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The Emerging Role of miR-375 in Cancer

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Mini Review

MicroRNAs (miRNAs) are evolutionarily conserved, small noncoding RNAs that are believed to play fundamental roles in various biological processes through regulation of gene expression at the level of posttranscription. MiR-375 was first identified as a pancreatic islet-specific miRNA regulating insulin secretion. However, further study revealed that miR-375 is a multifunctional miRNA participating in pancreatic islet development, glucose homeostasis, mucosal immunity, lung surfactant secretion and more importantly, tumorigenesis. Recently, miR-375 has been found significantly downregulated in multiple types of cancer, and suppresses core hallmarks of cancer by targeting several important oncogenes like *AEG-1*, *YAP1*, *IGF1R* and *PDK1*. The alteration of miR-375 in cancer is caused by a variety of mechanisms, including the dysregulation of transcription factors, aberrant promoter methylation and so on. Reduced expression of miR-375 in tissue or circulation may indicate the presence of neoplasia as well as a poor prognosis of many malignant cancers. Moreover, miR-375 stands for a promising direction for developing targeted therapies due to its capacity to inhibit tumor cell growth *in vitro* and *in vivo*. Here, we summarize the present understanding of the tumor suppressive role of miR-375 in cancer progression; the mechanisms underlying the dysregulation of miR-375; the potential use of miR-375 in prognosis and diagnosis and the therapeutic prospects of miR-375 in cancer.

MicroRNAs (miRNAs) are small approximately 22 nucleotide single stranded noncoding RNAs. The biosynthesis of miRNAs is involved in miRNA gene transcription by RNA polymerase II (Pol II) and ribonuclease III (RNase III) processing within and outside the nucleus by Drosha and Dicer, respectively.[1,2] The mature miRNAs function by incorporating into RNA-induced silencing complex (RISC) and binding to partially complementary sites in the 3′ untranslated region (3′ UTRs) of messenger RNAs (mRNAs), which causes translation repression and, in some cases, the degradation of the target mRNA.[3–5] It is estimated that miRNA genes regulate the expression of as much as 30% of all protein-coding genes.[4,6] The discovery of miRNAs has opened up an entirely new area of posttranscriptional regulation of gene expression in living organisms. MiRNAs are often considered as fine-tuning regulators and are likely to be involved in almost all biological processes. Therefore, dysregulation of miRNA is involved in the pathogenesis of many human diseases, including human malignancies.[7–9] Accumulating evidence suggests that miRNAs act as oncogenes or tumor suppressors by targeting genes involved in cell differentiation, proliferation, survival, apoptosis and metastasis.[10–12] First, identified from murine pancreatic β-cell line MIN 6, miR-375 is characterized as a pancreatic islet-specific miRNA and regulates glucose-induced insulin secretion.[13] Further studies confirmed miR-375 participates in glucose homeostasis by controlling the growth and morphogenesis of the pancreatic islet.[14–18] However, genome-wide miRNA expression profiling studies

Key words: miR-375, tumor suppressor, microRNA, cancer

Abbreviations: AEG-1: astrocyte elevated gene-1 protein; ASH1: achaete-scute homolog 1; ATG7: autophagy-related protein 7; 5AzadC: 5-aza-2′-deoxycytidine; BC: breast cancer; bHLH: helix-loop-helix; Chol-miR-375: cholesterol-conjugated miR-375; CTCF: CCCTC-binding factor; EA: adenocarcinoma of the esophagus; ER α: estrogen receptor α; ESCC: esophageal squamous cell carcinoma; GC: gastric cancer; HCC: hepatocellular carcinoma; HNC: head and neck cancer; HNSCC: head and neck squamous cell carcinomas; IGF: insulin-like growth factor; IGF1R: insulin-like growth factor 1 receptor; JAK2: Janus kinase 2; 4-PBA: 4-phenylbutyric acid; PC: prostate cancer; Pdx1: pancreas/duodenum homeobox protein 1; PDK1: 3-phosphoinositide dependent protein kinase-1; Pol II: RNA polymerase II; RASD1: RAS, dexamethasone-induced 1; miRNA: microRNA; NeuroD1: neurogenic differentiation factor 1; RISC: RNA-induced silencing complex; RNase III: ribonuclease III; SP1: Sp1 transcription factor; TF: transcription factor; 3′UTR: 3′ untranslated region; YWHAZ: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide

