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A Transcriptome Sequencing Analysis on Berberine-mediated Gene Expression in Nasopharyngeal Carcinoma cells

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KEYWORDS

Nasopharyngeal Carcinoma;
Berberine;
Transcriptome;
Gene Expression;
RNA Transcription

ABSTRACT

Nasopharyngeal carcinoma (NPC) is a highly prevalent head and neck malignant tumor in southern China and Southeast Asia. Traditional Chinese medicine treatment with Coptis chinensis as the main medicine is an important part of the comprehensive treatment of NPC with traditional Chinese medicine. The extract of Coptis chinensis, berberine, has good inhibitory effects on various solid tumors and hematological tumors, including nasopharyngeal carcinoma. However, its inhibitory effect and mechanism on tumors are unclear. This study used high-throughput sequencing technology to analyze the effect of berberine on RNA transcriptome of nasopharyngeal carcinoma cells to reveal the molecular mechanism of berberine anti-NPC. The Transcriptome Sequestration Analysis showed that berberine treatment significantly enriched differentially expressed genes in GO processes such as misfolded protein endoplasmic reticulum stress, DNA replication, cell apoptosis, and inflammatory response. Signal pathway analysis showed that differentially expressed genes were significantly enriched in tumor oncogenic and autophagic regulatory signaling pathways such as MAPK, Hippo, PI3K/AKT, TGF β, p53, etc. Berberine significantly altered the transcriptome expression profile of NPC cells, inhibited pro- tumor signals such as AKT/mTOR, thereby suppressing the growth, proliferation, migration, and invasion ability of NPC cells, inducing autophagic death of nasopharyngeal carcinoma cells, and inhibiting cell growth and malignant characteristics.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a common malignant tumor in the head and neck region. According to NPC histopathological type standards announced by the World Health Organization (WHO), it is divided into keratinizing squamous cell carcinoma (SCC) and non keratinizing (NK) carcinoma, the latter of which includes differentiated and undifferentiated cancers. The non keratinized NPC is the most common in China, and its important reason is the high correlation between non keratinized NPC and Epstein-Barr (EB) virus [2]. The non keratinized NPC is extremely sensitive to radiation, and radiotherapy (RT) is the basic treatment for nasopharyngeal carcinoma. The NPC patients at early stage can be treated with radiotherapy alone, with a five-year survival rate of up to 95%. The complex structure of the nasopharynx leads to the insidious onset of NPC, which has a high degree of malignancy and is difficult to distinguish between early symptoms and benign diseases. Therefore, it is prone to distant metastasis. The vast majority of patients are already in the middle and late stages of cervical lymph node invasion and distant metastasis when they seek medical attention. Therefore, it further increases the difficulty of the NPC treatment. The most commonly used treatment mode current-

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ly is radiotherapy combined with chemotherapy, and platinum based combination radiotherapy and chemotherapy is the recommended treatment for NPC patients. Although combination therapy such as radiotherapy and chemotherapy can achieve certain clinical benefits, there is still a high proportion of treatment failure rates and treatment-related side effects [1-4]. Therefore, the search for new therapeutic method that can improve the efficacy of combined radiotherapy and chemotherapy for advanced NPC and reduce treatment side effects has an important clinical value. Traditional Chinese medicine treatment with Coptis chinensis as the main medicine is an important part of the comprehensive treatment of tumors with traditional Chinese medicine[6-8].

In recent years, a large number of studies have shown that berberine, an effective component of Coptis chinensis, has important therapeutic value in digestive system diseases, cardiovascular and cerebrovascular diseases, diabetes and other metabolic diseases and malignant tumors. As the main active ingredient of Coptis chinensis, berberine is a quaternary ammonium salt derived from isoquinoline alkaloids, with a molecular weight of 336.36 g/mol. It is a light yellow crystalline powder with a typical alkaloid bitterness, odorless or slightly characteristic odor, and can be slightly soluble in water, ethanol, and methanol [4,5]. Berberine has a wide range of pharmacological activities, including inhibitory activity against various microorganisms such as viruses, chlamydia, bacteria, and worms. It has the ability to regulate inflammation and immunity in the body, and also has good anti-tumor effects.

Some studies reported that berberine has shown some therapeutic effects on solid malignant tumor cells, including NPC, breast cancer, esophageal cancer, colorectal cancer, neuroblastoma, ovarian cancer, lung cancer, endometrial cancer, bladder cancer, gastric cancer, liver cancer, pancreatic cancer, prostate cancer, melanoma, head and neck squamous cell carcinoma, thyroid cancer [6-8]. Simultaneously, berberine can enhance the sensitivity of tumor cells to chemotherapy and radiation therapy. The reported studies have shown that berberine can inhibit tumor proliferation through various mechanisms such as inducing cell cycle arrest, inhibiting telomerase activity, inducing mitochondrial and p53 dependent cell apoptosis, inducing reactive oxygen species stress (ROS), activating mitochondrial dependent necrosis, and inhibiting cytokines [9]. Meanwhile, berberine can inhibit tumor cell metastasis by suppressing the activity of signaling pathways such as PI3K-AKT, NF-κB, ERK/MAPK, and inhibiting the expression of metastasis related molecules such as VGFR, Slug, Snail, etc [9].

