

REVIEW

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# miR-144/451 in hematopoiesis and beyond

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## Abstract

MicroRNAs (miRNAs), a class of 18–25 nucleotide (nt) non-coding RNAs, usually inhibit the expression of their target genes. They are transcribed from endogenous genes and are processed for maturation by multiple pathways. miR-144/451, a bicistronic gene locus, encodes miR-144 and miR-451, both of which are highly conserved in evolution. These two miRNAs are on the same primary RNA molecule whose transcription is controlled by multiple nuclear proteins, including GATA1, GATA4, Myc, Oct1, Pax4, FXR, AP1, SMAD3 and SMAD4 depending on tissue types. They are abundant and almost exclusively exist in red blood cells, but low expression of both miR-144 and miR-451 are also detected in non-erythroid lineages. Interestingly, deletion of both miR-144 and miR-451 DNA sequences coding pre-miR-144/451 hairpins in mice leads only mild microcytic anemia but worsen upon a number of stresses including developmental stress, acute blood loss, phenohydrazine-induced hemolysis and precursor depletion by chemotherapeutic drug 5-FU. Such knockout animals older than 15 months also spontaneously develop malignant tumors including B-lymphoma and acute myeloid leukemia, indicating that miR-144/451 is a bona fide tumor-suppressing gene in non-erythroid cells, though its levels are much lower compared to that in red blood cells. Consistent with the findings in animals, disruption of miR-144/451 expression and their abnormal functions are observed in human hematopoietic and non-hematopoietic organs. Moreover, miR-451 is the only miRNA discovered so far whose maturation does not depend on Dicer, an enzyme required by all other miRNAs for maturation. This review focuses on the biogenesis, transcriptional regulation and biological roles of miR-144/451 in erythropoiesis, tumor initiation and other pathological conditions.

**Keywords:** miR-451, miR-144, Biogenesis, Erythropoiesis, Cancer, Transcription

## Background

microRNA (miRNA) is a class of endogenous non-protein-coding RNAs, only 18–25 nt in length, and was first discovered in *Caenorhabditis elegans* by Ambros' group [1]. Hundreds of miRNAs have been confirmed functional in mammals, and thousands of others are still with unknown functions. miRNAs are expected to host gene regulation in virtually all mammalian tissues and cells [2]. miRNAs are generated typically as monocistronic or polycistronic gene loci (the latter is about 50% [3]). These miRNA gene loci usually exist as independent units, but can be in introns of protein-coding genes as well (Fig. 1). Activating signals drive RNA polymerase II (Pol II) to transcribe miRNA genes into large primary hairpin-containing transcripts (pri-miRNAs) [4]. Pri-miRNAs

are processed in nucleus through the canonical pathway (Fig. 1) [5, 6] and the resultant hairpin precursors (pre-miRNA, usually 60–70 nt long) are further processed by RNase III enzyme Dicer [7, 8] to generate a miRNA duplex. miRNA duplex is then incorporated into a Argonaute-containing, RNA-induced silencing complex (RISC), where the functional mature miRNAs are produced. Besides the canonical pathway, other alternative pathways have been described for mature miRNA generation [9–13] (Fig. 1). Noncanonical pathways also produce miRNAs by processing endogenous short-hairpin RNAs (shRNAs) and chimeric hairpin RNAs (produced in tandem with or as part of small nucleolar RNAs, tRNA-like molecules, and small nuclear RNAs) [2]. Regardless of how miRNAs are generated, mammalian miRNAs typically inhibit large networks of downstream gene targets primarily through the Watson-Crick base-pairing of miRNA “seed” sequences to the specific binding sites in target RNAs, which leads to deadenylation,

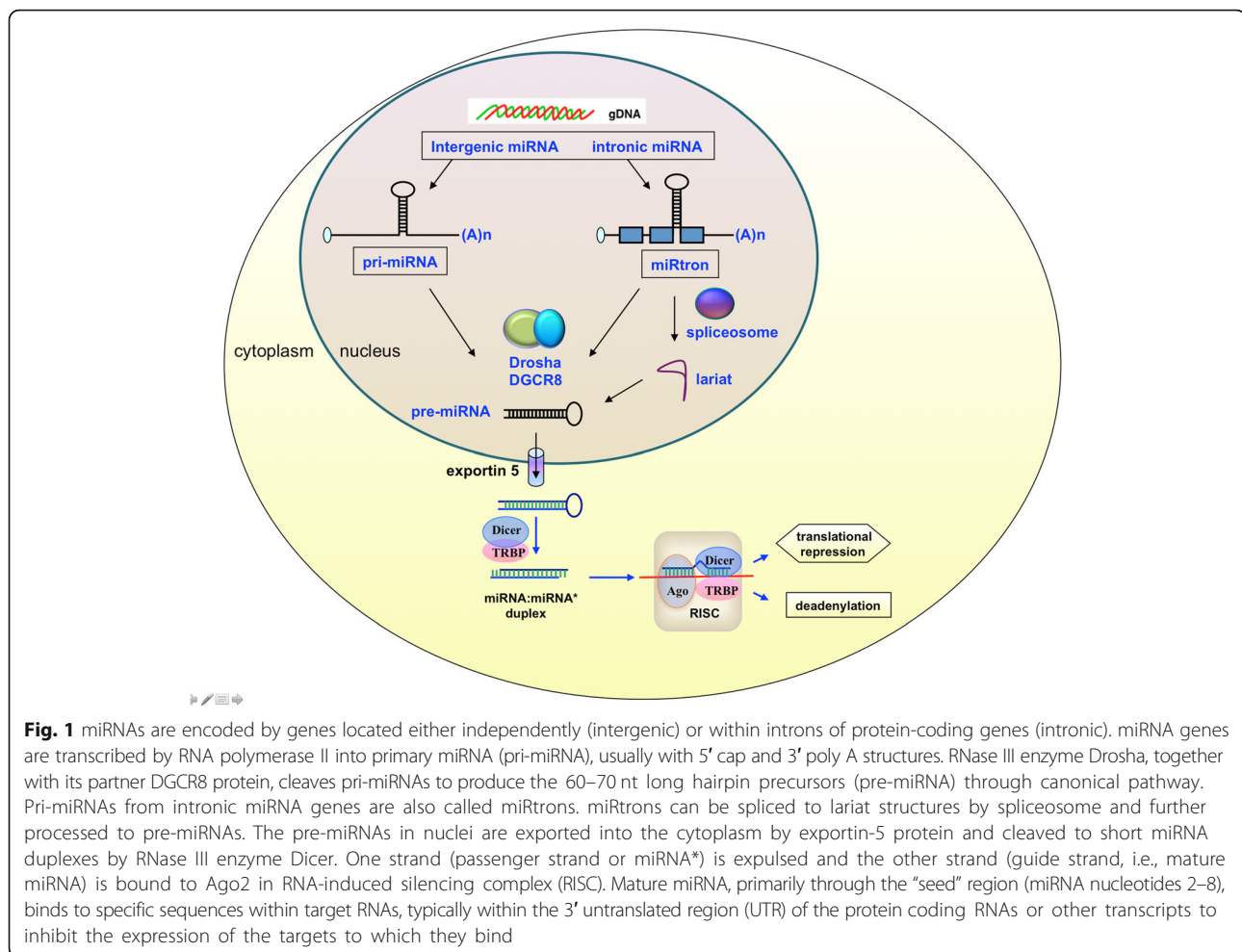
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RNA decay, and/or translational suppression [14]. The details about miRNA classification, biogenesis, function and target identification can be found in recent reviews [2, 15].

