Implications of miRNAs in Colorectal Cancer Chemoresistance

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Abstract

With the exponential growth of research efforts on non-coding microRNAs (miRNAs) in the past decade, miRNAs have been demonstrated to be important in many major human diseases, including diabetes, heart disease, and cancer. Due to the broad regulatory function of miRNAs, alterations of their expression can have profound consequences on multiple critical genes and pathways. One of the major issues related to the success of treating advanced colorectal cancer is chemoresistance. In this review, we will present some of the recent advancements in miRNA research related to chemoresistance mechanisms to 5-FU based chemotherapy in colorectal cancer and cancer stem cells. We believe that this miRNA-mediated resistance mechanism will offer novel strategies to develop future anti-cancer therapies.

Introduction

Colorectal cancer is the second leading cause of death from cancer in the Western World, responsible annually for an estimated 50,000 deaths in the United States and 500,000 worldwide (1). Recent studies have found that in addition to the genetic changes traditionally associated with carcinogenesis (MLH1, MLH2, MSH6, PMS2), epigenetic alterations (e.g. promoter methylation, miRNAs) also play key roles in colorectal cancer development (2, 3) and metastasis (4, 5). Additionally, there is mounting evidence indicating that post-transcriptional and translational controls mediated by various regulatory molecules, such as RNA binding proteins and non-coding miRNAs, are critically important in the development of various diseases, including cancer (6–8).

In 1993, Ambros et al. made a landmark discovery of a small non-coding RNA, lin-4, which impacted the development of Caenorhabditis elegans (9). This RNA turned out the be the first identified miRNA, a class of non-coding, single-stranded RNAs of ~22 nucleotides that modulate protein expression by promoting RNA degradation, inhibiting mRNA translation, and in some cases affecting transcription. Nearly a decade after the discovery of lin-4, the potential roles of miRNAs in cancer were first reported (8), profoundly changing our understanding of cellular networks and opening the door for new discoveries in cancer research.

To date, over 1000 mammalian miRNAs have been identifiedby cloning and sequencing approaches (4), including several hundred of human miRNAs (10). Although miRNA-mediated mRNA degradation occurs in mammals, most miRNAs are thought to use a secondary mechanism of gene regulation mainly via imperfect base-pairing to the 3′-untranslated regions (3′-UTRs) of their target mRNAs. This leads to the repression of target gene expression post-transcriptionally, likely at the translational level (9, 11, 12). Such

regulation can induce rapid changes in protein synthesis without the need for transcriptional activation and subsequent steps in mRNA processing, in effect acting as a new layer of controlling expression of a given protein that is more precise, immediate and energy-efficient than transcriptional regulation alone (13). Furthermore, miRNA-mediated translational regulation has the advantage of being readily reversible, providing the cell with great flexibility in responding to various genotoxic stresses and rapid changes in growth conditions. It has been estimated that over 30% of the protein coding genes were regulated by miRNAs. Clearly, it is essential to know not only the levels of individual mRNAs, but also the extent to which mRNAs are being translated into their corresponding proteins and the miRNAs that regulate these processes. Thus, post-transcriptional and translational control mediated by miRNAs has become a new frontier for cancer research.

miRNA and Colorectal Cancer

It has been well documented that a number of miRNAs play important roles in colorectal cancer (14). Michael et al. first reported that miR-143 and miR-145 was reduced in colorectal specimens in 2003 (15). The first large scale miRNA expression profiling was reported in 2006 by Cummins et al (10). Furthermore, studies from our group have demonstrated that miR-192 and miR-215 directly contribute to one mechanism of chemotherapy resistance to fluoropyrimidines and antifolates in colorectal cancer (16–20). In this review, we will cover some of the recent advancements of miRNA research in colorectal cancer with a focus on chemoresistance.

