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The role of microRNAs in the resistance to colorectal cancer treatments

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Abstract

Colorectal cancer (CRC) is one of the leading cancer-related causes of death in the world. Several approaches such as surgery, chemotherapy, radiotherapy, targeted therapy, or combinations thereof have been used to treat CRC patients. However, the fact that many patients develop a drug resistance during the course of the treatment is a major obstacle. Understanding the mechanisms underlying resistance is critical in order to develop more effective targeted treatments. Recently, several studies have reported on the regulatory role of microRNAs (miRNAs) in the response to anti-cancer drugs and suggested them as a source of predictive biomarkers for the purpose of patient stratification and for the prognosis of treatment success. For example, overexpressing miR-34a, a master regulator of tumor suppression attenuates chemoresistance to 5-FU by downregulating silent information regulator 1 (SIRT1) and E2F3. MRX34, a miR-34a replacement is the first synthetic miRNA mimic to enter clinical testing. MiR-34a antagonizes cancer stemness, metastasis, and chemoresistance processes that are necessary for cancer viability. This example shows that miRNAs are coming into focus for the design of enhanced cancer therapies that aim to sensitise tumor cells for anti-cancer drugs. In this review, we provide an overview on the role of miRNAs in the resistance to current colorectal cancer therapies. Furthermore, we discuss the value of miRNAs as biomarkers for predicting chemosensitivity and their potential to enhance treatment strategies.

Key words: Colorectal cancer, MicroRNA, Chemotherapy, Radiotherapy, Biomarker.

Introduction

Colorectal carcinoma is a frequent cause of cancer-related death mainly due to metastases that are resistant to conventional therapies (1). Five-fluorouracil (5-FU), a cytostatic drug, is the most frequently used for the treatment of metastatic CRC (mCRC). Recently, more effective chemotherapeutic agents such as oxaliplatin and the monoclonal antibodies cetuximab and panitumumab have been applied in clinical practice (2). Radiotherapy is another approach which is used to treat CRC patients. However, either acquired or inherent resistance to therapy is one of the main challenges in the treatment of CRC patients (3). Since patients show diverse responses to cancer treatments, the identification of biomarkers that predict the sensitivity or resistance toward designated chemotherapeutic agents is essential. In this way, the patients will receive a treatment that is suitable for their individual case and toxic side effects of unsuccessful treatments would be avoided. The identification of chemoresistance during or even before treatment would facilitate the design of more customized treatment regimens. Results of many studies show that the response to therapy is being controlled by particular genes, but it remains to be clarified how the resistance of cells to drug treatment is facilitated. Recent studies highlighted the role of miRNAs in controlling those genes which are involved in the cellular response to anti-cancer therapies, e.g. drivers of apoptosis, proliferation and drug efflux mechanisms (4). MiRNAs are endogenous small non-coding RNAs which are involved in biological pathways and negatively regulating gene expression at the post-transcriptional level (5). MiRNA-mediated gene regulation is also important in the context of the emergence and progression of human diseases (6). Causal effects induced by miRNAs in the context of a number of different human pathologies have been validated in e.g. autoimmune diseases, cardio-vascular diseases, neurodegenerative diseases, metabolic diseases and cancer. In cancer, miRNAs can act as tumor suppressors, oncogenes (a.k.a. oncomir) as well as mediators of metastasis (a.k.a. metastamir) (7-9). Discovering miRNA signatures in the response to cancer treatments could pave the path for the development of novel therapeutic strategies to overcome drug resistance. Thus, understanding biological processes and signaling pathways regulated by miRNAs in mechanisms of resistance to cancer therapies is essential when it comes to the development of strategies for improving treatment outcome. In this review, we focus on miRNAs involved in the response to CRC treatments (Figure 1), with special emphasis on those with potential predictive or prognostic function.

