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MicroRNA variants and colorectal cancer risk: a meta-analysis

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ABSTRACT. Colorectal cancer (CRC) is a multi-factorial disease, and genetic background may contribute to its etiology. Single nucleotide polymorphisms (SNPs) in microRNAs (miRNAs) may be used as specific markers of predisposition for CRC diagnosis and prevention.

In this review, we summarize and discuss recent publications evaluating the roles of miRNA SNPs in CRC. A meta-analysis was also carried out to assess the association between the five most frequently studied miRNA SNPs and CRC risk. No relationship was established between this disease and the three SNPs rs11614913, rs2910164, and rs3746444 in miR-196a-2, miR-146a, and miR-499, respectively. However, polymorphisms of miR-149 (rs2292832; CT *vs* TT: odds ratio [OR] = 0.816, 95% confidence interval [CI] = 0.691-0.963; CC+CT *vs* TT: OR = 0.834, 95%CI = 0.715-0.972) and pre-miR-27a (rs895819; GG *vs* AA: OR = 1.534, 95%CI = 1.148-2.049; GG+AG *vs* AA: OR = 1.324, 95%CI = 1.066-1.645) were found to be associated with CRC in our analysis. In conclusion, the SNPs rs2292832 in miR-149 and rs895819 in pre-miR-27a were associated with CRC susceptibility, whereas rs11614913, rs2910164, and rs3746444 in miR-196a-2, miR-146a, and miR-499, respectively, were not. Further studies should be carried out to validate these findings.

Key words: MicroRNA; Single nucleotide polymorphism; Colorectal cancer; Review; Meta-analysis

INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed malignancy in men and the second among women. In 2012, approximately 1.4 million cases were diagnosed, and 693,900 CRC-related deaths occurred (Torre et al., 2015). In China, an estimated 310,244 cases and 149,722 deaths were recorded in 2011, placing CRC among the top five malignancies for incidence and mortality (Chen et al., 2015). To date, the exact mechanisms responsible remain obscure, and efficient diagnostic methods and treatments for this disease are lacking. Thus, there exists an urgent need to develop new strategies to diagnose and target individuals at high risk of CRC.

CRC is a multi-factorial disease. Genetic background, environmental factors, and gene-environment interactions all contribute to its etiology. Many studies have been carried out to identify genetic variations that might be used for CRC diagnosis and prognostic assessment. Two single nucleotide polymorphisms (SNPs) in miR-608 and miR-219-1 are reported to be associated with CRC survival and recurrence, and thus might be useful in predicting therapy response (Pardini et al., 2015). In addition, a microarray analysis of 146 CRC cases revealed that the rs6707530 SNP in the fibronectin 1 gene may be helpful in diagnosing this condition (Kida et al., 2014). Zhi et al. (2015) also reported serum miR-29a level to be a promising CRC biomarker, with a diagnostic specificity of 0.89.

MicroRNAs (miRNAs) are small, non-coding, single-stranded RNAs of 21-24 nucleotides capable of binding complementary sequences within target mRNAs for post-transcriptional regulation of their functions as tumor suppressors or oncogenes. SNPs are common variants in the human genome and have been reported to influence disease susceptibility. SNPs in miRNAs may alter miRNA processing or expression, or influence mRNA function, potentially contributing to cancer susceptibility. Therefore, miRNA polymorphisms may be used as specific markers of predisposition for CRC diagnosis and prevention.

To date, several reviews analyzing the relationship between miRNA polymorphisms and cancer risk have been published (Du et al., 2013; Xie et al., 2014). However, these articles have focused on only one or a small number of selected miRNA SNPs. A comprehensive analysis of all reported CRC-related miRNAs is lacking. In this review, we aimed to assemble an extensive list of all miRNA SNPs with potential roles in CRC, carry out a meta-analysis of the most frequently studied miRNAs, and discuss the underlying mechanisms by which such polymorphisms affect CRC susceptibility.

