Investigation of the Treatment Mechanism of Cervical Cancer with Quercetin

MGI's DNBSEQ Sequencing Platform Facilitates the Discovery of Cancer Therapeutic Targets and Regulatory Networks

A research team led by Prof. Chao Xu from the Second Affiliated Hospital, Shaanxi University of Chinese Medicine utilized the RNA-seq technology based on MGI's DNBSEQ sequencing platform to investigate the treatment of cervical cancer with quercetin. They successfully constructed a possible regulatory network related to this treatment. This study provides reliable evidence for exploring the regulatory mechanisms and molecular targets of quercetin in the treatment of cervical cancer[1].

This research was published on the journal of *Onco Targets Ther* in 2021, titled with "Screening of Therapeutic Candidate Genes of Quercetin for Cervical Cancer and Analysis of Their Regulatory Network"[1].

Recommended application: Cancer Genomics (Cervical Cancer)

Recommended models: DNBSEQ-G400RS

- ### Data output is efficient and high-quality

  DNBSEQ sequencing technology has significant features such as high accuracy, low repeat sequence rate, and low index hopping rate, etc.

- ### Offer a complete product combination for experimental procedures

  Based on independently developed automatic solutions and analysis software, MGI provides a complete set of product combination from sample to result output.

- ### Suitable for various tumor genomics applications

  MGI offers a product portfolio that meets various practical application needs in cancer research, such as whole-genome sequencing, whole-exome sequencing, targeted sequencing, RNA sequencing, and methylation sequencing.

Background

Cervical cancer (CC) is a malignant tumor that poses a major threat to the health of women. It is the second most common reason for cancer-related mortality among women in developing countries and ranks fourth in the prevalence of female tumors worldwide[2]. Human papillomavirus (HPV) testing results for 99.7% of cervical cancer cases are positive, according to epidemiological case studies[3]. High-risk HPV is the primary cancer-causing factor in the development of cervical cancer. Currently, a vaccine against this virus has been developed, and it can stop up to 70% of infections caused by HPV. However, there are age constraints and safety concerns with the vaccination, and it cannot completely prevent all subtypes of HPV infection. Cervical cancer still ranks among the cancers that pose a hazard to the health of women since many people had HPV infections prior to taking the vaccine.

A plant flavonoid called quercetin (Que) has been found in both in vitro and in vivo test to have anti-cancer properties. Animal experiments and clinical trials have validated its safety and potential efficacy in the prevention and treatment of cancer. According to studies, quercetin activates the *P53* gene in cervical cancer cells without requiring the presence of E6 expression, leading to G2 phase cell cycle arrest and apoptosis[4]. It has been also reported that quercetin has a dose-dependent effect and can significantly suppress the growth of cervical cancer cells (HeLa and SiHa)[5]. Tumor molecular targeted therapy has been used in clinical practice because of the rapid development of tumor molecular biology, and its significance in the treatment of tumors is becoming more well understood[6]. Therefore, it is very important to search for molecular targets and related regulatory mechanisms of quercetin in the treatment of cervical cancer.

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Study description

Gene expression profiling analysis, as a subfield of bioinformatics, has demonstrated considerable promise for the study of cancer and has been widely used in studies related to molecular changes associated with tumor progression, diagnosis, treatment, and prognosis of various cancers, as well as the discovery of new therapeutic targets[1]. This study downloaded four cervical cancer mRNA expression datasets from the NCBI-GEO database（https://www.ncbi.nlm.nih.gov/geo/）. The research team identified differentially expressed genes (DEG), carried out functional enrichment analysis and protein-protein interaction studies, discovered candidate genes for the treatment of cervical cancer and their upstream miRNAs, lncRNAs, and circRNAs, and ultimately created a potential regulatory network for quercetin treatment of cervical cancer by analyzing the pertinent datasets and the RNA-Seq sequencing results[1]. This study offers a solid foundation for investigating the regulatory mechanisms and molecular targets of quercetin in cervical cancer treatment.

