Chapter

MicroRNAs (miRNAs) in Colorectal Cancer

Burcin Baran, Nazli-Mert Ozupek, Gizem Calibasi-Kocal and Yasemin Basbinar

Abstract

Colorectal cancer (CRC) is the third most common cancer in the world and third leading cause of cancer-related deaths in men and women as well. While early screening procedures and removal of small polyps improve the survival rates among the patients, there is still need for new diagnostic and therapeutic approaches for developing more effective treatments. MicroRNAs (miRNAs) are short noncoding RNA fragments, which involve in posttranscriptional regulation of gene expression, and they are shown to involve in tumorigenesis either targeting oncogenes or tumor suppressor genes. Based on the current studies, miRNAs are now suggested as potential biomarkers for CRC diagnosis, prognosis, and therapeutic responses. In this chapter, the latest findings on the role of miRNA in CRC in many aspects are reviewed: diagnosis (role of circular miRNAs in blood and miRNAs from tissue biopsies and their potential role in pathophysiology and diagnosis of CRC), prognosis (miRNAs related with metastasis, recurrence, and survival rates in CRC), and therapeutic responses (role of miRNAs both in chemotherapies and/or in targeted therapies in CRC). In conclusion, miRNAs are promising molecules for diagnosis, prognosis, and therapeutic responses of CRC.

Keywords: colorectal cancer, diagnosis, miRNA, prognosis, therapeutic response

1. Introduction

MicroRNAs are a subgroup of small noncoding RNAs containing 18–25 nucleotides, and they do not carry any genetic information for protein expression. They regulate the posttranslational gene expression by binding 3′ untranslated region (UTR) of the target messenger RNA (mRNA). Approximately 30% of protein coding genes are regulated by miRNAs, and they have important roles in cellular functions including proliferation, differentiation, apoptosis, signaling, metabolism, and tumorigenesis. Due to their effect on crucial processes, miRNAs are significant modifiers of transcription and translation of both oncogenes and tumor suppressor proteins. Hence, some of them are classified as oncomiR and tumor suppressor miRNA in the cellular processes of tumor [1].

First miRNA, lin-4, was discovered in *Caenorhabditis elegans* in 1993, and it had role on the regulation of larval development by the repression of a nuclear protein encoded by *lin-14*. The second discovered miRNA, let-7, is expressed in late development and complementary to the 3′ UTR of the several genes including lin-14,

lin-28, lin-41, lin-42, and daf-12. After the discovery of lin-4 and let-7, miRNAs were shown in other organisms including plants and animals [2, 3], and over 10,000 miRNAs have been identified in various organisms. In humans, over 2500 types of encoded miRNAs have been determined [4].

2. Biogenesis of miRNA

The biogenesis of miRNA is a complicated process, starting in the nucleus, following with posttranslational modifications, and finalized in the cytoplasm. Similar to gene encoding, biogenesis of primary miRNAs (pri-miRNAs) is starting with the transcription by RNA polymerase II or RNA polymerase III enzyme. In the nucleus, pri-miRNA is recognized and cleaved by Drosha enzyme to form precursor miRNA (pre-miRNA). The pre-miRNA is exported to cytoplasm by exportin-5. In the cytoplasm, pre-miRNA is bound to cytoplasmic RNase Dicer and RNA-induced silencing complex (RISC), which is composed of argonaute 2 (AGO2) and transactivation response (TAR) RNA-binding protein (TRBP). Firstly, AGO2 cleaves the pre-miRNA from its 3′ end, and the cleaved pre-miRNA is further cleaved by Dicer into mature miRNA duplex. Mature miRNA duplex is then unwounded; while one strand of the miRNA remains on AGO2 protein, and the other strand (passenger strand) is degraded. Mostly, miRNAs are recognizing the complementary sequence of 3′ UTR of mRNAs, hence directing RISC to cleave mRNAs and translational repression of mRNAs [5, 6] (**Figure 1**).