Differentially expressed miRNAs in CRC

Accumulating evidence strongly indicates that aberrant miRNA expression is an important feature of CRC. A recent study measured the differential expression of miRNAs in colorectal adenocarcinoma tissues from 28 patients, and analyzed their profiles at various differentiation stages. The levels of 1547 miRNAs were detected by quantitative real-time polymerase chain reaction, 93 of which were found to be significantly dysregulated in these malignant tissues. In particular, miR-1, miR-145, and miR-145* were shown by receiver operating characteristic analysis to be potential biomarkers for CRC diagnosis. Furthermore, 58 miRNAs demonstrated significantly altered expression between well- and moderately differentiated cancers, and 32 could be used to distinguish normal from cancerous tissues, as well as different levels of differentiation (Wu et al., 2015). Gu et al. (2014) proposed a gene module-based approach to infer the identity of key miRNAs involved in CRC. miR-101, miR-124, and miR-139 were found to be frequently down-regulated in this disease, and using this approach, the latter was determined to be a key tumor suppressor in early cancer development. Using a microarray, Yong et al. (2013) identified seven miRNAs differentially expressed in both CRC tissue and blood samples, and indicated that the triple miRNA classifier comprising miR-193a-3p, miR-23a, and miR-338-5p is a potential blood biomarker for early CRC detection. According to various miRNA-profiling studies, several miRNAs have been validated as potential oncogenes or tumor suppressor genes in CRC. SNPs in miRNAs and related loci are often located in miRNA primary (pri-) and precursor (pre-) sequences, seed sequences, and the 3'-untranslated regions of target genes (Landi et al., 2012). Thus, such polymorphisms might influence miRNA function in three ways: by altering transcription of the primary transcript, affecting pri- and pre-miRNA processing, and modifying miRNA-miRNA interactions (Ryan et al., 2010). SNPs in miRNAs may ultimately result in the alteration of their expression and/or maturation, with possible consequences for cancer development and progression. Furthermore, approximately half of miRNA genes are located in cancer-related regions (Garzon et al., 2010). Thus, variations in these sequences may result in significant functional consequences, making them ideal candidates for cancer risk prediction. Many have been validated as potential oncogenes or tumor suppressor genes in CRC, such as miR-143, miR-145, miR-21, and miR200c (Schetter and Harris, 2011). miRNA expression profile analyses indicate that these sequences play an etiological role in the initiation and progression of this condition. Therefore, the study of miRNAs may provide a better understanding of the molecular mechanisms responsible for CRC pathology, and assist in the development of CRC diagnosis and treatment approaches.

miRNA SNPs and their association with CRC

SNPs in miRNAs can affect hundreds of mRNAs, since it is estimated that mature

miRNAs regulate around 30% of human genes. Since the first report of their existence, several studies have been carried out to identify these variants and explore their associations with cancer.

Recently, numerous miRNA sequence variations have been implicated in CRC (Lv et al., 2013; Cao et al., 2014). However, the results of many such studies are conflicting and inconclusive. In the present study, we focused on the relationship between miRNA polymorphisms and CRC. A systematic review was carried out to comprehensively analyze and integrate all published studies concerning miRNA SNPs in relation to CRC risk.

MATERIAL AND METHODS

Selection of published studies

We searched the PubMed database (most recently on June 30, 2015) for all articles relevant to the association between miRNA SNPs and CRC. The following search terms were used: "miRNA polymorphism, miRNA SNP, or miRNA variant" and "colorectal cancer or colorectal tumor". Additional eligible studies were identified by a manual search of the references included in retrieved articles (Figure 1). Authors of the selected papers were not contacted.

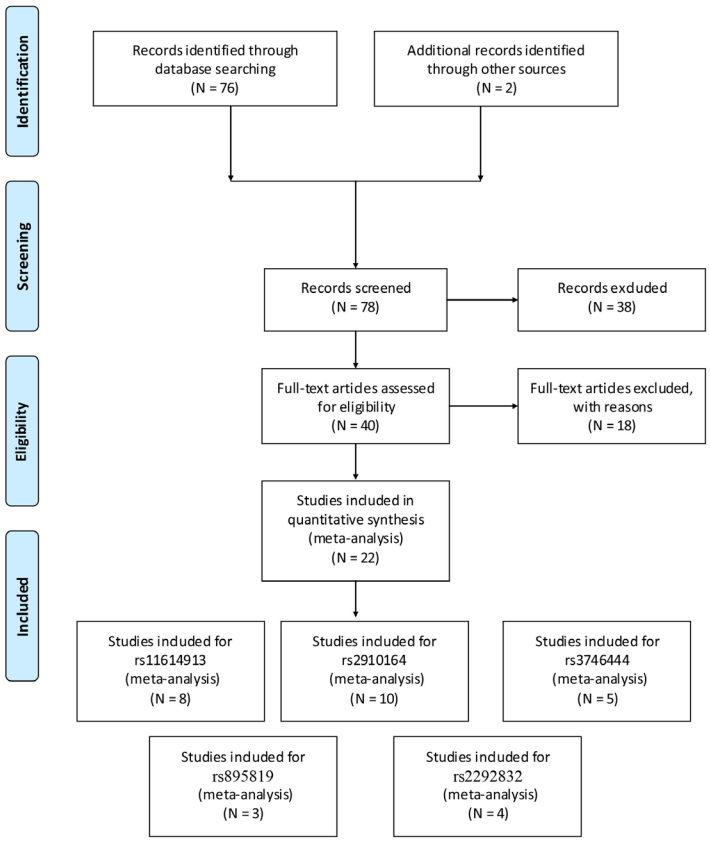

Figure 1. Flow diagram of the study inclusion process.

