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Review

Pathogenetic and Clinical Relevance of MicroRNAs in Colorectal Cancer

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Abstract. *Colorectal cancer (CRC) has been described as a multistep disease due to the progressive accumulation of mutations and chromosomal rearrangements involving critical oncogenes or oncosuppressors. MicroRNAs (miRNAs) are a class of small interfering RNAs frequently involved in the pathogenesis of cancer. Several genome-wide profiling studies have identified miRNAs deregulated in colorectal cancer. Many of these deregulated miRNAs contribute to CRC tumorigenesis and may help to understand CRC pathogenesis, prognosis and response to treatment. This review will focus on common mechanisms involved in miRNA alterations in CRC, their functional implication in CRC development and the potential use of miRNAs as prognostic and predictive surrogate markers for the management of CRC patients.*

Colorectal cancer (CRC) represents one of the most frequent causes of death for cancer. CRC has been recently defined as the third most common cancer expected to occur in both sexes in the USA in 2009 with more than 140,000 new cases and about 50,000 deaths in both sexes (1). Substantial success in the treatment of CRC has been achieved in the last decades. However, the median overall survival of patients affected by metastatic CRC is still poor (2), underlining the need for improved and more effective therapies. Thus, understanding the molecular genesis of colorectal cancer represents a fundamental step in the identification of novel molecular targets that might be useful to define CRC patients prognosis and tailor their therapy.

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MiRNAs are post-transcriptional regulators of gene expression which arise as transcripts from cognate genes in non coding regions of chromosomes. After nuclear and cytoplasmic processing, mature miRNAs bind to their target mRNA, inducing target protein down-regulation by translational inhibition, mRNA cleavage or degradation (3, 4). Some studies have also proposed a role for miRNAs in inducing target mRNA up-regulation (5) or interfering with gene transcription (6).

MiRNAs play a critical role in the pathogenesis of different solid and hematological malignancies (7). Aim of this review is to summarize recent advances in miRNA related CRC pathogenesis and the potential prognostic and predictive value of miRNA expression in the clinical management of CRC patients.

Frequent Causes for MicroRNAs Deregulation in Colorectal Cancer

The first miRNA profiling in CRC was performed by Cummins and collaborators in 2006 (8). 273,966 cDNA tags obtained from human colorectal cancers, matched normal colonic tissue and CRC cell lines were analyzed using the miRAGE (miRNA serial analysis of gene expression) technology. This analysis arose two main issues: first, the number of miRNAs present in the human genome was much higher then expected, with 133 new miRNAs and 122 previously uncharacterized. Second, miRNAs were aberrantly expressed in CRC tissues and cell lines compared to the normal counterparts, providing the first evidence for a role of miRNAs in colo-carcinogenesis. The new candidate miRNAs discovered by Velculescu's group (8) were also validated in CRC cell lines after modulation of Dicer, an enzyme involved in the cytoplasmic procession of miRNAs. Based on the assumption that Dicer-depleted cells contain reduced amounts of mature miRNAs (9), the authors

generated CRC cell lines where Dicer was inactivated because of the mutation of its helicase domain ($Dicer^{ex5}$) and compared their miRNA expression to the expression of wild-type (WT) Dicer cells. These studies resulted not only in a sharp trick to investigate the presence of new microRNAs, revealing reduced amounts of mature miRNAs and accumulation of miRNA precursors in $Dicer^{ex5}$ cells compared to their corresponding parental lines, but also strengthened the hypothesis that alterations of the miRNA machinery can play a central role in miRNA deregulation in cancer. However, this is not the only mechanism through which miRNAs may be deregulated in human cancers. Other theories have been postulated and experimentally verified over the last few years. Some miRNAs are located at fragile sites (10) in the human genome that can be lost during the chromosomal remodeling associated with the malignant transformation. Moreover, loss or gain of function of microRNA genes can be caused by impaired miRNA post-transcriptional regulation (11, 12), epigenetic silencing (13), miRNA transcriptional inhibition from oncogenic factors (14), unbalance between miRNA expression and levels of their putative target (15) or polymorphisms within micro-RNA-seed regions in their target 3'UTR (16).

