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"Multi-omics analysis jointly reveals the mechanism of matrine salicylate against acne"

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1. Introduction

Acne is a common skin disease that affects approximately 85% of adolescents and young adults. Acne tends to occur in areas where sebaceous glands are highly concentrated, such as face and upper back[1]. Currently, the occurrence of acne is considered to be closely related to factors such as excessive sebum secretion, excessive hair follicle keratinization, abnormal colonization of Cutibacterium acnes (C. acnes) and severe skin inflammation. Among them, excessive sebum secretion is one of the main causes of acne[2]. In addition to manifesting as skin lesions such as comedones, papules and pustules, acne can also cause sequelae such as pigmentation and scars, affecting the physical and mental health of patients[3]. Currently, acne are usually treated with drugs such as antibiotics and retinoids. Although their effects are significant, they all have certain side effects[4]. Therefore, there is still a huge demand for potential alternative treatments for acne and more and more cosmetic raw materials have been developed for the daily care of acne-prone skin.

Matrine salicylate (MSA) is a supramolecular ionic salt synthesized by the atom-economical quaternization reaction of salicylic acid with matrine. Our previous studies have shown that MSA has good water solubility and transdermal properties and its cytotoxicity and irritancy are both lower than those of salicylic acid. Moreover, MSA also has higher biological activity, manifested as better antioxidant and anti-inflammatory abilities compared to salicylic acid and matrine. The clinical efficacy study highlighted the excellent anti-acne performance of MSA, leading to significant decreases in sebum, pimples, porphyrin and red lesions on the human face[5]. However, the specific mechanism by which MSA exerts its anti-acne effect remains unknown.

Compared with traditional research methods, omics technologies exhibit higher sensitivity and resolution. Saeko N. et al. deciphered the pathogenesis of atopic dermatitis through the combination of multiple omics technologies such as proteomics and transcriptomics[6]. Zhu et al. elucidated the action mechanism of cryptotanshinone against acne by using proteomics and metabolomics[7]. It can be seen from the above that the combined analysis of multiple omics can not only uncover the potential therapeutic targets for skin diseases, but also elucidate the mechanism of active substances in treating skin diseases, providing a scientific basis and theoretical foundation for their application in the field of skin health[8].

Our study aims to elucidate the potential molecular mechanism of MSA against acne in two cell models (keratinocytes and sebocytes) through transcriptomics, proteomics and metabolomics and provide a theoretical basis for expanding its application in the field of skin care products in the future.

2. Materials and Methods

2.1. Materials

MSA (Shinesky, Shaoguan, China), Human sebocyte (C-reagent, Shanghai, China), HaCaT (Procell, Wuhan, China), MTT (ST1537-5g, Beyotime, Shanghai, China), Testosterone (T102169-1g, Aladdin, Shanghai, China), Nile Red (N121291-1g, Aladdin, Shanghai, China), Palmatine (B21646, Yuanye, Shanghai, China), EGF (10605-HNAE, SinoBiological, Beijing, China).

2.2. Nile red fluorescence staining

Human sebocytes were treated with MSA for 24 h and then treated with 100 μM testosterone. Meanwhile, the cells underwent a second treatment with MSA. After 24 h, the cells were stained with 200 nM nile red (MedChemExpress, Shanghai, China) for 30 min. Then the images were taken at the wavelength of 559/635 nm. The fluorescence intensity was analyzed with ImageJ software.

2.3. Scratch assay

HaCaT cells were cultured in 96-well plates at a density of 3×10^5/mL for 24 h. Using a 200 μL pipette tip, a scratch across the cell monolayer was introduced across the well diameter. After removal of the old media, the scratch was washed gently with 1 mL PBS twice. Then the cells were treated with MSA at various doses for 24 h. Photos were taken at 0 h and 24 h after administration respectively, and ImageJ software was used to measure the scratch area and calculate the migration area.

