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MicroRNAs are important regulators of drug resistance in colorectal cancer

Yang Zhang¹ and Jing Wang²

¹Department of Cancer Biology, University of Pennsylvania, Philadelphia, PA, 19104, USA

²Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE, 68198, USA

Abstract

Despite of continuous development of cancer treatment over the past decades, drug resistance is still one of the major hurdles of effective therapy for advanced colorectal cancer (CRC) worldwide and the understanding of its underlying mechanisms remains limited. Emerged data suggests that many microRNAs (miRNAs) may contribute to drug resistance in CRC. Major findings on miRNA functions in drug resistance of CRC are systemically reviewed here, with the goal of providing new updates to broaden our comprehension of its mechanisms and evidence to utilize miRNAs as potential therapeutic targets for CRC treatment.

Keywords

colorectal cancer; miRNAs; chemotherapy; drug resistance; chemoresistance; apoptosis

Colorectal cancer

Colorectal cancer (CRC) is the third most common cancer diagnosed and the second leading cause of cancer mortality in the United States. CRC develops as a benign adenomatous polyp initially, which gradually progresses to an advanced adenoma, an invasive carcinoma and eventually distant metastases. CRC is staged into four categories (stage I – IV) according to the tumor-node-metastasis (TNM) guidelines.

CRC and many other cancers are often the results of molecular alterations that lead to enhanced function of oncogenes or loss of function of tumor-suppressor genes. The development of majority of CRCs follows a classical pathway as described by Kinzler and Vogelstein. Disruption of the APC pathway may be sufficient to start a small adenomatous growth (Kinzler and Vogelstein, 1996). Mutation of a RAS gene often occurs among the next genetic events of progression.

Evidence also points to one or more of the genes, DCC, SMAD4, and SMAD2 in 18q21, as playing a tumor suppressor role in carcinogenesis. In addition, loss of functional p53 drives progression to carcinomas (Vogelstein and Kinzler, 2002).

In addition to the classical pathway, inherited mutations to mismatch repair (MMR) cause hereditary nonpolyposis colorectal cancer (HNPCC). This pathway accounts for only about 2–4% of all CRC cases. Defects in MMR usually leads to the observed fluctuations in microsatellite length named microsatellite instability (MSI). HNPCC follows the same morphological stages as tumors developed from the classical pathway, but with different mutations and rates of progression. HNPCC tumors have less loss of heterozygosity (LOH), similar K-RAS mutation frequency, fewer p53 mutations, and more mutations in various growth-related genes with repetitive sequences, including TGF β -RII, IGF-II, and BAX. The HNPCC pathway also lacks chromosomal instability and instead uses malfunction in DNA repair to raise the mutation rate (Jass et al., 2002a).

It is also proposed that some CRCs are caused by accumulated changes in gene expression of multiple pathways through hypermethylation of promoter regions, accounting for up to 40% of all CRC. Commonly hypermethylated genes in CRC include p14, p16, hMLH1, MGMPT, and HPP1 (Jass et al., 2002b). However, this model was based on limited sample sizes.

Common treatment for CRC includes surgery, chemotherapy, radiation therapy, immunotherapy, vaccine therapy and combination therapy, *etc.* Most patients (70%–80%) newly diagnosed with CRC have localized tumors that are generally treated with curative surgical resection. Other therapies are often combined with surgery to treat more advanced tumors of CRC patients. Adjuvant chemo- and/or radiation therapy is a standard clinical treatment for patients with stage III CRC after resection. The most invasive and metastatic CRC still remain incurable.

