Journal of Pharmaceutical and Medical Research
ISSN: 2663-1962
DOI: https://doi.org/10.61784/jpmr3016

PLANT MIR157 INHIBITS THE PROLIFERATION OF HEPATOMA CELLS HEPG2 BY TARGETING MTDH

Yang Lou, ShuYang Li, JingLing Li, YuTian Wang, HongWei Xue, Juan Lu*, Xi Chen*
Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, China.
**Juan Lu and Xi Chen are both the corresponding authors.*
**Corresponding Author: Juan Lu, Xi Chen; Email: chenxi@implad.ac.cn (X.C.); jlu@implad.ac.cn (J.L.)*

Abstract: Background: MicroRNAs (miRNAs) play key regulatory roles as oncogenes or anti-oncogenes at the posttranscriptional level in human cancers. Plant miRNAs can regulate mammalian systems across kingdoms. The present study investigated the effects of plant miRNA157 (miR157) on the viability and proliferation of human hepatoma cells and the underlying mechanisms. **Method:** The potential targets of miR157 were predicted by bioinformatics methods; the targeted regulatory relationship between miR157 and MTDH was detected by dual luciferase reporter assay; hepatoma cells HepG2 were cultured in vitro and divided into control group (transfected with negative control SNC mimics) and miR157 transfection group (transfected with miR157 mimics). After transfection, cell counting kit-8 and colony formation were used to detect cell proliferation ability, and RT-qPCR and Western blot were used to detect the effect of miR157 on MTDH expression in HepG2 cells. **Results**: miR157 had an inhibitory effect on the proliferative capacity of hepatoma cells HepG2 ($P<0.05$); dual-luciferase reporter gene assay showed that MTDH is a target gene of miR157; miR157 could be directly targeted to down-regulate the expression of MTDH ($P<0.01$). **Conclusion:** These preliminary pieces of evidence suggest that miR157 inhibits the proliferation of HepG2 cells by targeting MTDH.
Keywords: miRNA; MTDH; Hepatoma cells HepG2; Cross-kingdom regulation

1 INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common and lethal cancers worldwide, especially in Asia [1]. The high mortality rate of HCC is mainly attributed to the rapid progression of the disease and the lack of effective treatments. Although currently, surgical resection of the tumor is the primary treatment for liver cancer, postoperative recurrence and complications pose significant challenges. Therefore, exploring the molecular mechanisms underlying HCC is crucial for developing new treatment strategies.

MicroRNA (miRNA) is a class of endogenous non-coding small RNA molecules with a length of 20–26 nucleotides that negatively regulates gene expression at the transcriptional or posttranscriptional level by complementary pairing with the target mRNA. Moreover, miRNAs play a critical role in all organisms, including humans [2], plants [3], viruses, and bacteria [4,5], especially in the occurrence and development of diseases. miRNAs function by assembling into RNA-induced silencing complexes (RISCs) and targeting specific mRNAs toward degradation or translational repression [6]. Recent studies have revealed the cross-border regulatory role of miRNAs across species. A previous study has shown that rice miR168a can regulate human genes [7], while plant miRNAs could be detected in mammals and could be effectuated through blood circulation [8,9]. Another study found that food storage, processing, and cooking processes did not significantly reduce the stability of plant miRNAs, indicating their strong stability in the digestive system [10]. Thus, it can be deduced that the stability of plant miRNAs depends on their structural characteristics and the ability to form complexes with proteins in vivo [11,12].

Compared to animal miRNAs, plant miRNAs show significant differences in the recognition and binding of target mRNAs and can guide their degradation [13–15]. In vitro studies have shown that plant-derived miR167e-5p inhibits the proliferation of colorectal cancer cells by targeting b-catenin protein [16]. Additionally, plant miR159 detected in the serum of Western people can significantly inhibit breast cancer cell proliferation. miR159 downregulates the expression of the proto-oncogene MYC by targeting the transcription factor 7 gene encoding the Wnt signaling pathway, thereby suppressing the tumors. Interestingly, miR159 is highly expressed in various plants, such as broccoli, a widely accepted anti-cancer food. This study provides a theoretical basis for the anti-cancer mechanism of broccoli and evidence for the cross-border regulation of gene expression by plant miRNA[17].