2.4. Multiomics Analysis

The transcriptomics, proteomics and metabolomics analysis were conducted by OE Biotech Co., Ltd. (Shanghai, China)[9]. RNA was extracted by using the TRIzol reagent (Invitrogen, CA, USA) and sequenced on an Illumina Novaseq 6000 platform. Differential gene expression analysis was conducted using DESeq2 with adjusted $p<0.05$ and $|\log2(\text{fold change})|>0.58$ or 1 thresholds to identify significant differentially expressed genes (DEGs). Proteomic analysis was performed by an Nanoelute2 system (Bruker) that was coupled to a timsTOF HT mass spectrometer (Bruker Daltonics). Differentially expressed proteins (DEPs) were defined by $p<0.05$ and $|\log2(\text{fold change})|>0.58$ or 1 thresholds. An ACQUITY UPLC I-Class plus (Waters Corporation, Milford, USA) fitted with Q-Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to analyze the metabolic profiling. Differentially expressed metabolites (DEMs) were defined by $p<0.05$ and VIP>1 thresholds.

2.5. Statistical analysis

Data were statistically analyzed and graphed using GraphPad Prism 8 software, with results presented as mean ± SD. Student's t-test was employed to evaluate the significance of differences between two independent experimental groups, where the p-value indicates the significance of the difference. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Cell efficacy of MSA

To confirm the anti-acne effect of MSA, we tested its sebum production control and repairing efficacy in human sebocytes and HaCaT cells. Prior to this, we determined the safe concentration range of MSA for two cell lines. MSA showed no obvious toxicity to human sebocytes

at concentrations below 0.015625%. In HaCaT cells, obvious cytotoxicity of MSA only occurred at concentrations above 0.0625%. The results of Nile red fluorescence staining showed that after stimulation with testosterone, lipid synthesis in human sebocyte increased significantly, while treatment with MSA could substantially inhibit lipid synthesis. The sebum production control effect of a high dose of MSA was comparable to that of the positive drug palmatine(Fig. 1A&B). In addition, the results of the scratch assay showed that MSA could significantly promote the migration of HaCaT cells in a concentration-dependent manner, indicating that MSA is not only non-irritating but may also have the ability to repair skin lesions (Fig. 1C&D).

