Review

mirSNPs as Potential Colorectal Cancer Biomarkers: A Systematic Review

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Article

mirSNPs as Potential Colorectal Cancer Biomarkers: A Systematic Review

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Abstract: Colorectal cancer (CRC) is the third most common neoplasm in the world and the second with the highest mortality rate. Single nucleotide polymorphisms (SNPs) in miRNA genes (mirSNPs) may be related to dysregulated miRNA expression in several neoplasms. This systematic review aims to investigate studies that investigate SNPs located in regions of miRNA genes that influence their expression and are associated with CRC, as well as their potential as biomarkers for the disease, based on the available literature. For this, searches were performed in public databases, including MEDLINE/PubMed, Embase, Web of Science and Scopus. The rigorous review of the PRISMA 2020 guidelines and the methodological quality of the studies were assessed using the Newcastle-Ottawa scale and the Mixed Methods Assessment Tool. Of the 175 studies identified, 26 were considered eligible: 18 of them highlighted mirSNPs as potential biomarkers of risk and prognosis for CRC; 4 studies suggested a protective role; 1 study linked mirSNPs to treatment; and 3 studies found no relevant evidence. These results highlight the need for further research on this topic, since these variants have the potential to serve as biomarkers and may be useful in assessing risk, prognosis, and developing therapeutic strategies for patients with CRC.

Keywords: miRNA; SNP; MirSNP; colorectal cancer; biomarkers

Introduction

Colorectal cancer (CRC) is a malignancy affecting the large intestine and rectum and is notable for being the third most common cancer and the second leading cause of cancer-related deaths worldwide [1]. Most cases of CRC arise due to a combination of genetic and environmental factors, with several elements contributing to an increased risk of development. Modifiable risk factors include diet, obesity, physical inactivity, smoking, and alcohol consumption, while non-modifiable factors include gender, ethnicity, personal history of inflammatory bowel disease, personal or family history of polyps or cancer, inherited conditions such as Lynch syndrome, and genes involved in carcinogenesis [2]. Current methods of diagnosis and prognostic monitoring of CRC include colonoscopy and flexible sigmoidoscopy; however, these techniques are invasive, uncomfortable, and often suffer from poor patient compliance [3]. Alternative methods such as fecal occult blood tests, fecal immunochemical tests, carcinoembryonic antigen (CEA), and carbohydrate antigen 19–9

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(CA19–9) are plagued by high false-positive rates and low sensitivity. Each method has distinct advantages and limitations that impact clinical decision-making. Consequently, there is an increasing focus on research into individualized genetic risk profiles to improve the diagnosis and prognosis of CRC [4,5].

Numerous studies aim to identify molecular markers that may contribute to the development of personalized medicine by improving disease prediction and patient outcomes [6]. Advances in molecular biology techniques are increasingly aimed at developing less invasive methods to obtain accurate information about tumors [7]. Among the widely studied markers, microRNAs (miRNAs) hold significant promise due to their differential expression in tumors and their presence in various body fluids, along with their stability under different storage and handling conditions [8–11].

MiRNAs are small non-coding RNAs, typically approximately 22 nucleotides in length, that regulate gene expression by binding to complementary sites within the 3′-untranslated regions (3′-UTRs) of target mRNAs, thereby inhibiting mRNA translation [12]. These molecules play a crucial role in cancer biology, influencing processes such as cell proliferation, differentiation, migration, angiogenesis, and apoptosis [13]. Research indicates that dysregulated miRNA expression is essential in the development and metastasis of CRC, with miRNAs acting as tumor suppressors or oncogenes by regulating the expression of specific mRNA targets [14]. Single nucleotide polymorphisms (SNPs) in the 3′-UTR, known as mirSNPs, can affect the binding affinity between miRNAs and target mRNAs, leading to alterations in miRNA expression and maturation, as well as changes in the expression levels of target genes [15,16]. These genetic variants can occur in regions such as pre-miRNAs, mature miRNAs, seed region or miRNA binding sites, significantly impacting miRNA function and contributing to susceptibility to diseases, including cancer [17,18]. In this systematic review, we specifically focused on genetic variants in miRNA binding sites associated with CRC, exploring their potential as biomarkers for this disease, in alignment with the existing literature.

Methods

Protocol and registration

This systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Table S1) [19]. The review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under number CRD42024574543. Following registration, changes to the protocol were made and recorded and can be accessed at https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024574543.

Inclusion and exclusion criteria

Eligibility criteria were carefully formulated to identify studies addressing polymorphisms in miRNA binding binding sites in target genes associated with CRC. This review included observational and clinical studies published without date restrictions, peer-reviewed in English and published in scientific journals. Review studies, systematic reviews, books, case reports, short communications, editorials, letters to the editor, preprints, conference proceedings, dissertations, theses, preclinical studies and studies addressing polymorphisms in lcnRNAs were excluded.

Literature search strategy

We conducted a systematic search for original articles in the literature in July 2024, using the MEDLINE/PubMed, Embase, Scopus, and Web of Science databases. The search strategy employed a wide range of keywords, aligned with the study objectives, and Boolean operators (AND, OR, NOT) were strategically employed to refine the search terms. The following terms were used: “colorectal cancer mirSNP” OR “neoplasm colorectal mirSNP” AND “miRNA SNP colorectal cancer” OR “miRNA SNP colorectal neoplasm”. After the search, the articles were imported into the RAYYAN software, accessed at https://www.rayyan.ai, for duplicate removal. Two authors (KAS and LMCAS) independently screened the studies by evaluating titles and abstracts based on predefined eligibility criteria. After the initial screening, the full texts of the articles were reviewed by both researchers

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independently for final inclusion. In case of discrepancy between researchers, the opinion of a third author (KSCS) was requested.