Berberine can downregulate the phosphorylation level of Ezrin by inhibiting Rho kinase activity, thereby inhibiting the metastatic ability of NPC cells [10]. Meanwhile, berberine can inhibit the ability of tumor associated fibroblasts to activate STAT3 in EB virus positive NPC cell C666-1, thereby inhibiting cell growth in vitro and in vivo [11]. In addition, berberine can downregulate the expression of EB virus nuclear antigen 1 (EBNA1) in EB virus positive cells HONE and HK1-EBV, thereby inhibiting cell growth in vitro and in vivo [12]. Berberine inhibits the invasion and metastasis of NPC cells by suppressing the expression level of transcription factor SP1 and epithelial mesenchymal transition (EMT). Berberine also has a certain therapeutic effect on NPC patients. But berberine anti-NPC mechanism is still unclear.

Transcriptome sequencing (RNA-Seq) is a core technology that uses high-throughput sequencing technology to analyze whole RNAs (including mRNA and non coding RNA) in a specific cell or tissue in a certain state. This sequencing technology reveals information including gene expression, alternative splicing, and the discovery of new transcripts [13,14]. This technology at first extracts high-quality RNAs from cells, tissues, or blood, then mRNAs are enriched through polyA tail capture or rRNA removal strategies[14], cDNA synthesis and fragmentation are performed, reverse transcription is followed by connecting sequencing adapters, PCR amplification is used to construct libraries, and finally the libraries are subjected to sequencing [15]. High-throughput sequencing is performed with a read length of 2 × 150 bp, and it is recommended to have a data volume of ≥ 5G [16]. Then, comparative reference genomes (such as Hisat2), quantitative expression (such as EigenCounts), and differential gene analysis are conduced[14, 15]. This technology has been widely used in the studies including disease mechanism research, aging synthesis, and searching for new transcripts [16]. In the present study, high-throughput sequencing technology was used to investigate the effect of berberine on the overall transcriptome of NCP cell with berberine, providing a scientific theoretical basis for revealing the mechanism of berberine anti-NPC metastasis and exploring new effect molecules and signaling pathways in the future.

MATERIALS AND METHODS

Materials

The human NPC cell line S18 was purchased from the Cell Bank of Shanghai Institute of Cell Biology, and S18 cell line was validated by SRT. This cell line was derived from human NPC biopsy tissue specimens, the cells with high metastatic potential were established through in vitro culture and cell cloning [17]. For the NPC cell line, conventional culture is performed. Berberine was purchased from Selleck company with a purity of 99.70 %, a molecular weight of 336.36, and a molecular formula of C20H18NO4, also known as Umbellatine. Berberine storage conditions: The powder was stored for 3 years at -20 ℃, and the soluble in solvents was stored for 2 years at -80 ℃. berberine was dissolved in DMSO, and it was diluted in serum-free medium to the indicated concentration [18].

Cell Counting Reagent（CCK8 Assay Kit）

10 μl of berberine solutions with concentration gradients of 0, 2.5, 5, 10, 20, 40, 80, and 100 μm were added to a 96 well cell plate in sequence, and incubate in a 37 ℃, 5% CO2 incubator for 24 and 48 hours, respectively. 10 μl of CCK reagent was accurately added to each well. The added CCK cells were incubate in a 37 ℃, 5% CO2 incubator for 1-4 hours. The OD value was measured at 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader [19]. The non-toxic concentration can be calculated by using the X-axis as the concentration of berberine and the Y-axis as the obtained cell growth inhibition rate [18].

Collection and Processing of LongRNA Seq Cell Samples

EpiTM mini long RNA seq kit reagent was used to extract RNA from berberine treated NPC cells. Briefly, the cells were collected by centrifugation, and float washed 1-2 times with PBS. The sufficient TRIzol was used to thoroughly lyse the washed cells, and the lysate was transferred to an eppendorf (EP) tube. DNase I was added to the EP tube, digested at 37 ℃ for 30 minutes to remove residual DNA. The RNA samples were purified and recovered using magnetic beads. The ribosomal RNA and RNA fragmentation of the sample were removed. The first strand cDNA was synthesized. 2 × pfuMax HiFi PCR ProMix and sequencing primers were added to the first strand cDNA sample, the mixtures were mixed well, and amplified in a PCR instrument. The purification of PCR amplification products was performed using EpiTM DNA Clean Beams, and 300-400bp DNA fragments were recovered using magnetic beads at a ratio of 0.65/0.2. The second round of PCR amplification was performed on the purified product to enrich 300-400bp DNA fragments [20]. Bioptic Qsep100 Analyzer was used to perform quality inspection on the library and check whether the size distribution of the library conforms to the theoretical size.

Analysis of LongRNA Seq Data

Fastp software was used to filter sequencing data, remove adapters, N-bases, and low-quality bases, and evaluate overall data quality. Hisat2 (Hierarchical Indexing for Spliced Alignment of Scripts) was used for reference gene sequence alignment analysis. HTseq count was used to count the reads of some units on the genome, and HTseq was applied to calculate the reads of the genome. DEGseq was used to differentially screen to ensure the effectiveness of data analysis [20].

mRNA Gene Function and Signaling Pathway Analysis

The differentially expressed genes obtained from the analysis were annotated with GO from the BP, MF, and CC levels based on the database. All GO genes involved were analyzed using Fisher's test to calculate the significance level (P-Value) of each GO, and to screen for differentially expressed genes and significantly enriched GO genes with negative correlation [21].