Materials and Methods

1. Cell line culture and drug treatment

Human cervical cancer cell lines HeLa and SiHa were cultured in DMEM culture medium containing 10% FBS at 37°C in an incubator with 5% CO_2. Quercetin was dissolved at a concentration of 100 mM in DMSO, and then diluted using DMEM culture medium (DMSO as a control) to the appropriate dose (0, 6.25, 12.5, 25, 50, 100, or 200 μM). The cell presented 96-well plates were evaluated in accordance with the established protocol at 24, 48, 72, and 96 hours by measuring the absorbance at 490 nm. The data were used to calculate the inhibition rate, and curve fitting was performed to determine the IC50 value.

Cell viability assays: cells were plated in 96-well plates at a density of 1-2x10^3 cells per well and cultured with the DMEM solution containing quercetin. On days 1, 3, 5, and 7, the plates were tested by measuring the absorbance at 490 nm, which produced cell growth curves to measure cell proliferation.

2. RNA Extraction, Library Preparation, and Sequencing

Total RNA was extracted using TRIzol reagent, a third-party company was commissioned to complete the construction of the RNA-Seq sequencing library, and the DNBSEQ sequencing platform from MGI was used for library sequencing.

3. Data Analysis

From the NCBI Gene Expression Omnibus (GEO) database, four original human cervical cancer mRNA expression datasets were retrieved. Differentially expressed genes (DEGs) were found by combining GEO and analysis of RNA-seq data from cervical cancer cells treated with quercetin, and functional enrichment and protein-protein interactions were examined. Upstream miRNA, lncRNA, and circRNA of candidate genes were predicted. Finally, a regulatory network was constructed using Cytoscape software.

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Sample collection	Library preparation	Bioinformatics analysis	Result analysis
Human cervical cancer cell lines HeLa and SiHa were treated with quercetin and total RNA was extracted.	 MGIEasy RNA Library Preparation Kit  DNBSEQ-G400 genetic sequencer	Differential gene expression analysis was performed using the GEO database and candidate genes for quercetin treatment were predicted along with their upstream miRNA, lncRNA, and circRNA.	DEGs were identified and functional enrichment and protein-protein interaction analyses were performed. Upstream miRNA, lncRNA, and circRNA for candidate genes were predicted and a regulatory network was constructed.

Results

Quercetin inhibited the proliferation of cervical cancer cells

HeLa and SiHa cells were treated with various concentrations of quercetin to confirm the anti-tumor effects, and cell proliferation was measured using MTT assays. According to the findings, quercetin significantly and dose-dependently reduced the growth of cervical cancer cells (*P<0.001*) (Figure 1).

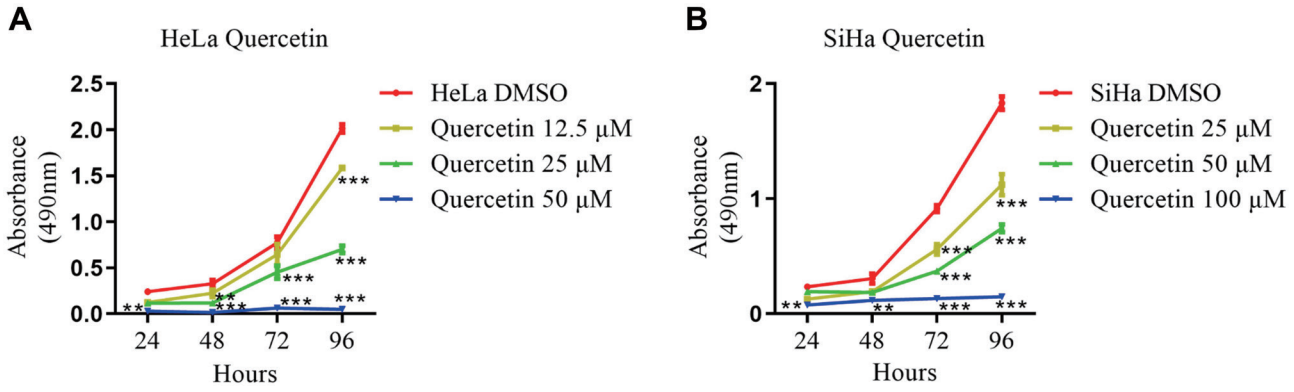

Figure 1. The proliferation of cervical cancer cells was inhibited by quercetin.

