Research Article

Analysis of the Mechanism of Maslinic Acid on Papillary Thyroid Carcinoma Based on RNA-Seq Technology

Rong Li,1 Yanjiao Zhang,2 Runqing Xiang,3 Aihe Lin,1 Zongxiao Xia,1 Xiaomei Long,1 Shuang Guo,1 Yuan Fan,1,4 and Zukun Chen1,4

1 Yunnan University of Chinese Medicine, Kunming 650500, Yunnan, China
2 Yunnan Union of Medicinal Herbs Cultivation, Kunming 650500, Yunnan, China
3 Haiyuan College, Kunming Medical University, Kunming 651700, Yunnan, China
4 The Second Affiliated Hospital of Yunnan University of Chinese Medicine, Kunming 650216, Yunnan, China

Correspondence should be addressed to Yuan Fan; 1647909799@qq.com

Received 19 June 2022; Revised 27 July 2022; Accepted 18 August 2022; Published 7 September 2022

Academic Editor: Talha Bin Emran

Copyright © 2022 Rong Li et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objective. This study analyzed gene sequence changes in the thyroid papillary carcinoma (PTC) cell line TPC-1 treated with the natural compound maslinic acid (MA) through RNA-sequencing (RNA-seq) and identified the necessary genes to provide a basis for the study of the molecular mechanism of action of MA in PTC treatment. Methods. RNA-seq technology was used to detect genetic differences between the normal cell group (Nthy-ori 3-1) and the TPC-1 cell group (N vs T). Then, gene ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, Venn diagram analysis of shared genes, and protein–protein interaction (PPI) network analysis were used to analyze the therapeutic effect of MA on TPC-1 cells. Real-time quantitative PCR (qRT-PCR) was used to verify six key genes. Results. GO and KEGG analyses showed that four crucial signaling pathways are related to PTC development: cytoplasmic molecule (cell adhesion molecules), neuroactive ligand–receptor interaction, tumor transcriptional disorder, and cytokine–cytokine interaction. The Venn diagram revealed 434 genes were shared between the MA vs T-group and 387 genes were shared between the MA vs T-group and N vs T groups. PPI and ClueGO showed that NLRP3, SERPINE1, CD74, EDN1, HMOX1, and CXCL1 genes were significantly associated with PTC, while CXCL1, HMOX1, and other factors were mainly involved in the cytokine–cytokine interaction. The qRT-PCR results showed that the expression of NLRP3, SERPINE1, CD74, EDN1, HMOX1, and CXCL1 genes was significantly upregulated in the TPC-1 group but significantly downregulated after MA treatment (p < 0.01). SERPINE1 and CD74 genes were not expressed in TPC-1 cells, whereas they were significantly upregulated after MA treatment (p < 0.01). Conclusions. This present study proves for the first time that MA can treat PTC, and the preliminary identification of key genes and rich signal transduction pathways provides potential biomarkers. It also provides potential biomarkers for the treatment of PTC with the natural compound MA and preliminarily discusses the therapeutic mechanism of action of MA against PTC, which is helpful for the further diagnosis and treatment of PTC patients.

1. Introduction

Thyroid carcinoma is the most common thyroid malignancy. According to the pathological type, it is mainly divided into five types: follicular thyroid carcinoma, papillary thyroid carcinoma (PTC), thyroid medullary carcinoma, anaplastic thyroid carcinoma, and undifferentiated carcinoma. PTC is the most common thyroid cancer that belongs to differentiated thyroid cancer, accounting for approximately 88% of thyroid cancer. The incidence rate of PTC is increasing year by year, mostly accompanied by lymph node metastasis [1]. Modern related research shows that the PTC involves multiple genes and targets. Researchers have usually found that the PTC-1 pathogenesis is associated with several classical pathways, including BRAF mutation, RET/PTC rearrangement [2], PI3K/AKT/mTOR [3, 4], and MAPK/ERK [5].

