mRNA-miRNA Transcriptomics Analysis of Mechanism of Quercetin Inhibiting Sune-1 Cell Activities

Qihuang LIN, Wenwen LU, Shuning YANG

Xiamen Medical College, Xiamen 361023, China

Abstract [**Objectives**] To further explore the mechanism of quercetin regulating the activity of Sune-1 cells. [**Methods**] High-throughput mRNA-miRNA transcriptome sequencing technology was used to screen miRNA in Sune-1 cells treated with quercetin. [**Results**] Statistical analysis showed that 1 264 miRNAs were differentially expressed in Sune-1 cells treated with quercetin, of which 716 were significantly up-regulated and 548 were significantly down-regulated; 191 miRNAs were differentially expressed in Sune-1 cells treated with quercetin, of which 129 were significantly up-regulated and 62 were significantly down-regulated. By comparing the expression differences of these mRNAs and miRNAs in different samples, six different expression patterns were clustered. The expression of the above miRNAs was verified by real-time quantitative PCR (qPCR), and the results were highly consistent with the transcriptome sequencing data. In addition, Gene Ontology annotation and functional enrichment analysis of miRNA target genes showed that CTGF, VHL and H19, which are related to the regulation of cell proliferation signal transduction, were predicted to be new targets of differential miRNAs such as miR494-3p and miR675-3p and may play an important regulatory role in the process of Quercetin inhibiting the proliferation of Sune-1 cells. [**Conclusions**] This study provides a basis for the rational use of anti-tumor functional components of traditional Chinese medicine, and also provides a theoretical basis for the targeted therapy of nasopharyngeal carcinoma.

Key words Quercetin, Sune-1 cells, miRNA transcriptome, mRNA transcriptome, Cell proliferation

1 Introduction

Nasopharyngeal carcinoma (NPC) is a unique head and neck cancer. It is common in Southeast Asia and South China, and brings huge economic pressure and health burden to patients[1]. Radiotherapy is the most commonly used method in routine clinical treatment, followed by chemotherapy. As a flavonoid, quercetin is ubiquitous in a variety of vegetables, fruits and Chinese herbal medicines[2]. Some studies have shown that quercetin can inhibit growth, induce apoptosis, interfere with cell cycle and signal transduction pathways, and reverse multidrug resistance in tumor cells[3]. However, most of the studies have focused on the association of quercetin with single cellular proteins or global signaling pathways, and there are still many uncertainties about the role of quercetin in the regulation of intracellular small molecules.

Among the non-coding RNAs, there is a class of regulatory non-coding small RNAs (microRNAs, miRNAs) with a length of about 20 bases, which are highly conserved and endogenous[4-5]. miRNA plays an important role in the regulation of gene expression after transcription in cells, which can specifically bind to the 3' untranslated region of mRNA, thereby degrading or translationally inhibiting mRNA[6]. In a variety of tumor cells, miRNA plays an important regulatory role in the occurrence, development and apoptosis of tumors. These miRNAs have been reported to act as proto-oncogenes or tumor suppressors in regulatory processes. For example, miR-137 down-regulates the expression of PAK2 protein, thereby inhibiting the proliferation of malignant melanoma cells[7]; miR-494 targets the expression of CDC2, thereby inducing apoptosis of glioma cells[8]; miR-125b shows distinct roles in hematologic malignancies and solid tumors, acting as a proto-oncogene and a tumor suppressor, respectively[9-10]. At present, a variety of miRNA regulatory effects have been found in lung cancer, such as miRNA-148a, miR-21 and miR-17-92[11-13], however, the miRNA involved in quercetin's inhibition of nasopharyngeal cancer and its regulatory mechanism are not yet clear, and related studies are relatively rare.

The present study was conducted in the Sune-1 cell line of nasopharyngeal cancer treated with quercetin, using the combined mRNA-miRNA transcriptomics method to conduct in-depth analysis of potential differential miRNAs and target genes to further reveal the important regulatory mechanism of quercetin on Sune-1 cells, so as to provide a theoretical basis for the future development and utilization of the potential functions of quercetin.

2 Materials and methods

2.1 Materials and reagents Human nasopharyngeal carcinoma cell line Sune-1 was purchased from ATCC (American type culture collection) and preserved by the Central Laboratory of Xiamen Medical College. Quercetin was purchased from Sigma Company (the USA); CCK-8 reagent was from Biosharp Company (China); DMEM cell culture medium was from Hyclone Company (the USA); fetal bovine serum was from Capricon Company (Germany); RevertAid RT Reverse Transcription Kit was from Thermo Fisher Scientific (the USA); TRIzol Reagent was from Beyotime Biotechnology Company (China); Tripure Isolation Reagent, FastStart Universal SYBR Green Master) was from Roche (Switzerland).

2.2 Instruments and equipment Inverted microscope (Nikon, Japan); multifunctional microplate reader (Thermo Fisher Scien-

Received: January 4, 2025 Accepted: March 6, 2025

Supported by Educational Research Project for Young and Middle-aged Teachers in Fujian Province (Science and Technology Category, JAT210477).