MicroRNA157 (miR157) is a highly conserved miRNA in plants. It was first discovered in *Arabidopsis thaliana*, and similar sequences have been identified in other plants such as rice, wheat, and corn. Moreover, miR157 affects plant growth and development, including flowering time and leaf morphology, via the target genes [18].

Herein, we hypothesized that plant miRNAs may be exogenous regulatory factors that affect animal hepatocyte function. The present study identified the potential targets of miR157 in humans through bioinformatics analysis and validated the role of miR157 in hepatoma cells (HepG2) through in vitro assays. The results indicated a positive impact of miR157 on human health by regulating the related genes, suggesting that plant miRNAs may be a new class of bioactive molecules involved in the epigenetic regulation in humans and animals.

2 MATERIALS AND METHODS

2.1 Cell Culture

The human hepatoma cell line HepG2 was obtained from the Cell Resource Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. The cells were cultured in DMEM medium (Solarbio, Beijing, China) supplemented with 10% fetal bovine serum (FBS; PAN, Adenbach, Germany) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) at 37 °C in an atmosphere containing 5% CO_2 under standard conditions. Subsequently, the cells were harvested for further experiments upon reaching 80% confluency. miR157 mimics and negative control SNC were synthesized by Shanghai GenePharma Co., Ltd (Shanghai, China).

2.2 Cell Transfection

1×10^5 HepG2 cells were seeded into 6-well plates for 24 h and transfected with 10 nM synthetic 2'-O-methylated miR157 (5'-UUGACAGAAGAUAGAGAGCAmC-3') mimics and synthetic 2'-O-methylated scrambled SNC mimics (negative controls) using Lipofectamine RNAiMAX transfection reagent (Invitrogen™, Shanghai, China), according to the manufacturer's instructions. Subsequently, the cells were harvested 48–72 h later for downstream assays.

2.3 Cell Proliferation and Colony Formation Assays

Cell viability was measured using the CCK-8 assay (Beyotime, Shanghai, China). HepG2 cells were seeded in 96-well plates at a density of 5×10^3/well (n = 6/group) and cultured for 1, 2, 3, 4, and 5 days, respectively, after transfection. Then, 100 μL of CCK-8 reagent was added to the cells, and the absorbance was measured at 450 nm on a microplate reader（Multiskan FC，Thermo Scientific） to calculate the number of viable cells. For colony formation assay, hepatoma cells were seeded in six-well culture plates at a density of 3000 cells/well (n = 3/group). After incubation at 37 °C for 14 days, the colonies were washed twice with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 30 min, and stained with 0.5% crystal violet. After drying, the images of the stained cell colonies were captured with a camera. The number of colonies was calculated using ImageJ software.

2.4 miR157 Target Prediction

The target genes of miR157 were predicted using the miRanda (http://www.microrna.org/) database.

2.5 Dual-luciferase reporter gene assay

The miRanda database predicted that the MTDH was directly regulated by miR157. For reporter gene assays, HepG2 cells were co-transfected with pmirGLO firefly luciferase reporter (MTDH WT/MTDH MUT) and miR157 mimics or negative control miR-SNC in 24-well plates using Lipofectamine RNAiMAX. The cells were harvested 48 h post-transfection and analyzed for relative luciferase activity using the luciferase assay kit (Solarbio) on a multifunction microplate reader (TECAN M1000). Firefly luciferase activity was normalized to renal luciferase activity in each well.

2.6 RT-qPCR Detection

Total RNA was extracted from HepG2 cells using a total RNA extraction kit (Tiangen, Beijing, China) and reverse transcribed into cDNA using the Hifair® III 1st Strand cDNA Synthesis SuperMix for qPCR (gDNA digester plus) reverse transcription system (Yeasen, Shanghai, China). Then, RT-qPCR was performed using ArtiCanA™ SYBR qPCR Mix (Tsingke, Beijing, China). miRNA was extracted using MiPure Cell/Tissue miRNA Kit (spin column type) (Vazyme, Nanjing, China), and cDNA was reverse transcribed using miRNA 1st Strand cDNA Synthesis Kit (by stem-loop) (Vazyme, Nanjing, China) and the reverse transcription primer sequence of GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTGCTC. The target gene was amplified using miRNA Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China). *U6* and *GAPDH* were the internal reference genes for miR-297 and mRNA, respectively. The relative gene expression was determined using the $2^{-\triangle\triangle CT}$ method. The primer sequence of the *MTDH* gene was F: GATGAAGGAGCCTGGGAAACTAA, R: CAGGAAATGATGCGGTTGTAAGT; the primer sequence of *GAPDH* gene was F: GGAGTCCACTGGCGTCTTCA, R: GTCATGAGTCCTTCCACGATACC; the forward primer sequences of *miR157* and *U6* were: CCGCGCGTTGACAGAAGATAGA and CTCGCTTCGGCAGCACATATA, respectively, and the reverse primer were provided by the kit.

