Downregulation of MicroRNA-455-3p Links to Proliferation and Drug Resistance of Pancreatic Cancer Cells via Targeting TAZ

Ting Zhan,1,2,3,4 Xiaodong Huang,1,4 Xia Tian,1 Xiaoli Chen,1 Yu Ding,2 Hesheng Luo,3 and Yadong Zhang2

1Department of Gastroenterology, Wuhan Third Hospital, Tongren Hospital of Wuhan University, Wuhan 430060, China; 2Key Laboratory for Molecular Diagnosis of Hubei Province, 26 Shengli Street, Wuhan 430014, China; 3Department of Gastroenterology, Renmin Hospital of Wuhan University, Wuhan 430060, China

Drug resistance is a major cause of treatment failure in pancreatic cancer. The limited evidence indicates the involvement of miR-455-3p in chemotherapy resistance of pancreatic cancer. Here we observed by qPCR that miR-455-3p was significantly decreased in pancreatic cancer tissues and cell lines. We then confirmed that the inhibition of miR-455-3p increased cell proliferation and gemcitabine resistance of pancreatic cancer, whereas forced overexpression of miR-455-3p had the opposite effect. Furthermore, we demonstrated that TAZ, which is associated with drug resistance of pancreatic cancer, is a new direct downstream target of miR-455-3p. Our present study suggests that miR-455-3p contributes to cell proliferation and drug resistance in pancreatic cancer cells via targeting TAZ.

INTRODUCTION

Pancreatic cancer is one of the most aggressive tumors with a high mortality rate and short survival as a result of the early metastasis and reduced chemotherapeutic efficacy.1 Drug resistance is one of the main causes of failure in the chemotherapeutic treatment of different types of cancer, including pancreatic cancer. The molecular mechanism underlying the drug resistance includes drug inactivation, drug target alteration, drug efflux, DNA damage repair, cell death induction, epithelial-mesenchymal transition, inherent cell heterogeneity, and epigenetic effects.2 Elucidation of the mechanism of drug resistance in pancreatic cancer is important for improving chemotherapeutic efficacy against pancreatic cancer.

MicroRNAs act as post-transcriptional regulators of gene expression, and they are involved in the regulation of genes related to various kinds of biological processes, such as development, differentiation, proliferation, and apoptosis of cells. Based on the different targeted genes by different microRNAs in different types of cells, microRNAs exhibit either a positive or negative effect on drug resistance. Accumulating data have demonstrated that microRNAs contribute to the development progression, metastasis, and drug resistance of pancreatic cancer.3–8 It has been reported that miR-455-3p is implicated in acquired temozolomide resistance in glioblastoma multiforme cells.9 Our recent microRNA microarray study showed that miR-455-3p was downregulated in human ovarian cancer cells (data not shown). However, it is not clear how miR-455-3p plays its role in drug resistance associated with pancreatic cancer.

Transcriptional co-activator with PDZ-binding motif (TAZ), also known as WW domain-containing transcriptional regulator 1 (WWTR1), is a key downstream component of the Hippo pathway.10–12 The expression of TAZ is upregulated in a variety of tumors, such as lung cancer,13 breast cancer,14 colorectal cancer,15 oral cancer,16 and pancreatic cancer.17,18 Growing evidence suggests that TAZ promotes resistance to various anti-cancer therapies, including cytotoxic chemotherapy.19 It has been known that many microRNAs, including miR-9-3p, miRNA-125a-5p, miRNA-141, and miRNA-338-3p, directly regulate the expression of TAZ.20–23 It is unclear whether miR-455-3p also exerts the post-transcriptional regulation of the TAZ gene.

In this study, we show that miR-455-3p is downregulated whereas TAZ is upregulated during the development of drug resistance in pancreatic cancer cells. Furthermore, we reveal that miR-455-3p acts as a direct post-transcriptional regulator of TAZ to contribute to the proliferation and drug resistance of pancreatic cancer cells. The data presented here provide new insights into the role of miR-455-3p and its downstream target TAZ in drug resistance of pancreatic cancer.

