



Transcriptome Analysis Reveals the Anti-Tumor Mechanism of Eucalyptol Treatment on Neuroblastoma Cell Line SH-SY5Y

Kai Gao¹ · Congying Wu² · Yanlong Li² · Jian Lu² · Yuwu Jiang¹

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Abstract

Eucalyptol (1.8-cineole), an active component in traditional Chinese medicine *Artemisia argyi* for moxibustion. Previous studies have shown that eucalyptol has anti-tumor effects on leukemia and colon cancer. Nonetheless, the effect and mechanism of eucalyptol on neuroblastoma remains unclear. In the present study, we intended to reveal the effect and mechanism of eucalyptol treatment on the neuroblastoma cell line SH-SY5Y through transcriptome analysis. In the group treated with eucalyptol, 566 brain genes were up-regulated, while 757 genes were down-regulated. GO function analysis showed that positive regulation of cell cycle was down-regulated in biological processes. Meanwhile, cancer-related pathways were identified in KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis, including pathways in cancer, PI3K-Akt signaling pathway, cAMP signaling pathway, TGF-beta signaling pathway, Hippo signaling pathway, p53 signaling pathway, and additional pathways. Furthermore, we found a key gene, such as *MYC*, by constructing a network of cancer related pathways with differentially expressed genes and transcription factor analysis. In conclusion, our research indicates that *MYC* might play a central role in the anti-tumor mechanisms of eucalyptol.

Keywords Eucalyptol · 1.8-cineole · Moxibustion · Anti-cancer · *MYC* · RNAseq

Introduction

Neuroblastoma is a developmental tumor of children from the neural crest. This disease is the primary cause of cancer-related death in children under 5 years of age [1]. Neuroblastoma is a heterogeneous pediatric tumor. Half of this disease is a high risk type and lacks effective cures [2]. SH-SY5Y cell line is a subclone of human neuroblastoma, originally derived from a child metastatic bone tumor biopsy [3].

This cell line is a common cell model to study neurotoxicity [4], neurodegenerative disease [5], neuron differentiation and neuroblastoma [6].

Eucalyptol is a cyclic ether and monoterpenoid, naturally produced by plants such as *Artemisia argyi* [7, 8]. It is an ingredient in some brands cough suppressants and is also used as a flavoring agent [9]. Eucalyptol has been reported to possess multiple pharmacological effects, including anti-inflammatory [10–12], antioxidant effects [13], pain reduction [14, 15], epilepsy inhibition [16], et al. In some *in vitro* studies, eucalyptol has anti-tumor effect on leukemia [17], ovarian cancer cells [18] and colon cancer cell line [19]. However, the anti-tumor effect of eucalyptol on neuroblastoma is still unknown.

In this research, we investigated the anti-tumor mechanism of eucalyptol by transcriptome sequencing and bioinformatic analysis. And we discovered that eucalyptol exerts anti-tumor activity on human neuroblastoma cell lines SH-SY5Y by regulating several cancer related pathways and genes. Our findings will be valuable for understanding the anti-tumor mechanism of eucalyptol in neuroblastoma cell proliferation and provide a new therapeutic candidate agent for neuroblastoma therapy.

Kai Gao and Congying Wu contributed equally.

✉ Jian Lu
lvjian2999@126.com

✉ Yuwu Jiang
jjiangyw@263.net

¹ Department of Pediatrics, Peking University First Hospital, No.1 Xi'an Men Street, West District, 100034 Beijing, China

² Department of acupuncture and moxibustion, Dongzhimen Hospital, Beijing University of Chinese Medicine, No.116 Cuiping West Street, Tongzhou District, 101121 Beijing, China

Methods

Cell Culture of SH-SY5Y

The SH-SY5Y was kindly given by Professor Yun Wang of the Neuroscience Research Institute, Peking University. Cell cultures of SH-SY5Y were cultured in DMEM/F12 (Sigma, Darmstadt, Germany) with 10% (v/v) fetal bovine serum (FBS) (Gibco, Grand Island, USA) and 1% Penicillin-Streptomycin Solution (Gibco, Grand Island, USA). All cultures were incubated in a Thermo CO₂ incubator at 37°C with 95% air and 5% CO₂ (v/v) and a humidity of 95%. The cell culture medium was changed twice a week. 70%–80% of confluent cultures used for passage to experiments.

Transcriptome Sequencing

Three pairs of cell samples were collected from untreated and 100 μM eucalyptol-treated SH-SY5Y cells for 6 days, and RNA was extracted for RNA-seq by Trizol (Invitrogen, Carlsbad, CA, USA). Two micrograms of RNA per sample were used as input material for the RNA sample preparations. Sequencing libraries were generated with the VAHTS mRNA-seq v2 Library Prep Kit for Illumina following the manufacturer's recommendations. Index codes were added to attribute sequences to each sample. Then libraries were sequenced using an Illumina NovaSeq platform to generate 150 bp paired-end reads according to the manufacturer's instructions.

Raw data of FASTQ format was processed first through primary quality control. In this step, clean data were obtained by removing read pairs that contain N more than 3 or the proportion of base with quality value below 5 is more than 20%, in any end, or adapter sequence was founded. The clean data of each sample was more than 6 GB. All the downstream analyses were based on clean data with high quality.

Differential Expression Analysis and Venn Diagrams

Alignment of paired-end clean reads to the reference genome was with TopHat (v2.1.1). Differential expression analysis between two conditions was performed using Cufflinks (v2.2.1). Differently expressed genes (DEGs) were defined as those for which the *P*-value below 0.01 and the absolute value of log₂(Fold change) more than 1. The Venn Diagrams were constructed by an interactive Venn diagram viewer [20].

