Hypoxia-induced *MIR155* is a potent autophagy inducer by targeting multiple players in the MTOR pathway

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Hypoxia activates autophagy, an evolutionarily conserved cellular catabolic process. Dysfunction in the autophagy pathway has been implicated in an increasing number of human diseases, including cancer. Hypoxia induces upregulation of a specific set of microRNAs (miRNAs) in a variety of cell types. Here, we describe hypoxia-induced *MIR155* as a potent inducer of autophagy. Enforced expression of *MIR155* increases autophagic activity in human nasopharyngeal cancer and cervical cancer cells. Knocking down endogenous *MIR155* inhibits hypoxia-induced autophagy. We demonstrated that *MIR155* targets multiple players in MTOR signaling, including *RHEB, RICTOR,* and *RPS6KB2. MIR155* suppresses target-gene expression by directly interacting with their 3′ untranslated regions (UTRs), mutations of the binding sites abolish their *MIR155* responsiveness. Furthermore, by downregulating MTOR signaling, *MIR155* also attenuates cell proliferation and induces G_1/S cell cycle arrest. Collectively, these data present a new role for *MIR155* as a key regulator of autophagy via dysregulation of MTOR pathway.

Introduction

Oxygen deprivation or hypoxia is a common feature in various physiological or pathophysiological states, including exercise, development, or cancer. Hypoxia has emerged as a pivotal factor in solid tumors due to overwhelming growth, inadequate blood supply, tumor progression, and resistance to therapy. Responses to hypoxia are orchestrated in part through activation of HIF (hypoxia-inducible factor). By controlling the transcription of hundreds of target genes, HIF regulates many cellular processes, such as angiogenesis, metabolism, cell migration, and tumor invasion.[1] Recent studies suggest that hypoxia activates autophagy, an evolutionarily conserved cellular catabolic process by which cells capture intracellular proteins and organelles and eliminate them through delivery to lysosomes.[2] Autophagy is vital in a range of physiological and pathological situations, and dysfunction in the

autophagy pathway has been implicated in an increasing number of human diseases, including cancer, neurodegenerative diseases, and infection.[3-5]

The mechanistic target of rapamycin (MTOR), integrates and transmits signals from a diverse array of signaling pathways to regulate cell growth, metabolism, and ribosomal biogenesis, as well as autophagy. MTOR is part of 2 different protein complexes: rapamycin-sensitive MTORC1 and rapamycin-insensitive MTORC2, along with the regulatory-associated proteins RPTOR (regulatory-associated protein of MTOR, complex 1) and RICTOR (RPTOR independent companion of MTOR, complex 2).[6,7] Under nutrient-rich conditions, activation of MTORC1 triggers a cascade of anabolic processes for cell growth and proliferation, mainly mediated by RPS6KB2 (ribosomal protein S6 kinase, 70 kDa, polypeptide 2/p70 S6 kinase) and EIF4EBP1 (eukaryotic translation initiation factor 4E binding
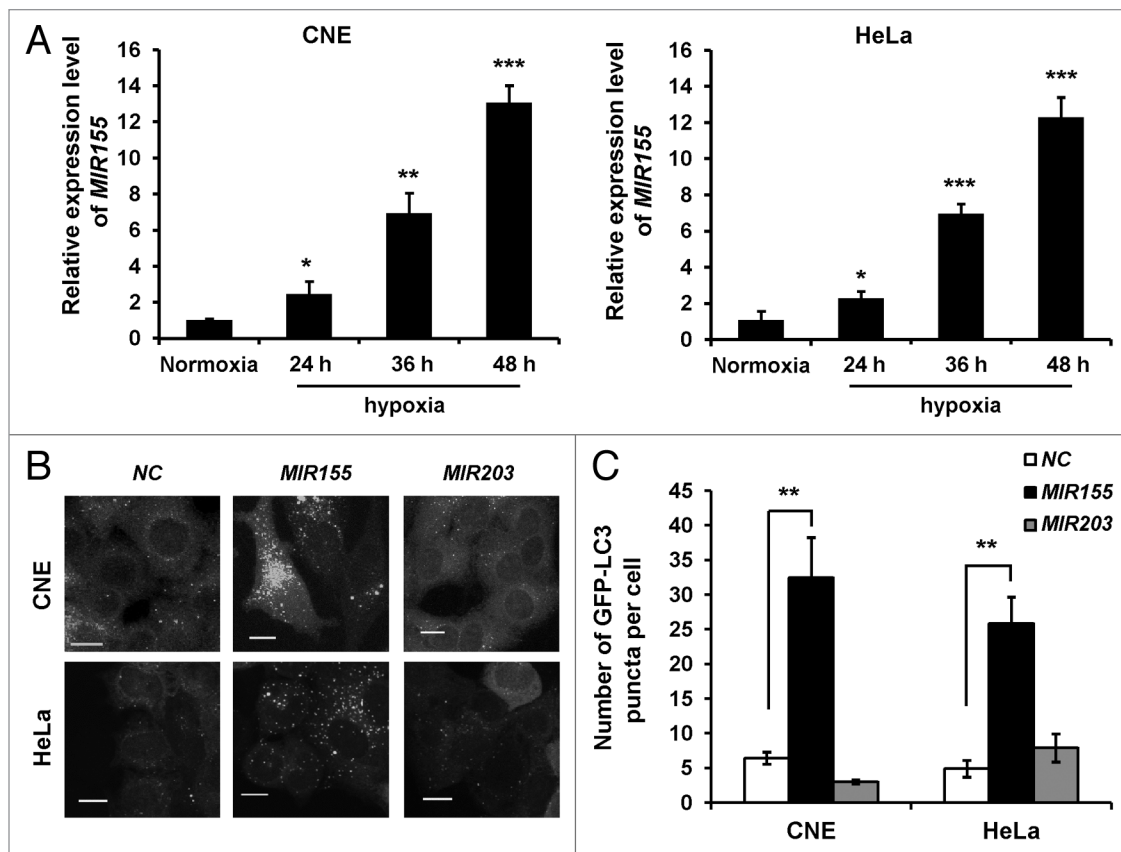
Figure 1. Hypoxia-induced *MIR155* promotes autophagosome accumulation. (**A**) Hypoxia induces *MIR155* expression. CNE or HeLa cells were exposed to 1% oxygen for 24, 36 and 48 h. Cells were collected for qRT-PCR to quantify the expression of *MIR155*. (mean ± s.d. of independent experiments, n = 4, *P < 0.05, **P < 0.01, ***P < 0.001, Student 2-tailed *t* test). (**B**) *MIR155* promotes GFP-LC3 translocation. CNE or HeLa cells stably expressing GFP-LC3 were transfected with negative control (*NC*), *MIR155* or *MIR203*. Cells were fixed 48 h post transfection. Representative images are shown. Scale bar: 10 μm. (**C**) Quantitative analysis of GFP-LC3 puncta in (**B**). At least 200 cells were examined in each experimental group. Data shown are means ± s.d. of four independent experiments. **P < 0.01, Wilcoxon rank sum test.

protein 1).[8] Concomitantly, activation of MTORC1 also inhibits autophagosome formation through phosphorylating and inhibiting the ULK1-ATG13-RB1CC1-C12orf44/ATG101 complex.[9-11] By contrast, MTORC2 is less well understood. It has been reported that MTORC2 activates AKT via phosphorylation and enhances the expression of HIF1A.[12,13]

MicroRNAs (miRNAs) are the most widely studied class of noncoding RNAs that regulate gene expression by blocking mRNA translation and/or mediating mRNA degradation.[14,15] miRNAs control diverse fundamental biological and pathological processes, including cell proliferation, apoptosis, differentiation, and metabolism, and are also associated with cancer.[14,16-18] miRNA expression is often deregulated in cancer and miRNAs can act as oncogenes or tumor suppressors.

