Deregulation of MicroRNA-375 Inhibits Proliferation and Migration in Gastric Cancer in Association With Autophagy-Mediated AKT/mTOR Signaling Pathways

Kai-Tao Yuan, MD, Bao-Xia Li, MD, Yu-Jie Yuan, MD, Min Tan, MD, Jin-Fu Tan, MD, Wei-Gang Dai, MD, Wei-Dong Feng, MD, and Ji-Dong Zuo, MD

Abstract
Gastric cancer is a deadly disease. Some microRNAs are involved in tumor invasion and metastasis. Underexpression of miR-375 has been correlated with tumorigenesis, treatment resistance, and poor prognosis. In this study, we first analyzed the profiles and prognostic values of miR-375 expression in gastric cancer tissues from a public database, and the expression level of miR-375 in gastric cancer samples and gastric cancer cell lines was then analyzed by quantitative real-time polymerase chain reaction. Significant underexpression of miR-375 was seen in all the gastric cancer samples compared to paired paracarcinoma tissues, and the expression level of miR-375 in the gastric cancer cell lines was negatively associated with the cell migration ability. A Cell proliferation (CCK-8) assay was performed to examine cell viability. Overexpression of miR-375 suppressed the proliferation of gastric cancer cells. A Western blot analysis was carried out to test protein expression. Overexpression of miR-375 inhibited autophagy through the AKT/ mammalian target of rapamycin signaling pathway. MiR-375 regulated invasion and migration via AKT/ mammalian target of rapamycin pathway-mediated epithelial-to-mesenchymal transition. Wound healing and migration assays were used to determine the motility of gastric cancer cells. A gastric cancer xenograft nude mouse model was used for an in vivo efficacy evaluation. Overexpression of miR-375 significantly suppressed cell proliferation in the established gastric cancer xenograft nude mouse model. Our results demonstrate that increasing the expression level of miR-375 suppresses proliferation in vitro and in vivo, and they provide a mechanistic and applicable rationale for the future clinical evaluation of miR-375 in gastric cancer treatment. Our findings provide not only new information about the molecular mechanism of microRNAs in regulating invasion and migration in gastric cancer but also a theoretical principle for a potential targeted therapy for gastric cancer.

Keywords
MiR-375, proliferation, invasion, migration, gastric cancer

Abbreviations
EMT, epithelial-to-mesenchymal transition; FBS, fetal bovine serum; miRNA, microRNA; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; NC, negative control; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; siRNA, small-interfering RNA.

Received: May 8, 2018; Revised: July 24, 2018; Accepted: August 31, 2018.

Introduction
Gastric cancer is one of the most common malignancies worldwide and is a major cause of cancer-related mortality in China. The occurrence of gastric cancer is a complex process of progressive development. Due to the increasing early detection of cancer and the widespread implementation of radical surgery, the overall survival of patients with gastric cancer has

1 Department of Gastrointestinal Surgery, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China

Corresponding Author:
Ji-Dong Zuo, MD, Department of Gastrointestinal Surgery, The First Affiliated Hospital of Sun Yat-sen University, No.58, Zhong Shan Er Lu, Guangzhou 510080, China.
Email: zuojd@126.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
In gastric cancer, cell invasion into the surrounding tissue is a crucial early step. The mechanisms of gastric cancer cell migration and metastasis are not fully understood; the prognosis of advanced gastric cancer remains poor, and safe and effective adjuvant therapy options are limited. Thus, exploring highly sensitive and low-cost, early-stage diagnostic methods is of great pragmatic value. MicroRNAs (miRNAs) are endogenous small noncoding RNAs that bind 3’-untranslated regions of targeted genes with high fidelity to suppress gene and protein production, thus exerting various regulatory functions on tissue and organ development in both animals and humans. MicroRNAs play a crucial role in the regulation of cell growth and differentiation. It has been demonstrated that the aberrant expression of miRNAs is involved in tumorigenesis and tumor development. Some miRNAs are involved in tumor invasion and metastasis. Studies have demonstrated that miR-375 inhibits tumor progression in gastric, prostate, breast, lung, and head and neck cancers. To date, a number of miRNAs have been found to be implicated in the progression of gastric cancer metastasis. Determining the association between specific dysregulated miRNAs and specific steps of gastric cancer metastasis will provide insight into the potential mechanisms of gastric cancer cell migration, invasion, and metastasis.

