Overview of microRNA-199a Regulation in Cancer

This article was published in the following Dove Press journal:
Cancer Management and Research

Qiwen Wang^{1,2,*}

Bingyu Ye^{1,2,*}

Ping Wang^{1,2}

Fenjie Yao^{1,2}

Chunyan Zhang^{1,2}

Guoying Yu^{1,2}

¹Henan International Joint Laboratory of Pulmonary Fibrosis, College of Life Science, Henan Normal University, Xinxiang 453007, Henan, People's Republic of China; ²State Key Laboratory Cell Differentiation and Regulation, College of Life Science, Henan Normal University, Xinxiang 453007, People's Republic of China

*These authors contributed equally to this work

Abstract: microRNAs (miRNAs) are a class of endogenous short, non-coding RNAs that regulate a multitude of genes at the post-transcriptional level. miR-199, which is a highly conserved miRNA family, consists of miR-199a and miR-199b. Researchers mainly focused on miR-199a over the past few years. Functional studies have demonstrated that mature miR-199a is a key player in the maintenance of normal homeostasis and in the regulation of disease pathogenesis. Here, we summarize the biological functions of miR-199a and review recent research on its roles in the physiological processes of cancer cells, such as proliferation, migration, invasion, apoptosis, autophagy and glycometabolism.

Keywords: microRNA-199a, target genes, carcinoma, biomarker, regulatory mechanisms

Introduction

miRNAs are conserved evolutionary single long non-coding small RNAs about 22 nt long, which regulate the expression of target genes by binding to the mRNA untranslated region (UTR) sequence in complete or incomplete complementary base pairing mode, resulting in restraint of translation or mRNA degradation.¹⁻⁴ MicroRNAs are involved in multiple cellular pathways, notably in fundamental processes, such as development, differentiation, proliferation, survival, and death. Combined with some classical markers, miRNAs are important indicators in evaluating the function of tissues and organs as well as the progression and prognosis of various cancers, such as hepatocellular carcinoma (HCC).⁵ Furthermore, recent studies have established that circulating miRNAs represent novel, predictable, and non-invasive biomarkers.^{6,7} As a member of the most important miRNA families, miR-199 has been reported to be implicated in a variety of carcinomas as either repressors or promoters. Recent evidence showed that miR-199 could even be used as biomarkers for the diagnosis and prognosis of cancer patients.^{8,9} This review focuses on biological functions of miR-199 and the mechanisms of miR-199 in cancer, such as the relationship with proliferation, apoptosis, autophagy and glucose metabolism. It also discusses challenges of miR-199 as biomarkers for diagnosis and prognosis or as therapeutic targets.

Overview of miR-199

Current studies have found two members of the miR-199 family: miR-199a and miR-199b.¹⁰ In 2003, Lagos-Quintana et al¹¹ cloned miR-199-s (from half of 5') and miR-199-as (from half of 3') from human osteoblast sarcoma cells and mouse skin tissues. Pre-miRNAs have two types in hsa-miR-199a: pre-miR-199a-1 (MI000242) and pre-miR-199a-2 (MI0000281), which are derived from chromosome 19 and chromosome 1, respectively, and are later renamed as miR-199a-5p

Correspondence: Chunyan Zhang;
Guoying Yu
College of Life Science, Henan Normal University, 46# East of Construction Road, Xinxiang, Henan 453007, People's Republic of China
Tel +86 373 3325654; +86 373 3326001
Fax +86 373 3326524
Email zcy1119sdc@163.com;
guoyingyu@htu.edu.cn

(MIMAT0000231) and miR-199a-3p (MIMAT0000232).¹² There are also two mature forms of hsa-miR-199b (derived from chromosome 9): miR-199b-5p (MIMAT0000263) and miR-199b-3p (MIMAT0004563) (miRBase, <http://www.miRbase.org>). At present, researchers mainly focus on the biological function of miR-199a. A large number of studies have indicated that the two mature types of miR-199a regulate the activities of normal cells to participate in corresponding physiological or pathological processes [Table 1](#). For example, miR-199a-5p is highly expressed in the breast, colon, and testis; relatively low in the thymus, liver, and kidney; and extremely low in the brain.¹³ miR-199a-5p is a negative regulator of proliferation of endometrial mesenchymal stem cells.¹⁴ In the striated muscle, overexpression of miR-199a-5p promotes myoblasts but not myotube proliferation, blunted the abnormal muscle fiber myogenic differentiation.¹⁵ However, in the lung, the high expression of miR-199a-5p promotes the formation of pulmonary fibrosis through the activation of the TGF- β signaling pathway by Caveolin-1.¹⁶ In cardiomyocytes, the functions of miR-199a mainly include: regulating cell size and proliferation. In regulating cell size, miR-199a-5p causes pathological hypertrophy of rat cardiomyocytes by down-regulating hypoxia-inducible factor 1 (HIF-1 α) and sirtuin1.¹⁷ However, endogenous silencing of miR-199a-5p causes hypertrophy through peroxisome proliferator-activated receptor γ co-activator 1 α (PGC1 α), but cardiac morphology and function are not affected.¹⁸ In terms of affecting cell proliferation, An et al found that after decellularization of the right atrium of mice, miR-199a-3p promotes the proliferation of neonatal cardiomyocytes and sinus nodal cells by inhibiting homeodomain-only protein (HOPX) and increasing GATA-binding

4 (Gata4) acetylation. After recellularization, miR-199a-3p mediates the enrichment of cardiomyocytes and sinus nodal cell population, restores the electrical activity as shown by normalization of electrocardiograph (ECG), and significantly improves myocardial function.¹⁹

Mechanism of miR-199a in Cancer miR-199a and Cell Proliferation, Migration and Invasion