MA is a common pentacyclic triterpene compound mainly found in olive fruits and leaves [6, 7], pomegranate flowers [8], and other natural plants. MA has various
pharmacological activities, including antitumor [9], anti-AIDS [10], antioxidant [11], and antidiabetes [12]. Through the PI3K/AKT/NF-κB signaling pathway, MA plays an anti-inflammatory role [13]. MA inhibits the growth of neuroblastoma cells by inducing apoptosis, activating caspase, inhibiting cell migration and invasion, and targeting the MAPK/ERK signaling pathway [14]. It exhibits an inhibitory effect on precancerous colon lesions in rats [15]. MA can significantly inhibit tumor formation in azomethane/dextran sodium sulfate mice and xenotransplantation mice and regulate the AMPK/mTOR pathway, thereby inhibiting the growth of colon tumors [16]. In addition, MA can induce apoptosis of pancreatic cancer cells [17] and act against breast cancer [18].

Gene sequencing technology can obtain the gene information of each sample through sample RNA extraction, primer design, sequencing, and bioinformatics sequence analysis, and can reflect the expression and regulation of genes in cells as a whole. Gene sequencing has become the most commonly used technology for studying the mechanisms of modern molecular pharmacology. It can widely and deeply mine the molecular genes of diseases to identify key target genes. Next-generation gene sequencing showed that the polycystic kidney disease and liver disease like protein 1 (PKHD1L1) may be a tumor suppressor gene in thyroid cancer [19]. Genomic DNA sequencing and mutation detection of PTC tumors showed that VEGF expression in PTC tissues was significantly lower than that in adjacent normal thyroid tissues, which was significantly related to invasive tumor behaviors such as lymph node metastasis and mutation [20]. Numerous studies on PTC have shown that in adjacent normal thyroid tissues, which was significantly lower than that in adjacent normal thyroid tissues, which was significantly related to invasive tumor behaviors such as lymph node metastasis and mutation [20].

Currently, no study has been reported on the role of the MA in PTC treatment. In this study, RNA-seq was used to detect TPC-1 cells and MA-treated TPC-1 cells to analyze the changes in the molecular function and signal transduction pathway and thus find target genes, offer broader information for the inhibition of TPC-1 proliferation, and provide more evidence for MA to treat PTC.

2. Materials and Methods

2.1. Cell Lines and Culture Conditions. The human PTC cell line TPC-1 and human normal thyroid cells (Nthy-ori3-1) cells were purchased from the American Type Culture Bank (USA). Both cells were cultured in RPMI/1640 medium containing 10% fetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin at 37°C in a 5% CO₂ incubator. The medium was changed regularly. The cells were divided into Nthy-ori3-1, TPC-1, and MA + TPC-1 cell groups. MA (purity>98%) was purchased from Sichuan Weikeqi Biological Technology Co., Ltd., China.

2.2. RNA Isolation and Sequencing. Nthy-ori3-1 and TPC-1 cells were cultured in RPMI/1640 medium for 24 h, and TPC-1 cells (66,700 cells/mL) were cultured in the medium containing 12, 24, and 48 μM MA for 48 h. The cells were washed with PBS and lysed using the TRIzol reagent (MRC, Germany). The RNA concentration (OD260/280 ratio) was detected using a nano-photometer spectrophotometer, and the RNA integrity was accurately detected using the Agilent 2100 Bioanalyzer (Agilent Technologies, USA). After passing the quality inspection, different libraries were pooled according to the effective concentration and target offline data volume and then sequenced using Illumina, and a 150-bp paired-end read was generated. Four fluorescently labeled dNTPs, DNA polymerase, and adaptor primers were added to the sequenced flow cell for amplification. When each sequencing cluster extends the complementary strand, each fluorescently labeled dNTP releases the corresponding fluorescence. The sequencer then captures the fluorescent light signal and converts it into a sequencing peak through computer software, thereby obtaining the sequence information of the fragment to be tested.

2.3. Differential Gene Expression analysis. Differentially expressed genes (DEGs) are genes whose expression levels have changed significantly. The gene expression differences between the T vs N, MATL vs T, MATM vs T, and MATH vs T groups were calculated. The DEGs were filtered and analyzed according to the following criteria: condition setting (|Fold change| > 2, p < 0.05).

