Expert Opinion on Therapeutic Targets

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EXPERT OPINION

MicroRNAs as potential drug targets for therapeutic intervention in colorectal cancer

Mohammad Alam Jafri, Syed Kashif Zaidi, Shakeel Ahmed Ansari, Mohammed Hussein Al-Qahtani & Jerry W Shay[†]

[†]*UT Southwestern Medical Center, Department of Cell Biology, Dallas, TX, USA and Centre of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia*

Introduction: MicroRNAs (miRNAs) are small (19 – 22 nucleotide), non-protein-coding RNA segments that function as master regulators of hundreds of genes simultaneously in both normal and malignant cells. In colorectal cancer (CRC) miRNAs are deregulated and have critical roles in initiation and progression of CRC by interacting with various oncogenes and tumor suppressor genes including *APC, KRAS* and p53, or by modulating downstream signal transduction pathways. Numerous promising miRNAs have emerged as potential drug targets for therapeutic intervention and possible candidates for replacement therapy in CRC.

Areas covered: In this review the authors summarize the available information on miRNAs and their role in CRC. The authors point out specific miRNAs as potential drug targets and those having a significant role in gene activation and gene silencing during the process of CRC development, to highlight their importance as possible therapeutic candidates for the treatment of CRC.

Expert opinion: Targeting miRNAs provides an emerging opportunity to develop effective miRNA-based replacement therapy or antagonists to alter expression in colon cancer patient tumors. However, the biggest challenge is to overcome obstacles associated with pharmacokinetics, delivery and toxicity in order to translate the potential of miRNAs into efficacious anticancer drugs.

Keywords: antagomirs, anti-microRNA oligonucleotides, colon cancer, genomic instability, microRNA dysregulation, microRNA mimics, microRNA silencing, replacement therapy

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1. Introduction

Colorectal cancer (CRC) is the third most common type of cancer in men and women causing ~ 610,000 deaths per year worldwide and 50,310 deaths in 2014 in the USA [1]. The management/treatment of CRC is extremely challenging for oncologists due to the highly complex multifactorial nature of the disease. Despite huge investment and intensive research on the diagnosis and drug discovery for CRC, the clinical results have not significantly improved for the majority of CRC patients. The advent of target-based drug design has led to the discovery of many promising anticancer drugs including monoclonal antibodies, but they also failed to achieve desired outcomes in providing extended disease-free survival for the vast majority of patients suffering from CRC. In addition, current CRC chemotherapy is also facing a number of challenges because of variability in pharmacological responses, unacceptable toxicities and the development of drug resistance. Thus, the overall clinical scenario of CRC treatment does not appear very encouraging and suggests an urgent need for new, safe and effective anti-CRC drugs that can address

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Article highlights.

- Targeting microRNAs (miRNAs) for colorectal cancer therapy constitutes a rational method of target-based therapeutic intervention.
- MiRNA-based therapies may be developed to restore cell-type specific normal expression patterns of miRNAs.
- MiR-135b and miR-200c are promising candidates for miRNA-based therapy in colorectal cancer.
- A novel, acid-stable, miRNA systemic delivery platform, termed peptide with low pH-induced transmembrane structure (pHLIP), may improve successful delivery of desired therapeutic miRNAs to the tumor microenvironment.
- Numerous dysregulated miRNAs are potential biomarkers for the diagnosis, prognosis as well as for chemoresistance in colorectal cancer.

This box summarizes key points contained in the article.

present clinical issues. The existing situation also necessitates extensive exploration of other options such as natural biomolecules that may be developed as safe and effective treatment for CRC management or as novel drug targets for target-based therapeutic interventions. An important class of biological molecules known as microRNAs (miRNAs) has emerged as master regulators of gene expression in both normal cells and in malignant cells. Rapid advances in identifying miRNA specifically altered in solid tumors have significantly added to our knowledge of tumorigenesis regulation in terms of molecular events responsible for initiation and progression of cancer. However, exactly how these miRNAs (which can affect 100s of genes) control processes of transformation of a normal cell into a malignant cell remains much less well understood [2]. miRNAs have been found to be highly dysregulated in all types of human cancers including CRC [3]. Therefore, current research efforts are being directed towards finding out if miRNAs can be utilized for the purpose of diagnosis, prognosis and/or the development of novel anticancer drugs [4].

In this review we have attempted to summarize the available information on the biosynthesis, transportation, biological functions and potential uses of miRNAs as relevant drug targets and possible therapeutic candidates with particular reference to CRC.

2. miRNA: biosynthesis and function

miRNAs are small (19 – 22 nucleotide), single-stranded, evolutionary conserved, non-coding RNAs that represent a class of endogenously expressed small RNAs associated with a novel post-transcriptional gene regulation mechanism [5]. miRNAs usually function to maintain overall genetic homeostasis inside the cell by post-transcriptional gene expression control and by influencing several fundamental molecular events such as cell differentiation, division, survival, self-defense, migration, apoptosis and stem cell maintenance [6]. They act as guide molecules in a wide range of diverse gene silencing pathways. A large number of miRNAs implicated in cancer invasion, progression and chemotherapeutic resistance have been discovered. Characteristics of miRNAs such as their small size and remarkable stability compared to most messenger RNAs (mRNA) in tissues and extracellular fluids make them suitable as diagnostic and prognostic biomarkers for cancer and other diseases [7]. Additionally, specific miRNAs may either serve as appropriate therapeutics or an attractive therapeutic target for the discovery of potentially safe and effective treatments.

The biogenesis of miRNAs, involves several sequential steps (**Figure 1**). miRNA encoding genes are first transcribed from genomic DNA by RNA polymerase II to form 1 – 3 kb long primary-miRNAs [8]. The primary-miRNAs are subsequently recognized and cleaved by Drosha (RNA polymerase III enzyme) and a protein called DiGeorge Syndrome Critical Region 8 protein (DGCR8) [9]. This step results in the formation of about 60 – 100 nucleotide long hairpin-shaped precursors of miRNA termed pre-miRNAs. The specific cleavage of primary-miRNAs by the Drosha–DGCR8 complex is a crucial step in the synthetic process of miRNAs as it leads to the generation of what is believed to be specific nucleotide sequences in miRNAs that are associated with the ability of miRNAs for gene silencing. This specific nucleotide sequence is the target area on mRNA and is complementary to the 2 – 7 positions of antisense miRNA oligonucleotides. The pre-miRNAs are transferred to the cytoplasm by an RNA-binding protein called Exportin-5 [10] and are further fragmented into 18 – 24 double-stranded oligonucleotides by the RNase-III enzyme Dicer to form mature double-stranded RNAs (dsRNAs) [11]. One of the double strands of dsRNA matures as a miRNA which is finally incorporated in the RNA-induced silencing complex (RISC) to form a miRNA-induced silencing complex (miRISC) [12]. The second strand of dsRNA is usually degraded or it may be involved in the maintenance of optimal level of miRNA in the cell. The miRISC complex pairs with its complimentary target sequence on mRNA in a perfect or most often in an imperfect manner, thereby hiding it from the translational machinery or causing complete degradation of these complementary sequences [13]. It has been reported that in the case of perfect complementarity of the miRNA: mRNA complex, the mRNA is usually degraded by a protein called Ago2 (Argonaute RISC Catalytic Component 2) whereas if the complementarity is not perfect the translation of target sequence on mRNA is suppressed [14]. The translational inhibition appears to be a major miRNA gene regulatory mechanism because the majority of miRNAs pair with their respective mRNA imperfectly [15].The extent of expression of miRNAs in the cell is believed to be precisely controlled at three important stages that is at the miRNA gene level, at the epigenetic level, and at the

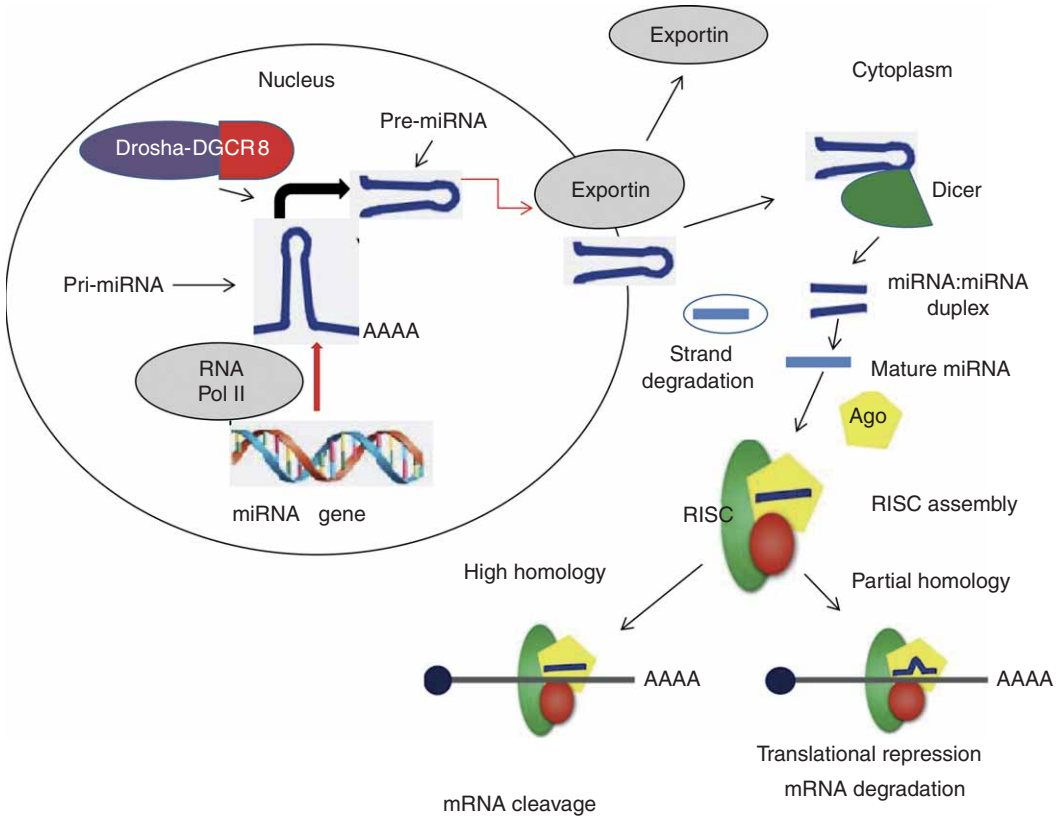

Figure 1. Biogenesis and function of miRNA: Genes of miRNA are first transcribed by RNA polymerase as Pri-miRNA. This nascent Pri-miRNA is then cleaved by a microprocessor composed of Drosha and DGCR8 in the nucleus, giving rise to precursor miRNA (Pre-miRNA). Pre-miRMA is transported to cytoplasm by a protein called Exportin 5. In cytoplasm Dicer removes the loop from pre-miRNA and helicase opens the pre-miRNA duplex forming the guide strand of mature miRNA. The mature miRNA is loaded with Argonaute (Ago) within the RISC complex and forms miRNA:RISC complex. The miRISC complex pairs with its complimentary target sequence on mRNA in a perfect or imperfect manner, there by hiding it from translational machinery or causes complete degradation of these complementary sequences.

transcriptional/post-transcriptional level to maintain normal genetic and physiological homeostasis (**Figure 2**) [16].