Growing evidence shows that the aberrant expression of miR-199a is tightly related to tumorigenesis and progression [Table 2](#). miR-199a serves different functions in different cancer cells. For example, samples were obtained from 52 patients with gastric cancer tissue; results showed that miR-199a-3p expression is upregulated in 36 (69.2%) patients. miR-199a-3p is also highly expressed in human gastric cancer cell lines AGS, MKN-45, MKN-28, SGC-7901, NCI-N87, and BGC-823. Further study showed that miR-199a-3p inhibits the expression of zinc fingers and homeoboxes 1 (ZHX1) by binding its 3'UTR, and promotes the growth and proliferation of cancer cells.²⁰ Recent studies have proved that klotho is a tumor suppressor which is negatively associated with lymph node metastasis and epithelial-mesenchymal transition.^{21,22} He et al²³ found that miR-199a-5p, another form of miR-199a, is highly expressed in gastric cancer tissues, and promotes their migration and invasion by targeting klotho, indicating that klotho may be involved in tumorigenesis as an anti-tumor molecule. In situ hybridization further confirmed that high expression of miR-199a-5p may be closely associated with the lymph node metastasis of gastric cancer. Zhang et al identified 39 miRNAs that can target Smad4 from 388 miRNAs and found that miR-199a-5p is

Table 1 miR-199a Biological Function

Normal Tissues	miRNA	Expression	Targets	Effect of miRNA	Ref.
Uterus	miR-199a-5p	Downregulated	VEGFA	Inhibits the proliferation, movement, and angiogenesis of ectopic endometrial mesenchymal stem cells and alleviates the endometriosis	14
Striated muscle	miR-199a-5p	Downregulated	–	Promotes myoblasts proliferation and inhibit myogenic differentiation	15
Lung	miR-199a-5p	Upregulated	Caveolin-1	Promotes lung fibroblast proliferation, and differentiation	16
Cardiomyocytes	miR-199a-5p	Upregulated	HIF-1 α , Sirtuin1, PGC1 α /HOPX, Gata4	Regulates cell size/proliferation	17–19

Table 2 miR-199a and Proliferation, Migration and Invasion of Cancer Cell

Cancer Types	miRNA	Expression	Targets	Effect of miRNA	Ref.
Colorectal cancer	miR-199a-5p	Downregulated	HIF-1 α /VEGF/DDR1	Inhibits the cell proliferation, migration and invasion	31,32
Lung cancer	miR-199a-5p	Downregulated	HIF1 α	Inhibits the cell proliferation	33
Gastric cancer	miR-199a-3p	Upregulated	ZHX1	Promotes the cell proliferation	20
	miR-199a-5p	Upregulated	Klotho/Smad4	Promotes migration and invasion/Inhibits cell growth	23,24
ADPKD	miR-199a-5p	Upregulated	CDKN1C/p57	Promotes the proliferation of cyst cells	25
Early/Advanced cervical cancer	miR-199a-5p	Upregulated/ Downregulated	–	Promotes malignant transformation and lymph node metastasis	27,28
Melanoma	miR-199a-5p/3p	Upregulated	ApoE/DNAJA4	Promotes melanoma metastasis and angiogenesis	30
Esophageal cancer	miR-199a-5p	Downregulated	MAP3K11	Inhibits the proliferation	34
Breast cancer	miR-199a-3p/5p	Downregulated	Ets-1	Inhibits cell proliferation and cycle progression/ Reduces the risk of breast cancer	35–37
Cutaneous squamous cell carcinoma	miR-199a-5p	Downregulated	BCAM/FZD6/DDR1	Inhibits the migration of keratinocytes	40
Bladder cancer	miR-199a-5p/3p	Downregulated	Integrin 3	Inhibits cell migration and invasion, and is a potential prognostic marker in bladder cancer	38
HCC	miR-199a-5p	Downregulated	RGS17/FZD7	Inhibits the cell proliferation	43,44
	miR-199a-3p	Downregulated	mTOR, c-Met/PAK4	Reduces invasive capability, enhanced susceptibility to hypoxia or doxorubicin/inhibit the cell growth	41,42

upregulated in 67% of gastric cancer tissues (10/15), negatively regulates SMAD family member 4 (Smad4), and inhibits transforming growth factor beta (TGF- β) induced gastric cancer cell growth arrest in vitro; thus, miR-199a-5p plays a carcinogenic role in human gastric tumorigenesis and predicts potential therapeutic targets for future gastric cancer patients.²⁴ In autosomal dominant polycystic kidney disease (ADPKD), miR-199a-5p is upregulated, which promotes the proliferation of cyst cells by inhibiting the expression of cyclin-dependent kinase inhibitor 1C (CDKN1C)/p57 gene and plays a key role in the proliferation of cyst epithelial cells.²⁵ Moreover, high expression levels of miR-199a-3p and miR-199a-5p in esophageal adenocarcinoma are associated with poor prognosis in patients.²⁶ In cervical cancer, Lee et al²⁷ found that miR-199a-5p is significantly overexpressed in early squamous cell carcinomas of the cervix (SCCC), and then transfection of anti-miR-199a-5p oligonucleotides into cervical cancer cell lines in vitro (Siha and ME180) inhibits cell growth. Thus, miR-199a-5p is presumed to promote the

growth of cervical cancer cell lines. For advanced SCCC, however, Huang et al²⁸ found that the significant down-regulation of miR-199a-5p is significantly associated with lymph node metastasis and decreases prognosis survival in patients with SCCC. It is suggested that miR-199a-5p may be an important indicator for the prognosis and diagnosis of patients with SCCC. Interestingly, both miR-199a-3p and miR-199a-5p significantly differentiate between high metastatic and low metastatic groups of patients with uveal melanoma, with the former having higher expression.²⁹ Pencheva et al further observed that miR-1908 and miR-199a-3/5p cannot promote tumor cell proliferation but can regulate melanoma metastatic invasion, angiogenesis, and endothelial cell recruitment by targeting Apolipoprotein E (ApoE) and the heat-shock factor DnaJ heat shock protein family (Hsp40) member A4 (DNAJA4).³⁰

miR-199a also plays a negative regulatory role in the proliferation, migration and invasion of cancer cells Table 2. HIF-1 α is regulated by phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT) pathways. Ye et al³¹ found that miR-

