

The Potential Role of miR-451 in Cancer Diagnosis, Prognosis, and Therapy

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Abstract

MicroRNAs (miRNA) are small noncoding RNAs that converge to maintain an intrinsic balance of various processes, including cell proliferation, differentiation, and apoptosis. Recent research efforts have been devoted to translating these basic discoveries into applications that could improve the early diagnosis and therapeutic outcome of patients with cancer. Early studies have shown that miRNA-451 (miR-451) is widely dysregulated in human cancers and plays a critical role in tumorigenesis and tumor progression. In this review, we summarize the potential use of miR-451 for cancer diagnosis, prognosis, and treatment. In addition, we discuss the possible mechanisms of miR-451 dysregulation and future challenges in development of miR-451 as a noninvasive biomarker and a potential therapeutic target in human cancers. *Mol Cancer Ther*; 12(7); 1153–62. ©2013 AACR.

Introduction

In recent years, it has become increasingly apparent that the nonprotein-coding portion of the genome is of crucial functional importance in both normal development and disease. Noncoding RNAs (ncRNA) can be divided into the following categories: microRNAs (miRNA), PIWI-interacting RNAs (piRNA), small nucleolar RNAs (snoRNA), long noncoding RNAs (lncRNAs; e.g., HOTAIR, lincRNAs, and T-UCRs), and other types of ncRNAs (1). The most widely studied class of ncRNAs is miRNA, which negatively controls gene expression. Through enormous high-throughput biochemical screens, more than 1,000 miRNAs in the human genome have been identified during the past few years. It is estimated that more than 60% of the protein-coding genes in the human genome are regulated by miRNAs. In human diseases, particularly in cancer, it has been shown that epigenetic and genetic alterations in miRNAs and their processing machinery are common hallmarks.

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miRNAs are a family of endogenous, small, nonprotein-coding RNAs approximately 19 to 25 nt in length, which mediate posttranscriptional gene silencing by translational repression and mRNA cleavage. Biogenesis of miRNAs takes place through a multistep process that involves the RNase III enzymes Drosha and Dicer and ultimately results in the production of mature miRNAs of approximately 22 nt. miRNAs have emerged as critical components of the complex functional networks involved in many cellular processes, including proliferation, differentiation, apoptosis, and development. Interestingly, more than 50% of miRNA genes are frequently located at fragile sites and genomic regions involved in cancers (2). Some miRNAs are highly evolutionarily conserved from species to species in animals and in plants, and function in a tissue- and time-specific manner.

In the human genome, the miRNA-451 (*miR-451*) gene is located on chromosome 17 at 17q11.2 (Gene ID: 574411), 100 bp downstream of the *miR-144* gene (Fig. 1A; ref. 3). The miR-144/451 loci reside in intergenic regions adjacent to the protein-coding gene *ERAL1* (Era G-protein-like 1), which is transcribed in a direction opposite to that of miR-144/451 (4). miR-451 is perfectly conserved among vertebrates (Fig. 1B; ref. 5). Broad miRNA expression profiling analyses have recurrently identified miR-144 and miR-451 for their high erythropoietic cell-restricted expression in several species, including zebrafish (4, 6, 7), mice (8–10), and human (5, 11, 12). It has been shown that miR-144 regulates α -hemoglobin expression in zebrafish by targeting the erythroid-specific transcription factor KLFD (13). Gain or loss of miR-451 function in murine erythroleukemia (MEL) cells leads to promotion or impairment of erythrocyte differentiation, respectively (11), consistent with a role for miR-451 as a positive regulator of erythroid maturation. Early studies have also highlighted the roles of miR-144/451 in human cancers. Many studies have