2.7 Western Blot Analysis

Total protein was extracted from cells in a high-performance RIPA lysis buffer (Solarbio). Proteins were separated by 10% SDS-PAGE and then transferred to PVDF membranes (EMD Millipore, MA, USA). Subsequently, the membranes were blocked with 5% skim milk in TBST buffer (pH 7.5; 100 mM NaCl, 50 mM Tris, and 0.1% Tween-20) for 2 h at room temperature, probed with the primary antibodies [MTDH (13860-1-AP, Proteintech, Rosemont, IL, USA) and

GAPDH (ab9485, Abcam, Cambridge, MA, USA)] overnight at 4 °C, and then incubated with appropriate secondary antibodies (ab6721, Abcam) for 2 h at room temperature. Finally, the immunoreactive bands were visualized by enhanced chemiluminescence (ECL, CWBIO, Jiangsu, China) and detected by e-blot.

2.8 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 26 and GraphPad Prism 8.0 software. Data are presented as mean ± standard deviation (SD) of at least three independent experiments. Statistical evaluation methods used one-way analysis of variance or two-tailed Student's t-test; $p < 0.05$ was considered significant, and $p < 0.01$ was considered highly statistically significant.

3 RESULTS

3.1 Overexpression of miR157 Inhibits the Proliferation of HepG2 cells

In order to elucidate the role of miRNAs in inhibiting cell proliferation, we analyzed the effect of miR157 on the proliferation of HepG2 cells. Cell counting kit-8 (CCK-8) assay showed that the cell survival rate in the miR157 transfection group was significantly lower than in the control group, as shown in Figure 1.

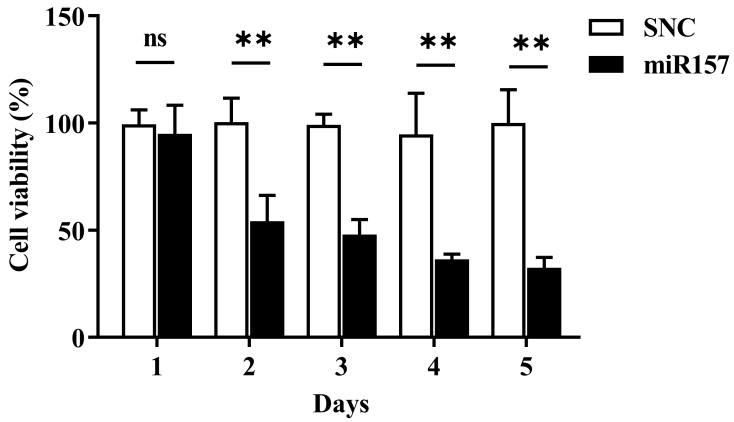

Figure 1 Effect of miR157 on the Proliferation Ability of HepG2 Cells. The Data are Expressed as Mean ± SD (n = 3/group). **$p < 0.01$, Compared to the Untreated Soybean Group; p, ns Indicates no Significant Differences between the Two Groups.

3.2 Overexpression of miR157 Inhibits the Colony Formation of HepG2 cells

The biological function of the cells was assessed based on the effect of miR157 on the colony formation of HepG2 cells. The number of cell colonies in the miR157 transfection group was significantly smaller than that in the control group (Figure 2). Colony formation is an effective method to detect cancer cell proliferation activity and determine the independent survival ability of the cancer cells. The results showed that miR157 effectively inhibits the growth of HepG2 cells.