Chemotherapy for Colorectal cancer

Chemotherapy, the use of chemicals to treat a disease, was first defined by the famous German chemist Paul Ehrlich in the early 1900s. Chemotherapy for CRC can be dated back to 1957, when Charles Heidelberger and colleagues correctly hypothesized that a fluorouracil (5-FU) analogue would block tumor cell division by inhibiting the conversion of deoxyuridine monophosphate (dUMP) to thymidylate (Heidelberger et al., 1957). Studies later demonstrated that the main mechanism of 5-FU inhibiting thymidylate synthase is the formation of 5-fluorodeoxyuridine monophosphate (FdUMP), a potent inhibitor of thymidylate synthase, from 5-FU via complex metabolic pathways (Whirl - Carrillo et al., 2012). Since then, antimetabolite 5-FU has been used in clinical oncology and continually served as an important agent in the treatment of CRC and many other cancers. In the early 1980s, physicians started to combine 5-FU with leucovorin to treat CRC patients, especially those with metastatic tumors, as preclinical data suggested that leucovorin enhanced cytotoxicity of 5-FU in cancer cells (Poon et al., 1989).

Major developments in chemotherapy accrued in the early 2000s with the introduction of the topoisomerase I inhibitor irinotecan, and the platinum containing agent Oxaliplatin (Oxal) as components for the treatment of metastatic CRC patients (Gustavsson et al., 2014). Like other platinum compounds, the cytotoxicity of Oxal is thought to result from inhibition of DNA synthesis in cells (Graham et al., 2004). Clinical trials of combination treatment of FOLFOX (5-FU/leucovorin with Oxal) (Hong et al., 2014; Qin et al., 2013) and FOLFIRI (infusional 5-FU/leucovorin and irinotecan) (Colucci et al., 2005) showed higher response rate, overall survival and reduced side effects for patients with metastatic CRC.

Though the idea of targeted cancer therapy by blocking angiogenesis was proposed in 1971 (Folkman et al., 1971), it was not until 2004 that the humanized monoclonal antibody, bevacizumab, which inhibits the action of vascular endothelial growth factor, was evaluated in the pivotal Avastin/Fluorouracil 2007 phase III trial (Hurwitz et al., 2004). Addition of bevacizumab to the combination chemotherapy further promotes response rate and overall survival of the patients (Giantonio et al., 2007). In 1983 and 1984, John Mendelsohn and colleagues recommended directly targeting EGFR for cancer therapy, as EGFR is frequently upregulated in many epithelial cancers (Gill et al., 1984; Kawamoto et al., 1983). However, the therapeutic strategy of adding anti-EGFR monoclonal antibodies cetuximab or panitumumab to FOLFIRI had only marginal improvement of the outcomes in metastatic CRC patients with wild type KRAS, and no improvement in those with mutated KRAS, compared with FOLFIRI alone (Heinemann et al., 2014; Van Cutsem et al., 2011). With the concept of combining cetuximab with FOLFIRI being continually tested in clinical trials (Janne et al., 2014), dramatic improvement in the outcomes of chemotherapy for CRC patients, especially those with advanced disease, is not expected in the near future as resistance to these anticancer agents can be intrinsic or developed over the course of treatment and our understanding of drug resistance remains limited.

Drug resistance

Chemotherapy for CRC has been continuously developed since 5-FU was introduced as an anticancer agent. Despite of the progress, resistance to chemotherapy is still one of the biggest obstacles against effective therapy for CRC patients. It is estimated that the failure of treatment in over 90% of patients with metastatic cancer is due to drug resistance (Hammond et al., 2016). Elucidating the mechanisms of drug resistance and developing more effective therapeutic agents or procedures are major challenges of cancer research.

Many mechanisms have been proposed to be responsible for drug resistance (Fig-1.). Multidrug resistance can be the result of limitation of accumulation of drugs within cells by reducing uptake, enhancing efflux, or affecting membrane lipids such as ceramide (Dany et al., 2016). Blocking apoptosis (Czabotar et al., 2014; Zhang et al., 2016), drug inactivation, activation of detoxification, repairing DNA damage, and alterations in the regulation of cell cycle and checkpoints may also contribute to drug resistance (Bouwman and Jonkers, 2012; Holohan et al., 2013). In addition, cancer stem cells (CSCs) have been implicated to be resistant to chemotherapeutic agents (Holohan et al., 2013). Recent studies have demonstrated that epigenetic mechanisms or metabolic abnormality may play significant

roles in mediating drug resistance in certain types of cancer (Brown et al., 2014; He et al., 2014).