Chromosomal Aberrations

Calin *et al*. reported that 52.5% of miRNA genes are located in cancer-associated genomic regions or in fragile sites frequently involved in translocation or chromosomal rearrangement (10). A clear example is represented by miR-15 and miR-16-1 down-regulation in B cell chronic lymphocytic leukemias (B-CLL). This cluster is in fact located at chromosome 13q14 which is deleted in more than 50% of B-CLLs (17). CRCs often harbor amplification of the region 20q13 (18) where miR 297-3 was found to be located (10). However, there is no evidence so far for the correlation between chromosomal aberrations and the location of the most frequently down-regulated miRNAs in CRC, such as miR-143 or miR-145 (19-23).

More recently another European study analyzed the correlation between the loci and the copy number of more than 250 miRNAs in prostate, bladder and colon cancers (24). CRC and prostate tumors showed a significant correlation between miRNA expression and gene copy number gain or loss. The same trend was unexpectedly not confirmed in bladder cancer leading the authors to speculate about the existence of a cancer-specific abnormality pattern as supposed previously also by Croce's group (10).

Moreover, no statistically significant relationship between miRNA copy number and expression level was found when the Danish database was compared to the published miRNA expression data (25, 26), suggesting that different mechanisms might be at the basis of miRNA deregulation.

So far the mechanism most frequently involved in miRNA deregulation in CRC seem to be related to miRNA processing alterations and epigenetic deregulation.

MiRNA Processing Alterations

The idea of an altered miRNA processing was first postulated by Michael and co-workers (19) who analyzed microRNA deregulation in CRC by cloning and northern blot analysis. Comparative analysis of CRC tissue and normal colonic mucosa led to the identification of 28 microRNA sequences that included three completely new miRNAs (miR-320, miR-321, and miR-200c), seven mouse-specific miRs and two miRNAs (miR-143 and miR-145) that were consistently down-regulated in all cancer specimens. In the attempt to find an explanation for the deregulation of these miRNAs they analyzed the expression of the RISC complex associated genes, Dicer and eIF2C2, by Real-time PCR (RT-PCR) analysis in a subset of matched tissue-RNA samples. No discrepancies in gene expression between normal and tumoral tissues were found hypothesizing that other factors might regulate miRNA expression in CRC.

More recently Esteller's group found that frameshift mutations in TARBP2 (TAR RNA-binding protein 2, an essential element of the DICER machinery) are very frequent in sporadic and hereditary CRCs associated with microsatellite instability. These mutations can cause TRBP protein expression reduction, destabilization of DICER1 protein and defect in the processing of miRNAs, providing a possible explanation for miRNA deregulation in colorectal cancers (27).

When Co115 cells (TARBP2 mutant CRC cells) were transfected with WT TARBP2, both TARBP2 and DICER1 protein expression was increased and the pre-miRNA processing capacity was restored, as shown by microarray and RT-PCR analysis of many mature oncosuppressor miRNAs such as let-7f, miR-205, miR-26a, miR-125a and miR-125b. Moreover Co115 TARBP2 transfected cells showed oncoproteins ERBB2 and EZH2 down-regulation, reduced cell proliferation rate and impaired tumor forming ability both *in vitro* and *in vivo*.

On the contrary SND1, another important component of the RISC complex, has been reported among the list of highly expressed genes in human colon cancers (28). Tsuchiya and co-workers, showed that SND1 mRNA is frequently up-regulated in human and mice cancers as well as in aberrant crypt foci, and can down-regulate APC protein expression by post-transcriptional regulation. Based on these findings the authors speculated that SND1 may control gene expression of APC or other cancer-related genes through the regulation of microRNA-induced translational repression.