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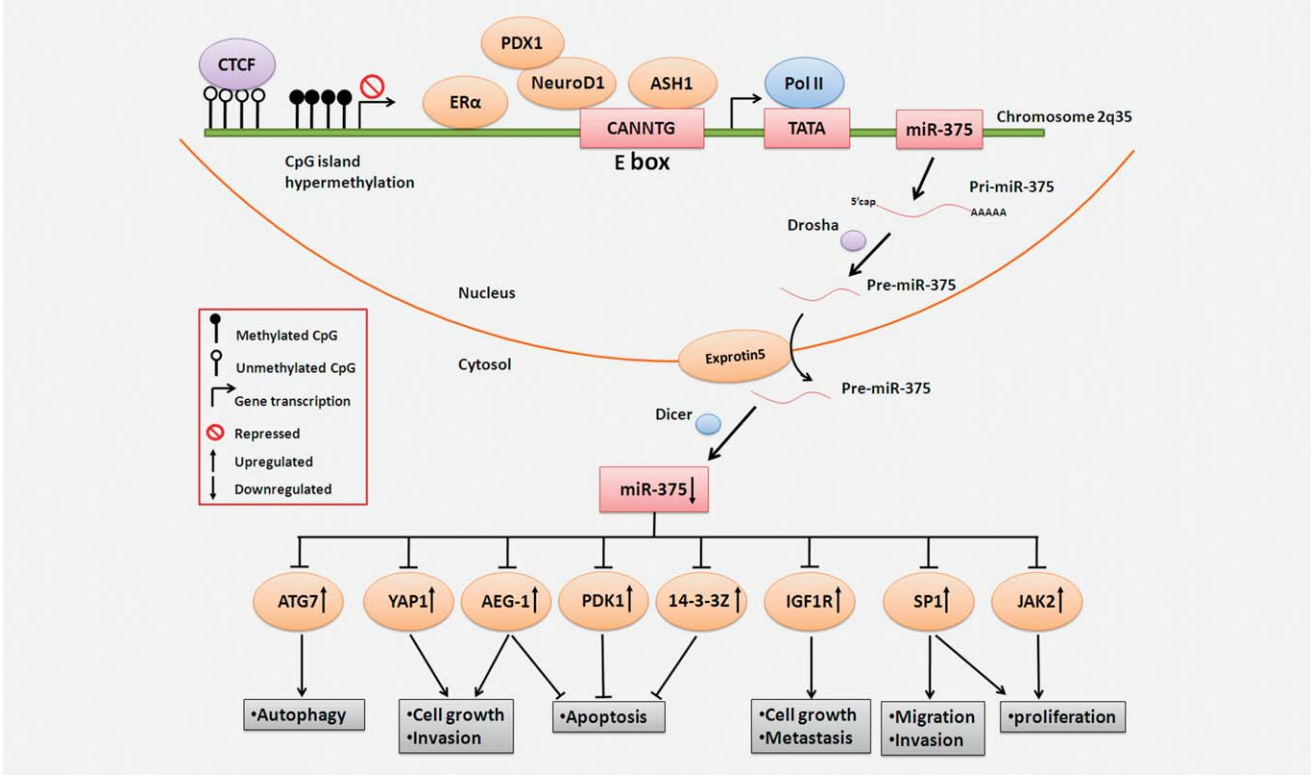

Figure 1. Tumor suppressive role and regulation of miR-375 in cancer. MiR-375 gene is transcribed by RNA polymerase II (Pol II) and then processed by ribonuclease III (RNase III) within and outside the nucleus by Drosha and Dicer, respectively. MiR-375 is significantly downregulated in multiple of cancers and acts as tumor suppressors by targeting important oncogenes, such as *AEG-1*, *PDK1*, *ATG7*, *IGF1R*, *JAK2*, *14-3-3Z*, *YAP1* and *SP1*, and moderates cancer-related processes such as cell proliferation, apoptosis, invasion and migration, metastasis and autophagy. DNA hypermethylation status of CpG islands embedded in miR-375 promoter and CTCF binding to unmethylated DNA block miR-375 transcription. Furthermore, miR-375 promoter contains essential TFs binding elements like TATA box and E-box. TATA box offers a binding site for pol II, which initiates miR-375 transcription. TFs characterized by bHLH motifs could bind to E-box and regulate miR-375 expression. For instance, ASH1 induces miR-375 expression in lung cancer, NeuroD1 and pancreas/duodenum homeobox protein 1 (pdx1) is responsible for miR-375 expression in pancreatic islet. ER α binds to miR-375 putative promoter and activates its transcription in breast cancer.

