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Review

microRNAs in colon cancer: A roadmap for discovery

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ABSTRACT

Cancer *omics* data are exponentially created and associated with clinical variables, and important findings can be extracted based on bioinformatics approaches which can then be experimentally validated. Many of these findings are related to a specific class of non-coding RNA molecules called microRNAs (miRNAs) (post-transcriptional regulators of mRNA expression). The related research field is quite heterogeneous and bioinformaticians, clinicians, statisticians and biologists, as well as data miners and engineers collaborate to cure stored data and on new impulses coming from the output of the latest Next Generation Sequencing technologies.

Here we review the main research findings on miRNA of the first 10 years in colon cancer research with an emphasis on possible uses in clinical practice. This review intends to provide a road map in the jungle of publications of miRNA in colorectal cancer, focusing on data availability and new ways to generate biologically relevant information out of these huge amounts of data.

1. Introduction

Research in the past decade has shown that several types of non-coding RNAs (ncRNAs, an RNA molecule that is not translated into a protein but has other important biological functions), including long ncRNAs and notably microRNAs (miRNAs), are involved in cancer development and progression [1]. NcRNAs are classified based on their size and known function. In this review we focus on miRNAs, since they are the most widely studied class of ncRNAs, although many issues about mining the data remain unsolved. We are still far from truly understanding the extent of the involvement of miRNAs in cancer, many results have been produced with the final aim of finding biomarkers for personalized (intended as the P5 concept in its entirety) [2] therapy, but still much must be done in integrating different *omics* data, validation of results in large cohorts of patients, evaluation of the treatments' risks, as well as socio-economic plans of what can be really done with the available resources [3]. Colorectal cancer (CRC) is one of the most common malignancies in the western world. Up to 90% of patients can be cured by surgery if the disease is detected at the early stage, but unfortunately it is often diagnosed only at an advanced stage and the prognosis is therefore poor. Synchronous metastases are present in 15–25% of CRC patients and since patients with synchronous colorectal liver metastases comprise at least 25% of patients reported from large resection series, determination of the optimal management of these patients, in terms of classifying subgroup of patients to avoid over- or under-treatment, possibly avoiding the progression of the disease, is of fundamental importance [4], and miRNAs have the potentiality to be the main actor in this schema, being them regulators of mRNAs, situated in fragile sites [5], involved in cancer biology and having decoy activity [6].

Abbreviations: miRNA, microRNAs; CRC, Colorectal cancer; mCRC, Metastatic colorectal cancer; ncRNA, Non-coding RNA; ISH, In situ hybridization; FFPE, Formalin-fixed paraffin-embedded; NGS, Next Generation Sequencing; MSI-H, Microsatellite instability (high); MSI-L, Microsatellite instability (low); MSS, Microsatellite stability; CIN, Chromosomal instability; HNPCC, Hereditary non-polyposis colorectal cancer; OS, Overall survival; ceRNAs, Competing endogenous RNAs; SRT, Surgically resected tissue; CCCL, Colon cancer cell line

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1.1. miRNA function and colon cancer

miRNAs are short (19-23-nucleotides) RNAs that are processed from hairpin loop structures and control the translation of mRNA into protein. The role of miRNAs in cell physiology and pathology is hard to understand due to their complex relation to biological function:

1. a typical miRNA can control translation of more than one messenger RNA, [7] perhaps as many as a few thousand messenger RNAs [8] and,
2. a single messenger can be controlled post-transcriptionally by more than 1 miRNA [9], perhaps dozens [8,10].

The literature related to miRNAs, has grown exponentially in the last decade, in summary we know that [11,12]:

- miRNAs can act as oncogenes or tumor-suppressor genes.
- miRNAs are involved in tumor progression and metastasis through their role in pathways that contribute to metastasis, including migration, invasion, cell proliferation, epithelial-to-mesenchymal transition (EMT), angiogenesis, and apoptosis.
- miRNAs might be useful as prognostic and predictive markers.

But how are these intermediate results linked together and with other *omics* data? Have they been analyzed associated to already known subgroups of patients like KRAS mutated, BRAF mutated [13], BRAF-like [14]? Are they particular for CRC or might they be general for cancer? How many studies failed or had discordant results on the same miRNAs when analyzed in different cohorts?

1.2. Public data

The development of miRNA microarrays, RT-PCR platforms and deep sequencing methodologies resulted in the acquisition of a growing number of miRNA profiling studies, and has paved the way to new approaches for biomarker discovery. Some of the published miRNA profiles are publicly available in the NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), ArrayExpress (http://www.ebi.ac.uk/arrayexpress/), miRNA body map (http://www.mirnabodymap.org), TCGA (http://cancergenome.nih.gov) and SMIRNADB (http://www.mirz.unibas.ch).

Two tools offer compact visualizations of uploaded miRNA expression differences:

1. The Geo profiles tool (http://www.ncbi.nlm.nih.gov/geoprofiles) among uploaded samples.
2. The Gene Expression Atlas (http://www.ebi.ac.uk/gxa/) per organism and per experimental condition.

The TCGA project (https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp) provides multiple *omics* data for the same set of samples. Mirnabodymap (http://www.mirnabodymap.org/) contains RT-qPCR data on cancer (but not yet for CRC).

For CRC, more than 1400 arrays are available in GEO, 873 arrays in ArrayExpress and 187 microRNASeq samples are downloadable from the TCGA database. SMIRNADB contains miRNA and other ncRNA expression data in large and small intestinal samples in the mouse.

1.3. MiRNA profiling

The main methods currently used for miRNA profiling are sequencing, microarray, real-time PCR-based approaches and in situ hybridization (ISH); all of them require standard procedures to be correctly processed and mined. Initially these techniques were applied on fresh-frozen tissue specimen, but recently reproducible profiles of comparable quality have been obtained also using formalin-fixed paraffin-embedded tissue samples (FFPE), making archived tumor tissue collections accessible for study [15,16].

Reis and colleagues reported in 2011 that with FFPE material the Nanostring technology might be preferable over RT-PCR [17] notably on older samples with more highly degraded RNA and DNA [18].

Deep sequencing or Next Generation Sequencing (NGS) platforms have recently emerged as powerful technologies that provide unprecedented insight into biological systems. Thanks to the development of NGS technologies, large portions of the human genome are being re-sequenced in many individuals, opening new opportunities to find out how changes in the genome are associated with disease.

This situation poses a number of new challenges to translational research scientists, especially in regard to requirements for advanced data management and data analysis procedures and for teamwork between clinicians, computer scientists, and molecular biologists.

Therefore, integrative analysis between different *omics* platforms has become an essential element in the experimental design of studies in the era of NGS genomics [19].

The field of Bioinformatics is undergoing a rapid evolution to create new tools for NGS data visualization, manipulation, and analysis in terms of alignment, assembly, quality control and variations detection.

However, there is a lack of mature high standard data analysis methods although several open-source applications and utilities start providing useful tools for data analysts, for example: Galaxy (http://www.galaxy.psu.edu/, web-based) provides an NGS analysis toolbox for quality control, mapping, SAM tools and several post-processing analyses.