199a-5p can inhibit the proliferation, migration and invasion of colorectal cancer cells via the HIF-1 α /vascular endothelial growth factor (VEGF) pathway. Another study identified that overexpression of miR-199a-5p results in the downregulation of discoidin domain receptor family, member 1 (DDR1), matrix metalloproteinase-2 (MMP2), N-cadherin and vimentin and up-regulation of E-cadherin, thereby reducing the colony formation, invasive and migratory capabilities of colorectal cancer cell lines LOVE1 and LOVO cells.³² miR-199a-5p also suppresses angiogenesis in hypoxic-treated non-small cell lung cancer cells by HIF1 α approach.³³ When overexpressed, miR-199a-5p can bind to mitogen-activated protein kinase kinase kinase 11 (MAP3K11), which results in the downregulation of MAP3K11, blunted G2/M arrest, and proliferation of esophageal cancer cells.³⁴ In breast cancer cells, miR-199a/b-3p is significantly decreased in highly metastatic breast cancer cells such as MDA-MB-231, CAL120, and HCC1395 compared with MCF-7, a weak metastatic breast cancer cell line. Further experiments confirmed that miR-199a/b-3p inhibits MDA-MB-231 cell proliferation and cell cycle progression by regulating the PAK4/MEK/ERK pathway.³⁵ Studies have shown that miR-199a-5p can downregulate the level of β -integrin by targeting the 3'-UTR of Ets-1 through the FAK/Src/Akt/mTOR signaling to alleviate breast cancer invasion.³⁶ Li et al also confirmed that miR-199a-5p inhibits the migration and invasion of MCF-7 and MDA-MB-231 by binding to Ets-1 in vitro.³⁷ Sakaguchi et al found that the expression levels of miR-199 family members (miR-199a-3p/-5p and miR-199b-3p/-5p) are downregulated in bladder cancer by targeting Integrin 3, and the survival rate of patients is significantly improved after the restoration of miR-199 expression.³⁸ Ecke et al further proposed that miR-199a-3p is an important indicator in evaluating the prognosis of patients with muscle-invasive bladder cancer after radical cystectomy.³⁹ The latest research shown that endogenous

miR-199a-5p targeted basal cell adhesion molecule (BCAM), frizzled class receptor 6 (FZD6) and DDR1, significantly inhibited the migration of skin keratinocyte instead of proliferation, indicating that miR-199a-5p also plays an important role in cutaneous squamous cell carcinoma.⁴⁰ It is basically a consensus that miR-199a presents downregulated tendency, and elevating levels of miR-199a may have therapeutic benefits and increase sensitivity to chemotherapy drugs in HCC.⁴¹ For example, miR-199a-3p can suppress HCC growth by targeting tumor-promoting the mammalian target of rapamycin (mTOR), tyrosine-protein kinase Met (c-met)⁴² and p21 (RAC1) activated kinase 4 (PAK4);⁴¹ Zhang⁴³ and Song⁴⁴ team also found that the overexpression of miR-199a-5p significantly suppresses the proliferation and survival of HCC cell by binding to the 3'-UTR of Regulators of G-protein signaling (RGS17) and frizzled type 7 receptor (FZD7), respectively. These studies suggest that two mature forms of miR-199a have different target genes in various tumors and exert different regulatory functions. miR-199 expression is an important cause of the proliferation, migration, or invasion of various tumor cells, but the regulatory effects of miR-199a are diverse and cannot be generalized. In addition, the role of SCCC is still controversial and needs further exploration. A variety of indicators, such as tolerance, different forms of miR-199a, duration of action, tissue cell specificity, drug resistance, and experimental standardization, must be considered before reaching a conclusion.

miR-199a and Apoptosis

The relationship between miR-199a and apoptosis is well documented; past studies showed that miR-199a can induce apoptosis either by up-regulating the level of pro-apoptotic protein or decreasing the expression of anti-apoptotic protein in most situations Table 3. Kim et al found that miR-199a-3p causes more pronounced apoptosis than miR-199a-5p in

Table 3 miR-199a and Apoptosis

Varieties	miRNA	Expression	Targets	Effect of miRNA	Ref.
DLBCL	miR-199a	Upregulated	–	No significant	45
A549	miR-199a-5p/3p	-	MET	Promote apoptosis	13
Bladder cancer	miR-199a-5p	Downregulated	MLK3	Promote apoptosis	46
Ovarian cancer	miR-199a-5p	-	mTOR	Promote apoptosis	47
HCC	miR-199a-5p	Downregulated	FZD7	Promote apoptosis	48
Cardiomyocyte	miR-199a-5p	Upregulated	HIF-1 α	Inhibit apoptosis	49
Colorectal cancer	miR-199a-3p	Upregulated	–	Inhibit apoptosis	50
SzS	miR-199a-3p	Upregulated	EVL	Inhibit apoptosis	51
UUO	miR-199a-3p	Upregulated	SOC57	Inhibit apoptosis	52

