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Transcriptome Sequencing Analysis of Nasopharyngeal Carcinoma 5-8F Cells Induced by Timosaponin A-III

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Abstract: *Objective:* To explore the differential gene expression of nasopharyngeal carcinoma cells induced by Timosaponin A-III and conduct biological information analysis. *Methods:* Total RNA was extracted from nasopharyngeal carcinoma cells treated with Timosaponin A-III for transcriptome sequencing using Illumina HiSeq 2500. Differentially expressed genes (DEGs) were screened by DESeq2, and their functions and signaling pathways were explored using GO enrichment analysis and KEGG pathway analysis. *Results:* Differential gene expression analysis revealed 3858 DEGs, including 1077 upregulated and 2781 downregulated genes. The heatmap and volcano plot showed significant changes in the gene expression profile. GO analysis indicated that the DEGs were enriched in biological processes such as regulation of apoptosis, autophagy, lipid metabolism, and redox processes, involving cellular components like cell membrane, cytoplasm, and mitochondria, as well as molecular functions including protein binding and protein kinase activity. KEGG pathway analysis revealed significant enrichment in signaling pathways such as apoptosis, p53, and TNF. *Discussion:* Timosaponin A-III may exert antitumor effects by activating apoptosis pathways and the p53 pathway. The interaction of these pathways mediates the inhibitory effect on nasopharyngeal carcinoma cells. *Conclusion:* This study reveals that Timosaponin A-III inhibits nasopharyngeal carcinoma cells through multi-gene and multi-pathway regulation, providing a theoretical basis for further experiments.

Keywords: Nasopharyngeal carcinoma; Timosaponin A-III; Transcriptome sequencing

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1. Research background and significance

Nasopharyngeal carcinoma (NPC) is a malignant tumor originating from the epithelial cells of the nasopharynx, closely related to the Epstein-Barr virus (EBV) [1]. It has significant regional clustering and a higher incidence rate in southern China, such as the Guangdong and Guangxi regions [2]. Due to its concealed early symptoms, about 70% of patients have already developed lymph node or distant metastasis at the time of diagnosis, posing significant challenges to treatment and seriously threatening patients' lives and health. There is an urgent need to analyze its metastasis mechanism and develop targeted treatment options in clinical practice [3]. Currently, the treatment of nasopharyngeal carcinoma mainly includes radiotherapy, chemotherapy, and surgical treatment. However, these treatment methods have certain limitations.

For example, radiotherapy may cause radiation injury, chemotherapy may cause severe adverse reactions, and surgical treatment is limited by tumor location and staging [4]. Therefore, finding new treatment methods and drugs has become an important direction in nasopharyngeal carcinoma research. In this study, we used the nasopharyngeal carcinoma cell line 5-8F as the research object and employed transcriptome sequencing technology to explore the changes in gene expression profiles of 5-8F cells induced by Timosaponin A-III. We screened differentially expressed genes and analyzed their functions, aiming to lay a foundation for understanding the antitumor mechanism of Timosaponin A-III and provide a theoretical basis for developing new drugs and methods for the treatment of nasopharyngeal carcinoma.

2. Materials and methods

2.1. Materials

The human highly metastatic nasopharyngeal carcinoma cell line 5-8F (purchased from Shanghai Fuheng Biotechnology Co., Ltd.) was isolated from lung metastases of a 58-year-old Chinese male patient with poorly differentiated squamous cell carcinoma of the nasopharynx in 1980. It was subcloned through passage in nude mice to obtain a highly metastatic subclone. ATCC standardized cell resources and supporting experimental protocols were provided to ensure the stability of cell quality and characteristics. Timosaponin A-III (purity ≥ 98%, CAS number: 41059-79-4) was purchased from Chengdu Desite Biotechnology Co., Ltd., with a molecular formula of C39H64O13 and a molecular weight of 740.9177. RPMI 1640 medium (Gibco, USA), high-quality fetal bovine serum (FBS, Gibco, USA), 0.25% trypsin-EDTA digestion solution (Gibco, USA), TRIzol reagent (Invitrogen, USA), RNAsimple Total RNA kit (Tiangen Biotech Co., Ltd.), PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa Bioengineering Co., Ltd.), and NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, USA) were used. Equipment included a CO2 incubator (Thermo Fisher Scientific, USA), a clean bench (Suzhou Purification Equipment Co., Ltd.), a high-speed frozen centrifuge (Eppendorf, Germany), a microplate reader (Bio-Rad, USA), a real-time fluorescent quantitative PCR instrument (Applied Biosystems, USA), and an Illumina HiSeq 2500 sequencing platform (Illumina, USA).