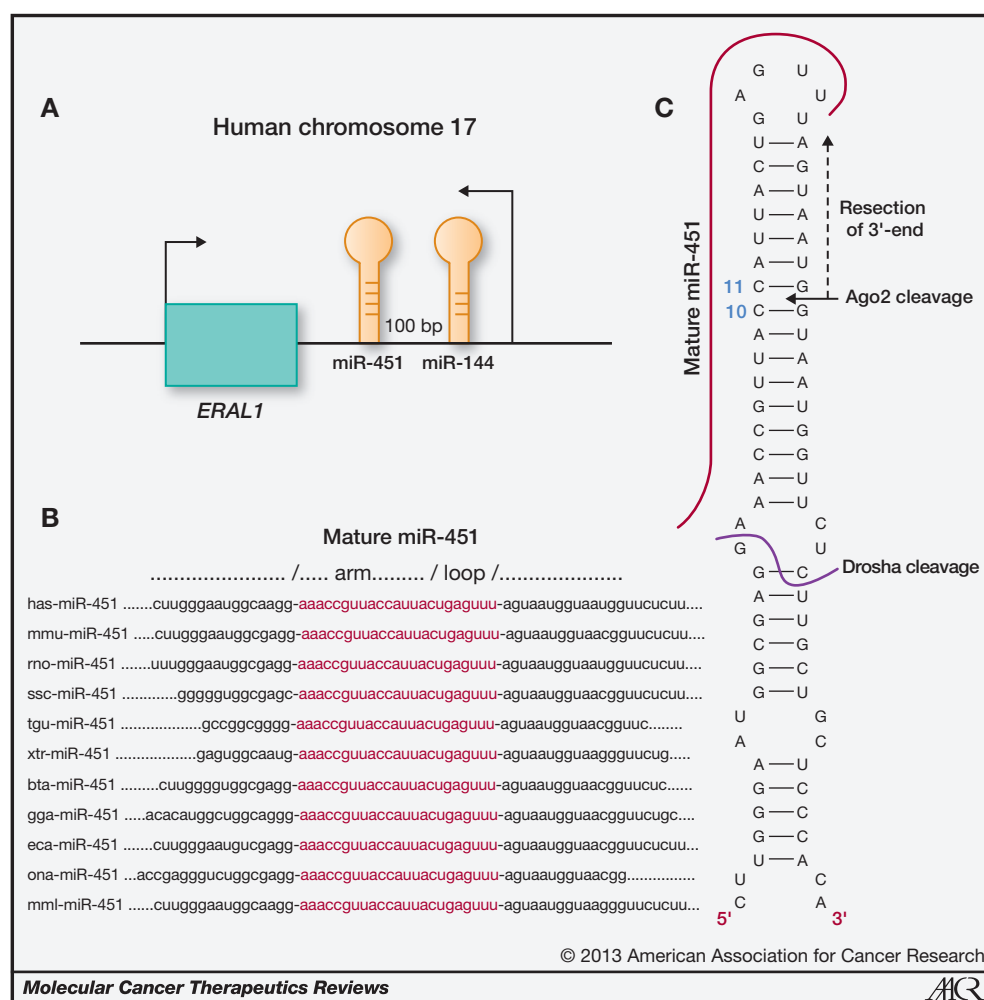


Figure 1. *miR-451* gene locus in human genome and the noncanonical biogenesis pathway. A, features of the *miR-144/451* locus and the adjacent *ERAL1* gene. B, atypical conservation of *miR-451* among vertebrates. C, model for *miR-451* processing.

established that miR-451 is widely dysregulated in human malignancies, including in lung cancer (14, 15), gastric cancer (16), breast cancer (17, 18), glioma (19–21), and leukemia (22), indicating that miR-451 might play a critical role in oncogenesis. In this review, we focus on the noncanonical biogenesis pathway for miR-451 and its roles in erythroid differentiation. We also review recent studies addressing the clinical applications of miR-451 as a diagnostic or prognostic biomarker and as a tool for sensitizing tumors to traditional chemotherapeutic agents.

Mechanisms of miR-451 Biogenesis

Canonical animal miRNA biogenesis occurs via a 2-step processing pathway, wherein Drosha initially cleaves the primary miRNA transcript to liberate a hairpin pre-miRNA about 55 to 70 nt in length. This hairpin pre-miRNA is exported to the cytoplasm with the help of Exportin 5 and cleaved by the Dicer RNase III enzyme to

yield a miRNA/miRNA* duplex, which is loaded onto the Argonaute (Ago) protein. The passenger strand (miRNA*) is removed through unknown mechanisms to yield a complex ready for target recognition. However, miR-451 is an exception to this mode of processing and is processed through a noncanonical pathway. Recent studies have identified several miRNA classes that bypass Drosha-mediated processing, including short hairpin introns (miRtrons), miRNAs derived from transfer RNAs, and snoRNAs (23–25). In contrast to Drosha, Dicer has been viewed as a central processing enzyme in the maturation of small RNAs.

Interestingly, mature miR-451 includes nucleotides from both sides of the pre-miRNA hairpin loop (Fig. 1C; ref. 26). As annotated, the 6 terminal nucleotides of mature miR-451 (23 nt) span the loop region and extend into the complementary strand of the hairpin precursor, which has not been shown to occur in other known miRNAs, so far. The 42-nt Drosha-cleaved miR-451 hairpin with a 17-nt stem does not possess a sufficiently long duplex

to be a Dicer substrate (>19 nt), suggesting that its maturation might bypass a requirement for Dicer (27). Recently, 3 studies found by coincidence that the maturation of miR-451 seemed refractory to loss-of-function mutations in Dicer (5, 7, 10). miR-451 accumulated effectively in embryonic stem cells carrying a conditional knockout of Dicer (10), in mouse embryonic fibroblasts (MEF) stably deleted for Dicer (5) and in MZdicer mutant zebrafish (7). However, other miRNAs were obviously reduced in Dicer mutants, providing evidence that unlike other miRNAs, the miR-451 hairpin does not mature via a Dicer-dependent pathway.

