MicroRNA gene polymorphisms and the risk of colorectal cancer

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Abstract. The present study was carried out to demonstrate the epidemiological value of microRNA (miRNA) in colorectal cancer (CRC) by investigating the association between miRNA gene polymorphisms and the susceptibility to CRC. Multiple meta-analyses of reported data were conducted, and odds ratio values and 95% confidence intervals were used to assess these associations. Stata 11.0 software was used to analyze the data and the modified Jadad quality score was employed to evaluate the quality of the retrieved studies. We retrieved 38 studies on the association between miRNA polymorphisms and risk of CRC, however only 15 met the requirements of the inclusion criteria. In conclusion, we identified a variety of miRNAs (miRNA-let-7, miR-34b/c, miR-146a, miR-603 and miR-149) gene polymorphisms that are associated with susceptibility to CRC. However, some miRNAs (miR-192a, miR-608 and miR-27a) are associated with CRC, but not susceptibility to CRC. The results have limitations given the relatively low number of studies available. Therefore, it is necessary to collect data from large sample-size studies to further validate the results.

Introduction

Colorectal cancer (CRC) is a common malignant tumor that occurs worldwide. According to statistics, both the incidence and mortality of CRC rank third among all forms of cancer in the United States, even though the mortality rates have declined. The vast majority of cases (90%) occur in individuals over 50 years of age (1). Apart from hereditary CRC, the development of this cancer type is poorly understood (2,3). Both germline and somatic genetic variations have been suggested as contributing factors in CRC development (4-6).

MicroRNA (miRNA) refers to a group of small non-coding RNAs that are ~22 (18-25) nucleotides in length and which regulate RNA expression at the translational level (7-9). miRNAs have been associated with a variety of diseases, including different forms of cancer. Increasing evidence has confirmed the importance of miRNAs in regulating common biological characteristics/processes of different tumors, such as: self-growth signals, insensitivity to anti-growth signals, abnormal apoptosis, unlimited replication potential, sustained induction of angiogenesis, invasion, and metastasis (10). Many researchers have identified tumor-specific miRNA signatures which accurately distinguish malignant tumors from several different parts of the benign tissue, and their results showed that some miRNAs are carcinogenic depending on other genetic mutations present in tumors (11). miRNA species can directly affect cell proliferation and apoptosis of tumor cell lines. Furthermore, many studies have confirmed the link between abnormal miRNA expression and abnormalities in intracellular signal transduction pathways and tumorigenesis (12-16). For example, miR-9 is activated by YC/MYCN, which induces cancer metastasis by regulating the expression of metastasis suppressor protein, E-cadherin (17), and miR-449a can cause retinoblastoma (Rb)-dependent cell cycle arrest and cellular senescence of prostate cancer (18).

miRNAs constitute a new class of molecules that through interaction with oncogenes and/or tumor suppressor genes, can promote the formation of cancer (19). However, different miRNAs regulate different signaling pathways and different target proteins/genes that affect the biological changes characteristic of cancer.

Previous findings showed that human miRNAs with single-nucleotide polymorphisms (SNPs) can be targets of genetic variation in human genomic DNA sequences and may be related to susceptibility of human disease (20). miRNA SNPs have inter-individual differences in disease diagnosis, treatment, and prognosis. Recent large-scale studies reported significant risks associated with different germline variations for development of CRC (5). In addition, novel studies suggest

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Table I. Characteristics of the 15 studies included in the meta-analysis.