revealed that miR-375 is widely present in various of tissues or organs and is significantly reduced in malignant cell, for instance, hepatocellular carcinoma (HCC), esophageal carcinoma, gastric cancer (GC), head and neck cancer (HNC), melanoma and glioma.[19–23] It is becoming increasingly clear that miR-375 is an important cancer-related miRNA. Here, we review the current knowledge about the role of miR-375 on cancer development, the molecular mechanisms leading to aberrant expression of miR-375 in cancer and the potential clinical applications of miR-375 for diagnosis, prognosis and cancer treatment.

MiR-375 as a Tumor Suppressor

An increasing body of evidence has demonstrated that miR-375 is frequently downregulated in multiple types of cancer and acts as a tumor suppressor by inhibiting malignant properties of cancer cells (Fig. 1).

Hepatocellular carcinoma

Our team analyzed miRNA expression profiles in cancerous and normal hepatocytes and identified that miR-375 is one of the most significantly downregulated miRNAs in HCC. Expression levels of miR-375 in 60 pairs of HCC and adjacent nontumor tissues were also determined and the results showed consistency with that in cell lines.[19] Ectopic expression of miR-375 decreases liver cancer cell growth, invasion and induces G1 arrest and apoptosis.[19] We further demonstrated astrocyte elevated gene-1 (AEG-1, also named as MTDH) is a downstream direct target of miR-375 signaling pathway.[19] AEG-1 was found overexpressed and acts as an important oncogene in multiple types of cancer including HCC.[24–27] Luciferase reporter assay, mRNA quantification and western blotting showed miR-375 could directly target AEG-1 and repress its expression. MiR-375 and AEG-1 expression levels are inversely correlated in primary HCCs. Overexpression of AEG-1 rescued miR-375 ectopic expression effects. *In vivo* studies, administration of cholesterol-conjugated miR-375 (Chol-miR-375) impaired the growth of hepatoma xenografts. Therefore, we have detailed the tumor suppressive role of miR-375 in HCC.[19] Interestingly, further investigation of the function of miR-375 in HCC, we found that miR-375 could inhibit hypoxia induced autophagy,

consequently impairing the survival of HCC cells under hypoxic conditions. Under hypoxic stress, autophagy is activated and acts as a protective mechanism by enabling cells to break down cellular organelles and recycling catabolites for essential biosynthesis and energy supply.[28,29] MiR-375 could suppress the conversion of LC3I to LC3II and hence block the formation of autophagosome, which is fused with lysosome for degradation and recycling. In addition, autophagy-related protein 7 (ATG7), which is an essential component for autophagy and mediates ATGs conjugation and autophagosome formation, was identified to be a direct target of miR-375.[30] These findings provide a new insight into the role of miR-375 in tumor progression. Downregulation of miR-375 facilitates the survival of HCC cells by derepressing protective autophagy under hypoxia stress. Autophagy is important for regulating both cellular and whole body metabolism and sustains cancer cell survival by reutilizing self-digested nutrients under metabolic stress which is commonly seen in tumor lesion with poor vascularization.[31,32] Our team disclosed the link between miR-375 and autophagy and thus the metabolic role of miR-375 in cancer. Additionally, Liu et al. also found that miR-375 was significant downregulated in HCC and overexpression of miR-375 decreased HCC cell invasion and proliferation by targeting another important oncogene *YAP1*.[33] Based on the above knowledge gained in HCC, we could reach a firm conclusion that miR-375 functions as a tumor suppressor in liver cancer.