Qihuang LIN, master's degree, senior experimenter.

tific, the USA); cell incubator and biosafety cabinet (ESCO, Singapore); real-time fluorescence quantitative PCR instrument (BIO-RAD, the USA); centrifuge (Beckman, the USA).

2.3 Methods

2.3.1 Cell culture. Penicillin (final concentration 100 U/mL) and streptomycin (final concentration 100 U/mL) were added to DMEM medium containing 10% fetal bovine serum to culture Sune-1 cells at 37 ℃ and 5% carbon dioxide. The cells were passaged every 3 – 4 d, and the cells in the logarithmic growth phase were taken during the experiment.

2.3.2 Quercetin treatment of Sune-1 cells. Sune-1 cells in logarithmic growth phase were inoculated with 5×10^3 cells in a 96-well culture plate. When the cell confluence reached 50% to 60%, different concentrations of quercetin were added to 8 groups (WT and each concentration group), and each group was repeated three times. According to the peak plasma concentration (PPC) reported in the literature and the results of the preliminary experiment, the concentration gradient of quercetin in the benchmark experiment was determined as 0, 1, 2.5, 6.25, 15.63, 39.06 and 97.66 μM[14].

2.3.3 CCK-8 experiment. After treated with quercetin for 48 h, 10 μL CCK-8 reagent was added to each well, and the absorbance at $OD_{450\ nm}$ of cells in each group was measured by a multifunctional microplate reader within 1 h. Cell inhibition rate and IC_{50} value were calculated. The calculation formula of cell inhibition rate is:

Cell inhibition rate = [(Cell control group – Drug group)/(Cell control group – Blank group)] × 100%.

2.3.4 Library construction and sequencing. Untreated and quercetin-treated Sune-1 cells were sequenced in 3 replicates. Total RNA was isolated using the Trizol protocol[15]. To obtain sufficient transcriptome sequences from 6 samples of each family, total RNA families were pooled and sequenced on Illumina's HiSeq platform (Illumina Inc, CA, the USA).

2.3.5 Data processing and analysis. Transcriptome sequencing and miRNA sequencing data were processed separately under the guidance of literature[16-17]. In the transcriptome data screening study, the original data came from the BGISEQ-500 sequencing platform, and were preprocessed by SoAPnuke software to filter out reads containing adaptors, reads with unknown base N content greater than 5%, and low-quality reads. HISAT was used to align the selected clean reads with the human genome sequence, and the alignment results were assembled by Cuf-links program to construct transcripts. In the miRNA sequencing data processing, blast or bowtie was used to compare the sRNA obtained by sequencing with the miRBase database, and the known miRNA was screened out.

2.3.6 Fluorescence quantitative PCR verification of microRNA and its targets. RNA-Seq differential expression was verified from the two sets of RNA samples used for sequencing. The cDNA was synthesized using the RevertAid RT Reverse Transcription Kit and the microRNA reverse transcription primers (Table 1), and the fluorescent quantitative PCR was performed using the SYBR Green quantitative PCR reaction mixture. The amplification primer sequences are shown in Table 1. Non-coding RNA U6 or GAPDH was used as an endogenous control for normalization, and fold changes were calculated by the ΔΔCt method.

Table 1 Sequences of microRNA reverse transcription primers, microRNA and its target PCR primers