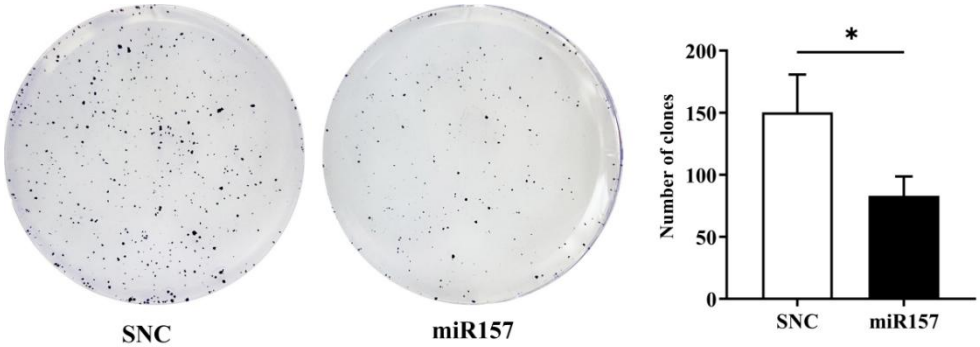

Figure 2 Effect of miR157 on Colony Formation of HepG2 Cells. The Data are Expressed as Mean ± SD (n = 3/group). *$p < 0.05$, Compared to the Untreated Soybean Group.

3.3 MTDH is a Downstream Target of miR157

A dual-luciferase reporter gene assay verified whether MTDH is a true downstream target of miR157, as predicted by

bioinformatics methods. Moreover, the *MTDH* gene is closely related to the occurrence of liver cancer. The putative binding sites of the gene and miR157 are shown in Figure 3A. In order to determine whether miR157 mediates the negative regulation of MTDH expression by binding to the putative site in the 3'-untranslated region (UTR) of *MTDH* mRNA, we fused the UTR containing the binding site downstream of the renal luciferase gene in the reporter plasmid and introduced point mutations into the corresponding sites as a control group. The renal and firefly luciferase genes were used as the reporter and reference genes, respectively (Figure 3B). The relative activity of renal luciferase was determined to assess the regulatory effect of miR157 on MTDH expression. As shown in Figure 3C, overexpression of miR157 significantly reduces the luciferase activity compared with the stable negative group (SNC) group. Importantly, the mutation in the binding site did not significantly inhibit the luciferase activity in the miR157 or the SNC group. These results suggested that plant miR157 directly binds to the 3'-UTR of the *MTDH* gene and mediates gene silencing.

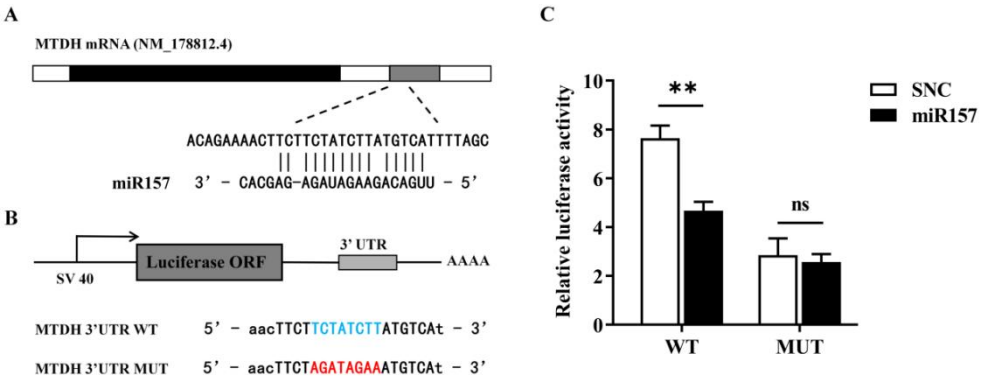

Figure 3 *MTDH* is one of the Target Genes of miR157. (A) Predicted Binding Sites Between miR157 and Target Gene *MTDH*. (B) Construction Diagram of Luciferase Reporter Plasmid Carrying Wild-type (WT) or Mutant (MUT) MTDH 3'-UTR. (C) Effect of miR157 on Luciferase Activity of Dual-Luciferase Reporter Gene. Data are Expressed as Mean ± SD (n = 3/group). $^{**}p < 0.01$ Indicates a Significant Difference between the two Groups; ns Indicates no Statistically Significant Difference between the Two Groups.