2.4. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Analyses. Volcano map and heat map analyses were performed on the four groups of DEGs to determine the changes in genes and enrichment of upregulated and downregulated genes. In the enrichment analysis of T vs N and MA vs T groups, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used for DEG analysis. The first 20 enrichment pathways and the first 10 meaningful functional genes were selected, and their similarities were analyzed.

2.5. Data Visualization Analysis to Identify Essential Gene Targets. The Venn diagram was used to determine the shared genes of MATH, MATM, MATL vs T, and T vs N groups, and the Java plug-in ClueGo in Cytoscape 3.8.0 (http://www.cytoscape.org) was used to compare the shared genes between MATH vs T, MATM vs T, MATL vs T, and MA administration groups and the T vs N group for enrichment analysis. In addition, the protein interaction (PPI) network analysis was performed using string (https://string-db.org/) to enrich the genes. The protein node map was imported into Cytoscape 3.8.0, data were visualized, key target genes were identified, degree values were combined, and gene enrichment was performed in ClueGO (showed genes that are enriched) and then the key genes were verified.
2.6. Verification of the Expression of Related Factors through qRT-PCR. After sequencing the same batch of RNA samples, total RNA quantification and reverse transcription were performed to synthesize cDNA, and the 2 × Power Up SYBR Green Master Mix kit was used to verify key genes according to the primer sequences listed in Table 1.

2.7. Data Analysis. The SPSS 23.0 software was used for repeated measurement analysis of variance (ANOVA). Differences between groups were analyzed using the LSD test and one-way ANOVA. If \( p < 0.05 \), the difference was considered significant. In addition, GraphPad Prism 8 (GraphPad Software, Inc.) was used to draw each group of bar graphs.

3. Results

3.1. N vs T and MA vs T differential expression analysis. According to the conditions of |Fold change| > 2 and \( p < 0.05 \) for the screening of genes, N vs T gene volcano map exhibited 4381 differential genes. Of these genes, 2202 were upregulated and 2179 were downregulated. The MATH vs T group exhibited 86 different genes including 27 upregulated and 2179 were downregulated. Of these genes, 2202 were upregulated and 2179 were downregulated.

3.2. GO and KEGG Analyses in the T vs N and MA vs T Groups. GO analysis found that the selected DEGs in the T vs N group were mainly involved in biological processes such as extracellular matrix organization, extracellular structure organization, and neuron projection guidance, and cell composition, including extracellular matrix, proteinaceous extracellular matrix, and receptor complex. In biological processes, cell composition and extracellular matrix, proteinaceous extracellular matrix, and neuron projection guidance, and cell composition, including matrix organization, extracellular structure organization, and negative regulation of cytokine production; cell composition, including the receptor complex, side of the membrane, and external side of the plasma membrane; and molecular functions such as cytokine activity, receptor ligand activity, and cytokine receptor binding. According to the KEGG analysis, pathways mainly enriched in the MA vs T group were the cytokine–cytokine receptor interaction, the IL-17 signaling pathway, and the TNF signaling pathway. The cytokine receptor interaction pathway was significantly enriched in the T vs N and MA vs T groups.

3.3. Analysis of DEGs shared by the MA vs T and T vs N Groups. Venn diagram analysis of MATH, M, L vs T, and T vs N groups revealed that 434 genes were shared between the MA vs T and T vs N groups. In total, 15 genes were shared between the MATL vs T and N vs T groups, 32 genes were shared between the MATM vs T and N vs T groups, and 387 genes were shared between the MATH vs T and N vs T groups (Figure 3(a)); the total gene PPI (TSV format) was calculated. The degree values are shown in Table 2. In addition, the analysis of the MATL vs T, MATM vs T, and MATH vs T groups by using ClueGO revealed that the MATH vs T group was most correlated with the N vs T group (Figure 3(b)). An analysis of the shared genes of the MA vs T and T vs N groups showed that their main molecular functions were related to cytokine secretion; this result is basically the same as that of the previous analysis (Figure 3(c)). We then visually analyzed the data, combined with the calculation of the shared gene degree values, and found that the genes with the highest correlation were mainly SERPINE1, NLRP3, CD74, and EDN1 (Figure 3(d); Table 2).