3. miRNA dysregulation in colorectal cancer

CRC is believed to be a consequence of mutational activation of oncogenes coupled with the mutational inactivation of tumor suppressor genes. The majority of CRCs arise from adenomatous polyps, which contain predisposed genetic mutations with the potential for malignant transformation. The development of CRC is characterized by an ordered series of events that are collectively known as the 'Adenoma-Carcinoma Sequence' (**Figure 3**). This sequence of events is primarily triggered by genomic instability which arises from the chromosomal instability, microsatellite instability and CpG island methylator phenotype. Genomic instability results in the loss of cell cycle control, thus, promoting unregulated cell growth and differentiation leading to tumorigenesis (**Figures 4** and **5**). Specific gene mutations also contribute to genomic instability. The inherited and somatic genetic mutations that are involved in the development of colorectal carcinogenesis include the mutations in Adenomatous Polyposis Coli (*APC*), *KRAS, Ctnn-Beta* (Catenin-Beta), *COX2* (Cyclooxygenase-2), Deleted In Colorectal Carcinoma (*DCC*), *SMAD4* (*SMA* and Mothers Against Decapentaplegic (*MAD*) Related Protein-4), *p53,* Transforming Growth Factor-Beta Receptor-Type II (*TGF-Beta R2*), Bcl2 Associated-X Protein (*BAX*), Matrix Metalloproteinase (*MMP*)-1/2/7/9/11/12/14, E2F Transcription Factor-4 (*E2F4*) and Mismatch Repair (*MMR*) genes such as, MutS Homolog-2 (*MSH2*), MutS Homolog-3 (*MSH3*), MutS Homolog-6 (*MSH6*) and MutL Homolog-1 (*MLH1*) [17]. These mutations result in dysregulation of miRNAs in the cells leading to aberrant activation of signaling pathways and their downstream effector molecules. This miRNA-mediated cascade results in altered patterns of cellular processes such as cell differentiation, division, invasion, migration and apoptosis. Therefore, the miRNA expression profile of a

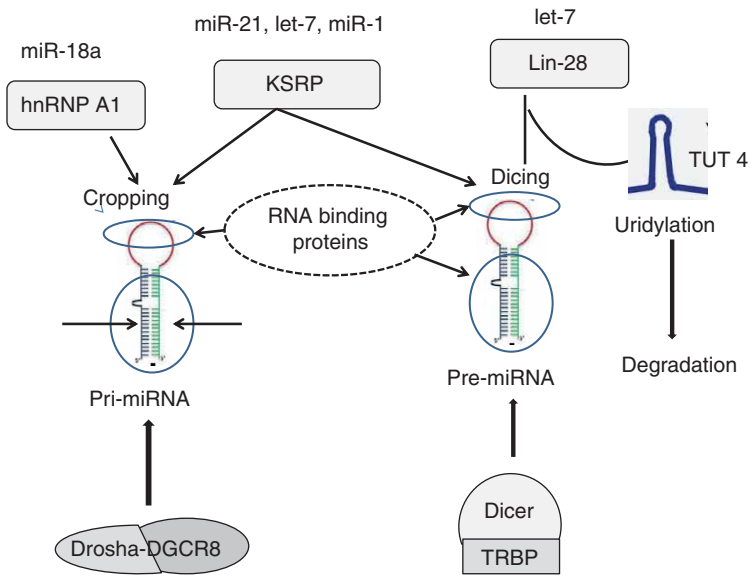

Figure 2. Posttranscriptional regulation of miRNA maturation by the action of RNA-binding proteins (hnRNP, KSRP, Lin-28) on pri- and pre-miRNA. These RNA-binding proteins interact with the terminal loop of the miRNAs and stimulate or retard their biogenesis.

hnRNP: Heterogenous nuclear ribonucleoprotein; KSRP: KH-type splicing regulatory protein; TRBP: Trans-activation response RNA-binding protein.

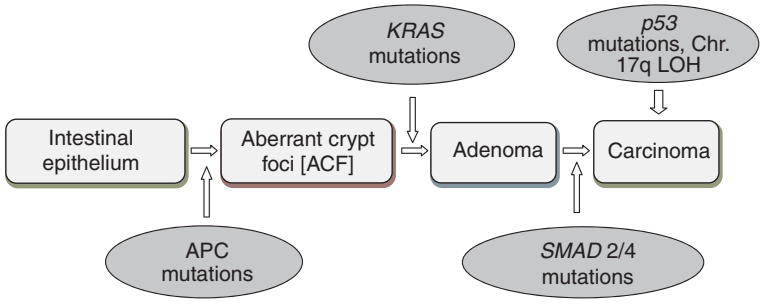

Figure 3. The adenoma-carcinoma sequence: a sequential transformation from normal epithelium to carcinoma due to a series of genetic changes.

particular cell may drastically affect the fate of the cell. Low expression levels of a specific miRNA may result in the upregulation of oncogenes whereas higher expression of others may cause downregulation of tumor suppressor genes. The extensive research on differential expression of miRNAs in CRC using a variety of molecular techniques such as deep sequencing, miRNA microarrays, and qRT-PCR to understand miRNA expression profile in CRC tumors has revealed that miRNA expression patterns in CRC tumors is considerably different compared to that observed in non-malignant tumors. This suggests that miRNAs play a crucial regulatory function and their dysregulation is associated with malignant transformation of normal cells [18]. In addition, recent studies have focused their attention on understanding the miRNA expression profile of different cell types in order to gain insights into their therapeutic potential. Attempts are being made to decipher single-cell mRNA expression patterns to understand their role in cell-specific gene expression regulation [19].

The first study on differential expression profile of miRNAs in CRC tumors and normal tissues showed that miR-143 and miR-145 were consistently downregulated at the adenoma and carcinoma stages compared to corresponding normal tissues [20]. Subsequent studies also confirmed these findings and established that *ERK5* (extracellular-signal-regulated kinase 5), a member of the MAPK family, is the target gene for miR-143 and it functions as a tumor suppressor through inhibition of *KRAS* translation. Similarly, miR-145 has also

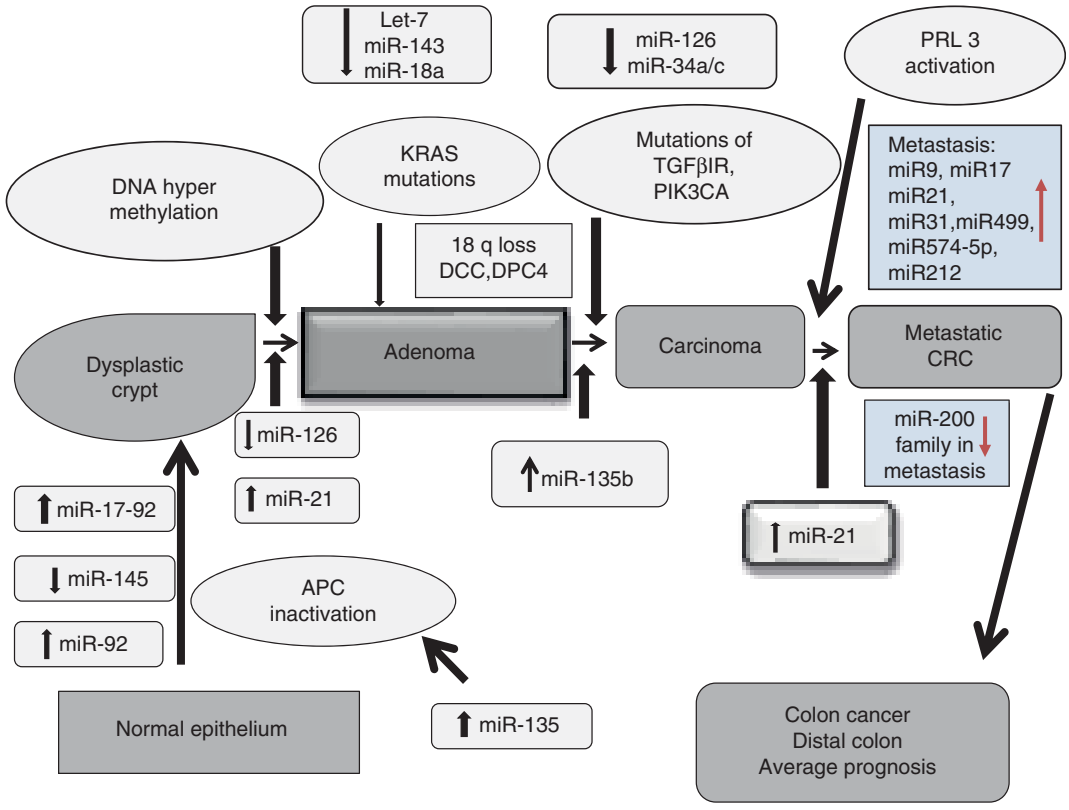

Figure 4. Chromosomal instability pathway (CIN) and expression of miRNAs in CRC. miRNAs are indicated as up- or downregulated.

been extensively studied as a tumor suppressor that regulates multiple cellular pathways including cell cycle, proliferation, apoptosis and invasion, by targeting multiple oncogenes. miRNA-145 could suppress cancer cell proliferation by targeting growth factor-related genes such as *IRS-1* (Insulin receptor Substrate 1), IGF-Receptor I (*IGF-IR*) or EGFR. Moreover, it induced cell apoptosis and cell cycle arrest by inhibiting DNA Fragmentation Factor 45 (*DFF45*), a gene encoding a 45 KDa protein involved in apoptotic cascade, *CBFB* gene (Core-Binding Factor Beta subunit, a transcription factor encoding gene), Clathrin Interactor 1 (*CLINT1*) gene (encodes epsin-like protein CLINT 1 that functions in transport via clathrin-coated vesicles from the trans-Golgi network to endosomes), *PPP3CA* (protein phosphatase 3 catalytic subunit, alpha isozyme) gene that encodes calcium-dependent, calmodulin-stimulated protein phosphatase or c-Myc (Myelocytomatosis) (a regulator gene that codes for a transcription factor) [21]. Furthermore, miR-145 has also been implicated in direct regulation of some oncogenes involved in cancer cell invasion and metastasis, such as Mucin 1, Cell-surface Associated (*MUC1*), Fascin Actin-Bundling Protein 1 (*FSCN1*), Neural Precursor Cell Expressed, Developmentally Down-Regulated 9 (*NEDD9*) and Sry-related HMG box 9 (*SOX9*) [22,23]. In addition, miR-145 plays an important role in regulating cell differentiation by targeting core reprogramming factors, including Octamer-Binding Transcription 4 (*OCT4*), *SOX2* and Krüppel-like Factor 4 (*KLF4*) [21]. However, further studies revealed that the miR-143 and miR-145 genes are closely located in a 1.6 kb region on chromosome 5q33.1. These miRNAs are usually co-expressed and are consistently reported as being downregulated in cancer cells including CRC [24]. In addition, *in vitro* functional studies indicated that miR-143 and miR-145 may perform opposite functions in different cell types. For example, they inhibit cell proliferation in mesenchymal cells of metastatic CRC but in fibroblasts they stimulate cell differentiation [25]. Furthermore, numerous recent studies suggest that the expression of miR-143 and miR-145 is highly cell-type specific. Chivukula *et al.* [26] demonstrated that miR-143/145 are expressed and function exclusively within the mesenchymal cells rather than epithelial cells. Additionally, in a miR-143/145 knockout mice, the development of intestine proceeded normally but regeneration after injury was severely impaired due to dysfunction of smooth muscle cells (SMCs) and fibroblasts. This was associated with derepression of the miR-143 target IGF Binding Protein 5 (*IGFB5*), which impaired IGF signaling after epithelial injury thus inhibiting cell growth and proliferation. Similar predominant expression of miRNA 143/145 in mesenchymal

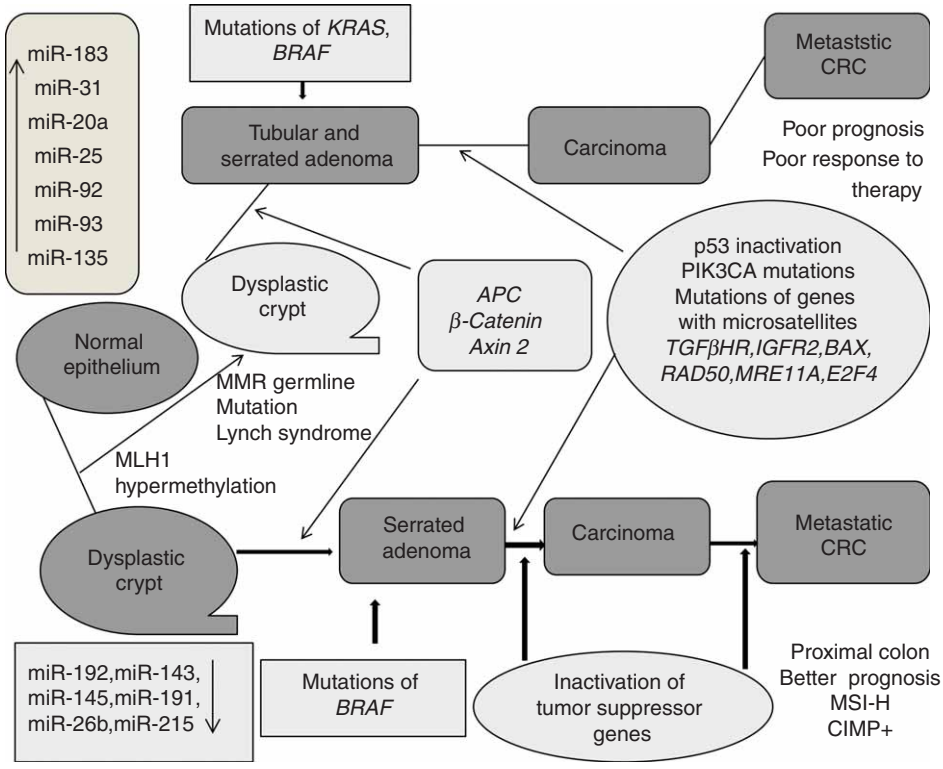

Figure 5. Microsatellite instability (MSI) pathway leading to CRC and association of miRNAs expression with MSI status in colorectal cancer. Upward arrow shows miRNAs significantly overexpressed in MSI positive tumors relative to non-neoplastic mucosa and downward arrow denotes miRNAs significantly underexpressed in MSI positive tumors.

cells including SMCs and fibroblasts has also been reported [24]. These studies highlight the importance of understanding the cellular pattern of miRNA expression in CRC initiation and progression in order to establish their therapeutic potential.