Gastric cancer

A large number of miRNAs have showed differential expression in GC tissues and have been characterized as proto-oncogenes or tumor suppressors for their impact on genes regulating cell cycle, apoptosis and metastasis.[34–36] MiR-375 was found among them. Ding et al. found that miR-375 is frequently downregulated in human GC and inhibits GC cell proliferation *via* targeting Janus kinase 2 (JAK2), a member of the Janus family of cytoplasmic nonreceptor tyrosine kinases.[20] Expression levels of JAK2 and miR-375 are inversely correlated in GC tissues. Inhibition of JAK2 activity by tyrphostin AG490 or silencing of JAK2 by RNAi suppresses GC cell proliferation, while overexpression of JAK2 dismisses the growth-suppressing effect of miR-375.[20] Tsukamoto et al. also found a tumor suppressive role of miR-375 in GC and identified two miR-375 targets in GC[37]: one is 3-phosphoinositide dependent protein kinase-1(PDK1 or PDPK1), which phosphorylates and activates protein kinase Akt and downstream signaling pathways and is firstly identified in pancreatic β-cells and mediates the repression of glucose induced insulin expression and β-cell proliferation by miR-375;[15,37] the other one is YWHAZ (also named 14-3-3Z), which belongs to the highly conserved 14-3-3 family of binding proteins and functions as an oncogene *via* associating with numerous proteins involved in mitosis, cell survival signaling, cell cycle and apoptosis.[38] Knockdown of YWHAZ and PDK1 by RNAi induces caspase activation in GC.[37]

Esophagus cancer

It is reported that miR-375 is one of the most downregulated miRNAs in esophagus cancers.[21,39] Mathé et al. detected the miRNA expression of cancerous and adjacent noncancerous tissue pairs in 100 adenocarinoma (ADC) and 70 squamous cell carcinoma (SCC) patients and found that miR-375 is most reduced miRNAs in cancerous tissue compared with noncancerous tissue, whereas, is sixfold higher in ADCs cancerous tissue compared with SCCs.[21] Kong et al. elucidated a tumor suppressive role of miR-375 *via* inhibiting cell proliferation of esophageal squamous cell carcinoma (ESCC), colony formation ability and metastasis *in vitro* and *in vivo* and identified a novel target insulin-like growth factor 1 receptor (IGF1R).[40] IGFs bind to IGF1R and induce receptor autophosphorylation activating downstream signaling pathways, for instance, PI3K-AKT/PKB signaling pathway. Moreover, Li et al reconfirmed PDK1 as a direct miR-375 target in ESCC.[39] Therefore, it is envisioned that miR-375 may be an important modulator of PI3K/Akt signaling pathway. As it has been proved that miR-375 is a critical regulator both in glucose metabolism and cancer biology, it is tempting to explore whether miR-375 is responsible for reprogrammed cancer cell metabolism. The most clarified reprogrammed cancer metabolism is known as the Warburg effect or aerobic glycolysis.[41–43] It is well studied that activation of PI3K-Akt signaling pathway promotes aerobic glycolysis by enhancing glucose uptake and the activity of glycolytic enzymes.[44] MiR-375 could directly repress PDK1, IGF1R and AEG-1, which all could activate PI3K-Akt signaling pathway,[45–47] and thus maybe suppress aerobic glycolysis that facilitate rapid growth of cancer cell.

Head and neck cancer

The HNC is a broad term including epithelial malignancies that derive from the paranasal sinuses, nasal cavity, oral cavity, pharynx and larynx. Almost all of the HNC are squamous cell carcinoma.[48] Substantial evidence has supported that miR-375 is one of the most downregulated miRNAs in head and neck squamous cell carcinoma (HNSCC),[22,49,50] which was summarized by Kinoshita et al.[51] Restoration of miR-375 expression suppresses cell proliferation and migration and invasion in HNSCC.[22,49,50] AEG-1 and LDHB have been proved as direct targets of miR-375 in HNC.[49,52] Therefore, all this findings highlight the tumor suppressive role of miR-375 in HNC.

Other tumors

Friboulet et al. reported that miR-375 is significantly reduced in excision repair cross-complementation group 1 (ERCC-1)-positive non–small-cell lung cancer (NSCLC) compared with ERCC-1-negative NSCLC.[53] Nishikawa et al. confirmed YAP1 is a miR-375 target in lung cancer, however, no clear growth inhibition effect is found in A549 cell line when stably introduced with a lentivirus expressing miR-375, implicating that

miR-375 function may vary in some cancers depending on the cellular context and transcriptomes.[54] In cervical cancer, Wang et al. observed 4.45-fold decrease in miR-375 expression compared with normal tissues and the expression of miR-375 and sp1 transcription factor (TF) (SP1) in cervical cancer tissues is inversely correlated. Further studies confirmed that miR-375 inhibits cell proliferation and cell migration and invasion, blocks G1-to-S cell-cycle transition in human squamous cervical cancer cell *via* repressing SP1.[55]