2.2. Cell culture

The 5-8F cells were thawed and inoculated into a T25 flask containing RPMI 1640 complete medium (with 10% FBS) and cultured in a 37℃, 5% CO2 incubator. When the cell density reached 80–90%, they were digested with 0.25% trypsin-EDTA for 2–3 minutes and passaged at a ratio of 1:3 to 1:5, twice a week. The experiment was divided into a control group and a Timosaponin A-III treatment group. The treatment group was treated with 7.5 μM Timosaponin A-III solution for 24 hours, while the control group received an equal amount of DMSO solvent. The experiment was repeated three times.

2.3. Transcriptome sequencing analysis

Total RNA was extracted from cells using TRIzol reagent and further purified using the RNAsimple Total RNA kit to remove impurities and genomic DNA contamination. RNA concentration was determined using a Nanodrop 2000 spectrophotometer, ensuring an A260/A280 ratio between 1.8 and 2.2. RNA integrity was checked using an Agilent 2100 Bioanalyzer, and RNA samples with a RIN value greater than 7.0 were used for subsequent experiments.

mRNA was reverse transcribed into cDNA using the PrimeScript RT reagent Kit with gDNA Eraser. Library construction was then performed using the NEBNext Ultra RNA Library Prep Kit for Illumina, including steps such as end repair, A-tailing, ligation of sequencing adapters, and PCR amplification. The constructed libraries were quantified using a Qubit 2.0 fluorometer, and the fragment size distribution was checked using an Agilent 2100 Bioanalyzer to ensure library quality.

Qualified libraries were sequenced on the Illumina HiSeq 2500 platform with paired-end 150 bp reads. During sequencing, standard operating procedures were followed, and sequencing data quality was monitored in real-time to ensure accurate and reliable data output. Raw sequencing data were obtained.

2.4. Differential gene screening and analysis

Gene expression levels were calculated using StringTie software and normalized using the FPKM (Fragments Per Kilobase of exon per Million reads mapped) method to obtain a gene expression matrix. Differential gene screening was performed using DESeq2 software, with a threshold of $|\log2FC| \geq 1$ and padj < 0.05 to determine differentially expressed genes between the Timosaponin A-III treatment group and the control group. GO (Gene Ontology) enrichment analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis were performed on the differentially expressed genes using the clusterProfiler package.

3. Results

3.1. Analysis of differentially expressed genes in the transcriptome

Transcriptome sequencing yielded a total of 45,435,638 and 46,317,472 reads for the control group and the treatment group, respectively. After filtering, the proportions of clean reads were 97.18% and 97.03%, respectively. A total of 58,825 genes were detected in this transcriptome sequencing. According to the differential expression screening criteria, 3,858 differentially expressed genes were obtained, including 1,077 upregulated genes and 2,781 downregulated genes. Heatmaps (**Figure 1A**) and volcano plots (**Figure 1B**) are shown in **Figure 1**.