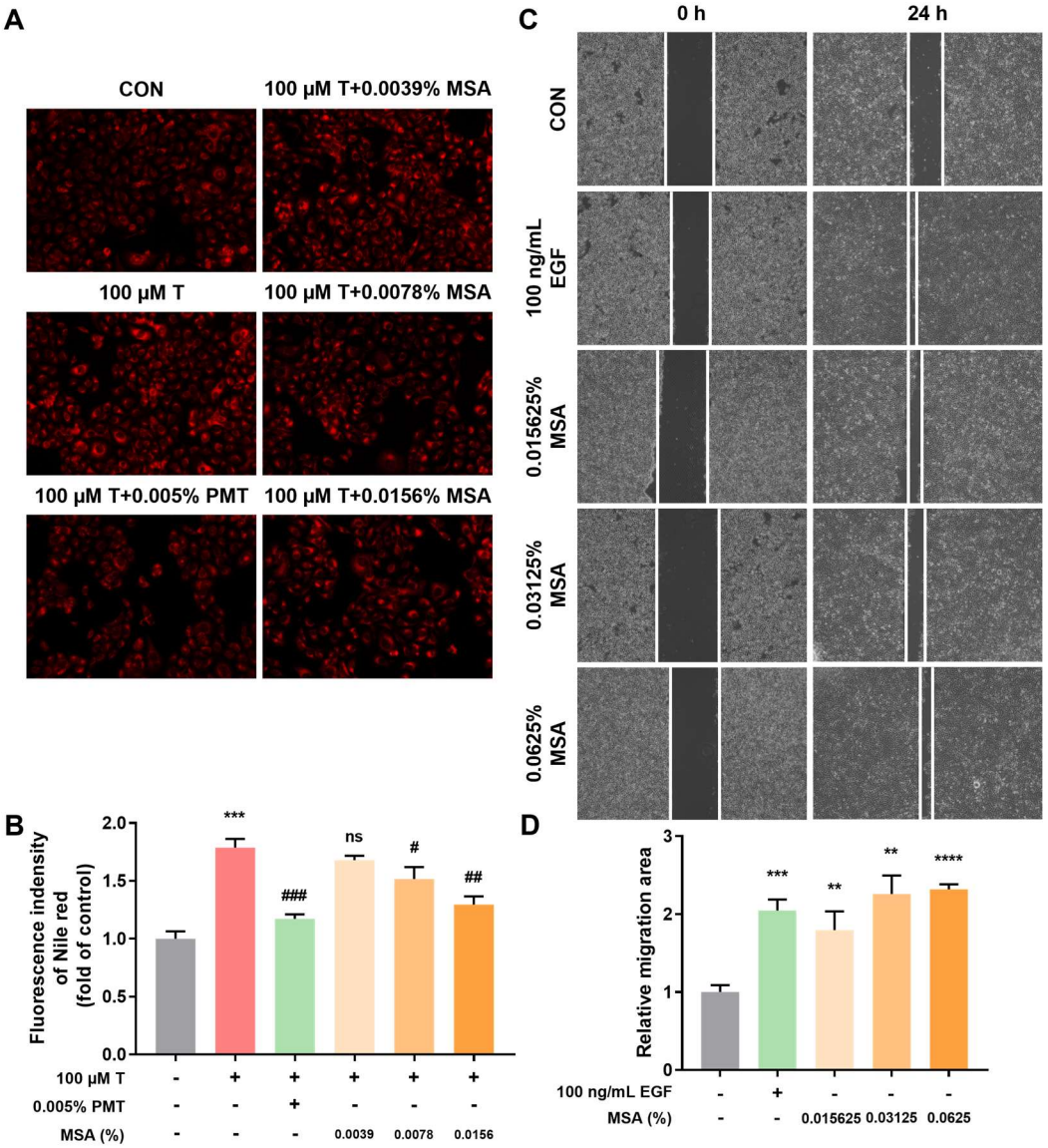
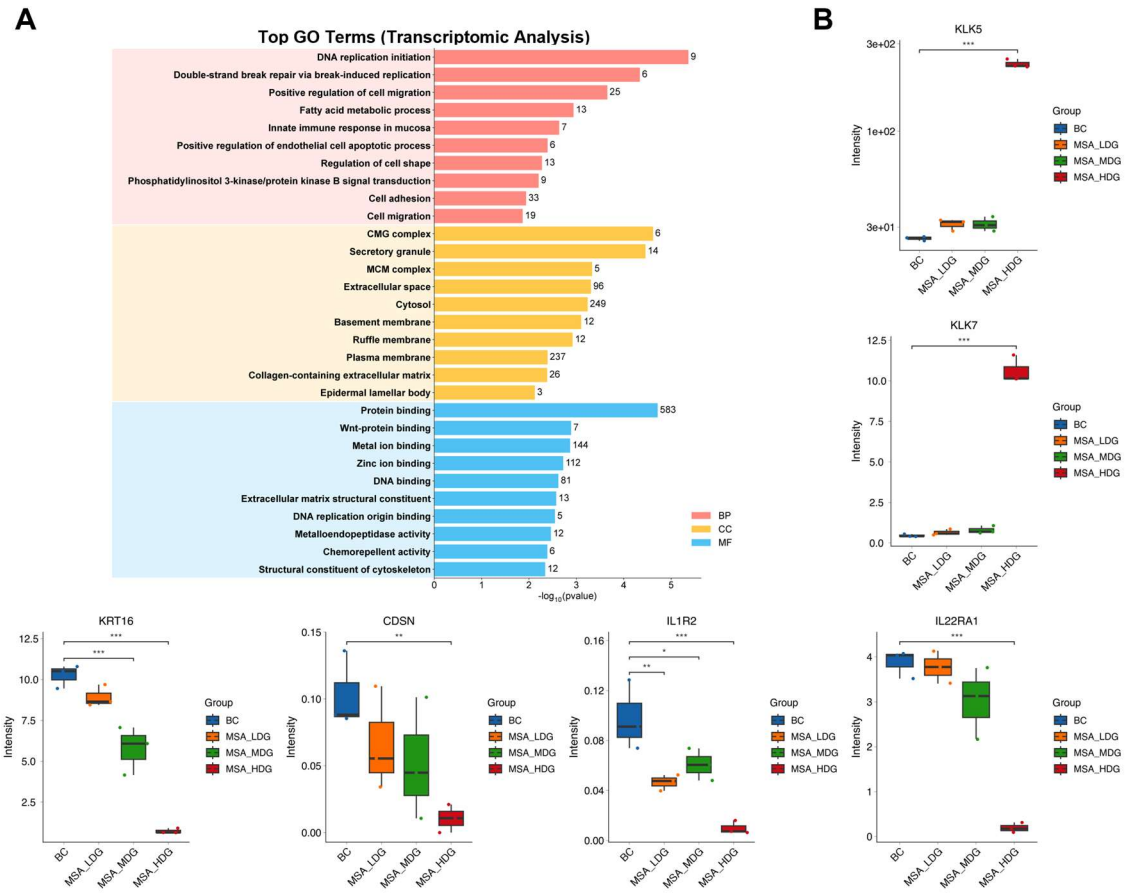