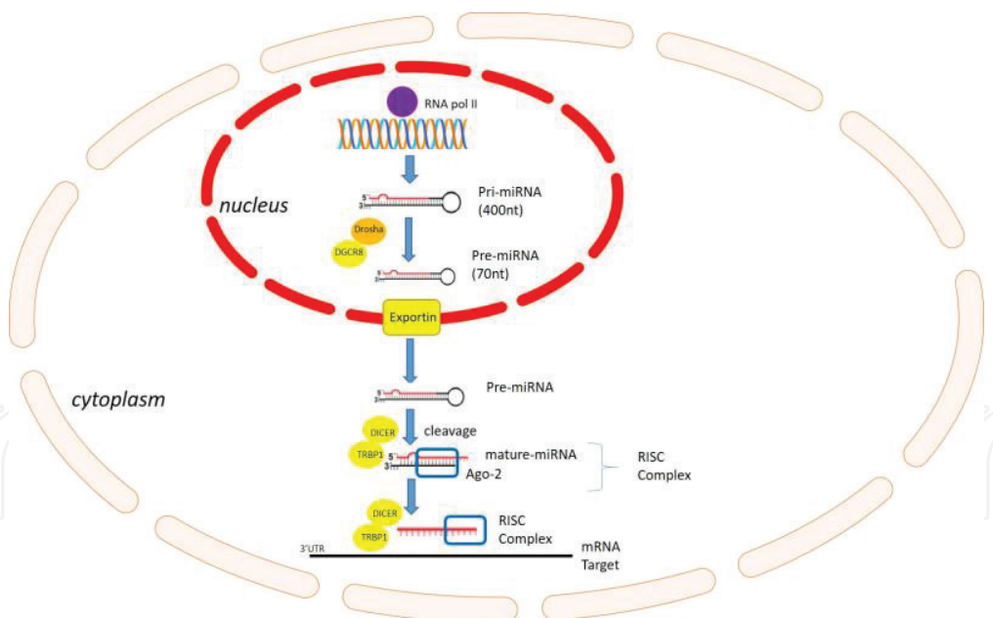

Figure 1.
miRNA biogenesis. The pathway starts miRNA transcription by RNA polymerase II or III to generate the primary transcripts (pri-miRNAs). Pri-miRNA is processed by the Drosha-DiGeorge syndrome critical region gene 8 (DGCR8, Pasha Pasha in Drosophila melanogaster and Caenorhabditis elegans) complex (also known as the microprocessor complex) that generates ~70 nucleotide (nt) pre-miRNAs. Pre-miRNA, which is recognized by the nuclear export factor exportin-5, is transferred to the cytoplasm. In the cytoplasm, the cytoplasmic RNase Dicer cleaves the pre-miRNA hairpin to its mature length. Dicer in complex with the transactivation response (TAR) RNA-binding protein (also known as TRBP and TARBP2) and argonaute (AGO) 1–4 mediate the processing of pre-miRNA and the assembly of the RISC (RNA-induced silencing complex). With the formation of this complex structure, one strand of the miRNA duplex is removed and single-stranded miRNA is generated. Interaction between microRNA complex and target mRNA induces post-transcriptional silencing by destabilization of mRNA and suppression of translation [7, 8].

3. Involvement of microRNAs in cancer

microRNA studies were began in *C. elegans*, as lin-4 and let-7 were identified as noncoding RNAs functioning in larval development. Soon after, the research groups focused on the function of these noncoding RNAs and discovered their homologs in vertebrates as well. The role of miRNAs in tumorigenesis was first reported in chronic lymphocytic leukemia (CLL) by two different groups in 2002. Hemizygous or homozygous loss of 13q14 chromosome was frequently observed among CLL patients [9]. Two different miR-15 and miR-16 expression levels were shown to be downregulated with the deletion of this locus [10]. Both miR-15/16 levels are inversely correlated with antiapoptotic B cell lymphoma-2 (Bcl-2) protein level in the cells. Introduction of miR-15/16 to the leukemic cell lines repressed Bcl-2 expression and induced apoptosis in these cells [11]. It is now very well established that aberrant miRNA expression contributes to cancer [12]. miRNAs are targeting the genes, which involve in cell proliferation, migration, invasion, and metastasis; hence dysregulation of these miRNAs leads to transformation and malignancy of cells [13, 14]. miRNA dysregulation in cancer cells can be result of genomic deletion, mutations, amplification, or epigenetic silencing [14]. A single miRNA can target a variety of mRNAs involved in different cell signaling pathways; interestingly, a single mRNA can be targeted by several miRNAs also [15], such as Let-7, which is one of the initially discovered miRNAs, targets human rat sarcoma (RAS), high-mobility group AT-hook 2 (HMGA2), and MYC mRNAs and downregulates their expression [16]. Phosphotensin homolog (PTEN), which is an important regulator of cell cycle, can be targeted by several different miRNAs including miR-21, miR-22, miR-106b-25, miR-17-92 [17].