Data collection

The bibliographic search in the databases resulted in 175 records. After eliminating 66 duplicates, 109 titles and abstracts were evaluated by two independent reviewers. Of these, 47 studies were excluded for not meeting the inclusion and exclusion criteria, leaving 62 studies for full reading. Among these, 36 studies were discarded for not meeting the eligibility criteria. As a result, 26 articles were considered eligible and included in the systematic review, as illustrated in the article screening process flowchart (Figure 1). Information about authors, year of publication, study type, study population, miRNA SNP location, altered miRNA, SNP rsID, target gene, and miRNA SNP effect on CRC were extracted from each study when available (Table 1).

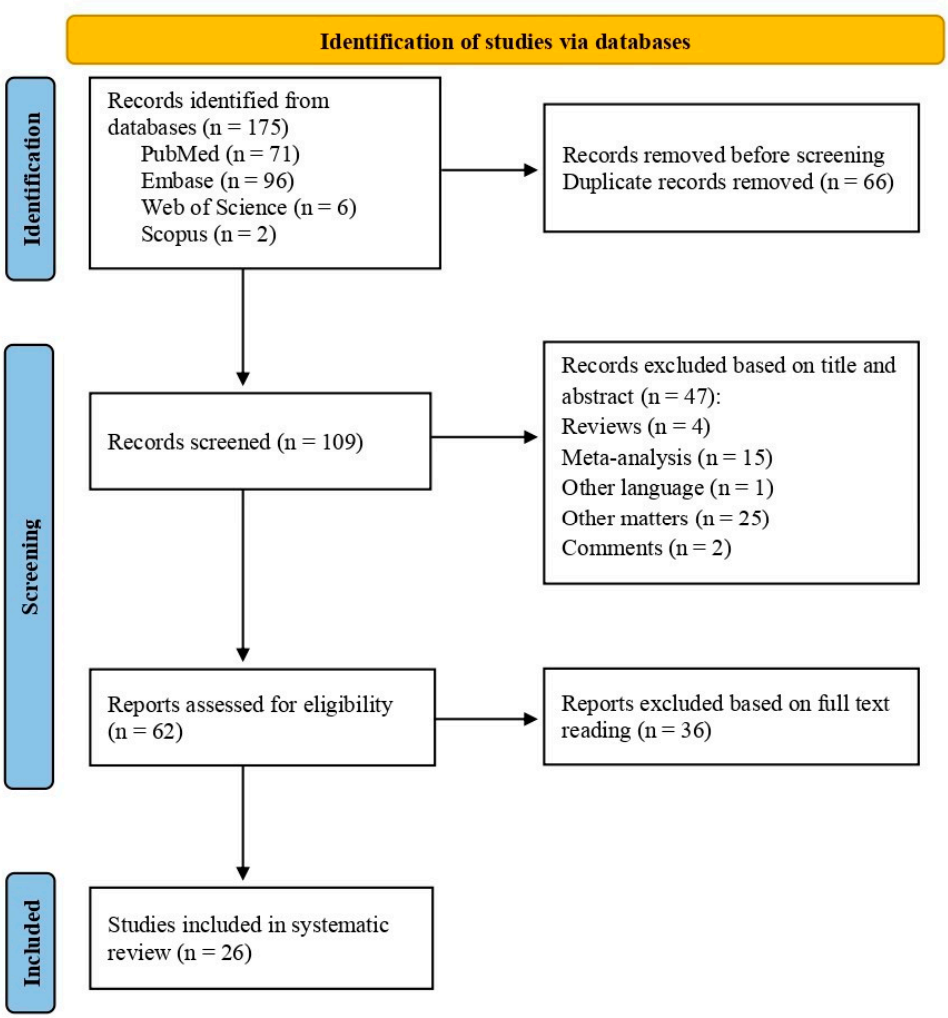

Figure 1. The flowchart illustrates the article selection methodology according to the recommendations of the PRISMA guidelines for systematic reviews [19].

Table 1. General characterization of included studies.

STUDY DESIGN	AUTHOR	POPULATION	SNP ID	ALLELE/ GENOTYPE	GENE ID	miR ID	ASSOCIATION OF mirSNPS WITH CRC
Case-control study	Landi et al.	Czech Republic	rs17281995 rs1051690	G/A	CD86 INSR	miR-337, miR-582, miR-200a, miR-184, and miR-212 miR-618 and miR-612	Increased risk
Case-control study	Ding et al.	Chinese	rs141178472	CC	PIK3CA	miR-520a	Increased risk
Case-control study	Ni et al.	Chinese	rs3814058	C>T	PXR	hsa-miR-129-5p	Increased risk
Case-control study	Xie et al.	Chinese	rs6504593	T	IGF2BP1	miR-21	Increased risk; cell proliferation and apoptosis
Case-control study	Ke et al.	Chinese	rs1062044	A	LAMC1	miR-423-5p	Increased risk; colon adenocarcinomas
Case-control study	Kang et al	Korea	rs2279398	G>A	DOK3	miR-370	Increased risk
Case-control study	Lee et al.	Korea	rs7930	AG	TOMM20	miR-4273-5p	rs7930 AG: Increased risk; Suppression of TOMM20 gene expression
Case-control study	Xicola et al.	Spain	rs868		TGFBR1	let-7	Increased risk