MiRNAs serve as markers for patient stratification

The discovery of suitable biomarkers for monitoring treatment success will lead to a reduction in health care cost which is of growing interest in the biomedical research and for clinical economics (10). Evidence demonstrates that miRNAs have cancer-specific expression patterns and the potential to be used as prognostic and predictive markers in the clinic (11). Circulating

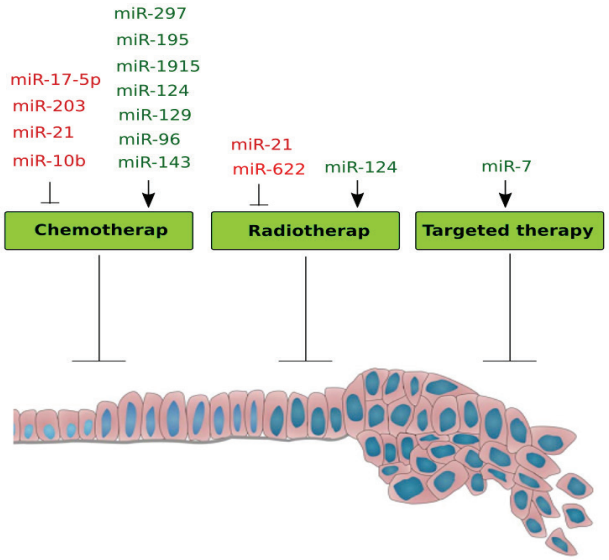

Figure 1. Role of miRNAs in CRC treatments. MiRNAs in Red are involved in causing resistance to treatments. MiRNAs which sensitize the response to treatments are in green.

miRNAs in particular can be easily and non-invasively collected and their high stability in serum and plasma make them ideal for diagnostic, prognostic or predictive screening. Prognostic biomarkers provide information on course and outcome of the disease regarding survival and recurrence regardless of the treatment applied, while predictive biomarkers provide information on the possibility of response to a certain treatment and help to direct cancer therapy towards a personalized treatment and to determine its chance of success (12). Recently, several miRNAs have been described as promising candidates to predict chemotherapy outcomes in CRC (Table 1).

MiRNAs associated with the response to chemotherapeutic agents

Chemotherapy is an important therapeutic strategy for cancer treatment. However, for a sizable proportion of the patients chemotherapy fails to eliminate all tumor cells because of drug resistance, which is the most common cause of tumor recurrence (13). Evasion of apoptosis, a hallmark of cancer is a main reason of resistance to chemotherapeutic agents (14) (Figure 2).

Five-FU, the most frequently applied component in chemotherapy is an anti-cancer drug that induces apoptosis through modulating Bcl-2 family proteins. However, resistance to 5-FU represents a major challenge to successful treatment outcome (15).

Increasing evidence demonstrates that some miRNAs can influence the therapeutic effect of drug-induced genotoxic stress in tumor cells. For example, Borralho et al. showed that enhanced expression of miR-143 in HCT116 human colorectal cancer cells results in increased cell death after exposure to 5-FU (18). They found that miR-143 increases the sensitivity of colon cancer cells to 5-FU, probably via expression suppression of the extracellular-regulated protein kinase 5 (ERK5), nuclear factor-κB (NF-κB) and B-cell lymphoma 2 (BCL2). ERK5, a mitogen-activated protein kinase which is involved in cell survival, differentiation and proliferation, can be activated by a wide range of mitogens and cellular stresses. NF-kB is a critical transcription factor involved in the suppression of apoptosis and associated with increased resistance to chemotherapeutic agents. Therefore, strategies like overexpression of miR-143 aimed at reducing ERK5 and NF-kB signalling may increase sensitivity to chemotherapeutic agents (16).

MiR-10b is a novel prognostic marker and a potential indicator of chemoresistance to the common 5-FU-based chemotherapy. *In vitro* studies demonstrated that overexpression of miR-10b induces chemoresistance by targeting pro-apoptotic BIM, a mediator of the apoptosis pathway that either activates BAX or inhibits BCL2 (17, 18). High expression levels of miR-10b are therefore associated with enhanced malignant potential and poor prognosis (19).