In tumorigenesis, miRNAs either act as tumor suppressor or as an oncogene; interestingly, their expression is repressed or induced by transcription factors such as p53 or MYC via their promoter regions. miR-145 is one of the initial examples of tumor suppressor miRNAs. miR-145 was found to be downregulated in a variety of tumors including colon, breast carcinomas [18, 19]. It is interesting that tumor suppressor protein p53 induces miR-145 expression via p53 response element in its promoter. Later, miR-145 targets c-Myc or insulin receptor substrate I (IGF-R1) protooncogenes and silences their expressions, hence preventing tumor cell proliferation [18, 20] . Furthermore miR-145 inhibits invasion and metastasis by targeting Fli-1 or Mucin-1 [20, 21]. miR-145 also targets estrogen receptor-α (ER-α) via its two complementary sites and downregulates ER-α expression [22]. miR-34 family is another target of p53 tumor suppressor protein [23]. Another important tumor suppressor miRNA is miR-34 family. miR-34 family comprises three members: miR-34a, miR-34b, and miR-34c. While miR-34a is ubiquitously expressed in every tissue, expression of miR-34b and miR-34c is restricted to fallopian tubes, lungs, and brain [24, 25]. miR-34a is a very potential tumor suppressor since it is targeting many mRNAs related with proliferation [such as cyclin-dependent kinase-4 (CDK4) and cyclin-dependent kinase-6 (CDK6)], cellular growth [such as Notch2, platelet-derived growth factor receptor A (PDGFRA)], antiapoptosis [Bcl-2, sirtuin 1 (SIRT1), survivin], invasion, and migration [MET, SNAIL, cluster of differentiation (CD44)] [26–28]. Downregulation of miR-34 is observed among many malignancies and associated with poor prognosis [29, 30]. As a result of its role as a tumor suppressor, miR-34 has been applied either alone or in combination with conventional therapies on several tumor cell lines and mouse tumor models and showed promising results [31–34]. miR-34 was first miRNA tested in human Phase I trial (NCT01829971). MRX34, liposomal miR-34 mimic, was tested among patients with

solid advanced tumors. While MRX34 treatment showed evidence of antitumor activity in a subset of patients, it exerts some toxicities in patients. Hence, there is need for further studies for improving tolerability among the patients [35, 36].

In addition to tumor suppressor miRNAs, miRNAs behave like oncogenes, called as "oncomiRs." mir-21 is the first miRNA identified as oncogenic; it is significantly upregulated in many tumors including colon cancer, breast cancer, hepatocellular carcinoma, and glioblastoma [37]. miR-21 overexpression contributes to cell proliferation and antiapoptotic responses by targeting important downstream proteins such as phosphotensin homolog (PTEN), programmed cell death protein 4 (PDC4), and tropomyosin I [38–40]. Besides this, miR-21 was shown to be bona fide oncogene by causing pre-B-cell lymphoma in mouse models by overexpression. When mir-21 expression was inactivated, tumors regressed completely in few days [41].

As the importance of miRNAs became evident, miRNA expression profiles for each tumor type have been studied with several methodologies including microarray, QRT-PCR, and next-generation sequencing [15, 42]. miRNA expression profiles can reflect embryonic or development origin of the tissue and able to classify the origin of tissue with high accuracy (>90%), even separate cell subtypes (stem cells vs. progenitor cells) in the same tissue [43–45]. These miRNA profiling studies open the way for biomarker studies. In the biomarker studies, it is aimed to find diagnostic, prognostic, and predictive markers for better characterization of the disease and therapy response as an outcome [46].