MiRNAs and cancers

MicroRNAs (miRNAs) are a class of small (22 bp ~24 bp) non-coding regulatory RNA molecules which regulate gene expression primarily by binding to the 3'-UTR of their target mRNA to inhibit their expression by sequence-specific cleavage of mRNA or inhibition of translation (Bartel, 2009). Since their discovery, miRNAs have been virtually linked to all known biological processes including cell cycle, proliferation, differentiation, metabolism, and apoptosis as well as various pathological processes including cancer (Lujambio and Lowe, 2012). miRNAs are predicted to regulate more than one third of human genes and majority of genetic pathways (Lewis et al., 2005). The regulation of expression of miRNAs involves transcription, processing by Drosha and Dicer, loading onto AGO (Argonaute) proteins and miRNA turnover (Lin and Gregory, 2015). Different machineries are applied to regulate each step, including the recruitment of transcription factors, RNA-binding proteins, protein-modifying enzymes, RNA-modifying enzymes, exoribonucleases and endoribonucleases (Ha and Kim, 2014).

A significant number of miRNAs are located at unstable genomic regions linked to cancer (Calin et al., 2004). Alterations in miRNA expression are associated with many human cancers (Lin and Gregory, 2015) and therefore miRNA profiling or signature in cancers can be potentially used to classify tumor subtypes, diagnose cancer, determine treatment plans and predict patient outcomes (Stahlhut and Slack, 2013).

MiRNAs mediate drug resistance in CRC

MiRNAs primarily execute their functions by suppressing expression of their target genes, some of which directly or indirectly modulate responses to chemotherapeutic agents in CRC treatment (figure-1). Thereby, a group of miRNAs mediating the response of CRC to chemotherapeutic agents were identified (table-1). MiRNAs whose target genes promote drug resistance would increase the sensitivity of CRC to chemotherapy whereas miRNAs whose target genes reduce drug resistance would enhance drug resistance in CRC.

Multidrug resistance (MDR)

Drug resistance or MDR in cancer cells can be attributed to many different mechanisms including reduction of drug accumulation in the cell due to enhanced efflux and reduced uptake, decreased drug cytotoxicity by detoxification or inactivation, alternations of regulation of cell cycle and checkpoints, resistance to apoptosis, enhanced DNA damage repair, epigenetic modifications *etc.* Membrane transporter proteins which involve drug efflux and uptake, often affect responses to multiple therapeutic agents. MiRNAs may alter sensitivity of CRCs to these agents by suppressing these membrane transport proteins. For example, miR-297 sensitizes CRC to multiple therapeutic agents *in vitro* and *in vivo* by down-regulating expression of MRP-2, which is an important MDR protein in platinum drug-resistant cells (Ke et al., 2012). MiR-451 represses the expression of ATP-binding