Another reason that miR-451 differs from other canonical miRNAs is the unusually perfect base-pairing in pre-miR-451 (Fig. 1C), whereas most other miRNA hairpins contain multiple unpaired nucleotides within the stem. The perfectly paired pre-miR-451 stem calls to mind the capacity of mammalian Ago2 to cleave highly paired canonical pre-miRNAs (28). Loading of an extensively paired pre-miRNA into Ago2 allows cleavage at nucleotides 10 and 11 across the 5'-end of the hairpin (29). Analysis of miRNA expression profiles using microarray-based methods revealed that miR-451 was the most markedly reduced miRNA in MZago2 mutant zebrafish embryos (7), and revealed a complete loss of intermediate and mature forms of miR-451 in the bone marrow of Ago2^{-/-} mice, which instead accumulated pre-miR-451 (5). In addition, the processing of a mutant *pre-miR-451^{mm10-11}* (with 2 mismatches in the predicted Ago2 cleavage site) was strongly reduced when *pre-miR-451^{mm10-11}* was incubated with Ago2 protein (7). Ago2 consists of a catalytic DDH triad that serves as a metal coordination site (30). Yang and colleagues showed that wild-type Ago2 immunoprecipitated with mature miR-451, whereas catalytic-dead Ago2 (D669A) proteins were associated exclusively with the hairpin precursor (5). On the other hand, histologic examination did not reveal any gross morphologic defects in mutant mice homozygous for Ago2^{ADH} (an Ago2 allele with ADH rather than DDH), as compared with wild-type mice, even though none of the mutant mice survived past the point of weaning (10). However, the appearance of the Ago2^{ADH} mice was strongly indicative of anemia, which raised the possibility that the Ago2 catalytic center might help to catalyze the maturation of miR-451, which promotes erythroid differentiation.

Then, the Ago2-cleaved pre-miR-451 (ac-pre-miR-451) hairpins were processed into approximately 30-nt intermediates, which may undergo nucleolytic trimming at the 3'-end to yield the mature product (Fig. 1C). Interestingly, Cifuentes and colleagues observed that Ago2 protected the approximately 30-nt slicer-cleaved intermediate from RNase I *in vitro*, resulting in a approximately 20- to 26-nt 3'-end trimmed product, similar to the observations by Yang and colleagues (5, 7, 31, 32). Recently, Yang and colleagues show that the degree of G:C content in the resected region governs the maturation efficiency of 3' resection and resultant

regulatory activity, such that a relatively low G:C content improves processing (31).

Furthermore, Dueck and colleagues reported that miR-451 is not only processed by Ago2, but also loaded exclusively into Ago2-containing RISCs (33). In RNA interference (RNAi), that siRNAs are loaded into all 4 Ago proteins. Because, in mammals, only Ago2 is catalytically active and needed for efficient gene knockdown, noncatalytical Ago1, Ago3, and Ago4, however, may cause unwanted off-target effects. Thus, a miR-451-like hairpin could be used for siRNA or ectopic miRNA expression to minimize the off-target effects (7, 10, 31, 33). To our disappointment, processing as well as silencing activity was less efficient compared with classical, Dicer-dependent hairpins (33). For this reason, further researches are demanded. Nevertheless, the unconventional structure of miR-451 provides a platform for Dicer-independent, Ago2-mediated noncanonical miRNA biogenesis. Indeed, miR-451 backbones should have an advantage over sh-miRNA vectors in Dicer-compromised cells and would be the only possibility for siRNA expression in Dicer-deficient cells.

miR-451 in Erythropoiesis

miR-451 is essential for maintenance and/or late-stage maturation of committed erythroid precursors and the expression of miR-451 is regulated directly by the critical hematopoietic transcription factor, GATA-1 (4). Overexpression of miR-451 augments dimethyl sulfoxide-induced erythroid differentiation in MEL cells (11). Conversely, loss-of-function of miR-144/451 impedes erythroid maturation in MEL cells (4), in zebrafish embryos (4, 6, 34), and in mice (9, 35). miR-144 has been shown to negatively regulate α -hemoglobin expression and form a feedback circuitry in fine-tuning of its expression (13). Nonetheless, restoration of miR-451, but not miR-144, rescues erythropoiesis in *meunier*, a zebrafish mutant with miR-144/451-deficient erythrocytes (6).

The level of miR-451 expression rapidly increases during erythroid maturation in human, and Bruchova and colleagues detected an approximately 35-fold increase in miR-451 expression in late normoblasts and some reticulocytes as compared with the miR-451 levels in immature early erythroid progenitors (36). The erythroid differentiation defect was observed in both *miR-451^{-/-}* mice and wild-type mice injected with a cholesterol-modified miR-451 antagomir, indicating that miR-451 plays a constitutive role in the differentiation of erythrocytes, rather than a role in embryonic development alone, and that both a chronic and transient loss of miR-451 function elicit the same phenotype (8). In zebrafish, miR-451 accelerates the rate of erythrocyte maturation, partly by repression of *gata2*, the gene encoding for GATA-2, a prostem cell transcription factor (6). One known mechanism for downregulating *gata2* is by directly binding to and repressing its promoter (37). Hence, at least, a 2-pronged GATA-1-driven regulatory mechanism is involved in relaxing GATA-2-mediated repression of erythroid maturation:

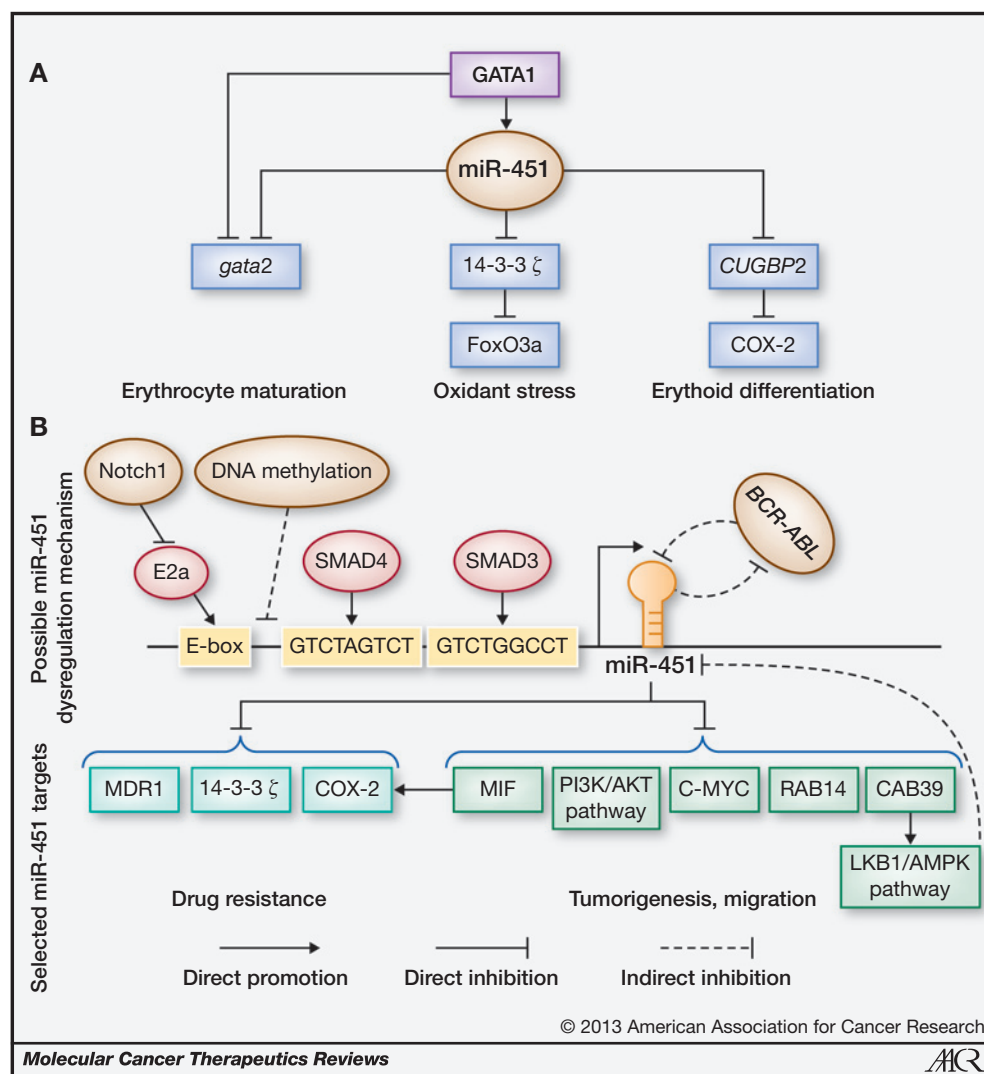


Figure 2. Functions of miR-451 based on the current literature. A, miR-451 was essential in erythropoiesis. B, the molecular mechanism involving miR-451, validated targets, and interactions of miR-451 with other factors/pathways in human cancer cells.

GATA-1 turns off transcription of *gata2* and activates transcription of a miRNA locus that further downregulates *gata2* transcripts (Fig. 2A). The predictions of this model were consistent with the findings of Dore and colleagues, such that overexpression of miR-144 and miR-451, either separately or together, cannot substitute for loss of GATA-1 (4).

Moreover, miR-451 ablation not only causes mild erythrocyte instability, but also increases susceptibility to damage after exposure to oxidizing drugs in mice and zebrafish. At least some protective activities of miR-451 stem from its ability to directly suppress production of 14-3-3 ζ , an intracellular regulator of cytokine signaling proteins that inhibits nuclear accumulation of the transcription factor FoxO3, a positive regulator of erythroid antioxidant genes (8, 9). The miR-451/14-3-3 ζ /FoxO3 regulatory axis might have potential implications beyond erythropoiesis. Conversely, a recent study showed that increased miR-

144 is associated with decreased glutathione regeneration and attenuated antioxidant capacity in homozygous sickle cell disease (HbSS) erythrocytes (38). Although miR-144 is part of a polycistronic precursor with miR-451, higher miR-144 expression is related to more severe anemia in patients with HbSS.