5-Fluorouracil (5-FU), miRNA, chemoresistance, and colon cancer stem cells

5-fluorouracil (5-FU) was one of the rationally-designed drugs that target thymidylate sythase (TYMS, TS), an enzyme that is responsible for the de novo synthesis of thymidylate, a necessary precursor for DNA biosynthesis. Nearly half a century after its introduction, 5-FU-based chemotherapy is one of the main treatment options for advanced metastatic colorectal cancer and other solid tumors. However, despite the steady improvement in response rate with various modulation strategies such as leucovorin (LV), methotrexate (MTX), FOLFOX4 (oxaliplatin at a dose of 85 mg/m2 as a 2-hour infusion on day 1, every two weeks, plus LV/5-FU), many patients still go through 5-FU based chemotherapy without any benefit, underscoring the need for a set of reliable predictive biomarkers that can guide personalized chemotherapy. To this end, tremendous effort has been put into searching for predictive and prognostic biomarkers in colorectal cancer. Despite extensive investigations of 5-FU and its target TS, the predictive power of TS for 5-FU-based chemotherapy is still under debate (21, 22), and studies evaluating TS, thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD); a catabolic enzyme of 5-FU) as predictive biomarkers were unable to confirm the high response rates reported by several retrospective studies (23). From these inconsistent results, it is clear that the search continues for biomarkers that can be used, either alone or in combination with existing biomarkers such as TS and DPD, to predict the likely response to 5-FU-based treatment. Chemoresistance is one of the major factors for the failure of chemotherapy. One effect behind chemoresistance is that chemotherapeutic agents, which primarily affect fast-

proliferating cells, are mostly ineffective against the more slowly-proliferating and/or quiescent cancer stem cells, allowing a tumor to reconstitute itself once therapy has ceased. Because of the broad influence and reversible nature of miRNA on gene expression, we believe that miRNAs may offer new insights to this resistance mechanism, in particular with 5-FU-based colorectal cancer chemotherapy. The information we gained may help to develop new strategies to overcome chemoresistance and better predict clinical outcomes.

miRNA, p53 and colorectal cancer p53 is one of the most frequently mutated and/or deleted tumor suppressor genes in colorectal cancer and other tumor types: nearly half of all colorectal cancer patients carry p53 mutations or deletions (24). Until recently, it has been thought that the effects of p53 loss were primarily related to its function in transcriptional regulation, but it is now appreciated that post-transcriptional and translational controls are equally important. Our own research efforts began by systematically investigating which miRNAs are influenced by the p53 tumor suppressor and, by extension, p53 loss. In 2006 we first reported a regulatory relationship between p53 and a number of miRNAs (16), and discovered that nearly half of the 328 miRNA putative promoter regions, including the promoters for miR-34s, miR-192/215, and miR-26a, contain one or more p53 binding sites (16). Subsequently, several groups have demonstrated that miR-34a, which acts as a tumor-suppressor by targeting several cell-cycle genes, is regulated directly by p53 (25–27). In addition, we and two other groups provided further direct evidence that miR-192 is another miRNA that is both regulated by p53 and capable of inducing cell-cycle arrest (17, 28, 29).

Our recent efforts have focused on miRNAs that suppress the expression of thymidylate synthase (TS) and dihydrofolate reductase (DHFR), two important chemotherapeutic targets. We have found that two miRNAs we have previously identified as being regulated by p53, miR-192 and miR-215, target both TS and DHFR mRNA (17, 30). Additionally, Mishra et al reported that miR-24 also regulates DHFR expression, and demonstrated that 829C→T, a naturally occurring SNP near the miR-24 binding site in the 3′ UTR of DHFR, interferes with miR-24 function and, resulting in DHFR overexpression and methotrexate resistance in colorectal tumors (31). Further studies revealed that miR-24 is a potential tumor suppressor capable of reducing tumor cell proliferation in a p53-independent fashion and mediating several key cell cycle related genes such as p21, E2F, Myc and other cell cycle control genes (32, 33). It is also tempting to reason that nature build such redundancy to adope various growth stress conditions by utilizing several different miRNAs to quickly modulate protein expression.

Another potential p53-regulated miRNA is miR-26a. We have demonstrated that miR-26a is down regulated after loss of p53, and that miR-26a may be directly regulated by tumor suppressor p53 in colon cancer (16). A recent report showed that miR-26a not only impact colorectal cancer, it also play key role in liver cancer both as potential therapeutic agent and prognosis biomarker (34, 35).