Identification of differentially expressed genes (DEGs) in the treatment of cervical cancer with quercetin

Online (GEO database) analysis of four cervical cancer microarray datasets (GSE7803, GSE9750, GSE63514, and GSE527) revealed 1887 differentially expressed genes, including 698 upregulated genes and 1189 downregulated genes, in normal cervical and cervical cancer tissue samples.

This study treated HeLa cells with quercetin since it dramatically reduced the growth of cervical cancer cells before running an RNA-seq investigation. There were 1122 genes that were differentially expressed, 688 of which were upregulated and 434 of which were downregulated.

Genes that are up- or down-regulated in the GEO database for cervical cancer relative to normal cervical tissue were found by merging the results of RNA sequencing analysis with the GEO database results. Genes were also classified as upregulated or downregulated if they showed opposite expression changes following quercetin treatment. 74 differentially expressed genes (DEGs) were discovered in total, as shown in Table 1. Of these, 21 were upregulated in the GEO database but downregulated after quercetin treatment, while 53 were downregulated in the GEO database but elevated after quercetin treatment.

A. 21 genes upregulated in geo, downregulated after quercetin
APOL2, BCCIP, BIRC3, C3, CCDC28B, CRIP1, EFNA1, GALNT2, GTPBP8, IL32, ISG15, KRT17, KRT18, KRT8, LIPE, LMO7, MUC1, PLAC8, SNRNP25, TMC5, UPF3B
B. 53 genes downregulated in geo, upregulated after quercetin
ADGRL2, ANK2, AR, BCHE, BHLHE40, CD44, CELSR2, CRYL1, CYP11A1, DAPK1, DUSP5, EGFR, EGR1, EPHX2, FCGBP, FOSB, FZD1, FZD10, GAB1, HIPK2, HMOX1, IER3, IRS2, JUN, KCTD15, KLF10, KLF4, KMT2D, LRRN2, MAFB, MAP2, NLRX1, NPAS2, NR1D1, NT5E, PER2, PHGDH, PIM1, PPP1R15A, RFX2, SLC16A6, SLC48A1, SNX19, SRD5A1, THSD4, TLE4, TMEM246, TST, WNK1, WNT5A, ZCCHC24, ZNF710, ZSCAN18

Table 1. The identification of 74 differentially expressed genes (DEGs) by combining GEO database and RNA sequence analysis

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GO function and KEGG pathway enrichment analysis of DEGs

The identified DEGs were analyzed in the Metascape database for Gene Ontology (GO, including biological process, cellular component, molecular function) and KEGG pathways enrichment analysis.

There were 861 enriched biological processes, 32 enriched cellular components, and 50 enriched molecular functions, as shown in Figure 2. Circadian rhythm, apoptotic signaling pathway, gland development, positive regulation of blood vessel development, adipocyte differentiation, and regulation of DNA-binding transcription factor activity are among the top 20 representative enriched terms of biological processes (Figure 2A). Transcription factor complexes, co-matrices, membrane rafts, apical plasma membranes, perinuclear cytoplasmic areas, and azurophil granule lumens are among the top eight cellular components (Figure 2B). Transcription factor binding, transcription co-factor binding, DNA-binding transcription activator activity, co-factor binding, Wnt-activated receptor activity, ATPase binding, and phosphatase binding are among the top 15 molecular functions (Figure 2C).

In addition, 56 KEGG pathways were enriched, with the top 8 clusters shown in Figure 3, including circadian rhythm, proteoglycans in cancer, microRNA in cancer, ErbB signaling pathway, AGE-RAGE signaling pathway in diabetic complications, and regulation of lipolysis in adipocytes.

The links between ethylene glycol's protein-protein interactions were examined and visualized using the STRING online tool. The remaining 42 node proteins were used to build a protein interaction network with 65 edges after 32 non-interacting proteins were buried in accordance with the set parameter conditions (Figure 4).