In this study, we have examined the association between a specific dysregulated miRNA and specific steps of gastric cancer metastasis, which will provide insight into the potential mechanisms of gastric cancer cell migration, invasion, and metastasis.

Many studies have shown that the overexpression of miR-375 plays a role in the regulation of autophagy and in the ability of miR-375 to regulate autophagy through the AKT/mammalian target of rapamycin (mTOR) signaling pathway. It has been confirmed that miR-375 plays a tumor suppressive role, inhibiting proliferation, invasion, and migration and promoting apoptosis via AKT signaling pathways. In our study, we found that miR-375 inhibits autophagy through the AKT/mTOR signaling pathway. The miRNAs that inhibit the autophagy of cancer cells might be developed as therapeutics. This approach is clinically challenging because of the complex mechanisms underlying these processes. Therefore, clarifying the molecular mechanisms is an important goal.

**Methods**

**Cell Cultures and Reagents**

Gastric cancer cell lines MKN-45 and GT3TKB were purchased from the American Type Culture Collection (ATCC, USA) and provided by Sparklebio (USA). MKN-45 cells were maintained in RPMI medium 1640 (Gibco, Carlsbad, California) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin, and GT3TKB cells were maintained in RPMI medium DMEM (Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin and. Cells were incubated in a 5% CO₂ humidified incubator at 37°C and collected using 0.05% trypsin EDTA following the specified incubation period. The following primary antibodies were used: E-cadherin (#3195 S), N-cadherin (#13116 S), Vimentin (#5741 S), Snail (#3879 S), AKT (#9272 S), p-AKT (#4060 S), mTOR (#2972 S), p-mTOR (#5536 S), and Slug (#9585 S; purchased from Cell Signaling Technology Inc, USA); GAPDH (#sc-47724) was from Santa Cruz Biotechnology (Shanghai, China). The data were collected from at least 3 independent experiments.

**Lentivirus Infection**

MiR-375 mimics and inhibitors and miRNA negative control (NC) were synthesized by RiboBio Co, Ltd (Guangzhou, China) (Table 1), and all recombinant lentiviruses were obtained from RiboBio Co, Ltd (Guangzhou, China), and used for hASC infection at an MOI of 80. The packaged lentiviruses used contained miR-375-mimics, NC, and miR-375 inhibitors. Infection was performed by exposing hASCs to dilutions of the viral supernatant in the presence of polybrene (5 mg/mL) and fresh medium for 24 hours, followed by selection with puromycin (Sigma-Aldrich) at 1 mg/mL. Transduction efficiency was evaluated by determining the percentage of GFP-positive cells observed under an inverted fluorescence microscope and reverse-transcriptase polymerase chain reaction (RT-PCR).

**Quantitative Real-Time RT-PCR**

Total RNA was extracted from 30 pairs of frozen tissue samples and gastric cancer cells, MKN-45 and GT3TKB, using TRIzol (Invitrogen, Carlsbad, California), according to the manufacturer’s protocol. Quantitative real-time RT-PCR analysis was performed using an Applied Biosystems 7500 Real-Time PCR System (Foster City, California). The primers used in quantitative real-time PCR analysis as follows: miR-375(F5’-TCGTTCCGAGGTATT-3’ (R3’-CTGCTTGTTCGTCG-5’). The results of U6 qRT-PCR gene expression by 2-DΔCt method were used as the control.