Inclusion and exclusion criteria

Studies testing the association between miRNA polymorphisms and CRC were included if all of the following conditions were met: a) the investigation consisted of a case-control design; b) the total number of cases and controls was made available; c) genotype frequencies in case and control groups were given; and d) the study was published in English. The principal exclusion criteria were as follows: a) data for calculation of odds ratios (ORs) was lacking and b) the retrieved document consisted of an abstract, comment or editorial.

A total of 78 relevant articles were identified finally. After screening of titles and abstracts, 38 studies clearly irrelevant to the subject of interest were excluded, and from the remaining 40 publications, 18 reviews were removed. Finally, after screening of method sections, 22 epidemiological investigations focusing on the importance of miRNA-related SNPs in CRC susceptibility were retained [Zhan et al., 2011; Chen et al., 2012; Hezova et al., 2012; Min et al., 2012; Ryan et al., 2012; Zhang et al., 2012; Zhu et al., 2012; Chae et al., 2013; Gao et al., 2013; Lv et al., 2013; Ma et al., 2013; Vinci et al., 2013; Cao et al., 2014; Hu et al., 2014; Li et al., 2013, 2014 (for unpublished results of Chen, 2013); Mao et al., 2014; Oh et al., 2014; Parlayan et al., 2014; Wang et al., 2014a,b; Dikaiakos et al., 2015; Table 1], including data from two unpublished studies extracted from review articles.

Table 1. List of the retrieved studies and the microRNA polymorphisms involved.

Reference (first author)	Ethnicity	Cases/Controls	Matching criteria	Source of controls	miRNA	SNP ID	Genotyping method
Zhan, 2011	Asian	252/543	Age, gender	HB	miR-196a-2	rs11614913	PCR-RFLP
Chen, 2012	Asian	126/407	Age, gender	HB	miR-196a-2	rs11614913	PCR-LDR
Hezova, 2012	Caucasian	197/212	Age	HB	pre-miR-27a	rs895819	TaqMan
Ryan, 2012		245/446	-	HB	miR-196a-2	rs11614913	TaqMan
Min, 2012	Mixed	446/502	-	HB	miR-146a	rs2910164	PCR-RFLP
Zhang, 2012	Asian	443/435	Age, gender, residence area	PB	hsa-miR-608	rs4919510	PCR-RFLP
Zhu, 2012	Asian	573/558	Age, gender	-	miR-196a-2	rs11614913	TaqMan
Chae, 2013	Asian	399/568	Residence area	HB	miR-146a	rs2910164	PCR-RFLP
Gao, 2013	Asian	347/488	Age, gender, ethnicity, residence area	HB	miR-149	rs2292832	PCR-RFLP
Li, 2013	Asian	242/283	-	HB	miR-499	rs3746444	PCR-RFLP
Lv, 2013	Asian	353/540	-	HB	miR-149	rs2292832	PCR-RFLP
Ma, 2013	Asian	1147/1203	Age, gender	HB	hsa-miR-605	rs2043556	TaqMan
Vinci, 2013	Asian	160/178	Age, gender	-	miR-196a-2	rs11614913	HRM
Chen, 2013[1]	Caucasian	547/561	Age, gender	-	miR-146a	rs2910164	SNPscan
Cao, 2014		254/238	Residence area	HB	miR-34b/c	rs4938723	PCR-RFLP
Hu, 2014	Asian	276/373	-	HB	miR-143/145	rs41291957	PCR-RFLP
Mao, 2014	Asian	554/566	Age, gender, residence area	PB	miR-196a-2	rs4705343	SNPscan
Oh, 2014	Asian	545/428	-	PB	miR-499	rs4705342	PCR-RFLP
Parlayan, 2014	Asian	524/116	-	-	miR-146a	rs353292	TaqMan
Wang, 2014a	Asian	205/455	-	HB	miR-149	rs353293	TaqMan
Wang, 2014b	Asian	102/204	Age, gender, ethnicity, residence area	HB	miR-146a	rs17723799	Sequenom MassARRAY
Dikaiakos, 2015	Asian	157/299	Age, gender	HB	miR-196a-2	rs17796757	
	Asian				miR-499	rs4705341	Genotyping platform
	Caucasian				miR-146a	rs4705340	PCR-RFLP
					miR-149	rs12659504	
					miR-146a	rs3733845	
					hsa-miR-27a	rs3733846	
					miR-499	rs11614913	
					miR-146a	rs3746444	
					miR-146a	rs2910164	
					miR-34b/c	rs2292832	
					miR-146a	rs2910164	
					miR-196a-2	rs11614913	
					pre-miR-27a	rs3746444	
					hsa-miR-603	rs2910164	
					miR-146a	rs2292832	
					miR-499	rs2910164	
					miR-196a-2	rs895819	
						rs3746444	
						rs2910164	
						rs2910164	
						rs4938723	
						rs2910164	
						rs11614913	
						rs895819	
						rs11014002	
						rs2910164	
						rs3746444	
						rs11614913	

miRNA = microRNA; SNP = single nucleotide polymorphism; HB = hospital-based study; PB = population-based study; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; PCR-LDR = polymerase chain reaction-ligase detection reaction; HRM = high-resolution melting. [1]Unpublished data recorded by Chen (2013), included in the review authored by Li et al. (2014).