The positive feedback mechanism between p53 and some of these miRNAs proved to be an important part of the regulatory function and networks of p53 mediated through miRNAs. Due to the broad impact of miRNA on regulating translation rate, miRNAs may be responsible for the fine tuning of the tumor suppressor function of p53 under acute growth

environmental changes including genotoxic stress. We have reasons to believe that modulation of miRNA will have a broad impact in colorectal and other cancer types.

miRNAs mediated chemoresistance

To discover miRNAs that may play a role in chemoresistance in colorectal cancer, we focused our efforts on miRNAs that may modulate the expression of key anti-cancer therapeutic targets such as TS, DHFR and histone deacetylases (HDACs). As mentioned briefly above, recent studies from our group have found that miR-215 directly targets the expression of both TS and DHFR (30). Strikingly, our studies found that that despite the reduction of TS and DHFR levels with elevated expression of miR-215, colon cancer cells became more resistant to the TS Tomudex (TDX) and DHFR inhibitor methotrexate (MTX). Further investigation found that this effect was mainly due to the enhanced G2/M cell cycle check point control and reduced cell proliferation. The enhanced G2 check point control was mediated by the suppression of denticleless (DTL), one of the components of CUL4A-DDB1 E3 ubiquitin ligase complex (36), and essential for the early G2/M checkpoint. We found that the induction of p53 and p21 was a result of DTL suppression by miR-215, and that siRNA knock-down of DTL conferred chemoresistance to TDX and also triggered G2/M arrest in colon cancer cells. Taken together, this suggests the presence of a positive feedback loop between miR-215 and p53, mediated through the suppression of DTL (Figure 1).

In another study, we identifiedthat one of the key targets of miR-140 is HDAC4 (37). Ectopic expression of miR-140 reduced colon cancer cell proliferation and triggers cell cycle arrest. miR-140 over-expression also rendered colon cancer cells more resistant to TDX and MTX treatment. Further studies from our laboratory revealed that colon cancer stem-like cell populations, known to be highly resistant to 5-FU treatment, contained high levels of miR-215 and miR-140 (37). By blocking the activity of miR-140 using anti-miR-140 LNA oligonucleotides, we were able to sensitize colon cancer stem cells to 5-FU treatment (37).

These findings raise an interesting point: previously, miRNAs have been classified as either pro- or anti-oncogenic. However, given the breadth of targets and pathways impacted by a given miRNA, it can target both oncogenes and tumor suppressor genes resulting in a net phenotypic outcome is rather complex. For example, miR-215 and miR-140 appear at first glance to act as tumor suppressors because they can slow tumor growth and reduce tumor size in vivo. However, despite their anti-proliferative effect, both miRNAs produced cells resistant to chemotherapy. This may be a reason that we can cure mice by shrink human tumor xenografts with various approaches, but most of these approaches will not work in human as we were unable to monitor tumor relapse from the small population of chemoresistant tumor cells due to the short life span of the mice. Innovative strategies have to be developed to overcome such difficulties.

miRNA as diagnostic, predictive, and prognostic biomarkers in colorectal cancer

The effort of discovering colorectal cancer related miRNAs begin with expression profiling of miRNAs using colorectal cancer cell lines and tumor specimens. Previously, our lab demonstrated that miRNAs are adequately stable in archival formalin fixed paraffin embedded (FFPE) tissue samples for expression analysis (38), allowing our own and numerous other labs to use large numbers of archival clinical specimens for miRNA-based cancer biomarker discovery. In addition to FFPE samples, circulating miRNAs in body fluids (e.g. blood) have been discovered to be potential cancer biomarker candidates for cancer detection (39). Several recent reports have demonstrated the potential of miRNAs as biomarkers in the diagnosis colorectal cancer (40, 41). Ng et al. revealed that the expression of miR-92 is significantly elevated in plasma of patients with colorectal cancer and can be a potential non-invasive molecular marker for colorectal cancer screening (41). Huang et al. also reported that miR-29a and miR-92a also have value for the diagnosis of advanced colorectal cancer (40). Table 1 summarizes some of the important miRNAs as potential biomarkers in colorectal cancer.