miR-144/451 is a bicistronic gene locus on chromosome 17 and chromosome 11 in human and mouse genomes, respectively. The miR-144/451 locus encodes two highly conserved miRNAs: miR-144-3p and miR-451a (unless otherwise indicated, miR-144 and miR-451 referred in this review are miR-144-3p and miR-451a, respectively) [16]. miR-144 and miR-451 are highly expressed in erythroid cells [16, 17], and knockout of miR-144/451 sequences that contain pre-miR-144/451 hairpins in mice disrupts erythroid homeostasis [18–20]. Interestingly, recent *in vivo* evidence reveals that miR-144/451 alone acts as a weak tumor suppressive miRNA locus that inhibits B-lymphomagenesis and tumorigenesis of myeloid lineage cells in mice [21], clearly indicating that miR-144/451 also functions in non-erythroid cells, though its levels are much lower than that in red blood cells. Moreover, miR-451, but not miR-144, is the only miRNA whose maturation is Dicer-independent [22–24]. Still, a lot about

miR-144/451 biology is yet to be defined. In this review, we focus on recent miR-144/451 studies to elucidate the biogenesis, transcriptional regulation and biological functions of miR-144/451 in erythropoiesis, cancer development and other pathological conditions. We believe that genetic and pharmacologic manipulations that change miR-144/451 levels could impact the development courses of various diseases.

#### Regulation of miR-144/451 expression at transcriptional levels

miR-144 and miR-451 are encoded by a single miRNA locus and their mature sequences, only 100 bp apart, are on the same primary RNA [16]. The miR-451 expression is largely restricted to red blood cells, though a low level of miR-451 is observed in non-erythroid lineages. Remarkably, about half of the miRNAs in mouse fetal liver (FL) erythroblasts are miR-451 [16, 17], while miR-144 levels are much lower than those of miR-451 in red cells [16, 20]. In addition, miR-144 expression is relatively more ubiquitous than the expression of miR-451 during embryonic development [25, 26], suggesting that not

only overlapping but also independent mechanisms regulate the levels of functional miR-144 and miR-451. Nevertheless, at least eight nuclear factors are involved in directly regulating the expression of miR-144/451 (Table 1) in various tissues. Whether the expression of miR-144/451 is activated or inhibited depends on the transcription factors and also probably cell types.

miR-144/451 expression is strongly induced upon differentiation of erythroid cells in zebrafish, mice, and humans [27–29]. Chromatin immunoprecipitation (ChIP) and gene promoter activity assays demonstrate that GATA1, a nuclear factor that potentially regulates more than 1 thousand of genes in erythroblasts [30, 31], binds the gene promoter region at ~2.8 kb from the transcription initiation site of the miR-144/451 locus and activates miR-144/451 transcription in erythroid cells [16].

miR-144/451 is strongly expressed during red cell development. However, numerous studies have shown disrupted levels of miR-144/451 in non-erythroid tumor cells [32–38], including diffuse large B-cell lymphomas (DLBCL) [21]. In many DLBCLs, the level of Myc, a protooncogenic protein that directly targets an estimate 15% of human genes, is altered [39]. Myc can directly inhibit a large set of miRNAs in B-lymphoma cells in a consensus “E-box” sequence-independent manner [40]. Consistent with this work, Myc can occupy the conserved DNA sequence located within the miR-144/451 gene promoter and directly repress the expression of miR-144/451 [21]. This explains why high MYC-expressing human and murine B-lymphoma cells usually show low abundance of miR-144 and miR-451 compared with normal B-lymphocytes.