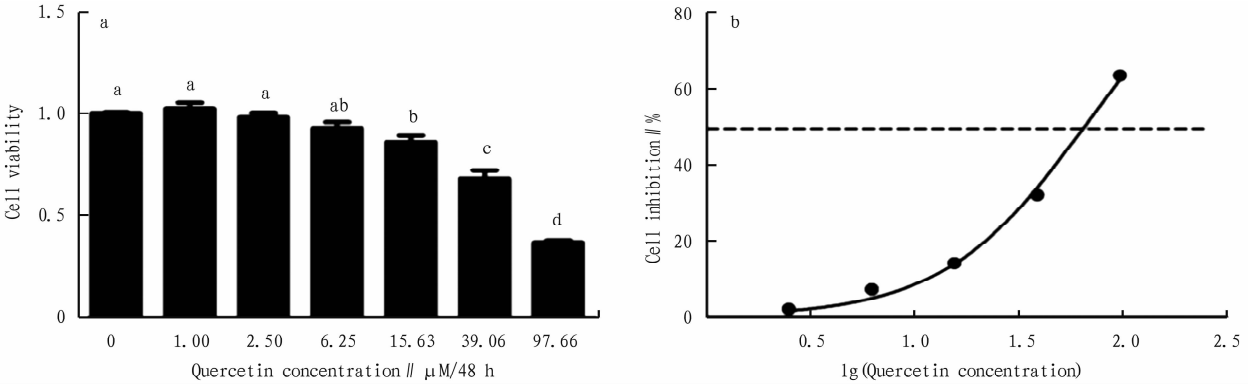
Primer	Name	Sequence (5′-3′)
microRNA reverse transcription primer	Homo-miR377 RT primer	GTCGTATCCAGTCGAGGGTCCGAGGTATTCCGACTGGATACGACACAAAA
	Homo-miR494 RT primer	GTCGTATCCAGTCGCAGGGTCCGAGGTATTCGCACTGGATACGACGAGGTT
	Homo-miR675 RT primer	GTCGTATCCAGTCGCAGGGTCCGAGGTATTCGCACTGGATACGACTGAGCG
	Homo-U6 RT primer	GTCGTATCCAGTCGCAGGGTCCGAGGTGCACTGGATACGACAAAATATGG
PCR primer for microRNA and its target	Homo-miR377-3p F	GGGCATCACACAAAGGCAA
	Homo-miR377-3p R	CCAGTCGAGGGTCCGAGGT
	Homo-miR494-3p F	CGGCACTTGAAACATACACGGG
	Homo-miR494-3p R	CCAGTCGAGGGTCCGAGGT
	Homo-miR675-3p F	CGGCACTCTGTATGCCCTCAC
	Homo-miR675-3p R	CCAGTCGAGGGTCCGAGGT
	Homo-H19 F	TGGACGTGCCCACCAG
	Homo-H19 R	CTCTGTCCTCGCCGTCA
	Homo-CTGF F	GCCTCTTCTGTGACTTCGGC
	Homo-CTGF R	TGCTCTGGAAGGACTCTCCG
	Homo-VHL F	GAAGAGTACGGCCCTGAAGAAG
	Homo-VHL R	GCGATTGCAGAAGATGACCTG
	Homo-U6 F	TGCGGGTGCTCGCTTCGGCAGC
	Homo-U6 R	GTGCAGGGTCCGAGGT
	Homo-GAPDH-F	CGGAGTCAACGGATTTGGTCGTAT
	Homo-GAPDH-R	AGCCTTCTCCATGGTGGTGAAGA

2.3.7 Statistical analysis of data. The experimental data were presented in the form of mean ± standard deviation, and statistical analysis such as one-way ANOVA was performed with the help of GraphPad Prism 5 software. When $P < 0.05$, the difference was

significant, and when $P<0.01$, the difference was extremely significant.

3 Results and analysis

3.1 Effects of quercetin on the proliferation of Sune-1 cells *in vitro* Sune-1 cells were first treated with different concentrations of quercetin (0, 1, 2.5, 6.25, 15.63, 39.06, and 97.66 μM) to evaluate the effect on cell growth. The results showed (Fig. 1) that the higher the concentration of quercetin, the lower the activity of Sune-1 cells, showing a significant dose-dependent effect, indicating that quercetin has a significant inhibitory effect on the growth of Sune-1 cells. Further calculation resulted in an IC_{50} value of 65.86 μM for quercetin in Sune-1 cells.



NOTE A. Sune-1 cell actuvities of the quercetin treated with the different concentrations; B. The lg drug concentration-inhibition rate curve of quercetin for Sune-1 cells.

Fig. 1 Effects of quercetin on the proliferation of Sune-1 cells *in vitro*

3.2 Gene expression level The number of genes in different expression amount (FPKM) intervals of each sample was counted (Table 2). There were 9 773, 9 819, 9 788 and 9 469 genes with FPKM≤1 in Hu1, Hu2, DMSO1 and DMSO2, respectively, and 11 011, 10 929, 10 763 and 10 985 genes with FPKM between 1 and 10, respectively; There were 4 793, 4 826, 5 023 and 5 123 genes with expression level≥10, respectively.

Table 2 Statistics of gene transcript coverage depth of samples

Coverage	Hu1	Hu2	DMSO1	DMSO2
0 – 1	9 773	9 819	9 788	9 469
1 – 10	11 011	10 929	10 763	10 985
≥10	4 793	4 826	5 023	5 123

3.3 Statistical analysis of differentially expressed genes

3.3.1 Variations between samples. According to the expression level of each sample gene, the significantly differentially expressed genes were selected under the condition of Fold Change≥2 and the corrected value of *P*-value (FDR) ≤0.001. The results showed that 716 differentially expressed genes were up-regulated and 548 differentially expressed genes were down-regulated.