MiR-1288 was also shown to be differentially expressed in CRC tumors. The majority of colorectal adenocarcinoma (CAC) tumors displayed reduced miR-1288 expression compared to colorectal adenomas (CA) and non-neoplastic tissues. However, the relative expression levels of miR-1288 were higher in distal CACs and in tumors of early stages [26]. Similarly, differential expression of many other miRNAS (miR-200c, miR-200a-3p, miR-1246, miR-92a-3p, miR-141 – 3p, miR-192 – 5p, miR-194 – 5p, miR-574 – 3p and miR-3651 – 5p) was demonstrated in tumor tissues and stroma of 51 patients with colorectal carcinoma [27]. High-throughput sequencing showed elevated expression of additional miRNAs (miR-135b-5p, miR-146a-5p, miR-148b-3p, miR-17 – 5p, miR-196a-5p, miR-200a-3p, miR-20a-5p, miR-21 – 5p, miR-223 – 3p, miR-27a-5p, miR-29b-3p, miR-30e-5p, miR-374b-5p, miR-4787 – 5p, miR-485 – 3p and miR-660 – 5p) in solid tumors of CRC patients [28]. Furthermore, in tissue samples of CRC patients, miR-455, miR-484, and miR-101 were found to be substantially downregulated and the overexpression of miR-455 in SW480 cells significantly inhibited their proliferation and invasion. It was also reported that overexpressed miR-455 decreased protein expression of the *RAF* proto-oncogene serine/threonine-protein kinase (*RAF1*) but had no effect on mRNA level suggesting that miR-455 regulated *RAF1* expression directly [29]. It was shown in a study involving 46 microsatellite stable stage II CRC patients that miR-375 expression was downregulated, and it exerted its proapoptotic function by directly targeting a potent oncogene Yes-associated protein-1 (*YAP1*) gene (associated with Hippo-tumor suppressor pathway) and downstream anti-apoptotic targets *BIRC5* (Baculoviral IAP Repeat-Containing 5) and *BCL2L1* (B-cell Lymphoma 2 Like 1). Interestingly, it has been shown that hypermethylation of miRNA promoter region inhibits miRNA gene transcription in cancer cell lines but in the case of miR-375, promoter hypermethylation does not appear to be a major mechanism of miR-375 downregulation under *in vivo* conditions because none of the CRC tissue samples demonstrated miR-375 promoter methylation. Overall, these results can be interpreted to suggest that yet unidentified molecular mechanism affects miR-375

expression in CRC [30]. Recently, Wang B *et al.* [31] have shown that tumor suppressive miR-194 was significantly downregulated in CRC tissues compared to that of corresponding noncancerous tissues. Decreased miR-194 expression correlated well with tumor size and tumor differentiation, as well as TNM stage. Overexpression of miR-194 inhibited cell proliferation both *in vitro* and *in vivo* by directly targeting *MAP4K4* and its downstream target *MDM2*. Thus, miR-194 appears to regulate the MAP4K4/c-Jun/MDM2 signaling pathway. A comparative analysis of miR-378 expression in CRC cell lines and 84 pairs of CRC and normal adjacent mucosa demonstrated that levels of miR-378 were significantly downregulated in CRC tissues and cell lines and it functions as tumor suppressor by directly targeting the 3′-UTR of vimentin [32]. However, miR-378 has been reported to be upregulated in many cancer types including glioblastoma, breast cancer and renal cell carcinoma. In these types of cancers, miR-378 seemed to be an oncogene, and enhanced tumor cell survival, promoted tumor growth and metastasis in some tumors via regulation of the target genes *SuFu* (Suppressor of Fused Homolog), *FUS1* (Fused In Sarcoma 1), *HMOX1* (Heme Oxygenase (Decycling)1), *ESRRG* (Estrogen-Related Receptor Gamma) and *GABPA* (GA Binding Protein Transcription Factor) [33-35]. On the contrary, other studies demonstrated that miR-378 was significantly downregulated in gastric cancer and oral cancer and may act as tumor suppressor by negatively regulating expression of Cyclin-Dependent Kinase 6 (CDK6) and VEGF [36]. Similarly, it has been reported that miR-378 is significantly down-regulated in CRC [37]. The downregulation of miR-378 in CRC is associated with large tumor size, advanced clinical stage, lymph node metastasis and shorter overall survival of the patients suggesting that it might be involved in CRC progression. Furthermore, over-expression of miR-378 could significantly inhibit cell proliferation and invasion *in vitro* and tumor growth *in vivo*. Overall, these studies suggest that the oncogenic or tumor suppressive function of miR-378 may be cancer-type or cell-type specific. Consequently, potential application of miR-378 in miRNA-based therapy for CRC treatment appears to be limited but it may be utilized as an independent diagnostic and prognostic biomarker.

3.1 Mechanisms of miRNA deregulation in CRC

Over the past few years, a large number of miRNAs have been discovered and specific miRNA gene targets of many newly identified miRNA have been identified, facilitating our understanding of miRNA-induced pathologies in CRC. This has generated hopes that miRNA-based therapies may be development to eventually rescue aberrant miRNA gene expression. To achieve this an in-depth understanding of the factors that govern miRNA gene regulation is extremely necessary. However, the exact mechanism of dysregulation of miRNAs in CRC remains complex and not well understood. Recently, several studies have shown that the regulation of miRNA expression and function may involve several diverse mechanisms. One of the most important mechanisms controlling miRNA abundance is the regulation of pri-miRNA transcription, which could be positively or negatively regulated by different factors such as transcription factors, enhancers, silencers and epigenetic modification in miRNA promoters. It has been indicated that the oncogenic transcription factor Myc acts as a miRNA transcriptional regulator, promoting the transcription of some oncogenic miRNAs as well as the transcriptional inhibition of tumor suppressor miRNAs. For example, transcription of oncogenic miR-17–92 is promoted when Myc binds to the E-box in the miR-17–92 coding sequence [38]. Additionally, several lines of evidence have recently emerged to suggest that miRNAs participate in regulatory loops that modulate their own expression. For example, tumor suppressor miR-145 coordinates with tumor suppressor p53 to induce the pro-apoptotic effect and p53 in turn stimulates miR-145 transcription. Thus, p53 also plays an important role in transcriptional regulation of miRNAs. It regulates gene transcription of the miR-34 family members (miR-31a, mir31b and miR-31c) through binding to p53 REs (response elements) in miR-34a and miR-34b/c promoters and activates transcription of the miR-34 family [39]. In addition to the miR-34 family, p53 also directly regulates the transcriptional expression of several additional miRNAs through binding to the p53 REs in their promoters, including miR-145, miR-107, miR-192 and miR-215 [40].The epigenetic processes including hypermethylation of promoter regions or histone modifications, significantly contribute to the transcriptional regulation of dysregulated expression of miRNA in CRC [41]. For example, extensive hypermethylation of promoters of miRNAs such as miR-9, miR-34a, miR-34b, miR-34c, miR-129 and miR-137 leads to their decreased expression in CRC tissues, suggesting that promoter methylation also contributes to transcriptional downregulation resulting in miRNA dysregulation. It has also been suggested that transcriptional regulation of miRNA gene expression may be due to the influence of epigenetic changes in the host gene regulatory apparatus, which is situated at a distance from the miRNA locus. For example miR-342 is silenced by CpG island methylation in CRC [42]. Inherited genetic variations leading to alterations in both miRNAs and their binding sites have also been implicated in miRNA dysregulation. Certain environmental conditions, such as hypoxia, have also been reported to upregulate transcription of miRNAs. Single nucleotide polymorphisms (SNPs) in miRNA gene might affect the transcription of primary miRNA. For example, a significant reduction in miR-499 expression in CRC has been linked to a specific genotype. However, SNPs in miRNA binding sites mainly contribute to the miRNA-mediated regulation of target gene expression by significantly altering miRNA–mRNA interactions. Similarly, SNPs in miRNA sequences may change miRNA expression and/or maturation consequently affecting abundance of miRNA in CRC tissues.

Table 1. Expression of the miRNAs with promising potential as therapeutic targets/therapy and biomarkers in CRC.

Identified miRNA	Target gene	Function	Ref.
Some of the upregulated miRNAs in CRC and their target genes			
miR-135/b	*APC*	Wnt-signaling pathway	[54,95]
miR-27	*APC*	Promotes growth and metastasis	[85]
miR-221	*RECK*	Stimulates invasion and metastasis	[99]
miR-210	*RBM3*	Promotes Angiogenesis	[83,84]
miR-21	*CDC25A*	Cell cycle regulation	[45]
miR-31	*RASA1*	Promotes cell proliferation	[47]
miR-182 and miR-503	*FBXW7*	Tumor growth and progression	[59]
miR-200c	*ZEB1* and *ZEB2*	Metastasis	[71]
miR-224	*PHLPP1, PHLPP1*	Proliferation	[49]
miR-301a	*TGFBR2*	Proliferation, migration	[61]
Some of downregulated miRNAs in CRC and their target genes			
miR-34a	*FRA-1*	Invasion, migration	[60]
miR-137	*Cdc-42,LSD-1*	Tumor growth	[133]
miR-141	*SIP1*	Inhibits cell invasion and migration	[134]
miR-149	*FOXM1*	Proliferation, migration	[62]
miR-185	*RHOA*	Cell cycle progression	[50]
miR-195	*CARMA3*	Promotes apoptosis	[52]
miR-215	*TYMS,DTL*	Cell migration and proliferation	[135]
miR-330	*CDC42*	Cell proliferation	[51]
miR-339 – 5p	*MDM2*	Invasion, migration	[64]
miR-375	*YAP1*	Apoptosis	[30]
miR-455	*RAF1*	Proliferation	[29]

In one study of 57 SNPs responsible for miRNA binding site variability, eight SNPs were found to be significantly associated with modified expression of miR-184, miR-212, miR-200a, miR-337 and miR-582 in CRC patients [43]. However, a subsequent study with 40 miRNA-related SNPs in 426 patients with adenocarcinoma could not establish a consistent correlation between SNPs that affect miRNA-binding sites and their dysregulation [44].

4. miRNA in the pathogenesis of CRC

Since the discovery of miRNAs as an important class of gene expression regulators, intensive research has been conducted to understand their precise role in cancer initiation and progression including CRC. These studies have strongly suggested their important and critical roles in cancer but no unifying theme has emerged. A large number of upregulated or downregulated miRNAs have been identified. Some of these miRNAs appear to function by interfering with normal activity of important genes including tumor suppressor and oncogenes (**Table 1**).