Functional Enrichment Analysis

GO and KEGG enrichment analysis of DEGs sets were executed by the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 [21]. GO terms and KEGG pathways with adjusted *P*-value below 0.05 were considered as significantly enriched by DEGs. The volcano map was drawn by R language with ggplot2. The bar and bubble graphs are plotted by the GPlot package in R. The network graph of cancer related pathways with DEGs was produced by Cytoscape 3.7.1 [22].

Transcription Factor Analysis

The target genes of *MYC* analyzed in this study were found by Gene Transcription Regulation Database (GTRD) [23] and Database of Human Transcription Factor Targets (hTF-target) [24]. We found *MYC* target genes from GTRD in *Homo sapiens* with the promoter setting from –1000 to +100. And we got target genes of *MYC* from hTFtarget with the default mode. Then we took the intersection from the above two lists for further analysis.

Result

Differential Expression Analysis of Eucalyptol Treatment

In order to identify the DEGs (up-regulated and down-regulated expression) in SH-SY5Y cells after eucalyptol treatment, we performed mRNA sequencing of normal SH-SY5Y and 100 μM treated SH-SY5Y on the 6th day. RNA-seq identification of DEGs was measured by TopHat and Cufflinks (See Methods). As the results shown in Fig. 1, a total of 1255 genes (1350 transcripts) were differentially expressed, including 566 up-regulated genes (593 transcripts) and 717 down-regulated genes (757 transcripts). There were 28 DEGs with both up-regulated transcripts and down-regulated transcripts. It can be shown from volcano map that anti-tumor genes *BAD3*, *TBX3* and *APC* were up-regulated genes, at the same time, oncogenes *LEF1*, *PDGFRB*, and *MYC* were down-regulated genes (Fig. 2).

GO Enrichment Analysis Identified the Biological Functions of DEGs in SH-SY5Y After Eucalyptol Treatment

To further evaluate the biological functions of these DEGs, GO enrichment analysis was performed on the experimental group. The results revealed that there was significant enrichment of GO terms, which are grouped into three categories:

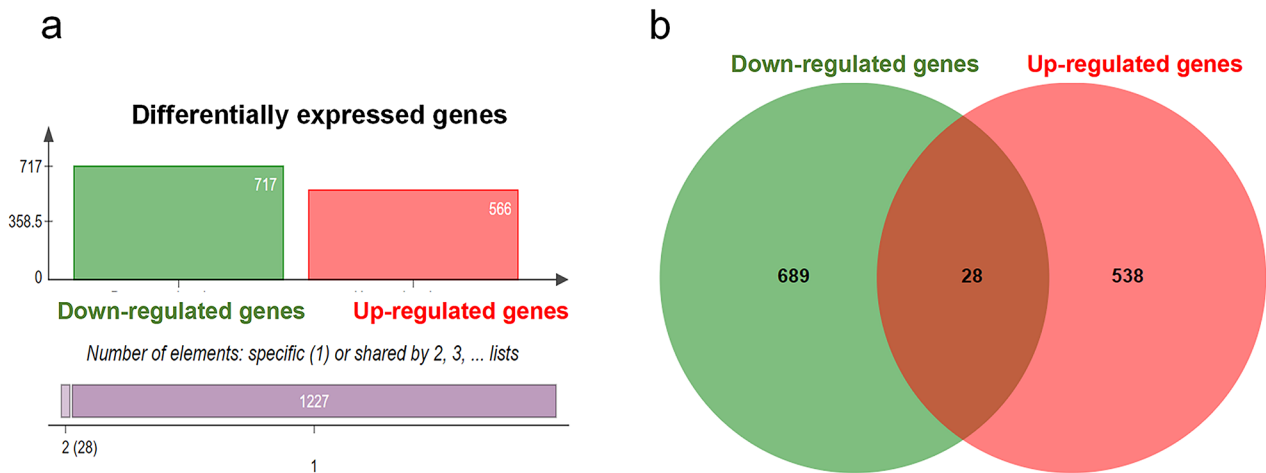


Fig. 1 Differentially expression genes of eucalyptol treated SH-SY5Y. a shows the classification of differentially expressed genes by bar graphs, b is the Venn Diagram of down-regulated genes and up-regulated genes