**Cell Viability Assay**

Cell viability was performed with the Cell proliferation assay of CCK-8 (Dojindo, Kumamoto, Japan) following the manufacturer’s instruction. The inhibition rate of cell proliferation was calculated for each well as (A450control cells – A450 treated cells)/A450control cells × 100%. Experiments were performed in

| Table 1. The Sequence of miR-375 Mimic and Inhibitor and Micro-RNA Negative Control. |
|-----------------------------------|-----------------|-----------------|
| miR-375 mimics                   | 5’-TTTTGGTTCGTTCCGCTCGCCGTGA-3’ |
| miR-375 inhibitors               | 5’-TACCGGAGCCGAACGAACAAA-3’     |
| Mimics negative control          | 5’-TTCTCCGAACGTGTCACGT-3’       |
| Inhibitors negative control      | 5’-TCGTTTCATGAACACATT-3’        |

Note: Experiments were performed in
The cells were plated on sterile coverslips for 24 hours and fixed in 4%
forgoing fixation, a permeabilization step was conducted with 0.25%
serum albumin for 1 hour at 37°C. The nucleus was stained with
4,10,10-trifluor-3,3-dimethylacridinium ethiodide (DAPI) (1 mg/mL) for
5 minutes at room temperature. Fluorescence images were then captured
by a confocal laser scanning microscope (LSM 700; Carl Zeiss, Oberkochen, Germany).

### Western Blot Assays

Cells were harvested in lysis buffer, and tumors were harvested in
RIPA buffer. After incubating on ice for 30 minutes, the cells were
centrifuged at 12,000g for 15 minutes at 4°C, and the supernatant was collected. Samples were then analyzed by
Western blot. Proteins were visualized by incubation with SuperSignal West pico reagents (NCI5079; Thermo), followed by exposure to radiograph film.

### Nude Mouse Xenograft Studies

Four-week-old BALB/c (athymic) nude mice were purchased from the Shanghai’ SIPP-R BK Laboratory Animal Co, Ltd. A total of 4 x 10⁶ cells were subcutaneously injected into the right flank of nude mice. Body weights and tumor volumes (V) were measured every 2 days. Tumor volumes were calculated using the formula: \( V = (length \times width²)/2 \).

### Statistical Analysis

All assays were performed in triplicate. Data are expressed as
the mean (SD). Statistical analyses were performed using an
analysis of variance with SPSS 13.0 software. Statistical sig-
nificance was set at 2-sided \( P < .05 \).
Gastric cancer cell lines (GT3TKB, MKN-45) were characterized. As shown in Figure 1F and G, wound healing assays were conducted to investigate migration. As shown in Figure 1H and I, invasion assays were conducted to investigate invasion. The results showed that the migration and invasion abilities of MKN-45 cells were greater than those of GT3TKB cells (**P < .01). Our results indicate that miR-375 might have a causal role in gastric cancer metastasis.

**Overexpression of miR-375 Suppressed the Proliferation of Gastric Cancer cells**

Gastric cancer cell lines (GT3TKB, MKN-45) were characterized. As shown in Figure 1F and G, wound healing assays were conducted to investigate migration. As shown in Figure 1H and I, invasion assays were conducted to investigate invasion. The results showed that the migration and invasion abilities of MKN-45 cells were greater than those of GT3TKB cells (**P < .01). Our results indicate that miR-375 might have a causal role in gastric cancer metastasis.

All recombinant lentiviruses were obtained from RiboBio Co, Ltd. The packaged lentiviruses used contained miR-375 mimics, NCs, and miR-375 inhibitors. Lentiviral transduction was performed following the manufacturer’s protocol. The transduction efficiency was evaluated by determining the percentage of GFP-positive cells observed under an inverted fluorescence microscope (Figure 2A and C). Reverse-transcriptase polymerase chain reaction was conducted in Eca-109-miR-375, Eca-109-miR-375-mimic, and Eca-109-miR-375-NC cells to assess the expression level of miR-375 (Figure 2B and D). Significant underexpression of miR-375 was seen in GT3TKB-miR-375-inhibitor compared to GT3TKB-LV3-NC (Figure 2B), and significant overexpression of miR-375 was seen in MKN-45-miR-375-mimic compared to MKN-45-LV3-NC (Figure 2D).