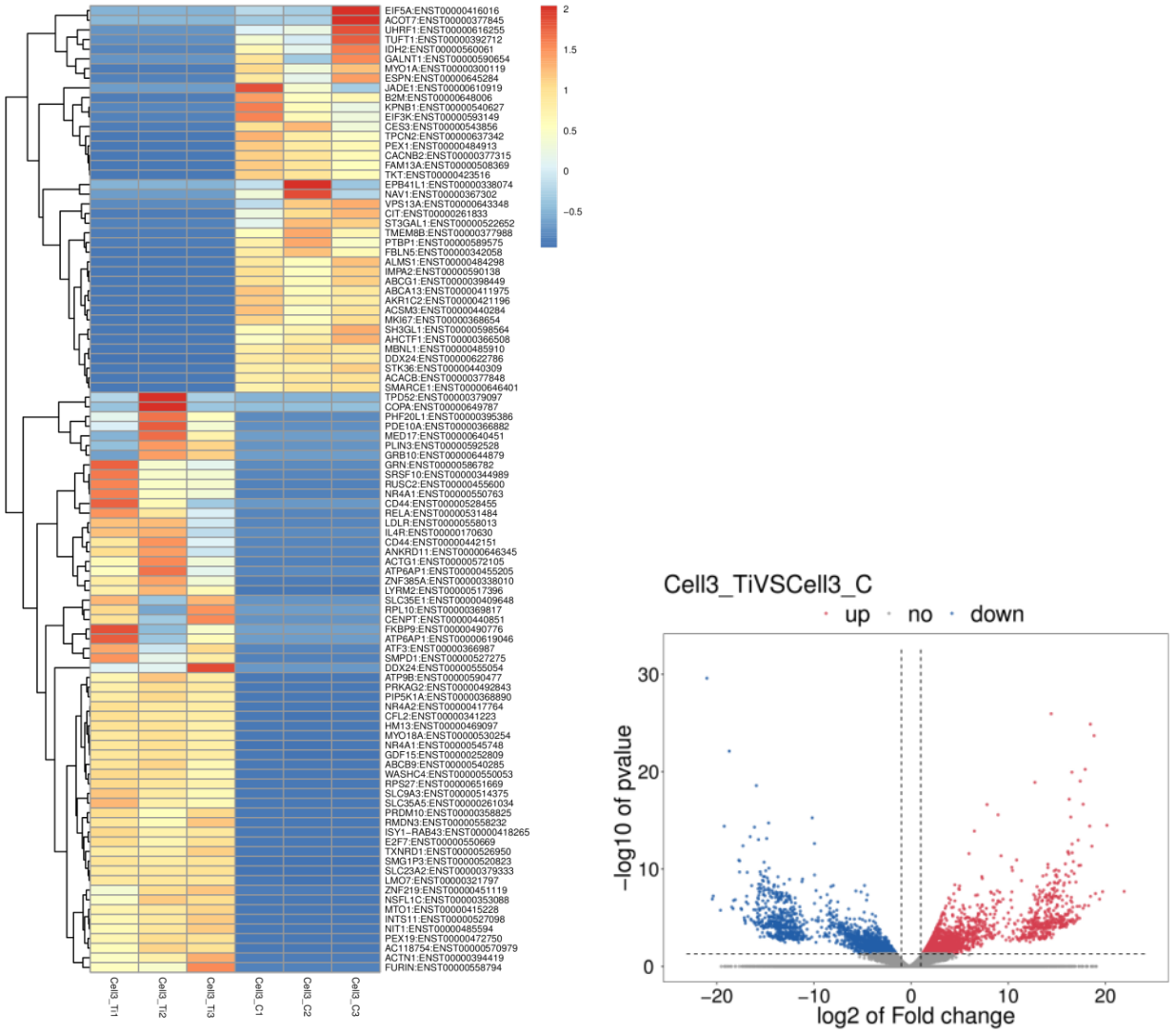

Figure 1. Heatmap (1A) and Volcano plot (1B) of differentially expressed genes.

3.2. Functional enrichment analysis of genes

To gain a deeper understanding of the functions of differentially expressed genes, GO enrichment analysis and KEGG pathway enrichment analysis were performed (**Figure 2** and **Figure 3**). The results of GO enrichment analysis showed that in biological processes, the differentially expressed genes were mainly enriched in biological processes such as regulation of apoptosis, autophagy, lipid metabolic process, and oxidation-reduction process. For example, during the regulation of apoptosis, multiple apoptosis-related genes such as BAX and CASP3 were upregulated [5], suggesting that Timosaponin A-III may inhibit the growth of nasopharyngeal carcinoma cells by activating the apoptotic pathway. In terms of molecular function, the differentially expressed genes were mainly enriched in molecular functions such as protein binding, protein kinase binding, and oxidoreductase activity. Among them, changes in genes related to protein kinase activity may affect the activation of intracellular signaling pathways, thereby regulating the biological behavior of cells. In terms of cellular components, the differentially expressed genes were mainly enriched in cellular structures such as the cell membrane, cytoplasm, and mitochondrial matrix, indicating that the effects of Timosaponin A-III on 5-8F cells involve multiple cellular levels.