RESULTS

miR-455-3p Is Downregulated in Pancreatic Cancer and Contributes to Cell Proliferation of Pancreatic Cancer

miR-455-3p has recently been implicated in acquired temozolomide resistance of glioblastoma multiforme cells and chondrogenesis of mesenchymal stem cells.9,24 Our recent microRNA (miRNA) microarray study showed that miR-455-3p was downregulated in human
miR-455-3p Decreases Gemcitabine Resistance in Pancreatic Cancer Cell Lines

Since our previous study showed that miR-455-3p was upregulated in drug-resistant human ovarian cancer cells (data not shown), we hypothesized that miR-455-3p might be involved in the drug resistance of pancreatic cancer. Gemcitabine has been widely used in the treatment of pancreatic cancer, and it is an antimetabolite that inhibits processes required for DNA synthesis phase. We conducted qPCR to detect the expression of miR-455-3p in pancreatic cancer cells, and we found that miR-455-3p was downregulated in both PANC1 and MIAPaCa2 compared with the normal pancreatic ductal epithelial cell line, HPDE6c7 (Figure 1B). To evaluate the effect of miR-455-3p on cell proliferation in pancreatic cancer, we transfected both PANC1 and MIAPaCa2 cells with miR-455-3p mimics, miR-455-3p inhibitors, or their negative control (NC), and then we measured the cell proliferation by CCK-8 assays and colony formation assay. We observed that the miR-455-3p overexpression decreased the proliferation of both PANC1 and MIAPaCa2, whereas the miR-455-3p inhibition increased the proliferation of both PANC1 and MIAPaCa2 (Figures 1C–1F). These data suggest that miR-455-3p is downregulated in pancreatic cancer and contributes to cell proliferation in pancreatic cancer.

miR-455-3p Participates in Cell Proliferation and Drug Resistance of Pancreatic Cancer Cells via Targeting TAZ

To determine whether TAZ is involved in the drug resistance of pancreatic cancer cells, we transfected both PANC1 and MIAPaCa2 with TAZ expression vector or TAZ small interfering RNA (siRNA) lentiviral vector, and then we treated the cells with gemcitabine. We showed that inhibition of TAZ decreased the IC50 values of gemcitabine and increased the number of apoptotic cells in pancreatic cancer cells (Figures 3F and 3H), whereas overexpression of TAZ increased the IC50 values of gemcitabine and decreased the number of apoptotic cells in pancreatic cancer cells (Figures 3G and 3I). These data indicate that TAZ is involved in the drug resistance of pancreatic cancer cells.

Figure 1. miR-455-3p Is Downregulated in Pancreatic Cancer

(A) Expression of miR-455-3p in pancreatic cancer tissue and the adjacent non-neoplastic tissues. (B) Expression of miR-455-3p in HPDE6c7, PANC-1, and MIAPaCa-2 cells via qPCR. (C and D) The effect of miR-455-3p on proliferation in PANC1 cells (C) and MIAPaCa2 cells (D) transfected with miR-455-3p mimics or inhibitors via CCK-8 assay. (E and F) The effect of miR-455-3p on proliferation in PANC1 cells (E) and MIAPaCa2 cells (F) transfected with miR-455-3p mimics or inhibitors via colony formation assay. The statistical significance was evaluated using Student’s t test or one-way ANOVA. All data are presented as the mean ± SD. *p < 0.05, **p < 0.01.