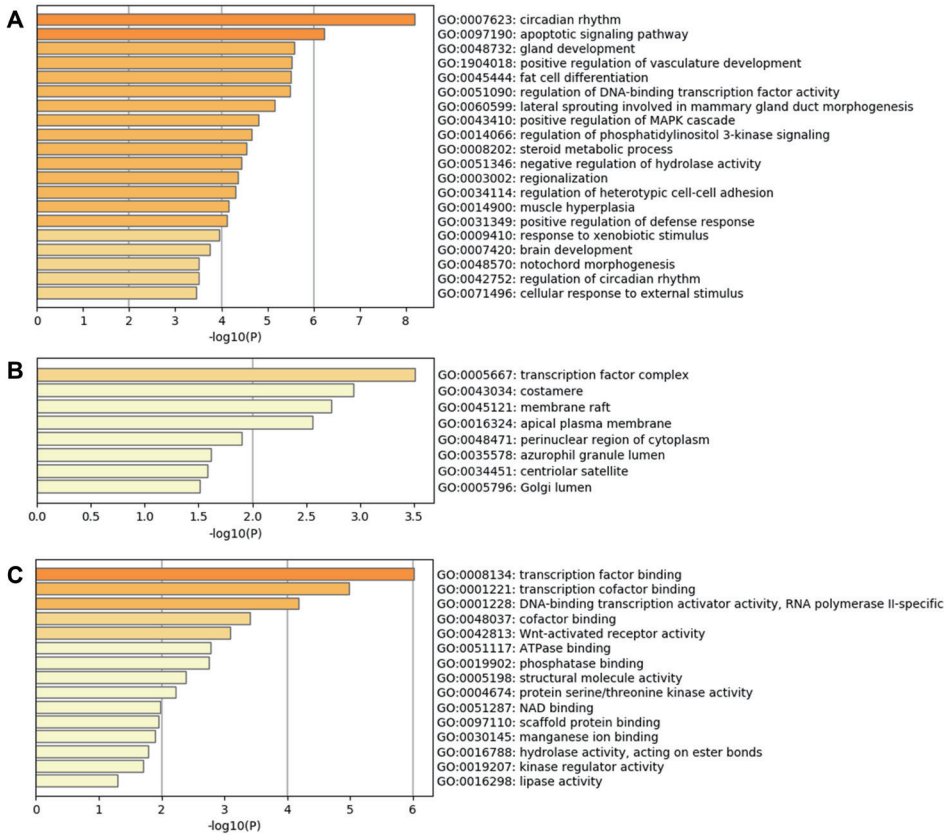

Figure 2. Results of DEGs' Gene Ontology (GO) enrichment analysis.