Epigenetic Deregulation

Two main pathways are involved in the epigenetic regulation of gene expression: DNA methylation and histone deacetylation or methylation. DNA methylation consists in the transfer of methyl groups to citidine guanine rich areas, called CpG islands usually located in the promoter region of a gene. This reaction is catalyzed by three different DNA methyltransferases, DNMT1 (constitutive DNMT), DNMT3a and DNMT3b (*de novo* DNMTs) (29). Histone deacetylation or methylation consist of acetyl groups removal or methyl group transfer to specific aminoacidic residues of histones respectively (30). Histones are the main protein components of chromatin and their epigenetic modulation results in the modification of the chromatin structure that makes the transcription and/or replication possible. Hypermethylation of CpG island promoters has been correlated with transcriptional silencing of microRNA genes in CRC. Esteller's group compared miRNA expression between methyltransferases (DNMT1 and DNMT3a) deficient HCT116 CRC cells and their WT counterpart (13). About 6% of the 320 analyzed miRNAs were up-regulated in the double knockout cells confirming how the methylation of CpG island exerts an important control of miRNAs expression. In the same analysis miR-124a, located at a CpG Island, was shown to be deregulated in different cancer types when compared to normal tissue and was shown to be involved in the regulation of Cyclin-dependent kinase 6 (CDK6) expression and CDK6-mediated retinoblastoma (Rb) phosphorylation.

These authors further studied the methylation-dependent miRNA regulation in CRC by profiling miRNA expression after treatment with the demethylating agent 5-aza-2'-deoxycytidine (5-Aza) (31). miR-148a, miR-34b/c, and miR-9 were re-expressed in metastatic CRC cell lines following 5-Aza treatment and bisulfite genomic sequencing confirmed the presence of hypermethylated CpG islands in the promoter region of these miRNA genes. Re-expression of epigenetically silenced miR-148a and miR-34b/c caused cell motility inhibition in cancer cell lines and reduced metastatic potential in xenograft models. More interestingly a statistically significant correlation was found between the hyper-methylation of the promoter of these three miRNA genes and the presence of extracolic metastasis in human CRC.

Another example of miRNA epigenetic regulation has been provided by Grady *et al*. (32) who showed that CpG island methylation of the EVL (Ena/Vasp-like) gene frequently occurs in adenoma and carcinoma and is associated with both EVL gene suppression and miR-342 down-regulation (located in a EVL intron). Restoration of miR-342, which is frequently down-regulated in CRC (8, 33) induced apoptosis in HT-29 CRC cells providing evidence for a connection between epigenetic miRNAs down-regulation and apoptosis inhibition.

The hypothesis that different epigenetic mechanisms may, at the same time, control microRNA regulation has also been studied by Bandres *et al*. (34) In this Spanish study the expression of miR-9, miR-129 and miR-137, that are frequently deregulated in CRC, was restored after the simultaneous treatment of CRC cell lines with both a methyltransferase and a histone deacetylase inhibitor.

A more intriguing example of how miRNA regulation can be articulated is represented by the interaction among miR-34 family, the epigenetic machinery and TP53 pathway. miR-34 is frequently down-regulated in CRC in comparison to normal paired tissue (33). TP53 was shown to transactivate miR-34 by acting as a transcription factor that recognizes specific binding sites in the miR-34 gene promoter (35). Loss of function in TP53 gene due to mutations occurs frequently in CRC multistep development (36) and can explain in part miR-34 down-regulation. Tazawa *et al*. (37) demonstrated that miR-34 induction by adriamycin in CRC cell lines is strictly dependent on WT TP53. miR34 stimulation by low dose adriamycin was time dependent and exerted oncosuppressive functions by inducing senescence-like growth arrest, down-regulation of E2F family and cell cycle progression genes (CDK4 and CDC25C) and over-expression of P53 and P21. In line with these findings Yamakuky *et al*. (38) evidenced a role for miR-34 on the control of silent information regulator 1 (SIRT1), a NAD dependent deacetylase that takes part in the apoptotic response to oxidative and genotoxic stress. SIRT1 has been frequently implicated in CRC pathogenesis through the epigenetic regulation of many target genes, and seems to play a critical role in tumor initiation, progression, drug resistance and neo-angiogenesis (39). Adriamycin stimulation of WT TP53 CRC HCT-116 cells induced SIRT 1 down-regulation, cell cycle arrest and apoptosis activation in a p53 dependent fashion as testified by the induction of P53, P21 and PUMA genes. The interaction among TP53, epigenetics and miR-34 is articulated. Epigenetics also contributes to miR-34 deregulation, by silencing the expression of both miR-34 and its host gene B-cell translocation gene 4 (BTG4) (40). In turns, miR-34 up-regulates directly and indirectly TP53 and the apoptosis pathway by inhibition of SIRT1 mediated methylation.