ISH is another extensively applied technique due to its ability to detect and localize specific miRNAs within tissue samples. Among many others, it has been performed while studying the *miR-200c* function in EMT in metastatic colorectal cancer (mCRC) [20]. After *miR-200c* expression was found lower in metastasis when compared to normal mucosa, ISH analysis of primary CRC, liver metastasis samples and adjacent hepatocytes suggested what might be the role of *miR-200c* during cancer progression, notably during the EMT-MET (mesenchymal-to-epithelial transition) switching: modulation of the *miR-200c* expression could influence the cell invasion and the cell proliferation functions during metastasis development.

2. miRNAs in colon cancer

Since the literature dedicated to miRNAs in colon cancer has grown considerably in the last decade, in Table 1 we report the updated (associations inserted by us are in bold) list of the causal associations between miRNAs and colon cancer taken from http://www.mir2disease.org.

The first definite association of miRNAs with CRC was the realization that in 2003 Human homologues of murine miRNA sequences, *miR-143* and *miR-145*, consistently display reduced steady-state levels of the mature miRNA at the adenomatous and cancer stages of CRC.

Since then, miRNA alterations have been observed in CRC (for a complete review of miRNAs in CRC diagnosis, prognosis and progression and possible mechanisms of action, see [21]), but in most cases the biological significance of the observation is not fully understood and still there is no clear division between those that are driver events and those that are passenger events without any physiological importance.

Table 1
miRNAs causal in CRC. Samples source was reported to specify whether CCCLs or SRTs (frozen or FFPE) were analyzed.

miRNA	Reference's title	Samples source	References
hsa-let-7a-1	let-7 miRNA functions as a potential growth suppressor in human colon cancer cells	SRTs	[51]
hsa-miR-124a	Genetic unmasking of an epigenetically silenced miRNA in human cancer cells	CCCLs	[52]
hsa-miR-126	The ncRNA, miR-126, suppresses the growth of neoplastic cells by targeting phosphatidylinositol 3-kinase signaling and is frequently lost in colon cancers.	CCCLs	[53]
hsa-miR-127	Specific activation of microRNA-127 with down regulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells	SRTs (fresh frozen), CCCLs	[54]
hsa-miR-135	Regulation of the adenomatous polyposis coli gene by the miR-135 family in CRC	SRTs (fresh frozen), CCCLs	[33]
hsa-miR-140	Mechanism of chemo resistance mediated by miR-140 in human osteosarcoma and colon cancer cells	SRTs (fresh frozen), CCCLs	[55]
hsa-miR-141	microRNA-141 Regulates Smad Interacting Protein 1 (SIP1) and inhibits migration and invasion of CRC cells	CCCLs	[56]
hsa-miR-143	Role of miR-143 targeting KRAS in colorectal tumorigenesis	SRTs	[51]
hsa-miR-143	microRNAs 143 and 145 are possible common onco-microRNAs in human cancers	SRTs	[57]
hsa-miR-143	microRNA-143 targets DNA methyltransferases 3A in CRC	SRTs (fresh frozen), CCCLs	[58]
hsa-miR-143	microRNA-143 reduces viability and increases sensitivity to 5-fluorouracil in HCT116 human CRC cells	SRTs	[36]
hsa-miR-145	Mechanism of growth inhibition by microRNA 145: the role of the IGF-I receptor signaling pathway	CCCLs	[59]
hsa-miR-145	microRNAs 143 and 145 are possible common onco-microRNAs in human cancers	SRTs	[57]
hsa-miR-145	microRNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells	CCCLs	[60]
hsa-miR-145	Diagnostic and prognostic miRNAs in stage II colon cancer	SRTs (fresh frozen)	[61]
hsa-miR-17-92	Augmentation of tumor angiogenesis by a Myc-activated miRNA cluster	SRTs (FFPE)	[62]
hsa-miR-18a*	The miR-18a* miRNA functions as a potential tumor suppressor by targeting on K-Ras	CCCLs	[63]
hsa-miR-192, has-miR-194	p53-Responsive microRNAs 192 and 215 are capable of inducing cell cycle arrest	CCCLs	[64]
hsa-miR-196a	High miR-196a levels promote the oncogenic phenotype of CRC cells	CCCLs	[65]
hsa-miR-21	microRNA-21 negatively regulates Cdc25A and cell cycle progression in colon cancer cells	CCCLs	[66]
hsa-miR-21	microRNA-21 (miR-21) post-transcriptionally down regulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in CRC	SRTs (fresh frozen), CCCLs	[67]
hsa-mir-200	A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancercells	CCCLs	[68]
hsa-miR-215	p53-Responsive microRNAs 192 and 215 are capable of inducing cell cycle arrest	CCCLs	[64]
hsa-miR-342	Epigenetic silencing of the intronic miRNA hsa-miR-342 and its host gene EVL in CRC	SRTs (fresh frozen, FFPE)	[69]
hsa-miR-34a	Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells	CCCLs	[70]
hsa-miR-34b, has-miR-34c	Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in CRC	SRTs, CCCLs	[71]
hsa-miR-451	microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells	SRTs	[72]

Luo and Brenner, performed literature mining and published a useful summary of manually and automatically curated information highlighting, which miRNAs have been studied and under which conditions [22].

In these studies the most promising miRNAs for a role in the pathogenesis in colon cancer appear to be *miR-20a*, *miR-21*, *miR-31*, *miR-92*, *miR-181b*, which are up-regulated in colon cancer and *miR-143* and *miR-145* which are frequently down-regulated in CRC without a significant association with tumor progression although associated with tumor size [23] and both with decreased expression in adenomas and carcinomas. Gao and colleagues reported that Evi1 is a transcriptional suppressor of *miR-143*: their data pointed to a pathway in which Evi1 suppresses *miR-143* gene transcription which in turn leads to elevated levels of K-Ras [24]; the study was performed on fresh frozen surgically resected primary tissue and cell lines.

miR-21 was found up-regulated in breast, oral, and CRC tumor tissues. Volinia et al. found *miR-21*,as well as *miR-17-5p*, *miR-191*, *miR-29b* and *miR-155* to be up-regulated at least in CRC. Schetter et al. found *miR-21* to be positively correlated with higher stage and of prognostic value [25]: expression profiling of colon adenocarcinoma and paired normal tissues was performed on a US training cohort by miRNA microarray and validated in a Chinese cohort by RT-PCR. Associations with tumor status, TNM staging, survival prognosis, and response to adjuvant chemotherapy were evaluated. High *miR-21* expression was associated with poor overall survival in both the training and validation cohorts, independent of TNM staging, and was associated with a poor therapeutic outcome (adjuvant chemotherapy). But still little is known about *miR-21* function in CRC, the overexpression in CRC samples has been confirmed several times, we also know it is correlated with advanced disease; the involvement of *miR-21* in different types of cancer suggests that it may have a general role in tumorigenesis, but still concrete explanations of its role have not been proposed [26].