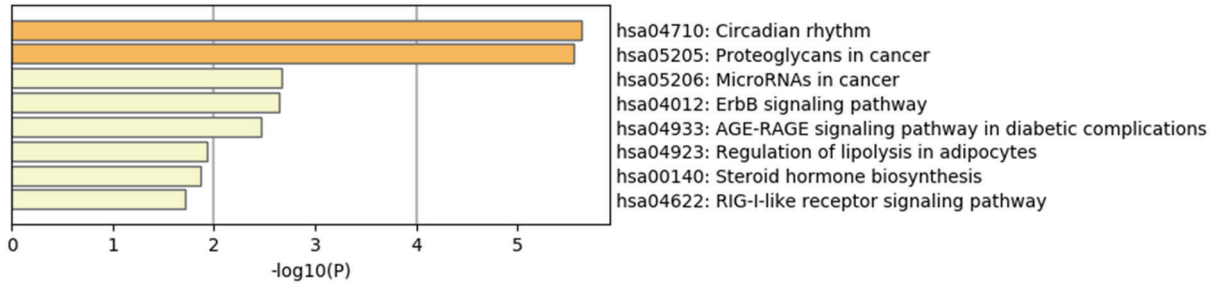

Figure 3. KEGG pathway enrichment analysis results of DEGs

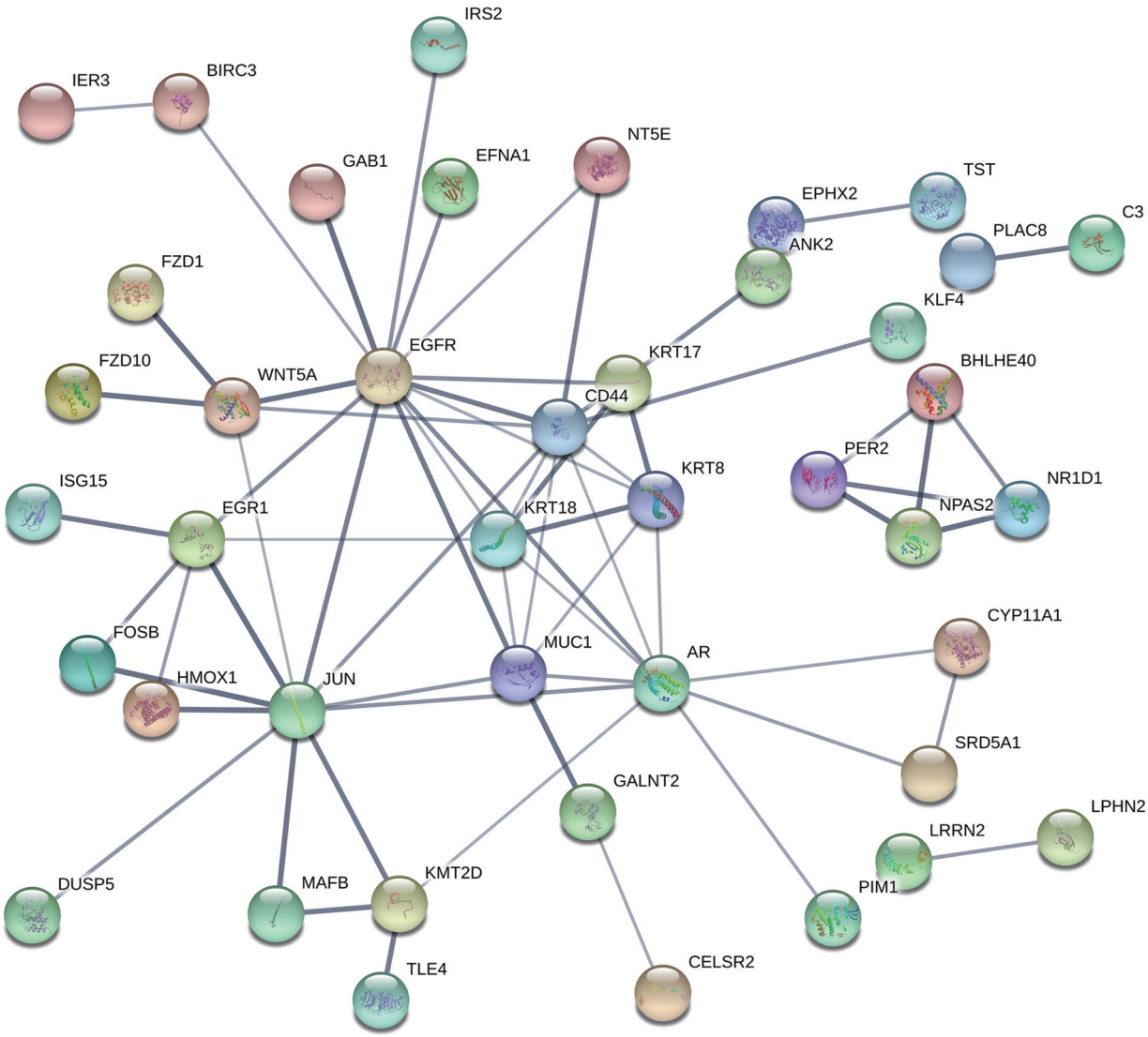

Figure 4. Protein-protein interaction analysis results of DEGs

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Identification of therapeutic candidate genes and analysis of upstream miRNAs, lncRNAs, and circRNAs

Using Cytoscape software and the MCODE plug-in, the aforementioned protein-protein interaction network's 7 core genes that make up the network's stable structure were found (Figure 5A, Table 2). 5 bottleneck genes and 6 hub genes were found using the Gentiscape2.2 plug-in (Table 2). The epidermal growth factor receptor (EGFR), Jun proto-oncogene (JUN), androgen receptor (AR), cluster of differentiation 44 (CD44), and mucin 1 (MUC1) were considered candidate genes because they are common core genes, hub genes, and bottleneck genes that are essential to the network (Figure 5B, Table 2). Additionally, a qRT-PCR experiment was employed to determine the level of candidate gene RNA expression in the HeLa control and quercetin groups. The results demonstrated that JUN expression was not significantly different between the two groups, and the expression of four genes (EGFR, AR, CD44, and MUC1) matched the findings of the RNA-seq study (Figure 5C). According to published research, quercetin targets the JUN gene by upregulating the expression of c-JUN and p-c-JUN in prostate cell lines in a dose-dependent manner.