Next, we explored the biological role of miR-375 at the cellular level. To explore the influence of miR-375 expression on cell survival, we used CCK-8 assay to investigate whether
miR-375 was involved in the suppression of cell proliferation. As shown in Figure 2E, a significant suppression of cell proliferation was found in GT3TKB-LV3-NC compared with GT3TKB-miR-375-inhibitors, and as shown in Figure 2F, a significant suppression of cell proliferation was found in MKN-45-miR-375-mimics compared to MKN-45-LV3-NC. The result suggests that overexpressed miR-375 has a significant inhibiting effect on the proliferation of gastric cancer cells. We explored the effect of miR-375 on the migration and invasion of gastric cancer cells. As shown in Figure 2G-J, the transwell invasion assay showed that the invasion of GT3TKB-miR-375-inhibitors cells was much higher than GT3TKB-miR-375-NC and MKN45-miR-375-NC was much higher than MKN45-miR-375-mimics. In consistent with these results, the scratch wound healing assay also revealed that the migration of GT3TKB-miR-375-NC when compared to GT3TKB-miR-375-inhibitors and MKN45-miR-375-mimics was much lower than and MKN45-miR-375-NC toward the wound area was significantly increased (Figure 2K-N). Collectively, these results indicate that overexpression of miR-375 is sufficient to inhibit both the migration and the invasion abilities of gastric cancer cells.

**Effect Quantitative of miR-375 on the Expression of Epithelial-to-Mesenchymal Transition-Related Proteins**

Quantitative RT-PCR was conducted to investigate the messenger RNA (mRNA) expression levels of the epithelial-to-mesenchymal transition (EMT)-related proteins E-cadherin, N-cadherin, vimentin, Snail, and Slug normalized to GAPDH.
mRNA expression levels. We detected an associated decrease in E-cadherin expression and an increase in N-cadherin, vimentin, Snail, and Slug expression at the mRNA level in GT3TKB-miR-375-inhibitor compared to their expression levels in GT3TKB-miR-375-NC, as shown in Figure 3A. Furthermore, we observed an associated increase in E-cadherin expression and a decrease in N-cadherin, vimentin, Snail, and Slug expression at the mRNA level in MKN-45-miR-375-mimic compared to their expression levels in MKN45-miR-375-NC, as shown in Figure 3B. These results demonstrated that miR-375 may inhibit EMT in gastric cancer cells. Next, Western blotting was conducted to investigate the expression levels of the EMT-related proteins E-cadherin, N-cadherin, vimentin, Snail, and Slug at the protein level. As shown in Figure 3C, GT3TKB-miR-375-inhibitor demonstrated a decreased expression of E-cadherin and an increased expression of N-cadherin, vimentin, Snail, and Slug compared to GT3TKB-miR-375-NC. As shown in Figure 3D, MKN-45-miR-375-mimic demonstrated an increased expression of E-cadherin and a decreased expression of N-cadherin, vimentin, Snail, and Slug compared to MKN45-miR-375-NC. The results indicated that in gastric cancer cells, E-cadherin, N-cadherin, vimentin, Snail, and Slug were the downstream proteins of miR-375 and that their expression levels were modulated by miR-375. Consistent with the previous results, the overexpression of miR-375 inhibited EMT in gastric cancer cells.