In a recent publication, it was shown that overexpression of the tumor suppressor miR-96 increases apoptosis and sensitivity to 5-FU in human CRC cells. MiR-96 indirectly targets the anti-apoptotic regulators X-linked inhibitor of apoptosis (XIAP) and UBE2N (20). UBE2N, also known as UBC13, is an E2 ubiquitin-conjugating enzyme which regulates the localization and stability of p53. Thus, inhibition of the genes causing enhanced apoptosis signaling can rescue sensitivity to 5-FU. This study demonstrated that transfection of

Table1. Predictive miRNAs which are involved in the response to conventional colorectal cancer treatments.

Therapy	miRNA	Up/Down	MiRNA target	Role of response	Reference
Chemotherapy	miR-143	Down	ERK5, NF-kß, BCL2	Resistance to 5-FU	(16)
	miR-10b	Up	BIM	Resistance to 5-FU	(19)
	miR-96	Up	XIAP, UBE2N	Sensitivity to 5-FU	(20)
	miR-129	Up	BCL2	Sensitivity to 5-FU	(21)
	miR-34a	Down	SIRT1, E2F3	Resistance to 5-FU	(22)
	miR-21	Up	hMSH2, TP, DPD	Resistance to 5-FU and radiation	(28)
	miR-203	Up	ATM	Resistance to Oxaliplatin	(29)
	miR-143	Down	IGF-IR	Resistance to Oxaliplatin	(30)
	miR-17-5p	Up	PTEN	Resistance to chemotherapy	(3)
	miR-195	Down	BCL2L2	Resistance to Doxorubicin	(50)
Radiotherapy	miR-124	Down	PRRX1	Resistance to radiation	(40)
	miR-622	Up	RB1	Resistance to radiation	(41)
	miR-100	Down	-	Resistance to radiation	(42)
Targeted therapy	miR-7	Down	EGFR, RAF-1	Resistance to Cetuximab	(46)

The genes which are indirectly regulated by miRNAs are in red.

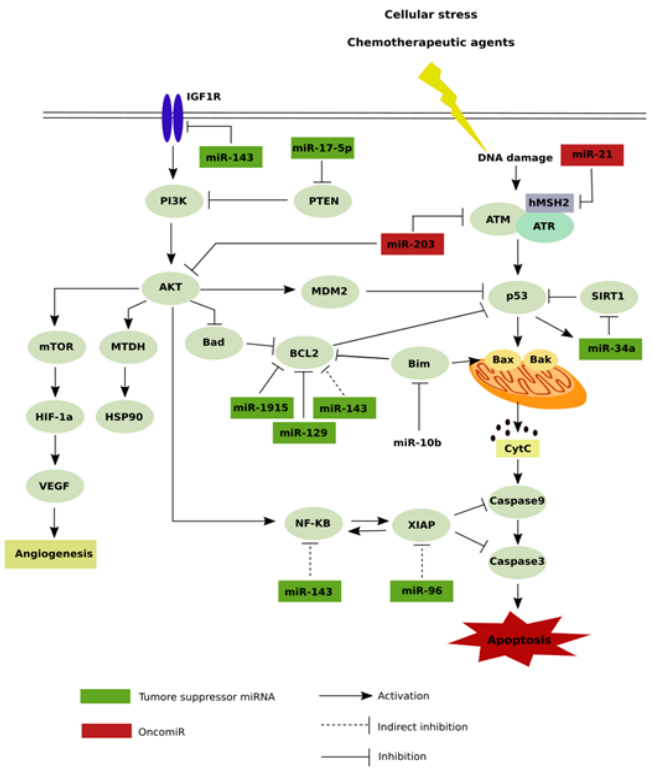

Figure 2. The role of miRNAs in the regulation of the apoptosis pathway in response to chemotherapeutic agents for the treatment of colorectal cancer. Tumor suppressor miRNAs (green) mediate apoptosis by inhibiting anti-apoptotic proteins (BCL2, IGF1R, NFKB, PTEN, SIRT1, XIAP), while oncomiRs (red) induce chemoresistance in cancer cells by inhibiting pro-apoptotic proteins (ATM, hMSH2).

miR-96 mimic into CRC cells results in the downregulation of XIAP and UBE2N, thereby promoting apoptosis (20).