cancer cells, such as A549, PC3, KB, and MCF7. In A549 cells, the apoptosis pathway induced by miR-199a-5p is caspase-dependent, whereas that induced by miR-199a-3p is caspase-independent. After transfection with miR-199a-5p/3p mimetics, MET proto-oncogene and its downstream effector mitogen-activated protein kinase 1 (ERK2) are downregulated.¹³ Another study identified that the expression of seven miRNAs, including miR-199a, is upregulated in Diffuse Large B-cell Lymphoma (DLBCL). However, high expression of miR-199a is associated with higher overall survival ($p = 0.007$); moreover, miR-199a significantly enhances the killing effect of rituximab, vincristine, and doxorubicin chemotherapy on cancer cells in a dose-dependent manner. Interestingly, overexpression of the miR-199a does not result in any difference of cell growth or apoptosis in four lymphoma cell lines.⁴⁵ Song et al,⁴⁶ reported that miR-199a-5p induces apoptosis by inhibiting the Mixed Lineage Kinase 3 (MLK3)/NF- κ B pathway in bladder urothelial carcinoma. Similarly, miR-199a-5p increases the sensitivity of ovarian cancer OV2008 cells to cisplatin and promotes apoptosis by inhibiting the expression of mTOR.⁴⁷ In addition, Shi et al⁴⁸ demonstrated that a long non-coding RNA, microvascular invasion in hepatocellular carcinoma (MVIH), is generally overexpressed in HCC, si-MVIH treatment inhibited cell viability and promoted apoptosis of HCC, but this effect was reversed by miR-199a inhibitor. This indicates that miR-199a promotes tumor cell apoptosis.

However, in some cases, miR-199a involves in the anti-apoptosis effect. Research has also shown that miR-199a-5p is down-regulated and apoptosis is increased on a decline in oxygen tension of cardiac myocytes. Dual-luciferase reporting system assay revealed that HIF-1 α is a targeted gene of miR-199a-5p. The results also showed that silent information regulator1 (Sirt1) is a direct target of miR-199a-5p and is responsible for downregulating prolyl hydroxylase 2, which is required for the stabilization of HIF-1 α . This result indicates that miR-199a can inhibit cardiac myocytes apoptosis under hypoxic conditions.⁴⁹ Wan et al⁵⁰ found that the transfection of SW480 cells with miR-199a-3p inhibitor causes

G0/G1 arrest, decreases the percentage of cells in the S and G2/M phases, and induces cell apoptosis. In patients with Sezary Syndrome (SzS, T-cell lymphoma), upregulation of miR-199a-3p inhibits the expression of Enah/VASP-like (EVL), which in turn mediates its anti-apoptotic effect by up-regulating TNF superfamily member 11(TNFSF11).⁵¹ A recent study has also indirectly illustrated the inhibitory effect of miR-199a-3p on apoptosis. Yang et al demonstrated that kidney biopsies with IgA nephropathy and diabetic nephropathy exhibit substantial activation of p53 and increase in miR-199a-3p. Interestingly, p53 knockout attenuates renal fibrosis, apoptosis, and inflammation, accompanied by the suppression of miR-215-5p, miR-199a-5p/3p, and signal transducer and activator of transcription 3 (STAT3) in unilateral urethral obstruction (UUO) mice model. Furthermore, overexpression of miR-199a-3p suppresses suppressor of cytokine signaling 7 (SOCS7) for STAT3 activation and renal fibrosis. These results demonstrate the novel p53/miR-199a-3p/SOCS7/STAT3 pathway is involved in renal interstitial fibrosis.⁵²

miR-199a and Autophagy

Autophagy is a catabolic process initiated by the accumulation of damaged organelles or protein aggregates under cellular stress conditions.⁵³ Since the first report revealing the link between miR-30a and autophagy in 2009,⁵⁴ more and more miRNAs have been found to play an important role in autophagy. For example, miR-26a/b can regulate the formation of Atg1/unc-51 like autophagy activating kinase (ULK1) complex to inhibit autophagy and promote the apoptosis of HCC cells.⁵⁵ miR-155 and miR-7 negatively regulate PI3K/AKT signaling pathway to induce autophagy.⁵⁶ At present, some literatures have reported about the relationship between miR-199a and autophagy Table 4. Overexpression of miR-199a-5p can impair autophagy and activate the mTOR/GSK3 β signaling pathway; inhibit the activity of proteins, such as Atg5, Atg12, BECN1, and LC3B; and induce cardiac hypertrophy in mice.⁵⁷ Yi et al⁵⁸ also observed that miR-199a-5p exerts bidirectional regulation in breast cancer MCF7 and MDA-MB-231 cells. In specific, miR-199a-5p

Table 4 miR-199a and Autophagy

Varieties	miRNA	Expression	Targets	Effect of miRNA	Ref.
Cardiomyocyte	miR-199a-5p	Upregulated	mTOR	Inhibit autophagy	57
Breast cancer (MCF7/MDA-MB-231)	miR-199a-5p	–	DRAM1/BECN1	Inhibit/Activate autophagy	58
Ovarian cancer	miR-199a-5p	–	mTOR	Inhibit autophagy	62
HCC	miR-199a-5p	Downregulated	Atg7	Activate autophagy	65

mimic treatment significantly suppresses radiation-induced autophagy in MCF7 cells, and enhances radiation-induced autophagy in MDA-MB-231 cells. However, the target genes are the same in both cells: DNA damage regulated autophagy modulator 1 (DRAM1) and BECN1. BECN1 can bind to cofactors, such as Atg14L, UVRAG, Bif-1, Rubicon, Ambra1, HMGB1, nPIST, VMP1, SLAM, IP3R, PINK, and survivin, to regulate lipid kinase Vps-34 activity and promote the formation of the Beclin 1-Vps34-Vps15 complex; BECN1 can also form a complex with Bcl-2 through the BH3 domain to participate in the regulation of autophagy.⁵⁹ The differences in the regulation of autophagy by miR-199a-5p between the two types of breast cancer cells may be due to the different regulation effects of miR-199a-5p on the cell cycle. Treatment of MDA-MB-231 cells with miR-199a-5p mimic increases the ratio of G2/M phase cells, but does not significantly affect the cell cycle of MCF7. This result may be due to the different cytogenetic background because MDA-MB-231 is a highly aggressive and estrogen receptor-negative breast cancer cell line, whereas MCF7 is a non-invasive and estrogen receptor-positive breast cancer cell line. These studies suggest that miR-199a-5p exerts cell-specific effects on DRAM1 and Beclin1.