Table 1: Gene primer sequences.

| Gene     | Primer sequence                  | Amplified product length |
|----------|----------------------------------|--------------------------|
| SERPINE1 | Forward, 5′-CTCATCAGCCACTGGAAAGGCA-3′  
Reverse, 5′-GACTCCTGGAAGTTCGCCAT-3′ | 154bp                    |
| NLRP3    | Forward, 5′-GGACCTGAGCACTGTGTGCA-3′  
Reverse, 5′-GGACCTGAGCACTGTGTGCA-3′ | 153bp                    |
| CD74     | Forward, 5′-AAGCCTGTGACAGATGCCGA-3′  
Reverse, 5′-AGCAGGGTCATCATCATGTGCT-3′ | 134bp                    |
| EDN1     | Forward, 5′-CTACTTCTGACCCTGGACATC-3′  
Reverse, 5′-TCAGGGTGCTGCTGTTGGC-3′ | 126bp                    |
| CXCL1    | Forward, 5′-AGCTTTGCCTCAATCTGCGATCC-3′  
Reverse, 5′-TCTCTTGGCAAGGACACGGCAGTGC-3′ | 119bp                    |
| HMOX1    | Forward, 5′-CCAGGCGAAGAATGCTGAGTTC-3′  
Reverse, 5′-AAAGACCTGGGGCTCTGTCGGTTC-3′ | 144bp                    |
| β-actin  | Forward, 5′-CACCAATGGCAATGACGCTGTC-3′  
Reverse, 5′-AGGTTTCTGCGGATGTCCACGT-3′ | 250bp                    |
Figure 1: Continued.
3; KRT5, Keratin 5; THBD, thrombomodulin; HMOX1, heme oxygenase 1; BMP2, bone morphogenetic protein 2; IL1A, interleukin 1 Alpha; MX2, MX dynamin-like GTPase 2; EDN1, endothelin 1; CD24, CD24 molecule; CD55, CD55 molecule; IL7R, interleukin 1 receptor; SERPINF2, serpin family F member 2; GSTA1, glutathione S-transferase alpha 1; CD74, CD74 molecule. Source: Genecards (https://www.genecards.org/).

3.4. qRT-PCR Verifies the Expression of Related Genes. SERPINE1, NLRP3, CD74, EDN1, HMOX1, and CXCL1 were verified as the key genes through qRT-PCR. The results showed that compared with normal cells (N), the NLRP3, EDN1, HMOX1, and CXCL1 genes were upregulated in the cancer cell group (T). Compared with the T group, the MATH group among the administration groups showed a downregulation trend (p < 0.01). However, the downregulation of NLRP3, HMOX1, and CXCL1 genes in the MATH group was significantly lower than that in the MATL and MATM groups (Figures 4(b), 4(d), 4(e), 4(f)); downregulated genes are SERPINE1 and EDN1, with significant differences (p < 0.01) (Figures 4(a) and 4(c)), and the MATH group was significantly upregulated (p < 0.01) (Figures 4(b), 4(d), 4(e), 4(f)).