3.4 Overexpression of miR157 in HepG2 Cells Downregulates the Expression of MTDH

To investigate the impact of miR157 on the in vitro growth of HepG2 cells, miR157 mimics and negative controls were transfected into the cells. Following transfection, miR157 expression in the liver cancer cell line notably increased compared to the SNC group (Figure 4A). The heightened miR157 expression led to downregulation of MTDH mRNA and protein levels in HepG2 cells. Real-time quantitative polymerase chain reaction (RT-qPCR) (Figure 4B) and Western blot (Figure 4C) results showed that the expression of MTDH in the miR157 group was significantly lower than in the control group.

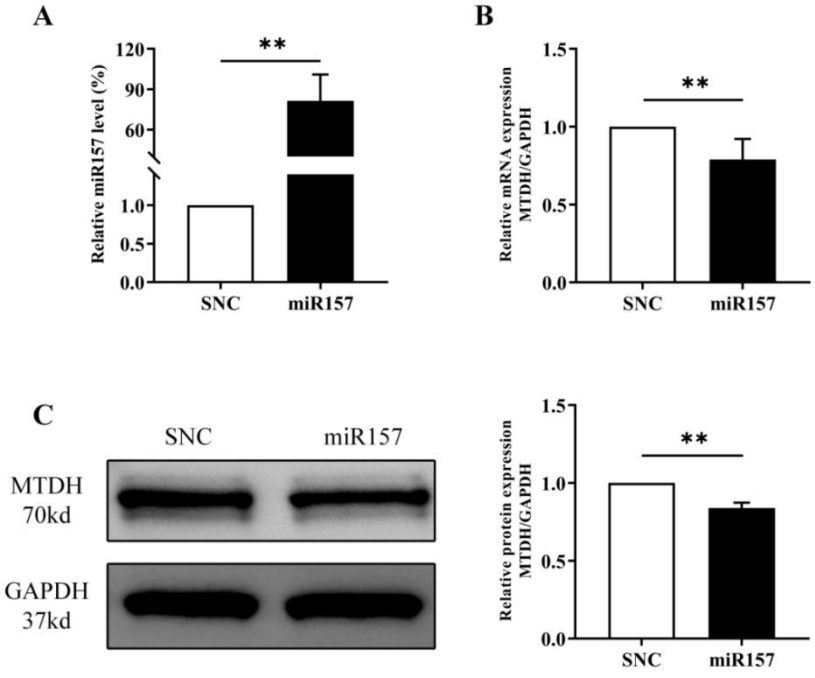

Figure 4 miR157 Inhibits the Growth of HepG2 Cells by Downregulating the Expression of MTDH. (A) The

Expression of miR157 was Measured by RT-qPCR. (B) Effect of miR157 on MTDH mRNA Level in HepG2 Cells. (C) Effect of miR157 on MTDH Protein Level in HepG2 Cells. Data are Expressed as Mean ± SD (n = 3/group). $^{**}p < 0.01$ Indicates a Significant Difference between the Two Groups.

4 DISCUSSION

The current results showed that miR157 significantly decreases the mRNA and protein levels of MTDH, thereby inhibiting the proliferation of liver cancer HepG2 cells. These findings provide new evidence for the cross-kingdom regulatory functions of plant miRNAs in mammals, especially in tumor suppression.

miRNAs are critical negative regulators in the physiological and pathological processes in plants and animals. The current results support the controversial cross-kingdom regulatory role of plant miRNAs in animals. Plant and animal miRNAs exhibit significant differences in processing and splicing methods, sequences, precursor structures, evolutionary origins, and biogenesis mechanisms [19–21]. For example, plant miRNAs are methylated on the ribose sugar of the 3' terminal nucleotide, stabilizing the naked plant miRNAs compared to animal miRNAs [22]. Methylation is mediated by HUA ENHANCER 1 (HEN1) [23], the methylated miRNA/miRNA* duplex is exported from the nucleus to the cytoplasm by Hasty (HST), a plant homolog of Exportin-5 and loaded into the cytoplasmic Ago protein [24]. Compared with animal miRNAs, plants consist of 10 Ago family members; among these, Ago1 is the main effector of miRNA-mediated silencing response [13,25]. Most other plant Ago proteins might also exhibit RNA cleavage activity [15]. In addition, plants package miRNAs into secreted plant-derived exosome-like nanoparticles (PELNs) [26] or exosomes [27], rendering miRNAs resistant to harsh internal conditions, such as the acidic stomach environment, varying temperatures, and nucleases [28]. This stability provides favorable conditions for the regulation of plant miRNAs in mammals.