Transcription factor paired box 4 (PAX4) binds to specific DNA sequence [41]. PAX4 is one of the PAX gene family members and its abnormal expression has been shown in many malignancies, including breast adenocarcinomas and neck squamous cell carcinomas [42]. Interestingly, ChIP and a promoter reporter assay demonstrate that PAX4, similar to Myc, transcriptionally inhibits the expression of miR-144 and miR-451, and

thus upregulating miR-144/451 target genes ADAMTS5 and ADAM10, two A disintegrin and metalloproteinase (ADAM) family proteins, both of which can promote cancer invasion and metastasis [42].

The fourth transcription factor that occupies the miR-144/451 promoter region is GATA4 [43]. Same as GATA1, GATA4 belongs to the GATA gene family that has six GATA members. Whereas GATA1, GATA2 and GATA3 are mainly expressed in blood cells, GATA4, GATA5 and GATA6 are usually expressed in cardiomyocytes and endodermal cells [44]. It has been demonstrated that GATA4 binds to two GATA sites in the miR-144/451 promoter sequence (at positions –512 and –826) in cardiomyocytes. Interestingly, GATA4 activated miR-144 and miR-451 coordinate the survival of cardiomyocytes [43].

Transcription factor OCT1 (POU2F1) also transcriptionally activates the miR-451 expression in glioblastoma multiforme (GBM) cells. In this case, metabolic stress, such as low glucose conditions, activates AMP-activated kinase (AMPK), which represses the activity of OCT1 protein by phosphorylation at serine 335. OCT1 activates miR-144/451 transcription by occupying multiple sites in the promoter region, and shutdown of OCT1 activity by AMPK reduces miR-451 expression, which further increases AMPK activity through the upregulation of miR-451 target gene *Cab39* [45]. Thus, in GBM cells, metabolic stress triggers a *Cab39*/AMPK-mediated positive feedback response between miR-451 and OCT1, which allows tumor cells to quickly adapt to variations of glucose concentrations in the tumor microenvironment. These findings in GBM cells uncover miR-451 as a major player in AMPK signaling, which is consistent with a recent finding in erythroid cells that miR-451 is an important effector that represses *Cab39*/AMPK activity [46]. The inconsistency is that deficiency of miR-451 in nucleated erythroid cells results in apoptosis, rather than adaptation for survival, in numerous stress conditions, including deprivation of glucose in culture medium ([46]; unpublished data).

**Table 1** Transcription factors that regulate miR-144/451 expression

TF	Activity	Region (~bp)	Target Cell	Reference
GATA1	Activation	–2800	Erythroid cell	[16]
Myc	Repression	–400	B-lymphoma cell	[21]
PAX4	Repression	–2000	Breast cancer cell	[42]
GATA4	Activation	–512, –826	Cardiomyocyte	[43]
OCT1	Activation	–6600~8200	Glioblastoma cell	[45]
FXR	Activation	–3000 (human) -12,000 (mouse)	Hepatocyte	[47]
AP1	Activation	–1000	Neuron	[51]
SMAD3/4	Activation	–1000	Glioblastoma cell	[52]

Farnesoid X Receptor (FXR) is a nuclear receptor and controls many aspects of lipid metabolism. FXR binds response elements in the upstream sequence of miR-144/451 locus to activate miR-144/451 transcription, which results in high levels of miR-144/451 in hepatocytes followed by inhibition of miR-144 target, hepatic ATP binding cassette transporter A1 (ABCA1) [47]. ABCA1 is a positive regulator of plasma high-density lipoprotein (HDL) levels and decrease of ABCA1 reduces plasma HDL levels. FXR/miR-144 thus lowers plasma HDL that is considered cardioprotective [48].

In addition to GATA1, Myc, PAX4, GATA4, OCT1 and FXR, activator protein-1 (AP-1) also transcriptionally activates miR-144/451. ADAM10 can reduce the production of amyloid  $\beta$ -peptide (A $\beta$ ), the essential protein that causes Alzheimer's disease (AD). However, the ADAM10 expression is repressed in AD [49]. Interestingly, miR-144 is one of the few miRNAs that is consistently increased in the brain tissues of elderly people and AD patients [50], suggesting that high levels of miR-144 might be detrimental to neurons in brain tissues. Notably, A $\beta$  activates AP-1, and the latter binds to the recognition sites in the miR-144/451 promoter and transcribes miR-144/451, both of the miRNAs are capable of inhibiting the expression of the ADAM10 protein [51]. Thus, AP1 transcribed miR-144/451 is implicated in AD pathogenesis by direct downregulation of neuron protective ADAM10.

Both SMAD3 and SMAD4 reduce the growth of GBM A172 cells in vitro [52], and the inhibitory effect might be due to the enhanced miR-451 transcription. miR-144/451 promoter region (1135 bp from the miR-451 mature sequence) has been identified to contain binding elements for SMAD3 and SMAD4, with a 157 bp interval. Promoter activity assay reveals enhanced reporter activity in the presence of SMAD3 or/and SMAD4 in multiple glioma cell lines, suggesting that miR-451 expression is transcriptionally activated by SMAD3 and SMAD4 [52].

In summary, GATA1, Myc, PAX4, GATA4, OCT1, FXR, AP-1 and SMAD so far have been identified as transcription factors that occupy the promoters of the miR-144/451 locus to either activate or inhibit the expression of miR-144 and miR-451 (Fig. 2).