In summary, the data discussed earlier indicate that miR-144/451 locus is a major downstream effector of GATA-1 and is important in the erythroid differentiation and homeostasis in erythroid cells of zebrafish (4, 13, 39). However, *miR-451* gene seems to be a positive regulator of erythropoiesis, whereas the physiologic function of *miR-144* gene in erythroid cells needs further understanding.

miR-451 in Cancer Diagnosis

Biomarkers are biologic indicators of disease states, used to define tumor subtypes or assess the efficacy of interventions. Ideally, biomarkers should be easily

Table 1. miR-451 as a diagnostic biomarker in human cancers

Cancer type	miR-451 expression	Sample	Functional target	References
Gastric cancer	Downregulation	Fresh gastroscopic biopsies	MIF	(16, 47)
		FFPE samples		
Colorectal cancer	Upregulation	Plasma	MIF	(51)
	Downregulation	FFPE samples		
NSCLC	Downregulation	FFPE samples	RAB14	(14, 15)
Renal cell carcinoma	Downregulation	Serum		(48)
Pre-B-ALL	Downregulation	Bone marrow		(49)
Glioblastoma multiforme	Upregulation	FFPE samples	CAB39	(20, 21)
Glioblastoma	Downregulation	Cell line		

accessible, such as in blood, so that they can be detected in samples obtained noninvasively. Conventional serum biomarkers include prostate-specific antigen, carcinoembryonic antigen, and α -fetoprotein, which are widely used in clinical diagnosis. However, these serum tumor markers lack sufficient sensitivity and specificity to facilitate early detection of cancer. miRNAs offer great potential as biomarkers for cancer detection due to their remarkable stability in blood and their characteristic expression in many cancers. More recently, the roles of miRNAs as tumor biomarkers have been investigated and developed (40). miRNA expression profiles could identify the tissue origin of tumors (41, 42), classify the subtypes of renal cell carcinomas (42), and facilitate the early-stage detection of breast, gastric, or lung cancers (43–45).

Studies conducted in the past few years have provided evidence for a crucial role of miR-451 in diagnosis of human cancer (Table 1). Early alterations in epithelial polarity are a hallmark of epithelial cell cancers or carcinomas and contribute to their uncontrolled growth or their progression to invasive adenocarcinomas. Tsuchiya and colleagues found that miR-338-3p and miR-451 contributed to the formation of basolateral polarity in epithelial cells (46). Ribeiro-dos-Santos and colleagues validated normal miRNA profiles of human gastric tissue to establish a reference profile for healthy individuals using high-throughput SOLiD sequencing technology (47). miR-29b, miR-29c, miR-19b, miR-31, miR-148a, and miR-451 were highly expressed in gastric tissue and could be considered as a part of the miRNA expression pattern of healthy gastric tissue (47). Likewise, another study detected miR-451 expression in epithelial cells by *in situ* hybridization and found that miR-451 expression was decreased in gastric and colorectal cancer tissues compared with non-tumor tissues (16). We previously also observed that miR-451 was significantly downregulated by 170.9-fold comparing non-small cell lung carcinoma (NSCLC) tissues with corresponding noncancerous tissues (14, 15). These results imply that miR-451 is required for the development and maintenance of normal tissues and might be downregulated during the transition to cancer.

Recently, several studies confirmed that circulating miR-451 may serve as a new, powerful, and noninvasive

biomarker for human malignancies. Combined detection of miR-378 and miR-451 in serum enabled the identification of renal cell carcinoma, with a sensitivity of 81%, specificity of 83%, and an area under the curve (AUC) value of 0.86 (48). miR-451 and miR-373* were dramatically downregulated in childhood B-cell precursor-acute lymphoblastic leukemia (pre-B-ALL) compared with samples from healthy individuals (49). Nan and colleagues showed that miR-451 was significantly downregulated in 3 glioblastoma cell lines, functioning as a tumor suppressor in human gliomas (50). However, different from the results described earlier, a recent study reported that the plasma concentrations of miR-451 and miR-486 were significantly decreased in postoperative patients as compared with preoperative gastric cancer patients (51). Moreover, the levels of both miRNAs were found to be markedly higher in patients in comparison with the levels in healthy control subjects. Another study also found that mature miR-451 was expressed at a higher level in glioblastoma multiforme tissues than in matched adjacent normal brain tissue (20, 21). These data indicated that the expression of miR-451 was associated with disease states and was cell-specific, supporting the clinical value of miR-451 as a diagnostic biomarker in human cancers.

miR-451 in Cancer Therapy

Drug resistance is a major obstacle in cancer therapy, which leads to therapeutic failure, uncontrollable disease, and mortality. It is well accepted that aberrant miRNA expression is associated with cancer, and miRNAs can influence the sensitivity of tumors to traditional antitumor therapy. Recently, several mouse models provide evidence that miRNAs perform crucial functions in cancer pathogenesis and progression (52–54). These observations imply that silencing an oncogenic miRNA or restoring a tumor-suppressive miRNA might serve as an effective antitumor therapy.