To date, miRNAs have been known to regulate the developmental and physiological processes, especially in tumors. A previous study indicated a connection between the abnormal expression of miRNA and intracellular signal transduction pathways and tumorigenesis, and the overexpression or silencing of miRNA can affect tumor progression [29].

HCC is the second leading cause of cancer-related deaths, with a continually increasing incidence worldwide. The traditional treatments include surgical resection and liver transplantation, but they are not sufficiently effective for many patients. Approximately 50% of HCC patients receive sorafenib or lenvatinib in the first line and regorafenib, cabozantinib, or ramucirumab in the second line systemic therapy [30,31]. Although emerging systemic therapies, including new molecular targeted monotherapies (for example, dorafenib), new immuno-oncology monotherapies (such as durvalumab), and new combination therapies (for instance, durvalumab plus tremelimumab), are beneficial, their efficacy in HCC is limited, thereby necessitating novel treatment approaches [32]. The ability of hepatocytes to readily absorb small nucleic acids has opened up new therapeutic avenues to combat HCC. For example, miRNAs, which play a regulatory role in cancer development, seem promising in miRNA-based treatments in HCC [33–35].

To the best of our knowledge, this study, for the first time, demonstrated the mechanism of plant miR157-mediated inhibition of liver cancer cell proliferation by targeting MTDH. miR157 is a conserved and highly expressed miRNA in the plant kingdom with potential biological functions. Previous studies have shown that other plant miRNAs, such as gma-miR159a and MIR156a, also show similar cross-kingdom regulatory capabilities in mammals [36–38].

Metadherin (AEG-1/MTDH/LYRIC) is a key oncogene in liver cancer cells. It is a transmembrane protein containing 582 amino acids, distributed throughout the cytoplasm, perinuclear region, nucleus and nucleolus, and endoplasmic reticulum (ER) and regulated by miRNA. The oncogenic function of Metadherin is effectuated by activating cell proliferation, survival, migration, and metastasis and through phosphatidylinositol-3-kinase/AKT (PI3K/AKT), NF-κB, mitogen-activated protein kinase (MAPK), and Wnt signaling pathways [39]. The overexpression of MTDH is associated with adverse clinical manifestations in various cancers [40]. For example, overexpression of MTDH has been observed in melanoma [41], glioma [41], neuroblastoma [42], breast cancer [43], prostate [44], esophagus [45], stomach [46], and liver [47]. Reportedly, MTDH is overexpressed in > 90% of human HCC compared to normal liver and plays a central role in regulating cancer pathogenesis [47]. In this study, miR157 significantly reduced the expression of MTDH, further confirming its ability to inhibit the proliferation of liver cancer cells.

5 CONCLUSION

This study provides new evidence supporting the theory that plant miRNAs exert cross-kingdom regulatory functions in mammals. We demonstrated that plant miR157 inhibits liver cancer cell proliferation by targeting MTDH. Together, these findings suggest that various plant miRNAs can be considered nutrients that have been overlooked for centuries and should be explored in-depth for disease prevention and treatment.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

FUNDING

This research was funded by CAMS Innovation Fund for Medical Science (CIFMS) (No. 2021-I2M-1-022) and Yunnan Province Innovation Guidance and Technology oriented Enterprise Cultivation Plan 202404BI090001.

REFERENCES

[1] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2018, 68(6): 394–424.

[2] La M, Pr M. MicroRNA: Biogenesis, Function and Role in Cancer. Curr Genomics, 2010 ;11(7).

[3] Zhang B, Pan X, Cobb GP, et al. Plant microRNA: A small regulatory molecule with big impact. Dev Biol, 2006, 289(1): 3–16.

[4] D B, P A. MicroRNA target and gene validation in viruses and bacteria. Methods Mol Biol Clifton NJ. 2014, 1107.