Quality assessment

The Newcastle-Ottawa Scale was used for quality assessment. Quality was judged based on three aspects of each case-control study: selection, comparability, and exposure. A "star" rating system was used, with scores ranging from 0 to 9. We assessed the quality of studies in a consensus meeting with all authors (**Table S1**). Those with a score ≥5 were considered to be of high quality.

Data extraction

Two researchers independently extracted data from each document using a predefined review form, and discrepancies were resolved by consensus among all investigators. The following information was recorded for each study: first author, year of publication, miRNA name, SNP ID, ethnicity of participants, number of cases and controls, genotyping method, matching criteria, source of controls, minor allele frequency among controls, and conformity to Hardy-Weinberg equilibrium (HWE; Table 1). Ethnicity was categorized as Asian, Caucasian, or mixed. However, no subgroup analysis by population was carried out, as the focus of the present study was on the relationship between miRNA polymorphisms and CRC risk in the overall patient group.

A list of the miRNA SNPs evaluated in regard to CRC is shown in Table 1. The most frequently studied of these were rs11614913 (miR-196a-2), rs2910164 (miR-146a), rs3746444 (miR-499), rs2292832 (miR-149), and rs895819 (pre-miR-27a).

Statistical analysis

Deviation from HWE in the control group was examined by the chi-square test, and $P < 0.05$ was considered statistically significant. The strength of relationships between miRNA polymorphisms and CRC risk was assessed using pooled ORs with 95% confidence intervals (CIs). Risk was estimated using allelic, homozygous, heterozygous, dominant, and recessive models. Heterogeneity was assessed with the chi-square-based *Q*-statistic. When this test returned P values <0.05, a random-effect model was applied (the DerSimonian and Laird method); otherwise, a fixed-effect model was employed (the Mantel-Haenszel method). Funnel plots and Egger's linear regression method were used to detect potential publication bias, and sensitivity analyses were performed to assess the stability of the results. All tests were performed with the Stata software, version 10.0 (StataCorp., College Station, TX, USA). All P values were two-sided.

RESULTS

Twenty-two case-control studies were included in this review. Most of these had NOS scores of 5, and all were of high quality in terms of selection and exposure (**Table S1**). Only the previously unpublished data recorded by Chen (2013), included in the review authored by Li et al. (2014), scored 4 stars, owing to a lack of information regarding experimental design.

In the following section, we discuss the SNPs rs11614913 (miR-196a-2), rs2910164 (miR-146a), rs3746444 (miR-499), rs2292832 (miR-149), and rs895819 (pre-miR-27a), and

present our meta-analysis evaluating the association between each of these and CRC risk. The relationship between this disease and other less frequently studied SNPs is also considered. We did not adjust our results for environmental effects, as our investigation concentrated on the influence of genetics on cancer development, nor did we carry out subgroup analyses.

DISCUSSION

rs11614913 in miR-196a-2 and its association with CRC

The rs11614913 polymorphism in miR-196a-2 has been associated with susceptibility to various cancers. The molecular basis for this remains obscure; however, it has been speculated that the T to C mutation in the pre-miRNA stem region may alter the expression level of mature miR-196a-2 and influence its binding to target mRNA (Hoffman et al., 2009). As elevated expression of miR-196a (the mature miRNA encoded by miR-196a-2 gene) can promote CRC cell migration and invasion, sequence variations leading to such increased transcription might raise CRC risk (Guo et al., 2012). We retrieved nine studies concerning the association between rs11614913 and this disease (Table 2). Three of these reported that the TC genotype increased CRC risk compared to the TT genotype, whereas three others demonstrated that it decreased susceptibility. Six studies showed individuals with the CC genotype to be at increased risk in comparison to those with the TT genotype, and one investigation established the opposite effect. Taking the CC genotype as a reference, Lv et al. (2013) and Vinci et al. (2013) obtained entirely different results. However, in a study of CRC tissues, Zhan et al. (2011) reported that miR-196a expression is significantly higher in patients carrying CC or TC genotypes than in TT carriers. In our meta-analysis of the association between this miR-196a-2 polymorphism and CRC risk, eight studies comprising 2264 cases and 3199 controls were included (Parlayan's study was excluded because the genotype distribution data of control is lacking). We found no relationship between rs11614913 and CRC under any genetic model (CC *vs* TT: OR = 0.852, 95%CI = 0.479-1.515; CT *vs* TT: OR = 0.946, 95%CI = 0.735-1.216; CC+CT *vs* TT: OR = 0.945, 95%CI = 0.689-1.298; C *vs* T: OR = 0.962, 95%CI = 0.752-1.230).