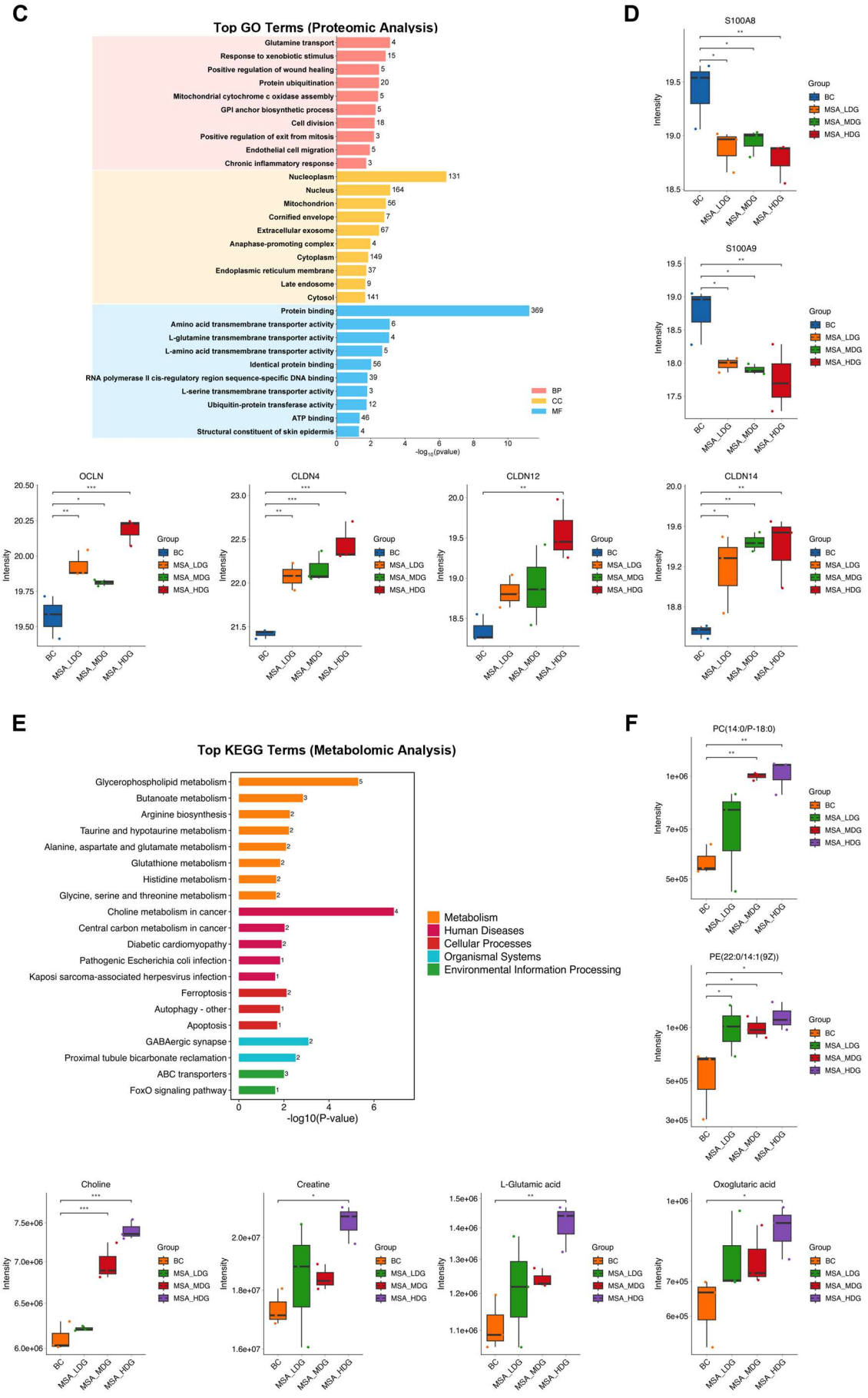