miRNA	SNP ID	Author (Refs.)	Year	Ethnicity	Study design	Genotyping method	Allele	Case no.	Control no.	HWE (P-value)	Jadad
miRNA-146a	rs2910164	Min *et al* (24)	2012	Asian	PB	PCR-RFLP	C/G	446	502	0.44	7
		Hezova *et al* (25)	2012	Caucasian	HB	TaqMan	C/G	197	212	0.41	7
		Ma *et al* (26)	2013	Asian	HB	TaqMan	C/G	1,147	1,203	0.075	7
		Lv *et al* (27)	2013	Asian	HB	PCR-RFLP	C/G	353	540	0.08	7
		Vinci *et al* (28)	2013	Caucasian	PB	HRM	C/G	160	178	0.11	6
		Hu *et al* (29)	2014	Asian	HB	PCR-RFLP	C/G	276	373	0.14	7
		Parlayan *et al* (30)	2014	Asian	HB	TaqMan	C/G	116	524	0.75	7
miRNA-149	rs2292832	Min *et al* (24)	2012	Asian	PB	PCR-RFLP	C/T	446	502	0.17	7
		Lv *et al* (27)	2013	Asian	HB	PCR-RFLP	C/T	353	540	<0.05	7
		Vinci *et al* (28)	2013	Caucasian	PB	HRM	C/T	160	178	0.91	6
miRNA-196a2	rs11614913	Min *et al* (24)	2012	Asian	PB	PCR-RFLP	T/C	446	502	0.63	7
		Hezova *et al* (25)	2012	Caucasian	HB	TaqMan	T/C	197	212	0.81	7
		Lv *et al* (27)	2013	Asian	PB	PCR-RFLP	T/C	353	540	<0.05	7
		Vinci *et al* (28)	2013	Caucasian	PB	HRM	T/C	160	178	0.09	6
		Parlayan *et al* (30)	2014	Asian	HB	TaqMan	T/C	116	524	0.78	7
		Zhu *et al* (31)	2012	Asian	HB	TaqMan	T/C	573	588	0.17	7
		Zhan *et al* (32)	2011	Asian	HB	PCR-RFLP	T/C	252	543	0.77	7
		Chen *et al* (33)	2012	Asian	HB	PCR-LDR	T/C	126	407	0.82	7
miRNA-27a	rs895819	Hezova *et al* (25)	2012	Caucasian	HB	TaqMan	G/A	197	212	0.78	7
		Wang *et al* (34)	2014	Asian	HB	TaqMan	G/A	205	455	<0.05	7
miRNA-34b/c	rs4938723	Gao *et al* (35)	2013	Asian	HB	PCR-RFLP	T/C	347	488	0.83	7
		Oh *et al* (36)	2014	Asian	PB	PCR-RFLP	T/C	545	428	0.4	7
miRNA-let-7	rs712	Pan *et al* (37)	2014	Asian	HB	PCR-RFLP	G/T	339	313	0.41	7
miRNA-603	rs11014002	Wang *et al* (38)	2014	Asian	HB	Sequenom mass	C/T	102	204	0.59	7
miRNA-608	rs4919510	Ryan *et al* (39)	2012	Caucasian	PB	TaqMan	C/G	245	446	0.94	7

PB, population-based case-control studies; HB, hospital-based case-control studies; PCR-RFLP, polymerase chain reaction with restriction fragment length polymorphism; HRM, high resolution melt; SNP, single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; Jadad, modified Jadad score.

a potential influence of miRNA SNPs on the risk of cancer development.

Therefore, the aim of the present study was to investigate the correlation between miRNA gene polymorphisms and the susceptibility to CRC by reviewing the literature.

Materials and methods

Screening and identification of relevant studies. We identified eligible and relevant studies by performing searches with the terms 'miRNA/microRNA', 'colorectal cancer', 'genotype', 'polymorphism', and 'variant' in the PubMed, Ovid, and Embase databases, as well as the Cochrane Library. The search was limited to English language studies and only published studies with full text were included. We evaluated potentially relevant publications by manually examining their titles and abstracts. The selected studies in our meta-analysis also matched the following inclusion criteria: i) Assessment of miRNA polymorphisms and the risks of suffering from CRC; ii) an individual case-control study in humans; iii) statistically sufficient genotype data by odds ratio (OR) values and 95% confidence intervals (CI); and iv) full-text search. Exclusion criteria were: i) Lack of controlled studies; ii) repeat of previous literature; iii) summaries, comments, reviews and editorials; and iv) focus on benign CRC tumors.

Data extraction and study characteristics. Two researchers independently extracted the data that met the above inclusion criteria and differences were resolved through discussion. For each study, the following information was extracted: Last name of first author, year of publication, ethnicity of patients in different studies, miRNA type, SNP ID, source of research, genotyping method, number of cases and controls, number of various genotypes among cases and controls, and the Hardy-Weinberg equilibrium of control subjects. If a study did not provide complete data, we sent a request to the corresponding author for the data. A total of 15 eligible studies met the inclusion criteria (Table I).