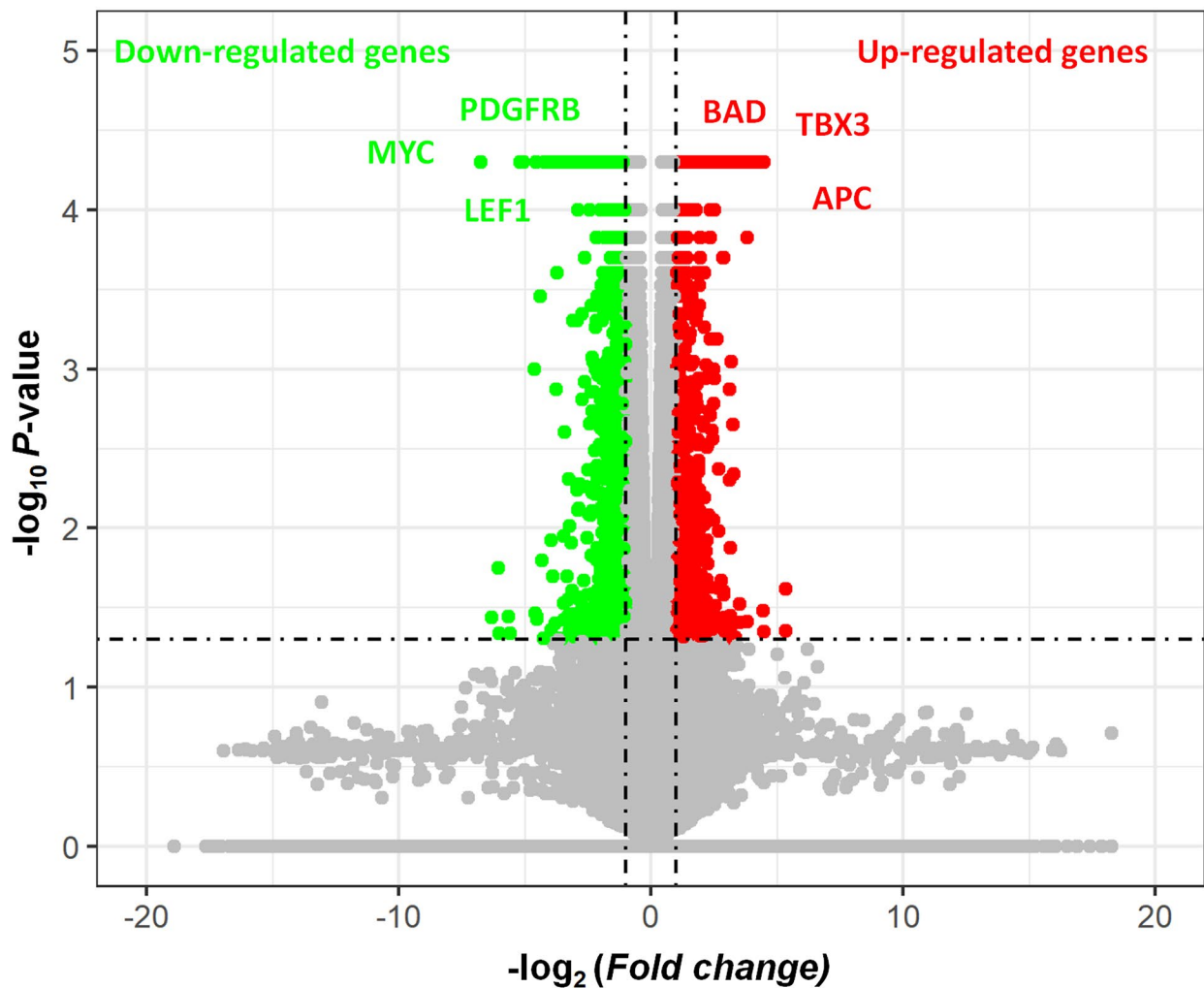


Fig. 2 Volcano map of gene expression changing after eucalyptol treatment. Red dots represent up-regulated genes. Green dots represent down-regulated genes

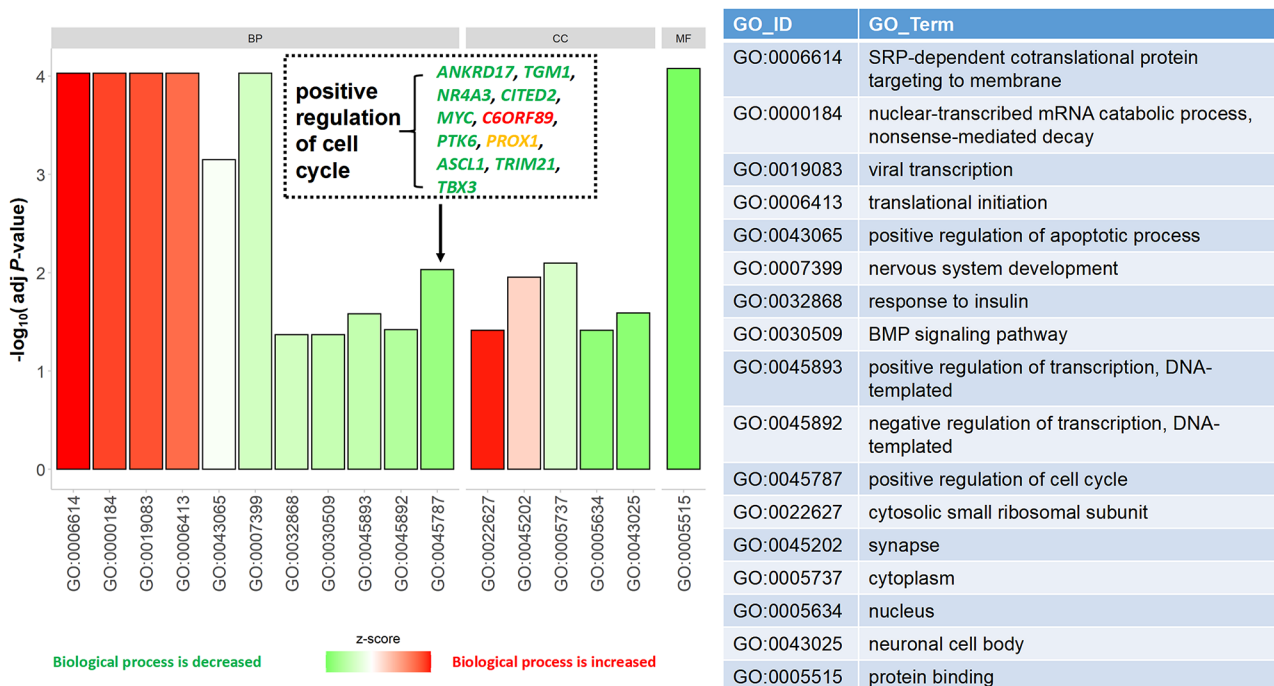


Fig. 3 Z-score coloured barplot of GO enrichment of DEGs. If the biological process (/molecular function/cellular components) is decreased, the colour of the bar is green. And If the biological process (/molecular function/cellular components) is increased, the colour of the bar is red

molecular function (MF), cellular component (CC) and biological process (BP). In the biological process, GO: 0007399: nervous system development, GO: 0000184: nuclear-transcribed mRNA catabolic process, nonsense-mediated decay, GO: 0006614: SRP-dependent cotranslational protein targeting to membrane, GO: 0006413: translational initiation, GO: 0019083: viral transcription, GO: 0043065: positive regulation of apoptotic process, GO: 0045787: positive regulation of cell cycle, GO: 0045893: positive regulation of transcription, DNA-templated, GO: 0045892: negative regulation of transcription, DNA-templated, GO:0032868: response to insulin, GO: 0030509: BMP signaling pathway had the most abundant GO function items. In the molecular function, GO: 0005515: protein binding had the most abundant GO function items. In the cellular component, GO: 0005737: cytoplasm, GO: 0045202: synapse, GO: 0043025: neuronal cell body, GO: 0022627: cytosolic small ribosomal subunit, GO: 0005634: nucleus (Fig. 3).