Our previous studies have shown that ectopic overexpression of miR-451 inhibits proliferation and triggers apoptosis of NSCLC cells by directly inhibiting its target, ras-related protein 14 (RAB14; Fig. 2B; refs. 14, 15). Upregulation of miR-451 could sensitize NSCLC A549 cells to cisplatin partly through inactivation of the Akt signaling

pathway (14). Likewise, Liu and colleagues found that c-myc reduced the expression of miR-144/451 in imatinib-resistant K562 (K562R) cells, and restoration of miR-144/451 might reverse the resistance of K562R cells to imatinib therapy. Their findings also help us understand the role of c-myc in drug resistance (55). Besides, Bandres and colleagues revealed that miR-451 overexpression reduced cell proliferation and sensitizes gastric and colorectal cancer cells to radiotherapy.

In breast cancer, many estrogen receptor (ER)-positive breast cancers respond well initially to endocrine therapies but often develop resistance to selective ER modulators (SERM), such as tamoxifen, and the resistance eventually contributes to disease progression. In a recent study, researchers found the parental MCF-7 cells expressed 3 times more miR-451 than did tamoxifen-resistant MCF7 cells. The regulation of miR-451 by tamoxifen induces downregulation of its target 14-3-3 ζ , a key proliferative and antiapoptotic factor in breast cancer that plays an important role in tamoxifen resistance. Overexpression of miR-451 restored the growth-inhibitory effectiveness of SERMs in endocrine-resistant cells (17). In addition, another study found that restoration of miR-451 in doxorubicin-resistant MCF-7 cells made them more sensitive to doxorubicin than did MCF-7 cells (18). Contrary to the data mentioned earlier, Zhu and colleagues showed that expressions of miR-27a and miR-451 were upregulated in MDR human ovarian and cervical cancer cells, compared with levels in the parental cells. The antagonists of miR-27 and miR-451 decreased the expression of P-glycoprotein and increased sensitivity to doxorubicin in MDR cancer cells (56).

The above evidence shows that miR-451 could reverse the resistance to chemoradiotherapy or endocrine therapy in many types of cancers. However, key studies evaluating their molecular mechanisms in the drug-resistant signal transduction network are still rare. Current researches are mostly preclinical, thus, systematic studies are demanded.

A small proportion of cells within a tumor are known to be capable of tumor growth, and these cells are called cancer stem cells (CSC). Many antitumor therapies affect rapidly dividing cells, whereas the rate of division of CSCs is slow. The ability of CSCs to renew pools of CSCs and to resist drugs perpetuates the tumor. Bitarte and colleagues showed that expression of miR-451 caused a decrease in self-renewal and tumorigenicity and caused chemoresistance to irinotecan in colonospheres that had properties of colorectal CSCs (57). Of note, the expression of miR-451 is downregulated in patients who did not respond to irinotecan-based first-line therapy compared with responding patients. A same study showed that miR-451 combined with imatinib mesylate had a cooperative effect on growth inhibition of glioblastoma stem cells (16). Similarly, higher expression of miR-451 and miR-15b in pretreatment osteosarcoma samples correlated with subsequent positive response to neoadjuvant chemotherapy (58). Thus, the *miR-451* gene could be used as a pivotal marker to predict

response to chemotherapy in patients with different carcinomas.

The regulation of miR-451 expression by glucose in glioma cells is of particular interest (20, 21, 59). Abundant glucose allows relatively high miR-451 expression, promoting cell growth. On the other hand, miR-451 levels decrease upon withdrawal of glucose, leading to decreased proliferation rates but increased migration and survival. The possible mechanism for these different effects may be attributed to increased survival of glioma cells under conditions of metabolic stress, forcing them to seek out favorable growth conditions. These data indicate that miR-451 plays a key role in modulating the balance of proliferation and invasion in response to metabolic stress within the microenvironment of the tumor, acting as an oncogene in gliomas. Thus, presumably, gliomas, in which miR-451 is upregulated, cannot be easily treated with miR-451 antagonists, as this would suppress tumor growth, but may promote invasion instead. However, targeting the response of gliomas to glucose deprivation may be an effective approach in combination with antiangiogenic treatment, for that inhibition of this pathway may lead to decreased survival and invasion. The contradictory results from these studies are consistent with the view that miRNAs function in a tissue- and cell-specific manner and more research is needed to elucidate the roles of miR-451 in cancer therapy.

miR-451 in Cancer Prognosis

Various reports have shown that miRNAs, including miR-451 (16), are associated with the clinical outcome of human patients with cancer, such as in lung cancer (60), hepatocellular cancer (61), esophageal squamous cancer (62), and leukemia (63). Bandres and colleagues showed that patients with gastric cancer with lower miR-451 expression had shorter disease-free survival (DFS) and overall survival (OS) than patients with higher miR-451 expression (16). In a previous study, we also examined the correlation between miR-451 levels and clinicopathologic factors in NSCLC (15). The downregulation of miR-451 was correlated with poor tumor differentiation, advanced pathologic stage, and lymph node metastasis. Moreover, low expression of miR-451 was an unfavorable independent factor associated with OS of patients with NSCLC.