Another actor, *miR-29a*, has been found to be up-regulated in CRC versus normal tissue (tested in plasma, primary tumor tissue and paired normal tissue) [27], and in 2012 it was proposed as a potential serum marker for early detection of CRC [28].

In a recent publication, based on fresh frozen and FFPE surgically resected tissues (SRTs), only one miRNA, *miR-150*, was found to be consistently and progressively deregulated in a collection of adenomas and carcinomas, with lower expression compared to normal tissue [29]. In two series of respectively 239 and 185 patients assessed by RT-PCR respectively by ISH, low expression of *miR-150* was associated with worse survival; curiously, a similar result was previously reported in a study of sepsis [30].

In regard of the physiology of CRC, it can be divided broadly into two groups: those exhibiting microsatellite instability and a high frequency of point mutations (MSI) and those with stable microsatellites (MSS), which almost invariably show chromosomal instability (CIN) with higher frequency of diploids and focal copy

number aberrations [31]. Microsatellite instability (high) (MSI-H) cases represent about 10–15% of all CRC and are associated with distinctive pathologic features, such as proximal location, poor differentiation, frequent mucinous and medullary phenotype, and marked peritumoral and intratumoral lymphocytic infiltration . MSI carcinomas have a more favorable clinical outcome than MSS (microsatellite stable) tumors and the survival advantage conferred by the MSI phenotype is independent of tumor stage and other clinical and pathological variables [31]. Members of the oncogenic miR-17-92 family (*miR-17-5p*, *miR-20*, *miR-25*, *miR-92-1*, *miR-92-2*, *miR-93-1* and *miR-106a*) were found significantly up-regulated in MSS *vs.*MSI fresh frozen tumor samples [32]; but these findings were only partially confirmed by subsequent studies on FFPE tissues [31].

Concerning the CIN pathway, in which the adenomatous polyposis coli (APC) gene plays a dominant role, Nagel et al. showed that *miR-135a* and *miR-135b* directly target the 3′ untranslated region of APC, suppress its expression and induce higher activity in the Wnt signaling pathway although without proof that this is relevant in vivo or during CRC oncogenesis by analyzing colon cancer cell lines (CCCLs), fresh frozen and FFPE CRC tissue and normal epithelium [33]. Nevertheless, *miR-135b* was also found over-expressed in inflammatory bowel disease, a condition that can lead to CRC. In mouse models, treatment with *anti-miR-135b* resulted well differentiated in tumors whereas those in the control groups showed low differentiation.

Along with studies of single or few miRNAs, it has become clear in the last years, that if we want to understand miRNAs biology and functional role, they must be studied in combination among them and with other *omics* data.

For example, the group of Volinia found networks of miRNAs in normal tissues and in their pathologic counterparts (more than 3000 tumor tissue samples were studied) which defined independently regulated miRNAs, and target genes of uncoordinated miRNAs involved in cancer-specific pathways [34]. Software Applications that can help scientists to study the interactions between genomics data and the pathways in which they are involved include MetaCore (http://www.genego.com/metacore.php), Ingenuity (http://www.ingenuity.com/), and Cytoscape (http://www.cytoscape.org/).

For our understanding of the role of miRNAs in cancer, it is essential to move on from reports of mere associations to knowledge of how single miRNAs are functionally related to each other and connected to the expression of proteins in the cell. For example, several methods have become available for identifying miRNA target sites (see Table 2), but the mere presence of a miRNA-binding site is insufficient for predicting target regulation. Regulation of targets by miRNAs is subject to various levels of control. The finding that any messenger RNA and RNA molecule can sequester miRNA molecules acting as "competing endogenous RNAs": ceRNAs has shown that targets can reciprocally manage the function of miRNAs. This interconnected regulation of miRNAs and target genes needs to be further clarified in order to understand to what extent miRNAs regulate genes.

This represents a way forward in the myriad of data, the integration of miRNA and target genes to identify "network" deregulation is still in its infancy, and there are few examples where we begin to unravel the possible role that miRNAs in tumour cells in tumorigenesis and tumour progression and metastasis.

Many are the open questions: it remains to be understood how miRNAs differentiate low from high microsatellite instability, how they are expressed in mutational subgroups like KRAS, BRAF and BRAF-like mutated patients. Among these subgroups, are miRNAs specific of a particular mutation? Each study should be ideally lead by a consortium of partners allowing miRNAs to be studied together with mutational profiles, clinical-pathological variables, mRNAs expression, verification of published subgroups and signatures to avoid cases like those reported by Roepman et al., in NSCLC where 8 previously published gene expression signatures were almost disjoint [35]. At least two large independent cohorts should be always available for discovery and validation. For example, Chen and colleagues [36] studied the role of *miR-143* as target of KRAS in CRC, the study provides the first evidences that *miR-143* is significant in suppressing CRC cell growth through inhibition of KRAS translation, but how *miR-143* able to distinguish between KRAS mutated and WT patients is? Only 13 paired samples were analyzed, what happens if we analyze a larger cohort, are there other sub-groups of patients? And if we have the opportunity to extract normal tissue from different sites, what are we going to discover [37]? Furthermore, if we consider that for FDA *omic* signatures are medical devices [38], should not we, as research community, have a register of negative results? In this framework, it is clear how the importance of consortiums, large heterogeneous team works, research networks like TCGA (http://cancergenome.nih.gov/) along with projects like p-medicine (http://www.p-medicine.eu) and VPH-NoE (http://www.vph-noe.eu/), that contribute to the integrative *omics* study, data sharing, data mining, privacy, access and ethical issues, toward research driven by interdisciplinary discussions, are going to lead the research field, not only in terms of transparency and scientific results but also in term of better prospective treatment, health and well-being for patients as well as decision support for clinicians.

3. miRNAs as candidate biomarkers for targeted therapies?

The ability to detect miRNAs in body fluids, such as serum and plasma, along with their role in cancer progression raised the question if they could be used as convenient diagnostic biomarkers and for monitoring therapy response. Wu and colleagues found that a mutation downstream of *let-7e* resulted in a significant reduction of its expression in vivo, suggesting that screening for genetic variations in miRNA genes in human cancers has potential for identification of molecular diagnostic and therapeutic targets [39]. They screened sequence variations in ~300 miRNAs from ~150 patients (mainly from human colon and prostate cancer patients) and in 20 human tumor cell lines. Interestingly, they found that a germ line mutation located downstream of the *pre-let-7e* miRNA led to a significant reduction of its expression. This underpins the potential use of circulating miRNAs as a biomarker for cancer detection although we would think that somatic mutations detected in blood might be indicating presence of a cancer, but population polymorphisms (germ line mutations) maybe indicate a heritable risk but not yet presence of cancer. Along these lines, *miR-144** has been found as a potential diagnostic biomarker for CRC in the feces [40].

There are cases in which the analysis's focus is on only one miRNA, especially when it is observed as a specific candidate that characterizes a sub-group of CRC patients, examples are the roles of *let-7a* and *miR-143* that were found to depend on KRAS mutation [41–43].

Anti-EGFR target therapy has been extensively used in all mCRC patients until unresponsiveness of KRAS mutated patients to this therapy was discovered [44].