Extraction of Total RNA

1ml Trizaol was added to the cell in six well plate, mixed well by blowing, and standed at room temperature for 5 minutes. The cell mixture was transferred to a 1.5 ml EP tube. 200 μ l of chloroform was added to the EP tube, mixed thoroughly upside down for 15 seconds, and standed on ice for 10 minutes. The mixture was centrifuged at 4 ℃ at 12900 rpm for 15 minutes, the clarified liquid was transferred from the upper layer to a new 1.5 ml EP tube, 1ml of pre cooled equal volume isopropanol was added and mixed well. The mixture was stayed at room temperature for 10 minutes, was centrifuged at 4 ℃ and 12900 rpm for 15 minutes. The supernatant was discarded. 1ml of 75% ethanol prepared with DEPC water added to wash the precipitate. The Centrifuge at 4 ℃ and 12900rpm for 5 minutes, and discard the supernatant. Add 75% ethanol for a second RNA wash, centrifuge at 12900rpm at 4 ℃ for 5 minutes, and discard the supernatant using a pipette. Open the lid and invert it onto the filter paper, then air dry the ethanol. Add 30-50 μ l of DEPC water, blow and mix repeatedly until fully dissolved.

RESULTS

Total RNA Quality Evaluation

The purity and integrity of RNA were detected by UV spectrophotometer and agarose gel electrophoresis, respectively. The results showed that the A260/A280 ratios of the control and berberine treatment groups were 1.954 and 1.924, respectively. They were ranged from 1.8 to 2.0, indicating that the quality of the control and berberine treatment groups meet the subsequent sequencing requirements. In addition, the agarose electrophoresis results of the control and berberine treatment groups showed that there was no obvious tailing in RNA electrophoresis, and the 28S, 18S, and 5S bands were integrated (**Figure 1**). The brightness of the 28S band was stronger than that of the 18S band, indicating that RNA integrity was good, no obvious contamination, and the quality could meet the subsequent sequencing requirements.

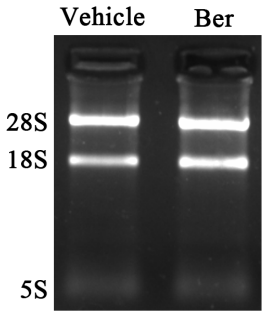

Figure 1 | Total RNA integrity of nasopharyngeal carcinoma cells with Berberine treatment

Transcriptome Sequencing Quality Control Results

The quality checks were conducted on the sequencing results, including quality assessment of the raw data through FastQC. By using Trimomatic for mass cutting, relatively accurate and effective data were obtained. HISAT2 was used to align the valid data of the sample to the reference genome, and the mapping information of the reference genome was calculated. Based on the alignment results, RSeQC was used to analyze redundant sequences and insert fragment distributions. Qualimap was used to perform homogeneity distribution checks and genomic structure distribution analysis based on the comparison results. To analyze the distribution of reads on chromosomes, BEDTools were used for statistical analysis of gene coverage and distribution of sequencing sequences on chromosomes. The reference genome alignment data maps were shown in **Figure 2A** and **2B**), the expression abundance distribution map (Density) was shown in **Figure 2C**, and the expression distribution map (Distribution) was shown in **Figure 2D**). There was no obvious chromosome aggregation in the reference genome alignment results, and both expression abundance and distribution met the requirements. The sequencing results were reliable.