Recently, miRNAs have been well characterized in the modulation of different autophagic stages. Autophagy induction is initiated by the ULK1 complex. *MIR20A* and *MIR106B* impede autophagy induction by leucine deprivation in C2C12 cells via directly targeting *Ulk1*.[19] Vesicle nucleation mainly involves the BECN1 (Beclin 1, autophagy related) complex. BECN1 itself is targeted by *MIR30A*, *MIR376B*, and *MIR519A*.[20-23] Additional regulators of this step are *MIR101*, *MIR630*, and *MIR374A*,

which target *RAB5A* and *UVRAG*, respectively.[23,24] Vesicle elongation is controlled by two ubiquitin-like conjugation systems. Several miRNAs, including *MIR30A*, *MIR181A*, *MIR374A*, *MIR630*, *MIR376B*, *MIR204*, *MIR375*, *MIR519A*, *MIR885–3P*, and *MIR-101*, have been identified to regulate this process by targeting the expression of some *ATG* (autophagy-related) genes.[25,26] To date, most of the miRNAs primarily inhibit the autophagic process and function as negative regulators. *MIR7* is the only example of an miRNA that may induce autophagy. *MIR7* has been characterized as a tumor suppressive miRNA through negative regulation of the phosphoinositide 3-kinase (PI3K)-AKT-MTOR pathway in hepatocellular carcinoma, which may account for the role in autophagy induction.[27]

In the present study, we report that hypoxia-induced *MIR155* is a potent inducer of autophagy. Overexpression of *MIR155* increases autophagic activity, while knocking down endogenous *MIR155* alleviates hypoxia-induced autophagy. Importantly, we demonstrated that 3 members of the MTOR pathway, *RHEB*, *RICTOR* and *RPS6KB2*, are direct targets of *MIR155*. By suppressing the activation of MTOR-AKT pathway, *MIR155* induces autophagy, decelerates cell proliferation and G_1/S cell cycle progression.
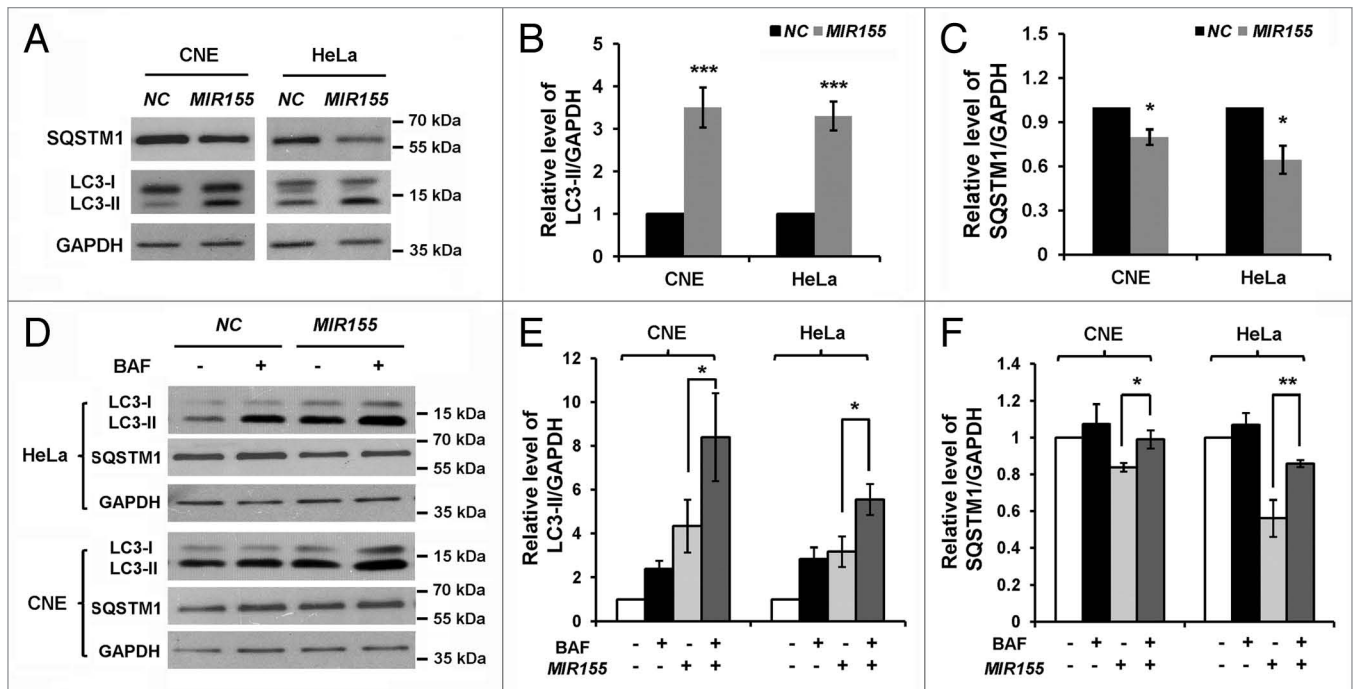
Figure 2. Overexpression of *MIR155* induces autophagic activity. (**A**) Overexpression of *MIR155* induces LC3 conversion and SQSTM1 degradation. Western blots of control (*NC*) and *MIR155*-transfected cells. (**B**) ImageJ densitometric analysis of the LC3-II/GAPDH or (**C**) SQSTM1/GAPDH ratios from immunoblots (mean ± s.d. of 4 independent experiments). *$P < 0.05$, ***$P < 0.001$, Student 2-tailed *t* test. (**D**) Overexpression of *MIR155* increases autophagic flux. CNE or HeLa cells were transfected with *MIR155* or *NC*, BAF was applied to the medium for 1 h at 47 h post transfection, cells were harvested for western blot. (**E**) ImageJ densitometric analysis of the LC3-II/GAPDH or (**F**) SQSTM1/GAPDH ratios from immunoblots (mean ± s.d. of 4 independent experiments). *$P < 0.05$, **$P < 0.01$, Student 2-tailed *t* test.

Results

Hypoxia induces *MIR155* upregulation

To investigate the function of *MIR155* in hypoxia-induced autophagy, first we examined the expression of *MIR155* in CNE and HeLa cells under hypoxic stress. As shown in **Figure 1A**, the expression level of *MIR155* was low in normal culture conditions (21% oxygen). Hypoxia (1% oxygen) treatment induced a sustained upregulation of *MIR155* in a time-dependent manner in both cell types. At 48 h after hypoxia treatment, more than a 12-fold increase of the *MIR155* expression level was detected.

Overexpression of *MIR155* induces autophagy

To explore the role of *MIR155* in autophagy, we performed a GFP-LC3 puncta-formation assay and an LC3 conversion assay. *MIR155* was transfected into CNE or HeLa cells that stably expressing GFP-LC3 fusion protein, the localization of GFP-LC3 was examined by confocal microscopy. GFP-LC3 puncta appear in the cytoplasm reflects the recruitment of LC3 proteins to autophagosomes. As shown in **Figure 1B**, there was a significant increase of GFP-LC3 puncta in *MIR155* transfected cells. *MIR203*, a control miRNA that has no predicted targets in the autophagy pathway, behaved similarly to the negative control (NC) miRNA. The quantification of GFP-LC3 dots per cell confirmed that overexpression of *MIR155* induced autophagosome accumulation in both CNE and HeLa cells (**Fig. 1C**). Next, we detected the conversion of LC3-I [cleaved, cytosolic form of MAP1LC3 (LC3)] to LC3-II (subsequently lipidated and

membrane-bound form) by western blot. Consistent with the GFP-LC3 puncta formation assay, *MIR155* overexpression led to a significant upregulation of lipidated LC3-II (**Fig. 2A and B**). Thus, both assays suggest that overexpression of *MIR155* induces autophagosome accumulation.

To distinguish whether autophagosome accumulation is due to autophagy induction or rather a block in downstream steps, we performed autophagic flux assays. SQSTM1/p62, a polyubiquitin binding protein is selectively incorporated into autophagosomes through direct binding to LC3 and efficiently degraded during autophagy, thus the total cellular levels of SQSTM1 reflects the autophagic activity. We examined the intracellular amount of SQSTM1 by western blot, overexpression of *MIR155* resulted in 20% to 40% reduction of SQSTM1 proteins in CNE and HeLa cells, suggesting that *MIR155* promotes autophagic degradation (**Fig. 2A and C**). Finally, we performed an LC3 turnover assay. Cells were treated with the lysosomotropic reagent bafilomycin A$_1$ (BAF) to block autophagic degradation. BAF treatment caused significant increase of LC3-II in both NC and *MIR155* transfected cells (**Fig. 2D and E**). In addition, the protein levels of SQSTM1 in *MIR155* transfected cells were also upregulated by BAF (**Fig. 2F**). Therefore, these data demonstrate that overexpression of *MIR155* increases autophagic activity.

Inhibition of endogenous *MIR155* represses hypoxia-induced autophagy

To document the physiological relevance of *MIR155* on autophagy, we inhibited the expression of endogenous *MIR155*

and repeated the above validation assays in both CNE and HeLa cells. LNA-derived *MIR155* inhibitor was used to inhibit the high level of endogenous *MIR155* during hypoxia treatment. Hypoxia-induced GFP-LC3 puncta accumulation was dramatically suppressed by LNA-155 in both HeLa and CNE cells (**Fig. 3A and B**). Compared with LNA-NC control, SQSTM1 degradation during hypoxia treatment was also reduced upon LNA-155 transfection, reflecting a decrease of autophagic activity (**Fig. 3C**). Hence, these results demonstrate the physiological relevance of endogenous *MIR155* on regulating autophagy process during hypoxia treatment.