#### **miR-144/451 in erythrocyte development and red cell diseases**

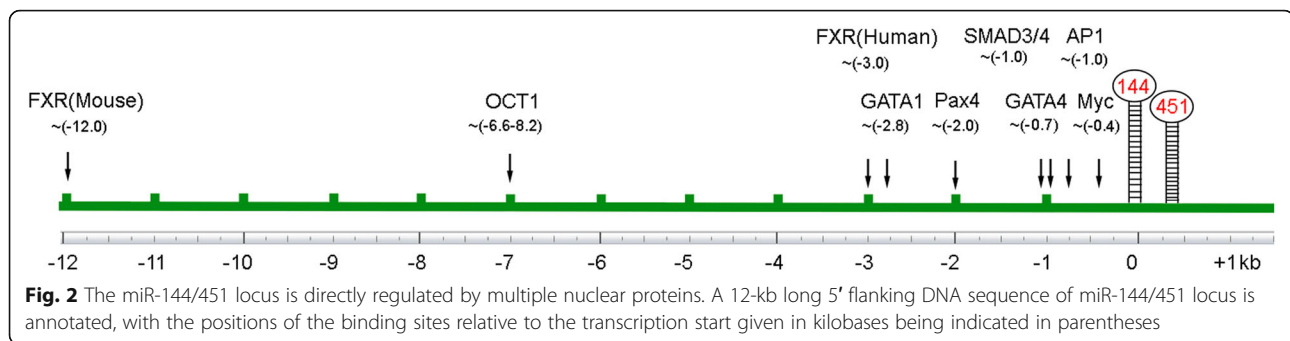
The levels of miR-144 and miR-451 are very abundant in erythroid cells [16, 29, 53, 54]. Over expression of miR-144 or miR-451 enhances maturation of murine erythroleukemic cells, whereas depletion of miR-451 has the opposite effect [29, 55]. Surprisingly, in vivo deletion of such abundantly expressed miR-144/451 genes only leads to mild hemolytic anemia [18–20, 46], partially

due to the erythrocytes' impaired capacity to remove reactive oxygen species (ROS) [19]. In animals, deletion of genomic miR-144/451 sequences elevates the level of miR-451 target gene (Ywhaz) product 14–3-3zeta, which sequesters the nuclear factor FoxO3 in the cytoplasm, thereby blocking transcription of anti-oxidant enzymes catalase (Cat) and glutathione peroxidase 1 (Gpx1) by FoxO3 [19]. This mechanism explains the hypersensitivity of miR-144/451 gene knockout erythrocytes to oxidants, since anemia is much worse upon oxidative stress generated by treating zebrafish with 1-phenyl-2-thiourea (PTU) [16, 19] and by treating mice with phenohydrazine (PHZ) [19, 46] or mature red blood cells with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Both PTU and PHZ are strong oxidants that induce rapid hemolysis of red cells.

A recent study demonstrates that erythroblasts isolated from miR-144/451 knockout mouse fetal liver or adult bone marrow and spleen exhibit more cell death during recovery from acute anemia, including PHZ-induced rapid destruction of erythrocytes, 5-fluorouracil (5-FU)-induced elimination of erythroid precursors, as well as acute blood loss by bleeding, suggesting that miR-144/451 is a main protector of erythroid cells in pathological conditions associated with not only oxidant stress but also other dramatically increased erythropoietic demand [46]. This effect is mediated by derepression of Cab39, a direct miR-451 target, followed by activation of the downstream effectors LKB1/AMPK. Interestingly, this anti-apoptotic effect of miR-451 is tumor suppressor p53-dependent. Ablation of p53 blocks the apoptosis of erythroblasts in miR-144/451 knockout animals [46]. However, miR-144/451 depletion via expression of decoy RNAs in adult mice [55] seems to inhibit erythropoiesis more severely than targeted deletion of the gene locus. This discrepancy is likely due to technical and/or biological differences between the two experimental approaches and illustrates the importance of using multiple modalities to assess miRNA function.

At least three groups have independently examined the effects of knockdown of miR-451 in zebrafish by injecting antisense morpholino (MO). Dore and Du et al. reported that treatment with anti-miR-451 MO caused severe anemia [16, 56], while Pase et al. found much milder erythroid phenotype with a similar treatment [28]. This discrepancy was not explained until Yu et al. demonstrated that deficiency of miR-451 expression in mice interferes FoxO3-mediated anti-oxidant signaling [19]. PTU is a melanin synthesis inhibitor that is used in Dore and Du's experiments to improve the visualization of zebrafish embryos by reducing the melanin in embryonic tissues. However, PTU is an oxidant that oxidizes hemoglobin in the embryos and thus synergizes the deleterious effect of miR-144/451 deficiency in zebrafish [19]. The enhanced loss of hemoglobin due to





PTU treatment confirms the anti-oxidative function of miR-451 in erythroid cells and also demonstrates that this function is well conserved in evolution.

miR-144 is coexpressed with miR-451 in a bicistronic primary transcript whose synthesis is directly activated by transcription factor GATA1 in erythroblasts [16]. However, the sequences of these two miRNAs are entirely different. Nevertheless, enforced expression of either miR-144 or miR-451 enhances maturation of murine erythroleukemic cells [29, 55], whereas depletion of either miR-144 or miR-451 using lentiviral-driven 'decoy' RNAs inhibits the maturation of hematopoietic stem/progenitor cells into mature erythroblasts [55]. More interestingly, miR-144 and miR-451 depletion additively inhibits erythropoiesis, suggesting the similar biological effects of miR-144 and miR-451, despite their different sequences, especially "seed" sequences that mediate the inhibition of distinct sets of target mRNAs [55]. However, this phenomenon has not yet been validated in gene knockout animals.

miR-144 also negatively regulates the expression of globin genes in zebrafish by targeting *klfd*, a Kruppel-like transcription factor that drives the transcription of embryonic alpha-globin. Specifically, miR-144 deficiency directly derepresses *klfd* expression, thereby promoting synthesis of embryonic alpha-globin [27, 56]. Interestingly, *klfd* inversely transactivates miR-144/451 expression in zebrafish, defining a feedback loop that appears to fine-regulate embryonic alpha-globin levels during erythropoiesis. Whether this regulatory axis also exists in mammalian erythropoiesis is unknown. A recent study demonstrates that high levels of miR-144 correlate with attenuated anti-oxidant capacity and thus more severe anemia in homozygous sickle cell disease (HbSS) erythrocytes [57]. This observation is certainly inconsistent with the findings that enforced expression of miR-144 enhances maturation of murine erythroleukemic cells [29], suggesting that miR-144 or miR-451 may function differently under different physiological and pathological conditions.