Activation of autophagy may be a necessary homeostasis process to remove damaged organelles and recover macromolecules to prevent cancer.^{60,61} Interestingly, it was observed that the expression levels of miR-199a-5p are higher in OV2008 cells (cisplatin-sensitive) compared with C13* cell (cisplatin-resistant), the reason was that miR-199a-5p increases the sensitivity of OV2008 cells to cisplatin by inhibiting mTOR, which is a negative regulator of autophagy.⁶² Similarly, Fornari et al⁴² confirmed that miR-199a-3p reduces invasive capability and enhances sensitivity to doxorubicin-induced apoptosis by targeting mTOR and c-Met in HCC cells. However, some researchers have shown that autophagy is also involved in the regulation of chemoresistance by attenuating the autophagy activity of cancer cells. Yoon et al⁶³ found that autophagy contributes to the sustained survival of breast cancer cells through DNA repair by the ATM-mediated activation of DNA-PKcs and PARP-1. In leukemia cells, endogenous high mobility group box-1 (HMGB1) activates autophagy and enhances the chemoresistance of leukemia cells through the PI3K/Akt/mTORC1/autophagy pathway.⁶⁴ The study found that miR-199a-5p expression is down-regulated in cisplatin-treated HCC patients. The possible reason is the reduction of miR-199a-5p enhances cell autophagy and promotes cell proliferation

by targeting Atg7/p62, thus increasing the development of drug resistance.⁶⁵ Overall, these findings provide evidence for the new role of miR-199a-5p in autophagy. Thus, this study may be used as a basis in understanding the autophagy networks regulated by miRNA and improving the strategies of cancer treatment.

miR-199a and Glucose Metabolism

Lactate overproduction is usually associated with poor prognosis. With the analysis of Genome Atlas and GSE10694 data, Guo et al⁶⁶ found that the expression of six types of glycometabolism-regulating miRNAs (miR-29c-3p, miR-126-3p, miR-148a-3p, miR-188-5p, miR-191-5p, and miR-199a-5p) is dysregulated in HCC. Moreover, only miR-199a-5p significantly inhibits lactate production in Huh-7, HepG2 and Hep3B cells by targeting hexokinase 2 (HK2). However, miR-199a-3p exerts no effect on lactate production in HCC cells. Conversely, up-regulation of HIF-1 α under hypoxia can inhibit the expression of miR-199a-5p and promote the glycolysis of HCC cells.⁶⁶ This study largely expanded our knowledge to further understand the role of miR-199a in the pathogenesis of cancer.

Problems and Prospects

The last ten years have brought a surfeit of new knowledge about how miRNAs are associated with disease states. Thus, we now understand better the links between the miR-199a and cancer. We also found that HIF-1 α is an important factor affecting the regulation of miR-199a-5p in cancer [Figure 1](#). This finding may be a breakthrough that can elucidate the mechanism of miR-199a in cancer, and future work is still needed.

Although considerable studies involving miR-199a have been conducted over the years, much research is still needed for the diagnosis and treatment of cancer and other diseases. One of the challenges is to identify the targets of miR-199a for each cancer type. At present, most studies only focused on the basic stage of miR-199a binding to target genes and their regulation. However, the studies *in vivo* are deficient, and the related signaling pathways and molecular mechanisms still need to be illustrated. In addition, miRNA has different UTR region binding sites for the target gene mRNA. Although miRNA mainly binds to the 3'-UTR of the target gene to inhibit its transcription level, it can also bind to the 5'-UTR of the target genes. This phenomenon may cause differences in miRNA-regulated target genes and affect their biological functions. With the increasing reports on the 5'-UTR binding of miRNAs target genes, relative bio-predictive tools have been

Figure 1 The relationship between HIF-1 α and miR-199a-5p.

developed successively.^{67,68} These tools are very helpful in exploring the mechanism of miR-199a in different contexts. Recently, great enthusiasm has evolved for extracellular vesicles, which are nanoscale membrane vesicles secreted by almost all cells, and can convey nucleic acids, proteins etc. from one cell/tissue to another.^{69,70} It has been demonstrated that differential expression of extracellular vesicles-derived miRNAs made them promising targets for detection and therapeutics to some diseases especially cancers.⁷¹ The latest study reported serum and extracellular vesicles miRNAs as biomarkers for pathology in acute graft-versus-host disease (aGvHD). Results showed that the miR-423, miR-199, and miR-93 levels of serum extracellular vesicles are lower at D14 in post hematopoietic stem cell transplantation patients who later developed aGvHD; conversely, these miRNAs show higher expression in D14 patients remaining aGvHD-free in the extracellular vesicles fraction.⁷² This study has largely expanded our horizons to further understand the pathogenesis of diseases and implies that miR-199a derived from extracellular vesicles is an important indicator in cancers. Future works using molecular biology techniques will be helpful to elucidate the mechanism of miR-199a in cancers and explore the potential targets of miR-199a for diagnosing and treating cancers.

Acknowledgments

This study was financially supported by Natural Science Foundation of China (31572270); the Key Scientific

Research Projects of Henan Higher Education (19A180019); the National Fostering Science Foundation Project of Henan Normal University (2016PL21), and the Postdoctoral research grant in Henan Province (001803040).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Tripathi A, Goswami K, Sanan-Mishra N. Role of bioinformatics in establishing microRNAs as modulators of abiotic stress responses. *Front Physiol.* 2015;6:286. doi:10.3389/fphys.2015.00286
2. Lai EC. Micro RNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat Genet.* 2002;30:363–364. doi:10.1038/ng865
3. Lee I, Ajay SS, Yook JI, et al. New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites. *Genome Res.* 2009;19:1175–1183. doi:10.1101/gr.089367.108
4. Lytle JR, Yario TA, Steitz JA. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5' UTR as in the 3' UTR. *Proc Natl Acad Sci U S A.* 2007;104:9667–9672. doi:10.1073/pnas.0703820104
5. Hayes C, Chayama K. MicroRNAs as biomarkers for liver disease and hepatocellular carcinoma. *Int J Mol Sci.* 2016;17:280. doi:10.3390/ijms17030280
6. do Amaral AE, Cisilotto J, Creczynski-Pasa T, de Lucca Schiavon L. Circulating miRNAs in nontumoral liver diseases. *Pharmacol Res.* 2018;128:274–287. doi:10.1016/j.phrs.2017.10.002
7. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov.* 2017;16:203. doi:10.1038/nrd.2016.246