However, as the more knowledge we gain about miRNA, the more we realize that miRNA functions rely tightly on their cellular context. Indeed, despite the well characterized role of miR-375 as a tumor suppressor, there are some contradictory results should be mentioned in other tumor types such as breast cancer and prostate cancer where miR-375 is upregulated. Szczyrba et al. found that miR-375 is upregulated 9.1-fold in primary prostate carcinoma compared with normal prostate tissue using miRNA deep sequencing technology. However, it is unclear what role miR-375 may have played in prostate carcinoma.[56] In breast cancer, de Souza et al. found that miR-375 is upregulated in estrogen receptor α (ER α)-positive breast cancer cell lines,[57] which was later verified in 204 lymph-node negative breast cancer tissues by Jonsdottir et al.[58] MiR-375 and ERα form a positive feedback loop. MiR-375 regulates ER α by targeting RAS, dexamethasone-induced 1 (RASD1), which is a negative regulator of ER α, and ER α upregulates miR-375 by binding to its putative promoter and activating transcription. Downregulation of miR-375 by antisense RNA inhibits the proliferation of breast cancer cell line MCF-7 without inducing apoptosis.[58] Giricz et al. found that miR-375 is upregulated during lobular neoplasia progression accompanied by the loss of mammary acinar polarity and acquisition of a hyperplastic phenotype.[59] Thus, the evidence we acquired so far likely indicates a tumor-promoting role of miR-375 in breast cancer regardless of the obvious disparity in other tumors. It is quite tempting to clarify the cellular context that causes the contradictory role of miR-375 in breast cancer and prostate cancer.

To sum up, characterization of miR-375 in cancers has indicated that miR-375 mainly acts as a tumor suppressor, especially in digestive tumors. MiR-375 exerts its antitumor effect by repressing many critical oncogenes such as *PDK1*, *JAK2*, *IGF1R*, *AEG-1* and so on, implying that miR-375 may serve as a promising new direction for developing therapeutics against cancer.

Regulation of miR-375

Similar to the protein-encoding genes, the dysregulation of miRNAs can be caused by various mechanisms, including deletions, amplifications or mutations of miRNA encoding genes, epigenetic alteration or the dysregulation of TFs that target specific miRNAs.[60] Similarly, the deregulation of miR-375 in cancer is involved in some of the mechanisms mentioned above as well (Fig. 1).

Transcription factors

MiRNA genes are mainly transcribed by RNA Pol II, and share similar transcription pattern to that of protein coding genes.[61] It is not hard to predict that miRNA expression is controlled by TFs that promote or block the recruitment of Pol II to promoters. Although hundreds of miRNAs have been characterized in cancer, little is known about the transcriptional regulation of miRNAs. Human miR-375 is located on chromosome 2 between the *CRYBA2* and *CCDC108* genes and is transcribed from its own promoter. The region upstream of the pre-miR-375 sequence is found highly conserved, implicating the possibility of these sequences for TF binding and gene expression control. Many studies have found that miR-375 upstream sequence contains TF binding sites such as TATA box and E-box. TATA box has the core DNA sequence 5′-TATAAA-3′ to which TFs bind and get involved in the process of transcription by RNA polymerase, while E-box contains a specific DNA sequence, typically CACGTG, which can be recognized and bound by TFs usually containing the basic helix-loop-helix(bHLH) motif and mediates transcription initiation. Keller et al. identified two Neurogenic differentiation factor 1 (NeuroD1) binding sites (E-box) 500 bp upstream and 1700 bp downstream from miR-375 5′ end. Chromatin immunoprecipitation (ChIP) assays showed that NeuroD1 and pancreas/duodenum homeobox protein 1 (Pdx1) directly bind to two of the E-box in mouse NIT-1 cells.[62] Avnit-Sagi et al. further characterized the miR-375 gene promoter intensively in mouse. They identified 768 bp upstream of the pre-miR-375 sequence is highly conserved between mouse and human, and a transcription start site is embedded in 259 bases upstream of the pre-miR-375 start, 24 bp downstream from a TATA box. Besides, three conserved E-box elements were found in this region. Mutational analysis showed that mutation of these indentified elements caused 47–71% reduction in luciferase activity, which suggests that they exert strong effects on miR-375 gene transcription.[63] So it is believable that TFs binding to two of the elements mentioned above may serve as potential transcription regulators of miR-375 gene, though they may differ due to the varying context of tissues. Indeed, Nishikawa et al., reported Achaete-scute homolog 1 (ASH1), which is a member of the basic helix-loop-helix (bHLH) family of TFs, acts as another miR-375 regulator by binding to two of the five E-boxes found upstream of miR-375 gene in human A549 cell line.[54] However, despite the knowledge we have gained, little is known about how the TFs regulate miR-375 in cancer. Both TFs and miRNAs are important regulators of gene expression and act in different regulatory layers, transcriptionally and posttranscriptionally. It is known that miRNAs and TFs could regulate mutually and both could coregulate the same downstream target genes. The abnormal TF-miRNA regulatory networks have added to the signaling networks related to cancer. If miR-375 involves in any TFs-miRNAs regulatory loops is still obscure so far. Further studies to elucidate the aberrant miR-375-TFs networks in cancer are very necessary for better understanding of miR-375 functions in cancer.