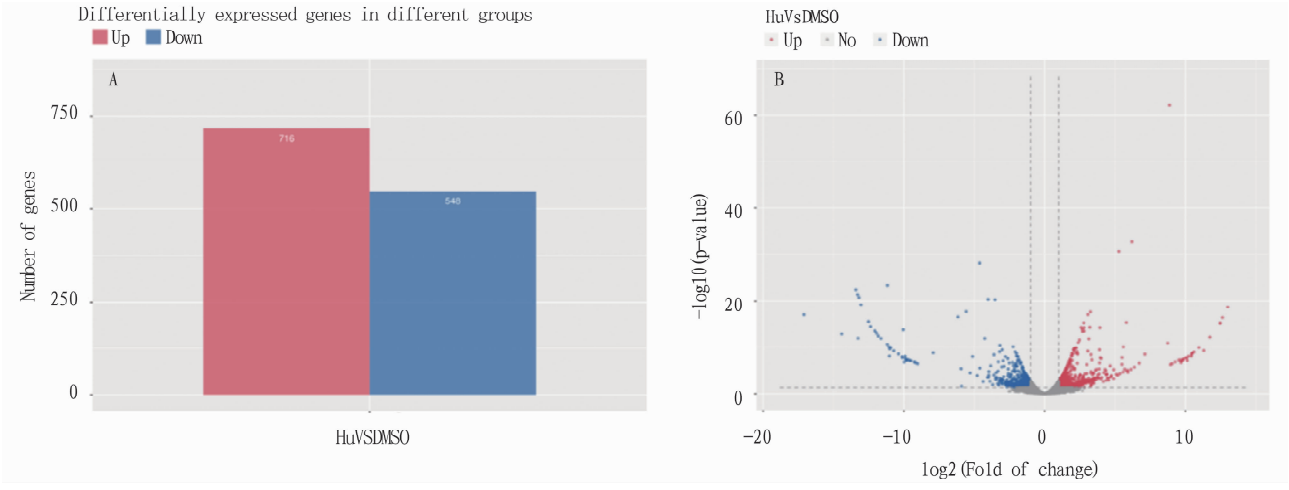
3.3.2 Go enrichment of differential genes. In the three comparison groups, the top 20 GO Terms with the smallest q-value were mapped, and the 20 GO enrichment functions (Fig. 3) of the differentially expressed genes were analyzed. The results showed that the differentially expressed genes involved in biological processes accounted for the largest proportion, followed by molecular functions, and cellular components. Among them, the map kinase tyrosine and the endoderm cell differentiation pathway are two particularly prominent enrichment pathways.

3.4 Differentially expressed miRNA

3.4.1 Screening of differentially expressed miRNAs. As shown in Fig. 4, 191 differentially expressed miRNAs were screened in the comparison group of Hu and DMSO, including 129 up-regulated miRNAs and 62 down-regulated miRNAs. Among the differentially up-regulated miRNAs, there were 11 miRNAs with $P<0.01$, 71 miRNAs with $P<0.05$, and 129 miRNAs with $P<0.1$; among the differentially down-regulated miRNAs, there were 3 miRNAs with $P<0.01$, 26 miRNAs with $P<0.05$, and 62 miRNAs with $P<0.1$. In the down-regulated differential miRNAs, miR675-3p was the most significant, while in the up-regulated differential miRNAs, miR-494-3p was the most significant.

3.4.2 KEGG enrichment of differentially expressed miRNA target genes. In general, the target genes of differentially expressed miRNAs in the three comparison groups were classified in biological processes (Fig. 5), with the largest number being Human diseases, followed by Organismal systems. In the classification of human diseases, the largest number was Cancer, in which the differential expression of CTGF, VHL and H19 was the most significant. Endocrine system and Immune system had the largest number in the classification of organic systems.

3.5 Verification of tumor-associated differential miRNAs and their target genes In the differential miRNA and target genes, several representative genes were screened and verified by qPCR technology. The results showed (Fig. 6) that the expression levels of miR675-3p, CTGF and H19 were significantly down-regulated, while the expression levels of miR-494-3p and VHL were significantly up-regulated in the Sune-1 cells treated with quercetin compared with the control group. This result was further verified by the sequencing results of RNA-seq and miRNA-seq, which confirmed that quercetin affected these differential miRNAs, and then affected the regulation of quercetin in the process of inhibiting nasopharyngeal carcinoma.



NOTE A. frequency of down regulation of significantly differentially expressed gene; B. volcano plot of differential gene expression levels.

Fig. 2 Statistics of differentially expressed genes

4 Discussion

It is reported that the five-year overall survival rate of NPC patients is only about 65%, and it is worth noting that the local recurrence rate of the disease is about 19%, and the distant metastasis rate is about 20%, both of which are at a high level[18]. Therefore, it is urgently necessary to find new drug treatment strategies to improve the survival and quality of life of NPC patients. In this study, it was observed that quercetin had a good inhibitory effect on SUNE-1 cells, which may be related to its regulation of miRNA in nasopharyngeal carcinoma cells. In addition, quercetin may affect cancer progression by targeting the MAPK and endodermal cell differentiation pathways in NPC.