[5] Kang S-M, Choi J-W, Lee Y, et al. Identification of microRNA-Size, Small RNAs in Escherichia coli. Curr Microbiol, 2013, 67(5): 609–613.

[6] To KKW, Fong W, Tong CWS, et al. Advances in the discovery of microRNA-based anticancer therapeutics: latest tools and developments. Expert Opin Drug Discov, 2020, 15(1): 63–83.

[7] Zhang L, Hou D, Chen X, et al. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res, 2012, 22(1): 107–126.

[8] Lukasik A, Brzozowska I, Zielenkiewicz U, et al. Detection of Plant miRNAs Abundance in Human Breast Milk. Int J Mol Sci, 2017, 19(1): 37.

[9] Witwer KW, McAlexander MA, Queen SE, et al. Real-time quantitative PCR and droplet digital PCR for plant miRNAs in mammalian blood provide little evidence for general uptake of dietary miRNAs: limited evidence for general uptake of dietary plant xenomiRs. RNA Biol, 2013, 10(7): 1080–1086.

[10] Philip A, Ferro VA, Tate RJ. Determination of the potential bioavailability of plant microRNAs using a simulated human digestion process. Mol Nutr Food Res, 2015, 59(10): 1962–1972.

[11] Yang J, Farmer LM, Agyekum AAA, et al. Detection of dietary plant-based small RNAs in animals. Cell Res, 2015, 25(4): 517–520.

[12] Zhou Z, Li X, Liu J, et al. Honeysuckle-encoded atypical microRNA2911 directly targets influenza A viruses. Cell Res, 2015, 25(1): 39–49.

[13] Baumberger N, Baulcombe DC. Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. Proc Natl Acad Sci U S A, 2005, 102(33): 11928–11933.

[14] Qi Y, Denli AM, Hannon GJ. Biochemical specialization within Arabidopsis RNA silencing pathways. Mol Cell, 2005, 19(3): 421–428.

[15] Vaucheret H. Post-transcriptional small RNA pathways in plants: mechanisms and regulations. Genes Dev, 2006, 20(7): 759–771.

[16] Li M, Chen T, He J-J, et al. Plant MIR167e-5p Inhibits Enterocyte Proliferation by Targeting β-Catenin. Cells, 2019, 8(11): 1385.

[17] Chin AR, Fong MY, Somlo G, et al. Cross-kingdom inhibition of breast cancer growth by plant miR159. Cell Res, 2016, 26(2): 217–228.

[18] Chen W, Kong J, Lai T, et al. Tuning LeSPL-CNR expression by SlymiR157 affects tomato fruit ripening. Sci Rep, 2015, 5: 7852.

[19] Gazzani S, Li M, Maistri S, et al. Evolution of MIR168 paralogs in Brassicaceae. BMC Evol Biol, 2009, 9: 62.

[20] Grimson A, Srivastava M, Fahey B, et al. Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals. Nature, 2008, 455(7217): 1193–1197.

[21] Hartig JV, Förstemann K. Loqs-PD and R2D2 define independent pathways for RISC generation in Drosophila. Nucleic Acids Res, 2011, 39(9): 3836–3851.

[22] Yu B, Yang Z, Li J, et al. Methylation as a crucial step in plant microRNA biogenesis. Science, 2005, 307(5711): 932–935.

[23] Chen X. MicroRNA biogenesis and function in plants. FEBS Lett, 2005, 579(26): 5923–5931.

[24] Park MY, Wu G, Gonzalez-Sulser A, et al. Nuclear processing and export of microRNAs in Arabidopsis. Proc Natl Acad Sci U S A, 2005, 102(10): 3691–3696.

[25] Qi Y, Denli AM, Hannon GJ. Biochemical specialization within Arabidopsis RNA silencing pathways. Mol Cell, 2005, 19(3): 421–428.

[26] Yi C, Lu L, Li Z, et al. Plant-derived exosome-like nanoparticles for microRNA delivery in cancer treatment. Drug Deliv Transl Res, 2024.

[27] Yan G, Xiao Q, Zhao J, et al. Brucea javanica derived exosome-like nanovesicles deliver miRNAs for cancer therapy. J Control Release Off J Control Release Soc, 2024, 367: 425–440.

[28] Li Z, Xu R, Li N. MicroRNAs from plants to animals, do they define a new messenger for communication? Nutr Metab, 2018, 15(1): 68.