4.1 miRNAs: cell cycle regulation in CRC

miRNAs have been reported to contribute to CRC tumorigenesis by disrupting critical regulatory check points in cell cycle. Oncogenic miRNAs that are frequently upregulated in CRC tumors stimulate cell cycle entry and cell division whereas as tumor suppressor miRNAs (downregulated in CRC) induce cell cycle arrest and thus inhibit cell proliferation. Several miRNA candidates, such as miR-21, miR-31, miR-135a&b and miR-224 that are frequently upregulated in CRC, play a predominant cell proliferation inhibitory role. For example miR-21 delays G1-S transition by targeting *CDC25A* (Cell Division Cycle 25A) in CRC cells [45]. This gene encodes a phosphatase (CDC25A) that activates the cyclin-dependent kinase CDC2 by removing two phosphate groups during progression from G1 to the S phase of the cell cycle. Xiong B *et al.* [46] demonstrated that miR-21 regulates numerous cellular processes including proliferation, invasion, migration and apoptosis by targeting PTEN/PI-3 K/Akt signaling pathway. Similarly, another upregulated oncogenic miRNA, miR-31 has been shown to stimulate CRC cell proliferation by targeting RASA1 (RAS P21 Protein Activator 1), a GTPase-activating protein [47]. MiR-135a, b target *APC* gene to induce cell cycle progression and stimulate cell proliferation [48]. Zhang *et al.* [49] reported that oncogenic miR-224 was significantly upregulated in CRC tissues and its overexpression in SW 480 cells promoted their proliferation. A tumor suppressor miR-185 (downregulated) inhibits division of CRC cells by targeting genes, *RHOA* (Ras Homolog Family Member A) and *CDC42,* involved in cell cycle progression; however, this mechanism appears to be cell-type specific [50]. Similarly, miR-330 expression is reported to be reduced in CRC tissues and its ectopic expression in SW1116 cells reduced their proliferative potential by negatively regulating expression of *CDC42* [51]. The expression of tumor suppressor miR-195 has been shown to be downregulated in CRC tissues. It inhibits cell

proliferation by targeting *CARMA3* (Caspase Recruitment Domain Family, Member) and an inverse correlation is noted between miR-195 expression and *CARMA3* protein expression [52].

4.2 Role of miRNAs in tumor initiation and progression

One of the most critical genes mutated in CRC is the *APC* gene located on human chromosome 5q21 and mutations in *APC* are considered to be one of the earliest events in the process of initiation of CRC. *APC* mutations have been found to correlate with increased expression of miR-135a/b in epithelial cells of colon [53]. In a recent study, it was demonstrated that deregulation of miRNAs in CRC is not a bystander molecular event but an actual driver of the tumor progression [54]. It was shown that over-expression of miR-135b is triggered by mutations in *APC* gene leading to PTEN/PI3K pathway deregulation. Inhibition of miR-135b in CRC mouse models reduces tumor growth by controlling genes involved in proliferation, invasion, and apoptosis. Additional somatic mutations accumulation in cells containing *APC* mutations contribute to further dysregulation of miRNAs leading to the emergence of aberrant downstream pathways. For example, miRNAs let-7, miR-18 and miR-143 have been reported to be associated with *KRAS* knockdown and activation of EGFR-MAPK pathway [55]. Similarly, miR-21 is involved in the stimulation of the PI3K pathway whereas miR-126 inhibits this pathway [56,57]. In addition, the miR-17 – 92 cluster (miR-17, miR-18a, miR-19a, miR-20a, miR-19b, miR-92a) stimulates transformation of adenomas to adenocarcinomas by up-regulation of *c-myc* [42]. Thus, activation or inhibition of these downstream pathways in CRC under the influence of miRNA-mediated gene silencing leads to increased cell proliferation, enhanced cell survival and initiation of angiogenesis. Additionally, the miRNA-34 family, which includes 34a, b, and c, are downregulated in CRC and contribute to the progression of the disease [58]. It has also been shown that miR-182 and miR-503 undergo sequential upregulation and drive the progression of colon adenoma to adenocarcinoma by cooperatively downregulating the tumor suppressor *FBXW7* (F-Box and WD Repeat Domain Containing 7) gene which encodes a protein called F-box/WD repeat-containing protein 7 involved in cell proliferation and differentiation. The increased expression of miR-182 has been found to be a regular feature of adenomas and further increase in miR-503 expression assists miR-182 to induce transformation of an adenoma to adenocarcinoma [59].

4.3 Role of miRNAs in invasion and migration

The mortality in CRC mainly results from cancer metastasis, a complex process that involves changes in the extracellular matrix to support invasion, increased cell motility and the ability of cells to initiate and maintain growth at a distant site. The molecular mechanisms underlying process of cell migration and invasion are highly complex and detailed insights are crucial for developing targeted therapy for CRC.

A large number of miRNAs have been implicated in regulation of CRC cell invasion and migration. miRNA-34a has been reported to play a significant role in the regulation of invassiveness and migratory abilities of CRC cells. It has been demonstrated that miR-34a expression is significantly decreased in metastatic/invasive colon cancer tissues when compared with the non-metastatic/non-invasive colon cancer tissues. The overexpression of miR-34a strongly inhibited the migration and invasion of HCT116 and RKO cells by inhibiting expression *FRA-1* (Fos-Related Antigen 1) through a direct action on its 3'-UTR [60]. *FRA-1* is involved in the progression of cancer and is upregulated in colon carcinomas. miRNA-143, which is downregulated in CRC cells, has been shown to target *MACC1* (metastasis-associated in colon cancer-1) to inhibit cell invasion and migration. *MACC1* is a CRC tumorigenesis and metastasis-related gene, which is overexpressed in CRC and promotes cell migration and invasion through trans-activating metastasis-inducing HGF/MET (Hepatocyte Growth Factor/Mesenchymal-Epithelial transition) signaling pathway. Another miRNA that has been implicated in regulation of migration and invasion is miR-301a, an oncogenic miRNA. It is up-regulated in lymph node metastatic CRC tissues and promotes CRC metastasis by targeting *TGFBR2* (Transforming Growth Factor Beta Receptor 2) that plays an important role in mesenchymal cell proliferation and migration [61]. Similarly, Xu K *et al.* [62] reported that miR-149 was highly downregulated in CRC tissues and low levels significantly correlated with lymph node or distant metastasis and advanced TNM stage. Gain and loss of function assays indicated that miR-149 significantly inhibited growth, migration and invasion of CRC cells by silencing *FOXM1* (Forkhead Box M1) gene, which encodes for a protein that functions as a transcriptional activator involved in cell proliferation. MiR-30c, a member of miR-30 family has been described as a tumor suppressor and downregulated in CRC. It inhibited cell migration and invasion ability of CRC cells by directly targeting *ADAM19* (A Disintegrin and Metalloprotease Domain) gene involved in cell–matrix interactions [63]. MiR-339 – 5p is downregulated in CRC and has been demonstrated to inhibit migration and invasion of CRC tumor cells (depending upon the p53 expression status) by repressing expression of *MDM2* (Murine Double Minute 2) [64]. Similarly, overexpression of miR-155 in CRC tissues compared to normal adjacent mucosa indicated extensive distant metastases, suggesting its involvement in the process of CRC cell migration and invasion. Further studies revealed that upregulation of miRNA-155 promotes the migration and invasion of CRC cells through the regulation of claudin-1 expression [65]. MiR-145 also participates in CRC cell migration and invasion by negatively regulating paxillin (adhesion protein) expression at the posttranscriptional level by binding to its 3′UTR. The paxillin is believed

to establish a structural link between the extracellular matrix and actin cytoskeleton to integrate multiple signals from the cell surface [66]. It has been reported that downregulation of miR-126 in CRC cells is associated with enhanced invasion and migration. Overexpression of miR-126 in CRC cell lines HT-29 and HCT-116 suppressed cell invasion and migration by downregulating *IRS-1* (Insulin Receptor Substrate 1) expression and suppressing AKT (serine/threonine kinase also known as protein kinase B or PKB) and ERK1/2 (Extracellular-signal-Regulated Kinase 1/2) activation [67].

4.4 Role of miRNAs in metastasis

Several miRNAs are associated with metastasis and effect downstream targets in the pathways leading to epithelial to mesenchymal transition (EMT). EMT is a complex process, which includes dissolution of cell–cell junctions and loss of normal cell polarity, resulting in the formation of migratory mesenchymal-shaped cells with invasive properties. During the EMT process, cancer cells lose the expression of cellular adhesion proteins such as E-cadherin and beta-catenin, and acquire expression of mesenchymal markers such as vimentin and N-cadherin. miRNAs are important mediators of EMT that can activate or attenuate EMT by targeting genes involved in EMT including *PTEN* (Phosphatase and Tensin homolog), *TWIST*, a transcriptional regulator and *ZEB-1* and *ZEB-2* (Zinc Finger E-Box Binding Homeobox-1 and 2). TGF-β/Wnt signaling, a prominent pathway in EMT, is regulated by the action of miR-21 and miR-31 by controlling downstream effectors of TGF-β in CRC. Similarly, the β-catenin expression is also controlled by miR-574 – 5p and miR-17 by downregulating Qkib/7 (Quaking homolog, KH domain RNA binding b/7) and P130 [68]. miRNAs such as miR-499 and miR-212 also function in regulating EMT by targeting *PDCD4* (Programmed Cell Death 4) gene and manganese superoxide dismutase [69]. However, the miRNA-200 family (miR-200 a,b,c, miR-141,miR-429) has been demonstrated to be a master regulator of epithelial phenotype, which is decreased in metastatic CRC. The miR-200 family represses EMT by targeting ZEB-1/2, which downregulates E-cadherin and up-regulates vimentin [70]. The increased expression of miR-200c results in the negative regulation of its gene targets (*ZEB1, ETS1*-V-Ets Avian Erythroblastosis Virus E26 Oncogene Homolog and *FLT1*-Fms-Related Tyrosine Kinase 1), which in turn regulate E-cadherin and vimentin expression to trigger an EMT switch in CRC cells [71]. MiR-9 was also reported to be important in the regulation of E-cadherin expression through *PROX1* (Prospero Homebox-1) which promotes EMT. *PROX1* binds to miR-9 promoter and triggers its expression to suppress E-cadherin 3'UTR and protein expression in CRC cells [70]. Recent reports highlight a connection between p53 and EMT through the modulation of some miRNAs that regulate EMT-TFs in different tumor models. The tumor suppressor p53 has been reported to maintain the epithelial phenotype by enhancing miR-34 expression that causes repression of *SNAIL1* that is involved in EMT. Importantly, a mutated form of p53 is unable to increase miR-34 expression, shifting the equilibrium toward a mesenchymal phenotype in CRC cells [72]. A positive feedback mechanism has been reported to exist between p53 and miR-34a. The increased expression (p53-induced) of miR-34a inhibits its target gene *SIRT1* (a histone deacetylase) and reduced activity of *SIRT1* (Silent mating type Information Regulation) leads to up-regulation of *p53* acetylation and the transcriptional activity of *p53* [73]. The enhanced expression of miR-34 has been shown to retard cell-cycle progression, cell invasion/migration and apoptosis [74].