The MAPK pathway plays a key role in cell proliferation, differentiation, and survival. In NPC, aberrant activation of MAPK signaling is associated with tumor progression and anti-apoptosis. Quercetin has been shown to modulate this pathway, thereby inhibiting tumor cell proliferation. Quercetin has been shown to induce

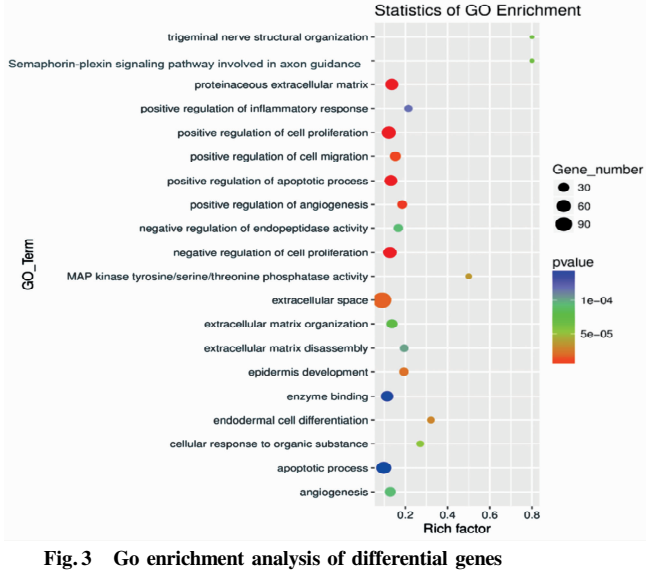
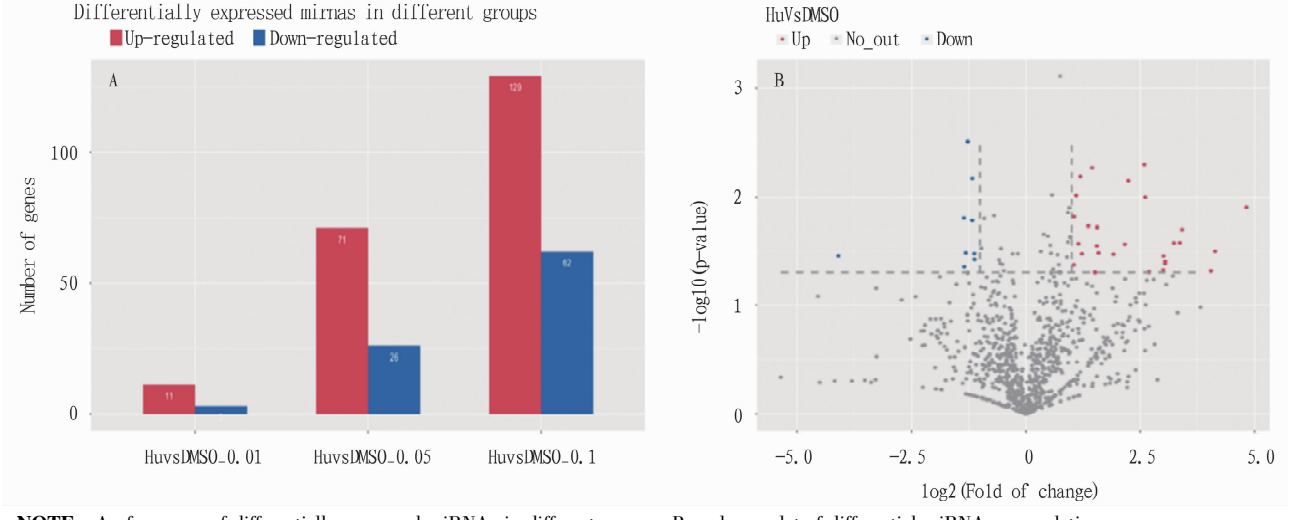


Fig. 3 Go enrichment analysis of differential genes



NOTE A. frequency of differentially expressed miRNAs in different groups; B. volcano plot of differential miRNA up-regulation.

Fig. 4 Statistical analysis of differential miRNA up-regulation

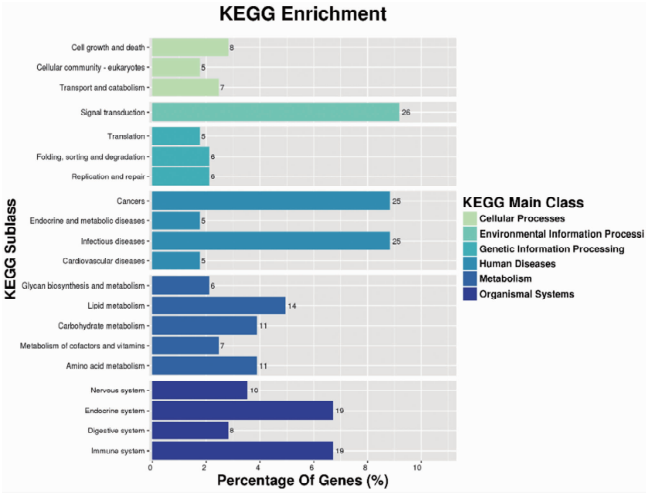


Fig. 5 Schematic diagram of correlation analysis

apoptosis by inhibiting MAPK and TRPM7 channels in gastric cancer cells, suggesting that a similar mechanism may operate in NPC cells[19]. In addition, quercetin has been reported to activate MAPK/ERK-mediated pathways leading to apoptosis in lung cancer cells, and this activation leads to the down-regulation of survivin, a protein that inhibits apoptosis, thereby promoting cancer cell death[20]. These findings suggest that the interaction of quercetin with the MAPK pathway may be a key mechanism for its anticancer effect in NPC. The effect of quercetin on the differentiation pathway of endodermal cells in nasopharyngeal carcinoma is not clear. However, quercetin has been shown to induce differentiation of various cancer cell types, resulting in a less aggressive phenotype. For example, quercetin treatment leads to differentiation of leukemic cells, reducing their proliferative capacity[21]. However, direct evidence is limited in NPC, suggesting that quercetin may promote differentiation pathways that may inhibit tumor progression.