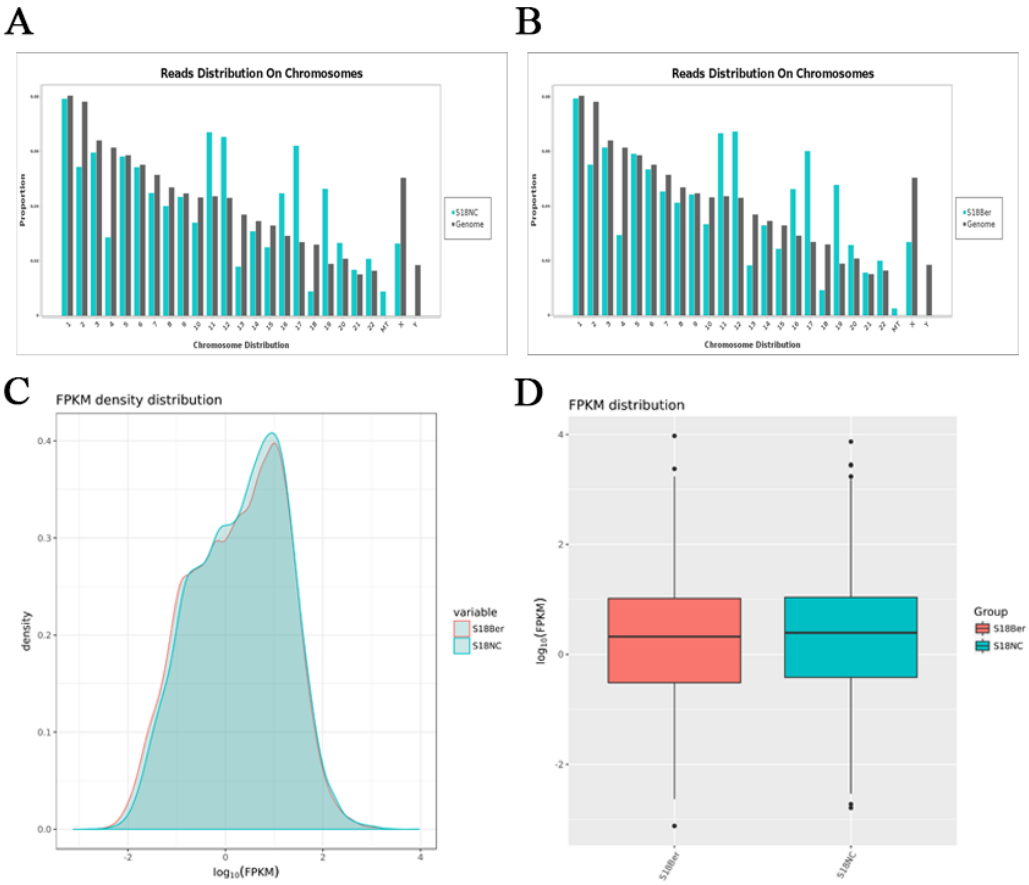

Figure 2 | Transcriptome sequencing data graph. A, Genome comparison data graph of control group; B, berberine genome alignment data graph; C, Distribution map of expression abundance; D, Expression level distribution map

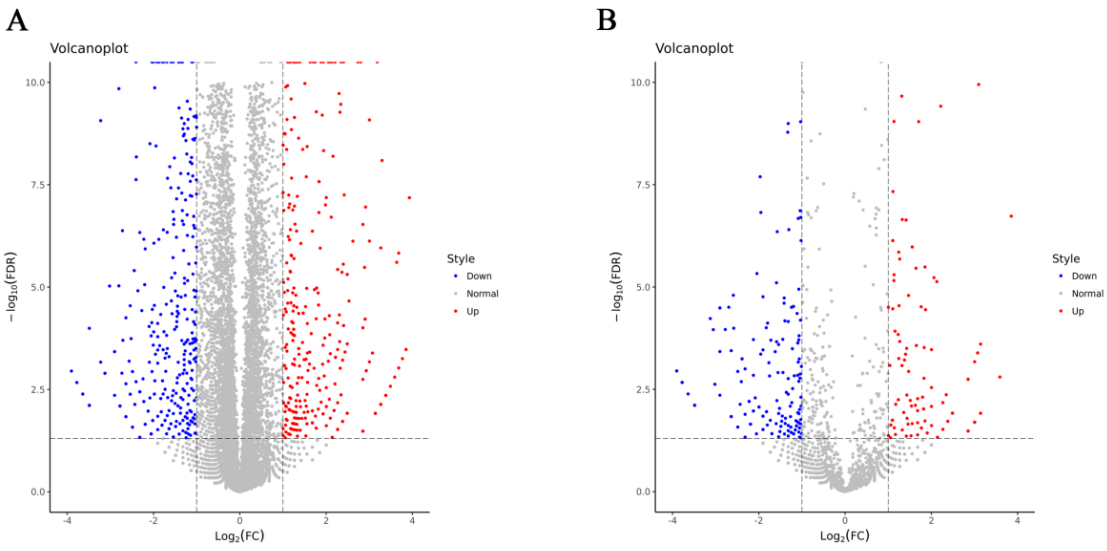

Figure 3 | Volcano plot of differently expressed mRNAs and lncRNAs in NPC cells with berberine treatment. A, Differential mRNA volcano plot; B, Differential lncRNA volcano plot. Red represents significant upregulation, blue represents significant downregulation, and gray represents no significant difference.

Table 1 | Top 20 upregulated LncRNAs from nasopharyngeal carcinoma cells with berberine

LncRNA name	log2FC	Pvalue	FDR	S18berberine	S18NC
AC110792.4	2.345037577	0.001536478	0.004281063	4.078372569	0.802635
AL844908.2	2.267035065	0.00251756	0.006681151	2.503019504	0.519968
AL049555.1	2.218125464	5.60E-11	3.82E-10	2.878968803	0.618689
AC015912.3	2.060584187	1.32E-06	5.91E-06	9.558145997	2.291047
AL355512.1	2.004000659	9.71E-05	0.000337559	4.587729282	1.143646
AC009090.1	2.004000659	0.000986981	0.002859688	4.466903211	1.113526
AC097372.3	2.004000659	0.003214628	0.008274934	2.745324205	0.684364
AL360091.2	1.819576088	0.003767815	0.00954531	2.165004904	0.613296
AL035458.2	1.762992559	7.40E-06	3.04E-05	7.360110814	2.168347
AL596325.2	1.706734618	1.39E-10	9.12E-10	14.9465235	4.578465
AC084824.4	1.682072564	0.001974081	0.005357641	2.450136131	0.763473
ARHGAP26-IT1	1.682072564	0.004175578	0.010485981	3.956663133	1.232914
MIR22HG	1.679600078	3.59E-45	1.04E-43	14.20114603	4.432731
AF131215.5	1.643598416	7.50E-07	3.47E-06	2.441200616	0.781248
AC090791.1	1.629605144	0.00297352	0.007744266	3.510332231	1.134348
AL031587.5	1.589915119	8.01E-28	1.34E-26	13.11148429	4.355096
AC148477.5	1.560766268	5.93E-17	5.86E-16	8.865811737	3.004962
AL021807.1	1.556541682	2.13E-07	1.05E-06	9.962854426	3.386695
AC103796.1	1.540053559	0.003128308	0.008075463	5.181607725	1.781641
AC007787.2	1.530069471	0.02051191	0.043084432	1.922185262	0.665513