In addition to its use as a diagnostic marker, it has also been shown that miRNA may useful in predicting the possible outcome of treatment. Our laboratory has investigated the potential of miRNAs as a predictive biomarker in colorectal cancer patients treated with 5-FU based chemotherapy, and also investigated the biomarker potential of miRNAs in colorectal cancer patients treated with S-1, the next generation oral 5-FU-based compound for treating colorectal cancer and other solid tumors (19), and have demonstrated that the expression of miR-181b and let-7g was significantly associated with chemoresponse to S-1.

A number of studies have also demonstrated the potential of miRNAs markers as prognostic markers in colorectal cancer. A recent microarray-based miRNA expression profiling study of 10 samples of normal mucosa and 49 stage II colon cancers revealed several differentially expressed miRNAs between normal tissue and tumor microsatellite subtypes, with miR-145 showing the lowest expression in cancer relative to normal tissue. They further demonstrated that a biomarker based on these miRNA expression profiles could not only correctly predict the microsatellite status for the majority of cancers, but also predict recurrence of disease with an overall performance accuracy of 81%, indicating a potential role of miRNAs in determining tumor aggressiveness (42). The expression levels of miR-320 and miR-498, both included in the predictive biomarker, correlated with the probability of recurrence-free survival by multivariate analysis (42).

Our group has discovered that miR-200c also has potential as a prognostic biomarker by providing experimental evidence that high levels of miR-200c were associated with poor prognosis in colorectal cancer (20). The close association of let-7g and miR-200c with clinical outcomes of colorectal cancer is no accident: it is now known that both let-7g and miR-200c plays roles in regulating epithelial-mesenchymal transition (EMT), a key event in cancer progression (43). In the case of miR-200c, this is accomplished by regulating the expression of ZEB1 and ZEB2 (44).

The predictive and prognostic potential of another miRNA, miR-21, has also been demonstrated to be closely associated with clinical outcome in colorectal cancer (45, 46). Shetter et al. found several miRNAs, including highly elevated miR-21, to be associated with low sensitivity and poor response to chemotherapy and poor survival.

Conclusion and Future Perspectives

Post-transcriptional control, miRNAs in particular, are now been recognized as important cellular processes, alteration of which can contribute to a number of human diseases. miRNA-mediated regulation allows cells to fine-tune the expression of multiple genes and pathways via an energy-efficient mechanism that can be both quickly effected and readily reversed, providing an incredible degree of flexibility constantly changing conditions. This highly-adaptive system, however, is also inherited by these cells that become cancerous, contributing to chemoresistance and other survival mechanisms. In the present review, we summarized the role of miRNAs in colorectal cancer and chemoresistance mechanism. The incredible complexity of the miRNA layer of regulation will certainly provide a host of future discoveries of new regulatory mechanisms ranging from the level of the individual genes to vast regulatory networks. With the continual advancement of knowledge in the domains of miRNA, mRNA, protein expression, and computational biology, we come ever closer to fully understanding the complex miRNA-mediated regulatory pathways and networks in both cancer and normal cells. This knowledge, in turn, will no doubt continually offer new therapeutic strategies and diagnostic/prognostic biomarkers for the treatment of colorectal cancer and other human diseases.