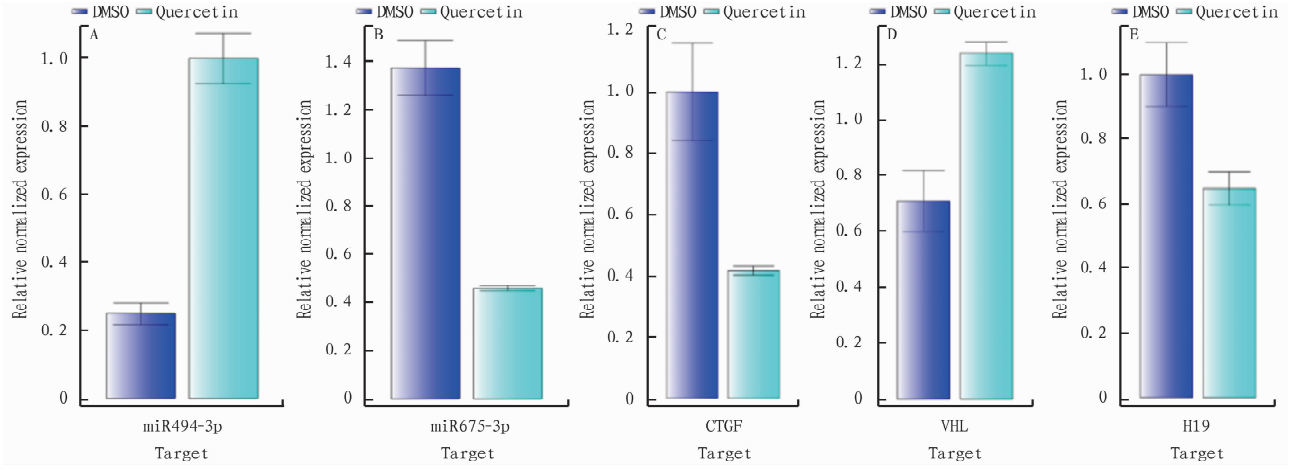


Fig. 6 Verification of tumor-associated differential miRNAs and their target genes

miRNAs can negatively regulate target genes at the post-transcriptional level[22]. Studies have shown that miR-494 and miR675-3p are involved in the regulation of a variety of tumor biological behavior. Down-regulation of miR-494 in different cancer cells is considered to play an important role as a tumor suppressor gene. For example, miR-494 affects the proliferation and apoptosis of lung cancer cells by targeting downstream genes[23-24]. Shen Pengfei[25] confirmed that in prostate cancer, CXCR4 is the target of miR-494. After MIR-494 is down-regulated, it acts on CXCR4, which is negatively correlated with the proliferation, metastasis and invasion of prostate cancer cells. In this study, through miRNA sequencing and qRT-PCR, it was found that the expression of miR-494-3p in quercetin-treated Sune-1 cells was significantly increased relative to the DMSO group, indicating that quercetin may affect the proliferation ability of Sune-1 cells by up-regulating miR-494-3p.

H19 (LncRNA H19), as a member of long non-coding RNA, located on chromosome 11, has been proved to play an important biological role in the occurrence, development, proliferation and migration of a variety of tumor cells and tumor tissues, especially in the process of tumor metastasis[26]. miR-675 is the cleavage product of precursor LncRNA H19. It is located in the first exon of LncRNA H19. It is a new miRNA related to the biological behavior of tumors, playing the role of both pro-tumor genes and tumor suppressor genes. In recent years, studies have found that miR-675 is up-regulated in human colorectal cancer[27], breast cancer[28-29], stomach cancer[30], glioma[31], and liver cancer[32]. Tsang *et al.*[27] pointed out through experiments that the high expression of miR-675 mediated by H19 in colorectal cancer tissues and cells is mainly due to the inhibition of the expression of RB and the promotion of the division and proliferation of colorectal cancer in an RB-dependent manner. Constance *et al.*[28] pointed out that lncRNA H19 can encode miR-675 in breast cancer cells, miR-675 reduces the expression of c-Cbl and Cbl-b in the E3 family by targeting, thereby increasing EGFR and c-Met, activating Akt and Erk, thereby promoting the occurrence of breast cancer, promoting its division and proliferation, and enhancing metastatic ability. It also found that H19/miR-675-3p in Sune-1 cells was significantly down-regulated by quercetin, which may be related to the inhibitory effect of quercetin on the proliferation of Sune-1 cells.