High miR-451 level is associated to the resistance of Malaria infection [58]. Malaria is one of the most

prevalent diseases in tropical and subtropical areas. Five species of the *Plasmodium* parasite can cause human malaria. World Health Organization (WHO) has reported that 99% of deaths in human malaria is caused by *Plasmodium falciparum* (*P. falciparum*) (World Malaria Report 2015, WHO). Human hemoglobin S (HbS) is a common hemoglobin variant in endemic areas of malaria and red blood cells containing HbS are confirmed resistant to malaria infection. Increased phagocytosis of sickle cells [59, 60] and decreased parasite invasion and growth [61] are among the factors that attribute to the resistance. Recent reports demonstrate that miR-451 is not only highly enriched in HbSS red cells [62] but also accumulated within the *P. falciparum* parasite [58, 63]. Moreover, high levels of miR-451 in sick erythrocytes contribute to malaria resistance. Specifically, high miR-451 levels in sickle red cells dramatically reduces the growth of *P. falciparum* through an unique non-canonical pathway in which miR-451 from host red cells enters *P. falciparum* and covalently forms a fusion transcript with mRNA of a subunit of the cAMP-dependent Protein Kinase (PKA-R) in parasites. The fused RNA impairs the translation of *P. falciparum* PKA-R, an essential enzyme for parasite growth [58]. These findings demonstrate that sickle erythrocytes can transfer their enriched miR-451 into parasites to suppress malaria infection. These findings, together with a recent report that dietary miR-451 (presumably from fish powder) is capable of passing through the gastrointestinal system into circulating blood to regulate the erythroid functions of mice [64], support the notion that miRNAs may perform cross-species gene regulation [65].

#### miR-144/451 in tumorigenesis

The levels of miR-144 or miR-451 in non-erythroid cells is very low; however, many studies have reported the deregulation of their expression and abnormal functions in various malignancies (Table 2). These two miRNAs participate in tumorigenesis with either oncogenic or tumor suppressive functions. The tumors associated with disrupted miR-144 or miR-451 expression and functions

**Table 2** The target genes and functions of miR-144 and miR-451 in tumorigenesis

miRNA	Tumor type	Target	Function	Reference
miR-451	B-cell lymphoma	c-Myc	suppressive	[21]
miR-144	B-cell lymphoma	BCL6	suppressive	[37]
miR-451	T cell acute lymphoblastic leukemia	c-Myc	suppressive	[34]
miR-451	multiple myeloma	TSC1	suppressive	[35]
miR-451	lung cancer	c-Myc	suppressive	[66]
miR-451	lung cancer	RAB14	suppressive	[67]
miR-451	lung cancer	Akt	suppressive	[68]
miR-451	lung cancer	c-Myc	suppressive	[32]
miR-451	glioblastoma	c-Myc	suppressive	[69]
miR-451	glioblastoma	Cab39	Conditional switching control	[70]
miR-451	glioblastoma	Akt1	suppressive	[71]
miR-451	glioblastoma	N/A <sup>a</sup>	suppressive	[52]
miR-451	breast cancer	14-3-3ζ	suppressive	[33]
miR-451	breast cancer	MDR1		[72]
miR-144	colorectal cancer	mTOR	suppressive	[73]
miR-451	colorectal cancer	MIF	suppressive	[74]
miR-451	colorectal cancer	MIF	suppressive	[75]
miR-451	colorectal cancer	14-3-3ζ	suppressive	[76]
miR-144	nasopharyngeal carcinoma	PTEN	oncogenic	[77]
miR-451	nasopharyngeal carcinoma	MIF	suppressive	[78]
miR-144	pancreatic carcinoma	Notch-1	suppressive	[79]
miR-144	insulinoma	PTEN	oncogenic	[80]
miR-451	liposarcoma		suppressive	[81]
miR-144/miR-451	breast cancer, head and neck carcinomas	ADAMs ADAMs	suppressive (invasion & metastasis)	[42] [42]
miR-451	ovarian and cervix cancers	MDR1	oncogenic	[82]

<sup>a</sup>Not available

include hematopoietic malignancies [21, 34, 35, 37], lung cancer [32, 66–68], glioblastoma [52, 69–71], breast cancer [33, 72], colorectal cancer [73–76], gastric cancer [74], nasopharyngeal cancer [77, 78], pancreatic cancer [79, 80], and other tumors [42, 81, 82]. However, no in vivo studies had confirmed such observations until a recent in vivo study demonstrated that the deficiency of miR-144/451 gene expression is a driver of B-lymphomagenesis though the tumor suppressive activity is weak by miR-144/451 alone [21]. Specifically, knockout of miR-144/451 locus initiates development of B-cell lymphoma and acute myeloid leukemia in aged mice with low incidence. The underlining mechanism is that the silenced oncogene Myc in normal hematopoietic cells is derepressed by the loss of miR-144/451 expression. Moreover, over-expressed Myc further maintains its own level by direct down regulation of miR-144/451, forming a miRNA-Myc feedback that is disrupted in B-cell lymphomas [21]. These data are consistent with the previous in vitro

or ex-in vivo findings that miR-144/451 are capable of inhibiting Myc expression [32, 34, 66]. The question remains whether such weak miRNAs could be a strong enhancer if other gene mutations exist. Inactivation of tumor suppressive genes and activation of pro-proliferative genes are usually required for rapid onset and high penetrance of many cancers [39]. Thus, it will be interesting to study whether loss of miR-144/451 sensitizes the deficiency of other gene expression for cancer development.