Since then, retrospective studies on patients KRAS-mutated treated with anti-EGFR have been very useful in understanding potential mechanisms that allow identifying KRAS-mutated patients that can still benefit from the anti-EGFR therapy. There is a first indication that among patients with mCRC (refractory to irinotecan) those with tumors KRAS mutated with a T > G base change in the let-7a KRAS mRNA binding site (rs61764370) might have higher chances of survival (overall and progression free) when *let-7a* is highly expressed [42].

Table 2
Commonly used miRNA target prediction software (in chronological order per category).

Category	Program	Main feature(s)	Website
Seed-based	miRanda	Identifies maximum local complementarity between the 3′ untranslated region and the miRNA, which is further filtered using the binding energy of the duplex and evolutionary conservation in multiple genomes	http://www.microrna.org/microrna/home.do
	PicTar	Identifies targets on the basis of sequence complementarity between single or multiple miRNAs and the 3′ untranslated region	http://pictar.mdc-berlin.de/
	TargetScan	Incorporates the "offset 6mer" miRNA binding site, probability of conserved targeting, and multiple miRNA "context scores" reflecting various features of miRNA–mRNA binding	http://www.targetscan.org/
	DIANA-microT 3.0	Identifies conserved and non-conserved miRNA recognition elements, providing confidence scores for each prediction that correlate with protein expression levels	http://diana.cslab.ece.ntua.gr/microT/
Pattern-based	Rna22	Identifies putative target islands on the basis of patterns that are conserved within the miRNA sequences of the same or different organisms, then examines whether the miRNA query sequence can form heteroduplexes with the identified target island that satisfy the user's parameter settings	http://cbcsrv.watson.ibm.com/rna22.html http://cm.jefferson.edu/rna22v1.0/
Machine learning	miTarget2	Support vector machine classifier, trained on multiple microarray datasets, that can identify miRNA–mRNA-degraded targets	http://mirdb.org
	NBmiRTar	Uses both "seed" and "out-seed" segments of the miRNA–mRNA duplex and can be used to further filter output files from miRanda	http://wotan.wistar.upenn.edu/NBmiRTar/login.php
	TargetMiner	Uses an experimentally validated (by protein and mRNA levels) negative training dataset to train the support vector machine classifier and identify unique miRNA–mRNA duplex features to use for target prediction	http://www.isical.ac.in/~bioinfo_miu/targetminer20.htm
Targets secondary structures	PITA	Focuses on target-site accessibility by calculating the difference between energy gained from the formation of the miRNA–mRNA duplex and energy required to unfold the miRNA binding site on the mRNA target	http://genie.weizmann.ac.il/pubs/mir07/

Furthermore, KRAS mutation is an indicator of only part of the unresponsive patients to EGFR-targeted therapy (approximately ~35–45%), and miRNAs became good candidates in the characterization of other patients that are KRAS wild type but still unresponsive to the anti-EGFR therapy [45].

miR-143 low expression has been shown to be predictive of poor prognosis (using cancer-specific survival as end-point) among patients that are KRAS wild type but unresponsive to EGFR targeted therapy, suggesting that *miR-143* might be a prognostic biomarker in this subgroup [43].

In other cases, patterns (modules) of miRNAs distinguish among classes of samples, like in a recent paper, where samples of non-neoplastic mucosa, low- and high-grade dysplasia in adenoma and invasive adenocarcinoma of the colon (FFPE) have been hybridized in microarrays to highlight modules of miRNAs that systematically change their regulation during tumor development and progression [46].

When studying the miRNAs functional involvement in cancer is also important to distinguish between mature sequences of the same step loop miRNA. For example, a study of the regulation of the expression of the miRNAs *miR-28-3p* and *miR-28-5p* has shown that they are diverse not only in the mRNAs they target but also in the regulation of their expression. *miR-28-5p* and *miR-28-3p* are down regulated in CRC whereas *miR-28-5p* altered the expression of CCND1 and HOXB3 and *miR-28-3p* bound NM23-H1. Overexpression of *miR-28-5p* reduced CRC cell proliferation, migration and invasion in vitro, whereas *miR-28-3p* increased CRC cell migration and invasion in vitro [47].

In unraveling these complex functional relations, robust methodology for measuring miRNAs and normalization of qRT-PCR data are essential [48].

3.1. Pros and cons

3.1.1. Diagnostic, prognostic and predictive applications

miRNAs potentially constitute effective diagnostic markers for cancer. Individual expression levels of particular miRNAs might be associated with risk of cancer relapse. In this regard, the stability of particular miRNAs in FFPE tissues, the need of little tissue (allowing assessment in small biopsies) and their presence in body fluids is a potential advantage [15]. However, standardized methods for predicting and sequencing miRNAs and miRNA targets remain to be developed. The biomedical and bioinformatics research community is working to fill in these gaps in miRNA research that remain so that the findings can be translated into clinical practice.

If miRNAs are intended to be used as biomarkers, therapeutic targets or therapeutic agents, consistency of results and methodological considerations will become increasingly important. The significance of these findings and potential roles as molecular classifiers or clinical biomarkers will require very well-supported validation in larger cohort studies.

3.1.2. Therapeutic applications

Given the role that certain miRNAs might have in driving disease, intervention on their expression may represent a rationale for new treatment modalities. Proof of principle has been provided in vitro for *miR-30b\c*, *miR-221* and *miR-222* which are involved in the modulation of gefitinib-induced apoptosis in Non-Small Cell Lung Cancer [49].

miRNAs need only partial sequence match to a target mRNA to repress gene expression. However, they do share the same gene-silencing machinery to silence target gene expression. Therapeutic approaches based on miRNA and siRNA have intrinsic similarities and differences. Therefore, the potential clinical benefits of modulating miRNAs can be explored from parallel studies of siRNA in cancer therapies, but with caution due to the intrinsic differences [50]. While the risk of toxicity might be reduced when we will have a better understanding the feedback mechanisms in the miRNA cellular processing, potential toxicity from off-target effects and immune activation during treatment (with miRNAs) appear to be relevant.

Therefore, when designing miRNA approaches, we need to use the most potent miRNA candidate at lowest concentration possible to interfere with tumor growth.

The fact that one miRNA usually regulates multiple genes adds a unique layer of complexity to miRNA therapy, which might render it difficult to control in practive. The use of tumor-specific delivery agents, such as tumor-specific nanoparticles or viral vectors, as has been demonstrated to work for siRNA in humans, may obviate at

least concerns regarding specific delivery. In patients with solid cancers, siRNA has been intravenously administered using targeted nanoparticles. Post treatment analysis showed that siRNA treatment remains effective after several cycles of administration which provides the first example of dose-dependent accumulation of targeted nanoparticle in human tumors. These data demonstrate that RNAi treatment is in principle feasible in patients using systemically delivered siRNA, and that siRNA can be used as a gene-specific therapeutic approach. With a better understanding of the role of miRNAs in tumor progression and a more sophisticated design of miRNA-modulating molecules, miRNA-mediated therapy will likely start providing new therapeutic options to be tested in clinical trials. It seems reasonable to postulate for the not so distant future that the analysis of cancer genome sequences and the use of *omics* based biomarkers will become important tools in the conduct of clinical trials and ultimately find their ways into daily clinical practice.