TAZ Is Associated with the Drug Resistance of Pancreatic Cancer Cells

To investigate how miR-455-3p regulates proliferation and drug resistance in pancreatic cancer cells, we predicted TAZ as one potential target of miR-455-3p using TargetScan (http://www.targetscan.org). TAZ is a key downstream component of the Hippo pathway, and it is associated with cancer cell proliferation, invasion, metastasis, epithelial-mesenchymal transition (EMT), and cancer stem cell maintenance. It has been reported that TAZ is markedly upregulated in pancreatic cancer cell and promotes the growth of pancreatic cancer cells. In this study, we examined the expression of TAZ in pancreatic cancer cell lines via qPCR and western blot, and we found significantly higher expression levels of TAZ mRNA and protein in both PANC1 and MIAPaCa2 compared to HPDE6c7 (Figures 3A and 3B). We also observed that the expression of TAZ mRNA and protein increased in both PANC1 and MIAPaCa2 treated with gemcitabine (Figures 3C–3E).

To verify whether miR-455-3p participates in cell proliferation and drug resistance of pancreatic cancer cells via targeting TAZ, we tested the cell proliferation and IC50 of gemcitabine in both PANC-1 and MIAPaCa2 cells, which were transfected with miR-455-3p mimics.
MicroRNAs are small non-coding RNAs of approximately 22 nt that act as post-transcriptional regulators of gene expression. It has been estimated that microRNAs regulate 30–60% of protein-coding genes. MicroRNAs are involved in the regulation of genes related to various kinds of biological processes, such as cell proliferation, apoptosis, and drug resistance. Drug resistance is the main cause of failure in the chemotherapeutic treatment of cancer, including pancreatic cancer. Several earlier reports suggested that a few microRNAs, such as miR-21, miR-141, miR-199a, miR-205, miR-214, and miR-421, contribute to drug resistance in cancer.27–30 Based on the different targeted genes by different microRNAs in different types of cells, microRNAs exhibit either a positive or negative effect on drug resistance. The limited data show that miR-455-3p has been implicated in cell proliferation, apoptosis, migration, and invasion of cancer cells.34–37 An earlier report demonstrated that miR-455-3p was upregulated in glioblastoma multiforme cells with temozolomide resistance and suppression of miR-455-3p led to a cell-killing effect of temozolomide.9 In this study, we reveal that downregulated miR-455-3p in pancreatic cancer is associated with the proliferation and drug resistance of pancreatic cancer cells. The paradoxical regulation of miR-455-3p on chemotherapy resistance may be due to different types of cancer cells and different drugs.

It has been reported that the direct downstream targets of the miR-455 family include RAB18,34 Runx2,35 UBE2B,36 and ZEB1.37 miR-455 plays a role in many biological processes via suppressing its downstream targets. Here we identified TAZ as a new direct downstream target of miR-455-3p. TAZ plays a role in many biological processes via suppressing its downstream targets. Here we identified TAZ as a new direct downstream target of miR-455-3p. TAZ as well as Yes-associated protein (YAP) is the major downstream effectors of the Hippo pathway, which regulates tissue homeostasis, organ size, regeneration, and tumorigenesis.38 Transcriptional upregulation of TAZ appears to be a critical mechanism in the development of drug resistance in cancer cells.18 Several microRNAs, including miR-9-3p, miRNA-125a-5p, miRNA-141, and miRNA-338-3p, directly regulate the expression of TAZ.20–23 The data presented in this study suggest miR-455-3p as a new regulator that participates in the growth and drug resistance of pancreatic cancer via directly suppressing TAZ.

Since many potential targets of miR-455-3p and other drug resistance-related genes exist, miR-455-3p may contribute to drug resistance of cancer through targeting other downstream genes. It is well known that YAP, which is similar to its homolog TAZ, functions as a transcriptional cofactor and the core of the Hippo-YAP-signaling pathway, which is implicated in the regulation of organ size, tissue growth, cell proliferation, migration, stem cell self-renewal, tissue regeneration, as well as drug resistance.39 Because no potential binding sites of miR-455-3p in the 3 ‘UTR of YAP were predictable, we did not examine the role of YAP in gemcitabine resistance of pancreatic cancer cells. However, we hypothesized that other potential microRNAs may lead to drug resistance via targeting YAP.