**MiR-375 Inhibits Autophagy Through the AKT/mTOR Signaling Pathway**

Autophagy was measured by light microscopic quantitation of cells transfected with GFP-LC3 as described or by Western blot analysis of the levels of LC3. To investigate whether miR-375 inhibits autophagy, we measured the expression levels of LC3, as shown in Figure 4A and B. The intensity of the LC3-II band increased and the conversion of LC3-I into LC3-II was evident, indicating the activation of autophagy. In our study, the results indicate that
Figure 4. MiR-375 inhibits autophagy through AKT/mammalian target of rapamycin (mTOR) signaling pathway in gastric cancer cells. A and B, The expression of LC3 was measured by Western blot. C and D, Cells were transfected with GFP-LC3 plasmids, then stained with DAPI, and analyzed by fluorescence microscopy. E and F, Statistical analysis of the percentage of LC3 puncta per cell. Columns, mean (n = 3); bars, standard deviation (SD). *P < .01 versus untreated group. LC3 puncta per cell was quantified. G, Cells were transfected with ATG7 small-interfering RNAs (siRNAs). Western blot was used to detect the expression of ATG7. H and I, Cells were transfected with ATG7 siRNAs, and Western blot was conducted using indicated antibodies. J and K, The protein levels of AKT/mTOR signaling pathway (AKT, p-AKT, mTOR, and p-mTOR) were assessed using Western blotting. L and M, Cells were transfected with ATG7 siRNAs. The expression levels of AKT, p-AKT, mTOR, and p-mTOR were analyzed by Western blot.
the overexpression of miR-375 inhibits autophagy. To further confirm these results, we examined LC3 punctate formation under a confocal microscope. LC3 is an autophagosome membrane marker, as demonstrated by accumulation of punctate LC3 in the cytoplasm of cells (shown in Figure 4C-F), suggesting the inhibition of autophagy by the overexpression of miR-375. In our study, siRNAs were designed to reduce ATG7 protein levels, and these siRNAs completely abolished the expression of ATG7 (shown in Figure 4G). ATG7 protein knockdown dramatically counteracted the autophagy inhibited by the overexpression of miR-375 (Figure 4H and I).

To investigate the effect of miR-375 on the AKT/mTOR signaling pathway, we examined the protein levels of AKT, p-AKT, mTOR, and p-mTOR by Western blotting. Figure 4J shows that miR-375 inhibitors increased the expression of p-mTOR and p-AKT, and Figure 4K shows that miR-375-mimics decreased the expression of p-mTOR and p-AKT. In our study, we next investigated the role of autophagy in miR-375-mediated AKT/mTOR signaling pathway induction; siRNAs were designed to reduce the ATG7 protein level. And then, we investigated the expression of AKT, p-AKT, mTOR, and p-mTOR after cells were transfected with ATG7 siRNAs, and the protein levels of AKT, p-AKT, mTOR, p-mTOR were partially observed, as shown in Figure 4L and M. These results indicating that miR-375 inhibits autophagy through AKT/mTOR signaling pathway.
Overexpression of miR-375 Suppressed Tumor Formation By Gastric Cancer Cells in Nude Mice

We then investigated the effect of miR-375 on in vivo gastric cancer tumorigenesis. Gastric cancer-bearing nude mouse models were generated by the subcutaneous inoculation of MKN-45-miR-375-mimic in the treatment group of nude mice and the subcutaneous inoculation of mock-carrier MKN45-miR-375-NC in the control group. The in vivo transplantation study was carried out for 4 weeks. Every 2 days, body weights and tumor volumes (V) were compared between the treatment group and the control group. As shown in Figure 5A to C, the results indicated that the overexpression of miR-375 significantly suppressed the proliferation of gastric cancer cells in vivo. Consistent with in vitro results, expressions levels of p-AKT and p-mTOR were markedly downregulated in MKN-45-miR-375-mimics group xenografts (Figure 5D and E).

Discussion

MicroRNAs have been demonstrated to play critical roles in cells during the progression of cancers in recent years. Studies have shown that miRNAs profoundly affect an array of normal biological processes and that they play important roles in cancer by regulating the expression of various oncogenes and tumor suppressors. Some miRNAs are involved in tumor invasion and metastasis. MicroRNAs have been suggested to be posttranscriptional repressors that inhibit target gene expression via translation inhibition or mRNA degradation. Moreover, the aberrant expression of miRNAs is significantly related to gastric cancer tumor stage, size, differentiation, and metastasis. MicroRNAs interrupt cellular signaling pathways and inhibit the activity of tumor suppressor genes in gastric cancer cells. The expression of miR-375 has been found to be significantly downregulated in multiple types of cancers, and miR-375 is believed to act as a cancer suppressor.