8. Li S-D, Zhang J-R, Wang Y-Q, Wan X-P. The role of microRNAs in ovarian cancer initiation and progression. *J Cell Mol Med*. 2010;14:2240–2249. doi:10.1111/j.1582-4934.2010.01058.x
9. Fan X, Zhou S, Zheng M, Deng X, Yi Y, Huang T. MiR-199a-3p enhances breast cancer cell sensitivity to cisplatin by downregulating TFAM (TFAM). *Biomed Pharmacother*. 2017;88:507–514. doi:10.1016/j.biopha.2017.01.058
10. Jiang X-P, Ai W-B, Wan L-Y, Zhang Y-Q, Wu J-F. The roles of microRNA families in hepatic fibrosis. *Cell Biosci*. 2017;7:34. doi:10.1186/s13578-017-0161-7
11. Lagos-Quintana M, Rauhut R, Meyer J, Borkhardt A, Tuschl T. New microRNAs from mouse and human. *RNA*. 2003;9:175–179. doi:10.1261/rna.2146903
12. Gu S, Chan WY. Flexible and versatile as a chameleon-sophisticated functions of microRNA-199a. *Int J Mol Sci*. 2012;13:8449–8466. doi:10.3390/ijms13078449
13. Kim S, Lee UJ, Kim MN, et al. MicroRNA miR-199a* regulates the MET proto-oncogene and the downstream extracellular signal-regulated kinase 2 (ERK2). *J Biol Chem*. 2008;283:18158–18166. doi:10.1074/jbc.M800186200
14. Hsu CY, Hsieh TH, Tsai CF, et al. Mirna-199a-5p regulates VEGFA in endometrial mesenchymal stem cells and contributes to the pathogenesis of endometriosis. *J Pathol*. 2014;232:330–343. doi:10.1002/path.4295
15. Alexander MS, Kawahara G, Motohashi N, et al. MicroRNA-199a is induced in dystrophic muscle and affects WNT signaling, cell proliferation, and myogenic differentiation. *Cell Death Differ*. 2013;20:1194–1208. doi:10.1038/cdd.2013.62
16. Lino Cardenas CL, Henaoui IS, Courcot E, et al. miR-199a-5p is upregulated during fibrogenic response to tissue injury and mediates TGF beta-induced lung fibroblast activation by targeting caveolin-1. *PLoS Genet*. 2013;9:e1003291. doi:10.1371/journal.pgen.1003291
17. Song XW, Li Q, Lin L, et al. MicroRNAs are dynamically regulated in hypertrophic hearts, and miR-199a is essential for the maintenance of cell size in cardiomyocytes. *J Cell Physiol*. 2010;225:437–443. doi:10.1002/jcp.22217
18. Li Z, Liu L, Hou N, et al. miR-199-sponge transgenic mice develop physiological cardiac hypertrophy. *Cardiovasc Res*. 2016;110:258–267. doi:10.1093/cvr/cvv052
19. An M, Kwon K, Park J, et al. Extracellular matrix-derived extracellular vesicles promote cardiomyocyte growth and electrical activity in engineered cardiac atria. *Biomaterials*. 2017;146:49–59. doi:10.1016/j.biomaterials.2017.09.001
20. Wang Z, Ma X, Cai Q, et al. MiR-199a-3p promotes gastric cancer progression by targeting ZHX1. *FEBS Lett*. 2014;588:4504–4512. doi:10.1016/j.febslet.2014.09.047
21. Zhu Y, Xu L, Zhang J, et al. Klotho suppresses tumor progression via inhibiting PI3K/Akt/GSK3 β /Snail signaling in renal cell carcinoma. *Cancer Sci*. 2013;104:663–671. doi:10.1111/cas.12134
22. Usuda J, Ichinose S, Ishizumi T, et al. Klotho is a novel biomarker for good survival in resected large cell neuroendocrine carcinoma of the lung. *Lung Cancer*. 2011;72:355–359. doi:10.1016/j.lungcan.2010.10.008
23. He XJ, Ma YY, Yu S, et al. Up-regulated miR-199a-5p in gastric cancer functions as an oncogene and targets klotho. *BMC Cancer*. 2014;14:218. doi:10.1186/1471-2407-14-218
24. Zhang Y, Fan KJ, Sun Q, et al. Functional screening for miRNAs targeting Smad4 identified miR-199a as a negative regulator of TGF- β signalling pathway. *Nucleic Acids Res*. 2012;40:9286–9297. doi:10.1093/nar/gks667
25. Sun L, Zhu J, Wu M, et al. Inhibition of MiR-199a-5p reduced cell proliferation in autosomal dominant polycystic kidney disease through targeting CDKN1C. *Med Sci Monit*. 2015;21:195–200. doi:10.12659/MSM.892141
26. Feber A, Xi L, Pennathur A, et al. MicroRNA prognostic signature for nodal metastases and survival in esophageal adenocarcinoma. *Ann Thorac Surg*. 2011;91:1523–1530. doi:10.1016/j.athoracsur.2011.01.056