In conclusion, our present study provides novel insights into the role of miR-455-3p and its post-transcriptional regulation to TAZ in the drug resistance of pancreatic cancer. Biological or pharmacological intervention based on miR-455-3p may be a promising new strategy to inverse the drug resistance and improve the chemotherapeutic efficacy in human pancreatic cancer cells.

**MATERIALS AND METHODS**

**Cell Lines and Cell Culture**

The human pancreatic cancer cell lines PANC-1 and MIAPaCa-2, human normal pancreatic ductal epithelial cell line HPDE6c7, and...
Figure 3. TAZ Is Associated with the Drug Resistance of Pancreatic Cancer Cells

(A and B) Expression of TAZ mRNA and protein in PANC-1 and MIAPaCa-2 cells was detected by qPCR (A) and western blot (B). (C–E) Expression of TAZ mRNA and protein in PANC-1 and MIAPaCa-2 cells treated with GEM (0, 20, 40, and 80 μM) for 6 hr was detected by qPCR (C) and western blot (D and E). (F and G) The IC50 values of GEM in PANC1 (F) and MIAPaCa2 cells (G) transfected with miR-455-3p mimics or lenti-TAZ for 72 hr using CCK-8 assay. (H and I) Flow cytometry assessment of apoptosis in PANC1 cells (H) transfected with lenti-siRNA/TAZ and MIAPaCa2 cells (I) transfected with lenti-TAZ, and both of them treated with 20 μM GEM for 48 hr. The total events shown in the lower right-hand and upper right-hand quadrants are apoptotic cells. The statistical significance was evaluated using Student’s t test or one-way ANOVA. All data are presented as the mean ± SD. *p < 0.05, **p < 0.01. GEM, gemcitabine.
HEK293T cells were provided by the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). All of the cells were cultured in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (NQBB, USA), 100 μg/mL streptomycin, and 100 IU/mL penicillin (Thermo Fisher Scientific, USA). Cells were incubated in a humidified incubator at 37°C with 5% CO₂.

 Patients and Tissue Samples
Human pancreatic cancer and corresponding noncancerous tissue used in this study were obtained from patients who underwent surgical resection. All patients whose tumor tissue was examined had not had any neoadjuvant chemotherapy prior to surgical resection. A total of 13 paired tissues involved in qPCR assay were from the Renmin Hospital of Wuhan University (n = 8) and the Central Hospital of Wuhan (n = 5). These samples were snap-frozen in liquid nitrogen immediately and stored at −80°C until RNA extraction. The use of tissues for this study was approved by the Ethics Committee of Renmin Hospital of Wuhan University and the Central Hospital of Wuhan.

Cell Transfection
Hsa-miR-455-3p mimics (sense: 5'-GCAGUCCAUGGCAUAACAG-3'; antisense: 5'-GUUAUGCCCAUGGACUGCUU-3'), hsa-miR-455-3p inhibitors (sense: 5'-GUGUAUAGGGUUUGUGUAGA-3'), hsa-miR mimics NC (sense: 5'-UUCUCCGAACGUGUCACGU TT-3'; antisense: 5'-UGAUCUCCGAACGUGUCACGU TT-3'), and hsa-miR inhibitors NC (5'-CA GUACUUUUGUAGAACAA-3') were synthesized by GenePharma (Shanghai, China). PANC-1 and MiaPaCa2 cells were transfected with miR-455-3p mimics (40 nM), miR-455-3p inhibitors (80 nM), or their NC using Lipofectamine 2000 (Invitrogen, USA), according to the manufacturer’s instructions. The TAZ lentiviral expression vector (lenti-TAZ) and TAZ siRNA lentiviral expression vector (lenti-siRNA/TAZ) were constructed by Shanghai GeneChem (Shanghai, China). The lentiviruses were transfected according to the manufacturer’s instructions.