It should be mentioned that GO: 0045787 (positive regulation of cell cycle) was negatively regulated for the most involved DEGs in this term were down-regulated. Those decreasing DEGs were *ANKRD17*, *TGM1*, *NR4A3*, *CITED2*, *MYC*, *PTK6*, *ASCL1*, *TRIM21*, *TBX3*.

KEGG Enrichment Analysis Identified Cancer Related Pathways

To analyse the DEGs, KEGG enrichment analysis was used for annotation. The top 20 enrichment KEGG pathways, including hsa03010: Ribosome, hsa05031: Amphetamine addiction, hsa04024: cAMP signaling pathway, hsa05200: Pathways in cancer, hsa04550: Signaling pathways regulating pluripotency of stem cells, hsa04510: Focal adhesion, hsa05205: Proteoglycans in cancer, hsa04115: p53 signaling pathway, hsa04728: Dopaminergic synapse, hsa04151: PI3K-Akt signaling pathway, hsa04520: Adherens junction, hsa04350: TGF-beta signaling pathway, hsa05210: Colorectal cancer, hsa04725: Cholinergic synapse, hsa05215: Prostate cancer, hsa04390: Hippo signaling pathway, hsa05231: Choline metabolism in cancer, hsa05202: Transcriptional misregulation in cancer, hsa05223: Non-small cell lung cancer, hsa05030: Cocaine addiction, were shown in Fig. 4.

The results demonstrated that the seven enriched pathways were directly cancer-related KEGG pathways, as shown in the top 7 pathways in Fig. 4. We also found that five cancer-related cellular signaling pathways, including Hippo signaling pathways, TGF-beta signaling pathways, PI3K-Akt signaling pathway, p53 signaling pathway, cAMP signaling pathway. These results suggest that eucalyptol

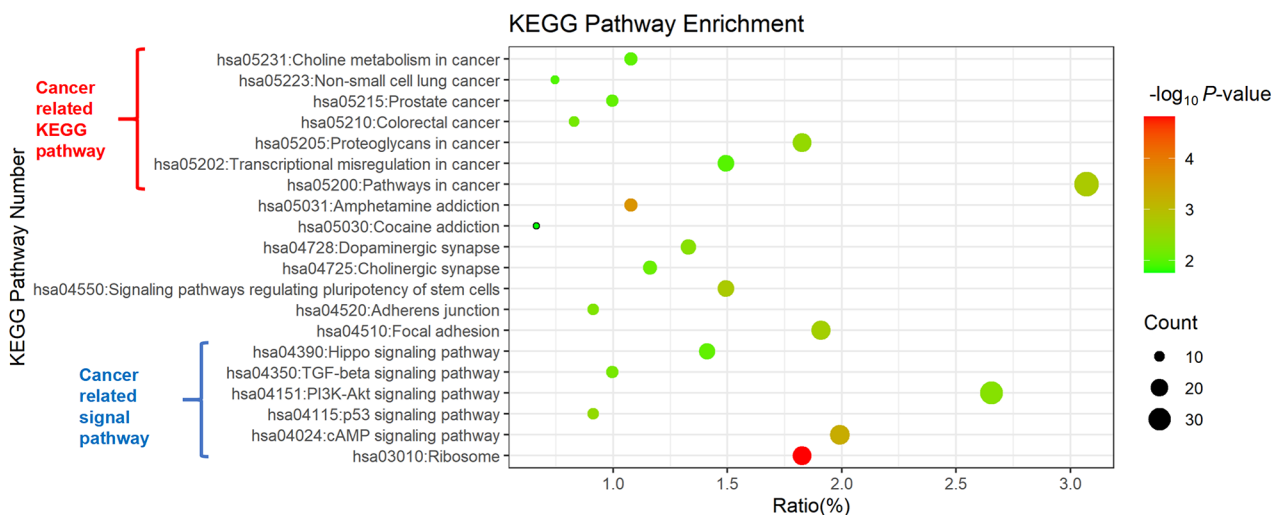


Fig. 4 Bubble map of KEGG pathway enrichment of DEGs. The size of bubbles represents the count of DEGs. The color of bubbles represents P -value