cassette drug transporter ABCB1, a member of a major class of energy-dependent transporters mediating MDR, and results in irinotecan sensitization. Thereby it is indicated as a novel candidate to circumvent recurrence and drug resistance in CRC and could be used as a marker to predict response to irinotecan in patients with CRC (Bitarte et al., 2011). Similarly, miR-519c suppresses resistance to 5-FU and irinotecan in CRC by targeting another MDR transporter ABCG2 and mRNA binding protein HuR (To et al., 2015). Other mechanisms including decreased drug cytotoxicity by detoxification or inactivation, alternations of regulation of cell cycle and checkpoints, resistance to apoptosis, enhanced DNA damage repair may also modify responses of CRCs to multiple therapeutic agents. Multiple studies by different groups identified miR-143, which sensitizes CRCs to 5-FU, oxaliplatin and cetuximab through repression of different target genes including ERK5, NF κ B, BCL2 and IGF1-R (Borrallho et al., 2009; Qian et al., 2013). In addition, miR-22 increases the sensitivity of CRCs to 5-FU by targeting gene B-cell translocation gene 1 (BTG1) and enhances the anti-cancer effect of paclitaxel in p53-mutated cells by activating PTEN mediated by unknown target of the miRNA (Li et al., 2011b; Zhang et al., 2015a). Furthermore, miR-203 sensitizes p53-mutated CRCs to paclitaxel by directly repressing AKT2 (Li et al., 2011a), but it confers CRCs resistance to oxaliplatin by targeting ATM (Zhou et al., 2014). These studies indicate that an individual miRNA may mediate the sensitivities of CRCs to different agents through different mechanisms. In additions, miR-222 suppresses MDR through modulating the expression of ADAM-17, a protein leading to growth factor shedding and growth factor receptor activation in CRC (Xu et al., 2012a). Furthermore, functional studies revealed that miR-153 upregulation increases CRC resistance to oxaliplatin and cisplatin both *in vitro* and *in vivo*, which was mediated directly by inhibiting expression of the Forkhead transcription factor Forkhead box O3a (FOXO3a) by the miRNA (Zhang et al., 2013b). Finally, miR-1915 mediates MDR in colorectal carcinoma cells in part by modulation of apoptosis via targeting BCL2 (Xu et al., 2013).

Apoptosis

Resistance to apoptosis is a hallmark of cancer (Hanahan and Weinberg, 2011). Cancer cells are not only resistant to apoptosis triggered by internal signaling molecules but also cytotoxic anti-cancer agents, which often leads to the failure of chemotherapy. Many miRNAs have been shown to regulate drug resistance by targeting signaling pathways of pro-apoptosis or anti-apoptosis. As discussed above, miR-153 upregulation increases CRC resistance to oxaliplatin and cisplatin both *in vitro* and *in vivo* by directly inhibiting expression of FOXO3a, a trigger for apoptosis through upregulation of pro-apoptotic genes such as *Bim* and *PUMA* (Zhang et al., 2013a) or downregulation of anti-apoptotic proteins such as FLIP (Zhang et al., 2013b). MiR-129, miR-139 and miR-1915 all can induce apoptosis by suppressing expression of a same key anti-apoptotic protein BCL2, thereby promoting apoptosis and enhancing chemosensitivity to 5-FU in CRC (Karaayvaz et al., 2013; Li et al., 2016; Xu et al., 2013). It is interesting to know if the three miRNAs have synergistic impact on repression of BCL2, which may justify a new strategy to more efficiently block expression of an individual gene by delivering multiple miRNAs. In addition, miR-143 enhances sensitivity of CRC to 5-FU by suppressing proliferation and survival signaling mediated by ERK5 and NF κ B, as well as anti-apoptotic signaling

mediated by BCL2 (Borrallho et al., 2009). Moreover, miR-497 and miR-143 increase sensitivity to apoptosis induced by various stimuli including chemotherapeutic drugs, cisplatin and 5-FU, by repressing IGF1-R expression, an important signaling molecule in CRC development, progression and resistance to treatment (Guo et al., 2013; Qian et al., 2013). Autophagy generally represses the induction of apoptosis, and apoptosis-associated caspase activation blocks the autophagic process. Autophagy or autophagy-relevant proteins may induce apoptosis or necrosis in special cases, and autophagy has been shown to degrade the cytoplasm, leading to 'autophagic cell death' (Mariño et al., 2014). MiR-22 represses expression of its target gene BTG1 thereby increasing the sensitivity of CRC cells to 5-FU treatment both *in vitro* and *in vivo* by inhibiting autophagy and promoting apoptosis (Zhang et al., 2015a). Finally, miR-195 sensitizes CRC cells to doxorubicin and enhances apoptosis by repression of BCL2L2 expression (Qu et al., 2015), of which the mechanism remains unknown.