Total RNA Extraction and qPCR Analysis
Total RNA from tissues and cell lines was extracted using TRIzol Reagent (Invitrogen, USA), and subsequent synthesis of cDNA was
carried out according to the manufacturer’s protocols (TOYOBO, Japan). To evaluate TAZ mRNA levels, the reverse transcription products were analyzed using UltraSYBR Mixture (ComWin Biotech, China) on an ABI StepOne Plus qPCR System (Applied Biosystems, USA). GAPDH mRNA was used as an endogenous control. The expression level of miR-455-3p was evaluated using the same qPCR system. Endogenous U6 small nuclear RNA (snRNA) was used for normalization. The primers for amplification were as follows: GAPDH (sense: 5′-TCTGACTTCAACGGACAC-3′; antisense: 5′-CCTTCGGGATTTGCGCTCA-3′); and TAZ (sense: 5′-GTCA CGGATTACAGC CAGGGTAAAAAG-3′). Primers for miR-455-3p and U6 were obtained from Ribobio (Guangzhou, China). The 2−ΔΔCT method was used to calculate changes in mRNA expression levels.

**Western Blot Analysis**

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitors (Beyotime, Shanghai, China), and the protein concentration was measured using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, USA). Soluble lysate was mixed with loading buffer and boiled for 10 min. Proteins were separated by 10% SDS-PAGE, transferred to NC membranes, and then incubated with loading buffer and boiled for 10 min. Proteins were separated by SDS-PAGE, transferred to NC membranes, and then incubated with primary antibodies against TAZ (1:1,000, CST, USA) or GAPDH (1:1,000, Santa Cruz Biotechnology, USA) overnight at 4°C. The membranes were further incubated with secondary antibodies (LI-COR Biosciences, USA) for 1 hr at 37°C, and the protein levels were detected by the enhanced chemiluminescence (ECL) Western Blot Analysis Detection System (Amersham, USA).

**Analysis of Cell Proliferation and IC50**

The Cell Counting Kit-8 Assay Kit (CCK-8, Dojindo, Japan) was used to determine the cell proliferation and drug resistance. Cells were seeded in 96-well plates at a density of 2 × 103 cells per well, and 10 μL CCK-8 solution was added to each well the next day. The cells were incubated for 2 hr at 37°C, and the absorbance at 450 nm was determined using EnSpire Multimode Plate Reader (PerkinElmer, USA).

**Colony Formation Assay**

Cells were seeded in 6-well plates at a density of 500 cells per well; visible cell colonies appeared after 2 weeks in complete medium at 37°C. The cell colonies were then fixed with methanol for 15 min and stained with 0.1% crystal violet for 30 min.

**Analysis of Cell Apoptosis**

Pancreatic cancer cells were transfected in 6-well plates and 20 mg gemcitabine (Sigma, USA) was added to each well the next day. After incubating for 48 hr, cells were stained using the Annexin V-PE Detection Kit (BD Biosciences, USA), according to the manufacturer’s protocols. All the samples were analyzed using the FACs Caliber II Sorter and Cell Quest FACs System (BD Biosciences, USA).

**Luciferase Activity Assay**

Cells were seeded in 24-well plates for 24 hr. The miR-455-3p mimics or mimics NC and the psiCHECK2-UTR vector (Promega, USA) were co-transfected into HEK293T cells using Lipofectamine 2000 (Invitrogen, USA) and cultured for 48 hr. Then the luciferase activities were measured using Dual-Luciferase Reporter Assay System (Promega, USA), according to the manufacturer’s protocols.