Quality assessment. Two researchers evaluated the uniform quality of studies which met the inclusion criteria, and

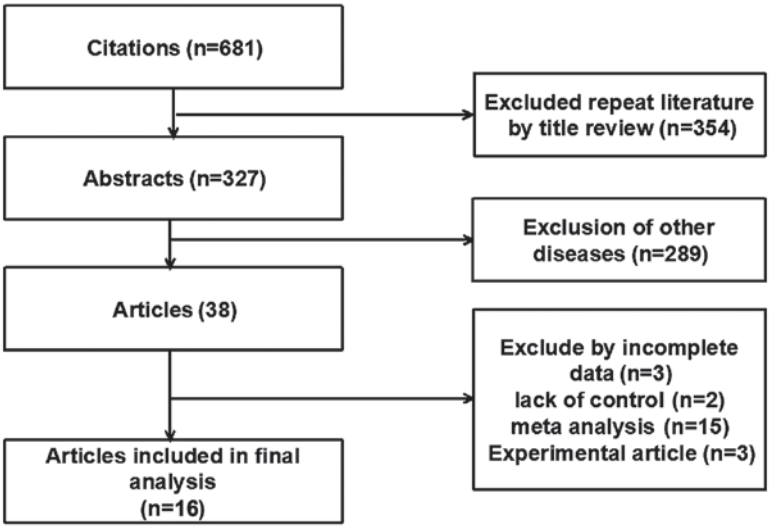

Figure 1. Flow chart of study selection.

cross-checked in case of disagreement, which was eventually resolved by discussion. The quality of the included studies was assessed using a modified Jadad score (21), where 1-3 points was considered a low quality study, and 4-7 points was considered high-quality research. Evaluation included whether to generate a random sequence by hiding the proper randomized allocation; whether the study was blinded, and whether the patients were lost or quit the study (Table I).

Statistical analysis. According to the genotype frequencies of cases and controls, the correlation between miRNA polymorphisms and risk of CRC was assessed via OR values with 95% CI. We performed statistical analyses of OR values and 95% CI by assessing five different genetic parameters: The allele, the dominant genetic model, the recessive genetic model, the homozygous comparison, and the heterozygous comparison. The Chi-square based Q statistic was used to assess heterogeneity between studies and $P<0.05$ was considered to indicate significant heterogeneity between studies. The I^2 index was expressed as a percentage for the total variability throughout the study. I^2 value of 25, 50 and 75% indicated low, medium, and high heterogeneity, respectively. Funnel plots were used to assess publication bias. When the effects were assumed to be homogeneous, the fixed-effects model was used (Mantel-Haenszel method). If heterogeneity was present, the random-effects model was applied (DerSimonian and Laird method) to account for inter-study heterogeneity instead of the fixed-effect model. Data were analyzed with Stata 11.0 software (StataCorp, College Station, USA) and all P-values were two-sided tests (22,23).

Results

Study characteristics. A total of 681 studies relevant to the search words were identified and only 38 studies were on the association between CRC and miRNA polymorphisms. According to the aforementioned inclusion and exclusion criteria, 16 publications (four using population-based controls and 11 using hospital-based controls) were included in the final meta-analysis (24-39) (Fig. 1). Of the 15 articles, seven related to miR-146a; three were on miRNA-149; eight were on miRNA-196a2; there were two each related to miRNA-27a and miRNA-34b/c; and there was one each on miRNA-let-7, miRNA-603, and miRNA-608. The main characteristics of the studies included in the meta-analysis are summarized in Table I.

Overall analyses. Table II shows that the overall analysis of all the studies revealed a statistically significant positive association between miRNA-let-7, miR-34b/c, miR-146a, and miR-149 polymorphisms and the risk of CRC. However, miR-196a2 (31-33), miR-27 (34), miR-603 (38) and miR-608 (39) did not correlate with the risk of CRC. Only significantly different results follow.