It has been speculated that miR-96 may control apoptosis and proliferation by modulating FOXO3 levels whereas it downregulates XIAP and UBE2N under cytotoxic insult conditions.

According to (21) ectopic expression of miR-129 promotes the intrinsic apoptosis pathway, inhibits cell proliferation and causes cell-cycle arrest in CRC cells by suppressing a key anti-apoptotic protein, BCL2. It has been reported that miR-129 sensitize CRC cells to the cytotoxic effect of 5-FU treatment both *in vitro* and *in vivo* in mice tumor xenografts. Restoration of miR-129 expression could be a future therapeutic strategy to modulate and enhance chemosensitivity to 5-FU treatment (21).

Another study showed downregulation of miR-34a in human colorectal cancer 5-FU-resistant DLD-1 cells compared with those in the parental DLD-1 cells (22). In this study chemoresistance to 5-FU could be attenuated by overexpressing miR-34a, which in turn downregulated the expression of silent information regulator 1 (SIRT1) and E2F3. E2F3 is a critical component of the apoptotic process which is required for DNA damage-induced apoptosis both *in vitro* and *in vivo* (23). MiR-34a, controls apoptosis in CRC by forming a positive feedback loop with p53. MiR-34a inhibits the SIRT1 which is an inhibitor of p53 and p53 increases the miR-34a transcription which results in enhanced activation of p53 (24).

Several studies have shown that miR-21 is significantly elevated in colorectal cancer. Valeri et al. (2010) showed that this enhanced expression of miR-21 significantly inhibits apoptosis, increases cell proliferation and invasion and enhances the resistance of tumor cells to 5-FU and X radiation in HT-29 colon cancer cells. Knockdown of miR-21 could reverse these effects. Additionally they showed that miR-21 applies its function by targeting the human mutS homolog2 (hMSH2), and indirectly regulating the expression of thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase (DPD) (25). TP and DPD which converts 5′-deoxy-5-fluorouridine (5′DFUR) to 5-FU and 5-FU to dihydrofluorouracil (DHFU) respectively, are involved in mechanism of 5-FU action (for more details see (26)). hMSH2 is a member of the mismatch repair (MMR) system which recognizes and repairs DNA mismatches. Additionally, there is evidence which shows their function in DNA damage signaling and consequent apoptosis. For example, Pabla et al. (2011) reported the role of hMSH2 in cisplatin-induced genotoxic stress and apoptosis by regulating ATR/Chk2/p53 signaling. They demonstrated hMSH2 as a critical factor which binds ataxia-telangiectasia and Rad3-related (ATR) and recruits it to the DNA damage site and thereby activates p53 and the apoptotic pathway in response to DNA-damaging chemotherapy drugs (27). Based on these findings, miR-21 can be a potentially useful marker for the prediction of the clinical response to 5-FU therapy, and may be a potential target for colorectal cancer therapy (28).

MiRNAs in regulating the response to other therapeutic regimens, such as oxaliplatin and paclitaxel, have also been detected. For example, Zhou et al. (2014) showed that miR-203 overexpression is a reason for acquiring resistance to oxaliplatin by targeting ataxia telangiectasia mutated (ATM), a primary mediator of the DNA damage response. They also indicated that knockdown of miR-203 can sensitize chemoresistant CRC cells to oxaliplatin. Therefore their results suggested miR-203 as a potentially predictive biomarker for therapy in regimens utilizing oxaliplatin (29).

In contrast to miR-203, miR-143 increased chemosensitivity to oxaliplatin treatment by negatively regulating IGF-IR, a known oncogene. They demonstrated that overexpression of miR-143 inhibits cell proliferation, migration, tumor growth and angiogenesis and sensitizes oxaliplatin treatment through the PI3K/AKT/HIF-1/VEGF pathway (30). In addition, another study introduced a novel function for IGF-1R in p53-dependent apoptosis through translational modulation of the p53-MDM2 feedback loop (31). Taken together, these findings suggest that miR-143 may be a useful biomarker for CRCs and provide new information for using miR-143/IGF-IR-based therapeutic strategies for CRC treatments in the future (30).