Table 2 | Top 20 downregulated LncRNAs from nasopharyngeal carcinoma cells with berberine

LncRNA name	log2FC	Pvalue	FDR	S18berberine	S18NC
AC090971.5	-3.125282358	1.50E-05	5.89E-05	0.654943	5.714325
AC008736.1	-2.777359055	7.23E-19	7.94E-18	2.027093	13.89633
AP002360.4	-2.777359055	2.89E-05	0.000109	0.531942	3.646623
HS1BP3-IT1	-2.59094593	3.72E-06	1.59E-05	0.639661	3.853541
AC008608.2	-2.487852437	0.000367	0.001155	0.615058	3.449788
LINC01843	-2.390335932	9.92E-23	1.33E-21	1.96463	10.29916
AC068025.2	-2.317927436	0.000314	0.001	0.763318	3.805653
AC002044.1	-2.317927436	0.00525	0.012866	1.214545	6.055324
AC084757.2	-2.125282358	0.000464	0.001434	0.710734	3.10055
AL133297.1	-2.125282358	0.004261	0.010683	0.633327	2.762867
AC012291.1	-2.125282358	0.004261	0.010683	0.59642	2.60186
LINC01411	-2.119023368	2.92E-16	2.79E-15	1.006139	4.370243
AL442067.1	-1.995999341	0.003339	0.00855	1.434216	5.720426
AC063943.3	-1.995999341	0.003339	0.00855	0.8984	3.583301
LINC01468	-1.982431576	9.37E-238	1.94E-235	42.05358	166.1622
AC087501.5	-1.970004133	3.44E-09	2.02E-08	0.565833	2.216545
AC084346.2	-1.955357357	0.000128	0.000436	0.606633	2.352367
LINC00513	-1.952642972	2.78E-08	1.51E-07	0.752076	2.910877
LINC02522	-1.902889937	4.09E-06	1.73E-05	3.498144	13.08045
AC078960.1	-1.902889937	0.005905	0.014299	1.806951	6.756647

Overall of Differentially Expressed Genes From NPC Cells With Berberine Treatment.

After quality control processing of sequencing data, differentially expressed genes were screened using DESeq method. The screening conditions for significantly differentially expressed genes were: | log2FC | > 1 and FDR < 0.05. The berberine treatment group showed downregulation of 579 protein coding genes and upregulation of 584 protein coding genes when compared with the control group. 188 lncRNAs were downregulated and 127 lncRNAs were upregulated. The overall expression differences were displayed in the form of a volcano plot (**Figure 3**), where red represents genes or lncRNAs significantly upregulated after berberine treatment, blue represents genes or lncRNAs significantly downregulated, and gray represents genes or lncRNAs with no significant changes at pre- and post-berberine treatment. As shown in **Table 1**, the top 20 significantly highly expressed and lowly expressed protein coding genes and lncRNAs are listed. To eliminate thelarge differences caused by low background expression, the protein coding genes with normalized expression values greater than 5 and lncRNA with normalized expression values greater than 0.5 were fil-