miR-144 and miR-451 as tumor biomarkers have been widely investigated. For example, miR-451 level is much lower in cancers from the digestive system [74, 83], non-small cell lung carcinoma (NSCLC) tissues [67, 68], and DLBCL [21]. While majority of studies indicate miR-144 or miR-451 as tumor suppressive miRNAs, several groups suggest that miR-144 or miR-451 inherits oncogenic activities [77, 80], which is consistent with the prognosis studies that glioblastoma patients with high miR-451 levels in tumor cells have significantly short

survival time [70] whereas patients with gastric cancer expressing lower miR-451 are associated with better outcomes [84]. These contradictory results imply that miR-451 may be a disease states-specific and tissue-specific tumor marker of diagnostic value. However, red blood cell contamination could be another explanation for these contradictory observations. Red blood cells express such high levels of miR-451 that miR-451 expression in non-erythroid cells is almost negligible. While any primary tumor is vascularized and contains large numbers of red cells, contamination of even a small number of red blood cells could affect the detection of miR-451 from cancer cells. Thus, serums may be a good source for miR-144 or miR-451 detection. Indeed, miR-451 has been investigated as diagnostic miRNA for human renal cell carcinoma [85], acute B-cell lymphoblastic leukemia [86] and gliomas [71].

Evidence has shown that miR-451 enhances the sensitivity of many types of malignancies to therapeutics. miR-451 restores the growth inhibitory effect of tamoxifen and doxorubicin in breast cancers [33, 72]. Treatment of osteosarcoma cells and NSCLC cells with miR-451 correlates with more positive response to conventional anti-cancer therapy [32, 68, 87]. However, contradictory results also have been reported that miR-451 decreases sensitivity of cancer cells to doxorubicin by increasing the levels of P-glycoprotein, the multidrug resistance 1 gene product that pumps chemotherapeutic drugs across the cell membrane to the outside of cancer cells [82]. Moreover, Decreased miR-451 expression by low glucose (a metabolic stress) inhibits cell proliferation [70, 88].

Another fundamental issue in miR-144/451 biology is that miR-144/451, especially miR-451, are considered erythroid lineage-specific genes whose expression are mainly limited to within erythrocytes and their precursors; in other words, whether low miR-144/451 expression can alter the path of normal development. A recent report has addressed this question by showing that a trace amount of miR-451 affects the clearance of ROS in red blood cells [64]. In this report, authors show that miR-144/451 gene knockout animals are capable of orally uptaking miR-451 to the blood stream from daily chow diet, presumably from fish powder in the food. Furthermore, these diet-derived miR-451 molecules, even at very low levels, can inhibit the expression of their target genes in mice. This result, together with the finding that miR-144/451 gene knockout animals develop non-erythroid malignancies including B-lymphoma, acute myeloid leukemia and benign liver adenoma [21], indicates clearly that large amounts of miRNAs are not necessary for sufficient regulation of cellular functions.

#### Function of miR-144/451 in additional tissues

Cardiac ischemia/reperfusion (I/R)-induced injury can be strongly protected by ischemic preconditioning (IPC). Interestingly, miR-144/451 are the most up-regulated miRNAs by IPC, suggesting a positive cardioprotective role of miR-144/451 [89]. Indeed, knockout of the miR-144/451 gene locus reduces IPC-linked cardioprotection [89]. This is similar to the findings that miR-144/451 protects cardiomyocytes against I/R-triggered or oxidative stress-induced cell death [43]. The latter function is particularly interesting in light of the similar functions in erythroid cells, which might occur through common mechanisms. However, uptake of high-fat diet (HFD) enriches miR-451 in mouse heart and the high levels of miR-451 contribute to the HFD-induced cardiac hypertrophy, a similar pathological alteration to diabetic cardiomyopathy, the cardiac hypertrophy and impaired cardiac function in type 2 diabetes in humans. The lipotoxicity of miR-451 in cardiomyocytes can be ameliorated by cardiomyocyte-specific miR-451 gene deletion in mice by directly activating the Cab39/LKB1/AMPK signaling [90]. The apparent opposite effects of miR-144/451 (alleviation of I/R-induced heart injury and promotion of HFD-induced cardiac hypertrophy) suggest that 1) miR-144 and miR-451, though produced from the same gene, might function differently; 2) the same miRNA could participate in gene regulation in a compartment-dependent or disease-dependent manner, where the latter is evidenced by our recent finding that miR-451 inhibits the same Cab39/LKB1/AMPK signaling pathway but with opposite cellular effects: protection of red cells against apoptosis [46] vs lipotoxicity in cardiomyocytes [90]. Another metabolism-related study demonstrates that HFD or diabetic condition induces the expression of hepatic miR-451 [91]. Glucose or insulin also upregulates miR-451 in hepatocytes, which leads to the inhibition of hepatic gluconeogenesis and thus low blood glucose levels [91]. Again, a negative feedback between glucose and miR-451 assures the maintenance of normal levels of blood glucose.