Ten miRNAs were discovered (Table 3) after 5 candidate genes were submitted to the MirDIP database to estimate their upstream interacting miRNAs and their veracity on TarBase v.8 online. The above-mentioned miRNAs were then input into the online ENCORI program to examine their upstream lncRNAs and circRNAs, yielding 2 miRNA-lncRNA pairings and 174 miRNA-circRNA pairs. One lncRNA and 71 circRNAs were discovered when duplicate genes were eliminated (Table 3).

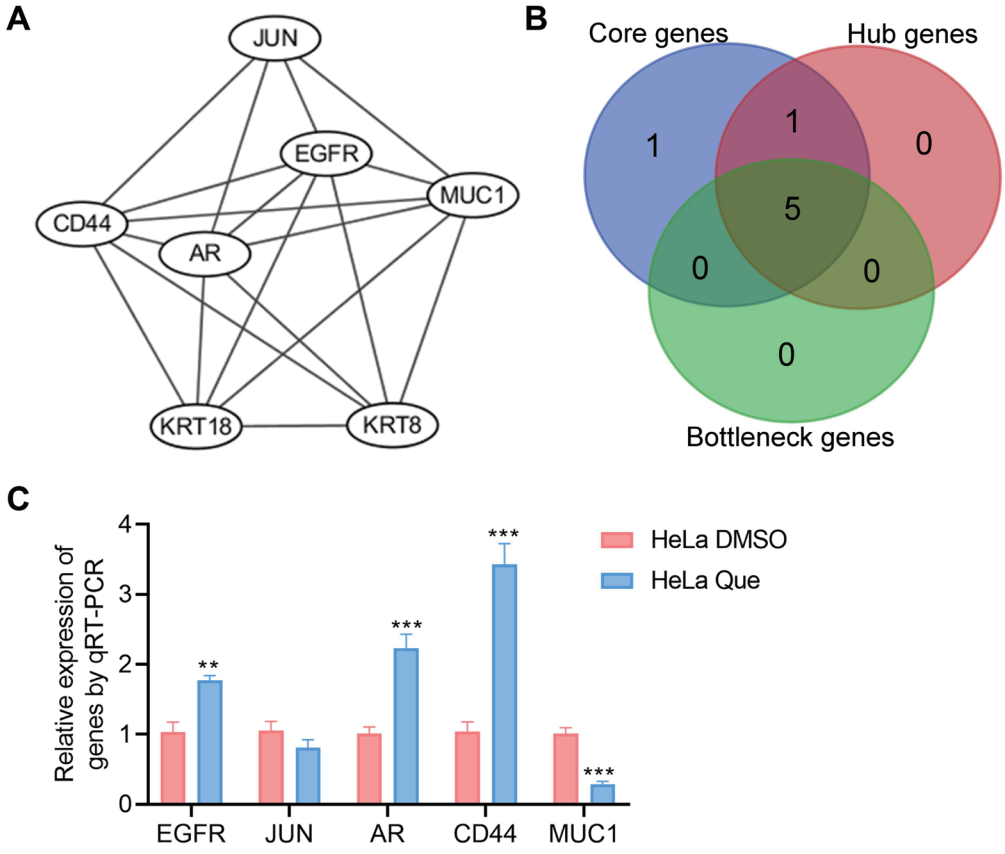

Figure 5. The screening outcomes for therapeutic candidate genes.

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Types	Names
Core genes Hub genes Bottleneck genes	***JUN, EGFR, MUC1***, *KRT8, KRT18,* ***CD44, AR*** ***EGFR, JUN, CD44, AR, MUC1***, *KRT18* ***EGFR, JUN, AR, CD44, MUC1***