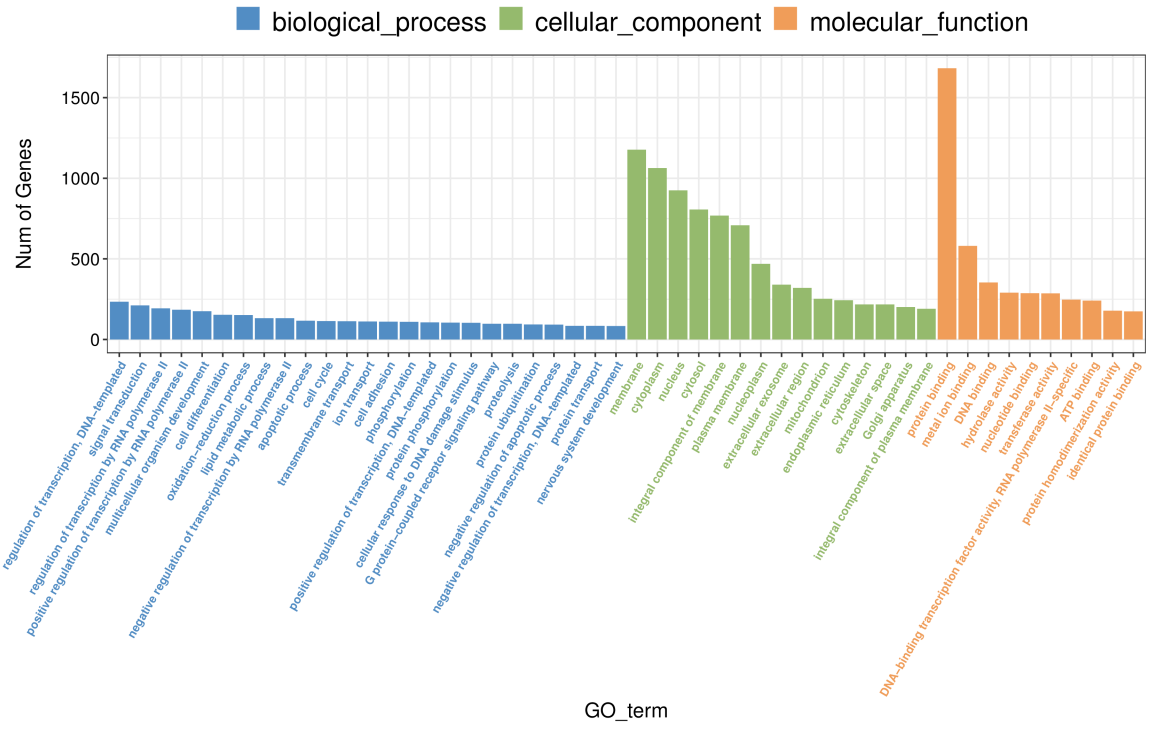

Figure 2. Bar chart of GO enrichment analysis.

The results of KEGG pathway enrichment analysis indicated that the differentially expressed genes were significantly enriched in multiple important signaling pathways, such as the apoptosis signaling pathway, p53 signaling pathway, and TNF signaling pathway. In the apoptosis signaling pathway, multiple key genes such as BCL-2 and IAP were upregulated, while BAX, BAK, and others were downregulated. Abnormal activation of this pathway is closely related to the proliferation, survival, and metastasis of tumor cells [6,7]. Inhibition of this pathway by Timosaponin A-III may be one of the important mechanisms by which it exerts its anti-tumor effects. In the p53 signaling pathway, the p53 gene and its downstream target genes such as PUMA and NOXA were upregulated. As an important tumor suppressor gene, p53

induces apoptosis by activating apoptosis-related genes [8], which is consistent with the results of apoptosis regulation in the GO enrichment analysis.