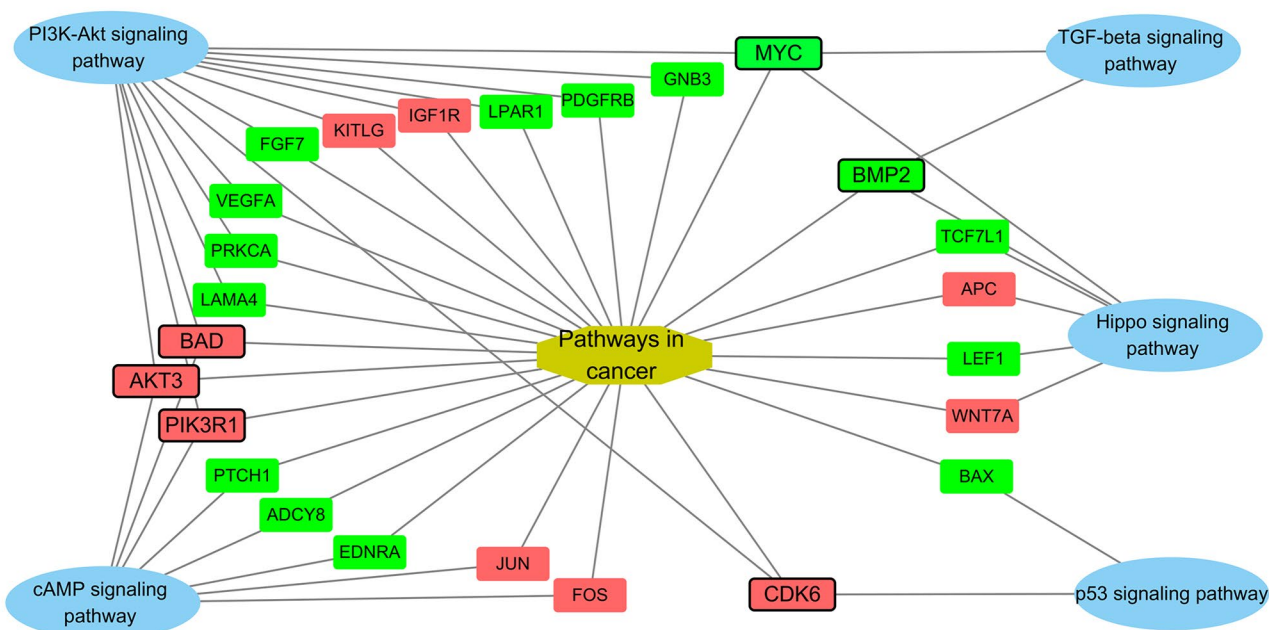


Fig. 5 The network of cancer-related KEGG pathways with DEGs. The up-regulated genes are in red boxes, while down-regulated genes are in green boxes

may exert antitumor effects through the above signaling pathways.

System Biological Analysis Identified the Key Genes in the Network of Cancer Related Pathways with DEGs

For clarify the mechanism of anti-proliferation, we constructed a network of the KEGG enriched cellular signaling

pathways (Hippo signaling pathways, TGF-beta signaling pathways, PI3K-Akt signaling pathway, p53 signaling pathway, cAMP signaling pathway) and Pathways in cancer with DEGs (Fig. 5). We found that *MYC*, *BMP2*, *CDK6*, *PIK3R1*, *AKT3* and *BAD* are linked with more than 3 pathways, demonstrating those genes are important roles in anti-proliferation effect of eucalyptol on SH-SY5Y.

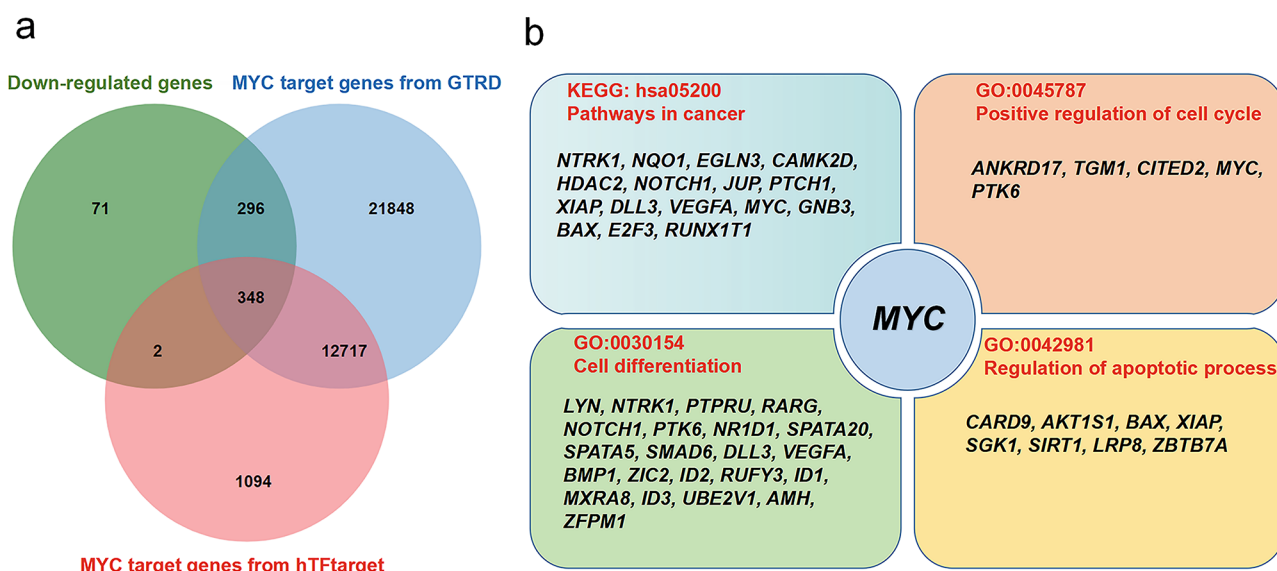


Fig. 6 Transcription factor analysis of *MYC* target gene. a shows the Venn Diagram of the list of down-regulated genes with the list of these two *MYC* target gene lists from GTRD and hTFtarget. b shows *MYC* targets genes of cancer related pathways by the KEGG and GO enrichment

Transcription Factor Analysis Showed Multiple Biology Functions of *MYC* in the Antitumor Mechanism of Eucalyptol

MYC is an important cancer-related transcription factor gene and sits on the most important gene node in the network (Fig. 5), suggesting that it plays an important role in the antitumor mechanism of eucalyptol. In this study, it was found that eucalyptol caused a pronounced down-regulation of *MYC* expression and then might result in the down-regulation of *MYC* target genes (MTGs). Therefore, we conducted a transcription factor analysis on *MYC*. First, we found 35,769 MTGs from GTRD and 14,741 MTGs from hTFtarget. Then we intersected the list of down-regulated genes with the list of these two MTG lists to determine the down-regulated genes regulated by *MYC* (Fig. 6a). After analysis, we found that about half of the down-regulated genes were *MYC* target genes (48.5%, 348/717). By KEGG and GO analysis of these MTGs, we found that these genes were enriched in KEGG: HSA05200 Pathways in cancer, GO:0045787 Positive regulation of cell cycle, GO:0030154 Cell differentiation, GO:0042981 regulates the passage of apoptotic process (Fig. 6b).