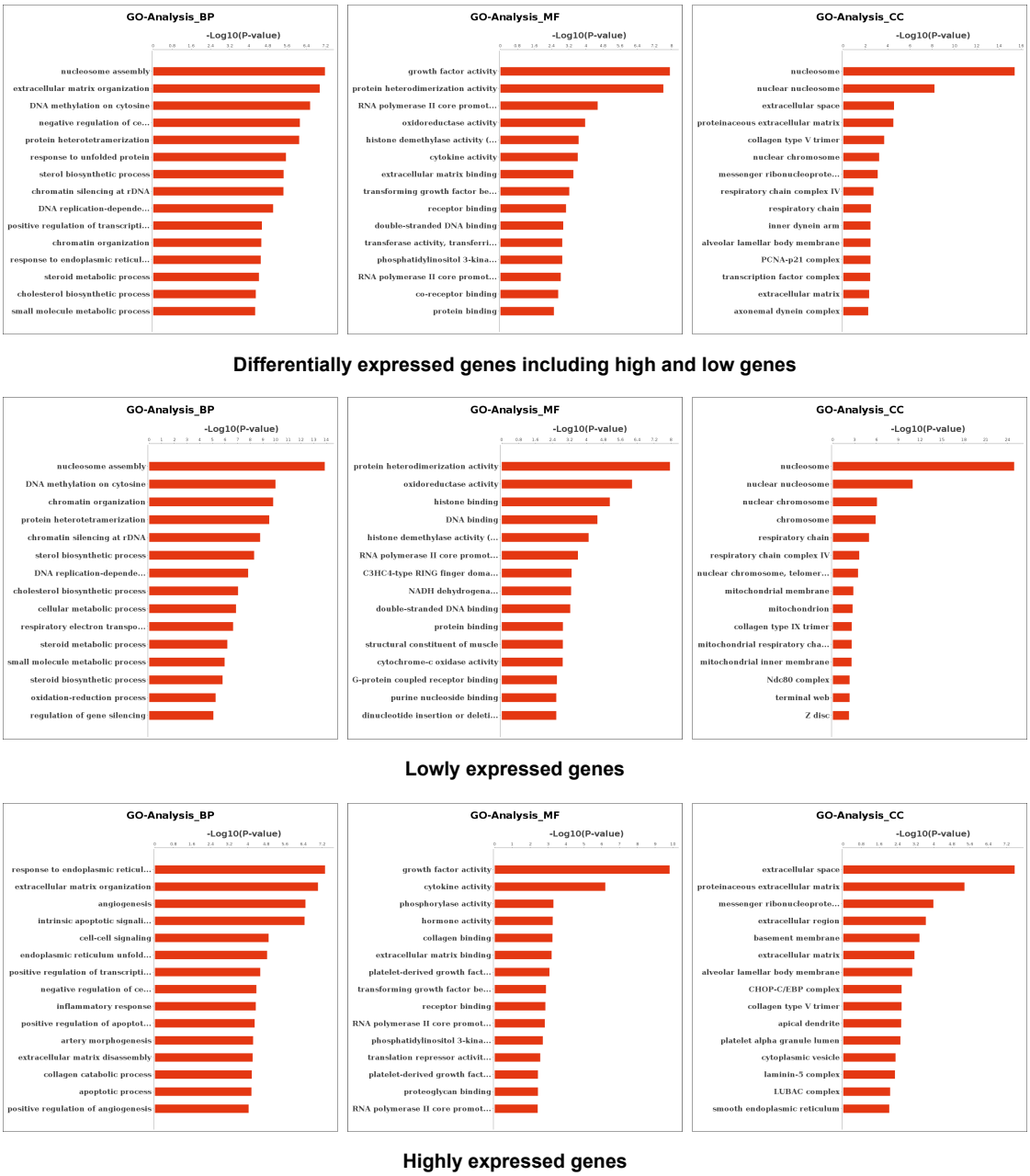

Figure 4 | GO analysis on significantly differentially expressed protein-coding genes

tered. The results showed that the expression levels of protein coding genes such as GDF15, UPP1, TRIB3, and MMP1, were significantly upregulated. The mitochondrial protein genes of histone gene sets, such as HIST1H4D, HIST1H4L, HIST1H2AL, MT-CO2, HIST1H2BG, and HIST1H2AB, were significantly downregulated. Meanwhile, the expressions of AC110792.4, AL844908.2, AL049555.1, and AC015912.3, were significantly upregulated. LncRNAs such as AC090971.5, AC008736.1, AP002360.4, and HS1BP3-IT1 were significantly downregulated.

GO Analysis of mRNA Gene Function

To probe the functions of the differentially expressed genes, this study annotated the differentially expressed protein coding genes from biological processes (BP), molecular functions (MF), and cellular components (CC), and obtained all GO genes involved. Fisher's test was used to calculate the significance level (P-Value) of each GO, significantly enriched GO genes and negatively correlated genes were screened. The results are shown in **Figure 4.** This study analyzed the significantly enriched GO genes from three directions: biological processes (BP), molecular functions (MF), and cellular components (CC). The results showed that differentially expressed genes were mainly involved in biological functions such as misfolded protein endoplasmic reticulum stress, DNA replication, cell apoptosis, and inflammatory response (**Figure 5**).

Differential Gene Signaling Pathway Analysis (KEGG)

To further explore the functions of differentially expressed genes in signaling pathways, significant signaling

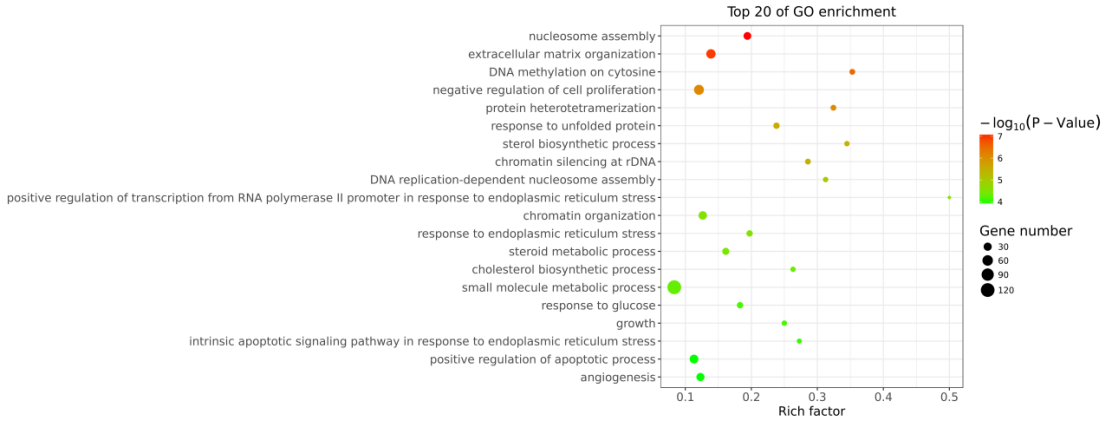

Figure 4 | GO enrichment analysis for the top 20 significantly differentially expressed genes of nasopharyngeal carcinoma with berberine treatment

pathways enriched by these genes were analyzed based on the KEGG database. Similar to GO analysis, both significantly upregulated and downregulated genes were examined in three directions for their enriched signaling pathways. As shown in Figure 5, the differentially expressed genes were primarily enriched in pathways such as MAPK, Hippo, PI3K/AKT, TGFβ, and p53 (**Figure 6**).