A retrospective study showed that miR-451 was markedly reduced in 21 patients with gastric cancer stage III, who were receiving postoperative chemoradiotherapy, and the lower levels were correlated with a higher risk of recurrence and death after resection of the primary tumor (16). Conversely, in another study, Brenner and colleagues reported that, lower, not higher, levels of miR-451 were associated with a better outcome (64). This discrepancy might be attributable to differences in study populations and methods for selecting and handling the tissues. Finally, and probably most importantly, the small sample sizes may explain the inconsistencies among these studies. In addition, patients with glioblastoma multiforme expressing high levels of miR-451 had significant

shorter survival time as compared with those expressing low levels of miR-451 (21). Taken together, miR-451 can serve as a candidate prognostic indicator in human malignancies.

Mechanisms Involved in the Regulation of miR-451 Expression

Much evidence has shown that miR-451 is significantly downregulated in human cancer tissues compared with its expression in adjacent noncancerous normal tissues. However, the mechanisms underlying the dysregulation of miR-451 are poorly understood. And, so far, there are few reports about genomic loss or mutations in the miR-451 locus in human cancers. DNA hypermethylation and/or histone modification has been recognized as an epigenetic mechanism in the silencing of tumor-suppressive miRNAs in cancer cells, similar to the epigenetic silencing of various protein-coding tumor-suppressive genes.

As we mentioned earlier, miR-451 is markedly reduced in various types of cancers, indicating that miR-451 exerts its biologic role as a tumor-suppressive gene. We previously investigated the relationship of miR-451 and DNA methylation and/or histone deacetylation in NSCLC cells to determine whether the downregulation of miR-451 is associated with epigenetic aberrations (15). Of note, after treatment with 5-aza-2'-deoxycytidine (5-aza-dc), sodium phenylbutyrate, or both agents, the expression of miR-451 was significantly increased by 2.5-, 4.2-, or 6.4-fold as compared with the expression in control cells, indicating that epigenetic mechanisms might be involved in miR-451 dysregulation. However, a search of the human genome sequence did not reveal any CpG islands within the region 2 kb upstream of the miR-451 transcription initiation site. Hence, we hypothesize that a long-distance region upstream (>2 kb) of the miR-451 promoter may need to be analyzed to find the CpG-rich region that may be involved in the epigenetic regulation of miR-451 expression. Alternatively, DNA demethylation or histone acetylation could activate the promoter of a gene whose product regulates miR-451 expression, indirectly increasing miR-451 expression. The exact role of epigenetic mechanisms in miR-451 downregulation needs to be studied further.

In addition, miRNAs are transcriptionally regulated by various transcription factors. We analyzed the transcription factor-binding sites (TFBS) within the miR-451 promoter (~2 kb) using the University of California, Santa Cruz (UCSC) Genome Browser [Human genome, February 2009 Assembly (hg19)], and discovered that this domain harbored several TFBS, such as those for HEY1, TAF1, STAT2, and PU.1. We also predicted TFBS using the TRANSFAC database (<http://www.gene-regulation.com/pub/databases.html/>). However, there were no TFBS common to the 2 different methods. Other groups have also described their findings with regards to the mechanism of miR-451 downregulation. In one study, chromatin immunoprecipitation assays showed that

endogenous E2a occupies and transcriptionally activates the promoter of miR-451 by binding to the E-box motifs, suggesting that miR-451 is a direct target of the tumor suppressor, E2a (22). In addition, repression of miR-451, by degradation of E2a, was shown to be essential for NOTCH1-driven oncogenesis in T-lineage ALL. Recently, Liu and colleagues revealed a regulatory pathway between myc and miR-144/451 (55). They found that there were 2 c-myc-binding sites located in the 1,000-bp promoter region (relative to the start site of the pre-miR-144), which mediated downregulation of miR-144/451. As we mentioned earlier, tamoxifen controls 14-3-3z levels through its regulation of the miR-451, which provides evidence for a novel mechanism in the development of resistance to endocrine therapy (17). They also observed that tamoxifen through transcription factor, ER- α markedly reduced the level of Pol II at the transcription start site of miR-451. However, tamoxifen had no effect on the regulation of miR-144, indicating the selectivity of tamoxifen for miR-451 regulation.

Future Direction of miR-451-Based Treatment

Personalized medicine is an emerging model that will revolutionize our current healthcare system, which promises prediction, prevention, and treatment of cancer that is targeted to individuals' needs. In the last decades, we have moved from a one-size-fits-all approach that emphasized cytotoxic chemotherapy to a personalized medicine strategy that focuses on the discovery and development of molecularly targeted drugs. An increasing number of successful personalized therapies have impacted the lives of a large number of patients with cancer, such as the BCR-ABL tyrosine kinase (ABL) inhibitor imatinib, EGF receptor (EGFR) kinase inhibitors gefitinib and erlotinib, HER-2 antibodies trastuzumab, and so on. Thus, it is urgent to find the driving factors during tumorigenesis. miR-34a mimics packaged into a lipid-based delivery of vehicle and given locally or systemically could block tumor growth in mouse models of NSCLC, implying the potential of miRNA replacement therapy in NSCLC (54). Similar results in another study were noted that systemic administration of miR-26 using adeno-associated virus in a mouse model of hepatocellular carcinoma resulted in dramatic suppression of tumor progression without toxicity (52).