4. Discussion

MA is a naturally occurring pentacyclic triterpene compound. Being an excellent pharmacological active product, MA has attracted people's interest because of its high-quality biological properties. MA has an inhibitory effect on various tumors. It targets the MAPK/ERK signaling pathway and inhibits cell migration and invasion. As the MA
| Category | GOID   | Description                                      | pvalue        | Count |
|----------|--------|--------------------------------------------------|---------------|-------|
| BP       | GO:0030198 | extracellular matrix organization               | 2.12E-12      | 98    |
| BP       | GO:0043062 | extracellular structure organization             | 2.15E-12      | 109   |
| BP       | GO:0097485 | neuron projection guidance                      | 1.25E-11      | 77    |
| BP       | GO:0048568 | embryonic organ development                      | 1.81E-11      | 114   |
| BP       | GO:0001655 | urogenital system development                    | 2.89E-11      | 90    |
| BP       | GO:0072001 | renal system development                         | 4.77E-11      | 82    |
| BP       | GO:0007411 | axon guidance                                    | 6.05E-11      | 75    |
| BP       | GO:0050900 | leucocyte migration                              | 4.67E-10      | 105   |
| BP       | GO:0098742 | cell–cell adhesion via plasmamembrane adhesion molecules | 4.94E-10      | 72    |
| BP       | GO:0001822 | kidney development                                | 5.80E-10      | 76    |
| CC       | GO:0031012 | extracellular matrix                             | 2.42E-17      | 138   |
| CC       | GO:0005578 | proteinaceous extracellular matrix               | 7.27E-17      | 115   |
| CC       | GO:0043235 | receptor complex                                  | 1.18E-10      | 100   |
| CC       | GO:0034703 | cation channel complex                           | 1.48E-09      | 65    |
| CC       | GO:0034702 | ion channel complex                              | 4.34E-09      | 80    |
| CC       | GO:1902495 | transmembrane transporter complex                | 2.38E-08      | 83    |
| CC       | GO:1990351 | transporter complex                              | 7.66E-08      | 83    |
| CC       | GO:0043025 | neuronal cell body                               | 5.96E-07      | 102   |
| CC       | GO:0098802 | plasma membrane receptor complex                 | 1.38E-06      | 50    |
| CC       | GO:0098793 | Presynapse                                       | 1.51E-06      | 88    |
| MF       | GO:0005261 | cation channel activity                          | 4.22E-11      | 89    |
| MF       | GO:0022836 | gated channel activity                           | 6.25E-11      | 93    |
| MF       | GO:0015267 | channel activity                                 | 6.47E-11      | 117   |
| MF       | GO:0022803 | passive transmembrane transporter activity       | 7.49E-11      | 117   |
| MF       | GO:0005216 | ion channel activity                             | 1.12E-10      | 109   |
| MF       | GO:0022838 | substrate-specific channel activity              | 1.65E-10      | 111   |
| MF       | GO:0022839 | ion gated channel activity                       | 2.10E-10      | 91    |
| MF       | GO:0046873 | metal ion transmembrane transporter activity     | 3.50E-10      | 114   |
| MF       | GO:0005244 | voltage-gated ion channel activity              | 8.33E-09      | 59    |
| MF       | GO:0022832 | voltage-gated channel activity                   | 8.33E-09      | 59    |

(a) Figure 2: Continued.
Figure 2: Continued.

(b) GeneRatio of different biological processes.

(c) GO terms with adjusted p-values.
concentration increases, the inhibition rate becomes stronger [14]. MA can also inhibit the proliferation of renal cancer cell lines and the angiogenesis of endothelial cells, thereby exhibiting an antirenal cancer effect [21]. It can also prevent the formation of colon tumors by inhibiting the AMPK/mTOR signaling pathway [22]. MA exhibits numerous biological activities. However, researchers are yet to unravel how MA therapeutically targets oncogenic cell signaling cascades in different cancers [23]. Moreover, the effect of MA on PTC and its mechanism of action require further study.