**Tumor Formation in Xenograft Model**

The 6-week-old male BALB/c nude mice were purchased from Beijing Vital River Laboratory Animal Technology (Beijing, China) and housed under specific pathogen-free conditions. 5 × 106 PANC1 cells suspended in 100 μL PBS-containing 25% Matrigel were subcutaneously injected into the flank of the nude mice. When the tumor volume reached approximately 100 mm3, miR-455-3p agomir and agomir NC (GenePharma, Shanghai, China) were respectively injected into the tumor at multiple sites three times every 5 days. Each treatment group had five animals. Tumor volume was measured every 5 days. All mice were sacrificed after 30 days. Tumor grafts from nude mice were dissected for immunohistochemistry (IHC) and qPCR analysis. Tumor volume was monitored by measuring the length (L) and width (W) with calipers and calculated using the following formula: tumor volume = L × W2/2.

**Histological Analysis**

IHC analysis was performed per standard protocols. The primary antibodies used for IHC analysis were a TAZ antibody (1:100, CST, USA) and Ki67 antibody (1:100, CST, USA).

**Statistical Analysis**

All statistical analyses were conducted using SPSS software and illustration data were performed by GraphPad Prism. The statistical significance was evaluated using Student’s t test or one-way ANOVA, and it is presented as the mean ± SD; p < 0.05 was considered statistically significant.

**AUTHOR CONTRIBUTIONS**

Y.Z. and T.Z. conceived and designed the experiments. T.Z., X.T., X.C., and Y.D. performed the experiments. Y.Z., T.Z., X.H., and H.L. analyzed data. Y.Z. and T.Z. wrote the manuscript.

Figure 5. miR-455-3p Participates in Cell Proliferation and Drug Resistance of Pancreatic Cancer Cells via Targeting TAZ

(A) qPCR analysis of TAZ in PANC1 and MIAPaCa2 cells transfected with miR-455-3p mimic or miR-455-3p mimic plus TAZ expression vector. (B and C) Western blot analysis of TAZ in PANC1 (B) and MIAPaCa2 cells (C) transfected with miR-455-3p mimic or miR-455-3p mimic plus TAZ expression vector. (D and E) The cell proliferation of PANC1 (D) and MIAPaCa2 cells (E) transfected with miR-455-3p mimic or miR-455-3p mimic plus TAZ expression vector. (F and G) The IC50 values of GEM in PANC1 (F) and MIAPaCa2 cells (G) transfected with miR-455-3p mimic or miR-455-3p mimic plus TAZ expression vector. The statistical significance was evaluated using one-way ANOVA. All data are presented as the mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.
Figure 6. miR-455-3p Functions In Vivo
(A–D) miR-455-3p significantly inhibited the growth of xenografts formed by PANC1 when compared with the NC group. (A) Representative images of tumor-bearing mice. (B) Representative images of tumors isolated from tumor-bearing mice. (C) Tumor volume in nude mice. (D) Tumor weight in nude mice. Each group contained five mice (n = 5). (E) Expression of miR-455-3p was detected by qPCR. (F) Expression of TAZ mRNA was detected by qPCR. (G) The protein expression of Ki67 and TAZ was detected by IHC. The statistical significance was evaluated using Student’s t test. All data are presented as the mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.
CONFLICTS OF INTEREST
The authors declare no competing financial interests.

ACKNOWLEDGMENTS
This study was partially sponsored by the National Natural Science Foundation of China (31373136 and 81671540 to Y.Z.), the Natural Science Foundation of Hubei Province in China (2013CFA060 to Y.Z. and 2015BHE022 to X.H.), the Commission for Science and Technology of Wuhan Municipal (2014070504020245 to Y.Z.), and the China Guanghua Science and Technology Foundation (2014093 to Y.Z.).