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Case-control study	Mullany et al.	United States	rs8176318 rs8905	AA GG	BRCA1 PRKAR1A	miR-525-5p miR-miR-214-3p	Increased risk
Case-control study	Chayeb et al.	Tunisian	rs2292832 rs2910164	C/T G/C		hsa-mir-149 hsa-mir-146a	Increased risk
Case-control study	Cao et al.	Chinese	rs895819	GG		miR-27a	Increased risk
STUDY DESIGN	**AUTHOR**	**POPULATION**	**SNP ID**	**ALLELE/ GENOTYPE**	**GENE ID**	**miR ID**	**ASSOCIATION OF mirSNPS WITH CRC**
Case-control study	Hu et al.	Chinese	rs2910164	G>C		miR-146a	Susceptibility and differentiation
Cohort Studies	Kim et al.	Korea	rs3757417 rs12373	T>G A>C	TPST1 PAUF	miR-571	Prognostic markers
Case-control study	Salem et al.	Iran	rs12904	AA/ A>G	EFNA1	miR-200C and miR-429	rs12904 AA: Pathogenesis and metástasis; rs12904 A>G: reduced disease progression; regulation of EFNA1 gene expression.
Cohort Studies	Smits et al.	Dutch	KRAS-LCS6	T>G	KRAS	let-7	Prognostic biomarker
Case-control study	Ryan et al.	Caucasian and African-American	rs4919510	GG		hsa-miR-608	Increased risk of death in Caucasians and a reduced risk in African Americans
Case-control study	Zhan et al.	Chinese	rs11614913	CC		miR-196a2	Increased susceptibility in patients who have undergone chemotherapy treatment

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Cohort Studies	Huang et al.	Chinese	rs2273626 rs202195689	A>C CCCCA>del		hsa-miR-4707-3p hsa-miR-4274	Predictive and prognostic biomarkers after chemoradiotherapy
Case-control study	Chen et al.	Chinese	rs2682818	AA and AC/AA		miR-618	Reduction of risk
Case-control study	Zhu et al.	Chinese	rs187960998	C>T	CHD5	miR-211	Decreased risk and protective factor for CC by preventing binding of the tumor suppressor gene CHD5
Case-control study	Radanova et al.	Bulgaria	rs2682818	AC		miR-618	Decreased risk of susceptibility to mCC
Case-control study	Jiang eta al.	Chinese	rs35301225	C/A	E2F1	miR-34a	Tumor suppression and protection by downregulating the E2F1 gene; rs35301225 C/A: upregulation of E2F1 expression, resulting in worse survival.
STUDY DESIGN	**AUTHOR**	**POPULATION**	**SNP ID**	**ALLELE/ GENOTYPE**	**GENE ID**	**miR ID**	**ASSOCIATION OF mirSNPS WITH CRC**
Randomized study	Sclafani et al.	Multicenter study	rs4919510	CC		miR-608	Prognostic biomarker
Case-control study	Chen et al.	Chinese	rs11614913	T>C		miR-196a2	No significant association with increased risk or disease progression was identified

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Case-control study	Ayadilord et al.	Iran	rs11614913	C/T		miR-196a2	No significant association was identified between the SNP rs11614913 in miR-196a2 and CRC
Case-control study	Simonian et al	Iran	rs12904		EFNA1	miR-200c	No significant association was demonstrated between rs12904 and CRC risk

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Quality assessment and risk of bias

Two reviewers (KAS and LMCAS) independently assessed the methodological quality and risk of bias of the studies included in the systematic review. The Mixed Methods Assessment Tool (MMAT) [20] was used for this assessment, as detailed in Table S2. The adapted Newcastle-Ottawa scale [21] was used to assess the risk of bias in case-control and cohort studies, with scores classified as very high risk of bias (0–3), high risk (4–6), or high methodological quality (7–9) (Table S3). Discrepancies in assessment were resolved through discussion between the reviewers or, if necessary, by consulting a third researcher.

Results

This review analyzed 26 studies conducted between 2008 and 2022 on SNPs that affect miRNA expression and their influence on the occurrence and progression of CRC in different populations. Most studies adopted a non-randomized quantitative design (96.15%), with emphasis on research conducted predominantly in the Chinese population [23–26,32,33,38–41,43,45]. The most frequently cited miRNA was miR-618 [22,40,42]. Of note, the SNP rs1051690 located in the miR-618 target gene region was associated with an increased risk of CRC in one study. In contrast, the SNP rs2682818 in mature miR-618 was correlated with a reduced risk. These discrepancies highlight that, in the case of miR-618, different SNPs (rs1051690 and rs2682818) may affect its expression and activity in distinct ways, resulting in opposite effects on CRC risk, as evidenced by the different results of the studies analyzed.