Experimental identification of *MIR155* targets

Having established the role of *MIR155* in autophagy, we next wanted to identify the direct targets of *MIR155*. We used FindTar, a prediction algorithm designed by our laboratory, to clarify the potential targets of *MIR155* in regulating autophagy.[28] FindTar predicted that several upstream or downstream regulators of the MTOR signaling pathway, including *RHEB*,

Figure 3. Knockdown of endogenous *MIR155* inhibits hypoxia-induced autophagy. (**A**) Inhibition of *MIR155* in hypoxia suppresses GFP-LC3 translocation. HeLa or CNE cells stably expressing GFP-LC3 were transfected with LNA-NC or LNA-155 and exposed to 1% oxygen. The number of GFP-LC3 puncta was quantified. (mean ± s.d. of independent experiments, n = 4, *P < 0.05,**P < 0.01, Wilcoxon rank sum test). (**B**) Representative images from the quantification are shown. Scale bar: 10 μm. (**C**) Knockdown of endogenous *MIR155* inhibits autophagy. HeLa or CNE cells transfected with LNA-NC or LNA-155 were cultured in 1% oxygen for 36 h, cells were harvested for western blot. Protein ratio of SQSTM1/GAPDH was calculated following ImageJ densitometric analysis. (3 independent experiments gave similar results).

RPTOR, *RICTOR*, *RPS6KB2*, and *MTOR* are potential *MIR155* targets. Besides, *BCL2* and *ATG3* were also putative *MIR155* targets. We performed qRT-PCR to examine the mRNA levels of *MIR155* putative targets. *MIR155* but not control miRNA, led to a significant attenuation of the mRNA levels of *RHEB*. Other putative *MIR155* target genes, however, did not show significant changes in both HeLa and CNE cells (**Fig. 4A and B**). Immunoblots with specific antibodies showed that the cellular levels of RHEB, RICTOR, RPS6KB2, and ATG3 proteins were decreased in *MIR155*-overexpressing cells (**Fig. 4C**). RHEB and RICTOR are reported to be positive regulators of MTORC1 and AKT; thus, we used phosphorylation site-specific antibodies to directly measure the activation of MTOR and AKT, including a phospho-Ser2448 antibody for MTOR and a phospho-Ser473 antibody for AKT. *MIR155* did not significantly change the total protein levels of MTOR and AKT; however, the phosphorylation status of these 2 proteins was significantly reduced (**Fig. 4C**). To validate, we also checked the mRNA and protein expression levels of *MIR155* targets *RHEB*, *RICTOR*, *RPS6KB2*, and *ATG3* in CNE and HeLa cells that stably expressing *MIR155* by lentiviral infection. Similar to transient expression, stable transfection of *MIR155* significantly suppressed *RHEB* mRNA levels in
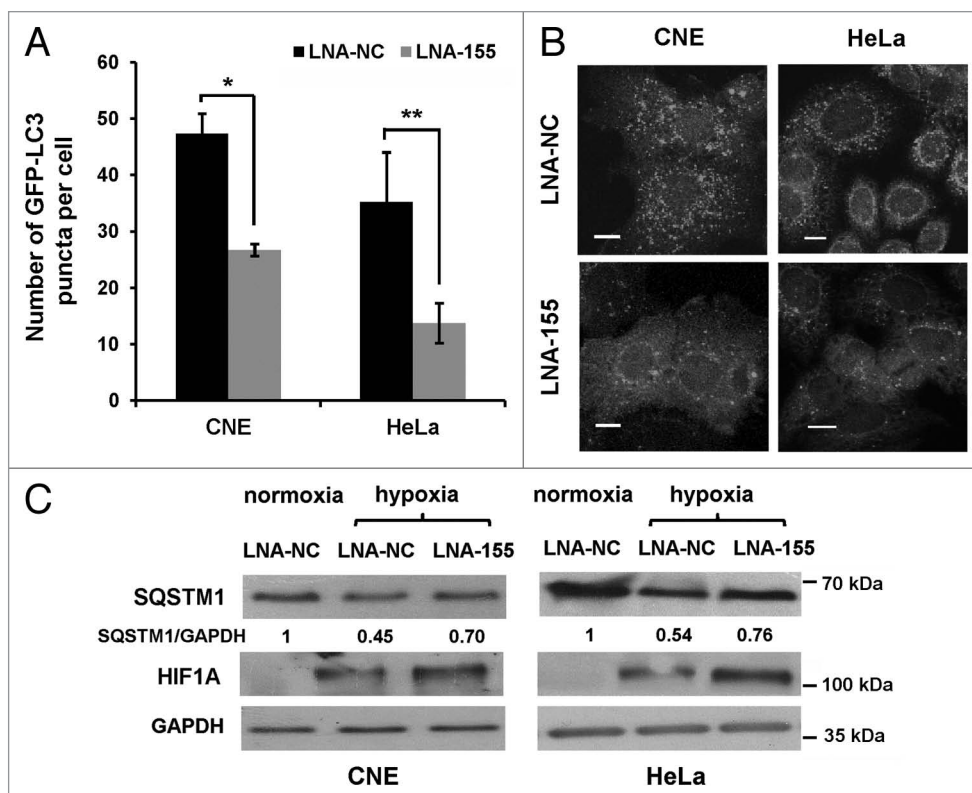
both CNE and HeLa cells. The mRNA expression of *RPS6KB2* was also downregulated by *MIR155* in HeLa cells (**Fig. S1A**). Stable transfection of *MIR155* suppressed the protein levels of RHEB, RPS6KB2, and RICTOR, as well as the phosphorylation of MTOR and AKT (**Fig. S1B**). Therefore, immunoblots from both transient and stable expression of *MIR155* confirmed that *MIR155* suppresses the expression of RHEB, RICTOR, RPS6KB2 and also downregulates MTOR-AKT signaling. Stable expression of *MIR155* did not change the protein levels of ATG3, thus subsequent studies will concentrate on RHEB, RICTOR, and RPS6KB2.

MIR155 is a hypoxia-inducible miRNA, thus we predicted that the protein levels of *MIR155* targets will be downregulated by hypoxia. As shown in **Figure S2**, hypoxia treatment dramatically attenuated the protein expression levels of RHEB, RICTOR and RPS6KB2 in both CNE and HeLa cells. The total protein levels of MTOR also decreased 23% and 54% in HeLa and CNE cells under hypoxic stress. Similarly, the phosphorylation of MTOR was reduced by hypoxia treatment (**Fig. S2**).