Construction and maintenance of epithelial cell polarity is a hallmark of normal tissue development. Disruption of cell polarity attributes to abnormal cell functions, including uncontrolled growth or progression towards malignancies [92]. One report has demonstrated that miR-451 is necessary for the development of epithelium polarity by promoting translocation of beta1 integrin to the basolateral membrane [93]. miR-451 also plays a role in autoimmune arthritis (RA). Neutrophils isolated from patients with RA contains lower miR-451 levels than those from healthy controls, and overexpression of miR-451 significantly inhibits neutrophil chemotaxis through p38 MAPK signaling and reduces the severity of arthritis in mouse RA models [94]. Erythropoietin

(EPO), a cytokine widely used for treating chronic anemia [95], limits the generation of tissue damage molecules and thus exerts remarkable neuroprotective effects in disease models including Alzheimer's disease (AD) and Parkinson's disease (PD) [96]. Interestingly, EPO treatment downregulates miR-451 expression, which enhances EPO-mediated anti-oxidant effects [97]. This is somewhat different from the finding in erythroid cells where miR-451 helps erythroid cells with reduction of oxidant stress [19]. This discrepancy may further confirm that miRNAs function in a tissue or cell compartment-specific manner.

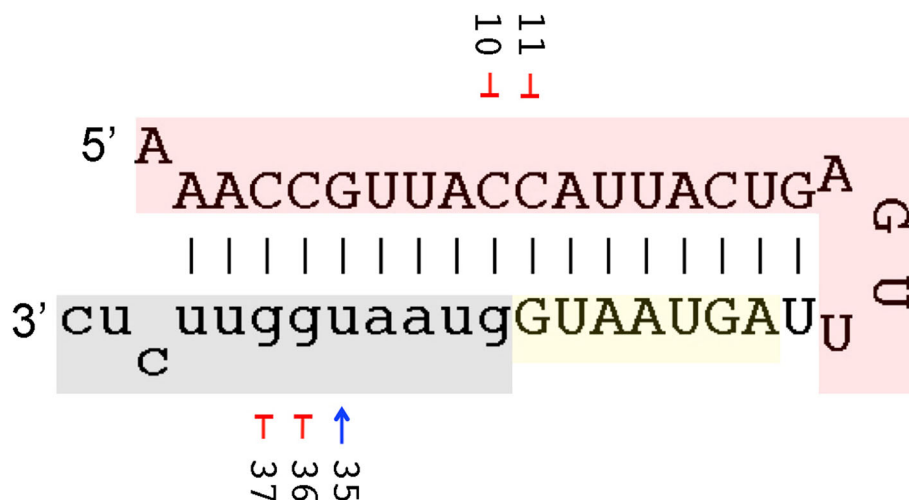
Like miR-451, miR-144 also involves the lipid metabolism [98]. The pathological characteristic of atherosclerosis is the accumulation of cholesterol in macrophages. Adenosine triphosphate-binding cassette transporter A1 (ABCA1) can facilitate the efflux of cholesterol to apolipoprotein A-I protein and increase HDL biogenesis in the liver to reduce cholesterol levels in macrophages. miR-144 directly inhibits hepatic ABCA1 expression both in vivo and in cultured cells, thereby attenuating cholesterol efflux from macrophages and reducing HDL levels in circulating blood [47, 98], suggesting its harmful role in the development of atherosclerosis. miR-144 also inhibits the antiviral response of animals. Ectopic expression of miR-144 in mouse lung epithelium facilitates increased replication of influenza virus, encephalomyocarditis virus and vesicular stomatitis virus [99]; ablation of miR-144/451 reduces the replication of influenza virus in the respiratory system and decreases the severity of pneumonia and bronchial damage [99]. Moreover, aberrant miR-144 expression is associated with the pathogenesis of psychiatric disorders. In fear extinction-impaired animals, over expression of miR-144

in the amygdala can successfully rescue fear extinction memory by targeting *Pten*, *Notch1* and *Spred1*, a group of genes important for the control of fear extinction [100], highlighting the significance of miR-144 for the memory-related activities.

### Biogenesis of miR-451 is dicer-independent

Mature miRNA generation proceeds via multiple pathways but shares a broad requirement for Dicer for trimming part of the hairpin sequence of the miRNA precursor. However, miR-451 seems to be the only miRNA whose precursors are cleaved by Ago2 rather than Dicer [22–24]. The primary transcript of miR-144/451 is a typical Drosha substrate, but the resultant miR-451 (but not miR-144) stem-loop precursor is too short (only 42 nt long in mice) that Dicer cannot cleave it. The hairpin instead directly enters Ago2 protein to produce a functional miR-451 molecule. miR-451 is dominant in erythroid cells and transcriptionally regulated by the nuclear protein GATA1. However, the miR-144 as a partner is not activated to the same level as miR-451 during erythroid maturation, suggesting that non-canonical biogenesis, together with differential stability of the miRNAs, may be the reason that the levels of miR-451 and miR-144 are different in erythroid cells.

This unique miRNA biogenesis pathway needs two requirements: first, the hairpin structure must be very short. Majority of miRNAs are about 60–70 nt long [2], but the miR-451 hairpin is only 42 nts in a diverse set of animals including humans, mice and zebrafish [101]; second, point mutations in 10C, 11C on 5' arm and 36G, 37G on 3' arm are strongly detrimental for mature miR-451 generation and function, while unpairing at the nt 35 U position on 3' stem enhances miR-451 function



**Fig. 3** Mutational analysis of miR-451 precursor. The pre-miR-451 hairpin contains the Ago2-cleaved byproduct (grey), the resected region (yellow), and the mature miR-451 (pink). The mutants are designated by their positions within the pre-miR-451 hairpin



[24, 101], indicating that a specific base-pairing in the hairpin structure is required for efficient splicing by Ago2 (Fig. 3).