[29] Cortez MA, Anfossi S, Ramapriyan R, et al. Role of miRNAs in immune responses and immunotherapy in cancer. Genes Chromosomes Cancer, 2019, 58(4): 244–253.

[30] Llovet JM, Castet F, Heikenwalder M, et al. Immunotherapies for hepatocellular carcinoma. Nat Rev Clin Oncol, 2022, 19(3): 151–172.

[31] Qin S, Chan SL, Gu S, et al. Camrelizumab plus rivoceranib versus sorafenib as first-line therapy for unresectable hepatocellular carcinoma (CARES-310): a randomised, open-label, international phase 3 study. Lancet Lond Engl, 2023, 402(10408): 1133–1146.

[32] Yang C, Zhang H, Zhang L, et al. Evolving therapeutic landscape of advanced hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol, 2023, 20(4): 203–222.
[33] Komoll R-M, Hu Q, Olarewaju O, et al. MicroRNA-342-3p is a potent tumour suppressor in hepatocellular carcinoma. J Hepatol, 2021, 74(1): 122–134.
[34] Kota J, Chivukula RR, O'Donnell KA, et al. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell, 2009, 137(6): 1005–1017.
[35] Lim L, Balakrishnan A, Huskey N, et al. MicroRNA-494 within an oncogenic microRNA megacluster regulates G1/S transition in liver tumorigenesis through suppression of mutated in colorectal cancer. Hepatol Baltim Md, 2014, 59(1): 202–215.
[36] Hou D, He F, Ma L, et al. The potential atheroprotective role of plant MIR156a as a repressor of monocyte recruitment on inflamed human endothelial cells. J Nutr Biochem, 2018, 57: 197–205.
[37] Liu J, Wang F, Weng Z, et al. Soybean-derived miRNAs specifically inhibit proliferation and stimulate apoptosis of human colonic Caco-2 cancer cells but not normal mucosal cells in culture. Genomics, 2020, 112(5): 2949–2958.
[38] Liu J, Wang F, Song H, et al. Soybean-derived gma-miR159a alleviates colon tumorigenesis by suppressing TCF7/MYC in mice. J Nutr Biochem, 2021, 92: 108627.
[39] Abdel Ghafar MT, Soliman NA. Metadherin (AEG-1/MTDH/LYRIC) expression: Significance in malignancy and crucial role in colorectal cancer. Adv Clin Chem, 2022, 106: 235–280.
[40] Hu G, Wei Y, Kang Y. The multifaceted role of MTDH/AEG-1 in cancer progression. Clin Cancer Res Off J Am Assoc Cancer Res, 2009, 15(18): 5615–5620.
[41] Kang D-C, Su Z-Z, Sarkar D, et al. Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. Gene, 2005, 353(1): 8–15.
[42] Lee S-G, Jeon H-Y, Su Z-Z, et al. Astrocyte elevated gene-1 contributes to the pathogenesis of neuroblastoma. Oncogene, 2009, 28(26): 2476–2484.
[43] Li J, Zhang N, Song L-B, et al. Astrocyte elevated gene-1 is a novel prognostic marker for breast cancer progression and overall patient survival. Clin Cancer Res Off J Am Assoc Cancer Res, 2008, 14(11): 3319–3326.
[44] Kikuno N, Shiina H, Urakami S, et al. Retraction Note: Knockdown of astrocyte-elevated gene-1 inhibits prostate cancer progression through upregulation of FOXO3a activity. Oncogene, 2022, 41(45): 4981.
[45] Yu C, Chen K, Zheng H, et al. Overexpression of astrocyte elevated gene-1 (AEG-1) is associated with esophageal squamous cell carcinoma (ESCC) progression and pathogenesis. Carcinogenesis, 2009, 30(5): 894–901.
[46] Jian-bo X, Hui W, Yu-long H, et al. Astrocyte-elevated gene-1 overexpression is associated with poor prognosis in gastric cancer. Med Oncol Northwood Lond Engl, 2011, 28(2): 455–462.
[47] Yoo BK, Emdad L, Su Z, et al. Astrocyte elevated gene-1 regulates hepatocellular carcinoma development and progression. J Clin Invest, 2009, 119(3): 465–477.