4.5 Role of miRNAs in angiogenesis

The generation of new network of blood vessels known as angiogenesis is a classical hallmark of cancer and an essential process for the growth and the contact with the bloodstream both in primary and metastatic CRC tumors. This critical step in development enables tumor expansion, local invasion, and dissemination to other tissues and organs. Angiogenesis is required for the delivery of oxygen, nutrients, and survival factors, production of growth factors that benefit tumor cells and formation of a route for tumor cells to spread. It is a well-controlled process that is regulated by angiogenic, growth, and survival factors that may act as activator (pro-angiogenic) or inhibitor (anti-angiogenic) molecules. More than a dozen different proteins have been identified as angiogenic activators, including VEGF, basic fibroblast growth factor (bFGF), angiogenin, TGF-α, TGF-β, tumor necrosis factor (TNF)-α, platelet-derived endothelial growth factor, granulocyte colony-stimulating factor, placental growth factor, interleukin-8, hepatocyte growth factor, and epidermal growth factor. The VEGF is a powerful angiogenic factor; therefore, VEGF family and their receptors (VEGFR) have been focus of anticancer drug discovery and many drugs are currently available in clinics that target VEGF (Bevacizumab, ramucirumab, and ziv-aflibercept) and VEGFR (Cetuximab and panitumumab). There are many naturally occurring proteins that can inhibit angiogenesis, including angiostatin, endostatin, interferon, platelet factor 4, thorombospondin, and prolactin. It is believed that miRNAs play an important role in regulation of process of angiogenesis in CRC and may exert pro-angiogenic or anti-angiogenic effects. MiR-194 significantly promotes angiogenesis in HCT116 cells by inhibiting the expression level of *THBS1* gene, which encodes thrombospondin-1 (TSP-1), an endogenous inhibitor of angiogenesis [75]. However, recent studies reported that miR-194 acts as a tumor suppressor in the colorectal carcinogenesis via targeting PDK1/AKT2/XIAP pathway [76] and MAP4K4/c-Jun/MDM2 signaling pathway [31]. In addition, downregulation of miR-194 has been reported in several cancer types including liver, lung and gastric cancer suggesting its tumor-suppressive function. Chiang *et al.* also confirmed miR-194 as a tumor-suppressor gene in CRC patients [77]. Similarly, tumor-suppressor miR-23b, which is downregulated in human CRC samples, has been reported to promote

angiogenesis *in vivo* [78]. Furthermore, the downregulation of miR-126 in CRC has been reported to be associated with enhanced angiogenesis through upregulation of *VEGF* expression [79] and enhanced cell proliferation, migration and invasion [67]. The polycistronic miR-17 – 92 cluster (miR-17, miR-18, miR-19a, miR-19b, miR-20a, and miR-92a) enhanced tumor angiogenesis by downregulating the mRNA of TSP-1 and connective tissue growth factor [80]. It has been reported that from the six members of the miR-17 – 92 cluster, all except miR-18a, showed significantly increased expression in colorectal tumors, which was associated with DNA copy number gain and overexpression of transcription factor c-myc. Thus, the expression of this cluster appears to be c-myc [81]. The Myc-activated miR-17 – 92 can stimulate tumor angiogenesis by attenuating the TGF-β signaling pathway, which provides an alternative target for miR-17 – 92 in addition to TSP-1 [82].These observations suggest that such miRNAs having dual role may not serve as potential candidates/targets for miRNA-based therapy in CRC. MiR-210, a well-known hypoxia-inducible miRNA, is overexpressed in CRC patients and correlates well with poor prognosis. The upregulated levels of miR-210 stimulate angiogenesis by enhancing *RBM3* (RNA Binding Protein 3) expression [83,84]. Ye *et al.* [85] demonstrated that miR-27b expression decreased in CRC tissues and its overexpression leads to inhibition of suppression of angiogenesis by targeting *VEGFC*. In addition, miR-143 functions as an anti-angiogenic regulator in CRC tumor growth. Overexpression of miR-143 in CRC cells led to reduced amount of microvessels in a CAM (Chick Chrolioallantoic Membrane) model and impaired tumor growth in a xenograft model in nude mice. Further studies indicated that miR-143 inactivated AKT and inhibited its downstream modulators, HIF-1α and VEGF, key regulators in angiogenesis and tumorigenesis. MiR-143 impairs tumor growth and angiogenesis through the PI3K/AKT/HIF-1/VEGF pathway [86]. The expression of miR-885 – 3p is also downregulated in CRC tumors. It inhibits angiogenesis in CRC xenograft models through disruption of BMPR1A (Bone Morphogenic Protein Receptor 1A) gene that regulates a pro-angiogenic factor ID-1 (Inhibitor of Differentiation 1) [87].

4.6 Role of miRNAs in regulation of immune response

It has been widely accepted that miRNAs play a crucial role in regulation of immune response in cancer. They perform multiple functions to negatively regulate various immunity-related processes triggered by rapidly proliferating malignant cells. For example, miR-146a regulates multiple immunological processes via downregulation of pro-inflammatory pathways, and its loss or decreased expression often results in adverse chronic inflammatory phenotypes [88,89]. In several cancer types such as metastatic breast cancer, prostrate cancer and cervical cancer, miR-146a/b has been reported to target *IRAK1* (Interleukin-1 Receptor-Associated Kinase 1) and *TRAF6* (TNF Receptor-Associated Factor 6) to negatively regulate activity of NF-κB (Nuclear Factor-kappa B), a pro-inflammatory signaling pathway [90]. However, the precise role of miR-146 in regulation of gut immune response in CRC is not well understood. MiR-21, an oncogenic miRNA, has been implicated as an inflammatory mediator and may promote inflammation-associated colon carcinogenesis [91]. Similarly, miR-301a, activated NF-κB and START3 (Signal transducer and activator of transcription 3), two pro-inflammatory pathways to promote tumorigenesis in a mouse model of CRC [92]. It is well known that genetic instability of cancer cells results in altered expression of surface antigenic patterns that may in turn lead to reduced recognition of the tumor by immune cells. Some of these alterations are largely influenced by the actions of different miRNAs. For example, miR-9, which is overexpressed in many types of cancers, is capable of down-regulating the transcription of the MHC class I gene, thereby preventing the recognition of tumor cells by the immune system [93]. Similarly, miR-222 and miR- 339 down-regulate the expression of Intracellular Cell Adhesion Molecule 1 on the surface of tumor cells [94].

5. miRNAs: diagnostic potential in CRC

A large number of miRNAs have been evaluated in order to identify a clinically relevant miRNA signature that can be utilized as a potential predictor of early tumors, recurrence, chemoresistance and long-term survival in CRC. In addition to being found in tissue, miRNAs have also been detected in feces, serum, plasma and urine making them potential good biomarkers using easily accessible specimens. Mutations in the *APC* gene, leading to reduced expression, are defined as an early event in CRC. MiR-135a and miR-135b are both overexpressed in adenomas and adenocarcinomas and one of their targets is APC, implying that the upregulation of miR-135 is an early event in CRC and might be used as an early detection biomarker [95]. It was reported that CRC-specific miRNAs, miR-23a, miR-134, miR-146A, miR-221 and miR-222 could be identified in the serum of CRC patients but not in serum from healthy subjects [96]. Since then several studies have been carried out to find out CRC-specific miRNAs in the serum of patients. Ng *et al.* [97] analyzed 95 miRNA and identified miR-17 – 3p, miR-92, miR-95, miR-135b, miR-122 that were upregulated in both serum and tissues of CRC patients and miR-29a and miR-92a were recognized by Huang *et al.* [98] in serum of CRC patients including those with advanced adenomas. Further reports demonstrated that miR-21, miR-221 and miR-222 were frequently detected in CRC patient serum but only miR-221 levels were sufficient enough to serve as a biomarker [99]. MiR-141 has also been identified as a potential biomarker as its upregulation correlated well with CEA levels and poor prognosis in CRC patients [100]. Kanaan *et al.* and Li *et al.* recognized miR-21 as a potential serum biomarker with high sensitivity and specificity and

further emphasized it to be a suitable target for therapeutic intervention [101,102]. A panel of 22 miRNAs was identified by Wang *et al.* (miR-10a, miR-19a, miR-22, miR-24, miR-92a, miR-125a-5p,miR-141, miR-150, miR-188 – 3p, miR-192, miR-210, miR-221, miR-224, miR-376a, miR-425, miR-495, miR-572, miR-601, miR-720, miR-760, miR-let-7a, and -let-7e) that were significantly downregulated or upregulated in serum samples of CRC patients. They demonstrated that miR-601and miR-760 were considerably down-regulated in CRC samples and could serve as markers for identification of serum samples of CRC patients and of healthy controls. These miRNAs also discriminated between the serum samples from patients with advanced adenomas and the serum of normal controls. Recently, Shivapurkar and colleagues [103] analyzed the expression of a panel of miRNAs (miR-15a, miR-103, miR-148a, miR-320a, miR-451 and miR-596) in serum samples from patients with early stage CRC. They found two miRNAs differentially expressed in the circulation that is miR-103 (downregulated) and miR-596 (up-regulated). A microarray analysis of miR-199a-3p expression using paired pre-operative and post-operative serum from 10 CRC patients revealed significantly decreased levels in the post-operative serum when compared to levels in the pre-operative serum. Also in serum samples from 84 CRC patients and 32 non-cancer patients, miR199a-3p expression was found to be significantly higher in the CRC patients than that in the non-cancer patients. Thus, miR-199a-3p can be used as a biomarker for CRC [104]. It has been reported that downregulation of miR-375 in plasma and tissue of CRC patients correlated well with disease progression and decreased survival chances [105]. Recently, Zheng *et al.* [106] have identified a panel of four miRNAs (miR-19a-3p, miR-223 – 3p, miR-92a-3p and miR-422a) by analyzing serum samples collected from 307 CAC patients, 164 CA patients and 226 healthy controls. The developed panel demonstrated high diagnostic accuracy for CAC and differentiated well-stage I/II CAC from controls. Additionally, this panel could also differentiate CA from CAC and healthy controls. An analysis of miRNAs in exosome-enriched fractions of serum samples from 88 primary CRC patients and 11 healthy controls demonstrated that serum exosomal levels of seven miRNAs (let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a) were significantly higher in primary CRC patients, even those with early stage disease, compared to healthy controls. The expression levels of these miRNAs were found to be significantly down-regulated after surgical resection of tumors. This panel of circulating miRNAs can be used for diagnosis of CRC [107]. Similarly, fecal miRNAs present in the stools of CRC patients may be used as diagnostic tools. miR-92a and miR-21 which have been studied extensively in plasma have also been observed to have higher expression levels in the stool of CRC patients [108]. The miR-17 – 92 cluster has also been investigated in patient stool samples and can predict CRC incidence with 69.5% sensitivity and 81.5% specificity [109]. Most recently, Ahmed *et al.* [110] found 12 miRNAs (miR-7, miR-17, miR-20a, miR-21, miR-92a, miR-96, miR-106a, miR-134, miR-183, miR-196a, miR-199a-3p and miR214) to be upregulated in the stool of CRC patients, and 8 miRNAs (miR-9, miR-29b, miR-127 – 5p, miR-138, miR-143, miR-146a, miR-222 and miR-938) to be downregulated in the stool of CRC patients. Using these 20 miRNAs, they could differentiate not only CRC incidences from healthy controls but also different TNM stages with high sensitivity and specificity. Currently, there are several miRNA candidates that seem to have promising CRC diagnostic potential but their large-scale clinical utility is yet to be established.

6. miRNAs-possible anti-CRC drug candidates

The rationale for using miRNAs as therapeutic intervention is based upon the idea that miRNA expression is dysregulated in malignant cells compared to normal cells and thus the cancer phenotype can be changed by targeting miRNA expression [111]. miRNAs can be grouped as tumor suppressive or oncogenic (oncomiR) depending upon the type of gene they target. The expression of tumor-suppressive miRNAs frequently retards tumor progression through silencing oncogene expression. In contrast, oncogenic miRNA normally inhibits tumor-suppression gene expression, resulting in accelerating carcinogenesis. Both tumor suppressive and oncogenic miRNAs are candidates for therapeutic purposes. The major objective of utilizing miRNAs for CRC therapy is to restore the levels of miRNAs that are downregulated and silencing the miRNAs that are upregulated. Two major approaches can be adopted to develop miRNAs as anti-CRC agents. The first approach is to develop them as antagonists to use them to inhibit miRNAs that are overexpressed and show a gain of function in tumor cells (gene-silencing therapy). The second approach is to generate mimics that can be used to restore the function of miRNAs that demonstrate a functional loss in malignant cells or to use miRNAs encoded in expression vectors (replacement therapy) (**Figure 6**).