Using Kaplan-Meier analysis, Fang et al. (2014) showed that CRC patients with higher levels of miR-17-5p had a reduced chance for survival, especially in patients who had previously received chemotherapy. They found chemotherapy leads to overexpression of miR-17-5p, which further repressed PTEN levels, contributing to the development of chemoresistance. MiR-17-5p is a predictive factor for chemotherapy response and a prognostic factor for overall survival in CRC, which is due to its regulation of PTEN expression (3).

Some other studies highlight the role of miRNAs in cancer therapeutic responses in patients with mutant p53. For example, Zhang et al. (2015) demonstrated that the p53/miR-520g/p21 signaling axis has a critical role in the response of CRC patients to chemotherapy. MiR-520g as a mediator of drug resistance in CRC, acts through downregulating p21. They showed while knockdown of p21 expression by two different shRNAs could enhance the resistance of HCT116 and RKO cells to 5-FU-induced apoptosis, its restoration almost completely restored the sensitivity of cells to 5-FU-induced apoptosis. Their results confirmed that p21 is essential for 5-FU-induced apoptosis and proposed that inhibition of miR-520g or restoration of p21 expression may have considerable therapeutic potential to overcome drug resistance in colorectal cancer patients, especially in those with mutant p53 which suppresses miR-520g (32).

Li and coworkers suggested in two separate studies that overexpression of miR-203 and miR-22 can reverse paclitaxel-induced chemoresistance in p53-mutated colon cancer cells by increasing cellular apoptosis and reducing cell proliferation and survival (34). They demonstrated that the tumor suppressive role of these two miRNAs was mediated by negatively regulating AKT protein expression which subsequently resulted in downregulation of Metadherin (MTDH), a downstream molecule of activated AKT. MTDH increased chemoresistance through regulating many downstream molecules, such as chaperone HSP90 which is directly involved in chemoresistance. They found that miR-22 in p53-mutated colon cancer cells negatively regulates AKT phosphorylation at Ser473 by activation of PTEN signaling (34). Furthermore, they reported that overexpression of miR-203 or miR-22 decreased anti-apoptotic Bcl-xL expression and increased pro-apoptotic BAX and active caspase-3 levels. The chemosensitive role of miR-203 and miR-22 suggests them as novel sensitizers for the chemotherapy in colon cancer (34, 35).

Some studies investigated the possible role of miRNAs in the development of multidrug resistance (MDR) in colorectal carcinoma cells. Their findings may guide further research towards predicting MDR in patients and designing personalized therapies for CRC patients. Xu et al. (2012) measured a lower abundance of miR-297 in HCT116/L-OHP cells compared to its parental cells. MiR-297 mimics could sensitize HCT116/L-OHP and HCT-8/VCR MDR cells to some anti-cancer drugs *in vitro* and *in vivo* by directly inhibiting multidrug resistance-associated protein 2 (MRP-2) (36). MRP2, a member of ATP-binding cassette (ABC) transporter family is involved in cancer MDR by catalyzing an ATP-dependent active transport of chemically unrelated compounds, including anti-cancer drugs outside the cell (37).

Using miRNA microarray, Xu et al. (2013) found that miR-1915 is the most strongly downregulated of all miRNAs in HCT116/L-OHP cells compared to their parental cells (HCT116). In their study they validated BCL2 as a target of a miR-1915. Furthermore, by transfection of miR-1915 mimic they could not only reduce the expression of BCL2 but also sensitize HCT116/L-OHP MDR cells to some anti-cancer drugs (38). Interestingly, another study by Nakazawa et al. (2014) showed that p53 negatively regulates BCL2 expression by inducing miR-1915 processing from primary into precursor miRNA (39).