The sequencing results showed that TPC-1 DEGs were significantly higher in the presence of MA, with the number of downregulated genes being significantly higher than that of upregulated genes. This suggests that MA mainly acts as an inhibitor of TPC-1 cells. The results of GO analysis of MA action on TPC-1 cells showed that MA and T groups were mainly involved in biological processes such as bacterial-derived molecular reactions, lipopolysaccharide reactions, and negative regulation of cytokine production. Cell composition analysis revealed correlations between the receptor complex side of the membrane, the outer side of the plasma membrane, cytokine activity, receptor ligand activity, and cytokine receptor binding. According to the KEGG analysis, the pathways mainly enriched were the cytokine–cytokine receptor interaction, the IL-17 signaling pathway, and the TNF signaling pathway, with the cytokine–cytokine receptor interaction significantly enriched in the T vs N and MA vs T groups. ClueGO analysis of MA action on TPC-1 revealed that the correlation between the MATH vs T, MATM vs T, and MATH vs T groups was the highest. Analysis of shared genes in the MAvs T and T vs N groups revealed that their main molecular functions were related to cytokine secretion. This result is essentially the same as that of the previous analysis (Figure 3(c)). Then, visual analysis of the data combined with calculation of the shared gene degree values, revealed that the SERPINE1, NLRP3, CD74, and EDN1 genes were closely related to these genes. In the present study, the bioinformatics function of the MA on TPC-1 cells was analyzed through next-generation gene sequencing. MA induced TPC-1 cells by triggering cytokine receptor interaction signals and through regulation of various cytokine functions. At 48 μMMA, the most common genes were shared with TPC-1 cells. Moreover, SERPINE1, NLRP3, CD74, EDN1, HMOX1, and CXCL1 were verified as the key genes by qRT-PCR. The results were consistent with those of gene sequencing. Importantly, gene enrichment analysis revealed that HMOX1, CXCL1, NLRP3, and other genes were found to be involved in the cytokine signaling pathway and jointly promote the anticancer effect of MA. Gene sequencing enables us to better understand the molecular mechanisms of the anti-TPC-1 effect of MA and provides a new clue for the further study of its pharmacological activity.

Figure 2: (a) GO analysis revealed the top 10 significantly enriched terms of the three major functions for the T vs N group; (b) KEGG analysis revealed the top 20 most significant genes and genomic pathways in this group; (c) GO analysis revealed the top 10 significantly enriched terms of the three major functions for the MA vs T group; (d) KEGG analysis revealed the top 20 most significant genes and genomic pathways in this group.
Figure 3: Continued.
Figure 3: (a) Venn diagram analysis of common genes of each group; (b) use of ClueGO in Cytoscape for analyzing the enrichment of MATH, TM, and TL vs T groups; c and d: analysis of common genes to determine the key genes.
Table 2: Degree values of genes shared between the MA vs T and T vs N groups.

| Nodes   | Degree | Nodes   | Degree |
|---------|--------|---------|--------|
| MMP9    | 20     | IL1A    | 8      |
| CCL5    | 18     | MX2     | 8      |
| CXCL1   | 15     | EDN1    | 8      |
| SERPINE1| 14     | CD24    | 7      |
| NLRP3   | 12     | CD55    | 6      |
| KRT5    | 12     | IL7R    | 6      |
| THBD    | 12     | SERPINF2| 5      |
| HMOX1   | 11     | GSTA1   | 5      |
| BMP2    | 9      | CD74    | 4      |

Table 4: Histograms of qRT-PCR results. (a) b, (c) d, (e), and f represent the relative expression of SERPINE1, NLRP3, CD74, EDN1, HMOX1, and CXCL1 genes respectively, p < 0.01.

**Data Availability**

The data used to support the findings of this study are included within the article.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

**Authors’ Contributions**

Rong Li and Yanjiao Zhang contributed equally to this work.

**Acknowledgments**

This study was supported by the Key Laboratory Project of Colleges and Universities in Yunnan Province (20190925), the Yunnan Provincial Science and Technology Department Science and Technology Program- Applied Basic Research Program (20211AZ010001-015), and the Yunnan Province “Ten Thousand People Plan” Famous Doctor Special program.

**References**

[1] A. K. Lam, “Papillary thyroid carcinoma: current position in epidemiology, genomics, and classification,” Methods in Molecular Biology, vol. 2534, pp. 1–15, 2022.

[2] P. Caria, T. Dettori, D. V. Frau et al., “Characterizing the three-dimensional organization of telomeres in papillary thyroid carcinoma cells,” Journal of Cellular Physiology, vol. 234, no. 4, pp. 5175–5185, 2019.
[3] C. Y. Wu, C. Zheng, E. J. Xia et al., "Lysophosphatidic acid receptor 5 (LPAR5) plays a significance role in papillary thyroid cancer via phosphatidylinositol 3-kinase/akt/mammalian target of rapamycin (mTOR) pathway," Medical Science Monitor, vol. 26, no. 26, Article ID e919820, 2020.