MiRNA Profilings in Colorectal Cancer Characterization

Human malignancies characterization by microRNA expression analysis has been extensively profiled in fresh (25, 26, 33), formalin fixed tissues (41) and in blood samples (42). These studies hypothesized a role for microRNA expression in cancer identification (26, 43), diagnosis and prognosis (33). The potential contribution of microRNA profiling in characterization and diagnosis of tumors of unknown origins has been investigated in many studies (26, 44, 45).

Lu *et al*. (26) used a flow cytometric miRNA expression profiling method to analyze the expression of more than 200 mammalian miRNAs on 334 samples of multiple human cancers. Specific miRNA signatures were able to identify the developmental lineage and the differentiation state of different tumors with higher accuracy than the mRNA profiling. Moreover, poorly differentiated tumors could be differentiated from the remainder ones with the same signature. In this analysis most miRNAs were down-regulated in cancer with respect to normal tissues. In 2005 Volinia *et al*. (25) identified a 21 miRNA cancer-specific signature by using a custom microarray platform. It included miR-21, miR-17-5p, miR-191, miR-29b, miR-223, miR-128b, miR-24, miR-155, miR-20a, miR-107, miR-32, miR-30c, miR-221 and miR-106a that were significantly up-regulated in CRC. Most of these miRNAs have been later confirmed to be up-regulated in CRC by other profiling studies (20, 22, 33, 46).

MiRNA profiling has also been used to classify CRC on the basis of their Microsatellite status. Microsatellite instability (MSI) is the hallmark of impairment in the DNA mismatch repair (MMR) system and is found in 100% of hereditary non-polyposis colorectal cancers (HNPCC) as well as in 15% of sporadic colorectal cancers (47). MSI screening using a panel of microsatellite markers is routinely used to asses MSI status of patients with clinical and familial suspect for HNPCC. If the screening is positive, patients are further studied for mutations or epigenetic silencing of MMR genes. Lanza and colleagues (48) showed that microRNA profiling clearly discerned between MSI and microsatellite stable (MSS) tumors. In the future, microRNA profiling might be used, together with the clinical evaluation and the MSI analysis, to better screen and select patients with suspected HNPCC, who deserve further and more expensive evaluation such as genotyping. More recently Schepeler *et al*. (49) used the same approach to classify a cohort of 49 tumor and 10 normal tissues from stage II CRC patients. The microRNA signature not only clearly differentiated MSI *versus* MSS tumors, but also showed the prognostic value of miR-320 and miR-498 in predicting recurrence-free survival. The correlation between microRNA expression and prognosis has been elucidated also in another study that analyzed 110 CRCs and found a statistically significant correlation between miR-106 expression, disease free (p=0.03) and over survival (p=0.04). In a further study by Bandres and colleagues (51), miR-451 was found down-regulated in CRC and gastric cancers and showed a statistically significant correlation with Disease Free Survival in stage III gastric cancer patients. MiR-451 was also identified as a regulator of the oncogene macrophage migration inhibitory factor (MIF) (51).

The use of microRNA profiling in specific subsets of CRC patients might be very useful to tailor patients' treatment and provide prognostic information. Indeed it may contribute to delineate the prognosis of stage II CRC patients as well as to identify MSI tumors that have a better prognosis but a limited benefit from 5-fluorouracil (5-FU) based chemotherapy (47).

In 2008 Harris's group (33) confirmed the promising clinical potential of microRNA profiling in clinical management of CRC patients by performing a microRNA microarray expression profiling in more than 200 paired tumor and non tumor tissues in two different cohorts of American and Chinese patients.

Among the thirty-seven miRNAs deregulated in the U.S. cohort, five (namely, miR-20a, miR-21, miR-106a, miR-181b, and miR-203) were selected on the basis of their up-regulation and confirmed in the Chinese cohort. MiR-21 was the most up-regulated miR in both cohorts and was correlated with tumor status, TNM staging, survival prognosis, and response to adjuvant chemotherapy. MiR-21 over-expression showed a statistically significant inverse correlation with overall survival (OS), correlated with TNM status and resulted a predictive marker for poor benefit from adjuvant fluoropyrimidine-based chemotherapy. None of the down-regulated miRNAs demonstrated a prognostic or predictive value.