The AKT/PI3K signaling pathway plays an important role in cell proliferation and anti-apoptosis in cancer cells, and alterations of its signaling affects not only tumor progression but also responses to chemotherapeutic agents. Several miRNAs modulate drug resistance by directly or indirectly regulating AKT/PI3K signaling in CRC. Overexpression of miR-203 significantly enhances the cytotoxicity of paclitaxel in p53-mutated colon cancer cells by negatively regulating AKT2 expression (Li et al., 2011a). Our studies have shown that miR-587 confers resistance in CRC cells to 5-FU-induced apoptosis *in vitro* and reduces the potency of 5-FU in the inhibition of tumor growth in a mouse xenograft model *in vivo*. Mechanistically, miR-587 modulates drug resistance through directly repressing the expression of PPP2R1B, a regulatory subunit of the PP2A complex, which negatively regulates AKT-mediated cell proliferation and anti-apoptosis signaling (Zhang et al., 2015c). Studies have shown that multiple miRNAs may have similar impact on drug responses of CRCs by regulating a same gene in AKT/PI3K signaling. MiR-17-5p promotes chemotherapeutic resistance and tumor metastasis of CRC by suppressing expression of another negative regulator of PI3K/AKT signaling --- PTEN (Fang et al., 2014). Conversely, by the activation of PTEN, overexpression of miR-22 enhances the anti-cancer effect of paclitaxel in p53-mutated cells through increasing apoptosis and reducing cell proliferation and survival (Li et al., 2011b). It is not clear how miR-22 mediates activation of PTEN only in muted but not wide-type p53 cells. Similarly, miR-199a-5p and miR-375 increase resistance to cetuximab in CRC partially due to their repression of a common target PHLPP1, a tumor suppressor that negatively regulates the AKT pathway (Mussnich et al., 2015). Studies have shown KRAS mutations are correlated with acquired resistance to anti-EGFR therapy in CRC (Misale et al., 2012). let-7a mediates responses to anti-EGFR therapy in KRAS-mutated CRC patients with chemotherapy-refractory metastatic colorectal carcinomas (Ruzzo et al., 2012). However, miR-143 and miR-145 increase sensitivity to cetuximab in CRC independently of KRAS status through downregulating BCL2 expression and enhancing caspase 3/7 activity, although their direct target genes have yet to be identified (Gomes et al., 2016).

DNA repair

Though the effects of DNA repair on drug resistance is not well appreciated, several miRNAs have been identified to play a role in DNA repair-mediated drug resistance. MiR-21 induces resistance to 5-FU in CRC cells by inhibiting the expression of hMSH2 and hMSH6, which belong to a core mismatch repair (MMR) recognition protein complex and play important role in DNA repair. High level of miR-21 expression significantly reduces 5-FU-induced G2/M arrest and apoptosis that is characteristic of defects in the core MMR component (Valeri et al., 2010). Furthermore, miR-203 induces oxaliplatin resistance in CRCs by negatively regulating expression of ATM Kinase, a primary mediator of the DNA damage response (Zhou et al., 2014); yet the study does not specify the status of p53 in CRCs as it has been shown that miR-203 sensitizes p53-mutated CRCs to paclitaxel (Li et al., 2011a).

Cell cycle and checkpoint

Cell cycle plays an important role in the resistance of cancer cells to a chemotherapeutic agent. It is common in combination chemotherapies, where one chemotherapeutic agent impacts the cell cycle such that the second chemotherapeutic agent given becomes less effective (Shah and Schwartz, 2001). MiRNAs modulating drug resistance in CRC by affecting cell cycle and checkpoint were also identified. MiR-140 regulates chemoresistance by inducing G1/G2 arrest mediated partially through the inhibition of HDAC4 expression in human colon cancer cells. Down regulation of HDAC4 may contribute to the induction of p21 by miR-140 in presence of wild-type p53 gene, which regulates cell proliferation and cell cycle control. (Song et al., 2009). In addition, we have identified a novel p53/miR-520g/p21 signaling axis that modulates the response of colon cancer cells to chemotherapeutic agents including 5-FU and Oxaliplatin. P53 suppresses miR-520g expression. MiR-520g confers drug resistance by inhibiting expression of a major cycle regulator p21, which is required for 5-FU-induced apoptosis (Zhang et al., 2015b). These studies indicate the level of P21 is fine tuned for normal cell cycle progression and alternations of its level may lead to cell cycle mediated resistance to anticancer agents.