Eighteen studies highlighted mirSNPs as potential biomarkers of CRC risk and prognosis. Among the mirSNPs associated with CRC risk, the following were identified: SNP rs17281995 (miR-337, miR-582, miR-200a, miR-184, and miR-212) and rs1051690 (miR-618 and miR-612) [22], rs141178472 (miR-520a) [23], rs3814058 (hsa-miR-129-5p) [24], rs6504593 (miR-21) [25], rs1062044 (miR-423-5p) [26], rs2279398 (miR-370) [27], rs7930 (miR-4273-5p) [28], rs868 (let-7) [29], rs8176318 (hsa-miR-525-5p) and rs8905 (hsa-miR-214-3p) [30], rs2292832 (hsa-mir-149) and rs2910164 (hsa-mir-146a) [31] and rs895819 (miR-27a) [32].

The highlighted mirSNPs associated with prognosis were: rs2910164 (miR-146a) [33], rs12373 (miR-571) [34], rs12904 (miR-200C and miR-429) [35], KRAS-LCS6 (let-7) [36], rs4919510 (hsa-miR-608) [37], rs11614913 (miR-196a2) [38], rs2273626 (hsa-miR-4707-3p) and rs202195689 (hsa-miR-4274) [39]. Furthermore, four studies identified mirSNPs as potential protective biomarkers: rs2682818 (miR-618) [40], rs187960998 (miR-211) [41], rs2682818 (miR-618) [42], and rs35301225 (miR-34a) [43]. One study reported a mirSNP associated with the treatment of CRC patients: rs4919510 (miR-608) [44]. Finally, three studies found nonsignificant associations between mirSNPs and CRC: rs11614913 (miR-196a2) [45,46] and rs12904 (miR-200c) [47] (Table 1).

mirSNPs associated with the risk and prognosis of CRC

Ten studies highlighted CRC risk-associated polymorphisms located in miRNA target gene regions [22–31] while one study investigated polymorphisms located in mature miRNA regions [32]. The earliest study, conducted by Landi et al. [22], included 697 CRC cases and 624 controls in the Czech Republic. This study identified two relevant SNPs: rs17281995 in the Cluster of Differentiation 86 (CD86) gene and rs1051690 in the insulin receptor (INSR). Both SNPs affect miRNA binding sites and are associated with an increased CRC risk. Furthermore, the G/A genotype in the 3' UTR of INSR demonstrated a significant biological impact on the target miRNA. In a study conducted by Ding et al. [23] with 386 cases and 394 controls in China, it was identified that the SNP rs141178472, located in the miR-520a binding site of the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene (PIK3CA), is associated with the risk of CRC. Individuals with the CC genotype had a significantly higher risk of CRC compared with those with the TT genotype. In the same year, Ni et al. [24] performed an in vitro experiment involving 1033 CRC cases and 1147 controls from the same Chinese population. The results suggested that the SNP rs3814058 C>T in the target site of hsa-miR-129-5p may influence the expression of the pregnane X receptor (PXR) gene, increasing the

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susceptibility to the development of CRC. Another study conducted in the Chinese population by Xie et al. [25] analyzed 1147 individuals with CRC and 1203 controls in a case-control study. The authors found that the SNP rs6504593, located in the miR-21 binding site in insulin-like growth factor 2 (IGF2BP1) mRNA-binding protein, may modify the expression of this gene and contribute to the risk of CRC. In vitro analysis revealed that the T allele of rs6504593 negatively regulates the post-transcriptional expression of IGF2BP1 by altering the binding affinity of miR-21. Inhibition of miR-21 led to decreased cell growth, while miR-21 mimetics promoted cell proliferation and suppressed apoptosis. In a study conducted by Ke et al. [26] with 2,347 cases and 3,390 Chinese controls, it was demonstrated that the rs1062044 SNP influences the interaction between miR-423-5p and Laminin Subunit Gamma 1 (LAMC1). Carriers of the A allele had a significantly higher risk of CRC. Additionally, a negative correlation between the expression levels of miR-423-5p and LAMC1 was observed in patients with colon adenocarcinomas. Another in vitro study by Kang et al. [27] in Korea suggested that the SNP rs2279398 G>A, located in the miRNA binding site, may impact the expression of the docking protein 3 (DOK3) gene. This influence is due to the direct binding of miR-370 to the 3'-UTR of the gene. These findings indicate that genetic variations in the DOK3 gene may play a significant role in the risk of CRC. In addition, another study conducted in Korea involving 325 CRC patients and 977 controls identified the SNP rs7930 in miR-4273-5p as a potential risk factor for CRC. These results highlight the importance of genetic variations in the predisposition to the development of this disease. The results of the study by Lee et al. [28] indicated that carriers of the rs7930 AG genotype had an increased risk of CRC compared to those with the AA genotype. This increased vulnerability is related to the interaction of miR-4273-5p specifically with the A allele of the rs7930 SNP, resulting in the suppression of the expression of the TOM20 mitochondrial import receptor subunit (TOMM20) gene. Thus, the A allele is considered protective, while carriers of the G allele have an increased risk of developing CRC. Additionally, a study conducted in Spain investigated the association between the rs868 polymorphism, located in the miRNA let-7 binding site in the 3'UTR of the transforming growth factor beta receptor 1 gene (TGFBR1). The results demonstrated that the presence of this genetic variant increases the binding of TGFBR1 to the miRNA let-7b-5p, resulting in decreased TGFBR1 expression and, consequently, increasing the risk of CRC. Xicola et al. [30] also explored the influence of altered miRNA expression in SNPs located in target gene regions and their impact on cancer risk. The study revealed that two specific SNPs, rs8176318 AA, located in the 3' UTR of the Breast Cancer 1 (BRCA1) gene, predicted to bind to miR-525-5p, and rs8905 GG, which targets the cAMP-dependent protein kinase type I regulatory subunit alpha (PRKAR1A) in miR-214-3p, were significantly associated with the risk of colon cancer in the United States population. Chayeb et al. [31] sought to clarify whether the genetic variants rs2292832 C/T in hsa-mir-149, rs2910164 G/C in hsa-mir-146a, and rs11614913 C/T in hsa-mir-196a2 are associated with the risk and prognosis of CRC. The study included 152 CRC patients and 161 Tunisian controls, and the results revealed a significant association between the SNPs rs2292832 C/T in hsa-mir-149 and rs2910164 G/C in hsa-mir-146a with the risk of CRC, considering ethnicity. However, no significant association was found for rs11614913C/T in hsa-mir-196a2. In a complementary study, carried out by Cao et al. [32] with 254 CRC patients and 238 healthy controls from the Han Chinese population, the relationship between the expression of the SNP rs895819 in miR-27a and the risk of CRC was examined. The results showed that patients with AG+GG genotypes had a significantly higher risk of CRC compared with those with the AA genotype. Furthermore, further analysis indicated that miR-27a was more highly expressed in the tumor tissue samples of patients with the GG genotype relative to those with the AA genotype.