27. Lee J-W, Choi CH, Choi J-J, et al. Altered microRNA expression in cervical carcinomas. *Clin Cancer Res*. 2008;14:2535–2542. doi:10.1158/1078-0432.CCR-07-1231
28. Huang L, Lin J-X, Yu Y-H, Zhang M-Y, Wang H-Y, Zheng M. Downregulation of six MicroRNAs is associated with advanced stage, lymph node metastasis and poor prognosis in small cell carcinoma of the cervix. *PLoS One*. 2012;7:e33762. doi:10.1371/journal.pone.0033762
29. Worley LA, Long MD, Onken MD, Harbour JW. Micro-RNAs associated with metastasis in uveal melanoma identified by multiplexed microarray profiling. *Melanoma Res*. 2008;18:184–190. doi:10.1097/CMR.0b013e3282feeac6
30. Pencheva N, Tran H, Buss C, et al. Convergent multi-miRNA targeting of ApoE drives LRP1/LRP8-dependent melanoma metastasis and angiogenesis. *Cell*. 2012;151:1068–1082. doi:10.1016/j.cell.2012.10.028
31. Ye H, Pang L, Wu Q, et al. A critical role of miR-199a in the cell biological behaviors of colorectal cancer. *Diagn Cytopathol*. 2015;10:65. doi:10.1186/s13000-015-0260-x
32. Hu Y, Liu J, Jiang B, et al. MiR-199a-5p loss up-regulated DDR1 aggravated colorectal cancer by activating epithelial-to-mesenchymal transition related signaling. *Dig Dis Sci*. 2014;59:2163–2172. doi:10.1007/s10620-014-3136-0
33. Ding G, Huang G, Liu HD, et al. MiR-199a suppresses the hypoxia-induced proliferation of non-small cell lung cancer cells through targeting HIF1 α . *Mol Cell Biochem*. 2013;384:173–180. doi:10.1007/s11010-013-1795-3
34. Byrnes KA, Phatak P, Mansour D, et al. Overexpression of miR-199a-5p decreases esophageal cancer cell proliferation through repression of mitogen-activated protein kinase kinase kinase-11 (MAP3K11). *Oncotarget*. 2016;7:8756–8770. doi:10.18632/oncotarget.6752
35. Shou-Qing L, Zi-Hang W, Xu-Guang M, Lei L, Yan T. MiR-199a/b-3p suppresses migration and invasion of breast cancer cells by down-regulating PAK4/MEK/ERK signaling pathway. *IUBMB Life*. 2015;67:768–777. doi:10.1002/iub.1433
36. Li W, Wang H, Zhang J, Zhai L, Chen W, Zhao C. miR-199a-5p regulates beta1 integrin through Ets-1 to suppress invasion in breast cancer. *Cancer Sci*. 2016;107:916–923. doi:10.1111/cas.12952
37. Wentong L, Hui W, Jinbao Z, Limin Z, Weijuan C, Chunling Z. miR-199a-5p regulates β 1 integrin through Ets-1 to suppress invasion in breast cancer. *Cancer Sci*. 2016;107:916–923. doi:10.1111/cas.12952
38. Sakaguchi T, Yoshino H, Yonemori M, et al. Regulation of ITGA3 by the dual-stranded microRNA-199 family as a potential prognostic marker in bladder cancer. *Br J Cancer*. 2017;116:1077–1087. doi:10.1038/bjc.2017.43
39. Ecker TH, Stier K, Weickmann S, et al. miR-199a-3p and miR-214-3p improve the overall survival prediction of muscle-invasive bladder cancer patients after radical cystectomy. *Cancer Med*. 2017;6:2252–2262. doi:10.1002/cam4.1161
40. Kim B, Kim I, Yoon S. Identification of miR-199a-5p target genes in the skin keratinocyte and their expression in cutaneous squamous cell carcinoma. *J Dermatol Sci*. 2015;79:137–147. doi:10.1016/j.jdermsci.2015.05.005
41. Hou J, Lin L, Zhou W, et al. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell*. 2011;19:232–243. doi:10.1016/j.ccr.2011.01.001
42. Fornari F, Milazzo M, Chicco P, et al. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocellular carcinoma cells. *Cancer Res*. 2010;70:5184–5193. doi:10.1158/0008-5472.CAN-10-0145
43. Zhang W, Qian S, Yang G, et al. MicroRNA-199 suppresses cell proliferation, migration and invasion by downregulating RGS17 in hepatocellular carcinoma. *Gene*. 2018;659:22–28. doi:10.1016/j.gene.2018.03.053
44. Song J, Gao L, Yang G, et al. MiR-199a regulates cell proliferation and survival by targeting FZD7. *PLoS One*. 2014;9:e110074. doi:10.1371/journal.pone.0110074