Analysis of miR-146a polymorphisms and susceptibility to CRC. i) Seven studies (24-30) reported the association between G/C alleles and susceptibility to CRC, there was no significant heterogeneity between studies (I^2=26.4%, P=0.227), and the fixed-effect model was used. The total analysis showed that individuals with the G allele are more susceptible to CRC than with C (OR=1.09, 95% CI=1.01-1.18, P=0.02). ii) Seven studies (24-30) reported the association between the recessive genetic model, GG/(CC+GC), and susceptibility to CRC, there was no significant heterogeneity between studies (I^2=28.9%, P=0.207), and the fixed-effect model was used. The total analysis showed that people with the recessive genetic model, GG, are more susceptible to CRC than (CC+GC) (OR=1.13, 95% CI=1.00-1.28, P=0.047). iii) Seven studies (24-30) reported the association between homozygous GG/CC and the susceptibility to CRC, there was no significant heterogeneity between studies (I^2=25.2%, P=0.237), and the fixed-effect model was used. The total analysis showed that people with homozygous GG are more susceptible to CRC than CC (OR=1.19, 95% CI=1.01-1.41, P=0.042).

Analysis of miRNA-149 polymorphisms and susceptibility to CRC. Five studies (24-28) reported the association between the recessive genetic model, TT/(TC+CC), and susceptibility to CRC, there was no significant heterogeneity between studies (I^2=0, P=0.582), and the fixed-effect model was used. The total

Table II. Analysis of the association between 8 miRNA gene polymorphism and risk of CRC.

miRNA	Contrast	N[a]	P-value[b]	OR (95% CI)	I^2 (%)	P-value[c]	P-value[d]
miRNA-146a		7					
	G/C		0.02	1.09 (1.01-1.18)	26.4	0.227	0.036
	GC+GG/CC		0.068	1.13 (0.99-1.27)	75.1	0.001	0.527
	GG/CC+GC		0.047	1.13 (1.00-1.28)	28.9	0.207	0.155
	GG/CC		0.042	1.19 (1.01-1.41)	25.2	0.237	0.141
	GC/CC		0.191	1.09 (0.94-1.24)	79.8	<0.001	0.646
miRNA-149		3					
	T/C		0.082	1.13 (0.96-1.3)	0	0.533	0.805
	TT+CT/CC		0.871	1.02 (0.79-1.31)	0	0.655	0.06
	TT/TC+CC		0.025	1.24 (1.03-1.5)	0	0.582	0.332
	TT/CC		0.241	1.19 (0.89-1.6)	0	0.591	0.4
	TC/CC		0.382	0.88 (0.67-1.16)	0	0.89	0.191
miRNA-196a2		8					
	T/C		0.747	1.01 (0.94-1.1)	89.2	<0.001	0.595
	TT+TC/CC		0.656	1.03 (0.9-1.17)	87.6	<0.001	0.011
	TT/TC+CC		0.898	1.01 (0.89-1.15)	83	<0.001	0.636
	TT/CC		0.763	1.03 (0.87-1.21)	90.2	<0.001	0.061
	TC/CC		0.49	1.05 (0.91-1.2)	84.7	<0.001	0.023
miRNA-27a		2					
	G/A		0.057	1.19 (0.99-1.43)	66.4	0.085	-
	(GG+GA)/AA		0.221	1.14 (0.9-1.55)	48.1	0.165	-
	GG/(GA+AA)		0.082	1.3 (0.97-1.74)	3.3	0.309	-
	GG/AA		0.071	1.38 (0.97-1.95)	39	0.201	-
	GA/AA		0.569	1.09 (0.81-1.47)	0	0.408	-
miRNA-34b/c		2					
	T/C		0.058	1.15 (1-1.33)	50.9	0.154	-
	TT+TC/CC		0.016	1.49 (1.08-2.06)	0	0.519	-
	TT/(TC+CC)		0.288	1.11 (0.92-1.33)	50	0.157	-
	TT/CC		0.015	1.52 (1.08-2.13)	0	0.342	-
	TC/CC		0.032	1.46 (1.03-2.05)	0	0.812	-
miRNA-let-7		1					
	T/G		0.003	1.49 (1.15-1.94)	-	-	-
	TT+TG/GG		0.015	1.48 (1.08-2.03)	-	-	-
	TT/TG+GG		0.015	2.52 (1.19-5.31)	-	-	-
	TT/GG		0.007	2.81 (1.32-5.98)	-	-	-
	TG/GG		0.074	1.35 (0.97-1.88)	-	-	-
miRNA-603		1					
	C/T		0.154	0.74 (0.50-1.12)	-	-	-
	CC+CT/TT		0.517	1.05 (0.59-1.87)	-	-	-
	CC/CT+TT		0.152	0.94 (0.69-1.29)	-	-	-
	CC/TT		0.398	1.02 (0.56-1.85)	-	-	-
	CT/TT		0.778	1.10 (1.60-2.02)	-	-	-
miRNA-608		1					
	C/G		0.829	0.97 (0.76-1.25)	-	-	-
	CG+CC/GG		0.869	1.05 (0.59-1.87)	-	-	-
	CC/CG+GG		0.716	0.94 (0.69-1.29)	-	-	-
	CC/GG		0.956	1.02 (0.56-1.85)	-	-	-
	CG/GG		0.769	1.10 (0.59-2.02)	-	-	-