Three studies have identified polymorphisms associated with CRC prognosis in miRNA target gene regions [33–35]. One study focused on complementary regions [36], while two analyzed mature miRNA regions [37,38], and another studied a SNP located in the seed region [39]. In a study conducted in China, Hu et al. [33] investigated the effects of rs2910164 and rs3746444 polymorphisms, belonging to miR-146a and miR-499, respectively, in 276 cases and 373 healthy Chinese individuals. The results demonstrated that the rs2910164 G>C SNP in miR-146a is associated with CRC susceptibility and differentiation, while the rs3746444 SNP in miR-499 showed no significant

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association with the disease. The authors highlighted the rs2910164 variant in miR-146a as a potential biomarker for CRC initiation and progression. In the same year, Kim et al. [34] conducted a study with 831 Korean individuals diagnosed with CRC. The analyses demonstrated that the SNPs rs3757417 T>G and rs12373 A>C in the 3′-UTR of Tyrosylprotein Sulfotransferase 1 (TPST1) and pancreatic adenocarcinoma upregulated factor (PAUF), respectively, are considered potential prognostic markers for patients undergoing surgical resection. The authors also reported that the genetic variant rs12373A>C increases the binding affinity of miR-571, resulting in the suppression of PAUF gene expression, which may impact the survival of CRC patients. In a case-control study conducted by Salem et al. [35] involving 146 CRC patients and 132 healthy individuals from Iran, it was identified that the rs12904 AA genotype plays a crucial role in the pathogenesis and metastasis of CRC. In contrast, patients carrying the rs12904 A>G genotype showed significantly reduced disease progression. The authors also observed that the rs12904 SNP in the ephrin A1 ligand gene (EFNA1) contains binding sites for the miRNAs miR-200C and miR-429, which may be important in regulating the expression of this gene. Another study, conducted by Smits et al. [36] with a significant number of Dutch individuals, evaluated a SNP located in the complementary site let-7 in the 3'-UTR of the KRAS gene (KRAS-LCS6). The analyses indicated that the T>G allele in the 3' UTR region is associated with improved survival in patients with early-stage CRC. Thus, the KRAS-LCS6 G variant may be considered a potential prognostic biomarker for early-stage CRC. The study by Ryan et al. [37] revealed that a SNP in the mature sequence of hsa-miR-608 may have a significant impact on the prognosis of CRC patients, analyzing 245 cases and 446 controls from Caucasian and African-American populations. Although the results did not show a significant association between the rs4919510 SNP and CRC risk, a correlation with survival was identified. The GG genotype was associated with an increased risk of mortality among Caucasians, while African-Americans had a reduced risk of death, suggesting that the effect of this SNP may vary by ethnicity. Another study conducted by Zhan et al. [38], which involved 252 CRC patients and 543 healthy individuals from the Chinese population, sought to determine whether the rs11614913 SNP in miR-196a2 is related to CRC progression and susceptibility. The results indicated that the CC genotype was more frequent among CRC patients compared with controls, and that this genotype, or any variant containing at least one C allele, was associated with higher levels of mature miR-196a and increased susceptibility to CRC. However, no association with disease progression was observed. The authors emphasized that these results were evident only in patients who received chemotherapy treatment. Huang et al. [39] observed that the SNP rs2273626 A>C in hsa-miR-4707-3p was significantly associated with a decreased risk of grade 2 leukopenia and the SNP rs202195689 hsa-miR-4274 with overall survival and free survival of disease in patients who received postoperative chemoradiotherapy. These results suggest these mirSNPs as potential predictive and prognostic biomarkers in chemoradiotherapy and clinical care of patients with rectal cancer.