Discussion

Moxibustion is an effective supportive cancer care in inhibiting tumor growth [25, 26] and alleviating side effects of chemotherapy and radiotherapy [27]. Such as, moxibustion can inhibit nausea and vomiting after chemotherapy

[28, 29]. In addition, moxibustion can also be used to treat cancer-related fatigue [30]. The mechanism of moxibustion therapy for cancer is still unclear. Eucalyptol is the main component of *Artemisia argyi* [11, 12]. Eucalyptol has been reported to inhibit the proliferation of many cancer cells [22, 23, 31–33]. In this study, we revealed the anti-tumor effect and mechanism of eucalyptol against human neuroblastoma SH-SY5Y cells by transcriptome sequencing.

The mechanism of the anti-tumor effect of eucalyptol is complex and has not been fully clarified. Suppression of growth by eucalyptol in leukemia, ovarian cancer cells and colorectal cell lines was reported to the induction of apoptosis [21–23, 32]. It was reported that eucalyptol also inhibited cell proliferation by promoting G0/G1 arrest in HepG2 cells [32]. In our study, we found that eucalyptol has a negative effect on “positive regulation of cell cycle (GO: 0045787)” by reducing the expression of most genes in this GO terms (Fig. 3), indicating that eucalyptol intervene cancer cell growth not only by inducing apoptosis but also with anti-proliferation.

Cancer is a complex disease characterized by excessive proliferation of cancer cells with selective growth advantage [34]. Many cell signaling pathways related to cancer development, such as PI3K-Akt signaling pathway [35, 36], Ras signaling pathway [37–40], STAT signaling [40–43], MAPK signaling pathway [35, 37], TGF-beta signaling pathway [44, 45], NOTCH signaling pathway [46–49], p53 signaling pathway [50–52], cAMP signaling pathway [53, 54], Hippo signaling pathway [55, 56], Wnt signaling pathway [57], and so on. In our study, we found that some cancer-related signaling pathways were enriched based on

DEGs, including Hippo signaling pathways, TGF-beta signaling pathways, PI3K-Akt signaling pathway, p53 signaling pathway, cAMP signaling pathway. This result suggests that eucalyptol can regulate multiple cancer-related signaling pathways to achieve its anti-cancer effect.

With system biological analysis of the network constructed by cancer related pathways and DEGs, we found that *MYC* is a key gene in the network of eucalyptol's anti-tumor mechanism. *MYC* is an important and well-known oncogene, which regulates cell growth and proliferation [58, 59]. A previous study reported that eucalyptol inhibited protein expression of *MYC* in AGE-treated podocytes and diabetic kidneys [60]. In our study, we found eucalyptol can down-regulated *MYC* transcription in neuroblastoma SH-SY5Y cell. And half of the down-regulated genes were *MYC* target genes. Some of those genes are related to positive regulation of cell cycle, cell differentiation, and apoptotic process, indicating that eucalyptol may be implicated in its anti-tumor effects by down-regulating *MYC* and its target genes involved in cell division, differentiation, and apoptosis pathways.

In conclusion, our findings demonstrate that eucalyptol can exert its anti-tumor activity by regulating multiple cancer-related cellular signal pathways in human SH-SY5Y cells *in vitro*. Eucalyptol shows promise as an effective and safe therapeutic agent for neuroblastoma.

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Authors' Contributions Jian Lu and Yuwu Jiang supervised, designed, carried out and wrote the manuscript. Kai Gao performed cell culture of SH-SY5Y, drug treatment and RNAseq. Congying Wu analyzed RNAseq data, GO and KEGG enrichment, and wrote the manuscript. Yanlong Li help to perform the data analysis and manuscript writing. All authors read and approved the final manuscript.

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Data Availability The original data presented in the study are included in the article materials, further inquiries can be directed to the author/ corresponding authors.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare that they have no competing interests.

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References

1. Louis CU, Shohet JM (2015) Neuroblastoma: molecular pathogenesis and therapy. *Annu Rev Med* 66:49–63
2. Whittle SB, Smith V, Doherty E, Zhao S, McCarty S, Zage PE (2017) Overview and recent advances in the treatment of neuroblastoma. *Expert Rev Anticancer Ther* 17:369–386
3. Kovalevich J, Langford D (2013) Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology. *Methods Mol Biol* 1078:9–21
4. Cheung YT, Lau WK, Yu MS, Lai CS, Yeung SC, So KF, Chang RC (2009) Effects of all-trans-retinoic acid on human SH-SY5Y neuroblastoma as in vitro model in neurotoxicity research. *Neurotoxicology* 30:127–135
5. Ramalingam M, Huh YJ, Lee YI (2019) The Impairments of alpha-Synuclein and Mechanistic Target of Rapamycin in Rotenone-Induced SH-SY5Y Cells and Mice Model of Parkinson's Disease. *Front Neurosci* 13:1028
6. Teppola H, Sarkanen JR, Jalonen TO, Linne ML (2016) Morphological Differentiation Towards Neuronal Phenotype of SH-SY5Y Neuroblastoma Cells by Estradiol, Retinoic Acid and Cholesterol. *Neurochem Res* 41:731–747
7. Zhang WJ, You CX, Yang K, Chen R, Wang Y, Wu Y, Geng ZF, Chen HP, Jiang HY, Su Y, Lei N, Ma P, Du SS, Deng ZW (2014) Bioactivity of essential oil of *Artemisia argyi* Levl. et Van. and its main compounds against *Lasioderma serricornis*. *J Oleo Sci* 63:829–837
8. Ge YB, Wang ZG, Xiong Y, Huang XJ, Mei ZN, Hong ZG (2016) Anti-inflammatory and blood stasis activities of essential oil extracted from *Artemisia argyi* leaf in animals. *J Nat Med* 70:531–538
9. Seol GH, Kim KY (2016) Eucalyptol and Its Role in Chronic Diseases. *Adv Exp Med Biol* 929:389–398
10. Juergens LJ, Worth H, Juergens UR (2020) New Perspectives for Mucolytic, Anti-inflammatory and Adjunctive Therapy with 1,8-Cineole in COPD and Asthma: Review on the New Therapeutic Approach. *Adv Ther* 37:1737–1753
11. Juergens UR (2014) Anti-inflammatory properties of the monoterpene 1,8-cineole: current evidence for co-medication in inflammatory airway diseases. *Drug Res (Stuttg)* 64:638–646