The miRNA antagonist antisense oligonucleotides that are fully or partially complementary to desired miRNA sequence have already been tested [112]. These antisense oligonucleotides act as competitive inhibitors of miRNAs and function by annealing to the mature miRNA guide strand thereby inducing destruction of functional miRNA. The precise hybridization of antagonist miRNA with the endogenous miRNA and its prevention from pairing with mRNAs is achieved by the introduction of nucleotide analogs such as 2'-O-methyl, 2'-O-methoxy ethyl or locked nucleic acids [113]. The pharmacokinetic properties of antisense oligonucleotides can be standardized by manipulating their length and chemical composition [114]. A novel miRNA antagonist of miR-122 is presently undergoing Phase II clinical trials for the treatment of infection caused by hepatitis C virus [115]. In several CRC cell lines, antisense oligonucleotide-based inhibition of miR-20a, miR-21, miR-31, miR-95 and miR-672 resulted in reduced cell

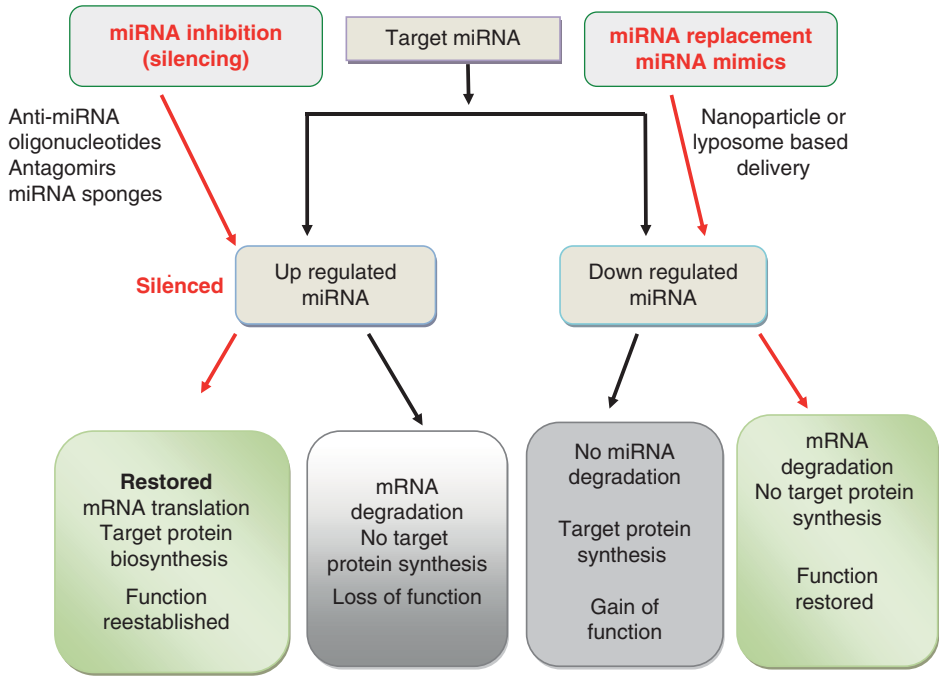

Figure 6. Strategy for miRNA-based therapy. Overexpressed miRNAs can be silenced by using Anti-miRNA oligonucleotides to neutralize functional gain. Similarly, underexpressed miRNAs can be replenished by delivering specific miRNA mimics utilizing nanoparticle or liposome-based delivery approaches.

division, invasion/migration and also an enhanced sensitivity of cells to undergo apoptosis in response to chemotherapeutic agents [116,117]. In addition, miR-135b has emerged as a target candidate and cumulative studies reveal that this miRNAs may be an attractive target to intervene the initiation and progression of human CRC.

MiRNA mimic approaches have gained much attention as this method provides an opportunity to replace the loss of function of tumor suppressor genes [118]. The basic requirement for the development of a miRNA mimic is the synthesis of a miRNA molecule that can readily enter the silencing complex and precisely target the same gene as the endogenous miRNA. The mature natural miRNA is a single-stranded small RNA molecule consisting of ~ 22-nucleotides and it readily associates with RISC, which is critical for its regulatory activity. The complete nucleotide sequence of endogenous miRNA can be easily synthesized as a single-stranded RNA molecule. However, the effectiveness of singled-stranded miRNA mimics is significantly lower compared to mimics that are double-stranded. There are several potential candidate miRNAs, which can be mimicked for the development of anti-CRC drug. The synthetic oligonucleotide mimics of silenced miRNAs (down-regulated) such as let 7a-1, miR133b, miR-137, miR-143, miR-145, miR-185, miR-192, miR-195, miR-196a, miR-200c, miR-215, miR-491 have been shown to restore normal tumor suppression activity in a variety of CRC cell lines [119-125].

7. Perspective on miRNA therapy in CRC

The emergence of miRNAs as possible targets for therapeutic intervention has provided an immense opportunity to develop better options for the treatment of cancer including CRC. There are several advantages of using miRNA therapy in CRC, first miRNAs are naturally produced molecules; thus they should have less toxicity. Second a single miR can influence multiple genes of the same pathway thus reducing the chances of developing resistance. These two outstanding features of miRNAs make them attractive target for strategic therapeutic intervention in CRC in order to enhance specificity and overcome drug resistance. The entire concept of using miRNAs as chemotherapeutic agents for the management of cancer including CRC is based upon their ability to enter the RISC to couple with mRNAs containing complementary sequences and subsequently repressing gene expression. Additionally, miRNA can also pair with mRNA in an imperfect manner to regulate activity of a large number of genes. Thus, single miRNA molecule can influence the function of several oncogenes and downstream processes in CRC cells. As cancer is a heterogeneous disease, a combination of several anticancer drugs may be needed for long-term and durable responses but miRNAs may potentially be used as a single agent for the cancer chemotherapy as each affects a large number of genes [113].

Though there are several miRNA candidates that can be exploited to induce antitumor action in CRC, miR-135b

which has been reported to be upregulated in CRC due to mutations in the *APC* gene (involved in adenoma to carcinoma transition) and miR-200c, also overexpressed and involved in the regulation of EMT are of special interest. The aberrant miR-135b expression appears to be a ubiquitous molecular event in the initiation and progression of not only CRC but also many other types of cancer including lung cancer, prostate cancer, pancreatic cancer and breast cancer [126]. The proof of concept with miR-135b has already been established in animal model of colon cancer. It has been reported that mice treated with anti-miR-135b oligonucleotides showed significant reduction in tumor size compared to untreated mice. Thus miR-135b may be a promising target that can be silenced by the development of anti-miR-135b oligonucleotides, antagomirs or RNA sponges for the treatment of CRC. Similarly, miR-200 family members (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) particularly miR-200c may be another promising candidate to target in CRC. miRNA-200c, which primarily regulates EMT by downregulating ZEB1/2 and upregulating E-cadherin, also plays a significant role in other cancer-related events including proliferation, cell cycle control, apoptosis and invasion. Moreover, miR-200c is a well-established prognostic and diagnostic marker in different cancer types including CRC. Analysis of miR-200c expression profiles has shown that it is overexpressed in CRC tumors compared to normal tissue but is downregulated in metastases compared to primary tumors suggesting a role for miR-200c in regulating colorectal tumor development. Additionally, miR-200c also regulates Sox-2 expression in a negative feedback loop in CRC and this regulation is associated with stemness, growth and metastatic potential of CRC. Furthermore, miR-200c has also been shown to function as an oncogene in CRC as its silencing leads to upregulation of the *Pten* and *p53* tumor suppressor genes. Thus miR-135b and miR-200c appear to be the most promising candidates for miRNA-based therapy in CRC at the present time. However, the major challenge in leveraging a meaningful benefit from miRNA-based CRC therapy is the development of a suitable vehicle for specific, efficient and safe systemic delivery of therapeutic miRNAs in human beings [127]. In recent years, systemic delivery of miRNAs has been achieved in animal models using various delivery systems such as neutral lipid emulsion [128], polymer nanoparticles [129], solid lipid nanoparticles [130] and liposomes [131]. Despite these *in vivo* studies, it is generally agreed that serum degradation and cellular barriers, non-specific tissue distribution and endolysosomal trafficking are major limiting factors for successful and effective delivery of miRNAs. Recently Cheng *et al.* [132] have reported a novel anti-miR delivery platform that remains stable in acidic tissue microenvironment. They attached anti-miR to a peptide with low pH-induced transmembrane structure and effectively silenced miR-155 in a lymphoma mouse model. However, effectiveness of this novel delivery system remains to be tested in human beings.

8. Expert opinion

The recent advances in genomics research have generated several potential new drug targets involved in cancer initiation, progression and metastasis. Several target-based compounds have emerged in recent years. Whereas most of these compounds are in preclinical testing, several are in clinical trials and a few have been approved by the FDA in the USA. Some cancer patients having tumors with specific oncogenic mutations, such as anaplastic lymphoma kinase (ALK) expression (tyrosine kinase receptor) in lung cancer or oncogenic Bcr-Abl in chronic myeloid leukemia, KIT expression or mutations in gastrointestinal stromal tumors, or EGFR mutation in lung cancer, HER2 amplification in breast cancer or MET overexpression in liver tumors, have greatly benefited from targeted agents. However, the vast majority of common tumors were found to be less responsive to these target-based drugs because most of the tumors do not depend on a single 'targetable' oncogenic activation. For example, ALK activations and EGFR mutations account for < 10% of lung adenocarcinoma and targeted agents are more efficacious than chemotherapy in oncogenic tumors. But the antitumor effects of target-based agents remain limited to a few months mostly due to rapid drug resistance development. As a result, the expected progression-free survival benefit from targeted therapy is often < 6 months. Therefore, for the vast majority of tumors, chemotherapy/radiation therapy alone remains the cornerstone of treatment with some added benefits by using monoclonal antibodies but only in a limited proportion of patients.

Combinations of several targeted agents have also been proposed to counteract potential resistance mechanisms, but in clinical trials combining targeted agents together is frequently associated with unacceptable toxicity rather than additive or synergistic efficacy. Currently, most patients with CRC are treated with 5-FU, oxaliplatin, irinotecan, bevacizumab cetuximab, panitumumab, aflibercept and regorafenib, but these drugs frequently encounter well-known clinical issues related to variable responses, toxicity and drug resistance. These facts highlight the urgent need for the development of novel anti-CRC drugs. As an important class of gene regulators miRNAs possess high potential for anti-CRC therapeutic development. Significant efforts have been made to establish miRNA expression profiling and their precise role in CRC initiation and progression. These efforts have successfully ascertained a few potential candidate miRNAs that may be more specific and targetable for CRC treatment, but the selection of an appropriate miRNA candidate remains to be a critical step as well as the targeted delivery to the tumor.

Thus, one of the biggest challenges is to discover a highly efficient miRNA delivery system for human applications. To address this challenge, nanoparticles and/or liposome-based approaches have been used as delivery vehicles for miRNAs. The nanoparticle-based approach appears to be particularly

attractive because nanoparticles can be coated with tumor-specific antibodies thus allowing tumor-specific delivery of miRNA of interest. Although a large amount of work has been done in the field of miRNAs, still more research is needed for an in-depth understanding of the complexities of the miRNA world. Therefore future approaches should be directed towards gaining additional insights into the genetic and epigenetic events that control miRNA genes and factors that lead to deregulation of miRNAs in order to use them as targets for anti-CRC drug development.