mirSNPs associated with reduced risk and protection of CRC

Three studies highlighted polymorphisms associated with reduced risk and protection against CRC, located in mature miRNA regions [40–42], and one study focused on target gene regions [43]. In 2018, Chen et al. [40] analyzed 878 CRC patients and 884 controls from the Chinese Han population of China, investigating the relationship of the SNP rs2682818 in miR-618 with CRC. The results showed that the AA and AC/AA genotypes of rs2682818 were associated with a reduced risk of CRC compared to the CC genotype. The following year, Zhu et al. [41] studied 685 patients with colon cancer (CC) and 618 healthy controls in the same population, evaluating the SNP rs187960998, located in miR-211, and its possible relationship with the occurrence of CC. The SNP was identified as a tumor suppressor, acting in the regulation of the target gene chromodomain helicase DNA binding protein 5 (CHD5). The authors found a significant association between the rs187960998 C>T genotypes and the presence of the T allele, which correlated with smaller tumor size, better differentiation and lower probability of metastasis. Thus, the results suggest that the SNP rs187960998 in miR-211 may be related to a reduced risk of CC, acting as a protective factor by inhibiting binding to the 3'UTR of the tumor suppressor gene CHD5. In Bulgaria, Radanova et al. [42] investigated the

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association of the rs2682818 SNP in miR-618 with susceptibility to metastatic colon cancer (mCC) in 104 patients before initiation of chemotherapy. The results showed that the risk of developing mCC was significantly lower in patients with the heterozygous AC genotype compared with those who had the homozygous CC and AA genotypes. On the other hand, Jiang et al. [43] conducted a study with 685 newly diagnosed CRC patients and 618 controls from the Chinese population, aiming to evaluate the association of the rs35301225 SNP in miR-34a with the risk of CRC. The authors found that the rs35301225 SNP in miR-34a may act as a tumor suppressor by downregulating the tumor-promoting gene E2F Transcription Factor 1 (E2F1). Thus, this variant can be considered a protective factor against CRC. However, the authors reported that the presence of the C/A genotype results in an increased expression of E2F1, which is associated with worse survival in patients with CRC.

mirSNPs associated with CRC treatment

Only one study has identified polymorphisms associated with CRC treatment located in mature miRNA regions. Sclafani et al. [44] investigated the role of the rs4919510 polymorphism in mature miR-608 through a retrospective study. The research evaluated the impact of perioperative treatment with neoadjuvant Capecitabine and Oxaliplatin (CAPOX), followed by chemoradiotherapy, surgery and adjuvant treatment with CAPOX and Cetuximab, in 155 patients with locally advanced rectal cancer (LARC). The results indicated that the CC genotype in miR-608 was associated with a worse prognosis compared with the CG/GG genotypes, in patients undergoing systemic chemotherapy, chemoradiotherapy, surgery and neoadjuvant chemotherapy.

mirSNPs not associated with CRC

Several studies have analyzed the association of mirSNPs with CRC and found no statistical significance in their results. Among them, two focused on polymorphisms located in miRNA target gene regions [45,47], while one study focused on regions of mature miRNAs [46]. Chen et al. [45] investigated the relationship between SNP rs11614913 in miR-196a2 and CRC susceptibility and progression. The research involved 126 CRC patients and 407 healthy individuals from the Chinese population, but no significant association with increased risk or disease progression was identified. In 2020, Ayadilord et al. [46] conducted a case-control study with 52 CRC patients and 120 healthy Iranian individuals to evaluate the correlation between SNP rs11614913 in miR-196a2 and CRC. Genotyping results showed no statistically significant association between this SNP and CRC. Another study conducted in Iran involving 152 CRC patients and 160 controls investigated the influence of the rs12904 SNP located in the 3'UTR region of the EFNA1 gene on the regulation of miR-200c expression and CRC risk. The results also did not demonstrate a significant association between the rs12904 SNP and CRC risk in the analyzed groups [47].

Quality assessment and risk of bias

The methodological quality of the studies included in the systematic review was carefully assessed and the results can be found in the Supplementary Material (Tables S3 and S4). Of the 22 case-control studies and 3 cohort studies analyzed, 17 case-control studies and 1 cohort study had scores between 7 and 9, reflecting high methodological quality. In contrast, 5 case-control studies and 2 cohort studies had scores of 4 to 6, raising concerns regarding the risk of bias and the validity of the results.

Discussion

In this systematic review, we performed a detailed analysis of miRNA genes (SNPs) associated with CRC, aiming to identify promising markers for early detection, risk prediction and prognosis of the disease. To achieve this objective, we reviewed 26 studies that met the established inclusion criteria.