DISCUSSION

Transcriptomics is a very important study in the post genomic era, which is an important pathway for exploring functional genes and plays a crucial role in regulating gene expression and mechanisms at the transcriptional level[27]. Transcriptome sequencing, also known as RNA sequencing (RNA Seq), has the following advantages: **1)** High sensitivity and accuracy; **2)** Full transcriptome analysis can be performed on any species; 3) Directly detect almost all transcript fragment sequences[23]. This study used transcriptome sequencing to comprehensively and systematically investigate tanti-NPC mechanism of berberine, and got some valuable data.

In current, a large number of studies have shown that berberine has broad-spectrum anti-tumor properties, and its inhibitory effects are mainly manifested in three aspects: inhibiting cell proliferation, migration and invasion, and enhancing chemoradiotherapy sensitivity. Moreover, the mechanism of its inhibitory effect in different tumors has certain tissue specificity. Berberine mainly exerts its inhibitory effects on cell growth and proliferation by inhibiting cell cycle progression, inducing apoptosis, and autophagy. Berberine can induce G1 phase arrest in A549 lung cancer cells by inhibiting the expression of cell cycle proteins CCND1 and CCNE1[24,25]. The combination of Hsp90 inhibitors and berberine inhibits cell growth by suppressing the expression of CDK4 and CCND1[26]. Berberine can also inhibit the expression of CCND1 and suppress the in vitro and in vivo growth of liver cancer cells. In addition, berberine inhibits tumor cell growth by suppressing CCNB1 expression and indirectly inhibiting CDC2 kinase activity. In chondrosarcoma, berberine inhibits the activity of PI3K/Akt and p38 signaling pathways[27], upregulates the expression of p53 and p21, leading to G2/M phase arrest[28]. In inducing cell apoptosis, berberine promotes cell apoptosis by activating Caspase enzyme activity [29]. In leukemia, berberine induces the expression of caspase-8 and caspase-9, and promotes cell apoptosis by activating caspase-3 and inhibiting Bcl-2 expression[30]. Simultaneously, berberine activates caspase [31], AMPK, and ROS production by increasing the level of cytochrome C, thereby activating apoptosis [32]. Berberine stimulation can increase the permeability of mitochondrial membranes, activate caspase cascade reactions, and induce apoptosis in liver cancer cells. It also activates JNK/p38 and enhances the phosphorylation of p53, thereby promoting the entry of apoptotic proteins Bax and Bim into mitochondria and activating mitochondrial apoptosis[33]. By increasing the acetylation of foxo1/3a and promoting the expression of Bim and Bax, apoptosis of liver cancer cells is promoted[34]. In colon cancer cells, berberine promotes apoptosis by increasing p53 transcriptional activity and inducing ATF3 protein expression[35]. This study used transcriptome sequencing to screen the differently expressed genes mediated by berberine, and conducted GO analysis and signal pathway enrichment analysis on these differential genes. The results showed that differentially expressed genes were significantly enriched in biological processes related to cell apoptosis, cell cycle regulation, and DNA replication, as well as mediating the expression of signaling molecules such as p53, FOXO1, PI3K-AKT to regulate cell cycle and apoptosis pathways. This is consistent with the reported mechanism of berberine induced cell apoptosis and cycle arrest.

Berberine not only inhibits cell proliferation and growth, but also has the effect of inhibiting tumor migration and invasion. In breast cancer, berberine inhibits the expression of MMP-2 and -9 by targeting Ephrin-B2 and TGF β, thereby inhibiting cell invasion and metastasis[36]. In melanoma, berberine can inhibit the EMT process by suppressing the PI3K/Akt signaling pathway, downregulating the expression of RAR α, and upregulating the expression of RAR β[37]. In triple negative breast cancer, berberine can inhibit EGFR/MEK/ERK signaling pathway, cell proliferation and invasion[38]. In addition, berberine can inhibit the COX-2/PGE2/JAK2/STAT3 signaling pathway and reduce the expression of MMP2 and MMP9[39]. Berberine can also induce phosphorylation of eEF2, inhibit the activity of HIF-1 α, reduce VEGF expression, and inhibit angiogenesis and metastasis[40]. In colorectal cancer, berberine inhibits cell