We reasoned whether knocking down functionally important target of *MIR155* could phenocopy the effect of *MIR155* on autophagy. Small GTP-binding protein RHEB is indispensable
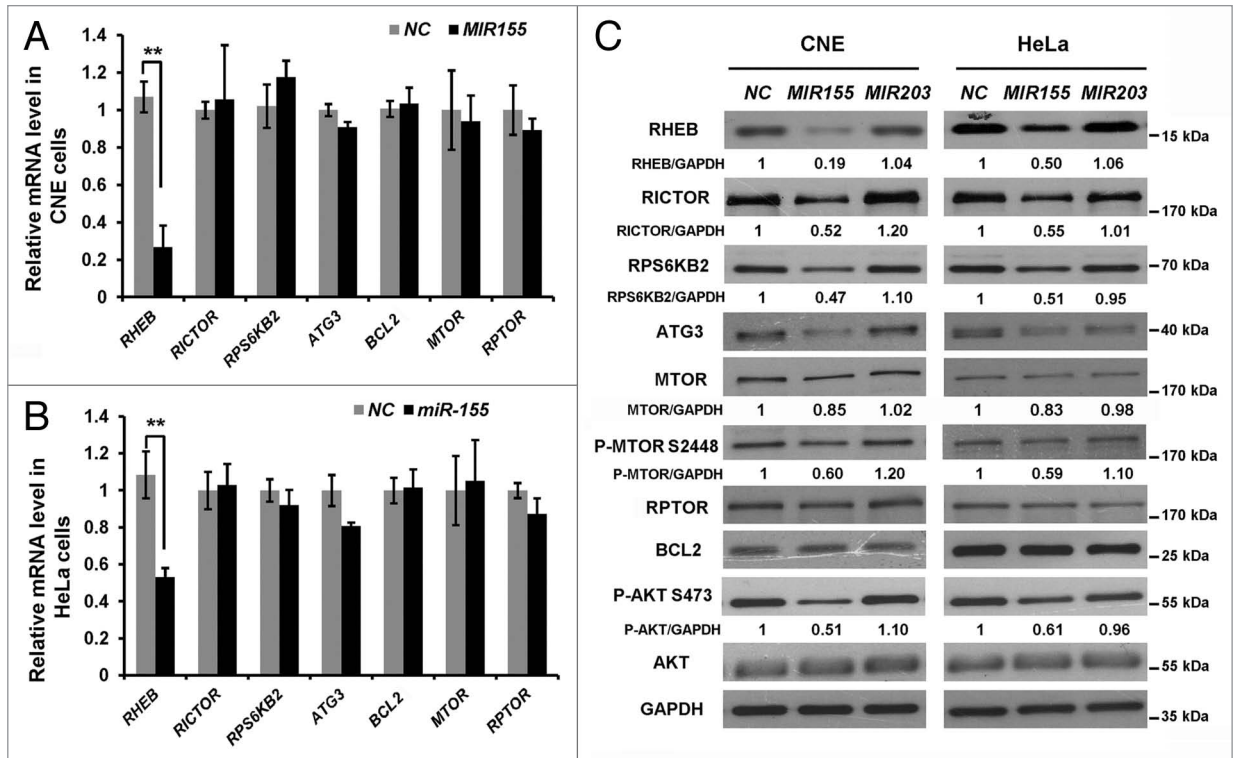
Figure 4. *MIR155* downregulates multiple players of MTOR signaling. (**A**) qRT-PCR analysis of mRNA expression levels of predicted targets of *MIR155*. CNE or (**B**) HeLa cells were transfected with *MIR155* or *NC*, cells were harvested at 48 h for qRT-PCR. (mean ± s.d. of independent experiments, n = 4, **$P <$ 0.01, ***$P <$ 0.001, Student 2-tailed *t* test). (**C**) Western blot analysis of RHEB, RICTOR, RPS6KB2, ATG3, MTOR, phospho-MTOR (Ser2448), RPTOR, BCL2, AKT, phospho-AKT (Ser473), and GAPDH proteins in CNE or HeLa cells transfected with *MIR155*, *MIR203*, or *NC*. Protein ratios were calculated following ImageJ densitometric analysis. (3 independent experiments gave similar results).
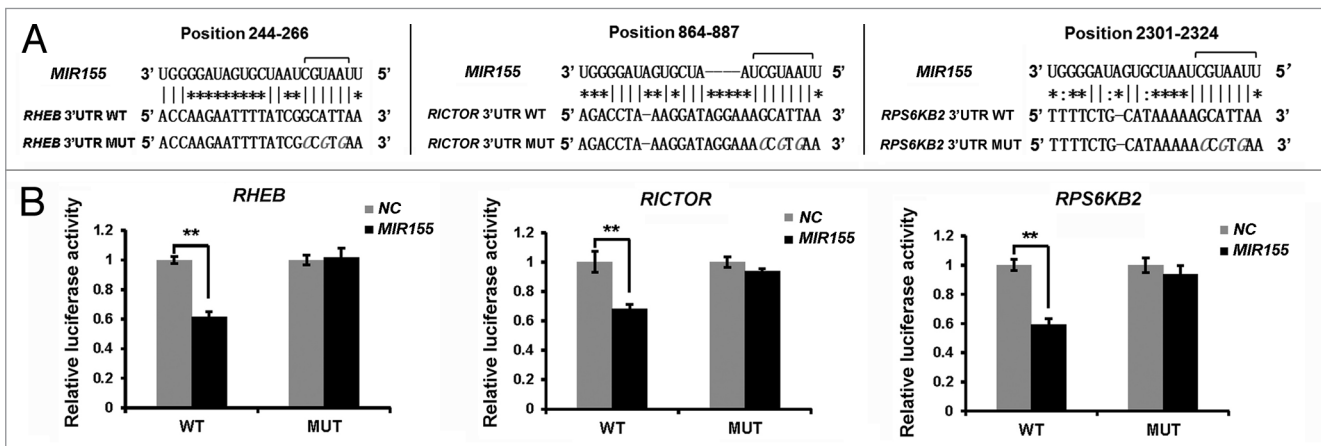

Figure 5. *MIR155* directly targets *RHEB*, *RICTOR*, and *RPS6KB2*. (**A**) Predicted binding sequences between *MIR155* and seed matches in *RHEB*, *RICTOR*, and *RPS6KB2* 3′ UTRs. (**B**) Luciferase reporter vectors were generated by inserting the wild-type or mutated 3′UTR fragments of *RHEB*, *RICTOR*, and *RPS6KB2* into pRL-TK plasmid. Luciferase reporter assays at 24 h after transfection with wild-type (WT) or mutated (MUT) plasmids, cotransfected with *NC* or *MIR155*. Data shown are means ± s.d. of independent experiments, n = 4, **$P <$ 0.01, ***$P <$ 0.001, Student 2-tailed *t* test.

for MTOR activity by direct binding and activating MTOR kinase.[29,30] Thus, we tested the effect of *RHEB* siRNA on autophagosome accumulation and LC3 conversion. Similar to *MIR155* overexpression, depletion of endogenous *RHEB* induced a significant increase of GFP-LC3 puncta and LC3 conversion (**Fig. S3**).

MIR155 directly interacts with the 3′ UTRs of *RHEB*, *RICTOR*, and *RPS6KB2*

To clarify if *MIR155* regulates the expression of target genes via direct interaction with their 3′ UTRs, we used 2 bioinformatics softwares TargetScan and FindTar to predict the potential binding sequences in the 3′ UTRs of target genes (**Fig. 5A**).

Then we designed pRL-TK-luciferase reporter constructs containing either the wild-type (WT) or mutated (MUT) *MIR155* binding sequences in the 3′ UTRs of *RHEB*, *RICTOR* and *RPS6KB2*. The pRL-TK-luciferase reporter constructs were cotransfected with either *MIR155* or NC control. Luciferase activity was determined at 24 h after transfection. *MIR155* significantly reduced luciferase activity when cells transfected with wild-type 3′ UTRs of *RHEB*, *RICTOR* and *RPS6KB2*. By contrast, the inhibitory effect of *MIR155* on luciferase activity was completely restored by the mutants (**Fig. 5B**). Moreover, the luciferase activity was also susceptible to knockdown of endogenous *MIR155*. Inhibition of *MIR155* could specifically relieve the repression of all 3 reporters (**Fig. S4**).

Inhibition of *MIR155* leads to an increase of target-gene expression

To test whether silencing endogenous *MIR155* would have an impact on target protein expression, we transfected cells with antagomir-155 (LNA-155) or nonspecific control antagomir (LNA-NC). Then, we checked the protein expression levels of RHEB, RICTOR, and RPS6KB2 by western blot. We showed that hypoxia induced downregulation of RHEB, RICTOR, and RPS6KB2 proteins. The amounts of all 3 proteins were increased when hypoxia-induced upregulation of *MIR155* was suppressed by LNA-155. In addition, there was a clear increase of in the phosphorylation of MTOR (Ser2448) (**Fig. 6**; **Fig. S5**). In the light of these results, we demonstrate that endogenous *MIR155* targets the expression of MTOR signaling, and hence the autophagic activity.