Declaration of interests

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2015. Ca Cancer J Clin 2015;65:5-29
2. Fang L, Yang N, Ma J, et al. microRNA-1301- mediated inhibition of tumorigenesis. Oncol Rep 2012;27:929-34
3. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6:857-66
4. Schetter AJ, Okayama H, Harris CC. The role of microRNAs in Colorectal Cancer. Cancer J 2012;18(3):244-52
5. Li T, Leong MH, Harms B, et al. MicroRNA-21 as a potential colon and rectal cancer biomarker. World J Gastroenterol 2013;19(34):5615-21
6. Esquela-Kerscher A, Slack FJ. Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer 2006;6(4):259-69
7. Mazeh H, Mizrahi I, Ilyayev N, et al. The Diagnostic and Prognostic Role of microRNA in Colorectal Cancer–a Comprehensive review. J Cancer 2013;4(3):281-95
8. Lee Y, Kim M, Han J, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO J 2004;23(20):4051-60
9. Han J, Lee Y, Yeom KH, et al. Molecular basis for the recognition of primary microRNAs by Drosha-DGCR8 complex. Cell 2006;125:887-901
10. Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 2004;10(2):185-1891
11. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 2000;404:293-6
12. Gregory RI, Chendrimada TP, Cooch N, et al. Human RISC couples microRNA biogenesis and post-transcriptional gene silencing. Cell 2005;123:631-40
13. Liu J, Valencia-Sanchez MA, Hannon GJ, et al. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. Nat Cell Biol 2005;7(7):719-23
14. Tokarz P, Blasiak J. The role of microRNA in metastatic colorectal cancer and its significance in cancer prognosis and treatment. Acta Biochimia Polonica 2012;59(4):467-74
15. Dong H, Lei J, Ding L, et al. MicroRNA: Function, Detection and Bioanalysis. Chem Rev 2013;113:6207-33
16. Jiang R, Li Y. Regulation of Mirna pathways and roles of Micrornas in tumorigenesis and metastasis. Human Genet Embryol 2013.S2. Available from: http//dx.doi.org/10.4172/2161-0436.S2-007
17. Wang Z, Cummins JM, Shen D, et al. Three classes of genes mutated in colorectal cancers with chromosomal instability. Cancer Res 2004;64:2998-3001
18. Oberg AL, French AJ, Sarver AL, et al. miRNA expression in colon polyps provides evidence for a multihit model of colon cancer. PLoS One 2011;6(6):e20465
19. Jaitin DA, Kenigsberg E, Keren-Shaul H, et al. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. Science 2014;343:776-9
20. Michael MZ, SM OC, van Holst Pellekaan NG, et al. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol cancer res: MCR 2003;1:882-91
21. Cui SY, Wang R, Chen LB. MicroRNA-145: a potent tumour suppressor that regulates multiple cellular pathways. J Cell Mol Med 2014;18(10):1913-26
22. Sachdeva M, Mo Y. MicroRNA-145 Suppresses Cell Invasion and Metastasis by Directly Targeting Mucin 1. Cancer Res 2010;70(1):378-87
23. Feng Y, Zhu J, Ou C, et al. MicroRNA-145 inhibits tumour growth and metastasis in colorectal cancer by targeting fascin-1. Br J Cancer 2014;110:2300-9
24. Oliver A, Kent I, Matthew N, et al. Lessons from miR-143/145: the importance of cell-type localization of miRNAs. Nucleic Acids Res 2014;42(12):7528-38
• **This article highlights importance of understanding of cell-type specific miRNA expression.**
25. Arndt GM, Dossey L, Cullen LM, et al. Characterization of global microRNA expression reveals oncogenic potential of miR-145 in metastatic colorectal cancer. BMC Cancer 2009;9:374
26. Chivukula RR, Shi G, Acharya A, et al. An Essential Mesenchymal Function for miR-143/145 in Intestinal Epithelial Regeneration. Cell 2014;157(5):1104-16
• **Explains differential expression of miR-143 and miR-145 in mesenchymal and epithelial cells and their specific functions in these cells.**
27. Scarpati GDV, Calura E, Marino MD, et al. Analysis of differential

miRNA expression in primary tumor and stroma of colorectal cancer patients. BioMed Res Int 2014;2014:840921

28. Sun Y, Wang L, Guo SC, et al. High–throughput sequencing to identify miRNA biomarkers in colorectal cancer patients. Oncol lett 2014;8:711-13
29. Chai J, Wang S, Han D, et al. MicroRNA-455 inhibits proliferation and invasion of colorectal cancer by targeting RAF proto-oncogene serine/threonine-protein kinase. Tumor Biol 2015;36(2):1313-21
30. Christensen LL, Holm A, Rantala J, et al. Functional Screening Identifies miRNAs Influencing Apoptosis and Proliferation in Colorectal Cancer. PLoS One 2014;9(6):e96767
31. Wang B, Shen ZL, Gao ZD, et al. MiR-194, commonly repressed in colorectal cancer, suppresses tumor growth by regulating the MAP4K4/c-Jun/MDM2 signaling pathway. Cell Cycle 2015;14(7):1046-58
32. Zhang G, Zhou H, Xiao H, et al. MiR-378 is an independent prognostic factor and inhibits cell growth and invasion in colorectal cancer. BMC Cancer 2014;14:109
33. Lee DY, Deng Z, Wang CH, et al. MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression. Proc Natl Acad Sci USA 2007;104(51):20350-5
34. Chen LT, Xu SD, Xu H, et al. MicroRNA-378 is associated with non-small cell lung cancer brain metastasis by promoting cell migration, invasion and tumor angiogenesis. Med Oncol 2012;29(3):1673-80
35. Eichner LJ, Perry MC, Dufour CR, et al. miR-378 mediates metabolic shift in breast cancer cells via the PGC-1beta/ERR gamma transcriptional pathway. Cell Metab 2010;12(4):352-61
36. Deng H, Guo Y, Song H, et al. MicroRNA-195 and microRNA-378 mediate tumor growth suppression by epigenetical regulation in gastric cancer. Gene 2013;518(2):351-9
37. Faltejskova P, Svoboda M, Srutova K, et al. Identification and functional screening of microRNAs highly deregulated in colorectal cancer. J Cell Mol Med 2012;16(11):2655-66
38. O'Donnell KA, Wentzel EA, Zeller KI, et al. c-Myc-regulated microRNAs modulate E2F1 expression. Nature 2005;435:839-43
39. Tazawa H, Tsuchiya N, Izumiya M, et al. Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. Proc Natl Acad Sci USA 2007;104:15472-7
40. Sachdeva M, Zhu S, Wu F, et al. p53 represses c-Myc through induction of the tumor suppressor miR-145. Proc Natl Acad Sci USA 2009;106:3207-12

• **Demonstrates role of tumor suppressor p53 in transcriptional regulation of miR-145.**

41. Bandres E, Agirre X, Bitarte N, et al. Epigenetic regulation of microRNA expression in colorectal cancer. Int J Cancer 2009;125:2737-43
42. Grady WM, Parkin RK, Mitchell PS, et al. Epigenetic silencing of the intronic microRNA hsa-miR-342 and its host gene EVL in colorectal cancer. Oncogene 2008;27(27):3880-8
43. Landi D, Gemignani F, Naccarati A, et al. Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer. Carcinogenesis 2008;29(3):579-84
44. Lee HC, Kim JG, Chae YS, et al. Prognostic impact of microRNA-related gene polymorphisms on survival of patients with colorectal cancer. J Cancer Res 2010;136:1073-8
45. Wang P, Zou F, Zhang X, et al. MicroRNA-21 negatively regulates CDC25A and cell cycle progressionin colon cancer cells. Cancer Res 2009;69:8157-65
46. Xiong B, Cheng Y, Ma L, et al. MiR-21 regulates biological behavior through the PTEN/PI-3 K/Akt signaling pathway in human colorectal cancer cells. Int J Oncol 2013;42(1):219-28
47. Sun D, Yu F, Ma Y, et al. MicroRNA-31 activates the Ras pathway and functions as an oncogenic microRNA in human colorectal cancer by repressing RAS p21 GTPase activating protein 1 (RASA1). J Biol Chem 2013;288:9508-18
48. Nagel R, le Sage C, Diodado B, et al. Regulation of the adenomatous polyposis coli gene by miR-135 family in colorectal cancer. Cancer Res 2008;68:5795-802
49. Zhang G, Zhou H, Xiao H, et al. Up-regulation of miR-224 promotes cancer cell proliferation and invasion and predicts relapse of colorectal cancer. Cancer Int 2013;13:104
50. Liu M, Lang N, Chen X, et al. miR-185 targets RhoA and cdc42 expression and inhibits the proliferation potential of human colorectal cells. Cancer lett 2011a;301:151-60
51. Li Y, Zhu X, Xu W, et al. miR-330regulates the proliferation of colorectal cancer cells by targetingCdc42. Biochem Biophys Commun 2013;431:560-5
52. Wang L, Qian L, Li Q, et al. MicroRNA–195 inhibits colorectal cancer cell proliferation, colony–formation and invasion through targeting CARMA3. Mol Med Reports 2014;10(1):473-8
53. Nagel R, le Sage C, Diosdado B, et al. Regulation of the adenomatous polyposis coli gene by the miR-135 family in colorectal cancer. Cancer Res 2008;68:5795-802
54. Valeri N, Braconi C, Gasparini P, et al. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. Cancer Cell 2014;25(4):469-83

•• **Shows role of miR-135b in initiation and progression of CRC.**

55. Chen X, Guo X, Zhang H, et al. Role of miR-143 targeting KRAS in colorectal tumorigenesis. Oncogene 2009;28:1385-92
56. Krichevsky AM, Gabriely G. miR-21: a small multi-faceted RNA. J Cell Mol Med 2009;13:39-53
57. Guo C, Sah JF, Beard L, et al. The noncoding RNA, miR-126, suppresses the growth of neoplastic cells by targeting phosphatidylinositol 3-kinase signaling and is frequently lost in colon cancers. Genes Chrom Can 2008;47:939-46
58. Akao Y, Noguchi S, Iio A, et al. Dysregulation of microRNA-34a expression causes drug-resistance to 5-FU in human colon cancer DLD-1 cells. Cancer Lett 2011;300:197-204
59. Li L, Sarver AL, Khatri R, et al. Sequential expression of miR-182 and miR-503 cooperatively targets FBXW7,

contributing to the malignant transformation of colon adenoma to adenocarcinoma. J Pathol 2014;234(4):488-501

60. Wuy J, Wu G, Lv L, et al. MicroRNA-34a inhibits migration and invasion of colon cancer cells via targeting to Fra-1. Carcinogenesis 2012;33(3):519-28

61. Zhang W, Zhang T, Jin R, et al. MicroRNA-301a promotes migration and invasion by targeting TGFBR2 in human colorectal cancer. J Exp Clin Cancer Res 2014;33:113

62. Xu K, Liu X, Mao X, et al. MicroRNA-149 suppresses colorectal cancer cell migration and invasion by directly targeting forkhead box transcription factor FOXM1. Cell Physiol Biochem 2015;35:499-515

63. Zhang Q, Yu L, Qin D, et al. Role of microRNA-30c targeting ADAM19 in colorectal cancer. PLoS One 2015;10(3):e0120698

64. Zhang C, Liu J, Wang X, et al. MicroRNA-339-5p inhibits colorectal tumorigenesis through regulation of the MDM2/p53 signaling. Oncotarget 2014;5(19):9106-17

65. Zhang GJ, Xiao HX, Tian HP, et al. Upregulation of microRNA-155 promotes the migration and invasion of colorectal cancer cells through the regulation of claudin-1 expression. Int J Mol Med 2013;3:1375-80

66. Qin J, Wang F, Jiang H, et al. MicroRNA-145 suppresses cell migration and invasion by targeting paxillin in human colorectal cancer cells. Int J Clin Exp Pathol 2015;8(2):1328-40

67. Zhou Y, Feng X, Liu Y, et al. Down-regulation of miR-126 is associated with colorectal cancer cells proliferation, migration and invasion by targeting IRS-1 via the AKT and ERK1/2 signaling pathways. PLoS One 2013;8(1):e81203

68. Ji S, Ye G, Zhang J, et al. miR-574-5p negatively regulates Qki6/7 to impact b-catenin/Wnt signalling and the development of colorectal cancer. Gut 2013;62:716-26

69. Zhou JJ, Zheng S, Sun LF, et al. MicroRNA regulation network in colorectal cancer metastasis. World J Biol Chem 2014;5(3):301-7

70. Zaravinos A. The regulatory role of MicroRNAs in EMT and cancer. J Oncol 2015;2015:865816

71. Hur K, Toiyama Y, Takahashi M, et al. MicroRNA-200c modulates epithelial-to-mesenchymal transition (EMT) in human colorectal cancer metastasis. Gut 2013;62(9):1315-26

•• **Highlights role of miR-200c in CRC metastasis.**

72. Yamakuchi M, Lowenstein CJ. MiR-34, SIRT1 and p53: the feedback loop. Cell Cycle 2009;8:712-15

73. Yamakuchi M, Ferlito M, Lowenstein CJ. MiR-34a repression of SIRT1 regulates apoptosis. Proc Natl Acad Sci USA 2008;105:13421-6