Figure 1. Cell efficacy of MSA. (A) Neutral lipid level of human sebocytes examined by Nile red fluorescence staining. (B) The quantitative analysis of neutral lipids fluorescence intensity. (C) Microscopic images of scratch area (within the white boundary) during the scratch assay on HaCaT cells. (D) The quantitative analysis of migration area.

3.2. The changes in the transcriptome, proteome, and metabolome of HaCaT cells after MSA treatment

To further elucidate the mechanism of MSA against acne, we conducted RNA-seq, proteomics and metabolomics analyses on HaCaT cells treated with MSA. Through GO enrichment analysis of DEGs and DEPs, we found that in HaCaT cells, MSA was mainly involved in biological processes such as positive regulation of cell migration, cell adhesion, glutamine transport, positive regulation of wound healing and chronic inflammatory response (Fig. 2A&C). Notably, after MSA treatment, the expression of kallikreins KLK5 and KLK7, which promoted cell desquamation, were significantly upregulated, while the expression of cell adhesion-related protein CDSN and hyperkeratosis marker KRT16 were decreased (Fig. 2B). Furthermore, MSA induced an upward trend in the expression of tight junction proteins OCLN and CLDNs, which were crucial for maintaining the normal structure and function of skin barrier (Fig. 2D). Conversely, the expression of certain pro-inflammatory cytokines, such as IL1R2 and IL22RA1, as well as inflammatory response proteins S100A8/A9, were significantly suppressed (Fig. 2B&D). In addition, KEGG analysis of metabolome showed that MSA could activate glycerophospholipid metabolism pathway and amino acid metabolism pathways such as those of glycine, proline and arginine (Fig. 2E&F). By regulating these pathways, MSA could accelerate the synthesis of cell membranes and the production of the extracellular matrix (ECM), thereby promoting the regeneration of human keratinocytes. These above results suggested MSA could improve hyperkeratosis in acne affected areas by enhancing keratin desquamation, as well as inhibited inflammatory response and promoted the healing of skin lesions.