[a]Number of comparison. [b]P-value of test for overall effect. [c]P-value of heterogeneity. [d]Egger's test. miRNA, microRNA; CRC, colorectal cancer; OR, odds ratio; CI, confidence interval.

analysis showed that people with the recessive genetic model, TT, are more susceptible to CRC than (TC+CC) (OR=1.24, 95% CI=1.03-1.5, P=0.025).

Analysis of miRNA-34b/c polymorphisms and susceptibility to CRC. i) Two studies (35,36) reported the association between the dominant genetic model, TT+TC/CC, and susceptibility to CRC, there was no significant heterogeneity between studies (I^2=0, P=0.519), and the fixed-effect model was used. The total analysis showed that people with the dominant model, TT+TC, are more susceptible to CRC than CC (OR=1.49, 95% CI=1.08-2.06, P=0.016). ii) Two studies (34,35) reported the association between the homozygous model, TT/CC, and susceptibility to CRC, there was no significant heterogeneity between studies (I^2=0, P=0.342), and the fixed-effect model was used. The total analysis showed that people with the homozygous model, TT, are more susceptible to CRC than CC (OR=1.52, 95% CI=1.08-2.13, P=0.015). iii) Two studies (34,35) reported the association between the heterozygous model, TC/CC, and susceptibility to CRC, there was no significant heterogeneity between studies (I^2=0, P=0.812), and the fixed-effect model was used. The total analysis showed that people with the heterozygous model, TC, are more susceptible to CRC than CC (OR=1.46, 95% CI=1.03-2.05, P=0.032).

Analysis of miRNA-let-7 polymorphisms and susceptibility to CRC. i) One study (37) reported the association between the allele, T/G, and susceptibility to CRC. The analysis showed that people with allele T are more susceptible to CRC than G (OR=1.49, 95% CI=1.15-1.94, P=0.003). ii) One study (37) reported the association between the dominant genetic model, TT+TG/GG, and susceptibility to CRC. The analysis showed that people with the dominant genetic model, TT+TG, are more susceptible to CRC than GG (OR=1.48, 95% CI=1.08-2.03, P=0.015). iii) One study (37) reported the association between the recessive genetic model, TT/GG+TG, and susceptibility to CRC. The analysis showed that people with the recessive genetic model, TT, are more susceptible to CRC than GG+TG (OR=2.52, 95% CI=1.19-5.31, P=0.015). iv) One study (37) reported the association between the homozygous model, TT/GG, and susceptibility to CRC. The analysis showed that people with the homozygous model, TT, are more susceptible to CRC than GG (OR=2.81, 95% CI=1.32-5.98, P=0.007).

These results indicate that miRNA-let-7, miR-34b/c, miR-146a, and miR-149 polymorphisms were positively correlated with CRC.

Statistical sensitivity. Statistical sensitivity where one study was removed and the rest were analyzed, the pooled relative risks (RRs) were similar with the overall pooled RRs (data not shown), supporting the robustness of our results.