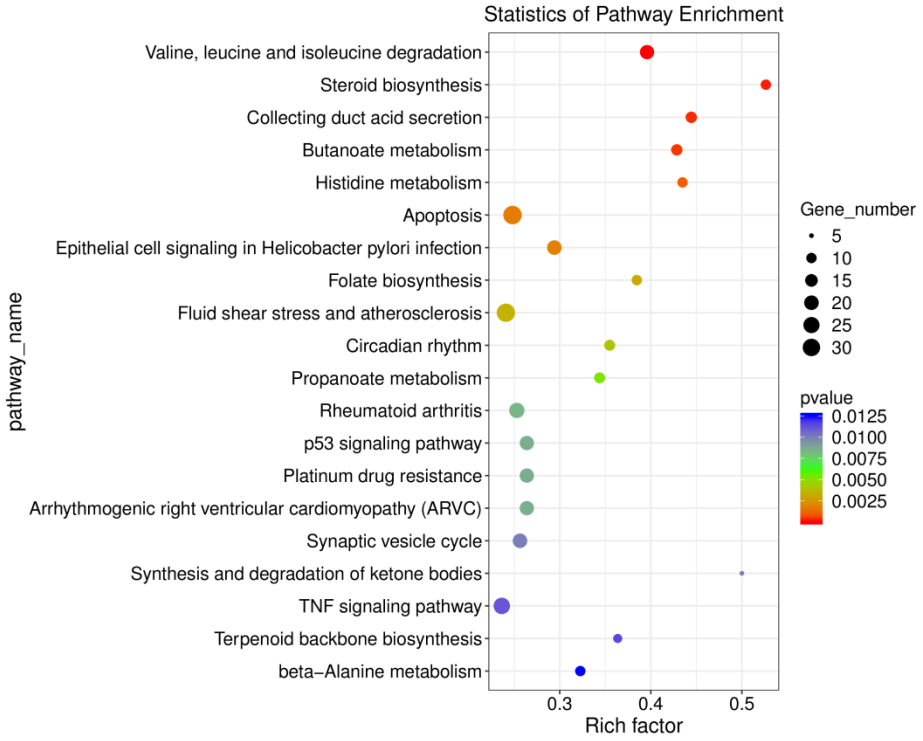

Figure 3. Bubble chart of KEGG pathway enrichment analysis.

4. Discussion

In this study, transcriptome sequencing analysis comprehensively revealed significant changes in gene expression in 5-8F cells treated with Timosaponin A-III. Among the differentially expressed genes, multiple genes closely related to tumor development and progression showed altered expression. For example, the upregulation of apoptosis-related genes such as BAX and CASP3 suggests that Timosaponin A-III may induce apoptosis in nasopharyngeal carcinoma cells by activating the endogenous apoptotic pathway, promoting the release of cytochrome C from mitochondria, and activating the Caspase cascade reaction. Downregulation of cell cycle-related genes such as CCND1 and CDK4 indicates that Timosaponin A-III may arrest the cell cycle in the G1 phase, inhibiting the transition from G1 to S phase and thus suppressing cell proliferation. These changes in gene expression provide important clues for a deeper understanding of the molecular mechanism of Timosaponin A-III's anti-nasopharyngeal carcinoma activity.

The results of KEGG pathway enrichment analysis showed significant changes in multiple key signaling pathways in 5-8F cells treated with Timosaponin A-III. These pathways play crucial roles in tumor initiation, development, and metastasis.

The apoptotic signaling pathway is one of the important signal transduction pathways in cells, and its abnormal activation is closely related to the proliferation, survival, migration, and invasion of tumor cells. In this study, Timosaponin A-III treatment led to changes in the expression of key genes such as BAX and CASP3 in the apoptotic signaling pathway, indicating that Timosaponin A-III may exert its antitumor effect by affecting this pathway and blocking the transmission of cell proliferation and survival signals.

The p53 signaling pathway, as an important tumor suppressor pathway, plays a key role in maintaining genomic stability, inducing apoptosis, and cell cycle arrest. Upregulation of p53 and its downstream target genes such as PUMA

and NOXA after Timosaponin A-III treatment suggests that Timosaponin A-III may activate the p53 signaling pathway, inducing apoptosis and cell cycle arrest, thereby inhibiting the growth of nasopharyngeal carcinoma cells [9]. Mutations or functional deletions of the p53 gene are common in various tumors, leading to evasion of apoptosis and uncontrolled proliferation of tumor cells [10–12]. Therefore, activating the p53 signaling pathway is one of the important strategies for tumor treatment.

These key signaling pathways may have complex interactions and regulatory networks that mediate the inhibitory effect of Timosaponin A-III on nasopharyngeal carcinoma cells. Further investigation of the interactions between these signaling pathways will help to fully reveal the molecular mechanism of Timosaponin A-III's anti-nasopharyngeal carcinoma activity, providing a more solid theoretical foundation for developing new treatment strategies.

5. Conclusion

In this study, transcriptome sequencing analysis of nasopharyngeal carcinoma cells 5-8F induced by Timosaponin A-III revealed that Timosaponin A-III can significantly alter the gene expression profile of 5-8F cells. The differentially expressed genes are mainly enriched in biological processes such as apoptosis regulation, cell cycle regulation, and signal transduction, as well as key signaling pathways such as MAPK and p53. These results suggest that Timosaponin A-III may exert its anti-nasopharyngeal carcinoma effect through multiple mechanisms, such as activating apoptotic pathways and inhibiting key signaling pathways. This study provides an important theoretical basis for further exploring the molecular mechanism of Timosaponin A-III's anti-nasopharyngeal carcinoma activity and lays a foundation for developing new treatment strategies based on Timosaponin A-III for nasopharyngeal carcinoma.

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Disclosure statement

The author declares no conflict of interest.

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