DNA methylation

Epigenetic regulation is another important mechanism governing miRNA expression. It was reported that the promoter of miR-375 encoding gene harbors CpG islands (CGIs) where cytosines can be methylated to form 5-methylcytosine and leads to gene silencing. de Souza et al. identified two CGIs in human. The first CGI (CGI 1) lies about 2,000 bp upstream of miR-375 gene and has a size of approximately 700 bp, and the second CGI (CGI 2) runs across transcription start site and spans approximately 850 bp. Bisulfite sequencing showed that active transcription of miR-375 is characterized by a hypermethylated CGI 1, a hypomethylated CGI 2. CpG 18 of CGI 1 hypomethylation is responsible for repressed transcription of miR-375. Their further analysis found consensus binding sites for the CCCTC-binding factor (CTCF) in this region.[57] CTCF is a multifunctional zinc finger protein, and one of its activities is acting as a transcriptional activator or repressor. CTCF could bind to unmethylated DNA, and CGI methylation disrupts its binding effect.[64,65] Therefore, the theory is that the binding of CTCF to unmethylated DNA in CGI 1 blocks miR-375 transcription, while dissociation of CTCF from CGI 1 due to DNA methylation activates miR-375 transcription. This may also explain why miR-375 is upregulated in breast cancer.[58] Furuta et al. explored the miRNAs regulated by CGI methylation in HCC. They examined the expression levels of 11 frequently hypermethylated mature miRNAs and the methylation status of their CGIs in 19 HCC cell lines and noncancerous liver tissues.[66] The results showed that DNA methylation status around miR-375 is inversely correlated with the expression patterns of miR-375 in HCC cell lines.[66] Treating the HCC cell lines with 5-aza-2′-deoxycytidine (5AzadC), which is a hypomethylating agent, significantly restored miR-375 level.[66] CGIs around miR-375 is in low methylation pattern in nontumorous liver tissues where miR-375 is comparatively highly expressed, while methylation around miR-375 gene is not frequent in primary HCCs, with only 34.8% of 23 primary HCCs.[66] However, it should be mentioned that miR-375 gene has a promoter more than 700 bp, while Furuta et al. focused on the CGIs located within 500 bp around miR-375, in this reason, some important information was probably missed by them. Further studies to explore the regulatory role of CGIs in miR-375 promoter are required to clarify the epigenetically silenced miR-375 in HCC. Mazar et al. compared the miR-375 promoter methylation levels between normal skin and melanoma patient tissues, melanocytes, keratinocytes and melanoma cells, and found that DNA hypermethylation may account for the downregulation of miR-375 in melanoma. Treating a melanoma cell line WM1552C with 5AzadC and/or 4-phenylbutyric acid (4-PBA), shows significant upregulation of miR-375.[67] In GC, Du et al. reported 28.6–57.1% miR-375 promoter methylation of 94 specimens while Kong et al. reported 57.8% of 45 specimens in ESCC.[40,68] Therefore, these studies suggest that aberrant promoter methylation add to the reasons why miR-375 is frequently deregulated in different cancer types.