4. Conclusions

miRNAs have several properties that could make them effective diagnostic markers for cancer, for example, measuring a patient's expression levels of specific miRNAs could help a clinician decide whether the patient is at risk for developing cancer or whether the patient's tumor has metastasized. The stability of miRNAs in FFPE tissues and body fluids is advantageous for biomarker discovery and validation; furthermore miRNAs can be extracted from small biopsy specimens. In addition, miRNAs are potential therapeutic agents for personalized cancer management. The research community is working toward switching from potentiality to reality.

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References

[1] Mattick, J.S. and Makunin, I.V. (2006) Non-coding RNA. Hum. Mol. Genet. 15 (Spec No 1), R17–29.
[2] Gorini, A. and Pravettoni, G. (2011) P5 medicine: a plus for a personalized approach to oncology. Nat. Rev. Clin. Oncol. 8, 444.
[3] Rossi, S., Christ-Neumann, M., Rüping, S., Buffa, F., Wegener, D., Mc Vie, G., Coveney, P., Graf, N. and Delorenzi, M. (2011) p-Medicine: from data sharing and integration via VPH models to personalized medicine. Ecancermedicalscience 5, 218.
[4] Gustavsson, B. (2012) Simultaneous surgery for primary colorectal cancer and metastatic lesions? Scand. J. Gastroenterol. 47, 269–276.
[5] Calin, G.A., Sevignani, C., Dumitru, C.D., Hyslop, T., Noch, E., Yendamuri, S., Shimizu, M., Rattan, S., Bullrich, F., Negrini, M. and Croce, C.M. (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc. Natl. Acad. Sci. U.S.A. 101, 2999–3004.
[6] Poliseno, L., Salmena, L., Zhang, J., Carver, B., Haveman, W.J. and Pandolfi, P.P. (2010) A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature 465, 1033–1038.
[7] Krek, A., Grün, D., Poy, M.N., Wolf, R., Rosenberg, L., Epstein, E.J., MacMenamin, P., da Piedade, I., Gunsalus, K.C., Stoffel, M. and Rajewsky, N. (2005) Combinatorial microRNA target predictions. Nat. Genet. 37, 495–500.
[8] Miranda, K.C., Huynh, T., Tay, Y., Ang, Y.-S., Tam, W.-L., Thomson, A.M., Lim, B. and Rigoutsos, I. (2006) A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. Cell 126, 1203–1217.
[9] Mattes, J., Yang, M. and Foster, P.S. (2007) Regulation of microRNA by antagomirs: a new class of pharmacological antagonists for the specific regulation of gene function? Am. J. Respir. Cell Mol. Biol. 36, 8–12.
[10] Tay, Y., Zhang, J., Thomson, A.M., Lim, B. and Rigoutsos, I. (2008) MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 455, 1124–1128.
[11] Nicoloso, M.S., Spizzo, R., Shimizu, M., Rossi, S. and Calin, G.A. (2009) MicroRNAs – the micro steering wheel of tumour metastases. Nat. Rev. Cancer 9, 293–302.
[12] Lujambio, A. and Lowe, S.W. (2012) The microcosmos of cancer. Nature 482, 347–355.
[13] Bosman, F.T., Yan, P., Tejpar, S., Fiocca, R., Van Cutsem, E., Kennedy, R.D., Dietrich, D. and Roth, A. (2009) Tissue biomarker development in a multicentre trial context: a feasibility study on the PETACC3 stage II and III colon cancer adjuvant treatment trial. Clin. Cancer Res. 15, 5528–5533.
[14] Popovici, V., Budinska, E., Tejpar, S., Weinrich, S., Estrella, H., Hodgson, G., Van Cutsem, E., Xie, T., Bosman, F.T., Roth, A.D. and Delorenzi, M. (2012) Identification of a poor-prognosis BRAF-mutant-like population of patients with colon cancer. J. Clin. Oncol. 30, 1288–1295.
[15] Xie, Y., Xiao, G., Coombes, K.R., Behrens, C., Solis, L.M., Raso, G., Girard, L., Erickson, H.S., Roth, J., Heymach, J.V., Moran, C., Danenberg, K., Minna, J.D. and Wistuba, I.I. (2011) Robust gene expression signature from formalin-fixed paraffin-embedded samples predicts prognosis of non-small-cell lung cancer patients. Clin. Cancer Res. 17, 5705–5714.
[16] Weng, L., Wu, X., Gao, H., Mu, B., Li, X., Wang, J.-H., Guo, C., Jin, J.M., Chen, Z., Covarrubias, M., Yuan, Y.-C., Weiss, L.M. and Wu, H. (2010) MicroRNA profiling of clear cell renal cell carcinoma by whole-genome small RNA deep sequencing of paired frozen and formalin-fixed, paraffin-embedded tissue specimens. J. Pathol. 222, 41–51.
[17] Reis, P.P., Waldron, L., Goswami, R.S., Xu, W., Xuan, Y., Perez-Ordonez, B., Gullane, P., Irish, J., Jurisica, I. and Kamel-Reid, S. (2011) mRNA transcript quantification in archival samples using multiplexed, color-coded probes. BMC Biotechnol. 11, 46.
[18] Ribeiro-Silva, A., Zhang, H. and Jeffrey, S.S. (2007) RNA extraction from ten year old formalin-fixed paraffin-embedded breast cancer samples: a comparison of column purification and magnetic bead-based technologies. BMC Mol. Biol. 8, 118.
[19] Bell, D., Berchuck, A., Birrer, M., Chien, J., Cramer, D.W., Dao, F., Dhir, R., DiSaia, P., Gabra, H., Glenn, P., Godwin, A.K., Gross, J., Hartmann, L., Huang, M., Huntsman, D.G., Iacocca, M., Imielinski, M., Kalloger, S., Karlan, B.Y., Levine, D.A., Mills, G.B., Morrison, C., Mutch, D., Olvera, N., Orsulic, S., Park, K., Petrelli, N., Rabeno, B., Rader, J.S., Sikic, B.I., Smith-McCune, K., Sood, A.K., Bowtell, D., Penny, R., Testa, J.R., Chang, K., Dinh, H.H., Drummond, J.A., Fowler, G., Gunaratne, P., Hawes, A.C., Kovar, C.L., Lewis, L.R., Morgan, M.B., Newsham, I.F., Santibanez, J., Reid, J.G., Trevino, L.R., Wu Y-Q., Wang, M., Muzny, D.M., Wheeler, D.A., Gibbs, R.A., Getz, G., Lawrence, M.S., Cibulskis, K., Sivachenko, A.Y., Sougnez, C., Voet, D., Wilkinson, J., Bloom, T., Ardlie, K., Fennell, T., Baldwin, J., Gabriel, S., Lander, E.S., Ding, L., Fulton, R.S., Koboldt, D.C., McLellan, M.D., Wylie, T., Walker, J., O'Laughlin, M., Dooling, D.J., Fulton, L., Abbott, R., Dees, N.D., Zhang, Q., Kandoth, C., Wendl, M., Schierding, W., Shen, D., Harris, C.C., Schmidt, H., Kalicki, J., Delehaunty, K.D., Fronick, C.C., Demeter, R., Cook, L., Wallis, J.W., Lin, L., Magrini, V.J., Hodges, J.S., Eldred, J.M., Smith, S.M., Pohl, C.S., Vandin, F., Raphael, B.J., Weinstock, G.M., Mardis, E.R., Wilson, R.K., Meyerson, M., Winckler, W., Getz, G., Verhaak, R.G.W., Carter, S.L., Mermel, C.H., Saksena, G., Nguyen, H., Onofrio, R.C., Lawrence, M.S., Hubbard, D., Gupta, S., Crenshaw, A., Ramos, A.H., Ardlie, K., Chin, L., Protopopov, A., Zhang, J., Kim, T.M., Perna, I., Xiao, Y., Zhang, H., Ren, G., Sathiamoorthy, N., Park, R.W., Lee, E., Park, P.J., Kucherlapati, R., Absher, D.M., Waite, L., Sherlock, G., Brooks, J.D., Li, J.Z., Xu, J., Myers, R.M., Laird, P.W., Cope, L., Herman, J.G., Shen, H., Weisenberger, D.J., Noushmehr, H., Pan, F., Triche Jr, T., Berman, B.P., Van Den Berg, D.J., Buckley, J., Baylin, S.B., Spellman, P.T., Purdom, E., Neuvial, P., Bengtsson, H., Jakkula, L.R., Durinck, S., Han, J., Dorton, S., Marr, H., Choi, Y.G., Wang, V., Wang, N.J., Ngai, J., Conboy, J.G., Parvin, B., 13 Feiler, H.S., Speed, T.P., Gray, J.W., Levine, D.A., Socci, N.D., Liang, Y., Taylor, B.S., Schultz, N., Borsu, L., Lash, A.E., Brennan, C., Viale, A., Sander, C., Ladanyi, M., Hoadley, K.A., Meng, S., Du, Y., Shi, Y., Li, L., Turman, Y.J., Zang, D., Helms, E.B., Balu, S., Zhou, X., Wu, J., Topal, M.D., Hayes, D.N., Perou, C.M., Getz, G., Voet, D., Saksena, G., Zhang, J., Zhang, H., Wu, C.J., Shukla, S., Cibulskis, K., Lawrence, M.S., Sivachenko, A., Jing, R., Park, R.W., Liu, Y., Park, P.J., Noble, M., Chin, L., Carter, H., Kim, D., Karchin, R., Spellman, P.T., Purdom, E., Neuvial, P., Bengtsson, H., Durinck, S., Han, J., Korkola, J.E., Heiser, L.M., Cho, R.J., Hu, Z., Parvin, B., Speed, T.P., Gray, J.W., Schultz, N., Cerami, E., Taylor, B.S., Olshen, A., Reva, B., Antipin, Y., Shen, R., Mankoo, P., Sheridan, R., Ciriello, G., Chang, W.K., Bernanke, J.A., Borsu, L., Levine, D.A., Ladanyi, M., Sander, C., Haussler, D., Benz, C.C., Stuart, J.M., Benz, S.C., Sanborn, J.Z., Vaske, C.J., Zhu, J., Szeto, C., Scott, G.K., Yau, C., Hoadley, K.A., Du, Y., Balu, S., Hayes, D.N., Perou, C.M., Wilkerson, M.D., Zhang, N., Akbani, R., Baggerly, K.A., Yung, W.K., Mills, G.B., Weinstein, J.N., Penny, R., Shelton, T., Grimm, D., Hatfield, M., Morris, S., Yena, P., Rhodes, P., Sherman, M., Paulauskis, J., Millis, S., Kahn, A., Greene, J.M., Sfeir, R., Jensen, M.A., Chen, J., Whitmore, J., Alonso, S., Jordan, J., Chu, A., Zhang, J., Barker, A., Compton, C., Eley, G., Ferguson, M., Fielding, P., Gerhard, D.S., Myles, R., Schaefer, C., Mills Shaw, K.R., Vaught, J., Vockley, J.B., Good, P.J., Guyer, M.S., Ozenberger, B., Peterson, J. and Thomson, E. (2011) Integrated genomic analyses of ovarian carcinoma. Nature 474, 609–615.
[20] Hur, K., Toiyama, Y., Takahashi, M., Balaguer, F., Nagasaka, T., Koike, J., Hemmi, H., Koi, M., Boland, C.R. and Goel, A. (2012) MicroRNA-200c modulates epithelial-to-mesenchymal transition (EMT) in human colorectal cancer metastasis. Gut.
[21] Melo, S.A. and Esteller, M. (2011) Dysregulation of microRNAs in cancer: playing with fire. FEBS Lett. 585, 2087–2099.