Beside the clinical relevance of these findings, this study highlighted the role of miR-21 in CRC pathogenesis, showing for this miR a clear involvement in tumor progression and metastasis. In fact, miR-21 was found up-regulated in adenomas and carcinomas when compared with normal paired tissue and its expression was progressively increasing in the adenoma-carcinoma-advanced carcinoma sequence. These results were recently confirmed by a Japanese study (52). The analysis of 39 surgically excised colorectal tumors and 34 endoscopically resected colorectal polyps by immunohistochemistry (IHC) with locked nucleic acid (LNA)/DNA probe showed an overexpression of miR-21 in adenoma, carcinoma and cancer-associated stromal fibroblasts when compared to normal paired tissue, whereas its expression was found negative in non neoplastic polyps.

As suggested by Schetter's study (33) miRNAs could also be used as surrogated markers to predict chemotherapy response. The interaction between miRNAs and drug treatment in CRC has been investigated in several studies. Rossi *et al*. (53) profiled miRNA expression after treating two different clones derived from HCT-116 and HT-29 CRC cells with 5-FU and generated interesting conclusions. First, 5-FU might exert its therapeutical effect by down-regulating specific miRNAs such as miR-200b, which is frequently up-regulated in CRC and exhibits an oncogenic effect. Secondly, the 5-FU-induced up-regulation of some miRNAs with mitogenic function like miR-21 could represent a mechanism of tumor resistance to the drug.

This hypothesis is supported also by another clinical study that investigated miRNA expression before and after capecitabine (an oral antimetabolite converted by the liver in

5-FU) and radiotherapy as neoadjuvant treatment for rectal cancer (54). Beside the up-regulation of different miRNAs (miR10a, miR21, miR145, miR212, miR339, miR361) two miRNAs, miR125b, miR137, were found to be increased after preoperative treatment and, most interestingly, showed a statistically significant correlation with the tumor regression grade assessed on the surgical specimen.

In another American study (55), the potential role of miRNAs as predictive tools for chemotherapy response was retrospectively analyzed in 46 patients with recurrent or residual colon cancer lesions treated with 5-FU-based anti-metabolite S-1. Hsa-let-7g and hsa-miR-181b expression showed a statistically significant (P 0.03 and 0.02 respectively) correlation with clinical response.

The potential role of circulating miRNAs in screening for CRC has been elucidated in an interesting study by Ng and co-workers (46). The analysis was divided in 3 different steps: identification of a subgroup of potential candidate miRNAs analyzed on plasma, cancer biopsy and normal adjacent tissue, validation by RT-PCR on a small group of patients and confirmation of the candidate miRNAs on plasma from an independent cohort of 90 CRC patients, 20 gastric cancer patients, 20 patients with inflammatory bowel disease (IBD) and 50 healthy controls. miR-92 up-regulation demonstrated the best predictive value for CRC with a sensitivity of 89% and a specificity of 70% . Moreover miR-92 expression showed to be independent from inflammation or other gastrointestinal cancer and was decreased after tumor resection demonstrating a CRC specificity.

From MiRNA Profilings to Target Identification

CRC development has been linked to the progressive acquisition of mutations in genes with a crucial role in cell growth, proliferation and programmed cell death (36). As shown in many different studies, miRNAs might perfectly fit and integrate this model initially postulated by Vogelstein by controlling several pathways involved in CRC development (Figure 1).

Type 1 insulin-like growth factor receptor (IGF-IR) and its physiological co-activator insulin receptor substrate-1 (IRS-1) are important regulators of proliferation and differentiation. IGF-IR activation by IRS-1 triggers a strong mitogenic and anti-differentiation stimulus for the cell. IRS-1 up-regulation has been proved to promote cell transformation both *in vitro* and *in vivo* (56). IGF-IR and IRS-1 are both regulated by miR-145 (57, 58), a microRNA frequently down-regulated in CRC (19-23). *In vitro* experiments showed that miR-145 transfection of 3T3 mouse embryo fibroblasts shut down IRS-1 expression of about 50% and induced inhibition of cell proliferation, without influencing apoptosis. On the contrary miR-145 caused no growth inhibition of BT-20 mammary cancer cells that do not express IRS-1, leading the authors to conclude an IRS-1 mediated action of miR-145 in CRC cell proliferation. miR-145 may represent a physiological controller of colon cells. Loss of miR-145 in CRC may account for the unbalance between proliferation and differentiation in CRC cells inducing an uncontrolled mitogenic stimulus IGF-1R and IRS-1 mediated.