In addition, this study also found that the expression of CTGF in Sune-1 was significantly down-regulated and the expression of VHL was significantly up-regulated under quercetin treatment. Studies have reported that CTGF plays a regulatory role in the expression of ANGPT2, which is a key factor in tumor angiogenesis,

and CTGF down-regulation can significantly affect tumor proliferation and angiogenesis *in vivo*[33-34]. VHL has the function of tumor suppressor gene and can play an anti-tumor role through a variety of signaling pathways[35-36]. This study found that CTGF and VHL were the potential target genes of miR-494-3p and miR-675-3p through the prediction of miRNA target genes, suggesting that quercetin may regulate CTGF and VHL by acting on miR-494-3p and miR-675-3p, thereby inhibiting the proliferation of Sune-1 cells.

However, there are still some shortcomings in this study. Although the target genes of miRNAs have been predicted, the direct regulatory relationship between key miRNAs and mRNAs has not been experimentally verified. In addition, normal cells and other nasopharyngeal carcinoma cells were not designed as controls because of the previous focus on the effect of quercetin on the Sune-1 cell line. These shortcomings will be improved in the follow-up study.

References

[1] CHEN YP, CHAN ATC, LE QT, *et al*. Nasopharyngeal carcinoma[J]. The Lancet, 2019, 394(10192): 64-80.

[2] SUGANTHY N, DEVI KP, NABAVI SF, *et al*. Bioactive effects of quercetin in the central nervous system: Focusing on the mechanisms of actions[J]. Biomedicine & Pharmacotherapy, 2016, 84: 892-908.

[3] KASHYAP D, MITTAL S, SAK K, *et al*. Molecular mechanisms of action of quercetin in cancer: Recent advances[J]. Tumor Biology, 2016, 37(10): 12927-12939.

[4] VISHNOI A, RANI S. MiRNA biogenesis and regulation of diseases: An overview[J]. Methods in Molecular Biology, 2017, 1509: 1-10.

[5] BACKES C, MEESE E, KELLER A. Specific miRNA disease biomarkers in blood, serum and plasma: Challenges and prospects[J]. Molecular Diagnosis & Therapy, 2016, 20(6): 509-518.

[6] RUPAIMOOLE R, SLACK FJ. MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases[J]. Nature Reviews Drug Discovery, 2017, 16(3): 203-222.

[7] HAO S, LUO C, ABUKIWAN A, *et al*. miR-137 inhibits proliferation of melanoma cells by targeting PAK2[J]. Experimental Dermatology, 2015, 24(12): 947-952.

[8] ZHANG L, NIU T, HUANG Y, *et al*. Compound 331 selectively induces glioma cell death by upregulating miR-494 and downregulating CDC20 [J]. Scientific Reports, 2015, 5: 12003.

[9] SHAHAM L, BINDER V, GEFEN N, *et al*. MiR-125 in normal and malignant hematopoiesis[J]. Leukemia, 2012, 26(9): 2011-2018.

[10] SUN YM, LIN KY, CHEN YQ. Diverse functions of miR-125 family in different cell contexts[J]. Journal of Hematology & Oncology, 2013, 6: 6.

[11] CHEN Y, MIN L, REN C, *et al*. miRNA-148a serves as a prognostic factor and suppresses migration and invasion through Wnt1 in non-small cell lung cancer[J]. PLoS One, 2017, 12(2): e0171751.

[12] ABU-DUHIER FM, JAVID J, SUGHAYER MA, *et al*. Clinical significance of circulatory miRNA-21 as an efficient non-invasive biomarker for the screening of lung cancer patients[J]. Asian Pacific Journal of Cancer Prevention, 2018, 19(9): 2607-2611.

[13] ZHANG X, LI Y, QI P, *et al*. Biology of miR-17-92 cluster and its progress in lung cancer[J]. International Journal of Medical Sciences, 2018, 15(13): 1443-1448.

[14] CHEN ZW, ZENG Z, ZHU SS, *et al*. Effects of different combined regimens of cisplatin, metformin, and quercetin on nasopharyngeal carcinoma cells and subcutaneous xenografts[J]. Scientific Reports, 2021, 11(1): 1040.

[15] SALEM M, PANERU B, AL-TOBASEI R, *et al*. Transcriptome assembly, gene annotation and tissue gene expression atlas of the rainbow trout [J]. PLoS One, 2015, 10(3): e0121778.

[16] SONG Q, HAWKINS GA, WUDEL L, *et al*. Dissecting intratumoral myeloid cell plasticity by single cell RNA-seq[J]. Cancer Medicine, 2019, 8(6): 3072-3085.

[17] REN H, YU X, SHEN G, *et al*. miRNA-seq analysis of human vertebrae provides insight into the mechanism underlying GIOP[J]. Bone, 2019, 120: 371-386.