Table 2. Characteristics of studies concerning the miR-196a-2 rs11614913 polymorphism in colorectal cancer.

Reference (first author)	Nation	Sample size (cases/ controls)	Genotype distribution (cases/controls)			C allele frequency (controls)	Risk in original publication [OR (95%CI)]	HWE P value
			TT	TC	CC			
Zhan, 2011	China	252/543	56/163	128/263	68/113	0.453618	TC/TT: 1.394 (0.947-2.052)	0.716
Chen, 2012	China	126/407	35/107	64/206	27/94	0.484029	CC/TT: 1.743 (1.112-2.731)	0.788
Hezova, 2012	Czech Republic	197/212	82/87	89/103	26/22	0.346698	TC/TT: 1.053 (0.656-1.691)	0.291
Min, 2012	Korea	446/502	125/148	201/254	120/100	0.452191	CC/TT: 1.139 (0.642-2.021)	0.633
Zhu, 2012	China	573/558	130/172	303/295	140/121	0.456633	TC/TT: 0.95 (0.62-1.45)	0.790
Lv, 2013	China	353/540	114/91	223/331	10/109	0.483051	CC/TT: 1.32 (0.69-2.54)	<0.05
Vinci, 2013	Italy	160/178	12/11	86/84	62/83	0.297753	TC/TT: 0.94 (0.69-1.27)	0.087
Parlayan, 2014	Japan	524/116	43/--	59/--	23/--	NA	CC/TT: 1.42 (0.99-2.03)	0.156
Dikaiakos, 2015	Greece	157/299	69/117	69/149	19/33	0.359532	TC/TT: 1.36 (1.03-1.80)	
							CC/TT: 1.53 (1.10-2.14)	
							TC/CC: 7.34 (3.76-14.34)	
							TT/CC: 13.66 (6.76-27.6)	
							TC/CC: 0.721 (0.46-1.13)	
							TT/CC: 0.587 (0.25-1.38)	
							NA	
							TC/TT: 0.78 (0.52-1.86)	
							CC/TT: 0.98 (0.51-1.85)	
Overall		2264/3199					CC/TT: 0.852 (0.479-1.515); P: 0.585	
							CT/TT: 0.946 (0.735-1.216); P: 0.664	
							CC+CT/TT: 0.945 (0.689-1.298); P: 0.728	
							C/T: 0.962 (0.752-1.230); P: 0.758	

OR = odds ratio; CI = confidence interval; HWE = Hardy-Weinberg equilibrium; NA = not applicable.

rs2910164 in miR-146a and its association with CRC

miR-146a was first identified in mice, and plays a crucial role in tumorigenesis by promoting cell proliferation and colony formation. Its deregulation has been reported in tumor tissue in several studies, implying a tumor-suppressor function, and it has also been shown to suppress metastasis. The rs2910164 G>C polymorphism at position +60 of the gene encoding miR-146a results in a G:U to C:U leader sequence change, causing a base-pair mismatch (Chae et al., 2013). The C allele leads to a less stable secondary structure and decreased production of mature miR-146a (Li et al., 2010). This SNP has been associated with a variety of cancers. We collated 11 studies testing the association between rs2910164 and CRC (Table 3); however, their results were conflicting. Compared to the GG genotype, five investigations supported a link between the GC genotype and decreased CRC risk, while three found it to increase susceptibility. Seven studies suggested that the CC genotype lowered the likelihood of developing CRC compared to the GG genotype, whereas two suggested that it raised this risk. In our meta-analysis evaluating the link between this miR-146a polymorphism and CRC, 10 studies were included, representing 4236 cases and 5002 controls. We failed to detect a correlation between rs2910164 and CRC under any of the genetic models tested (CC *vs* GG: OR = 0.986, 95%CI = 0.778-1.250; GC *vs* GG: OR = 1.002, 95%CI = 0.823-1.221; CC+GC *vs* GG: OR = 0.995, 95%CI = 0.831-1.192; C *vs* G: OR = 0.969, 95%CI = 0.864-1.086).

Table 3. Characteristics of studies concerning the miR-146a rs2910164 polymorphism in colorectal cancer.