Table 2. The screening outcomes of therapeutic candidate genes

Types	Names
miRNA	*hsa-miR-200b-3p, hsa-miR-139-5p, hsa-miR-7-5p, hsa-miR-200c-3p, hsa-miR-429, hsa-miR-495-3p, hsa-miR-32-5p, hsa-miR-454-3p, hsa-miR-92a-3p, hsa-miR-138-5p*
lncRNA	*MALAT1*
circRNA	*DHX9, NRBP1, RAI14, G3BP1, POM121C, KIAA1432, PSAP, SPTSSA, NOP56, XPO1, CCT5, RPS23, LAPTM4B, SCD, LUC7L3, SLC39A6, RCC2, RPS7, CTDSP1, CCNI, C11orf10, AHNAK, ARPP19, SRRM2, TBC1D10B, SEPW1, CBS, TAGLN2, NCK2, hsa_circ_001859, CNBP, MRFAP1, TMEM165, SEPT11, TMED9, HNRNPA2B1, SEC61G, CALU, hsa_circ_0089761, HCFC1, OTUD3, ATP5G3, C5orf24, ZDHHC5, NOB1, RECQL5, PTP4A2, 7-Mar, MYO10, C8orf38, RABGAP1, TAF1D, DAD1, STRN3, NEMF, RPS29, HNRNPL, NCL, PTMA, PPP2CA, VTA1, DNAJB6, RAD21, EEF1D, PRRC2B, GAPDH, FBRSL1, CKB, POLR2A, RPS28, NGFRAP1*

Table 3. Analysis of miRNAs, lncRNAs, and circRNAs interacting upstream of candidate genes

lncRNA/circRNA-miRNA-mRNA pathway regulatory network

The lncRNA/circRNA-miRNA-mRNA pathway regulatory network, which included a total of 90 nodes (4 candidate genes, 4 KEGG pathways, 10 miRNAs, 1 lncRNA, and 71 circRNAs), and 116 paths, was visualized using Cytoscape software using the candidate genes and their associated KEGG pathways, as well as all interaction relationships involved in the upstream miRNAs, lncRNAs, and circRNAs.