[22] Luo, X., Burwinkel, B., Tao, S. and Brenner, H. (2011) MicroRNA signatures: novel biomarker for colorectal cancer? Cancer Epidemiol. Biomarkers Prev. 20, 1272–1286.
[23] Slaby, O., Svoboda, M., Fabian, P., Smerdova, T., Knoflickova, D., Bednarikova, M., Nenutil, R. and Vyzula, R. (2007) Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. Oncology 72, 397–402.
[24] Gao, J.-S., Zhang, Y., Tang, X., Tucker, L.D., Tarwater, P.M., Quesenberry, P.J., Rigoutsos, I. and Ramratnam, B. (2011) The Evi1, microRNA-143, K-Ras axis in colon cancer. FEBS Lett. 585, 693–699.
[25] Schetter, A.J., Leung, S.Y., Sohn, J.J., Zanetti, K.A., Bowman, E.D., Yanaihara, N., Yuen, S.T., Chan, T.L., Kwong, D.L.W., Au, G.K.H., Liu, C.-G., Calin, G.A., Croce, C.M. and Harris, C.C. (2008) MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA 299, 425–436.
[26] Schetter, A.J., Okayama, H. and Harris, C.C. (2012) The role of microRNAs in colorectal cancer. Cancer J. 18, 244–252.
[27] Ng, E.K.O., Chong, W.W.S., Jin, H., Lam, E.K.Y., Shin, V.Y., Yu, J., Poon, T.C.W., Ng, S.S.M. and Sung, J.J.Y. (2009) Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut 58, 1375–1381.
[28] Wang, L.-G. and Gu, J. (2012) Serum microRNA-29a is a promising novel marker for early detection of colorectal liver metastasis. Cancer Epidemiol. 36, e61–67.
[29] Ma, Y., Zhang, P., Wang, F., Zhang, H., Yang, J., Peng, J., Liu, W. and Qin, H. (2011) miR-150 as a potential biomarker associated with prognosis and therapeutic outcome in colorectal cancer. Gut.
[30] Vasilescu, C., Rossi, S., Shimizu, M., Tudor, S., Veronese, A., Ferracin, M., Nicoloso, M.S., Barbarotto, E., Popa, M., Stanciulea, O., Fernandez, M.H., Tulbure, D., Bueso-Ramos, C.E., Negrini, M. and Calin, G.A. (2009) MicroRNA fingerprints identify miR-150 as a plasma prognostic marker in patients with sepsis. PLoS ONE 4P, e7405.
[31] Earle, J.S.L., Luthra, R., Romans, A., Abraham, R., Ensor, J., Yao, H. and Hamilton, S.R. (2010) Association of microRNA expression with microsatellite instability status in colorectal adenocarcinoma. J. Mol. Diagn. 12, 433–440.
[32] Lanza, G., Ferracin, M., Gafà, R., Veronese, A., Spizzo, R., Pichiorri, F., Liu, C., Calin, G.A., Croce, C.M. and Negrini, M. (2007) mRNA/microRNA gene expression profile in microsatellite unstable colorectal cancer. Mol. Cancer 6, 54.
[33] Nagel, R., le Sage, C., Diosdado, B., van der Waal, M., Oude Vrielink, J.A.F., Bolijn, A., Meijer, G.A. and Agami, R. (2008) Regulation of the adenomatous polyposis coli gene by the miR-135 family in colorectal cancer. Cancer Res. 68, 5795–5802.
[34] Volinia, S., Galasso, M., Costinean, S., Tagliavini, L., Gamberoni, G., Drusco, A., Marchesini, J., Mascellani, N., Sana, M.E., Abu Jarour, R., Desponts, C., Teitell, M., Baffa, R., Aqeilan, R., Iorio, M.V., Taccioli, C., Garzon, R., Di Leva, G., Fabbri, M., Catozzi, M., Previati, M., Ambs, S., Palumbo, T., Garofalo, M., Veronese, A., Bottoni, A., Gasparini, P., Harris, C.C., Visone, R., Pekarsky, Y., de la Chapelle, A., Bloomston, M., Dillhoff, M., Rassenti, L.Z., Kipps, T.J., Huebner, K., Pichiorri, F., Lenze, D., Cairo, S., Buendia, M.-A., Pineau, P., Dejean, A., Zanesi, N., Rossi, S., Calin, G.A., Liu, C.-G., Palatini, J., Negrini, M., Vecchione, A., Rosenberg, A. and Croce, C.M. (2010) Reprogramming of miRNA networks in cancer and leukemia. Genome Res. 20, 589–599.
[35] Roepman, P., Jassem, J., Smit, E.F., Muley, T., Niklinski, J., van de Velde, T., Witteveen, A.T., Rzyman, W., Floore, A., Burgers, S., Giaccone, G., Meister, M., Dienemann, H., Skrzypski, M., Kozlowski, M., Mooi, W.J. and van Zandwijk, N. (2009) An immune response enriched 72-gene prognostic profile for early-stage non-small-cell lung cancer. Clin. Cancer Res. 15, 284–290.
[36] Chen, X., Guo, X., Zhang, H., Xiang, Y., Chen, J., Yin, Y., Cai, X., Wang, K., Wang, G., Ba, Y., Zhu, L., Wang, J., Yang, R., Zhang, Y., Ren, Z., Zen, K., Zhang, J. and Zhang, C.-Y. (2009) Role of miR-143 targeting KRAS in colorectal tumorigenesis. Oncogene 28, 1385–1392.
[37] Yap, T.A., Gerlinger, M., Futreal, P.A., Pusztai, L. and Swanton, C. (2012) Intratumor heterogeneity: seeing the wood for the trees. Sci. Transl. Med. 4, 127ps10.
[38] Baggerly, K.A. and Coombes, K.R. (2011) What information should be required to support clinical ''Omics'' publications? Clin. Chem..
[39] Wu, M., Jolicoeur, N., Li, Z., Zhang, L., Fortin, Y., L'Abbe, D., Yu, Z. and Shen, S.-H. (2008) Genetic variations of microRNAs in human cancer and their effects on the expression of miRNAs. Carcinogenesis 29, 1710–1716.
[40] Kalimutho, M., Del Vecchio Blanco, G., Di Cecilia, S., Sileri, P., Cretella, M., Pallone, F., Federici, G. and Bernardini, S. (2011) Differential expression of miR-144* as a novel fecal-based diagnostic marker for colorectal cancer. J. Gastroenterol. 46, 1391–1402.
[41] Graziano, F., Canestrari, E., Loupakis, F., Ruzzo, A., Galluccio, N., Santini, D., Rocchi, M., Vincenzi, B., Salvatore, L., Cremolini, C., Spoto, C., Catalano, V., D'Emidio, S., Giordani, P., Tonini, G., Falcone, A. and Magnani, M. (2010) Genetic modulation of the Let-7 microRNA binding to KRAS 3'-untranslated region and survival of metastatic colorectal cancer patients treated with salvage cetuximab-irinotecan. Pharmacogenomics J. 10, 458–464.