MIR155 attenuates cell proliferation and cell cycle progression

Numerous studies demonstrate that MTOR signaling is essential for cell growth and proliferation. In this study, we discovered that overexpression of *MIR155* could suppress the activation of MTORC1 and AKT. Thus, it is interesting to check whether *MIR155* regulate cell proliferation and cell cycle progression. We observed that overexpression of *MIR155* attenuated cell proliferation in both CNE and HeLa cells (**Fig. 7A**). Next, we examined the effect of *MIR155* on cell cycle progression. HeLa cells transfected with NC or *MIR155* were synchronized at G_1/S boundary by treatment with hydroxyurea. At 4 h after release from G_1 block, more than 70% of the control cells finished G_1 and entered S phase. Similarly, *MIR885-5P*, a control miRNA had no effect on G_1/S cell cycle progression. By contrast, both *MIR155* and *RHEB* siRNA induced significant G_1 phase arrest (**Fig. 7B**). Then we tested whether the effect of *MIR155* on autophagy contributes to G_1 phase cell cycle arrest. Cells were cotransfected with *MIR155* and *ATG5* siRNA. Interestingly, depletion of *ATG5* relieved the inhibitory effect of *MIR155* on G_1/S cell cycle progression (**Fig. 7B**; **Fig. S6**). These data indicate a possible link between autophagy and cell cycle progression.
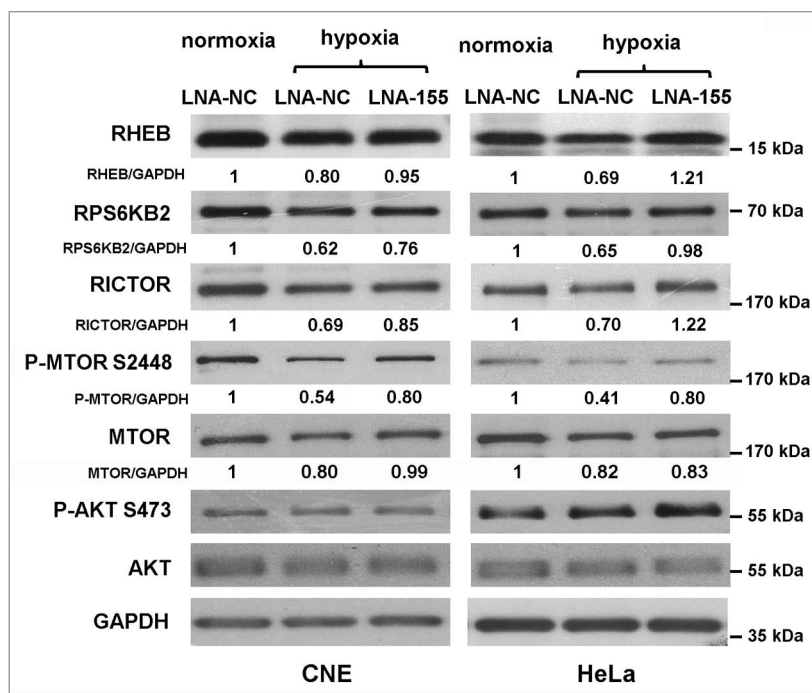

Figure 6. Blockage of endogenous *MIR155* led to an increase in RHEB, RICTOR, and RPS6KB2 protein levels. Western blot analysis of RHEB, RICTOR, RPS6KB2, MTOR, phospho-MTOR (Ser2448), and GAPDH in CNE or HeLa cells transfected with LNA-NC or LNA-155. Protein ratios were calculated following ImageJ densitometric analysis. (3 independent experiments gave similar results).

Discussion

The tumor microenvironment plays a critical role in tumor cell proliferation and progression. As the tumor grows, it rapidly outgrows its blood supply, leaving tumor cells been deprived of oxygen. HIF is a key transcription factor that allows rapid adaptation to hypoxic stress. Hypoxia regulates the expression of a group of miRNAs in a cell type- and tissue-specific manner.[31-34] In the present study, we identified hypoxia-induced *MIR155* as a potent inducer of autophagy. We discovered that multiple players of MTOR signaling are novel *MIR155* targets, including *RHEB*, *RICTOR*, and *RPS6KB2*. By suppressing the activation of MTOR-AKT pathway, *MIR155* induces autophagy, and attenuates cell proliferation and cell cycle progression.

MTOR functions as a central sensor of cellular energy that responses to many oncogenic and metabolic events as well as the control of autophagy. Extensive studies indicate that regulation of MTOR pathway by miRNAs plays a major role in cancer progression. miRNAs either directly interact with *MTOR* itself or target key players within the pathway, such as *IGF1R* (insulin-like growth factor 1 receptor), *PI3K*, *AKT*, and *PTEN*. For instance, *MIR99A*, *MIR100*, *MIR101*, and *MIR199-3P* inhibit tumor cell migration and cell growth, induce G_1 phase cell cycle arrest by directly binding to *MTOR* 3′ UTR and suppress *MTOR* expression.[35-38] By contrast, some miRNAs, including *MIR21*, *MIR221*, *MIR222*, *MIR26*, and *MIR19* can function as oncomiR by targeting the expression of *PTEN* and promotes cancer cell
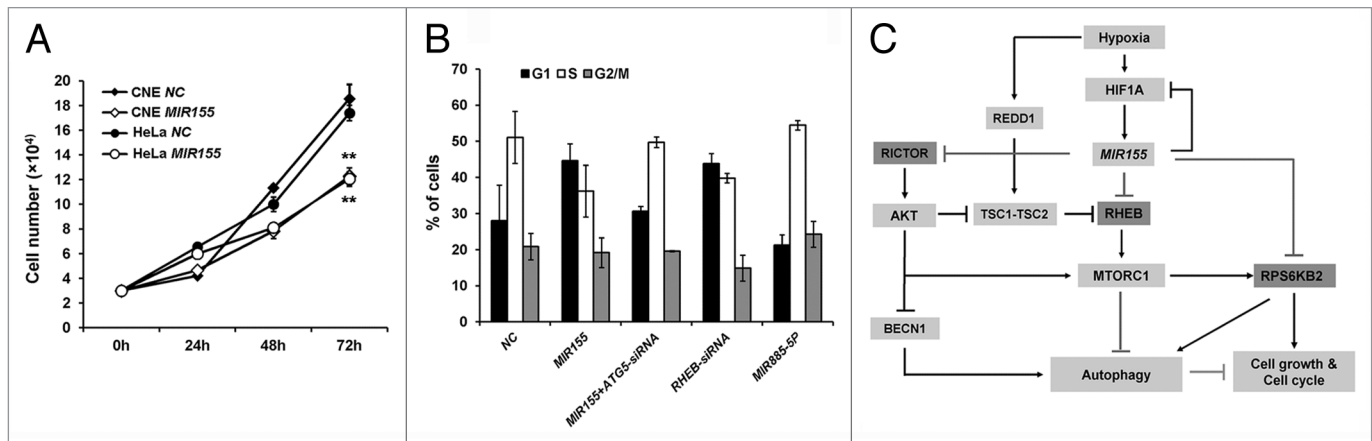
Figure 7. *MIR155* decelerates cell proliferation and cell cycle progression. (**A**) Overexpression of *MIR155* inhibits cell proliferation. CNE or HeLa cells transfected with *MIR155* or *NC* were cultured for 24, 48, and 72 h to determine cell proliferation rates. Data shown are means ± s.d. of independent experiments, n = 4, **$P < 0.01$, Student 2-tailed t test. (**B**) *MIR155* induces G_1/S cell cycle arrest. HeLa cells transfected with indicated miRNAs or siRNAs were synchronized at G_1/S boundary by treatment with hydroxyurea. Cells were released from hydroxyurea block for 4 h, fixed and stained with propidium iodide (PI) for flow cytometry. Percentage of G_1, S, and G_2/M phase cells was quantified from flow cytometry analysis. Data shown are means ± s.d. from 3 independent experiments. (**C**) Proposed model of hypoxia-induced *MIR155* in regulating autophagy, cell proliferation, and cell cycle progression. *MIR155* is a hypoxia-inducible miRNA. *MIR155* targets the expression of *RHEB*, *RICTOR*, and *RPS6KB2*, which in turns suppresses the activation of AKT and MTORC1. By deregulating the MTOR-AKT pathway, *MIR155* induces autophagy and decelerates cell proliferation and cell cycle progression.

growth, proliferation, and invasion.[39-44] *MIR155* targets, *RHEB* and *RICTOR*, are essential upstream regulators of MTORC1. The activity of MTORC1 is controlled through RHEB, a GTP-binding protein, which stimulates MTORC1 when it is in the GTP-bound state.[45,46] RICTOR is scaffold protein that regulating the assembly and substrate binding of MTORC2.[47] It has been reported that MTORC2 activates AKT via phosphorylation and enhances the expression of HIF1A.[12,13] In addition, AKT activates RHEB by negative phosphorylation of TSC1/TSC2 complex. A recent study by Wang et al. also reports that AKT-mediated BECN1 phosphorylation functions in autophagy inhibition.[48] Thus, *MIR155* is a potent autophagy inducer by suppressing RICTOR- and RHEB-mediated MTORC1-AKT activity (**Fig. 7C**).