REFERENCES
1. Von Hoff, D.D., Ervin, T., Arena, F.P., Chiorean, E.G., Infante, J., Moore, M., Seay, T., Tuelandin, S.A., Ma, W.W., Saleh, M.N., et al. (2013). Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N. Engl. J. Med. 369, 1691–1703.
2. Galluzzi, L., Senovilla, L., Vitale, I., Michels, J., Martins, I., Kepp, O., Castedo, M., and Kroemer, G. (2012). Molecular mechanisms of cisplatin resistance. Oncogene 31, 1869–1883.
3. Ebrahimi, S., Hosseini, M., Ghasemi, F., Shahidzadeh, S., Maftouh, M., Akbarzade, H., Parizadeh, S.A., Hassanian, S.M., and Avan, A. (2016). Circulating microRNAs as potential diagnostic, prognostic, and therapeutic targets in pancreatic cancer. Curr. Pharm. Des. 22, 6444–6450.
4. Feng, H., Wang, Y., Su, J., Liang, H., Zhang, C.Y., Chen, X., and Yao, W. (2016). Tjulandin, S.A., Ma, W.W., Saleh, M.N., et al. (2013). Increased survival in pancreatic cancer and related to the proliferation and metastasis of colon cancer cells. PLoS ONE 8, e65539.
5. Li, Z., Wang, Y., Zhu, Y., Yuan, C., Wang, D., Zhang, W., Qi, B., Qiu, J., Song, X., Ye, J., et al. (2015). The Hippo transducer TAZ promotes epithelial to mesenchymal transition and cancer stem cell maintenance in oral cancer. Mol. Oncol. 9, 1091–1105.
6. Xie, D., Cui, J., Xia, T., Jia, Z., Wang, L., Wei, W., Zhu, A., Gao, Y., Xie, K., and Quan, M. (2015). Hippo transducer TAZ promotes epithelial mesenchymal transition and supports pancreatic cancer progression. Oncotarget 6, 35949–35963.
7. Morvardi, S., Dhall, D., Greene, M.L., Pandol, S.J., and Wang, Q. (2015). Role of YAP and TAZ in pancreatic ductal adenocarcinoma and in stellate cells associated with cancer and chronic pancreatitis. Sci. Rep. 5, 16759.
8. Kim, M.H., and Kim, J. (2017). Role of YAP/TAZ transcriptional regulators in resistance to anti-cancer therapies. Cell. Mol. Life Sci. 74, 1457–1474.
9. Higashi, T., Hayashi, H., Ishimoto, T., Takeyama, H., Kaiada, T., Arima, K., Taki, K., Sakamoto, K., Kuroki, H., Okabe, H., et al. (2015). miR-9-3p plays a tumour-suppressor role by targeting TAZ (WWTR1) in hepatocellular carcinoma cells. Br. J. Cancer 113, 252–258.
10. Yuan, J., Xiao, G., Peng, G., Liu, D., Wang, Z., Liao, Y., Liu, Q., Wu, M., and Yuan, X. (2015). MRNA-125a-5p inhibits glioblastoma cell proliferation and promotes cellular differentiation by targeting TAZ. Biochem. Biophys. Res. Commun. 457, 171–176.
11. Zuo, Q.F., Zhang, R., Li, B.S., Zhao, Y.L., Zhuang, Y., Yu, T., Gong, L., Li, S., Xiao, B., and Zou, Q.M. (2015). MicroRNA-141 inhibits tumor growth and metastasis in gastric cancer by directly targeting transcriptional co-activator with PDZ-binding motif TAZ. Cell Death Dis. 6, e1623.
12. Liu, P., Zhang, H., Liang, X., Ma, H., Luan, F., Wang, B., Bui, F., Gao, L., and Ma, C. (2015). HBV preS2 promotes the expression of TAZ via miRNA-338-3p to enhance the tumorigenesis of hepatocellular carcinoma. Oncotarget 6, 2948–2959.
13. Zhang, Z., Hou, C., Meng, F., Zhao, X., Zhang, Z., Huang, G., Chen, W., Fu, M., and Liao, W. (2015). MiR-455-3p regulates early chondrogenic differentiation via inhibiting Runx2. FEBS Lett. 589, 3671–3678.