Though the biogenesis of miR-451 occurs independently of Dicer and Ago2 cut the 3' stem of the miR-451 precursor, how the Ago2-cleaved intermediates (26-nt, 29-nt in length in human cells) are trimmed to the 22-nt long mature miR-451 is not clear. One recent observation reveals that poly A specific ribonuclease (PARN), an enzyme controlling the poly A tail length and mRNA stability in eukaryotes [102], is responsible for the shortening of Ago2-cleaved miR-451 precursor. Surprisingly, without shortening, the Ago2-cleaved miRNA intermediates still silence the target gene expression, indicating that once cleaved by Ago2, modification is dispensable for target silencing [103]. Ago2 belongs to a protein family that includes four Ago members, but only Ago2 is endonucleolytically active in mammalian cells [104]. Surprisingly, eIF1A as a translation initiation factor directly augments the Dicer-independent pre-miR-451 cleavage by Ago2, suggesting that eIF1A also facilitates miR-451 biogenesis [105].

#### Future perspective

Although strong evidence has shown the participation of miR-144/451 in erythropoiesis and tumorigenesis, our understanding of these two miRNAs during the last several years has not accelerated. Considering their pervasiveness, abundance and capability targeting diverse genes, we strongly believe that miR-144/451 must have more functions that we need but have yet to define. For example, it is commonly accepted that miRNAs are considered as “tuning” rather than “switching” genes. However, whether two different “tuning” miRNAs, especially from a single gene locus, are additive to become a “switching” gene, or whether two different “tuning” miRNAs from the same gene locus inherit completely opposite activities is still not clear. In addition, whether low levels of miR-144 or miR-451 coordinate with other gene products to dramatically alter the gene regulation capacities is also not known. Most likely, normal erythropoiesis and cell development of other tissues require dynamic regulation of miR-144/451 together with other genes at specific developmental stages or under particular physiological conditions. Addressing these questions will illustrate new general principles of miRNA biology. In this regard, comparison of the phenotypes in miR-144 and miR-451 single knockout mice with those of miR-144/451 double knockout animals upon various stresses or disease conditions will provide ample evidence for new functions of the miR-144/451 gene locus. Moreover, understanding how miR-144/451 impact disease development should identify new cellular pathways that are amenable to manipulation by standard

pharmacologic approaches. In addition, miR-144 or miR-451 shows promise as diagnostic biomarkers of the onset, progression and prognosis of many diseases, as long as the contamination of erythrocytes when accessing the miR-144 or miR-451 levels is excluded. miR-144 and miR-451 are also emerge as potential therapeutic candidates, which could benefit for not only particular anemias [106, 29], but also non-erythroid diseases [107, 108]. We conclude that miR-144 and miR-451, no matter the levels, regulate homeostasis and affect disease development both temporally and spatially.

#### Abbreviations

5-FU: 5-fluorouracil; ABCA1: Adenosine triphosphate (ATP)-binding cassette transporter A1; AD: Alzheimer disease; ADAM: A disintegrin and metalloproteinase; Ago: Argonaute; AMPK: AMP-activated kinase; AP-1: Activator protein-1; BCL6: B-cell lymphoma 6; Cat: Catalase; ChIP: Chromatin immunoprecipitation; DGCR8: DiGeorge Syndrome Critical Region 8; DLBCL: Diffuse large B-cell lymphomas; EPO: Erythropoietin; FL: Fetal liver; FXR: Farnesoid X receptor; GATA1: GATA binding protein 1; GATA4: GATA binding protein 4; GBM: Glioblastoma multiforme; Gpx1: Glutathione peroxidase 1; HbS: Human hemoglobin S; HbSS: Homozygous sickle cell disease; HDL: High-density lipoprotein; HFD: High-fat diet; I/R: Ischemia/reperfusion; IPC: Ischemic preconditioning; klf4: Krüppel-like transcription factor 4; MDR1: Multidrug resistance 1; MIF: Migration inhibitory factor; miRNA: microRNA; MO: Morpholino; mTOR: mammalian target of rapamycin; NSCLC: Non-small cell lung carcinoma; Oct1(POU2F1): Organic cation transporter 1 (POU domain, class 2, transcription factor 1); PARN: Poly(A)-specific ribonuclease; Pax4: Paired box gene 4; PD: Parkinson's disease; PHZ: Phenohydrazine; pre-miRNA: Precursor microRNA; pri-miRNA: Primary microRNA; PTEN: Phosphatase and tensin homolog; PTU: 1-phenyl-2-thiourea; RAB14: Ras-related protein Rab-14; RISC: RNA-induced silencing complex; ROS: Reactive oxygen species; shRNA: short-hairpin RNA; SMAD: Similar to mothers against decapentaplegic homolog; TSC1: Tuberous sclerosis complex 1; UTR: Untranslated region; YWHAZ: 14-3-3 protein zeta (14-3-3ζ)

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#### Availability of data and materials

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#### Authors' contributions

TW and FW searched the literature and participated in the preparation of the manuscript. DY wrote the manuscript. All authors read and approved the final manuscript.

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DY obtained his Ph.D. in Developmental Biology at the University of Wisconsin-Madison and finished his post-doctoral training at the University of Pennsylvania. DY has published over 50 peer-reviewed research articles including the ones listed as reference #16, 19, 21, 30, 31, 40, 46, 64 and 77 in this review and now is the Director & professor of the Jiangsu Key Laboratory of Experimental & Translational Non-coding RNA Research at the Yangzhou University School of Medicine, Jiangsu Province, China.

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