Polymorphisms in miRNAs represent a promising class of genetic variations that deserve detailed exploration as markers for individual susceptibility to complex diseases, prognosis, and

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clinical decision-making [48]. These genetic variants can influence miRNA-mediated post-transcriptional regulation, resulting in changes in the expression of important genes and, consequently, contributing to interindividual variability in cancer risk [49–51]. Furthermore, SNPs located in miRNA target regions in genes relevant to CRC may play a significant role in its etiopathogenesis [52]. SNPs in the pri-miRNA and pre-miRNA regions can affect miRNA processing and maturation, impacting primary transcription and miRNA-target interactions, which may lead to altered expression of mature miRNA [40,53]. Similarly, SNPs in the mature sequence or seed region of the miRNA can modify interactions with target sites, altering the binding affinity between miRNAs and their target mRNA [37].

rs4919510 in miR-608

Two studies [37,44] found significant associations between the rs4919510 SNP in mature miR-608 and the prognosis of CRC patients. The study by Xing et al. [53] demonstrated that rs4919510 in pre-miR-608 is associated with changes in recurrence-free survival in patients undergoing chemotherapy. In contrast, another study indicated that the G allele and GG genotype are associated with decreased susceptibility to CRC, observed in codominant (CG vs. CC; GG vs. CC), dominant (GC/GG vs. CC), and recessive (GG vs. CC/CG) analyses, suggesting a protective effect among stage 0-II cases in the Chinese population [54]. This evidence corroborates what was observed in the study by Pardini et al. [55], which revealed that individuals carrying the rs4919510G variant in miR-608 had a reduced risk of recurrence compared to those carrying the wild-type genotype. However, further studies are needed to investigate the role of the rs4919510 SNP in miR-608 in relation to CRC risk and prognosis.

rs2682818 and rs1051690 in miR-618

Two studies have associated the rs2682818 SNP in mature miR-618 with reduced risk of CRC, especially in patients with the AC genotype [40,42]. In contrast, in the study by Landi et al. [22], the rs1051690 SNP in the miR-618 binding site was associated with an increased risk of CRC, especially in patients with the G/A genotype. Furthermore, Shao et al. [34] demonstrated that the rs2682818 SNP is associated with CRC risk, observing that individuals with the GG/TG genotypes had worse overall survival and reduced expression of mature miR-618 compared with those with the homozygous TT genotype. To date, no other studies have been identified that explored the rs1051690 SNP in the context of miR-618. Therefore, further studies are needed to investigate the functional effects of these genetic variants on miR-618 and their relationship with CRC susceptibility, aiming to improve risk stratification and develop better disease screening and prevention strategies.

rs11614913 in miR-196a2

Three studies found no significant association between the rs11614913 SNP in mature miR-196a2 and CRC risk, especially in individuals with the TC genotype, in two distinct populations (31,45,46). In contrast, Zhan et al. [38] reported that Chinese individuals with the CC genotype or carrying at least one C allele had higher levels of mature miR-196a and were associated with a higher risk of CRC, although no association with cancer progression was observed. Furthermore, another study indicated that the CT genotype of the rs11614913 SNP, located in the hsa-mir-196a2 pre-miRNA, was associated with an increased risk of CRC compared with the CC genotype [56]. On the other hand, a meta-analysis suggested that the TT genotype of the rs11614913 SNP might be associated with a reduced cancer risk, especially for CRC and lung cancer, in Korean and Indian populations [57]. These contradictory findings highlight the need for further investigation to better understand the impact of rs11614913 on miR-196a2 and its relationship with CRC risk.

rs2292832 in miR-149

Chayeb et al. [31] reported that the rs2292832 C/T variant in miR-149 and rs2910164 G/C variant in miR-146a are associated with CRC risk. A meta-analysis indicated that individuals with the TT

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genotype have a higher risk of CRC compared with those who carry at least one C allele [58]. Furthermore, another study suggested that individuals with the rs2292832 variant and the TT genotype have an increased risk of breast cancer. This polymorphism in pre-mir-149 may affect miR-149 processing, resulting in changes in the abundance of the mature form of the miRNA. These changes may influence both tumor progression and metastasis [59].

rs2910164 in miR-146a

The rs2910164 G>C SNP in the miR-146a region has been associated with CRC risk and progression in two studies [31,33]. Iguchi et al. [60] reported a significant association between the CC/CG genotypes in pre-miR-146a and liver metastasis. Another study analyzing four SNPs in the pre-miRNA region demonstrated that the rs2910164 GC genotype was associated with an increased risk of CRC, while the CC genotype was associated with a decreased risk [56]. Furthermore, a meta-analysis revealed that the C allele of rs2910164 is associated with a decreased risk of cancer in the Chinese population [57]. Another study in the same population indicated that the rs2910164 GG genotype or the G allele was associated with an increased risk of CRC, especially in men, when compared with the CC genotype or the C allele [52]. These results suggest that miR-146a may play distinct roles depending on an individual's genotype, highlighting the need for further research to fully understand these variations.

rs895819 in miR-27a

The SNP rs895819 is located in the terminal loop of pre-miR-27a and would affect the secondary structure of pre-miR-27a resulting in the abnormal expression of hsa-miR-27a [61]. Cao et al. [32] demonstrated in their study that the GG genotype of rs895819 in miR-27a was significantly associated with the risk of CRC and metastasis compared to the AA genotype in the Chinese population. A similar finding in an independent study performed in the same population suggested that the GG genotype of rs895819 was significantly associated with the risk of CRC and that the G allele was associated with a greater risk of progression. In contrast, patients with the A allele (AA/GA) were significantly associated with decreased risk of CRC [62]. Wang et al. [63] demonstrated that individuals with the GG genotype and G allele exhibited a significantly increased risk of CRC in the same population. On the other hand, a study carried out in the European population showed no association between the rs895819T>C polymorphism in miR-27a and the risk of CRC [64], demonstrating the need for further studies including other populations of different ethnic origins.