Cancer stem cells

It has been proposed that CSCs maintain the malignant potential and determine the resistance to therapy (Vidal et al., 2014). Cancer drug resistance seems to be closely related to many intrinsic or acquired properties of CSCs, such as quiescence, specific morphology, increased DNA repair ability, overexpression of anti-apoptotic proteins, enhanced drug efflux and detoxification (Vinogradov and Wei, 2012). Many miRNAs which regulate properties of CSCs thereby modulating drug resistance have been identified. MiRNA microarray analysis revealed that miR-328 represses stem cell like phenotype and decreases the population of CSCs in CRC, and that ectopic expression of miR-328 reverses drug resistance and inhibits cell invasion of CSCs.

Mechanistically, miR-328 is able to directly target matrix metalloproteinase 16 (MMP16) and ATP-binding cassette subfamily G2 (ABCG2), a member of ABC transporter family

mediating MDR (Xu et al., 2012b). It is also suggested that miR-451 suppresses MDR in CRCs partially through repressing CSC phenotypes. Mechanistically, cyclooxygenase-2 (Cox-2) is identified as an indirect target of miR-451, involved in CSC growth. Macrophage migration inhibitory factor (MIF), which is directly targeted by miR-451, is also involved in the regulation of Cox-2 expression. Cox-2 mediates Wnt activation, an essential signaling molecule for CSC growth (Bitarte et al., 2011). Finally, miR-215 enhances chemoresistance of colorectal CSCs to methotrexate and tomudex by inhibiting cell proliferation and inducing G2 arrest through the suppression of denticleless protein homolog (DTL) expression. Enhanced G2-arrest and reduced cell proliferation mediated by knock-down of DTL depends on p53 and p21 induction (Song et al., 2010).

Aberrant metabolism

Abnormal metabolism is a newly appreciated hallmark of cancer cells (Hanahan and Weinberg, 2011). Emerging data suggested that abnormal metabolism may also contribute to drug resistance (Rahman and Hasan, 2015; Zhao et al., 2011). One study has shown that overexpression of miR-122 re-sensitizes 5-FU-resistant colon cancer cells to the drug through the inhibition of PKM2 expression, which contributes to repression of glucose metabolism *in vitro* and *in vivo* (He et al., 2014). In addition, miR-34a re-sensitizes CRC cells to 5-FU by directly targeting the expression of lactate dehydrogenase A (LDHA), a primary isoform of LDH enzymes expressed in cancer tissues, which mediates the conversion of pyruvate to lactate during the glycolytic process (Li et al., 2015). The metabolic abnormalities in cancer cells have been shown to confer aberrant survival capacity under stress conditions, which implies that it may contribute to drug resistance of cancer cells (Carr et al., 2016; Rahman and Hasan, 2015). It is very likely that more miRNAs which mediate drug resistance by modulating metabolism of cancer cells will be identified.

Other mechanisms

Epigenetic mechanisms including DNA methylation and histone modification have been shown to modulate drug resistance in cancers (Brown et al., 2014). However, no miRNAs have been known to affect therapeutic resistance in CRC through epigenetic modifications. These miRNAs may be identified by global screening of DNA methylation or histone modification-related drug resistance in CRC, or by search of potential miRNAs targeting the known genes that mediate DNA methylation or histone status, thereby affecting drug responses in CRCs.