4. miRNA and colorectal cancer

Colorectal cancer (CRC) is the second most common cancer among the women and third most common cancer among men. In 2016, more than 1.4 million men and women in the USA have been diagnosed with CRC [47]. Despite the availability of successful treatment options such as surgery, chemotherapy, and radiotherapy, the prognosis of CRC is not promising. Relapse or metastatic spread occurs after surgery in many CRC patients. Colorectal cancer is divided into two phenotypes according to mutational status. In chromosomal instability phenotype (CIN), high rate of inactivating mutations in adenomatous polyposis coli (*APC*) and tumor protein P53 (*TP53*) genes are found as well as activating mutations in Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene. However mutations in DNA repair genes, transforming growth factor-beta receptor II *(TGFBRII) gene, Bcl2- associated C protein (BAX) and BRAF* genes are commonly existed in microsatellite instability-high tumors (MSI-H) [48]. Certainly, genomic background affects the miRNA expression in CRC, such as *TP53* mutations affect miR-145 expression levels, which is downregulated among many CRC patients [49, 50]. Furthermore, miR-193a-3p expression was found as specifically downregulated in *BRAF*-mutated CRC cases [51]. The distinction between these phenotypes became more prominent in disease progression and therapy response, which will be discussed in the following sections. In CRC, to date, totally, 1870 original studies were retrieved in PubMed (as of May 2018), in which 38 of them were clinical trials investigating miRNA expression patterns in both CRC tissue specimen and plasma samples and compared them with normal samples. Bunch of miRNAs were found to be dysregulated in CRC samples in these studies [52–54]. While some of these miRNAs are related with early stages of tumorigenesis and can be used as diagnostic markers, the others are associated with therapeutic response, resistance to chemotherapy, and survival prognosis, hence aiding the physician in making therapeutic decisions as prognostic and predictive biomarkers [55].

4.1 miRNAs in colorectal cancer diagnosis

Early diagnosis is essential for CRC patients since they have more favorable prognosis. Fecal blood test and colonoscopy techniques are being currently used for early screening. However, fecal blood tests are not very efficient for detecting early carcinoma formation. Colonoscopy is a gold standard technique, it reduces cancer risk about 30–75%, yet it is invasive and expensive technique and highly uncomfortable for a patient [56]. Therefore, noninvasive and inexpensive screening and diagnostic methods or biomarkers are needed. miRNAs are promising candidates for noninvasive biomarker diagnosis. Diagnostic miRNAs can be isolated from blood or stool samples as well as tumor tissues [57] (**Table 1**).

There are different miRNA profiling studies comparing CRC samples with normal healthy tissue samples; however, each study emphasized on different set of miRNAs in CRC diagnosis and progression. According to miRNA profile study, miR-18a, -20a, -21, -29a, -92a, -106b, -133a, -143, and -145 expression levels were found to be significantly changed in CRC patients when compared with normal patients, and these markers can be used for CRC diagnosis [59]. In a systematic review, miR-106a, -30a-3p, -139, -145, -125a, and -133a were proposed as diagnostic biomarkers [60]. In another study, miR-143, -145, -21, -320, -126, -484-5p, -143, -145, -16, -125b, -21, and -106 were found to be candidate for diagnostic biomarkers [57]. While studies share some common miRNAs (such as miR143, miR145, miR106, miR21), they are differing in their list of miRNAs. In fact, the type of miRNAs can be differed due to the type of sample (blood or stool), experimental procedures, and used microRNA platforms. Another handicap of these studies is that they have been conducted with a small number of samples. Larger sample studies and additional meta-analyses are need for better determination of CRC-related diagnostic markers. Still, it can be said that miRNAs are very promising noninvasive markers for tumor diagnosis.

