.195-11.235),0.704

**HR*(95%*CI*) adjusted by age, sex, tumor stage

Figures

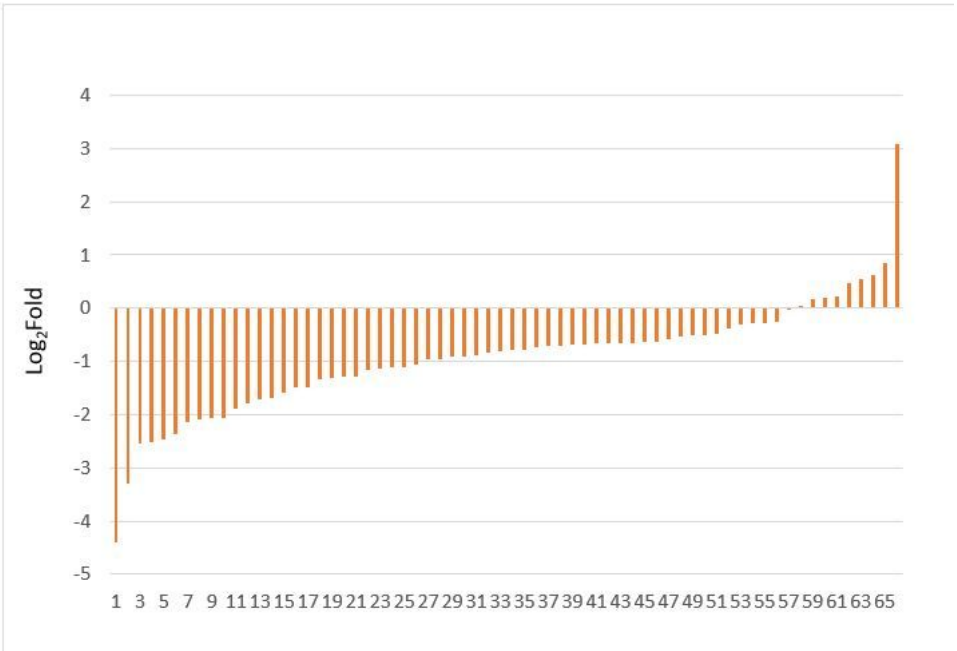

Figure 1

Fig.1 miR-143 expression in rectal cancer and matched normal mucosa in the rectal cancer study identified from the GEO profile database(accession number GSE38389). Of the 66 Tumor biopsies and corresponding matched mucosa sample, 50 were under-expressed (log2-fold difference<-0.5) and 4 highly expression(log2-fold difference>0.5) in rectal cancer tissue compared with adjacent normal controls(P value for paired t test <0.001)

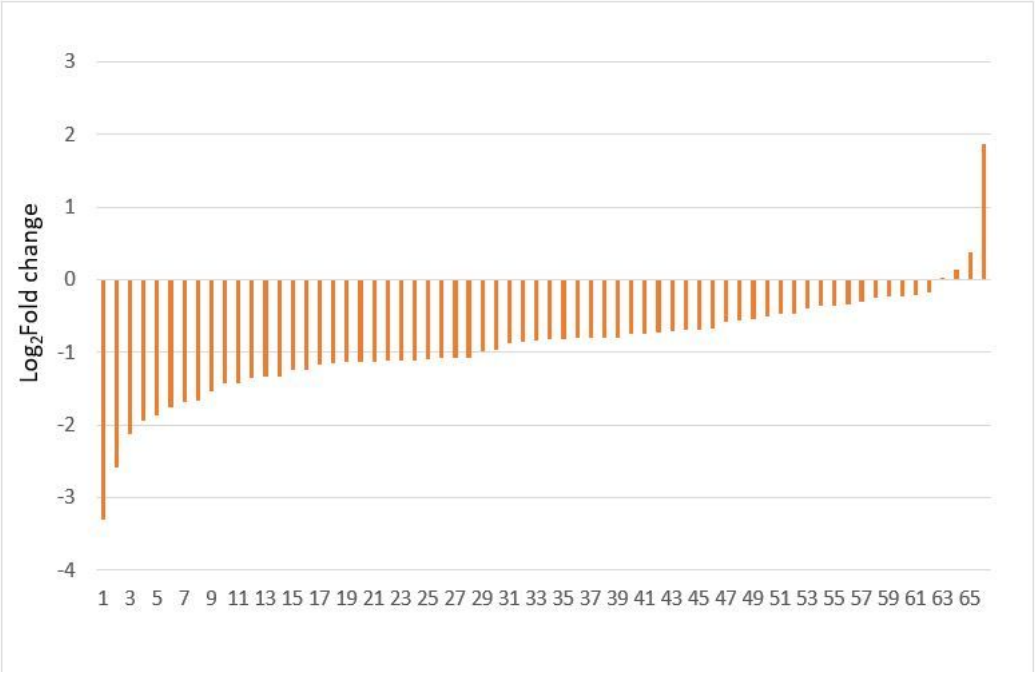

Figure 2

MiR-145 Expression in Pairs Samples for Rectal cancer - Normal Mucosa

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- DescriptionsofselectedSNPsofmiR.docx