MiRNAs as biomarkers, therapeutic targets or agents for drug resistance of colorectal cancer

In normal cells, multiple miRNAs converge to maintain a balance of various processes including proliferation, differentiation and cell death. MiRNA dysregulation can have profound consequences, especially because individual miRNAs regulate multiple mRNAs (Kasinski and Slack, 2011). As reviewed here, many miRNAs regulate drug resistance in CRC. Therefore, these miRNAs may be useful in predicting resistance to therapies or serve as potential targets or agents to overcome drug resistance in CRC patients. Studies of

expression of miRNAs in CRC patient specimens provide important information for patient prognosis and treatment. Table-2 summaries the aberrant expression of miRNAs in CRC patient specimens. However, due to small sample size, more studies are needed to determine whether these miRNAs can be used as prognosis biomarkers or therapeutic targets.

MiRNAs also represent promising therapeutic agents and many pharmaceutical companies have miRNA therapeutics in their developmental pipelines. Antisense antimiRs, (LNA), LNA-antimiR constructs, antagomirs, and miRNA sponges are currently major classes of inhibitory-miRNA therapeutic agents. Some have proven to be effective not only *in vitro* but also *in vivo* (Shah et al., 2016). For an example, MRX34, a liposome-formulated mimic of the tumor suppressor, miR-34, developed by Mirna Therapeutics, produced complete tumor regression in orthotopic mouse models of liver cancer with no observed immunostimulatory activity or toxicity to normal tissues. In a Phase I clinical trial with patients with advanced solid tumors (N= 99), a standard dose escalation trial of MRX34 infused IV on a biweekly or daily schedule were given. Though miR-34a has been shown to sensitize CRC cells to 5-FU by directly targeting LDHA, not many patients with CRC (3 subjects) were included in the trial. Despite of strong evidence of activity in hepatocellular carcinoma, renal cell carcinoma and melanoma (Bader, 2012), phase I trial of MRX34 has been suspended due to multiple immune-related severe adverse events observed in patients dosed with MRX34. Nevertheless, miRNAs as clinical therapeutic agents are still at early stage of development and no other miRNAs involved in drug resistance discussed above have been tested in clinical trials yet.

Closure remarks

Much effort has been made to understand the roles of miRNAs in cancer and the potential of manipulating miRNAs for cancer therapy. MiRNA-based anticancer therapies are being developed, either alone or in combination with targeted therapies, with the goal of overcoming drug resistance, improving patient response and survival rates. The advantage of using miRNA as therapeutic approaches is based on miRNA's ability to concurrently target multiple effectors of pathways involved in cell differentiation, proliferation and survival (Rahman and Hasan, 2015). Therefore, identification and characterization of new miRNAs and their downstream effectors will ultimately provide hints to utilize miRNAs as potential therapeutic targets for CRC. Furthermore, mechanisms of aberrantly expressed miRNAs in CRC are still largely unknown. Determination of those mechanisms would provide additional approaches to regulate miRNA expression as therapeutic strategies to overcome drug resistance.

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Figure 1. Drug resistance (or MDR) of cancer cells or CSCs

Drug resistance or MDR in cancer cells can be attributed to many different mechanisms such as reduction of drug accumulation in the cell due to enhanced efflux and reduced uptake, decrease of drug cytotoxicity by detoxification or inactivation, alternations of regulation of cell cycle and checkpoints, resistance to apoptosis, enhanced DNA damage repair, epigenetic modifications and aberrant metabolism etc. miRNAs mediate drug responses of CRC by targeting the pathways contributing to chemotherapeutic resistance through respective machinery.