Deregulation of *MIR155* has been reported in a wide range of malignancies, including various forms of lymphoma and carcinomas of breast, lung, pancreas, head and neck, nasopharyngeal, and kidney.[49-54] Several lines of evidence demonstrate that *MIR155* functions as an "OncomiR" and plays a role in tumor development. In human breast cancer cells, *MIR155* induces cell proliferation and chemosensitivity by targeting the *SOCS1* (suppressor of cytokine signaling 1) and *FOXO3* (forkhead box O3) genes.[55,56] In human lung cancer cells, *MIR155* targets the expression of *APAF1* (apoptotic peptidase activating factor 1) and *FOXO3*, and downregulation of *MIR155* enhances the sensitivity of lung cancer cells to cisplatin treatment or radiotherapy.[57] However, contradictory results exist concerning the oncogenic feature of *MIR155*. In some circumstances, *MIR155* could exert a tumor suppression function. For example, *MIR155* is downregulated in the majority of melanoma cell lines, overexpression of *MIR155* significantly inhibits proliferation and induces apoptosis.[58] *MIR155* inhibits the growth of lung cancer 95D cells through the induction of G_0/G_1 cell cycle arrest.[59] *MIR155* also

inhibits proliferation and migration in human extravillous trophoblast derived HTR-8/SVneo cells via negative regulation of the CCND1-CDKN1B pathway.[60] Here we demonstrated that overexpression of *MIR155* inhibits cell proliferation and G_1/S cell cycle progression. This tumor-suppressive effect could be due to *MIR155*-mediated downregulation of RPS6KB2. Therefore, the function of *MIR155* in physiological processes such as tumor development and tumor growth might be determined by its wide variety of targets in a cell type and tissue-specific manner.

There is growing evidence for the role of autophagy in the control of cell proliferation, however, less is known about the relationship between autophagy and the cell cycle. It has been demonstrated autophagy regulatory protein ATG7 could bind to the tumor suppressor TP53 to regulate the transcription of cell cycle inhibitor CDKN1A [cyclin-dependent kinase inhibitor 1 (p21, Cip1)]. Starved MEFs lacking the *Atg7* gene have impaired TP53-mediated cell cycle arrest.[61] In U87 glioma cells, pharmacological inhibition of resveratrol-induced autophagy abrogates S-G_2/M cell cycle arrest and significantly reduces the levels of CCNA, CCNE, CCNB, and phosphorylation of RB1 (Ser807/811) and CDC2 (Y15).[62] Here, we show that knockdown of the essential autophagy regulator *ATG5* could reverse *MIR155*-induced G_1 phase arrest, indicating that *MIR155*-induced delay of cell cycle progression might be related to autophagy. More work is required to define the mechanisms of autophagy in the control of cell cycle progression.

Materials and Methods

Cell culture and hypoxia treatment

CNE (human nasopharyngeal cancer) or HeLa (human cervical cancer) cells were grown in Dulbecco's modified Eagle's medium (Gibco/Invitrogen Ltd, 12800-017) containing 10%

fetal bovine serum (PAA, A15-101), 10 U/ml penicillin-strep-tomycin (Gibco/Invitrogen Ltd, 15140-122) in a 5% CO_2-humidified incubator at 37 °C. Hypoxic conditions were induced by incubating cells in a modular incubator chamber flushed with a gas mixture containing 1% O_2, 5% CO_2, and 94% N_2 at 37 °C. For experiments involving hypoxia, cells were maintained in culture medium supplemented with 25 mM HEPES buffer.

Target prediction

The conventional online programs, including Targetscan (http://www.targetscan.org) and Findtar (http://bio.sz.tsinghua.edu.cn) were used to predict the targets of *MIR155*.[28] The targets predicted by these 2 programs were further analyzed and demonstrated by the following biological experiments.

MiRNAs, LNAs, siRNAs, and transfections

All synthetic miRNAs (*MIR155, MIR203, MIR885-5P*), siRNAs and the negative control (*NC*) were purchased from Shanghai GenePharma Co. Ltd. LNA-NC and LNA-155 were purchased from Exiqon. For transfection, HeLa or CNE cells were transiently transfected with lipofectamine™ 2000 (Invitrogen, 11668-019) according to the manufacturer's protocol. The sequences of siRNAs are as follows: *ATG5*: 5′-GCAACUCUGG AUGGGAUUGTT-3′, 5′-CAAUCCCAUC CAGAGUUGCTT-3′, *RHEB*: 5′-GAAAGGGUGA UCAGUUAUGTT-3′, 5′-CAUAACUGAU CACCCUUUC TT-3′.

Plasmid construction and lentivirus production

A fragment of pre-*MIR155* encompassing the stem-loop was amplified from CNE genomic DNA and cloned into lentiviral vector pBabi-globin-EGFP, a plasmid developed by our laboratory. MicroRNA fragment was inserted into the globin intron flanking the promoter and *EGFP*. A lentiviral vector expressing a scrambled RNA was used as control. To produce viruses, the pre-*MIR* expression plasmid and the backbone plasmids were cotransfected into 293LTV cells using the calcium phosphate method. Supernatant containing the viruses were harvested at 48 h. Cells were infected with the viruses along with 8 ug/ml polybrene. The transfection efficiency was determined by monitoring EGFP expression using a fluorescence microscope (Leica DMI 6000B).

GFP-LC3 stable cell lines and quantitative GFP-LC3 analyses

HeLa GFP-LC3 stable cell line was established by transient transfection of pEGFP-LC3 plasmid following screening with G418 (Shanghai Sangon Biotech Co. Ltd, GDJ958). To obtain CNE GFP-LC3 stable cell line, we cloned CMV-GFP-LC3 to the pMSCV-neo^r and generated lentiviruses, CNE cells were infected with the viruses and selected stable clones with G418. CNE GFP-LC3 or HeLa GFP-LC3 stable cell lines were transfected with individual miRNAs or LNAs and fixed in dry methanol. GFP-LC3 puncta formation in normoxia or hypoxia conditions was determined by capturing images using Olympus FV1000 confocal microscope (Olympus). The number of GFP-LC3 dots per cell was counted from at least 200 cells.

qRT-PCR for mRNA and miRNA quantification

Total RNA was isolated using RNA iso Plus (Takara, D9108B). Real-time qRT-PCR was performed using reverse transcription kit (Takara, D6130) and SYBR Green PCR Master Mix (Toyobo, QPK-201). mRNA level were normalized to *GAPDH*. miRNA qPCR were performed according to manufacturer's instructions using the Hairpin-it™ miRNA qPCR kit (Shanghai GenePharma Co. Ltd, QPM-091 for *U6 snoRNA*, QPM-040 for *MIR155*). Briefly, 4 μg total RNAs were reverse transcribed using mRNA specific RT primer, miRNA qPCR were performed using miRNA specific primer set to detect expression of mature *MIR155*. miRNA expression levels were normalized by Livak's method and *U6* snoRNA was used as endogenous control. Primers used in the present study were listed in **Table S1**.

Construction of luciferase plasmids and reporter assay

The 3′ UTRs of *RHEB*, *RICTOR,* and *RPS6KB2* were amplified by PCR from CNE genomic DNA and cloned into pRL-TK reporter vector (Promega, E2241). The predicted target site was mutated by site-directed mutagenesis. For luciferase reporter assays, the WT or MUT luciferase plasmids and miRNAs were cotransfected into HeLa cells and each experiment was repeated in triplicate. Transfected cells were lysed 30 h after transfection and luciferase activities were assayed by a Dual-Luciferase Reporter System (Promega, E1960). Total protein concentration of cell lysate was determined using Bradford assay (Bio-Rad, 500-0006) at 595 nm on a spectrophotometer (Thermo Scientific). The luciferase activity was normalized by total protein content.

Cell synchronization and flow cytometry

For synchronization, cells were treated with 2 mM hydroxyurea (Sigma, H8627) for 16 h. The synchronous cells were washed 3 times with PBS to release from G_1 block. At 4 h after release, cells were fixed in 70% ethanol in PBS and counterstained with 10 μg/ml propidium iodide (Sigma, P4170) and analyzed for DNA content by use of a BD Influx™ flow cytometer (BD Biosciences).

Western blotting

Cells were lysed in ice-cold whole cell extract buffer (50 mM TRIS-HCl, pH 8.0, 4 M urea and 1% Triton X-100), supplemented with complete protease inhibitor mixture (Roche Diagnostics, 04693132001). Cell extracts were resolved by SDS-PAGE, and analyzed by western blotting. Antibodies used for western blotting were as follows: MAP1LC3B/LC3B (Sigma, L7543), SQSTM1/p62 (BD Bioscience, 610832), HIF1A (BD Bioscience, 610958), GAPDH (Proteintech, 10494-1-AP), RHEB (Cell Signaling, 4935), RPS6KB2/p70S6K (Cell Signaling, 2708), RICTOR (Cell Signaling, 2114), phospho-MTOR (Ser2448) (Cell Signaling, 5536), MTOR (Cell Signaling, 2972), ATG3 (Cell Signaling, 3415), ATG5 (Cell Signaling, 8540), phospho-AKT (Ser473) (Cell Signaling, 9271), AKT (Cell Signaling, 4691), BCL2 (Cell Signaling, 2870), RPTOR (Cell Signaling, 2280). Following incubation with horseradish peroxidase-coupled secondary anti-mouse (KPL, 074-1806) or anti-rabbit antibodies (KPL, 474-1506), protein bands were visualized using ECL Blotting Detection Reagents (KPL, 54-61-00).