MiRNAs associated with radiotherapy

Yin *et al*. identified significant changes in the expression of at least 855 genes in mouse brain 4 h after low-dose ionising irradiation. As miRNAs are able to target many genes simultaneously, this result suggests a rapid response of miRNAs and the subsequent regulation of a large number of target genes.

Recent findings introduce miRNAs as important players in the response to cellular stress caused by radiotherapy. For example, Zhang et al. (2014) found that miR-124 can sensitize human colorectal cancer cells to ionising irradiation *in vitro* and *in vivo* by directly inhibiting Paired related homeobox 1 (PRRX1) which is an EMT inducer and stemness regulator. Furthermore, PRRX1 knockdown could increase CRC radiosensitivity similar to the effects caused by miR-124. This indicates that miR-124 is an attractive prognostic/predictive biomarker, which may also be useful as therapeutic agent for CRC patients (40).

In another study overexpression of miR-622 could induce the radioresistance *in vitro* by directly targeting RB1-3′ UTR, while Rb overexpression through the Rb-E2F1-P/CAF complex could activate pro-apoptotic genes and reverse miR-622-induced radioresistance. MiR-622 is a potential biomarker for non-responders to radiotherapy and a potential therapeutic target (41).

Another regulator of CRC radiosensitivity is miR-100. It has been demonstrated that upregulation of miR-100 significantly increased X-ray-induced apoptosis of CCL-244 cells and regulated the expression of apoptosis-related proteins. An important mechanism of radiation-induced cell damage is the induction of DNA double-strand breaks (DSBs) (42). From the work of Yang et al. (2015) miR-100 modulates the sensitization of CCL-244 cells to irradiation by augmenting irradiation-induced DSBs.

MiRNAs associated with targeted therapies

Recently, targeted molecular treatments using antibodies, such as anti-vascular endothelial growth factor (VEGF) antibody and anti-epidermal growth factor receptor (EGFR) antibody, have also been employed for CRC treatment. EGFR participates in signaling pathways which are commonly deregulated in cancer cells including CRC cells. Cetuximab is a monoclonal antibody directed against EGFR and commonly used to block mitogenic factors that promote cancer growth. For this treatment wild-type KRAS status (wt-KRAS) is a necessary condition (43). Mutations of KRAS, BRAF, PI3K, PTEN and amplification of HER2 have been identified as negative predictors of response to anti-EGFR therapies (44). However, 10-20% of patients, negative for all these genetic lesions, do not properly respond to anti-EGFR therapy and the mechanism of resistance in these patients remains to be uncovered. Great effort has been made to identify new predictive biomarkers to anti-EGFR therapy, and it seems that miRNAs have this capability and could enable a personalized approach (45). Suto et al. (46) reported miR-7 as a tumor sup-

pressor in CRC that targets EGFR and RAF-1 and consequently, regulates the EGFR signaling pathway. Using multivariate analysis they indicated low expression of miR-7 as an independent prognostic factor for poor survival. Furthermore, these data indicated that miR-7 precursor, alone or in combination with cetuximab, may be useful in therapy against CRC. Their results suggest miR-7 as a promising candidate for targeted therapy in CRC patients whose tumors are resistant to EGFR-directed antibodies (46). Additionally, enhanced expression of miRNA-200b, another KRAS targeting miRNA could improve progression free survival (PFS) in KRAS mutated tumors. According to (44), it seems that not only the genetic status of KRAS but also the level of KRAS post-transcriptional regulators is another important factor for Cetuximab response in CRC that should come into consideration. The dysregulation of KRAS targeting miRNAs may explain why a few patients with KRAS mutation can still benefit from anti-EGFR therapy.

MiRNA targets are associated with cellular responses to DNA damaging stimuli

To get further insight into the biological functions of dysregulated miRNAs and their targets in CRC, the Database for Annotation, Visualization and Integrated Discovery (DAVID, v6.726) was used to identify over-represented gene ontology (GO) terms associated with the miRNA targets. We used REVIGO to visualize the GO terms in semantic similarity-based scatterplots (47). As can be seen in Figure 3 several GO term clusters with related functions were identified. Some of them are implicated in cellular responses to DNA damaging stimuli and the programmed cell death.