rs187960998 in miR-211

Only one study identified the SNP rs187960998 in mature miR-211 as a tumor suppressor, demonstrating that it inhibits the proliferation and invasion of CC cells through the upregulation of CHD5 [41]. Other studies have focused on the expression of miR-211 and its role in CRC, without establishing a clear association with the presence of the genetic variant [65,66]. Therefore, further investigations are needed to clarify the impact of the polymorphism in miR-211 in the context of cancer.

rs2273626 in miR-4707 and rs202195689 in miR-4274

The current literature on the rs2273626 variant in miR-4707 and rs202195689 in miR-4274 in the context of cancer is limited. Huang et al. [39] associated these variants, located in miRNA seed regions, with a reduced risk of grade 2 leukopenia, as well as improved overall survival and disease-free survival. Ghanbari et al. [67] demonstrated that the rs2273626 SNP in miR-4707 influences the interaction with the glaucoma-related target gene Caspase Recruitment Domain Family Member 10 (CARD10), resulting in elevated levels of this gene. Additionally, Natalia et al. [68] suggested that the rs1553867776 variant in miR-4274 is associated with susceptibility to intestinal gastric cancer.

rs8176318 in miR-525-5p

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The study by Mullany et al. [30] highlighted that the SNP rs8176318 AA, located in the 3′ UTR of BRCA1 and predicted to bind to miR-525-5p, is associated with an increased risk of CC. In contrast, a study conducted in Pakistan revealed that women with the GG genotype in this same region had an increased risk of developing breast cancer, while those with the TT genotype had a reduced risk compared with those carrying the GG genotype [69]. However, the results of Cao et al. [70] demonstrated that the GT and GT + TT genotypes of rs8176318 in the 3′-UTR of BRCA1 could be associated with an increased risk of breast cancer compared with the GG genotype. These divergent results suggest that further research is needed to clarify the role of miR-525-5p and the rs8176318 variant in the 3'-UTR region of BRCA1, as well as their impact on different cancer types.

rs12904 in miR-200c

A study in the Iranian population revealed that the rs12904 AA genotype is associated with CRC pathogenesis and metastasis, while the rs12904 A>G genotype is related to reduced disease progression. Furthermore, the rs12904 SNP in the EFNA1 gene influences the expression of this gene, mediated by the microRNAs miR-200c and miR-429a [35]. In contrast, Simonian et al [47] found no association between the rs12904 SNP and the risk of CRC in the same population. On the other hand, a study carried out in the Chinese population identified that the rs12904 SNP located in the miR-200c binding site of the EFNA1 gene is associated with an increased risk of developing gastric cancer, with the AA genotype correlating with a higher expression of EFNA1 in gastric tissues, although it has no effect on the expression of the miRNA [71].

KRAS-LCS6 and rs868 in let-7

Studies by Smits et al. [36] and Xicola et al. [29] investigated the genetic variants KRAS-LCS6 of the KRAS gene and rs868 of the TGFBR1 gene in the miRNA let-7 binding site. The results showed that the KRAS-LCS6 variant is associated with the prognosis of CRC, while the rs868 SNP is related to the risk of the disease. Furthermore, another study indicated that patients with metastatic colorectal cancer (mCRC) harboring the LCS6 variant had a better response to therapy with anti-EGFR monoclonal antibodies (moAbs) [72]. On the other hand, Christensen et al. [73] demonstrated that patients with head and neck squamous cell carcinoma harboring the KRAS-LCS6 variant had significantly reduced survival time, suggesting that this variant may alter the phenotype or therapeutic response in this condition. The rs868 SNP, located in the 3′UTR of the TGFBR1 gene, has been shown to have a regulatory effect on miRNA/mRNA interaction by influencing negative RNA correlation in patients with hepatitis C virus-associated end-stage liver disease. Furthermore, this variant affects the expression of miRNAs let-7 and miR98 after liver transplantation, with a particularly significant effect in carriers of the rs868 AG genotype. Despite the associations identified between KRAS-LCS6 and rs868 variants in different aspects of CRC and other diseases, several open questions remain. Additional studies are needed to better understand the mechanisms by which these variants influence risk, prognosis, and response to treatment [74].

This review presents some limitations that should be considered when interpreting the results. In addition to the scarcity of studies focused on genetic variants in specific miRNA regions for CRC, we observed significant heterogeneity among the included studies. Most participants were predominantly Caucasian or Asian, which may introduce genetic and geographic variations that influence the findings. Despite these limitations, a notable advantage of this systematic review is the ability to evaluate, based on the existing literature and studies of high methodological quality, the hypothetical associations of SNPs in miRNA regulatory regions and their potential as biomarkers for CRC.

Conclusion

In recent years, the study of polymorphisms in miRNA regions has aroused great interest in the search for new promising, less invasive, sensitive and specific biomarkers for the early detection of CRC. This systematic review provides valuable insights into studies related to the diagnosis, prognosis and treatment of CRC, focusing on mirSNPs and contributing to a better understanding of

the pathways involved in the development of the disease. Our findings highlight the potential of polymorphisms in miRNAs as important tools in the early identification and management of CRC.

Declarations

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Authors' contributions K.A.S. was responsible for the concept and design of the original study. K.A.S, L.M.C.A.S, K.S.C.S and O.M.A performed a systematic search in the literature of original articles. A.D.L. and V.N.S. were responsible for the critical review of the manuscript. All authors read and approved the final version of the manuscript.

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