Acknowledgments

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Biography

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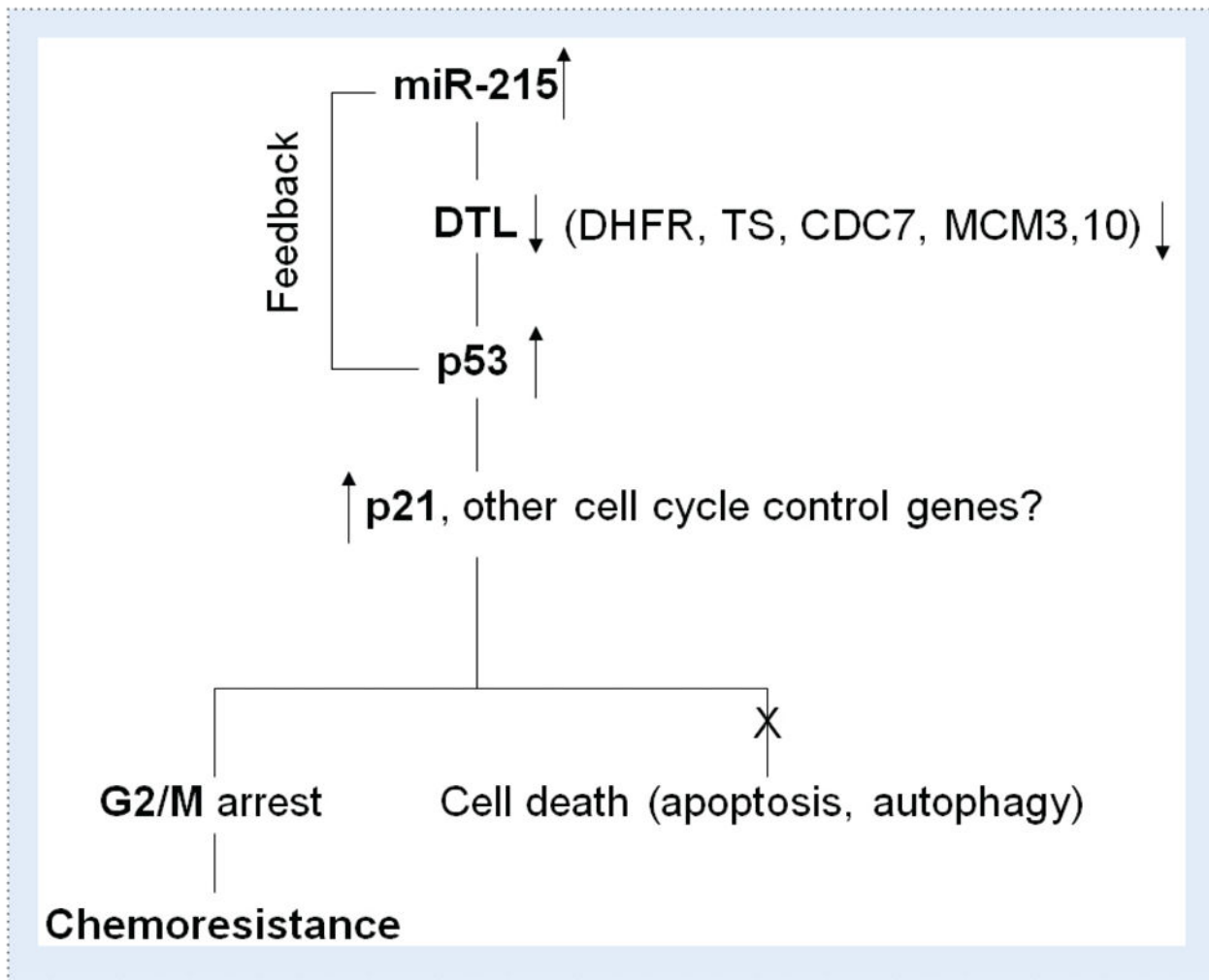
Figure 1.
Feedback regulatory mechanism of miR-215 and p53 network in cell cycle control and chemoresistance. The expression of p53 was induced by the suppression of DTL, a critical component of th e CUL4A-DDB1 E3 ubiquitin ligase complex, along with the p53 down stream gene p21. The elevated p53 in turn up-regulates the expression of miR-215. In addition, to DTL, miR-215 also suppresses the expression of TS, DHFR, CDC7, MCM3, MCM10. miR-215 triggers cell cycle arrest at the G2/M phase without triggering cell death, which contribute to chemoresistance to TS and DHFR inhibitors.

TABLE 1

miRNAs	References
Diagonstic marker	
miR-29a	40
miR-92a	41
Key targets	
TYMS, TS (miR-192, miR-215)	17,30
DHFR (miR-192, miR-215, miR-24)	17,30,31
Bcl-2 (miR-34)	25–27
HDAC4 (miR-140)	18
ZEB1, ZEB2 (miR-200c)	43,44
Predictive Biomarker	
miR-181b	19
let-7g	19
Prognosis	
miR-145	42
miR-21	45,46
miR-200c	20
miR-320	42
miR-498	42

Biomarker candidates (diagnostic, predictive and prognostic) of miRNAs in colorectal cancer.