Statistical analyses

Statistical significance of differences observed in samples was determined using the Wilcoxon rank sum test or the Student 2-tailed *t* test. Data were shown as means ± s.d. of at least 3

independent experiments. Values of $P < 0.05$ were considered significant.

Supplemental Materials

Supplemental materials may be found here:
www.landesbioscience.com/journals/autophagy/article/26534

References

1. Mazure NM, Pouysségur J. Hypoxia-induced autophagy: cell death or cell survival? Curr Opin Cell Biol 2010; 22:177-80; PMID:20022734; http://dx.doi.org/10.1016/j.ceb.2009.11.015

2. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008; 132:27-42; PMID:18191218; http://dx.doi.org/10.1016/j.cell.2007.12.018

3. Ravikumar B, Sarkar S, Davies JE, Futter M, Garcia-Arencibia M, Green-Thompson ZW, Jimenez-Sanchez M, Korolchuk VI, Lichtenberg M, Luo S, et al. Regulation of mammalian autophagy in physiology and pathophysiology. Physiol Rev 2010; 90:1383-435; PMID:20959619; http://dx.doi.org/10.1152/physrev.00030.2009

4. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008; 451:1069-75; PMID:18305538; http://dx.doi.org/10.1038/nature06639

5. Mah LY, Ryan KM. Autophagy and cancer. Cold Spring Harb Perspect Biol 2012; 4:a008821; PMID:22166310; http://dx.doi.org/10.1101/cshperspect.a008821

6. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell 2002; 110:163-75; PMID:12150925; http://dx.doi.org/10.1016/S0092-8674(02)00808-5

7. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol 2004; 14:1296-302; PMID:15268862; http://dx.doi.org/10.1016/j.cub.2004.06.054

8. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. Nat Rev Cancer 2004; 4:335-48; PMID:15122205; http://dx.doi.org/10.1038/nrc1362

9. Kroemer G, Mariño G, Levine B. Autophagy and the integrated stress response. Mol Cell 2010; 40:280-93; PMID:20965422; http://dx.doi.org/10.1016/j.molcel.2010.09.023

10. Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, Kundu M, Kim DH. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol Biol Cell 2009; 20:1992-2003; PMID:19225151; http://dx.doi.org/10.1091/mbc.E08-12-1249

11. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 2011; 13:132-41; PMID:21258367; http://dx.doi.org/10.1038/ncb2152

12. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005; 307:1098-101; PMID:15718470; http://dx.doi.org/10.1126/science.1106148

13. Toschi A, Lee E, Gadir N, Ohh M, Foster DA. Differential dependence of hypoxia-inducible factors 1 alpha and 2 alpha on mTORC1 and mTORC2. J Biol Chem 2008; 283:34495-9; PMID:18945681; http://dx.doi.org/10.1074/jbc.C800170200

14. Ambros V. The functions of animal microRNAs. Nature 2004; 431:350-5; PMID:15372042; http://dx.doi.org/10.1038/nature02871

15. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136:215-33; PMID:19167326; http://dx.doi.org/10.1016/j.cell.2009.01.002

16. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010; 11:597-610; PMID:20661255

17. Ventura A, Jacks T. MicroRNAs and cancer: short RNAs go a long way. Cell 2009; 136:586-91; PMID:19239879; http://dx.doi.org/10.1016/j.cell.2009.02.005

18. Mendell JT. MicroRNAs: critical regulators of development, cellular physiology and malignancy. Cell Cycle 2005; 4:1179-84; PMID:16096373; http://dx.doi.org/10.4161/cc.4.9.2032

19. Wu H, Wang F, Hu S, Yin C, Li X, Zhao S, Wang J, Yan X. MiR-20a and miR-106b negatively regulate autophagy induced by leucine deprivation via suppression of ULK1 expression in C2C12 myoblasts. Cell Signal 2012; 24:2179-86; PMID:22781751; http://dx.doi.org/10.1016/j.cellsig.2012.07.001

20. Zou Z, Wu L, Ding H, Wang Y, Zhang Y, Chen X, Chen X, Zhang CY, Zhang Q, Zen K. MicroRNA-30a sensitizes tumor cells to cis-platinum via suppressing beclin 1-mediated autophagy. J Biol Chem 2012; 287:4148-56; PMID:22157765; http://dx.doi.org/10.1074/jbc.M111.307405

21. Zhu H, Wu H, Liu X, Li B, Chen Y, Ren X, Liu CG, Yang JM. Regulation of autophagy by a beclin 1-targeted microRNA, miR-30a, in cancer cells. Autophagy 2009; 5:816-23; PMID:19535919

22. Korkmaz G, le Sage C, Tekirdag KA, Agami R, Gozuacik D. miR-376b controls starvation and mTOR inhibition-related autophagy by targeting ATG4C and BECN1. Autophagy 2012; 8:165-76; PMID:22248718; http://dx.doi.org/10.4161/auto.8.2.18351

23. Huang Y, Guerrero-Preston R, Ratovitski EA. Phospho-ΔNp63α-dependent regulation of autophagic signaling through transcription and microRNA modulation. Cell Cycle 2012; 11:1247-59; PMID:22356768; http://dx.doi.org/10.4161/cc.11.6.19670

24. Frankel LB, Wen J, Lees M, Høyer-Hansen M, Farkas T, Krogh A, Jäättelä M, Lund AH. microRNA-101 is a potent inhibitor of autophagy. EMBO J 2011; 30:4628-41; PMID:21915098; http://dx.doi.org/10.1038/emboj.2011.331

25. Fu LL, Wen X, Bao JK, Liu B. MicroRNA-modulated autophagic signaling networks in cancer. Int J Biochem Cell Biol 2012; 44:733-6; PMID:22342941; http://dx.doi.org/10.1016/j.biocel.2012.02.004

26. Frankel LB, Lund AH. MicroRNA regulation of autophagy. Carcinogenesis 2012; 33:2018-25; PMID:22902544; http://dx.doi.org/10.1093/carcin/bgs266

27. Fang Y, Xue JL, Shen Q, Chen J, Tian L. MicroRNA-7 inhibits tumor growth and metastasis by targeting the phosphoinositide 3-kinase/Akt pathway in hepatocellular carcinoma. Hepatology 2012; 55:1852-62; PMID:22234835; http://dx.doi.org/10.1002/hep.25576

28. Ye W, Lv Q, Wong CK, Hu S, Fu C, Hua Z, Cai G, Li G, Yang BB, Zhang Y. The effect of central loops in miRNA:MRE duplexes on the efficiency of miRNA-mediated gene regulation. PLoS One 2008; 3:e1719; PMID:18320040; http://dx.doi.org/10.1371/journal.pone.0001719

29. Findlay GM, Harrington LS, Lamb RF. TSC1-2 tumour suppressor and regulation of mTOR signalling: linking cell growth and proliferation? Curr Opin Genet Dev 2005; 15:69-76; PMID:15661536; http://dx.doi.org/10.1016/j.gde.2004.11.002

30. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev 2004; 18:1926-45; PMID:15314020; http://dx.doi.org/10.1101/gad.1212704

31. Kulshreshtha R, Ferracin M, Negrini M, Calin GA, Davuluri RV, Ivan M. Regulation of microRNA expression: the hypoxic component. Cell Cycle 2007; 6:1426-31; PMID:17582223; http://dx.doi.org/10.4161/cc.6.12.4410

32. Hua Z, Lv Q, Ye W, Wong CK, Cai G, Gu D, Ji Y, Zhao C, Wang J, Yang BB, et al. MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia. PLoS One 2006; 1:e116; PMID:17205120; http://dx.doi.org/10.1371/journal.pone.0000116

33. Donker RB, Mouillet JF, Nelson DM, Sadovsky Y. The expression of Argonaute2 and related microRNA biogenesis proteins in normal and hypoxic trophoblasts. Mol Hum Reprod 2007; 13:273-9; PMID:17327266; http://dx.doi.org/10.1093/molehr/gam006