12. Yin C, Liu B, Wang P, Li X, Li Y, Zheng X, Tai Y, Wang C, Liu B (2020) Eucalyptol alleviates inflammation and pain responses in a mouse model of gout arthritis. *Br J Pharmacol* 177:2042–2057
13. Ciftci O, Ozdemir I, Tanyildizi S, Yildiz S, Oguzturk H (2011) Antioxidative effects of curcumin, beta-myrcene and 1,8-cineole against 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced oxidative stress in rats liver. *Toxicol Ind Health* 27:447–453
14. Zheng XB, Zhang YL, Li Q, Liu YG, Wang XD, Yang BL, Zhu GC, Zhou CF, Gao Y, Liu ZX (2019) Effects of 1,8-cineole on neuropathic pain mediated by P2X2 receptor in the spinal cord dorsal horn. *Sci Rep* 9:7909
15. Zhang YL, Liu YG, Li Q, Wang XD, Zheng XB, Yang BL, Wan B, Ma JM, Liu ZX (2018) 1,8-cineole decreases neuropathic pain probably via a mechanism mediating P2X3 receptor in the dorsal root ganglion. *Neurochem Int* 121:69–74
16. Bahr TA, Rodriguez D, Beaumont C, Allred K (2019) The Effects of Various Essential Oils on Epilepsy and Acute Seizure: A Systematic Review. *Evid Based Complement Alternat Med* 2019:6216745
17. Moteki H, Hibasami H, Yamada Y, Katsuzaki H, Imai K, Komiya T (2002) Specific induction of apoptosis by 1,8-cineole in two human leukemia cell lines, but not a in human stomach cancer cell line. *Oncol Rep* 9:757–760
18. Abdalla AN, Shaheen U, Abdallah QMA, Flamini G, Bkhaitan MM, Abdelhady MIS, Ascrizzi R, Bader A (2020) Proapoptotic Activity of Achillea membranacea Essential Oil and Its Major Constituent 1,8-Cineole against A2780 Ovarian Cancer Cells. *Molecules* 25
19. Murata S, Shiragami R, Kosugi C, Tezuka T, Yamazaki M, Hirano A, Yoshimura Y, Suzuki M, Shuto K, Ohkohchi N, Koda K (2013) Antitumor effect of 1, 8-cineole against colon cancer. *Oncol Rep* 30:2647–2652
20. Bardou P, Mariette J, Escudie F, Djemiel C, Klopp C (2014) jvenn: an interactive Venn diagram viewer. *BMC Bioinformatics* 15:293
21. Jiao X, Sherman BT, Huang da W, Stephens R, Baseler MW, Lane HC, Lempicki RA (2012) DAVID-WS: a stateful web service to facilitate gene/protein list analysis. *Bioinformatics* 28:1805–1806
22. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 13:2498–2504
23. Kolmykov S, Yevshin I, Kulyashov M, Sharipov R, Kondra-khin Y, Makeev VJ, Kulakovskiy IV, Kel A, Kolpakov F (2021) GTRD: an integrated view of transcription regulation. *Nucleic Acids Res* 49:D104–D111
24. Zhang Q, Liu W, Zhang HM, Xie GY, Miao YR, Xia M, Guo AY (2020) hTFtarget: A Comprehensive Database for Regulations of Human Transcription Factors and Their Targets. *Genomics Proteom Bioinf* 18:120–128
25. Wang C, Yang M, Fan Y, Pei X (2019) Moxibustion as a Therapy for Breast Cancer-Related Lymphedema in Female Adults: A Preliminary Randomized Controlled Trial. *Integr Cancer Ther* 18:1534735419866919
26. Wang B, Huang J, Li S, Pan Z, Guo Y, Yang Y, Li L, Wang C, Gong Y, Wang J, Lu S, Xu Z, Guo Y (2020) The Combinatorial Effect of Cisplatin and Moxibustion on Tumor Growth Inhibition with Special Reference to Modulation of the Immune Microenvironment in Lewis Lung Cancer Mice. *Evid Based Complement Alternat Med* 2020:3170803
27. Zhang HW, Lin ZX, Cheung F, Cho WC, Tang JL (2018) Moxibustion for alleviating side effects of chemotherapy or radiotherapy in people with cancer. *Cochrane Database Syst Rev* 11:CD010559
28. Lee MS, Choi TY, Park JE, Lee SS, Ernst E (2010) Moxibustion for cancer care: a systematic review and meta-analysis. *BMC Cancer* 10:130
29. Zhang L, Wang YL, Fu RY, Li JX, Guo XQ, Xu B, Feng SH, Guan XJ (2020) [Ginger-partitioned moxibustion in the prevention of nausea and vomiting induced by chemotherapy in lung cancer randomized controlled trial]. *Zhen Ci Yan Jiu* 45:574–577
30. Han K, Kim M, Kim EJ, Park YC, Kwon O, Kim AR, Park HJ, Park YC, Cho JH, Kim JH, Lee JH (2021) Moxibustion for treating cancer-related fatigue: A multicenter, assessor-blinded, randomized controlled clinical trial. *Cancer Med* 10:4721–4733
31. Rodenak Kladniew B, Polo M, Montero Villegas S, Galle M, Crespo R, Garcia de Bravo M (2014) Synergistic antiproliferative and anticholesterogenic effects of linalool, 1,8-cineole, and simvastatin on human cell lines. *Chem Biol Interact* 214:57–68
32. Rodenak-Kladniew B, Castro A, Stärkel P, Galle M, Crespo R (2020) 1,8-Cineole promotes G0/G1 cell cycle arrest and oxidative stress-induced senescence in HepG2 cells and sensitizes cells to anti-senescence drugs. *Life Sci* 243:117271
33. Santos FA, Rao VS (2000) Antiinflammatory and antinociceptive effects of 1,8-cineole a terpenoid oxide present in many plant essential oils. *Phytother Res* 14:240–244
34. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW (2013) Cancer genome landscapes. *Science* 339:1546–1558
35. Laha D, Nilubol N, Boufraquech M (2020) New Therapies for Advanced Thyroid Cancer. *Front Endocrinol (Lausanne)* 11:82
36. Chalhoub N, Baker SJ (2009) PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol* 4:127–150
37. Masliah-Planchon J, Garinet S, Pasmant E (2016) RAS-MAPK pathway epigenetic activation in cancer: miRNAs in action. *Oncotarget* 7:38892–38907
38. Moore AR, Rosenberg SC, McCormick F, Malek S (2020) Author Correction: RAS-targeted therapies: is the undruggable drugged? *Nat Rev Drug Discov* 19:902
39. Vaseva AV, Yohe ME (2020) Targeting RAS in pediatric cancer: is it becoming a reality? *Curr Opin Pediatr* 32:48–56
40. Prior IA, Hood FE, Hartley JL (2020) The Frequency of Ras Mutations in Cancer. *Cancer Res* 80:2969–2974
41. Zou S, Tong Q, Liu B, Huang W, Tian Y, Fu X (2020) Targeting STAT3 in Cancer Immunotherapy. *Mol Cancer* 19:145
42. Rani A, Murphy JJ (2016) STAT5 in Cancer and Immunity. *J Interferon Cytokine Res* 36:226–237
43. Zhang Y, Liu Z (2017) STAT1 in cancer: friend or foe? *Discov Med* 24:19–29
44. Massague J (2008) TGFbeta in Cancer. *Cell* 134:215–230
45. Neuzillet C, Tijeras-Raballand A, Cohen R, Cros J, Favre S, Raymond E, de Gramont A (2015) Targeting the TGFbeta pathway for cancer therapy. *Pharmacol Ther* 147:22–31
46. Vinson KE, George DC, Fender AW, Bertrand FE, Sigounas G (2016) The Notch pathway in colorectal cancer. *Int J Cancer* 138:1835–1842
47. Kontomanolis EN, Kalagasidou S, Pouliliou S, Anthoulaki X, Georgiou N, Papamanolis V, Fasoulakis ZN (2018) The Notch Pathway in Breast Cancer Progression. *ScientificWorldJournal* 2018:2415489
48. Li L, Tang P, Li S, Qin X, Yang H, Wu C, Liu Y (2017) Notch signaling pathway networks in cancer metastasis: a new target for cancer therapy. *Med Oncol* 34:180
49. Aster JC, Pear WS, Blacklow SC (2017) The Varied Roles of Notch in Cancer. *Annu Rev Pathol* 12:245–275
50. Zhang C, Liu J, Xu D, Zhang T, Hu W, Feng Z (2020) Gain-of-function mutant p53 in cancer progression and therapy. *J Mol Cell Biol* 12:674–687
51. Lacroix M, Riscal R, Arena G, Linares LK, Le Cam L (2020) Metabolic functions of the tumor suppressor p53: Implications in