Glucose and cAMP

MiR-375 is enriched in pancreatic β-cells and regulates glucose-induced insulin secretion.[13] El Ouaamari et al. found evaluated glucose represses miR-375 expression in INS-1E cells, although the precise mechanism remains unclear. It is understandable that miR-375 may act as a glucose sensor and regulate insulin secretion and blood glucose. Keller et al. reported cyclic adenosine monophosphate (cAMP), an important modulator of glucose homeostasis, represses expression of pre-miR-375 at transcriptional level by activating PKA pathway. They believed that cAMP and glucose share some signaling pathways downstream of PKA in miR-375 regulation.[69] Nevertheless, it is unknown whether PKA pathway is responsible for the deregulation of miR-375 in cancer, as deregulated PKA pathway and the aberrantly activated downstream genes is linked to the growth of some cancers.[70–72]

MiR-375 as Diagnostic and Prognostic Biomarker

MiRNAs are differentially expressed in cancer which enables us to use them for diagnosis. MiRNA signatures provide an accurate method for cancer classification.[73–75] We have discussed the prevalent decrease in miR-375 in cancer, which indicates that miR-375 may serve as an ideal biomarker. Screening of primary tumors and biopsy samples have confirmed that miR-375 can be used to distinguish between malignant and benign hyperplasia in several organs. Avissar et al. reported the ratio of miR-221/miR-375 can accurately distinguish between nondiseased head and neck epithelial tissue and HNSCC tissue, with a sensitivity of 92% and specificity of 93%.[22] Wang et al combined miR-375, miR-424 and miR-92a to differentiate carcinomas from adenomas or high-grade intraepithelial neoplasms in colorectal specimens and found a satisfactory accuracy (89–94%).[76] Although tissue miRNA profiling could be an excellent diagnosis method, invasive procedures such as endoscopic biopsy, aspiration biopsy or even surgical biopsy are required to obtain specimens. Circulating miRNAs are stable and can be detected directly despite the existed ribonuclease in serum.[77] Recent studies have elucidated the stable existence of miRNAs in circulation. MiRNAs could be enveloped in microvesicles called exosomes protecting miRNAs from degradation.[78] MiRNAs could associate with Ago2, a component of RISC, and thus remain in extracellular space with high stability.[79] Circulating miRNAs could associate with proteins in plasma, for instance, high-density lipoprotein that assists miRNAs transportation in circulation.[80] Accumulating evidence has implied the possibility of miRNAs as diagnosis or prognosis biomarkers in cancer.[81–85] Then, it is of great significance to determine whether circulating miR-375 has a potential use in diagnosis and prognosis. Komatsu et al. analyzed serum miRNA expressions in 50 patients with ESCC and 20 healthy volunteers and found the circulating miR-21/miR-375 ratio might be a diagnostic marker in ESCC.[86] To develop circulating miRNA biomarkers for HCC, Li et al. found serum miR-25, miR-375 and let-7f as biomarkers to separate HBV-positive HCCs from

Table 1. Examples of miR-375 as prognostic biomarkers in human cancers

Cancer types	Prognostic miRNAs	Sources	Samples number	expression of miR-375	Prognosis or outcome of low miR-375	References
ESCC	miR-21, miR-375	Plasma	50	Down	Poor	89
PC	miR-375, miR-141	Serum	119	Up	Good	90
EA with Barrett's	miR-21, miR-375	Tissue	170	Down	Poor	91
ESCC	miR-375	Tissue	300	Down	Poor	92
GC	miR-375/miR-142-5p	Tissue	65	Down	Good	93
Glioma	miR-375	Tissue	138	Down	Poor	23
HNSCC	miR-375	Tissue	123	Down	Poor	50

Abbreviations: PC, prostate cancer; ESCC, esophageal squamous cell carcinoma; EA, adenocarcinoma of the esophagus; GC, gastric cancer; HNSCC, head and neck squamous cell carcinomas.