Reference (first author)	Nation	Sample size (cases/ controls)	Genotype distribution (cases/controls)			C allele frequency (controls)	Risk in original publication [OR (95%CI)]	HWE P value
			GG	GC	CC			
Hezova, 2012	Czech Republic	197/212	115/124	70/79	12/9	0.228774	GC/GG: 0.93 (0.61-1.41)	0.415
Min, 2012	Korea	446/502	62/69	233/245	151/188	0.381474	CC/GG: 1.31 (0.52-3.27)	0.443
Chae, 2013	Korea	399/568	61/121	182/282		0.538732	GC/CC: 1.18 (0.90-1.57)	0.980
Lv, 2013	China	353/540	54/96	230/274	156/165	0.545809	GG/CC: 1.12 (0.75-1.68)	0.080
Ma, 2013	China	1147/1203	444/397	534/614		0.414796	GC/GG: 1.079 (0.885-1.821)	0.075
Vinci, 2013	Italy	160/178	86/100	57/65	47/143	0.255618	CC/GG: 1.865 (1.278-2.722)	0.590
Chen, 2013[1]	China	547/561	34/44	82/187	169/192	0.631367	GC/GG: 1.49 (1.02-2.18)	0.137
Hu, 2014	China	276/373	70/85	291/271	17/13	0.606952	CC/GG: 0.58 (0.37-0.93)	0.768
Mao, 2014	China	554/566	12/--	50/--	84/142	NA	GC/GG: 0.78 (0.65-0.93)	NA
Parlayan, 2014	Japan	524/116	101/158	48/120	186/205	0.270903	CC/GG: 0.8 (0.62-1.03)	0.782
Dikaiakos, 2015	Greece	157/299	70/85	291/271	58/--	0.606952	GC/GG: 1.015 (0.64-1.60)	0.768
					8/21		CC/GG: 0.681 (0.31-1.48)	
							GC/GG: 0.567 (0.338-0.952)	
					186/205		CC/GG: 0.766 (0.454-1.291)	
							NA	
							NA	
							GC/GG: 0.63 (0.41-0.95)	
							CC/GG: 0.59 (0.25-1.39)	
							GC/GG: 0.81 (0.51-1.29)	
							CC/GG: 0.71 (0.36-1.42)	
Overall		4236/5002					CC/GG: 0.986 (0.778-1.250); P: 0.908	
							GC/GG: 1.002 (0.823-1.221); P: 0.980	
							CC+GC/GG: 0.995 (0.831-1.192); P: 0.598	
							C/G: 0.969 (0.864-1.086); P: 0.589	

OR = odds ratio; CI = confidence interval; HWE = Hardy-Weinberg equilibrium; NA = not applicable. [1]Unpublished data recorded by Chen (2013), included in the review authored by Li et al. (2014).

rs3746444 in miR-499 and its association with CRC

rs3746444 is an important SNP located in the seed sequence (nucleotides 2-8) of hsa-miR-499, consisting of an A to G base change (Akkiz et al., 2011). The seed region at the 5'-end of this sequence is important for miRNA-miRNA binding, thus the rs3746444 polymorphism might affect interactions between miR-499 and other miRNAs to influence cancer susceptibility. We identified five studies regarding the association between this SNP and CRC (Table 4), with contradictory results. Three of these asserted that the GG genotype decreased CRC risk compared to the AA genotype, while three proposed that the AG

genotype increased susceptibility, also in comparison to the AA genotype. Five investigations, consisting of 1392 cases and 1892 controls, were included in our meta-analysis, which found no association between rs3746444 and CRC risk using any genetic model (AG *vs* AA: OR = 1.011, 95%CI = 0.862-1.185; GG *vs* AA: OR =1.022, 95%CI = 0.783-1.335; GG+AG *vs* AA: OR = 1.019, 95%CI = 0.811-1.179; C *vs* G: OR = 1.023, 95%CI = 0.908-1.154).

Table 4. Characteristics of studies concerning the miR-499 rs3746444 polymorphism in colorectal cancer.

Reference (first author)	Nation	Sample size (cases/ controls)	Genotype distribution (cases/controls)			G (C) allele frequency (controls)	Risk in original publication [OR (95%CI)]	HWE P value
			AA (TT)	AG (TC)	GG (CC)			
Min, 2012	Korea	446/502	292/334	142/154	12/14	0.181	AG/AA: 1.06 (0.80-1.39)	0.453
Lv, 2013	China	353/540	258/366	88/138	54/96	0.275	GG/AA: 0.98 (0.45-2.15)	<0.05
Vinci, 2013	Italy	160/178	93/105	32/56	35/17	0.253	NA	<0.05
Hu, 2014	China	276/373	157/282	49/81	5/10	0.135	AG/AA: 0.663 (0.39-1.11)	0.162
Dikaiakos, 2015	Greece	157/299	85/182	64/99	8/18	0.226	GG/AA: 2.931 (1.49-5.77)	0.361
							TC/TT: 1.087 (0.725-1.629)	
							CC/TT: 0.898 (0.302-2.674)	
							AG/AA: 1.38 (0.92-2.08)	
							GG/AA: 0.95 (0.39-2.28)	
Overall		1392/1892					AG/AA: 1.011 (0.862-1.185); P: 0.892	
							GG/AA: 1.022 (0.783-1.335); P: 0.872	
							GG+AG/AA: 1.019 (0.811-1.179); P: 0.801	
							C/G: 1.023 (0.908-1.154); P: 0.706	