However, the expression and role of miR-375 in gastric cancer still need to be further studied. By qRT-PCR, the underexpression of miR-375 was demonstrated in gastric cancer tumor tissues compared to normal paracarcinoma tissues. Next, we successfully constructed the miR-375-inhibitor, miR-375-mimic, and miR-375-NC lentiviral expression vectors, hence creating the basis for a subsequent study involving the function of miR-375. Cell differentiation is a complex process governed by the interplay of several signaling pathways. We explored the influence of miR-375 expression on cell proliferation. Our results suggest that the overexpression of miR-375 has a significant inhibitory effect on the proliferation of gastric cancer cells. The EMT is a process where epithelial cells lose their characteristics and plays a critical role in many aspects of cancer behavior, including the phenotypic conversion that is implicated in the initiation of metastasis in gastric cancer progression. Furthermore, our results showed that overexpression of miR-375 was sufficient to inhibit both the migration and invasion abilities of gastric cancer cells. Previous studies have found that miR-375 regulates the progression of cancers through signaling pathways related to the EMT process in multiple cancers. The EMT is critical in many biological behaviors of cancer, such as migration and invasion. During EMT, the expression of some epithelial cell markers such as E-cadherin decreases, while the expression of vimentin increases. In the present study, we found that miR-375 regulated the EMT process by regulating the expression of E-cadherin, vimentin, Snail, and Slug in gastric cancer.

The multifaceted miR-375-mediated anticancer effects play a causal but unclear role in mammalian oncogenesis. The AKT/mTOR pathway is major pathway involved in the regulation of cell proliferation and differentiation, and it plays a significant role in regulating cell proliferation and differentiation. Some antitumor drugs can induce the early autophagy and late apoptosis of tumor cells through the AKT/mTOR signaling pathway. In this study, we found that miR-375 inhibits autophagy through the AKT/mTOR signaling pathway. Studies have demonstrated that miR-375 inhibits tumor progression in gastric, prostate, breast, lung, and head and neck cancers. Overexpression of miR-375 significantly suppressed proliferation in an established gastric cancer xenograft nude mouse model. Our findings provide not only new information about the molecular mechanism of miRNAs in regulating invasion and migration in gastric cancer but also a theoretical principle for a potential targeted therapy for gastric cancer.

Authors’ Note

Kai-Tao Yuan and Bao-Xia Li contributed equally to this study. Four-week-old BALB/c (athymic) nude mice were purchased from the Shanghai SIPPR-BK Laboratory Animal Co, Ltd. The animal study protocol was approved by Institutional Animal Care and Use Committee (IACUC) at Sun Yat-Sen University. Ethical number: IACUC-APS-EF-105. All animal experiments were performed at Ascentage. This study was performed with the approval of the local ethical committee and all the experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Bao-Xia Li, MD http://orcid.org/0000-0001-7113-3327

References

1. Smith MG, Hold GL, Tahara E, El-Omar EM. Cellular and molecular aspects of gastric cancer. World J Gastroenterol. 2006;12(19):2979-2990.
2. Ishihara R. Infrared endoscopy in the diagnosis and treatment of early gastric cancer. Endoscopy. 2010;42(8):672-676.
3. Tanizawa Y, Terashima M. Lymph node dissection in the resection of gastric cancer: review of existing evidence. *Gastric Cancer*. 2010;13(3):137-148.

4. Xu Y, Jin J, Liu Y, et al. Snail-regulated MiR-375 inhibits migration and invasion of gastric cancer cells by targeting JAK2. *PloS One*. 2014;9(7):e99516.

5. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116(2):281-297.

6. Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol*. 2014;15(8):509-524.

7. Pritchard CC, Cheng HH, Te wari M. MicroRNA profiling: approaches and considerations. *Nat Rev Genet*. 2012;13(5):358-369.

8. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol Med*. 2017;9(6):852.

9. de Souza Rocha Simionini P, Breiling A, Gupta N, et al. Epigenetically deregulated microRNA-375 is involved in a positive feedback loop with estrogen receptor alpha in breast cancer cells. *Cancer Res*. 2010;70(22):9175-9184.

10. Nishikawa E, Osada H, Okazaki Y, et al. miR-375 is activated by DGCR8, and AGO2, in Asian Korean papillary thyroid carcinoma levels of miRNA machinery-related genes, dicer, drosha, DGCR8, and AGO2, in Asian Korean papillary thyroid carcinoma and comparative validation using the cancer genome atlas. *Int J Genomics*. 2017;2017:5789769.

11. Pritchard CC, Cheng HH, Te wari M. MicroRNA profiling: approaches and considerations. *Nat Rev Genet*. 2012;13(5):358-369.

12. Nishikawa E, Osada H, Okazaki Y, et al. miR-375 is activated by DGCR8, and AGO2, in Asian Korean papillary thyroid carcinoma levels of miRNA machinery-related genes, dicer, drosha, DGCR8, and AGO2, in Asian Korean papillary thyroid carcinoma and comparative validation using the cancer genome atlas. *Int J Genomics*. 2017;2017:5789769.

13. Ding L, Xu Y, Zhang W, et al. MiR-375 frequently downregulated in gastric cancer inhibits cell proliferation by targeting JAK2. *Cell Res*. 2010;20(7):784-793.

14. Zhao X, Dou W, He L, et al. MicroRNA-7 functions as an anti-metastatic microRNA in gastric cancer by targeting insulin-like growth factor-1 receptor. *Oncogene*. 2013;32(11):1363-1372.

15. Yao Q, Cao Z, Tu C, Zhao Y, Liu H, Zhang S. MicroRNA-146a acts as a metastasis suppressor in gastric cancer by targeting WASF2. *Cancer Lett*. 2013;335(1):219-224.

16. Zheng L, Qi T, Yang D, et al. microRNA-9 suppresses the proliferation, invasion and metastasis of gastric cancer cells through targeting cyclin D1 and Ets1. *PloS One*. 2013;8(1):e55719.

17. Chang Y, Lin J, Tsung A. Manipulation of autophagy by MiR375 generates antigut effects in liver cancer. *Autophagy*. 2012;8(12):1833-1834.

18. Chang Y, Yan W, He X, et al. miR-375 inhibits autophagy and reduces viability of hepatocellular carcinoma cells under hypoxic conditions. *Gastroenterology*. 2012;143(1):177-87.e8.

19. Luo J, Wu J, Li Z, et al. miR-375 suppresses IGF1 R expression and contributes to inhibition of cell progression in lar- yngeal squamous cell carcinoma. *Biomed Res Int*. 2014;2014:374598.
pathway-mediated epithelial-mesenchymal transition. Cancer Lett. 2014;343(2):258-267.

39. Hansen CG, Moroishi T, Guan KL. YAP and TAZ: a nexus for Hippo signaling and beyond. Trends Cell Biol. 2015;25(9):499-513.

40. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012;149(2):274-293.

41. Hansen CG, Ng YL, Lam WL, Plouffe SW, Guan KL. The Hippo pathway effectors YAP and TAZ promote cell growth by modulating amino acid signaling to mTORC1. Cell Res. 2015;25(12):1299-1313.

42. Dibble CC, Cantley LC. Regulation of mTORC1 by PI3 K signaling. Trends Cell Biol. 2015;25(9):545-555.

43. Kumar D, Shankar S, Srivastava RK. Rottlerin-induced autophagy leads to the apoptosis in breast cancer stem cells: molecular mechanisms. Mol Cancer. 2013;12(1):171.

44. Rabinowitz JD, White E. Autophagy and metabolism. Science. 2010;330(6009):1344-1348.