The epidermal growth factor receptor (EGFR) is an other important growth factor implicated in proliferation, differentiation, and development. EGFR overexpression is frequently involved in CRC pathogenesis and resistance to chemotherapy (59). Two different studies showed that miR-7 and miR-128 are able to down-regulate EGFR and its downstream pathways in breast, glioblastoma and lung cancer cell lines and in lung cancer patients (60-61). Even though miR-128 and miR-7 have been shown to be deregulated in CRC (22, 46) no study support a role for their down-regulation in EGFR over-expression. Ras is a guanine nucleotide-binding protein situated downstream of the growth factor receptors that controls cell proliferation, cell adhesion, and apoptosis. Different isoforms have been identified, H-Ras K-Ras and N-Ras. Activating point mutations in the Ras oncogene occur in 50% of CRC (62). Three different miRNAs, let-7 (63), miR-143 (23) and miR-18a* (64), are able to down-regulate RAS and thus to affect cell proliferation as well as anchorage-independent growth in cancer cells (Figure 2). Interestingly, all these miRNAs were observed down-regulated in CRC (23, 33, 63, 64). miR-143 also demonstrated to reduce phosphorylation of the ERK1/2 system situated downstream of K-RAS in the phosphorilative cascade (23). Guo and colleagues (65) showed that miR-126 can deregulate phosphatidylinositol 3-kinase (PI3K), by targeting the PI3K regulatory subunit beta (p85b), and its downstream signaling effector phospho-AKT. This effect results in cell proliferation inhibition and impairment of the colony forming ability in CRC cells *in vitro*. A critical role for proliferation control and tumor forming ability has been recently attributed to DCAMKL1, a microtubule associated kinase expressed in the intestinal lumen, and in $APC^{+/-}$ adenomas. This new oncogene was found up-regulated in CRC in respect to normal adjacent tissues and showed an inverse correlation with let-7 (66). Knocking down DCAMKL1 expression in xenograft models by using selective siRNA induced an increase in let-7 expression and a down-regulation of its target c-myc, resulting in a stimulus for cell differentiation.

APC exerts its oncosuppressive function by regulating cell proliferation and differentiation in the intestinal crypts. Loss of APC translates in Wnt pathway activation due to β-catenin release from the APC, axin, and glycogen synthase 3 complex. This leads to β-catenin transcription factors (c-Myc or cyclin D1) activation and cell proliferation deregulation (Figure 1). Agami's group observed an inverse correlation between miR-135a and miR-135b and APC mRNA expression in a group of