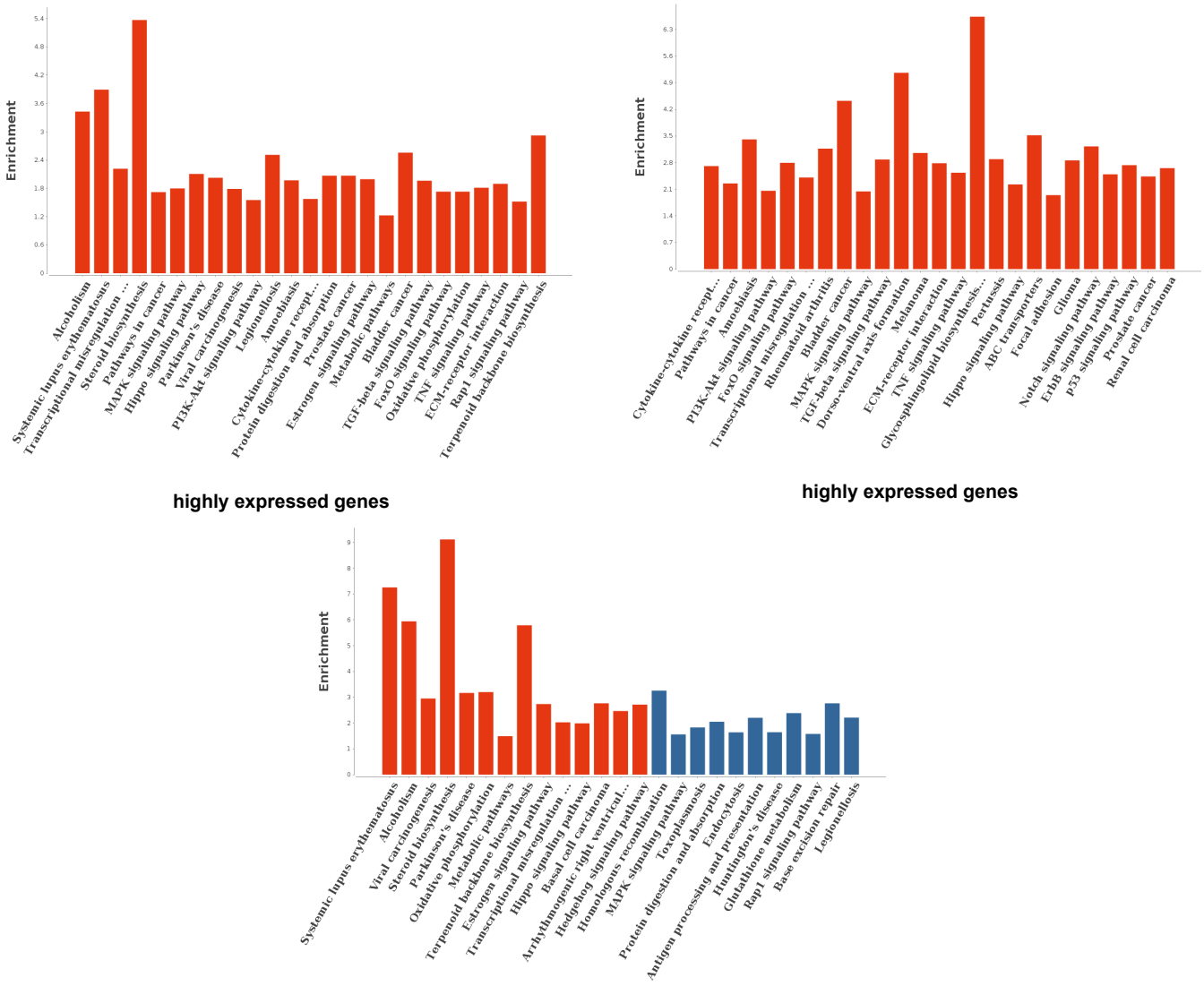

Figure 5 | KEGG analysis for the top 20 significantly differentially expressed genes

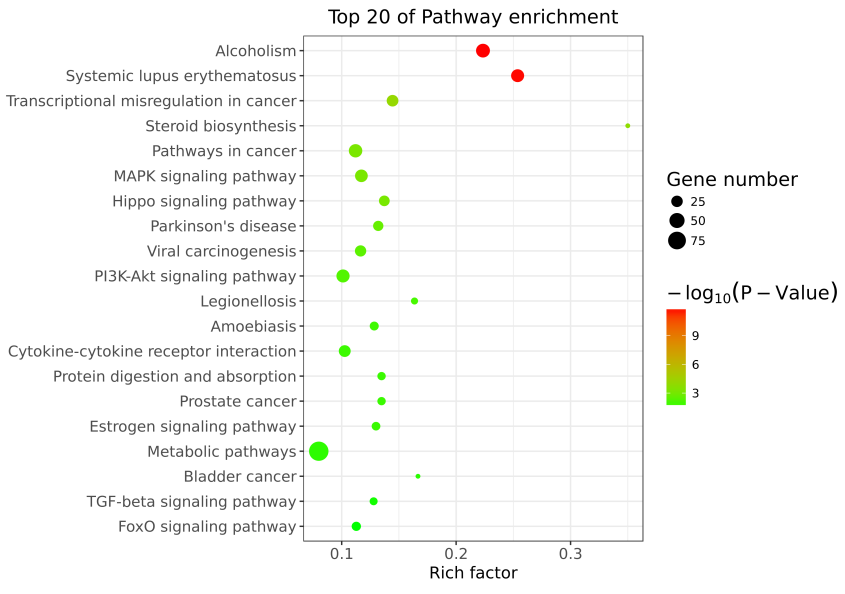

Figure 6 | Top 20 signaling pathways with significant enrichment of the differentially genes from nasopharyngeal carcinoma with berberine.

proliferation and migration by downregulating GRP78 expression[41]. In addition, berberine can inhibit the expression of tumor metastasis transcription factor Snail1 and suppress tumor metastasis. In this study, it was found that differentially expressed genes were significantly enriched in pathways closely related to metastasis regulation such as TGF β, PI3K-AKT, VEGF, MAPK, and Hippo, indicating that berberine inhibits the migration and invasion of nasopharyngeal carcinoma cells in a similar manner.

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