4.2 miRNAs in colorectal cancer prognosis

Taking part in CRC diagnosis, miRNAs are also affecting prognosis and therapeutic response. As mentioned before, the expression and deregulation of miRNAs in CRC patients are affected by chromosomal abnormalities and microsatellite instability [61, 62]. In CRC, miRNA expression dysregulation is shown especially in microsatellite instability (MSI-high) tumors. MSI-high groups are distinct population among CRC patients, which accounts for 15% of all cases, observed in hereditary cases such as Lynch syndrome or in sporadic cases mostly as a result of hypermethylation or inactivation of mismatch repair (MMR) genes [63]. These MSI tumors characterized by distinct behavior are associated with proximal tumor localization and high infiltration of lymphocytes. These phenotypes showed less distant organ metastasis than MSI stable tumors and have better prognosis [64]. Several miRNAs have been shown in participating in inactivation of several DNA mismatch repair genes, such as miR-155 downregulates mutL protein homolog 1 (MLH1), mutS homolog 2 (MSH2), and mutS homolog 6 (MSH6) mRNAs expression, whereas miR-21 targets MSH2 and MSH6 mRNA and inactivates them [65, 66]. Overall 94 miRNAs are differently expressed in microsatellite stable and in microsatellite instable tumors [67]. Upregulation (miR-17, miR-20, miR-25, miR-31, miR92, miR-93, miR-133b, miR-135a, miR-183, miR-203, and miR-223) and downregulation (miR-16, miR-26b, miR-143, miR-145, miR-191, miR-192, miR-215, and let-7a) are generally observed in MSI-high tumors [68]. miRNA expression is also differed among *TP53* and *KRAS* mutated tumors as well. miR-125p targets 3′ UTR region of p53 and represses p53 expression and accelerates the tumor growth;

miRNAs	Expression	Target genes
miR-15a	Upregulate	*Bcl-2*
miR-17-3p	Upregulate	*E2F*, *CDKN1A*
miR-18a	Upregulate	*SMAD4*, *KRAS*
miR-19a/miR-19b	Upregulate	*PTEN*
miR-20a	Upregulate	*BECN1*, *ATG16L1*, *SQSTM1*
miR-21	Upregulate	*PDCD4*, *PTEN*, *SPRY2*, *TPMI*
miR-24	Downregulate	*Topoisomerase-1*
miR-29a	Upregulate	*DNMT3*
miR-31	Downregulate	*WNT*, *β-catenin*
miR-34a	Downregulate	*SMAD4*, *FRAT1*, *Bcl-2*, *c-Met*
miR-92a	Upregulate	*PHLPP2*, *VHL*, *Bim*
Let-7g	Upregulate	*KRAS*, *Cdk6*, *Cdc25*, *HMGA2*
miR-106b	Upregulate	*P21*, *E2F1*
miR-133a	Downregulate	*MCL1*, *BCL2L2*
miR-143	Downregulate	*Erk5*, *DNMT3*, *KRAS*
miR-145	Downregulate	*EGFR*, *IRS-1*
miR-181b	Downregulate	*ATM*
miR-203	Downregulate	*ABL1*, *TP63*
miR-223	Upregulate	*STMN1*
miR-302	Upregulate	*GAB2*, *AKT2*
miR-320a	Downregulate	*VDAC*, *STAT3*, *SOX4*
miR-335	Upregulate	*RASA-1*
miR-375	Downregulate	*SLC7A11*, *IGFR1*, *SEC23A*
miR-422a	Downregulate	*TGF-β*, *CD73*
miR-423-5p	Downregulate	*RFVT3*
miR-601	Downregulate	*PTP4A1*
miR-760	Downregulate	*PHLPP2*

Abbreviations: Bcl-2, B cell lymphoma-2; E2F, E2F transcription factor 1; CDKN1A, cyclin-dependent kinase inhibitor 1A; KRAS, Kirsten rat sarcoma viral oncogene homolog; PTEN, phosphotensin homolog; BECN1, Beclin 1; ATG16L1, autophagy-related 16 like 1; SQSTM1, sequestosome 1; PDCD4, programmed cell death 4; SPRY2, sprouty RTK signaling antagonist 2; DNMT3, DNA methyl transferase 3; FRAT1, WNT signaling pathway regulator; PHLPP2, PH domain leucine-rich repeat protein phosphatase 2; VHL, von Hippel-Lindau tumor suppressor; Cdk6, cyclin-dependent kinase 6; Cdc25, cell division cycle 25A; HMGA2, high-mobility group gene; P21, CDKN1A, cyclin-dependent kinase inhibitor 1A; E2F1, E2F transcription factor 1; MCL1, BCL2 family apoptosis regulator; BCL2L2, BCL2 like 2; EGFR, epidermal growth factor receptor; IRS-1, insulin receptor substrate 1; ATM, ataxia telangiectasia mutated; ABL1, v-abl Abelson murine leukemia viral oncogene homolog 1; TP63, tumor protein p63; STMN1, stathmin 1; GAB2, GRB2-associated binding protein 2; AKT2, v-akt murine thymoma viral oncogene homolog 2; VDAC, voltage-dependent anion channel; SOX4, SRY (sex-determining region Y)-box 4; SLC7A11, solute carrier family 7 member 11; IGFR1, insulin-like growth factor 1 receptor; TGF-β, transforming growth factor-beta; CD73, cluster of differentiation 73; RFVT3, known as SLC52A3 (solute carrier family 52 member 3); PTP4A1, protein tyrosine phosphatase 4a1.