OR = odds ratio; CI = confidence interval; HWE = Hardy-Weinberg equilibrium; NA = not applicable.

rs895819 in pre-miR-27a and rs2292832 in miR-149

The gene encoding miR-27a has been reported to be involved in the development of gastrointestinal cancer. rs895819 is located at position +40 relative to the first nucleotide of miR-27a. This A to G mutation has been shown to increase miR-27a expression in tumor tissues (Cao et al., 2014). We examined three publications, involving 656 cases and 905 controls (Table 5). Only one study indicated that the AG genotype increased CRC risk compared to the AA genotype, with two demonstrating that the GG genotype elevates susceptibility. Our meta-analysis showed that rs895819 does increase CRC risk under homozygous (GG *vs* AA: OR = 1.534, 95%CI = 1.148-2.049, P = 0.004), dominant (GG+AG *vs* AA: OR = 1.324, 95%CI = 1.066-1.645, P = 0.011), and allelic models (C *vs* G: OR = 1.280, 95%CI = 1.102-1.486, P = 0.001), but not under the heterozygous model (AG *vs* AA: OR = 1.230, 95%CI = 0.971-1.558, P = 0.087).

Table 5. Characteristics of studies concerning the pre-miR-27a rs895819 polymorphism in colorectal cancer.

Reference (first author)	Nation	Sample size (cases/ controls)	Genotype distribution (cases/controls)			C allele frequency (controls)	Risk in original publication [OR (95%CI)]	HWE P value
			AA	AG	GG			
Hezova, 2012	Czech Republic	197/212	88/93	86/94	23/25	0.340	AG/AA: 0.98 (0.64-1.49)	0.867
Cao, 2014	China	254/238	92/114	113/93	49/31	0.326	GG/AA: 1.04 (0.54-1.98)	0.089
Wang, 2014a	China	205/455	48/138	68/157	89/160	0.524	AG/AA: 1.506 (1.021-2.220)	<0.05
							GG/AA: 1.959 (1.156-3.318)	
							AG/AA: 1.245 (0.806-1.923)	
							GG/AA: 1.865 (1.052-2.430)	
Overall		656/905					GG/AA: 1.534 (1.148-2.049); P: 0.004	
							AG/AA: 1.230 (0.971-1.558); P: 0.087	
							GG+AG/AA: 1.324 (1.066-1.645); P: 0.011	
							C/G: 1.280 (1.102-1.486); P: 0.001	

OR = odds ratio; CI = confidence interval; HWE = Hardy-Weinberg equilibrium.

miR-149 has been described as both a tumor suppressor and an oncogene in the development of various tumors (Zhang et al., 2012). Use of real-time polymerase chain reaction has revealed that miR-149 mRNA levels are increased in the tumor tissues of patients carrying rs2292832 CC or CT genotypes. We retrieved four articles, including a total of 1402

cases and 1655 controls (Table 6). None of these studies established an association between this polymorphism and CRC risk. However, our meta-analysis of this relationship suggested that rs2292832 might decrease CRC risk under heterozygous (CT *vs* TT: OR = 0.816, 95%CI = 0.691-0.963) and dominant models (CC+CT *vs* TT: OR = 0.834, 95%CI = 0.715-0.972), but not under allelic (C *vs* T: OR = 0.906, 95%CI = 0.809-1.015) or homozygous models (CC *vs* TT: OR = 0.885, 95%CI = 0.693-1.130).

Table 6. Characteristics of studies concerning the miR-149 rs2292832 polymorphism in colorectal cancer.