34. Hebert C, Norris K, Scheper MA, Nikitakis N, Sauk JJ. High mobility group A2 is a target for miRNA-98 in head and neck squamous cell carcinoma. Mol Cancer 2007; 6:5; PMID:17222355; http://dx.doi.org/10.1186/1476-4598-6-5

35. Li D, Liu X, Lin L, Hou J, Li N, Wang C, Wang P, Zhang Q, Zhang P, Zhou W, et al. MicroRNA-99a inhibits hepatocellular carcinoma growth and correlates with prognosis of patients with hepatocellular carcinoma. J Biol Chem 2011; 286:36677-85; PMID:21878637; http://dx.doi.org/10.1074/jbc.M111.270561

36. Nagaraja AK, Creighton CJ, Yu Z, Zhu H, Gunaratne PH, Reid JG, Olokpa E, Itamochi H, Ueno NT, Hawkins SM, et al. A link between mir-100 and FRAP1/mTOR in clear cell ovarian cancer. Mol Endocrinol 2010; 24:447-63; PMID:20081105; http://dx.doi.org/10.1210/me.2009-0295

37. Merkel O, Hamacher F, Laimer D, Sifft E, Trajanoski Z, Scheideler M, Egger G, Hassler MR, Thallinger C, Schmatz A, et al. Identification of differential and functionally active miRNAs in both anaplastic lymphoma kinase (ALK)+ and ALK- anaplastic large-cell lymphoma. Proc Natl Acad Sci U S A 2010; 107:16228-33; PMID:20805506; http://dx.doi.org/10.1073/pnas.1009719107

38. Fornari F, Milazzo M, Chieco P, Negrini M, Calin GA, Grazi GL, Pollutri D, Croce CM, Bolondi L, Gramantieri L. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res 2010; 70:5184-93; PMID:20501828; http://dx.doi.org/10.1158/0008-5472.CAN-10-0145

39. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007; 133:647-58; PMID:17681183; http://dx.doi.org/10.1053/j.gastro.2007.05.022

40. Ng R, Song G, Roll GR, Frandsen NM, Willenbring H. A microRNA-21 surge facilitates rapid cyclin D1 translation and cell cycle progression in mouse liver regeneration. J Clin Invest 2012; 122:1097-108; PMID:22326957; http://dx.doi.org/10.1172/JCI46039

41. Chun-Zhi Z, Lei H, An-Ling Z, Yan-Chao F, Xiao Y, Guang-Xiu W, Zhi-Fan J, Pei-Yu P, Qing-Yu Z, Chun-Sheng K. MicroRNA-221 and microRNA-222 regulate gastric carcinoma cell proliferation and radioresistance by targeting PTEN. BMC Cancer 2010; 10:367; PMID:20618998; http://dx.doi.org/10.1186/1471-2407-10-367

42. Garofalo M, Di Leva G, Romano G, Nuovo G, Suh SS, Ngankeu A, Taccioli C, Pichiorri F, Alder H, Secchiero P, et al. miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. Cancer Cell 2009; 16:498-509; PMID:19962668; http://dx.doi.org/10.1016/j.ccr.2009.10.014

43. Huse JT, Brennan C, Hambardzumyan D, Wee B, Pena J, Rouhanifard SH, Sohn-Lee C, le Sage C, Agami R, Tuschl T, et al. The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. Genes Dev 2009; 23:1327-37; PMID:19487573; http://dx.doi.org/10.1101/gad.1777409

44. Olive V, Bennett MJ, Walker JC, Ma C, Jiang I, Cordon-Cardo C, Li QJ, Lowe SW, Hannon GJ, He L. miR-19 is a key oncogenic component of mir-17-92. Genes Dev 2009; 23:2839-49; PMID:20008935; http://dx.doi.org/10.1101/gad.1861409

45. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol 2003; 13:1259-68; PMID:12906785; http://dx.doi.org/10.1016/S0960-9822(03)00506-2

46. Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev 2003; 17:1829-34; PMID:12869586; http://dx.doi.org/10.1101/gad.1110003

47. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012; 149:274-93; PMID:22500797; http://dx.doi.org/10.1016/j.cell.2012.03.017

48. Wang RC, Wei Y, An Z, Zou Z, Xiao G, Bhagat G, White M, Reichelt J, Levine B. Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. Science 2012; 338:956-9; PMID:23112296; http://dx.doi.org/10.1126/science.1225967

49. Eis PS, Tam W, Sun L, Chadburn A, Li Z, Gomez MF, Lund E, Dahlberg JE. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. Proc Natl Acad Sci U S A 2005; 102:3627-32; PMID:15738415; http://dx.doi.org/10.1073/pnas.0500613102

50. Kluiver J, Poppema S, de Jong D, Blokzijl T, Harms G, Jacobs S, Kroesen BJ, van den Berg A. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. J Pathol 2005; 207:243-9; PMID:16041695; http://dx.doi.org/10.1002/path.1825

51. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005; 65:7065-70; PMID:16103053; http://dx.doi.org/10.1158/0008-5472.CAN-05-1783

52. Liu X, Chen Z, Yu J, Xia J, Zhou X. MicroRNA profiling and head and neck cancer. Comp Funct Genomics 2009; 2009:837514; PMID:19753298; http://dx.doi.org/10.1155/2009/837514

53. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006; 103:2257-61; PMID:16461460; http://dx.doi.org/10.1073/pnas.0510565103

54. Du ZM, Hu LF, Wang HY, Yan LX, Zeng YX, Shao JY, Ernberg I. Upregulation of MiR-155 in nasopharyngeal carcinoma is partly driven by LMP1 and LMP2A and downregulates a negative prognostic marker JMJD1A. PLoS One 2011; 6:e19137; PMID:21541331; http://dx.doi.org/10.1371/journal.pone.0019137

55. Jiang S, Zhang HW, Lu MH, He XH, Li Y, Gu H, Liu MF, Wang ED. MicroRNA-155 functions as an OncomiR in breast cancer by targeting the suppressor of cytokine signaling 1 gene. Cancer Res 2010; 70:3119-27; PMID:20354188; http://dx.doi.org/10.1158/0008-5472.CAN-09-4250

56. Kong W, He L, Coppola M, Guo J, Esposito NN, Coppola D, Cheng JQ. MicroRNA-155 regulates cell survival, growth, and chemosensitivity by targeting FOXO3a in breast cancer. J Biol Chem 2010; 285:17869-79; PMID:20371610; http://dx.doi.org/10.1074/jbc.M110.101055

57. Babar IA, Czochor J, Steinmetz A, Weidhaas JB, Glazer PM, Slack FJ. Inhibition of hypoxia-induced miR-155 radiosensitizes hypoxic lung cancer cells. Cancer Biol Ther 2011; 12:908-14; PMID:22027557; http://dx.doi.org/10.4161/cbt.12.10.17681

58. Levati L, Alvino E, Pagani E, Arcelli D, Caporaso P, Bondanza S, Di Leva G, Ferracin M, Volinia S, Bonmassar E, et al. Altered expression of selected microRNAs in melanoma: antiproliferative and pro-apoptotic activity of miRNA-155. Int J Oncol 2009; 35:393-400; PMID:19578755

59. Qin A, Zhou Y, Sheng M, Fei G, Ren T, Xu L. [Effects of microRNA-155 on the growth of human lung cancer cell line 95D in vitro]. Zhongguo Fei Ai Za Zhi 2011; 14:575-80; PMID:21762626

60. Dai Y, Qiu Z, Diao Z, Shen L, Xue P, Sun H, Hu Y. MicroRNA-155 inhibits proliferation and migration of human extravillous trophoblast derived HTR-8/SVneo cells via down-regulating cyclin D1. Placenta 2012; 33:824-9; PMID:22858023; http://dx.doi.org/10.1016/j.placenta.2012.07.012

61. Lee IH, Kawai Y, Fergusson MM, Rovira II, Bishop AJ, Motoyama N, Cao L, Finkel T. Atg7 modulates p53 activity to regulate cell cycle and survival during metabolic stress. Science 2012; 336:225-8; PMID:22499945; http://dx.doi.org/10.1126/science.1218395

62. Filippi-Chiela EC, Villodre ES, Zamin LL, Lenz G. Autophagy interplay with apoptosis and cell cycle regulation in the growth inhibiting effect of resveratrol in glioma cells. PLoS One 2011; 6:e20849; PMID:21695150; http://dx.doi.org/10.1371/journal.pone.0020849