[42] Ruzzo, A., Graziano, F., Vincenzi, B., Canestrari, E., Perrone, G., Galluccio, N., Catalano, V., Loupakis, F., Rabitti, C., Santini, D., Tonini, G., Fiorentini, G., Rossi, D., Falcone, A. and Magnani, M. (2012) High let-7a microRNA levels in KRAS-mutated colorectal carcinomas may rescue anti-EGFR therapy effects in patients with chemotherapy-refractory metastatic disease. Oncologist 17, 823–829.
[43] Pichler, M., Winter, E., Stotz, M., Eberhard, K., Samonigg, H., Lax, S. and Hoefler, G. (2012) Down-regulation of KRAS-interacting miRNA-143 predicts poor prognosis but not response to EGFR-targeted agents in colorectal cancer. Br. J. Cancer 106, 1826–1832.
[44] Allegra, C.J., Jessup, J.M., Somerfield, M.R., Hamilton, S.R., Hammond, E.H., Hayes, D.F., McAllister, P.K., Morton, R.F. and Schilsky, R.L. (2009) American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J. Clin. Oncol. 27, 2091–2096.
[45] Bardelli, A. and Siena, S. (2010) Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J. Clin. Oncol. 28, 1254–1261.
[46] Bartley, A.N., Yao, H., Barkoh, B.A., Ivan, C., Mishra, B.M., Rashid, A., Calin, G.A., Luthra, R. and Hamilton, S.R. (2011) Complex patterns of altered MicroRNA expression during the adenoma-adenocarcinoma sequence for microsatellite-stable colorectal cancer. Clin. Cancer Res. 17, 7283–7293.
[47] Almeida, M.I., Nicoloso, M.S., Zeng, L., Ivan, C., Spizzo, R., Gafà, R., Xiao, L., Zhang, X., Vannini, I., Fanini, F., Fabbri, M., Lanza, G., Reis, R.M., Zweidler-McKay, P.A. and Calin, G.A. (2012) Strand-specific miR-28-5p and miR-28-3p have distinct effects in colorectal cancer cells. Gastroenterology 142, 886–896.
[48] D'haene, B., Mestdagh, P., Hellemans, J. and Vandesompele, J. (2012) miRNA expression profiling: from reference genes to global mean normalization. Methods Mol. Biol. 822, 261–272.
[49] Garofalo, M., Romano, G., Di Leva, G., Nuovo, G., Jeon, Y.-J., Ngankeu, A., Sun, J., Lovat, F., Alder, H., Condorelli, G., Engelman, J.A., Ono, M., Rho, J.K., Cascione, L., Volinia, S., Nephew, K.P. and Croce, C.M. (2011) EGFR and MET receptor tyrosine kinase-altered microRNA expression induces tumorigenesis and gefitinib resistance in lung cancers. Nat. Med. 18, 74–82.
[50] Davis, M.E., Zuckerman, J.E., Choi, C.H.J., Seligson, D., Tolcher, A., Alabi, C.A., Yen, Y., Heidel, J.D. and Ribas, A. (2010) Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature 464, 1067–1070.
[51] Akao, Y., Nakagawa, Y. and Naoe, T. (2006) let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. Biol. Pharm. Bull. 29, 903–906.
[52] Lujambio, A., Ropero, S., Ballestar, E., Fraga, M.F., Cerrato, C., Setién, F., Casado, S., Suarez-Gauthier, A., Sanchez-Cespedes, M., Git, A., Gitt, A., Spiteri, I., Das, P.P., Caldas, C., Miska, E. and Esteller, M. (2007) Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res. 67, 1424–1429.
[53] Guo, C., Sah, J.F., Beard, L., Willson, J.K.V., Markowitz, S.D. and Guda, K. (2008) 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 Chromosomes Cancer 47, 939–946.
[54] Saito, Y., Liang, G., Egger, G., Friedman, J.M., Chuang, J.C., Coetzee, G.A. and Jones, P.A. (2006) Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell 9, 435–443.
[55] Song, B., Wang, Y., Xi, Y., Kudo, K., Bruheim, S., Botchkina, G.I., Gavin, E., Wan, Y., Formentini, A., Kornmann, M., Fodstad, O. and Ju, J. (2009) Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells. Oncogene 28, 4065–4074.
[56] Hu, M., Xia, M., Chen, X., Lin, Z., Xu, Y., Ma, Y. and Su, L. (2010) MicroRNA-141 regulates Smad interacting protein 1 (SIP1) and inhibits migration and invasion of colorectal cancer cells. Dig. Dis. Sci. 55, 2365–2372.
[57] Akao, Y., Nakagawa, Y. and Naoe, T. (2006) MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. Oncol. Rep. 16, 845–850.
[58] Ng, E.K.O., Tsang, W.P., Ng, S.S.M., Jin, H.C., Yu, J., Li, J.J., Röcken, C., Ebert, M.P.A., Kwok, T.T. and Sung, J.J.Y. (2009) MicroRNA-143 targets DNA methyltransferases 3A in colorectal cancer. Br. J. Cancer 101, 699–706.
[59] La Rocca, G., Badin, M., Shi, B., Xu, S.-Q., Deangelis, T., Sepp-Lorenzinoi, L. and Baserga, R. (2009) Mechanism of growth inhibition by MicroRNA 145: the role of the IGF-I receptor signaling pathway. J. Cell. Physiol. 220, 485–491.
[60] Shi, B., Sepp-Lorenzino, L., Prisco, M., Linsley, P., deAngelis, T. and Baserga, R. (2007) Micro RNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells. J. Biol. Chem. 282, 32582–32590.
[61] Schepeler, T., Reinert, J.T., Ostenfeld, M.S., Christensen, L.L., Silahtaroglu, A.N., Dyrskjøt, L., Wiuf, C., Sørensen, F.J., Kruhøffer, M., Laurberg, S., Kauppinen, S., Ørntoft, T.F. and Andersen, C.L. (2008) Diagnostic and prognostic microRNAs in stage II colon cancer. Cancer Res. 68, 6416–6424.
[62] Dews, M., Homayouni, A., Yu, D., Murphy, D., Sevignani, C., Wentzel, E., Furth, E.E., Lee, W.M., Enders, G.H., Mendell, J.T. and Thomas-Tikhonenko, A. (2006) Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat. Genet. 38, 1060–1065.
[63] Tsang, W.P. and Kwok, T.T. (2009) The miR-18a* microRNA functions as a potential tumor suppressor by targeting on K-Ras. Carcinogenesis 30, 953–959.
[64] Braun, C.J., Zhang, X., Savelyeva, I., Wolff, S., Moll, U.M., Schepeler, T., Ørntoft, T.F., Andersen, C.L. and Dobbelstein, M. (2008) p53-Responsive micrornas 192 and 215 are capable of inducing cell cycle arrest. Cancer Res. 68, 10094–10104.