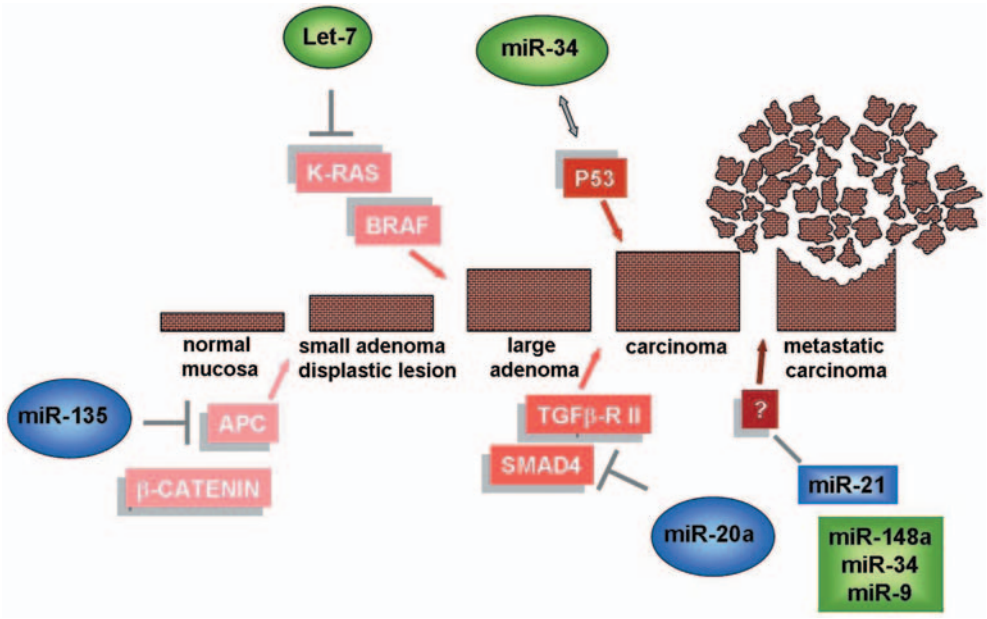

Figure 1. *Links between multistep CRC carcinogenesis model36 and microRNAs. According to the model proposed by Vogelstein different genes are involved in each step of the progression from normal mucosa to metastatic cancer. Further knowledge of the mechanism of action of microRNAs in CRC made the picture more articulated with the addition of specific microRNAs that modulate selective targets at each step of this model. On the basis of their expression and their targets, microRNAs may behave as oncogenes (blue circle) or onco-suppressors (green circle). Although it is not yet clear which genes play the main role in the progression from carcinoma to metastatic carcinoma, aberrant expression of additional miRNAs (in the squares) have been observed in metastatic CRC. Further studies are warranted to establish the connection between these miRNAs and metastasis related target genes.*

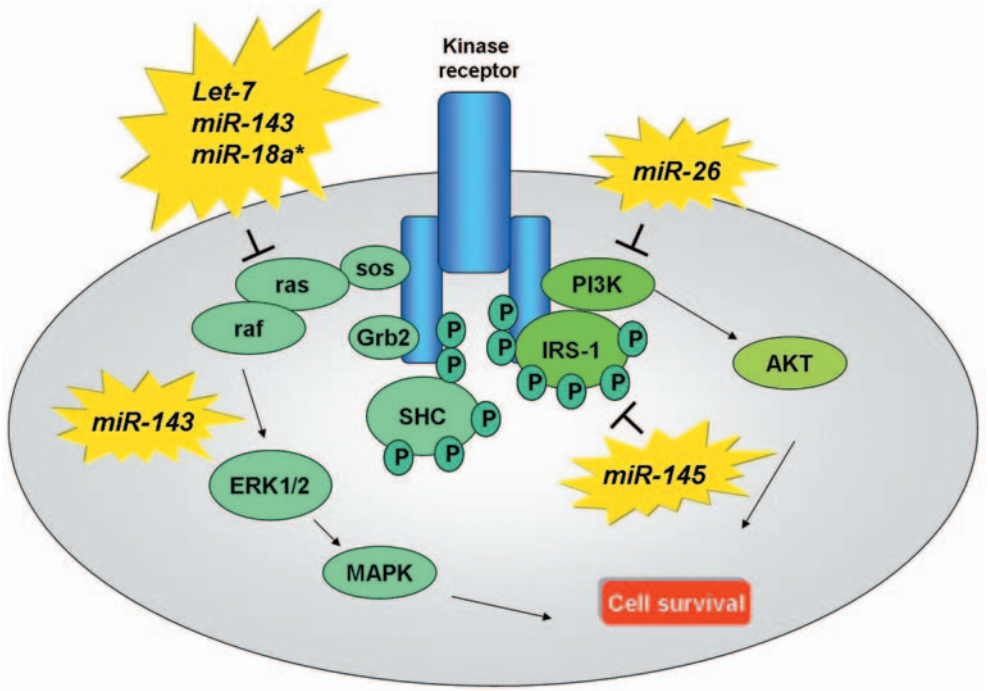

Figure 2. *miRNAs contribute to the regulation of growth factor receptors signaling. Ras-MAPK and PI3K-AKT pathways are common to several growth factor receptors-mediated signaling. Different miRNAs are involved in the regulation of these pathways through the modulation of multiple targets. The loss of these miRNAs in CRC may account for the dysregulated enhancement of survival signaling.*

43 sporadic colon adenomas and carcinomas. Moreover, both the isoforms of miR-135 can down-regulate APC expression *in vitro* by targeting its 3'UTR (68).