[4] P. Bian, W. Hu, C. Liu, and L. Li, "Resveratrol potentiates the anti-tumor effects of rapamycin in papillary thyroid cancer: PI3K/AKT/mTOR pathway involved," Archives of Biochemistry and Biophysics, vol. 689, no. 689, Article ID 108461, 2020.

[5] X. Lin, H. Zhang, J. Dai et al., "TFF3 contributes to epithelial-mesenchymal transition (EMT) in papillary thyroid carcinoma cells via the MAPK/ERK signaling pathway," Journal of Cancer, vol. 9, no. 23, pp. 4430–4439, 2018.

[6] E. Giménez, M. E. Juan, S. Calvo-Melía, J. Barbosa, V. Sanz-Nebot, and J. M. Planas, "Pentacyclic triterpene in Olea europaea L: a simultaneous determination by high-performance liquid chromatography coupled to mass spectrometry," Journal of Chromatography A, vol. 1410, no. 1410, pp. 68–75, 2015.

[7] I. Breakspear and C. Guillaume, "A quantitative phytochemical comparison of olive leaf extracts on the Australian market," Molecules, vol. 25, no. 18, p. 4099, 2020.

[8] Y. X. Yang, F. L. Yan, and X. Wang, "Chemical constituents from Punica granatum flowers," Zhong Yao Cai, vol. 37, no. 5, pp. 804–807, 2014.

[9] X. Lin, U. Ozbey, U. Y. Sabitliyevich et al., "Maslinic acid as an effective anticancer agent," Cellular & Molecular Biology, vol. 64, no. 10, pp. 87–91, 2018.

[10] H. X. Xu, F. Q. Zeng, M. Wan, and K. Y. Sim, "Anti-HIV triterpene acids from Geum japonicum," Journal of Natural Products, vol. 59, no. 7, pp. 643–645, 1996.

[11] A. Pérez-Jiménez, E. E. Rufino-Palomares, N. Fernández-Gallego et al., "Target molecules in 3T3-L1 adipocytes differentiation are regulated by maslinic acid, a natural triterpene from Olea europaea," Phytotherapy Research, vol. 23, no. 12, pp. 1301–1311, 2016.

[12] B. N. Mkhwanazi, F. R. van Heerden, G. A. Mavondo, M. V. Mabandla, and C. T. Musabayane, "Triterpene derivative improves the renal function of streptozotocin-induced diabetic rats: a follow-up study on maslinic acid," Renal Failure, vol. 41, no. 1, pp. 547–554, 2019.

[13] Y. L. Chen, D. Y. Yan, C. Y. Wu et al., "Maslinic acid prevents IL-1β-induced inflammatory response in osteoarthritic via PI3K/AKT/NF-kB pathways," Journal of Cellular Physiology, vol. 236, no. 3, pp. 1939–1949, 2020.

[14] Y. Liu, H. Lu, Q. Dong, X. Hao, and L. Qiao, "Maslinic acid induces anticancer effects in human neuroblastoma cells mediated via apoptosis induction and caspase activation, inhibition of cell migration and invasion and targeting MAPK/ERK signaling pathway," AMB Express, vol. 10, no. 1, p. 104, 2020.

[15] M. E. Juan, G. Lozano-Mena, M. Sánchez-González, and J. M. Planas, "Reduction of preneoplastic lesions induced by 1,2-dimethylhydrazine in rat colon by maslinic acid, a pentacyclic triterpene from olea europaea L," Molecules, vol. 24, no. 7, p. 1266, 2019.

[16] Q. Wei, B. Zhang, P. Li, X. Wen, and J. Yang, "Maslinic acid inhibits colon tumorigenesis by the AMPK-mTOR signaling pathway," Journal of Agricultural and Food Chemistry, vol. 67, no. 15, pp. 4259–4272, 2019.

[17] Y. Tian, H. Xu, A. A. Farooq et al., "Maslinic acid induces autophagy by down-regulating HSPA8 in pancreatic cancer cells," Phytoterapy Research, vol. 32, no. 7, pp. 1320–1331, 2018.