45. Troppan K, Wenzl K, Pichler M, et al. miR-199a and miR-497 are associated with better overall survival due to increased chemosensitivity in diffuse large b-cell lymphoma patients. *Int J Mol Sci*. 2015;16:18077–18095. doi:10.3390/ijms160818077
46. Song T, Zhang X, Yang G, Song Y, Cai W. Decrement of miR-199a-5p contributes to the tumorigenesis of bladder urothelial carcinoma by regulating MLK3/NF-kappaB pathway. *Am J Transl Res*. 2015;7:2786–2794. doi:10.1107/S0021889898010152
47. Wang Z, Liu Q, Gao Q, Luo Y, Li Q, Chen J. Involvement of miR-199a in cisplatin resistance of ovarian cancer cell through modulating expression of mTOR. *Natl Med J China*. 2015;95:2705–2708. doi:10.3760/cma.j.issn.0376-2491.2015.33.013
48. Shi Y, Song Q, Yu S, Hu D, Zhuang X. Microvascular invasion in hepatocellular carcinoma overexpression promotes cell proliferation and inhibits cell apoptosis of hepatocellular carcinoma via inhibiting miR-199a expression. *Oncotargets Ther*. 2015;8:2303–2310. doi:10.2147/OTT.S86807
49. Rane S, He M, Sayed D, et al. Downregulation of miR-199a derepresses hypoxia-inducible factor-1alpha and sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. *Circ Res*. 2009;104:879–886. doi:10.1161/CIRCRESAHA.108.193102
50. Wan D, He S, Xie B, et al. Aberrant expression of miR-199a-3p and its clinical significance in colorectal cancers. *Med Oncol*. 2013;30:378. doi:10.1007/s12032-012-0378-6
51. Ballabio E, Mitchell T, van Kester MS, et al. MicroRNA expression in sezary syndrome: identification, function, and diagnostic potential. *Blood*. 2010;116:1105–1113. doi:10.1182/blood-2009-12-256719
52. Yang R, Xu X, Li H, et al. p53 induces miR199a-3p to suppress SOCS7 for STAT3 activation and renal fibrosis in UUO. *Sci Rep UK*. 2017;7:43409. doi:10.1038/srep43409
53. Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: the clash between damage and metabolic needs. *Cell Death Differ*. 2015;22:377–388. doi:10.1038/cdd.2014.150
54. Zhu H, Wu H, Liu X, et al. Regulation of autophagy by a beclin 1-targeted microRNA, miR-30a, in cancer cells. *Autophagy*. 2009;5:816–823. doi:10.4161/auto.9064
55. Jin F, Wang Y, Li M, et al. MiR-26 enhances chemosensitivity and promotes apoptosis of hepatocellular carcinoma cells through inhibiting autophagy. *Cell Death Dis*. 2017;8:e2540. doi:10.1038/cddis.2016.461
56. Frankel LB, Lund AH. MicroRNA regulation of autophagy. *Carcinogenesis*. 2012;33:2018–2025. doi:10.1093/carcin/bgs266
57. Li Z, Song Y, Liu L, et al. miR-199a impairs autophagy and induces cardiac hypertrophy through mTOR activation. *Cell Death Differ*. 2017;24:1205–1213. doi:10.1038/cdd.2015.95
58. Yi H, Liang B, Jia J, et al. Differential roles of miR-199a-5p in radiation-induced autophagy in breast cancer cells. *FEBS Lett*. 2013;587:436–443. doi:10.1016/j.febslet.2012.12.027
59. Kang R, Zeh HJ, Lotze MT, Tang D. The beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ*. 2011;18:571. doi:10.1038/cdd.2010.191
60. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature*. 2008;451:1069–1075. doi:10.1038/nature06639
61. Jing Z, Han W, Sui X, Xie J, Pan H. Interaction of autophagy with microRNAs and their potential therapeutic implications in human cancers. *Cancer Lett*. 2015;356:332–338. doi:10.1016/j.canlet.2014.09.039
62. Wang Z, Ting Z, Li Y, Chen G, Lu Y, Hao X. microRNA-199a is able to reverse cisplatin resistance in human ovarian cancer cells through the inhibition of mammalian target of rapamycin. *Oncol Lett*. 2013;6:789–794. doi:10.3892/ol.2013.1448
63. Yoon JH, Ahn SG, Lee BH, Jung SH, Oh SH. Role of autophagy in chemoresistance: regulation of the ATM-mediated DNA-damage signaling pathway through activation of DNA-PKcs and PARP-1. *Biochem Pharmacol*. 2012;83:747–757. doi:10.1016/j.bcp.2011.12.029
64. Yang L, Yu Y, Kang R, et al. Up-regulated autophagy by endogenous high mobility group box-1 promotes chemoresistance in leukemia cells. *Leuk Lymphoma*. 2012;53:315–322. doi:10.3109/10428194.2011.616962
65. Xu N, Zhang J, Shen C, et al. Cisplatin-induced downregulation of miR-199a-5p increases drug resistance by activating autophagy in HCC cell. *Biochem Biophys Res Commun*. 2012;423:826–831. doi:10.1016/j.bbrc.2012.06.048
66. Guo W, Qiu Z, Wang Z, et al. MiR-199a-5p is negatively associated with malignancies and regulates glycolysis and lactate production by targeting hexokinase 2 in liver cancer. *Hepatology*. 2015;62:1132–1144. doi:10.1002/hep.27929
67. Guo Z-W, Xie C, Yang J-R, Li J-H, Yang J-H, Zheng L. MtiBase: a database for decoding microRNA target sites located within CDS and 5'UTR regions from CLIP-seq and expression profile datasets. *Database*. 2015;2015:1–9. doi:10.1093/database/bav102
68. Da Sacco L, Masotti A. Recent insights and novel bioinformatics tools to understand the role of microRNAs binding to 5' untranslated region. *Int J Mol Sci*. 2012;14:480–495. doi:10.3390/ijms14010480
69. Han L, Lam EWF, Sun Y. Extracellular vesicles in the tumor micro-environment: old stories, but new tales. *Mol Cancer*. 2019;18:59. doi:10.1186/s12943-019-0980-8
70. McAndrews KM, Kalluri R. Mechanisms associated with biogenesis of exosomes in cancer. *Mol Cancer*. 2019;18:52. doi:10.1186/s12943-019-0963-9
71. Kulkarni B, Kirave P, Gondaliya P, et al. Exosomal miRNA in chemoresistance, immune evasion, metastasis and progression of cancer. *Drug Discov Today*. 2019;24:2058–2067. doi:10.1016/j.drudis.2019.06.010
72. Crossland R, Norden J, Kralj Juric M, et al. Serum and extracellular vesicle MicroRNAs miR-423, miR-199, and miR-93* as biomarkers for acute graft-versus-host disease. *Front Immunol*. 2017;8:1446. doi:10.3389/fimmu.2017.01446

Cancer Management and Research

Publish your work in this journal

Cancer Management and Research is an international, peer-reviewed open access journal focusing on cancer research and the optimal use of preventative and integrated treatment interventions to achieve improved outcomes, enhanced survival and quality of life for the cancer patient.

Submit your manuscript here: <https://www.dovepress.com/cancer-management-and-research-journal>

Dovepress

The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.