- normal physiology, metabolic disorders, and cancer. *Mol Metab* 33:2–22
52. Duffy MJ, Synnott NC, Crown J (2017) Mutant p53 as a target for cancer treatment. *Eur J Cancer* 83:258–265
 53. Bergantin LB (2019) Diabetes and cancer: Debating the link through Ca(2+)/cAMP signalling. *Cancer Lett* 448:128–131
 54. Bergantin LB (2020) A Hypothesis for the Relationship between Depression and Cancer: Role of Ca2+/cAMP Signalling. *Anti-cancer Agents Med Chem* 20:777–782
 55. Zeng X, Ju D (2018) Hedgehog Signaling Pathway and Autophagy in Cancer. *Int J Mol Sci* 19
 56. Luo J, Yu FX (2019) GPCR-Hippo Signaling in Cancer. *Cells* 8
 57. Gartel AL (2017) FOXM1 in Cancer: Interactions and Vulnerabilities. *Cancer Res* 77:3135–3139
 58. Dang CV (2012) MYC on the path to cancer. *Cell* 149:22–35
 59. Stine ZE, Walton ZE, Altman BJ, Hsieh AL, Dang CV (2015) MYC, Metabolism, and Cancer. *Cancer Discov* 5:1024–1039
 60. Kim DY, Kang MK, Lee EJ, Kim YH, Oh H, Kang YH (2018) Eucalyptol Inhibits Advanced Glycation End Products-Induced Disruption of Podocyte Slit Junctions by Suppressing Rage-Erk-C-Myc Signaling Pathway. *Mol Nutr Food Res* 62:e1800302

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