Reference (first author)	Nation	Sample size (cases/ controls)	Genotype distribution (cases/controls)			C allele frequency (controls)	Risk in original publication [OR (95%CI)]	HWE P value
			TT	TC	CC			
Min, 2012	Korea	446/502	221/232	177/219	48/51	0.320	TC/TT: 0.85 (0.65-1.11)	0.948
Zhang, 2012	China	443/435	203/187	190/202	50/46	0.337	CC/TT: 0.99 (0.64-1.53)	0.431
Lv, 2013	China	353/540	253/308	64/103	30/48	0.217	TC/TT: 1.6 (0.88-2.59)	<0.05
Vinci, 2013	Italy	160/178	23/17	58/75	79/86	0.306	CC/TT: 1.41 (0.66-3.04)	0.912
							TC/CC: 0.99 (0.57-1.73)	
							TT/CC: 1.31 (0.81-2.14)	
							TC/CC: 1.176 (0.74-1.87)	
							TT/CC: 0.628 (0.31-1.29)	
Overall		1402/1655					CC/TT: 0.885 (0.693-1.130); P: 0.327	
							CT/TT: 0.816 (0.691-0.963); P: 0.016	
							CC+CT/TT: 0.834 (0.715-0.972); P: 0.021	
							C/T: 0.906 (0.809-1.015); P: 0.089	

OR = odds ratio; CI = confidence interval; HWE = Hardy-Weinberg equilibrium.

Other miRNA SNPs in CRC

Our review also identified other miRNA SNPs reported in only one or two investigations to influence CRC risk (Table 7). Owing to the limited number of articles in question, meta-analysis was not performed for these variants. Further studies of these polymorphic sites need to be carried out to explore their roles in CRC.

Table 7. Characteristics of studies concerning other microRNA single nucleotide polymorphisms in colorectal cancer.

Reference (first author)	Nation	miRNA SNP ID	Sample size (cases/ controls)	Genotype distribution (cases/controls)			Risk in original publication [OR (95%CI)]
				Homozygous	Heterozygous	Homozygous	
Ryan, 2012	USA	hsa-mir-608	245/446	124/231	96/166	19/36	GC/CC: 1.28 (0.89-1.84)
Gao, 2013	China	rs4919510	347/488	175/216	144/210	28/62	GG/CC: 0.96 (0.51-1.82)
Li, 2013	China	miR-34b/c	242/283	NA	NA	NA	TC/TT: 0.64 (0.39-1.05)
Oh, 2014	Korea	rs4938723	545/428	272/216	233/171	40/41	CC/TT: 0.56 (0.34-0.91)
Zhang, 2012	China	miR-143/145	443/435	219/199	152/190	63/61	NA
		miR-34b/c					NA
		rs4938723					NA
		hsa-miR-605					
		rs2043556					

miRNA = microRNA; SNP = single nucleotide polymorphism; OR = odds ratio; CI = confidence interval; NA = not applicable.

Study limitations

Certain limitations to our investigation should be considered. First, inconsistencies were observed between the findings of single studies and our final analysis. In the present study, we concentrated on the effects of miRNA SNPs in the overall human population, finding that certain genotypes may affect cancer risk, while others do not. However, environmental factors may also contribute to oncogenesis. In future, to explore the influence of miRNA SNPs on cancer susceptibility in different environmental backgrounds, we should take such factors into account, and carry out subgroup analysis by population or lifestyle variables. Second, the

limited number of studies included in our analysis may also have affected its results. Although we included most of the relevant reports published to date, and no fewer than previous meta-analyses performed by other groups, this issue may have introduced bias into our overall analysis. Third, the sample sizes of the datasets used in our investigation ranged from 100 to 1000. However, to confirm our results, more studies with larger sample sizes should be carried out and be incorporated into our analysis to increase its power.

Heterogeneity and publication bias are major concerns in meta-analyses. The former was detected among studies in the overall comparisons for rs11614913 in miR-196a-2 and rs2910164 in miR-146a. However, tests of sensitivity indicated that our results were statistically reliable in each meta-analysis. The omission of any study made no significant difference to the pooled OR. Publication bias was assessed using Begg's funnel plot and the Egger test. None was observed in our examinations of rs11614913 and rs2910164, with the shape of the corresponding funnel plots revealing no obvious asymmetry. Publication bias was not tested for rs3746444 (miR-499), rs895819 (pre-miR-27a), or rs2292832 (miR-149) because of the small number of studies involved.

In this review, we summarized and discussed current knowledge of the role of miRNA SNPs in CRC. Furthermore, meta-analysis was carried out to evaluate associations between the five most frequently studied of these and CRC risk.

rs11614913, rs2910164, and rs3746444 in miR-196a-2, miR-146a, and miR-499, respectively, are frequently reported as being related to this disease. However, in our meta-analysis, no correlation between these three polymorphic sites and CRC risk was established. Population differences may account for this negative result, as one SNP may exert divergent effects on cancer risk in separate populations. The limited number of studies might also explain this finding, and our results should be interpreted with caution. In addition, miRNA SNPs may influence a single gene's function at the molecular level, but when considered together in epidemiological investigations, multiple genes might be affected, resulting in different associations with cancers from a phenotypic perspective.

The polymorphisms rs2292832 in miR-149 and rs895819 in pre-miR-27a were found to be associated with CRC risk in our analysis. Further studies should be carried out to validate this conclusion.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

Table S1. Assessment of the quality of the studies included in our meta-analysis.