Table 1.
Simplified list of diagnostic miRNA markers for colorectal cancer (modified from Refs. [58, 59]).

hence, expression levels of miR-125p are associated with poor survival among CRC patients [69]. However, miR34 expression is a good prognostic marker. miR-34 is one of the targets of p53 protein and it increases miRNA expression. miR-34 then suppresses the expression of WNT pathway and epithelial mesenchymal transition (EMT)-related genes. Increase of miR-34b and miR-34c levels in stromal tissue is

leading to poor prognosis in colon cancer [70–72]. miR-122, miR-214, miR-372, miR-15b, let-7e, and miR-17 are other dysregulated miRNAs found in *TP53* mutated tumors [73]. miR-148-b and miR-221 are also important diagnostic markers associated with *p53* mutational status, and their overexpression is associated with worse prognosis [74, 75]. miR-143 and miR-145 are frequently downregulated in CRC and their one of the targets is KRAS mRNA; hence, they are important prognostic and predictive biomarkers in CRC [76, 77]. Let-7 role is one of the well-studied tumor suppressor miRNAs, which targets RAS. Let-7a expression is higher in *KRAS* mutated metastatic samples than normal mucosa or nonmetastatic disease [78]. Decrease Let-7b expression is worse prognostic marker, which is associated with recurrence and low overall survival of patients [79]. Furthermore, decrease in miR-487b levels is associated with liver metastasis in CRC patients [80]. Not only KRAS-associated miRNAs act as tumor suppressor, some of them are acting oncogenic in prognosis. miR-200 and miR-221 are downstream miRNAs of RAS pathway, and high expression of these miRNAs is related with worse prognosis [81].

Furthermore, exosome-containing miRNAs (miR-17/92 cluster and miR-19a cluster) are evaluated as biomarkers for early diagnosis and high recurrence in patients with CRC [82]. miR-21-5p, miR-29-3p, and miR-148-3p levels were studied in CRC samples and show that dysregulation in these miRNAs is associated with high mortality risk [83].

4.3 miRNAs in treatment response prediction of colorectal cancer

A variety of therapeutic advances are existed for CRC treatment such as conventional chemotherapy (5-fluorouracil, capecitabine, irinotecan, oxaliplatin), immunotherapy, radiotherapy, and chemoradiotherapy. miRNAs play an important role in the regulation of effectiveness and resistance to these therapies and prediction of personalized therapy response [84, 85]. Resistance to therapy is still the biggest challenge for defeating cancer. It may be caused by a variety of reasons such as reduction in transportation and intracellular accumulation of drugs by modulating the activity of drug transporters such ATP-binding cassette subfamily B (ABCB)/multidrug resistance (MDR) transporters (which is reviewed in reference [86]), dysregulation in DNA damage repair mechanisms, insufficient or oncogenic immune response, blockage of apoptosis, emergence of inflammation, and altered expression of oncogenes and tumor suppressor genes related with therapy response. miRNAs are actively participating in all of these resistance mechanisms [87, 88].