DNA reparation impairment has also been linked to CRC development and progression. A link between micro-environmental factors, miRNAs and DNA repair system has been highlighted by Crosby *et al*. in breast and cervical cancer cell lines. miR-210 and miR-373 expression can be induced by the hypoxia inducible factor 1 alpha (HIF1α). This transcription factor can bind to microRNA gene promoters and induce their expression. miR-210 and miR-373 in turn down-regulate two major key players in DNA repair system RAD52 and RAD23B respectively implicated in homology-dependent repair (HDR) and nucleotide excision repair (NER). Although this interaction has not been shown in CRC yet, it might contribute to colon tumorigenesis.

So far we analyzed miRNAs with oncosuppressor function whose loss might represent a critical step in proliferation and transcription factors deregulation. Among the up-regulated miRNAs (also called oncomiRs), miR-21 seems to play a critical role in CRC pathogenesis. miR-21 is responsible for the modulation of Pdcd4, a tumor suppressor gene that inhibits TPA-induced tumor transformation and progression (70). Pdcd4 showed an inverse correlation with miR-21 levels in 10 different CRC cell lines and in a cohort of 22 patients. Moreover Pdcd4 down-regulation was associated with cell invasion and metastatic potential increase *in vitro* assays (70). miR-21 is also responsible for phosphatase and tensin homologue (PTEN) PTEN deregulation in hepatocellular carcinoma (71), even though the same effect has never been confirmed in CRC. miR-21 is also responsible for cell migration and microvillus–like formation in CRC cell lines by targeting Sprouty2 (SPRY2), an inhibitor of branching morphogenesis and neurite outgrowths. Knocking down miR-21 expression in colon cancer SW480 cells with high miR-21 basal expression caused disappearance of their microvillus-like protrusions accompanied by SPRY2-dependent inhibition of cell migration (72).

Cyclooxygenase-2 (COX-2) catalyzes the production of Prostaglandin E2, one of the most important products of the arachidonate metabolism. COX-2 overexpression seems to exert an important function in CRC progression by stimulating cells invasion and tumor metastatization (73, 74). miR-101 frequently down-regulated in CRC showed an inverse correlation with COX-2 in CRC cell lines and in a small cohort of human CRC tissues. *In vitro* miR-101 could down-regulate COX-2 protein and mRNA expression through a direct effect (75).

Monzo *et al*. (76) analyzed and compared the expression of miRNAs in embryonic colon tissue, in colorectal cancer and paired normal colon tissue. Eleven embryos and 44 colorectal samples were analyzed by RT-PCR and miRNA *in situ* hybridization. A common microRNA signature was detected between embryonic colonic mucosa and colorectal cancer. Interestingly, miR-17-92 cluster and its target E2F1 showed an identical pattern of expression in human colon development and colonic carcinogenesis, highlighting its role as controller of the proliferation in the crypt progenitor compartment.

Conclusion

In the last five years miRNAs have been implicated in colon carcinogenesis, leading many authors to hypothesize a potential value of miRNA-based tools in CRC patients management. This has been true for many other prognostic or predictive markers that failed when transposed from basic research to clinical use (as example *c-myc, p53, DCC, smad4, nm23, bcl-2, BAX, thymidylate synthase, thymidine, Phosphatase, TGF, VEGF,* p27, p21, p16, cd44, E-cadherin, ICAM-1, MMPs, urokinase-type plasminogen, Activator, Superoxide dismutase, GST-pi) (2). To succeed where many others have failed we believe that prospective randomized trials in the future should include the evaluation of miRNAs as predictive biomarkers in their endpoints. Furthermore, as suggested also by other authors (77), the problematic of the variability in miRNAs detection should be overcome by using standardized techniques for tissue isolation and preparation, platform and software analysis in order to avoid selection bias.

If future prospective trials lead to the identification of a well defined prognostic and predictive miRNA signature in CRC patients, RT-PCR based validation of selected "hot" miRs could represent an easy and cost effective instrument to define CRC patient prognosis, and select target specific treatments as already proved for other targets (EGFR, Her2/neu). The validation of so far published data in a prospective context might also help to measure the real weight of many miRs and their potential role in CRC treatment eliminating false myths and exerting that caution that Filipowicz's group recommended when translating *in vitro* observations to cancer patient management (78).

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