14. Voutsadakis, I.A. (2011). Molecular predictors of gemcitabine response in pancreatic cancer. World J. Gastrointest. Oncol. 3, 153–164.
15. Chan, S.W., Lim, C.J., Guo, K., Ng, C.P., Lee, I., Hunziker, W., Zeng, Q., and Hong, W. (2008). A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells. Cancer Res. 68, 2592–2598.
16. Meng, F., Hensom, R., Lang, M., Webb, H., Maheshwari, S., Mendell, J.T., Jiang, J., Schmittgen, T.D., and Patel, T. (2006). Involvement of human micro RNA-200 in growth and response to chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology 130, 2113–2129.
17. Seki, N. (2011). A commentary on MicroRNA-141 confers resistance to cisplatin induced apoptosis by targeting YAP1 in human esophageal squamous cell carcinoma. J. Hum. Genet. 56, 339–340.
18. Tung, M.C., Lin, P.L., Cheng, Y.W., Wu, D.W., Yeh, S.D., Chen, C.Y., and Lee, H. (2016). Reduction of microRNA-184 by E6 oncoprotein confers cisplatin resistance in lung cancer via increasing Bcl-2. Oncotarget 7, 32362–32374.
19. Zhang, H., Zou, Q., Zhang, L., Hu, Y., Gao, H., Li, B., Yang, M., Li, Y., and Lu, X. (2013). miRNA-199a is able to reverse cisplatin resistance in human ovary cancer cells through the inhibition of mammalian target of rapamycin. Oncol. Lett. 6, 789–794.
20. Cai, J., Fang, L., Huang, Y., Li, R., Yuan, J., Yang, Y., Zhu, X., Chen, B., Wu, J., and Li, M. (2013). miR-205 targets PTEN and the enhanced Akt signaling and drive malignant phenotypes in non-small cell lung cancer. Cancer Res. 73, 5402–5415.
21. Yang, H., Kong, W., He, L., Zhao, J., O’Donnell, J.D., Wang, J., Wenzham, R.M., Coppola, D., Kruk, P.A., Nicosia, S.V., and Cheng, J.Q. (2008). MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res. 68, 425–433.
22. Ge, X., Liu, X., Lin, F., Li, P., Li, K., Geng, R., Dai, C., Lin, Y., Tang, W., Wu, Z., et al. (2016). MicroRNA-421 regulated by HIF-1α promotes metastasis, inhibits apoptosis, and induces cisplatin resistance by targeting E-cadherin and caspase-3 in gastric cancer. Oncotarget 7, 24466–24482.
34. Liu, J., Zhang, J., Li, Y., Wang, L., Sui, B., and Dai, D. (2016). MiR-455-5p acts as a novel tumor suppressor in gastric cancer by down-regulating RAB18. Gene 592, 308–315.

35. Qin, L., Zhang, Y., Lin, J., Shentu, Y., and Xie, X. (2016). MicroRNA-455 regulates migration and invasion of human hepatocellular carcinoma by targeting Runx2. Oncol. Rep. 36, 3325–3332.

36. Cheng, C.M., Shiah, S.G., Huang, C.C., Hsiao, J.R., and Chang, J.Y. (2016). Up-regulation of miR-455-5p by the TGF-β-SMAD signalling axis promotes the proliferation of oral squamous cancer cells by targeting UBE2B. J. Pathol. 240, 38–49.

37. Li, Y.J., Ping, C., Tang, J., and Zhang, W. (2016). MicroRNA-455 suppresses non-small cell lung cancer through targeting ZEB1. Cell Biol. Int. 40, 621–628.

38. Moroishi, T., Hansen, C.G., and Guan, K.L. (2015). The emerging roles of YAP and TAZ in cancer. Nat. Rev. Cancer 15, 73–79.

39. Morvaridi, S., Dhall, D., Greene, M.L., Pandol, S.J., and Wang, Q. (2015). Role of YAP and TAZ in pancreatic ductal adenocarcinoma and in stellate cells associated with cancer and chronic pancreatitis. Sci. Rep. 5, 16759.