4.3.1 Chemotherapy

Although there are advances in cytotoxic and targeted therapy in CRC, drug resistance is one of the most important obstacles in front of successful chemotherapy [89]. Fluoropyrimidine-based chemotherapy (5-FU or capecitabine), vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor (VEGFR)-targeted, and epidermal growth factor receptor (EGFR)-targeted therapies are the main therapeutic methods for CRC [87]. miRNAs have role in chemotherapy resistance in terms of deregulation of drug metabolism-related enzymes, increased efflux of chemotherapeutics, impairment of chemotherapeutic-induced apoptosis, modulation of DNA damage repair, and autophagy [87].

miR-92b-3p, miR-3156-5p, miR-10a-5p, and miR-125a-5 were found to be related with progression-free survival in meta;static CRC patients treated with 5-FU/oxaliplatin/bevacizumab regime [90]. A negative relationship was found between miR-27b, miR-148a, and miR-326 expression levels and progression-free

survival in metastatic colorectal cancer patients receiving first-line oxaliplatin-based treatment [91]. The expression of miR-326 was related with decreased overall survival. These results proposed that plasma miRNAs can be used as noninvasive biomarkers for evaluating drug response in metastatic CRC patients who are treated with 5-FU and oxaliplatin-based chemotherapy [91] (**Table 2**).

4.3.2 Immunotherapy

Since chemo/radio therapies and surgery have limitations, immunotherapy is a good alternative to treat CRC patients. Immunotherapy aimed to evoke immune system to eliminate tumors either using immune stimulatory cytokines (vaccines, etc.) or checkpoint inhibitors [such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed death 1 (PD-1) receptor, and its ligands (PD-L1/2)] [92]. Interestingly, immune cell filtrates more in MSI-high CRC, and these subtypes are responding better to immunotherapies [93].

miRNAs	Therapy	Expression	Target genes
miR-7	EGFR-targeted	Downregulate	*EGFR*, *RAF-1*
miR-10b	5-FU	Upregulate	*BIM*
miR-20a	Oxaliplatin	Upregulate	*BNIP2*
miR-21	5-FU	Upregulate	*MSH2*
miR-22	5-FU	Downregulate	*BTG-1*
miR-23a	5-FU	Upregulate	*APAF-1*, *ABCF-1*
miR-27a, miR-27b	5-FU	Downregulate	*DPYD*
miR-133b	EGFR-targeted	Downregulate	*EGFR*
miR-139-5p	5-FU	Downregulate	*Bcl-2*
miR-143	Oxaliplatin	Downregulate	*IGF-1R*
miR-153	Oxaliplatin	Upregulate	*FOXO3a*
miR-199-5p, miR-375	EGFR	Upregulate	*PHLPP1*
miR-203	5-FU	Downregulate	*TYMS*
miR-203	Oxaliplatin	Upregulate	*ATM*
miR-204	5-FU	Downregulate	*HMGA2*
miR-218	5-FU	Downregulate	*TYMS*, *BIRC5*
miR-302, miR-369, miR-200c	5-FU	Upregulate	*MRP8*
miR-409-3p	Oxaliplatin	Downregulate	*Beclin-1*
miR-425-5p	5-FU	Upregulate	*PDCDIO*
miR-494	5-FU	Downregulate	*DPYD*
miR-519c	5-FU	Downregulate	*ABCG2*, *HuR*
miR-520g	Oxaliplatin	Upregulate	*P21*

Abbreviations: 5-FU, 5-fluorouracil; EGFR, epidermal growth factor; RAF-1, Raf protooncogene; BNIP2, BCL2-interacting protein 2; MSH2, human mutS homolog 2; BTG-1, BTG antiproliferation factor 1; APAF-1, apoptotic peptidase-activating factor 1; ABCF-1, ATP-binding cassette subfamily D member 1; DPYD, dihydropyrimidine dehydrogenase; Bcl-2, B cell lymphoma-2; IGF-1R, insulin-like growth factor 1 receptor; FOXO3a, forkhead box class O3; PHLPP1, Phlpp1 PH domain and leucine-rich repeat protein; TYMS, thymidylate synthase; ATM, ataxia telangiectasia mutated; HMGA2, high mobility group AT-hook 2; BIRC5, baculoviral IAP repeat containing 5; MRP8, myeloid-related protein 8; ABCG2, ATP-binding cassette subfamily G member 2; P21, cyclin-dependent kinase inhibitor 1A.