Table 2. The dysregulated expression and target genes of miR-375 in cancer

Type of cancer	miR-375 expression	Reasons for dysregulation in cancer	Validated target genes	Reference
HCC	Down	Hypermethylation	*AEG-1, ATG7, YAP1*	19, 30, 33, 66
GC	Down	Hypermethylation	*JAK2, PDK1, YWHAZ*	20, 38
ESCC	Down	Hypermethylation	*IGF1R,PDK1*	39, 40
HNSCC	Down	Hypermethylation	*AEG-1, LDHB*	49, 52, 96
Cervical cancer	Down	Hypermethylation	*SP1*	55, 97
Lung cancer	Down	ASH1	*YAP1*	54
Pancreatic cancer	Down	UD	*UD*	98
Colorectal cancer	Down	UD	*UD*	99
Melanoma	Down	Hypermethylation	*UD*	67
BC	Up	Methylation, ERα, CTCF	*RASD1*	57
PC	Up	UD	*Sec23A*	56

Abbreviations: ASH1: achaete-scute homolog 1; UD, undetermined; BC, breast cancer; PC, prostate cancer; HCC, hepatocellular carcinoma; GC, gastric cancer; ESCC, esophageal squamous cell carcinoma; HNSCC, head and neck squamous cell carcinomas; CTCF, CCCTC-binding factor.

healthy controls.[87] Yu et al. explored four miRNAs(miR-21, miR-486, miR-375 and miR-200b) in sputum to detect lung adenocarcinoma.[88] In addition, plenty of studies have focused on the potential use of miR-375 as prognostic biomarker, which is summarized in Table 1. Low levels of miR-375 in tissue and serum always indicates a poor prognosis in a majority of cancers. Of note, the circulating miRNAs levels do not strictly reflect the expression levels in tissue, as circulating miRNAs could also derive from other organs or even dead cells instead of primary tumor lesion.[79,94] These results indicate maybe a combination of a panel of miRNAs with miR-375 is better for diagnosis and prognosis prediction, improving the accuracy and specificity. However, the knowledge we have gained so far is still not enough to come to a conclusion whether miR-375 could be used as a potent biomarker for diagnosis or prognosis. Further characterization of miR-375 is urgently needed to realize its full clinical potential.

Therapeutic Potential

Therapeutics based on miRNAs replacement or antagonizing have shown incredible promise in the fight against cancer. Unlike RNAi, miRNAs are endogenously produced small size regulators of global gene expression, with the lowest side effect caused by introduction of exogenous nucleic acid. Furthermore, therapeutic modulation of a single miRNA may affect many pathways simultaneously to achieve excellent clinical benefit. Abundant evidence have disclosed that miR-375 is one of the most significantly downregulated miRNAs and plays an important tumor suppressive role in multiple types of cancer and miR-375 could inhibit cancer cell growth *in vitro* and *in vivo*.[20,30,40] Our team has explored the therapeutic potential of miR-375 in a HCC mouse model. We prepared cholesterol-conjugated 2′-O-methyl-modified miR-375 mimics (Chol-miR-375), which we demonstrated is chemically stable and could efficiently enter HCC cells independent of transfection agent. Administration of the chemically stabilized Chol-miR-375 could significantly inhibit the growth of tumor.[19] Moreover, we also showed that lentivirus mediated miR-375 overexpression could inhibit autophagy, impair the viability of HCC cells, and thus slowed down the growth of hepatoma xenografts in mice.[30] Ding et al. found that forced expression of miR-375 by eukaryotic expression plasmid with the help of transfection agent significantly inhibited GC cell proliferation *in vitro* and *in vivo*.[20] In ESCC, Kong et al.

demonstrated that stably transfected miR-375 precursor into ESCC cells could inhibit tumor formation in nude mice.[40] Therefore, the results provide a strong rationale for applying miR-375 analogues to treat cancer in the future. Even though, miRNAs based therapeutics has opened up broad prospects for cancer treatment, there is still a long way to go. Novel miRNA-based delivery systems should be established to overcome the low stability of synthetic RNA *in vivo*, and ensure tumor-specific delivery, and prolong the drug effect and reduce possible side-effects.[95]

Conclusion and Future Directions

So far, it has been established that miR-375 is frequently downregulated and functions mainly as a tumor suppressor in various cancers as summarized in Table 2. Many critical target genes of miR-375 have been indentified but the signaling pathways miR-375 involved in are still not fully elucidated. In the future, a comprehensive analysis of miR-375 target genes and the regulatory networks with the help of bioinformatics methods will be of great significance to further clarify the function of miR-375. Given that miR-375 regulates glucose metabolism, it is interesting to explore whether miR-375 contribute to reprogrammed cancer cell metabolism, for instance, Warburg effect. Besides, recent studies highlighted the diagnostic and prognostic potential of miR-375 in cancer, while whether miR-375 could be used as a therapeutic agent is still unclear, so one of our orientations in the future should concentrate on the clinical potential of miR-375.

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