Therapeutic approaches

One of the most attractive features of miRNAs as therapeutic agents, and probably the most important advantage over the approaches targeting single genes, is their capability to target multiple molecules, commonly in the context of a network, making them tremendously efficient in regulating distinct biological processes relevant to normal and malignant cell biology. Another important application of miRNAs is their ability to target genes related to drug sensitivity and thereby result in the altered sensitivity of cancer cells to anti-cancer drugs. Therefore, manipulating miRNAs can be considered as

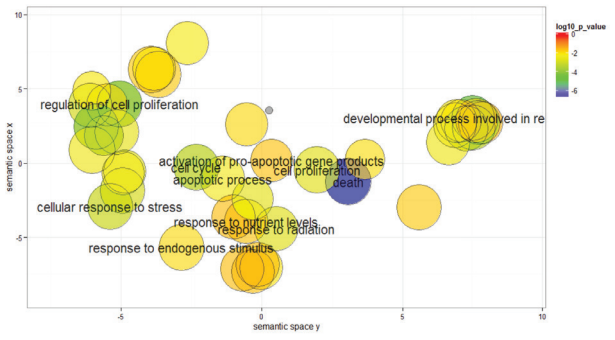

Figure 3. Genes targeted by dysregulated miRNAs were subjected to GO enrichment analysis, and then visualized as a scatterplot using the REVIGO.

new strategy to increase the effectiveness of therapeutic purposes.

Depending on miRNA function, miRNA therapeutic approaches can be divided into two different categories: (1) miRNA inhibition therapy using synthetic antisense oligonucleotides, miRNA sponges or miRNA-masks to inhibit oncogenic miRNAs that acquire a gain of function in human disease and (2) miRNA replacement therapy using miRNA mimics to restore miRNAs that show a loss of function. The aim of these approaches is to regulate miRNA expression in cancers, including inactivation of oncogenic miRNAs, activation of tumor suppressor miRNAs, and targeting specific miRNAs to restore drug sensitivity. Targeting miRNAs is a promising strategy for treatment optimization aiming to enhance inhibition of cancer cell proliferation and/or to increase the sensitivity of cancer cells to anti-cancer drugs.

Future Perspective and Conclusion

Resistance to anti-cancer drugs remains a major challenge in the clinic and is a main reason for recurrence in CRC patients. Choosing the right treatment for a patient is the biggest concern in the clinic, otherwise improper treatment will lead to cancer progression and irrecoverable damage. MiRNAs with their ability to predict the response to conventional anti-cancer therapies and minimize exposure of non-responders to the side effects of ineffective therapies are exciting molecular entities. They may change current therapeutic approaches and make them more directed, while more and more studies show the direct involvement of miRNAs in the response to cancer treatments, such findings provide a strong reason for the development of miRNA-based therapeutic strategies aiming to overcome cancer cell drug resistance and re-sensitize cancer cells to the effects of chemotherapy, radiation and targeted therapies. One possible application for miRNAs is to serve as predictive biomarkers to direct therapies that are available on the market today and hence improve treatment response and outcomes.

They can also be used in drug development for new, direct therapies that are currently in making progress on the research front, particularly as miRNA mimics or antagomirs (48).

There is a particular trend towards the utilization of miRNAs as the next generation of biomarkers for chemoresistance, which will help guiding patient-specific treatments for optimal results and minimum toxicity. The aim is to identify miRNAs as therapic targets in a personalized medicine context and identify patients that could take benefit from this therapy. Recent advances in the development of miRNA-based anti-cancer therapies yielded interesting results. However, delivering synthetic miRNAs to the right tissue in a targeted manner challenges the use of miRNAs as therapeutic tool. Upon understanding more deeply the mechanism by which miRNAs act in anti-cancer drug resistance and by developing more effective delivery technologies, these small RNAs may fulfill their promise as valuable therapeutics in prevailing anti-cancer drug resistance (49).

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Other articles in this theme issue include references (51-62).

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