[18] MAO CG, WAN LJ, SHEN M, *et al*. Statistics and analysis of survival rate and prognosis of patients with nasopharyngeal carcinoma after treatment[J]. Chinese Journal of Otorhinolaryngology, 2015, 50(5): 386-389. (in Chinese)

[19] KIM MC, LEE HJ, LIM B, *et al*. Quercetin induces apoptosis by inhibiting MAPKs and TRPM7 channels in AGS cells[J]. International Journal of Molecular Medicine, 2014, 33(6): 1657-1663.

[20] DEMIROGLU-ZERGEROGLU A, ERGENE E, AYVALI N, *et al*. Quercetin and cisplatin combined treatment altered cell cycle and mitogen-activated protein kinase expressions in malignant mesothelioma cells [J]. BMC Complementary and Alternative Medicine, 2016, 16(1): 281.

[21] MAHBUB AA, LE MAITRE CL, HAYWOOD-SMALL SL, *et al*. Differential effects of polyphenols on proliferation and apoptosis in human myeloid and lymphoid leukemia cell lines[J]. Anti-Cancer Agents in Medicinal Chemistry, 2013, 13(10): 1601-1613.

[22] OW SH, CHUA PJ, BAY BH. miR-149 as a potential molecular target for cancer[J]. Current Medicinal Chemistry, 2018, 25(9): 1046-1054.

[23] OLARU AV, GHIAUR G, YAMANAKA S, *et al*. MicroRNA down-regulated in human cholangiocarcinoma control cell cycle through multiple targets involved in the G1/S checkpoint[J]. Hepatology, 2011, 54(6): 2089-2098.

[24] OHDIRA H, SEKIGUCHI M, MIYATA K, *et al*. MicroRNA-494 suppresses cell proliferation and induces senescence in A549 lung cancer cells[J]. Cell Proliferation, 2012, 45(1): 32-38.

[25] SHEN PF, CHEN XQ, LIAO YC, *et al*. MicroRNA-494-3p targets CXCR4 to suppress the proliferation, invasion, and migration of prostate cancer[J]. The Prostate, 2014, 74(7): 756-767.

[26] MATOUK IJ, HALLE D, RAVEH E, *et al*. The role of the oncofetal H19 lncRNA in tumor metastasis: Orchestrating the EMT-MET decision [J]. Oncotarget, 2016, 7(4): 3748-3765.

[27] TSANG WP, NG EK, NG SS, *et al*. Oncofetal H19-derived miR-675 regulates tumor suppressor RB in human colorectal cancer[J]. Carcinogenesis, 2010, 31(3): 350-358.

[28] VENNIN C, SPRUYT N, DAHMANI F, *et al*. H19 non-coding RNA-derived miR-675 enhances tumorigenesis and metastasis of breast cancer cells by downregulating c-Cbl and Cbl-b[J]. Oncotarget, 2015, 6(30): 29209-29223.

[29] ZHAI LL, WANG P, ZHOU LY, *et al*. Over-expression of miR-675 in formalin-fixed paraffin-embedded (FFPE) tissues of breast cancer patients[J]. International Journal of Clinical and Experimental Medicine, 2015, 8(7): 11195-11201.

[30] ZHUANG M, GAO W, XU J, *et al*. The long non-coding RNA H19-derived miR-675 modulates human gastric cancer cell proliferation by targeting tumor suppressor RUNX1[J]. Biochemical and Biophysical Research Communications, 2014, 448(3): 315-322.

[31] SHI Y, WANG Y, LUAN W, *et al*. Long non-coding RNA H19 promotes glioma cell invasion by deriving miR-675[J]. PLoS One, 2014, 9(1): e86295.

[32] LYU J, MA L, CHEN XL, *et al*. Downregulation of LncRNA H19 and miR-675 promotes migration and invasion of human hepatocellular carcinoma cells through AKT/GSK-3β/Cdc25A signaling pathway[J]. Journal of Huazhong University of Science and Technology Medical Sciences, 2014, 34(3): 363-369.

[33] YANG HY, MA D, LIU YR, *et al*. Impact of hormone receptor status and distant recurrence-free interval on survival benefits from trastuzumab in HER2-positive metastatic breast cancer[J]. Scientific Reports, 2017, 7(1): 1134.

[34] CAPPARELLI C, WHITAKER-MENEZES D, GUIDO C, *et al*. CTGF drives autophagy, glycolysis and senescence in cancer-associated fibroblasts via HIF1 activation, metabolically promoting tumor growth[J]. Cell Cycle, 2012, 11(12): 2272-2284.

[35] ZHANG J, ZHANG Q. VHL and hypoxia signaling: Beyond HIF in cancer[J]. Biomedicines, 2018, 6(1): 35.

[36] GUO J, CHAKRABORTY AA, LIU P, *et al*. pVHL suppresses kinase activity of Akt in a proline-hydroxylation-dependent manner[J]. Science, 2016, 353(6302): 929-932.