[65] Schimanski, C.C., Frerichs, K., Rahman, F., Berger, M., Lang, H., Galle, P.R., Moehler, M. and Gockel, I. (2009) High miR-196a levels promote the oncogenic phenotype of colorectal cancer cells. World J. Gastroenterol. 15, 2089–2096.
[66] Wang, P., Zou, F., Zhang, X., Li, H., Dulak, A., Tomko Jr., R.J., Lazo, J.S., Wang, Z., Zhang, L. and Yu, J. (2009) microRNA-21 negatively regulates Cdc25A and cell cycle progression in colon cancer cells. Cancer Res. 69, 8157–8165.
[67] Asangani, I.A., Rasheed, S.A.K., Nikolova, D.A., Leupold, J.H., Colburn, N.H., Post, S. and Allgayer, H. (2008) MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 27, 2128–2136.
[68] Burk, U., Schubert, J., Wellner, U., Schmalhofer, O., Vincan, E., Spaderna, S. and Brabletz, T. (2008) A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep. 9, 582–589.
[69] Grady, W.M., Parkin, R.K., Mitchell, P.S., Lee, J.H., Kim, Y.-H., Tsuchiya, K.D., Washington, M.K., Paraskeva, C., Willson, J.K.V., Kaz, A.M., Kroh, E.M., Allen, A., Fritz, M., Markowitz, S.D. and Tewari, M. (2008) Epigenetic silencing of the intronic microRNA hsa-miR-342 and its host gene EVL in colorectal cancer. Oncogene 27, 3880–3888.
[70] Tazawa, H., Tsuchiya, N., Izumiya, M. and Nakagama, H. (2007) Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. Proc. Natl. Acad. Sci. U.S.A. 104, 15472–15477.
[71] Toyota, M., Suzuki, H., Sasaki, Y., Maruyama, R., Imai, K., Shinomura, Y. and Tokino, T. (2008) Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. Cancer Res. 68, 4123–4132.
[72] Bandres, E., Bitarte, N., Arias, F., Agorreta, J., Fortes, P., Agirre, X., Zarate, R., Diaz-Gonzalez, J.A., Ramirez, N., Sola, J.J., Jimenez, P., Rodriguez, J. and Garcia-Foncillas, J. (2009) microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. Clin. Cancer Res. 15, 2281–2290.