74. Raver-Shapira N, Marciano E, Meiri E, et al. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. Mol Cell 2007;26:731-43

75. Sundaram P, Hultine S, Smith LM, et al. p53-responsive miR-194 inhibits thrombospondin-1and promotes angiogenesis in colon cancers. Cancer Res 2011;71:7490-501

76. Zhao HJ, Ren LL, Wang ZH, et al. MiR-194 deregulation contributes to colorectal carcinogenesis via targeting AKT2 pathway. Theranostics 2014;4(12):119301208

77. Chiang Y, Song Y, Wang Z, et al. microRNA-192, -194 and -215 are frequently downregulated in colorectal cancer. Exp Ther Med 2012;3:560-6

78. Zhang H, Hao Y, Yang J, et al. Genome-wide functional screening of miR-23b as a pleiotropic modulator suppressing cancer metastasis. Nat Commun 2011;2:554

79. Zhang Y, Wang X, Xu B, et al. Epigenetic silencing of miR-126 contributes to tumor invasion and angiogenesis in colorectal cancer. Oncol Rep 2013;30:1976-84

80. Dews M, Homayouni A, Yu D, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat Genet 2006;38:1060-5

81. Diosdado B, van de Wiel MA, Terhaar Sive Droste JS, et al. MiR-17-92 cluster is associated with 13q gain and c-myc expression during colorectal adenoma to adenocarcinoma progression. Br J Cancer 2009;101:707-14

82. Zhou JJ, Zheng S, Sun LF, et al. MicroRNA regulation network in colorectal cancer metastasis. World J Biol Chem 2014;5(3):301-7

83. Wang J, Zhao J, Shi J, et al. Elevated expression of miR-210 predicts poor survival of cancer patients: a systematic review and meta-analysis. PLoS One 2014;9(2):e89223

84. Ramalingam S, Subramaniam D, Anant S, et al. RBM3 drives tumor angiogenesis by modulating miR-210. In: Proceedings of the 104th Annual Meeting of the American Association for Cancer Research; 2013 Apr 6-10; Washington, DC. Philadelphia (PA): AACR. Cancer Res 2013;73(8 Suppl):abstract nr 3901

85. Ye J, Wu X, Wu D, et al. miRNA-27b targets vascular endothelial growth factor C to inhibit tumor progression and angiogenesis in colorectal cancer. PLoS One 2013;8(4):e60687

86. Qiana X, Yuab J, Yinac Y, et al. MicroRNA-143 inhibits tumor growth and angiogenesis and sensitizes chemosensitivity to oxaliplatin in colorectal cancers. Cell Cycle 2013;12(9):1385-94

87. Xiao F, Qiu H, Cui H, et al. MicroRNA-885-3p inhibits the growth of HT-29 colon cancer cell xenografts by disrupting angiogenesis via targeting BMPR1A and blocking BMP/Smad/Id1 signaling. Oncogene 2015;34(15):1968-78

88. Williams AE, Perry MM, Moschos SA, et al. Role of miRNA-146a in the regulation of the innate immune response and cancer. Biochem Soc Trans 2008;36:1211

89. Li L, Chen XP, Li YJ, et al. MicroRNA-146a and human disease. Scand J Immunol 2010;71:227-31

90. Rusca N, Monticelli M. MiR-146a in immunity and disease. Mol Biol Int 2011;2011:437301

91. Okayama H, Schetter AJ, Harris CC, et al. MicroRNAs and inflammation in pathogenesis and progression of colon cancer. Dig Dis 2012;30(Suppl 2):9-15

92. Ma X, Yan F, Deng Q. Modulation of tumorigenesis by the pro-inflammatory microRNA miR-301a in mouse models of lung cancer and colorectal cancer. Cell Discov 2015;1:15005

93. Gao F, Zhao ZL, Zhao WT, et al. miR-9 modulates the expression of interferon-regulated genes and MHC class I molecules in human nasopharyngeal carcinoma cells. Biochem Biophys Res Commun 2013;431:610-16

94. Ueda R, Kohanbash G, Sasaki K, et al. Dicer-regulated microRNAs 222 and 339 promote resistance of cancer cells to cytotoxic T-lymphocytes by down-regulation of ICAM-1. Proc Natl Acad Sci USA 2009;106:10746-51

95. Remco Nagel R, Sage CL, Diosdado B, et al. Regulation of the adenomatous polyposis coli gene by the miR-135 family in colorectal cancer. Cancer Res 2008;68(14):5795-802

96. Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;18:997-1006

97. Ng EK, Chong WW, Jin H, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut 2009;58:1375-81

98. Huang Z, Huang D, Ni S, et al. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. Int J Cancer 2010;127:118-26

99. Pu XX, Huang GL, Guo HQ, et al. Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. J Gastroenterol Hepatol 2010;25:1674-80

100. Cheng H, Zhang L, Cogdell DE, et al. Circulating plasma MiR-141 is a novel biomarker for metastatic colon cancer and predicts poor prognosis. PLoS One 2011;6:e17745

101. Kanaan Z, Rai SN, Eichenberger MR, et al. Plasma miR-21: a potential diagnostic marker of colorectal cancer. Ann Surg 2012;256:544-51

102. Li T, Leong MH, Harms B, et al. MicroRNA-21 as a potential colon and rectal cancer biomarker. World J Gastroenterol 2013;19(34):5615-21

103. Shivapurkar N, Weiner LM, Marshall JL, et al. Recurrence of early stage colon cancer predicted by expression pattern of circulation microRNAs. PLoS One 2014;9(1):e84686

104. Nonaka R, Nishimura J, Kagawa Y, et al. Circulating miR-199a-3p as a novel serum biomarker for colorectal cancer. Oncol Rep 2014;32(6):2354-8

105. Xu L, Li M, Wang M, et al. The expression of microRNA-375 in plasma and tissue is matched in human colorectal cancer. BMC Cancer 2014;14:714

106. Zheng G, Du L, Yang X, et al. Serum microRNA panel as biomarkers for early diagnosis of colorectal adenocarcinoma. Br J Cancer 2014;111:1985-92

107. Ogata-Kawata H, Izumiya M, Kurioka D, et al. Circulating exosomal microRNAs as Biomarkers of Colon Cancer. PLoS One 2014;9(4):e92921

108. Wu CW, Ng SSM, Dong YJ, et al. Detection of miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps. Gut 2012;61:739-45

109. Koga Y, Yasunaga M, Takahashi A, et al. MicroRNA expression profiling of exfoliated colonocytes isolated from feces for colorectal cancer screening. Cancer Prev Res 2010;3:1435-42

110. Ahmed FE, Ahmed NC, Vos PW, et al. Diagnostic microRNA markers to screen for sporadic human colon cancer in stool: I. Proof of Principle. Cancer Genomics Proteomics 2013;10:93-113

111. Dassow H, Aigner A. MicroRNAs (miRNAs) in colorectal cancer: from aberrant expression towards therapy. Curr Pharm Des 2013;19(7):1242-52

112. Esau C, Davis S, Murray SF, et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 2006;3:87-98

113. Garzon R, Marcucci G, Croce CM. Targeting microRNAs in cancer: rationale, strategies and challenges. Nat Rev Drug Discov 2010;9:775-89

114. Petri A, Lindow M, Kauppinen S. MicroRNA silencing in primates: towards development of novel therapeutics. Cancer Res 2009;69:393-5

115. Lanford RE, Hildebrandt-Eriksen ES, Petri A, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 2009;327:198-201

116. Chai H, Liu M, Tian R, et al. miR-20a targets BNIP2 and contributes chemotherapeutic resistance in colorectal adenocarcinoma SW480 and SW620 cell lines. Acta Biochim Biophys Sin (Shanghai) 2011;43(3):217-25

117. Wang CJ, Stratmann J, Zhou ZG, et al. Suppression of microRNA-31 increases sensitivity to 5-FU at an early stage, and affects cell migration and invasion in HCT-116 colon cancer cells. BMC Cancer 2010;10:616

118. Bader AG, Brown D, Winkler M. The promise of microRNA replacement therapy. Cancer Res 2010;70:7027-30

119. Akao Y, Nakagawa Y, Naoe T. let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. Biol Pharm Bull 2006;29:903-6

120. Hu G, Chen D, Li X, et al. miR-133b regulates the MET proto-oncogene and inhibits the growth of colorectal cancer cells in vitro and in vivo. Cancer Biol Ther 2010;10(2):190-7

121. Liu M, Lang N, Qiu M, et al. miR-137 targets Cdc42 expression, induces cell cycle G1 arrest and inhibits invasion in colorectal cancer cells. Int J Cancer 2011;128(6):1269-79

122. Zhang J, Guo H, Zhang H, et al. Putative tumor suppressor miR-145 inhibits colon cancer cell growth by targeting oncogene Friend leukemia virus integration 1 gene. Cancer 2011;117(1):86-95

123. Liu M, Lang N, Chen X, et al. miR-185 targets RhoA and Cdc42 expression and inhibits the proliferation potential of human colorectal cells. Cancer Lett 2011;301(2):151-60

124. Boni V, Bitarte N, Cristobal I, et al. miR-192/miR-215 influence 5-fluorouracil resistance through cell cycle-mediated mechanisms complementary to its post-transcriptional thymidilate synthase regulation. Mol Cancer Ther 2010;9(8):2265-75

125. Liu L, Chen L, Xu Y, et al. microRNA-195 promotes apoptosis and suppresses tumorigenicity of human colorectal cancer cells. Biochem Biophys Res Commun 2010;400(2):236-40

126. Lin CW, Hong TM, Yang PC. MicroRNA 135b as therapeutic target in cancers. RNA Dis 2014;1:e410

127. Chen Y, Gao DY, Huang L. In vivo delivery of miRNAs for cancer therapy: challenges and strategies. Adv Drug Deliv Rev 2015;81:128-41

128. Trang P, Wiggins FG, Daige CL, et al. Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. Mol Ther 2011;19(6):1116-22

129. Baber IA, Cheng CL, Booth CJ, et al. Nanoparticle-based therapy in an in vivomicroRNA-155 (miR-155)-dependent mouse model of lymphoma. Proc Natl Acad Sci USA 2012;109(26):E1695-704

130. Shi S, Han L, Gong T, et al. Systemic delivery of microRNA-34a for cancer stem cell therapy. Angew Chem Int Ed 2013;52(14):3901-5

131. Takahashi YE, Negishi Y, Nakamura A. Systemic delivery of miR-126 by miRNA-loaded Bubble liposomes for the treatment of hindlimb ischemia. Scientif Rep 2014;4:3883

132. Cheng CJ, Bahal R, Baber IA, et al. MicroRNA silencing for cancer therapy targeted to the tumor microenvironment. Nature 2015;518(7537):107-10

•• **Reports a novel delivery platform for systemic delivery of miRNAs.**

133. Balaguer F, Link A, Lozano JJ, et al. Epigenetic silencing of miR-137 is an early event in colorectal carcinogenesis. Cancer Res 2010;70:6609-18

134. Hu M, Xia M, Chen X, et al. MicroRNA-141 regulates Smad interacting protein 1 (SIP1) and inhibits migration and invasion of colorectal cancer cells. Dig Dis Sci 2010;55:2365-72

135. Karaayvaz M, Pal T, Song B, et al. Prognostic significance of miR-215 in colon cancer. Clin Colorectal Cancer 2011;10:340-407

Affiliation

Mohammad Alam Jafri[1], Syed Kashif Zaidi[1], Shakeel Ahmed Ansari[1], Mohammed Hussein Al-Qahtani[1] & Jerry W Shay[†1,2]
[†]Author for correspondence
[1]King Abdulaziz University, Center of Excellence in Genomic Medicine Research, Jeddah, Saudi Arabia
[2]UT Southwestern Medical Center, Department of Cell Biology, Dallas, TX, USA
Tel: +1 214 648 4201;
Fax: +1 214 648 5814;
E-mail: Jerry.Shay@UTSouthwestern.edu