Table 1

MiRNAs mediate drug resistance in CRC

Machenism involved	miRNAs	Identified target genes or pathway	Impact of miRNAs on drug responses on CRCs	References
Multidrug resistance	miR-297	MRP-2	sensitized to multiple therapeutic agents	Ke et al., 2012
	miR-451	ABCB1	sensitized to irinotecan and others	Bitarte et al., 2011
	miR-519c	ABCG2 and HuR	sensitized to 5-FU and irinotecan	To et al., 2015
	miR-222	ADAM-17	sensitized to multiple therapeutic agents	Xu et al., 2012a
	miR-1915	BCL2	sensitized to multiple therapeutic agents	Xu et al., 2013
	miR-153	FOXO3a	resistant to oxaliplatin and cisplatin	Zhang et al., 2013b
Apoptosis	miR-153	FOXO3a	resistant to oxaliplatin and cisplatin	Zhang et al., 2013b
	miR-129	BCL2	sensitized to 5-FU	Karaayvaz et al., 2013
	miR-139	BCL2	sensitized to 5-FU	Li et al., 2016
	miR-1915	BCL2	sensitized to L-OHP, vincristine, DOX, 5-FU, and mitomycin	Xu et al., 2013
	miR-143	ERK5, NFκB and BCL2	sensitized to 5-FU	Borralho et al., 2009
	miR-497	IGF1-R	sensitized to cisplatin and 5-FU	Guo et al., 2013
	miR-143	IGF1-R	sensitized to oxaliplatin	Qian et al., 2013
	miR-22	BTG1	sensitized to 5-FU	Zhang et al., 2015a
	miR-203	AKT2	sensitizes p53-mutated CRCs to paclitaxel	Li et al., 2011a
	miR-587	PPP2R1B	resistant to 5-FU	Zhang et al., 2015c
	miR-17-5p	PTEN	resistant to chemotherapeutic	Fang et al., 2014
	miR-22	PTEN	sensitizes p53-mutated CRCs to paclitaxel	Li et al., 2011b
	miR-199a-5p	PHLPP1	resistant to cetuximab	Mussnich et al., 2015
	miR-375	PHLPP1	resistant to cetuximab	Mussnich et al., 2015
	miR-143	BCL2 and other	sensitized to cetuximab	Gomes et al., 2016
	miR-145	BCL2 and other	sensitized to cetuximab	Gomes et al., 2016
	let-7a	kRAS	sensitize KRAS-Mutated CRCs to Anti-EGFR Therapy	Ruzzo et al., 2012
	miR-195	BCL2L2	sensitized to doxorubicin	Qu et al., 2015
DNA repair	miR-21	hMSH2 and hMSH6	resistant to 5-FU	Valeri et al., 2010
	miR-203	ATM	resistant to oxaliplatin	Zhou et al., 2014
Cell cycle & checkpoint	miR-140	HDAC4	resistant to methotrexate (MTX) and 5-FU	Song et al., 2009
	miR-520g	P21	resistant to 5-FU and oxaliplatin	Zhang et al., 2015b
Cancer stem cells	miR-328	MMP16 and ABCG2	sensitized to multiple therapeutic agents	Xu et al., 2012b
	miR-451	Cox-2 and MIF	sensitized to multiple therapeutic agents	Bitarte et al., 2011
	miR-215	DTL	resistant to methotrexate and tomudex	Song et al., 2010
Metabolism	miR-122	PKM2	sensitized to 5-FU	He et al., 2014
	miR-34a	LDHA	sensitized to 5-FU	Li et al., 2015

Table 2

The expression of miRNAs mediating drug resistance in colorectal cancer patients.

miRNA studied in human clinical CRC specimens	Information from the studies for clinic treatment
miRNA-17-5p	correlated with chemoresistance of CRC patients and reduces overall survival in CRC
miR-140	significantly reduced in colorectal cancer tissues compared to adjacent normal mucosa
miR-195	significantly lower in Clinic DOX-resistant colon cancer tissues
miR-297	significantly down-regulated in a panel of human colorectal carcinoma tissues
miR-519c	lower in CRC specimens from unresponsive patients to chemotherapy
miR-587	significantly higher in CRC specimens from unresponsive patients to chemotherapy
miR-520g	significantly higher in CRC specimens from unresponsive patients to chemotherapy