Table 2.
The expression profile of miRNAs that have role on chemotherapy response in colorectal cancer (modified from Ref. [85]).

miRNAs are essential in regulation of the immune response as well. The role of miR-34 has been mentioned earlier. Upregulation of miR-34a elicits the activation of tumor-infiltrating $CD8^+$ T cells by targeting PD-L1 [94]. miRNAs are also involved in innate immunity by macrophages and NK cells, and adaptive immunity by B cells, T cells, and dendritic cells. miR-124 modulates signal transducer and activator of transcription 3 (STAT3) pathway and enhances the T cell-mediated immune clearance [95]. miR-491 regulates the proliferation and apoptosis of $CD8^+$ T cells [96]. miR-491 inhibits the activation of $CD8^+$ T cells and promotes its apoptosis via targeting B-cell lymphoma-extra-large (Bcl-xL), cyclin-dependent kinase-4 (CDK4), and T cell factor 1 (TCF1), hence aiding tumor cells escaping from immune system. Tumor-derived TGF-β also induces the miR-491 expression. Thus, miR-491 can be evaluated as a new immunotarget for CRC treatment [96].

miR-196b, miR-378a, and miR-486-5p are evaluated as predictive biomarkers for the efficacy of the vaccine treatment in CRC [97]. miRNAs were enrolled in Phase II studies. In 16 patients, high expression of miR-196b-5p and low expression of miR-378a-3p and miR-486-5p are associated with better prognosis after vaccine treatment. Hence, these miRNAs can be determined as novel biomarkers for prediction of outcome responses of patients [97].

4.3.3 Potential candidates

miRNAs are also involving in radiotherapy responses. The expression of miRNA-processing enzymes Drosha and Dicer was found to be upregulated in radioresistant cell lines when compared with radiosensitive cell lines [98]. The role of miRNAs in radiotherapy response was evaluated further in the study cited as reference [87]. In the study, biomarkers for the prediction of chemoradiotherapy response in CRC were identified by using integrative and systematic bioinformatics analysis. The unique target genes of miR-198 and miR-765 were altered significantly upon transfection of specific miRNA mimics in the radiosensitive cell line. Thus, it could be said that miR-198, miR-202, miR-371-5p, miR-513a-5p, miR-575, miR-630, and miR-765 could be used for predicting the response of CRC to preoperative chemoradiotherapy [87]. Still, further studies are needed to understand the miRNA role in radiotherapy/radiochemotherapy prediction.

5. Concluding remarks and limitations

By the discovery of miRNAs, a significant number of studies have been conducted to indicate the utility of miRNAs. According to the highlighted studies, miRNAs in body fluids have potential to be predictive, diagnostic or prognostic biomarkers; and also they can be therapeutic targets due to their inducer ability on tumorigenesis. Basically, miRNAs offer promising practice for screening, diagnosis, prognosis, and treatment of cancer. Therefore, these noncoding RNA fragments may be used alone or combined with other protocols to screen, diagnose, prognose, and treat cancer. However, their clinical importance is still not conclusive, and validation studies are needed for routine-based clinical application.

Evidences showed that inhibition of oncomiRs or replacement of tumor suppressive miRNAs could be used to develop innovative treatment approaches. Further studies are needed to reveal the molecular mechanisms on the regulation of miRNA biogenesis. Determination of miRNA target genes, molecular interactions between target mRNA and miRNAs, and signaling pathways will help to interpret molecular mechanisms of cancer. Besides investigations on miRNA expression patterns and

their molecular mechanisms, studies on technological developments for reliable and cost-effective miRNA applications are also extremely important to enhance minimally invasive routine miRNA applications. Methodological variability among different clinical centers is the biggest limitation for the successful combination of miRNAs in cancer management. Standardization and normalization of essential steps of miRNA applications, such as miRNA extraction, processing, biobanking, and quantitation, eliminate the clinical facility-based variations. Using internal controls and enrollment of the laboratory accreditation/validation programs may present benefits for standardization. miRNAs have potential to be therapeutic targets and treatment options. But determination of mRNAs and miRNAs interactions and obtaining the large population-based multicenter cohorts are essential to use miRNAs in therapy. Especially before the implementation of miRNAs in clinics, evaluation of miRNA panels on large patient cohorts must be achieved.

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