Publication bias. Egger's test was used to assess the publication bias of the studies (Table II). The results show some evidence of publication bias in some comparisons.

Discussion

At present, miRNA gene polymorphisms and susceptibility to a variety of tumors have been reported. De Ruyck *et al* found that miRNA-let-7 polymorphisms in the KRAS 3'-UTR are a prognostic factor of oropharyngeal cancer (40). Another study showed that miR-146aG>C and miR-196a2C>T polymorphisms were associated with the risk of hepatocellular carcinoma (HCC) in patients in China, especially in patients with hepatitis B virus infection (41). Palmieri *et al* found that miR-146a polymorphisms are not associated with tumor development in oral squamous cell carcinoma. However, a slight increase in the frequency of the variant allele was observed in stage II tumors (42).

There are also several meta-analyses that statistically correlated with miRNAs and cancer risk. Some meta-analysis studies evaluated the correlation between one miRNA and a single cancer susceptibility. Wang *et al* confirmed the association of the polymorphism, miR-196a2 rs11614913, with the risk of CRC, but not with tumor stage and grade (43). Some meta-analysis studies evaluated the correlation between one miRNA and a variety of cancer susceptibilities. Tao *et al* suggested that the hsa-miR-34b/c rs4938723 polymorphism may play opposite roles in different types of cancer based on the present studies, and a subgroup analysis revealed that the variant CT genotypes were associated with an increased risk of HCC compared with the wild-type TT genotype. However, a decreased risk of CRC was found in the genetic model of CC/TT and CC/CT+TT (44). Li *et al* reported that the miR-146a rs2910164 polymorphism may decrease the susceptibility of digestive system cancers, especially in the Asian population (45). Some meta-analysis studies evaluated the correlation between a variety of miRNAs and a variety of cancer susceptibilities. For example, one meta-analysis provided evidence that the miR-196a2 rs11614913 polymorphism is associated with an increased cancer risk and rs2910164 in miR-146a may be associated with susceptibility to papillary thyroid carcinoma and cervical cancer (46). In addition, some studies analyzed the correlation between a variety of miRNAs and a specific cancer susceptibility. Dikeakos *et al* investigated the association of the miR-146aC>G, miR-149T>C, and miR-196a2T>C polymorphisms with the risk of gastric cancer and survival in the Greek population (47). They found that the risk of gastric cancer was significantly higher in carriers of miR-149 rs2292832CC and miR-196a2 rs11614913CC genotypes, as well as for carriers of the rs2910164/rs2292832/rs11614913 CCC and GTC haplotype. The rs2910164/rs2292832/rs11614913 CTT and CCT haplotypes appear to have a protective role against the development of gastric cancer. Their data demonstrate that specific miRNA SNPs are associated with gastric cancer susceptibility in the Greek population (47).

In the present study, we performed statistical analyses of the relationships between miRNA polymorphisms and CRC. We investigated the effects of miRNA-196a2, miRNA-146a, miRNA-27a, miRNA-34b/c, miRNA-let-7, miRNA-603, miRNA-608 and miRNA-149 on CRC susceptibility. This was a more comprehensive statistical analysis of the various miRNAs that affect the risk of CRC. However, regardless of whether the aforementioned miRNA and risk of CRC risk are highly relevant, they are combined with other factors such as *Helicobacter pylori* (48), smoking (49,50), age (49,50), and drugs, which can lead to increased cancer risk. Therefore, stratified analysis is necessary. In addition, the limitation of this study is that most enrolled studies involved Asian

poplations, and the results may not be generalized to the global population. Therefore, we need a larger number of samples for statistical analysis, and more data on the relationship between miRNA gene polymorphisms and CRC among non-Asian populations, to make more reliable conclusions.

In conclusion, we statistically analyzed the relationship between miRNA gene polymorphisms and CRC prevalence through a meta-analysis of several publications. miRNA-let-7, miR-34b/c, miR-146a, miR-603, and miR-149 gene polymorphisms can significantly increase the risk of CRC, while miR-192a and miR-27a polymorphisms are not related to the risk of CRC. Analyses of a larger number of samples are required.

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