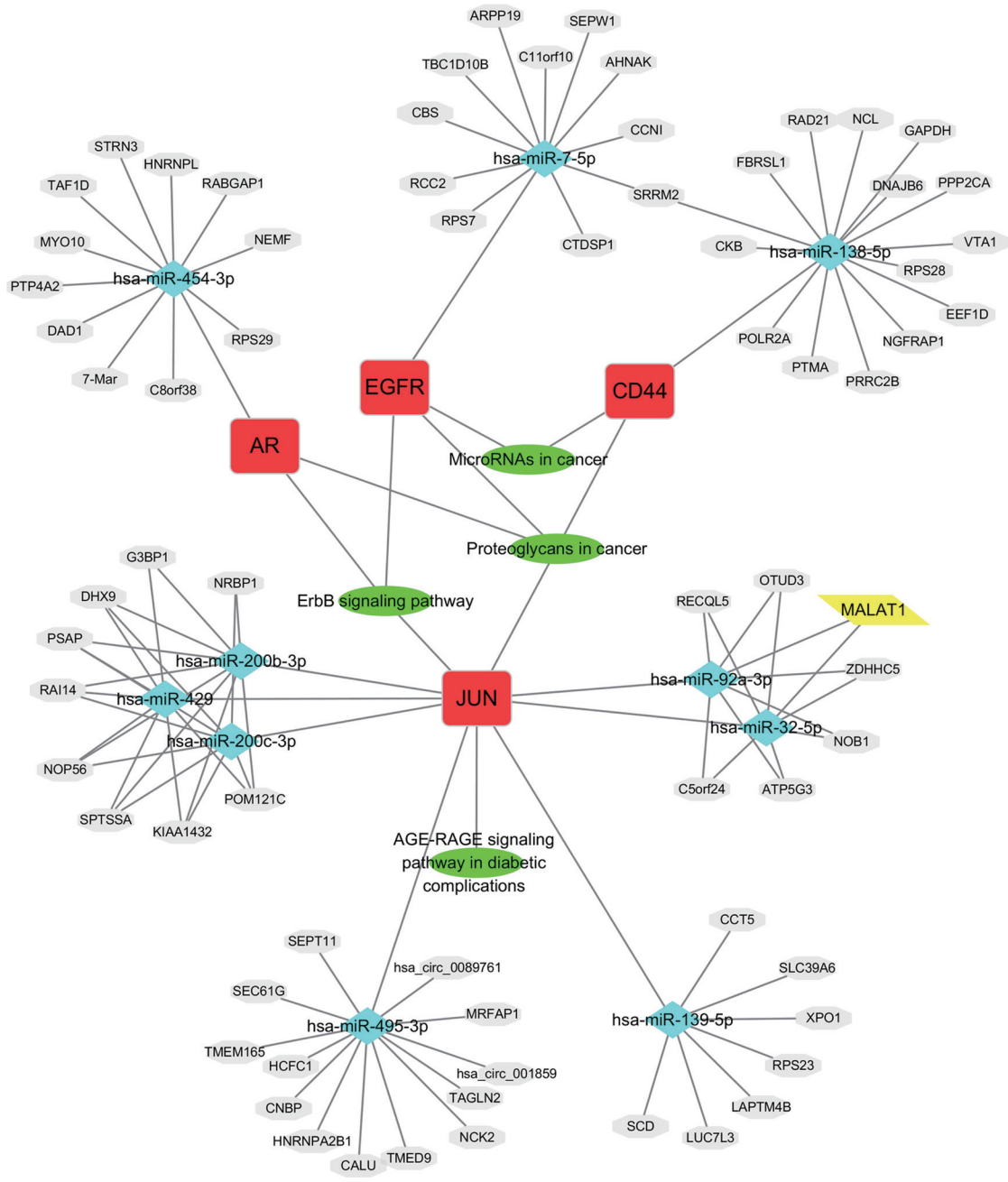

Figure 6. lncRNA/circRNA-miRNA-mRNA pathway regulatory network

Summary

In this study, the research team treated cervical cancer HeLa and SiHa cells with quercetin and performed RNA sequencing using the DNBSEQ sequencing platform from MGI. By jointly analyzing the GEO database and RNA-Seq sequencing results, differentially expressed genes (DEGs) were identified (i.e., genes upregulated/downregulated in cervical cancer compared to normal cervix in the GEO database, as well as genes downregulated/upregulated after quercetin treatment). Functional enrichment and protein-protein interaction analysis were performed on ethylene glycol. The upstream miRNAs, lncRNAs, and circRNAs of the candidate genes were predicted and a regulatory network was constructed. This study can provide a theoretical basis for targeted therapy of cervical cancer.

The high-throughput multiple parallel sequencing(MPS) technology based on the MGI DNBSEQ sequencing platform can provide high-quality, simple, and fast gene testing solutions for the prevention, diagnosis, and treatment of tumors.

DNBSEQ-G400 Genetic Sequencer

References

1. Li, Y., Kou, J., Wu, T., Zheng, P. & Chao, X. Screening of Therapeutic Candidate Genes of Quercetin for Cervical Cancer and Analysis of Their Regulatory Network. *Onco Targets Ther* **14**, 857-866, doi: 10.2147/OTT.S287633 (2021).
2. Bray, F. *et al.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **68**, 394-424, doi:10.3322/caac.21492 (2018).
3. Chen, W. *et al.* Cancer incidence and mortality in China, 2013. *Cancer Lett* **401**, 63-71, doi:10.1016/j.canlet.2017.04.024 (2017).
4. Jana, N., Bretislav, G., Pavel, S. & Pavla, U. Potential of the Flavonoid Quercetin to Prevent and Treat Cancer - Current Status of Research. *Klin Onkol* **31**, 184-190, doi:10.14735/amko2018184 (2018).
5. Clemente-Soto, A. F. *et al.* Quercetin induces G2 phase arrest and apoptosis with the activation of p53 in an E6 expression-independent manner in HPV-positive human cervical cancer-derived cells. *Mol Med Rep* **19**, 2097-2106, doi:10.3892/mmr.2019.9850 (2019).
6. Shen, S., *Zhang, S., Liu*, P., Wang, J. & Du, H. Potential role of microRNAs in the treatment and diagnosis of cervical cancer. *Cancer Genet* **248-249**, 25-30, doi:10.1016/j.cancergen.2020.09.003 (2020).

Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	Genetic Sequencer DNBSEQ-G400RS	900-000170-00
	MGISP-100RS Automated Sample Preparation System	900-000206-00
	MGISP-960RS Automated Sample Preparation System	900-000146-00
Softwares	MegaBOLT Bioinformatics analysis accelerator	900-000555-00
Library Prep reagents	MGIEasy RNA Library Prep Set（16 RXN）	1000006383
Sequencing Reagents	DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100)	1000016950

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