Discovery of the critical role of miR-451-modulating gene expression has not only changed our concept of gene expression regulation, but has also offered a new opportunity for designing anticancer strategies and therapies. miR-451 is significantly downregulated in multiple malignancies, contributing to tumor formation, maintenance, and metastasis. On the basis of such evidence, we and other groups focus on miR-451 in promising clinical implications as biomarkers of drug-sensitive/-resistant prediction, therapeutic targets to overcome drug resistance, cancer diagnosis, and prognosis. However, as of now, *in vivo* research is still needed.

Because of their capacity to simultaneously target multiple functionally related genes, miRNAs potentially influence cellular behavior through the regulation of extensive gene expression networks. Therapeutic modulation of a single miRNA may therefore affect many pathways simultaneously to achieve clinical benefit and in some cases have toxic consequences. Thus, more studies are needed to elucidate the biologic function and the regulatory network of miR-451.

From the current view, the critical issues for miRNA-based therapy are tumor-specific delivery, potency of the therapy, and elimination of off-target effects. Lipid- and nanoparticle-based delivery systems were shown to execute effective delivery to lung cancer cells or to animals locally and/or systematically (54, 65, 66). The double-stranded miRNA mimics promise a safe and cost-effective source of functionally mature miRNAs with less nonspecific effects as compared with viral-based miRNA-expressing vectors or chemically synthesized pre-miRs (66, 67).

Encouragingly, the first miRNA-targeted drug to enter clinical trials—LNA-antimir-122 (SPC3649) is successfully undergoing phase II trials. Phase I trial in 77 healthy volunteers established that LNA-miR-122 is safe and identified no dose-limiting toxicities. What we mentioned earlier provides evidence of promising miR-451-based novel therapies for human cancer in the future.

Challenges in the Use of miR-451 as a Biomarker

Despite data supporting the potential value of miR-451 as a biomarker in solid and hematologic human cancers, many challenges remain. First, as of now little is known about the origin of circulating miRNAs and the factors that influence the levels of circulating miRNAs, especially of miRNAs with biomarker potential. And, most of the studies so far have evaluated the expression of miRNAs using formalin-fixed paraffin-embedded (FFPE) samples. Pritchard and colleagues found that 58% of the solid tumor-associated circulating miRNA biomarkers were highly expressed in blood cells, and levels of these biomarkers were correlated with blood counts (68). Therefore, they hypothesized that the levels of circulating miRNAs might reflect blood counts, rather than correlate with a cancer-specific origin. Furthermore, one premise for using extracellular miRNAs to diagnose disease is the notion that the abundance of the miRNAs in body fluids reflects their abundance in the abnormal cells causing the disease. About this hypothesis, Pigati and colleagues compared intracellular and extracellular miRNA populations in breast cancer cell lines and found that about 66% of the released miRNAs are at an abundance that closely reflects the cellular miRNA abundance, whereas several other miRNAs (miR-451 and miR-107) were over-represented (69). In addition, 13% of the miRNA species were retained within cells, which made them nearly undetectable in the released population. They further investigated whether miR-451 was excessively released into the medi-

um in breast cancer cells; however, the release of miR-451 from nontumorigenic MCF10A cells and an unrelated normal fibroblast cell line (IMR90) was much lower, which indicated that the release of miR-451 was cell type-specific.

Second, although the circulating miRNAs are stably included in microvesicles (70) or exosomes (71), the exact secretory mechanism remains largely unclear. Considering that exosomes and microvesicles are evident in other types of body fluids, recent studies showed the presence of miRNAs in human saliva (72) and breast milk (73), indicating dietary intake of miRNAs by infants.

Third, nowadays, the detection of miRNA expression is conducted by microarray analysis and validated by real-time PCR methods. The selection of an appropriate reference gene (usually *RNU6B*, *RNU44*, *RNU48*, or *U6*) and the normalization process contributes to uncertainty in determining the presence and extent of dysregulation in miRNA expression (74). The dysregulation of snoRNAs, a relatively new area of research in cancer initiation and progress, for instance, could introduce bias when determining miRNA expression.

Nonetheless, further investigation of the potential of circulating miR-451 as a noninvasive biomarker is definitely warranted.

Conclusion

In this review, we have discussed convincing data showing that miR-451 is significantly dysregulated in human malignancies and propose that there is great promise that miR-451 will aid in the early diagnosis, evaluation, prognosis, and development of personalized therapies of human cancer. However, further research into miR-451 biogenesis, upstream regulatory mechanisms, along with the functional target identification will be necessary before the proper application of miR-451 as a molecular biomarker in human cancers. A better understanding of the complex regulatory network involved in miR-451 expression and function will definitely help elucidate mechanisms underlying the development and progression of human cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: X. Pan, R. Wang, Z.-X. Wang

Development of methodology: R. Wang

Writing, review, and/or revision of the manuscript: X. Pan, R. Wang, Z.-X. Wang

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