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Top GO Terms (Proteomic Analysis)
Glutamine transport 4
Response to xenobiotic stimulus 15
Positive regulation of wound healing 5
Protein ubiquitination 20
Mitochondrial cytochrome c oxidase assembly 5
GPI anchor biosynthetic process 5
Cell division 18
Positive regulation of exit from mitosis 3
Endothelial cell migration 5
Chronic inflammatory response 3
Nucleoplasm 131
Nucleus 164
Mitochondrion 56
Cornified envelope 7
Extracellular exosome 67
Anaphase-promoting complex 4
Cytoplasm 149
Endoplasmic reticulum membrane 37
Late endosome 9
Cytosol 141
Protein binding 369
Amino acid transmembrane transporter activity 6
L-glutamine transmembrane transporter activity 4
L-amino acid transmembrane transporter activity 5
Identical protein binding 56
RNA polymerase II cis-regulatory region sequence-specific DNA binding 39
L-serine transmembrane transporter activity 3
Ubiquitin-protein transferase activity 12
ATP binding 46
Structural constituent of skin epidermis 4
BP
CC
MF
-log10(pvalue)
D
S100A8
S100A9
OCLN
CLDN4
CLDN12
CLDN14
Intensity
Group
BC
MSA_LDG
MSA_MDG
MSA_HDG
E
Top KEGG Terms (Metabolomic Analysis)
Glycerophospholipid metabolism 5
Butanoate metabolism 3
Arginine biosynthesis 2
Taurine and hypotaurine metabolism 2
Alanine, aspartate and glutamate metabolism 2
Glutathione metabolism 2
Histidine metabolism 2
Glycine, serine and threonine metabolism 2
Choline metabolism in cancer 4
Central carbon metabolism in cancer 2
Diabetic cardiomyopathy 2
Pathogenic Escherichia coli infection 1
Kaposi sarcoma-associated herpesvirus infection 1
Ferroptosis 2
Autophagy - other 1
Apoptosis 1
GABAergic synapse 2
Proximal tubule bicarbonate reclamation 2
ABC transporters 3
FoxO signaling pathway 1
-log10(P-value)
Metabolism
Human Diseases
Cellular Processes
Organismal Systems
Environmental Information Processing
F
PC(14:0/P-18:0)
PE(22:0/14:1(9Z))
Choline
Creatine
L-Glutamic acid
Oxoglutaric acid

Figure 2. GO enrichment analysis and KEGG pathway analysis of DEGs, DEPs and DEMs. (A) GO enrichment analysis of DEGs in MSA-HDG and BC. The x-axis represented -log10(p value). The y-axis represented the Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). The column numbers represented gene counts. (B) Boxplots of typical DEGs. (C) GO enrichment analysis of DEPs in MSA-HDG and BC. (D) Boxplots of typical DEPs. (E) KEGG pathway analysis of DEMs in MSA-HDG and BC. The y-axis represented KEGG terms. (F) Boxplots of typical DEMs.

3.3. Multi-omics analysis reveals the molecular mechanism of the role of MSA in HaCaT cells

Three omics analysis jointly revealed significant changes in multiple signaling pathways (such as FoxO signaling pathway, PI3K-Akt signaling pathway and IL-17 signaling pathway) and metabolic pathways (such as glycerophospholipid metabolism) at different omics levels (Fig. 3A). Previous researches have reported the abnormal activation of PI3K/AKT and IL-17 signaling pathway is closely associated with the development of acne[10]. Based on the results of our previous GO enrichment and KEGG pathway analysis, we hypothesized that MSA could promote the expression of PTEN, inhibit the activation of PI3K and suppress the expression and activation of its downstream molecule AKT. Ultimately, this would control the hyperkeratinization of keratinocytes and promote the regeneration of the normal epidermal barrier. Additionally, MSA might block the initiation of chronic inflammatory response by inhibiting the expression of the IL-17 receptor, and the production of downstream pro-inflammatory cytokines such as IL-23 and IL-1 (Fig. 3B).

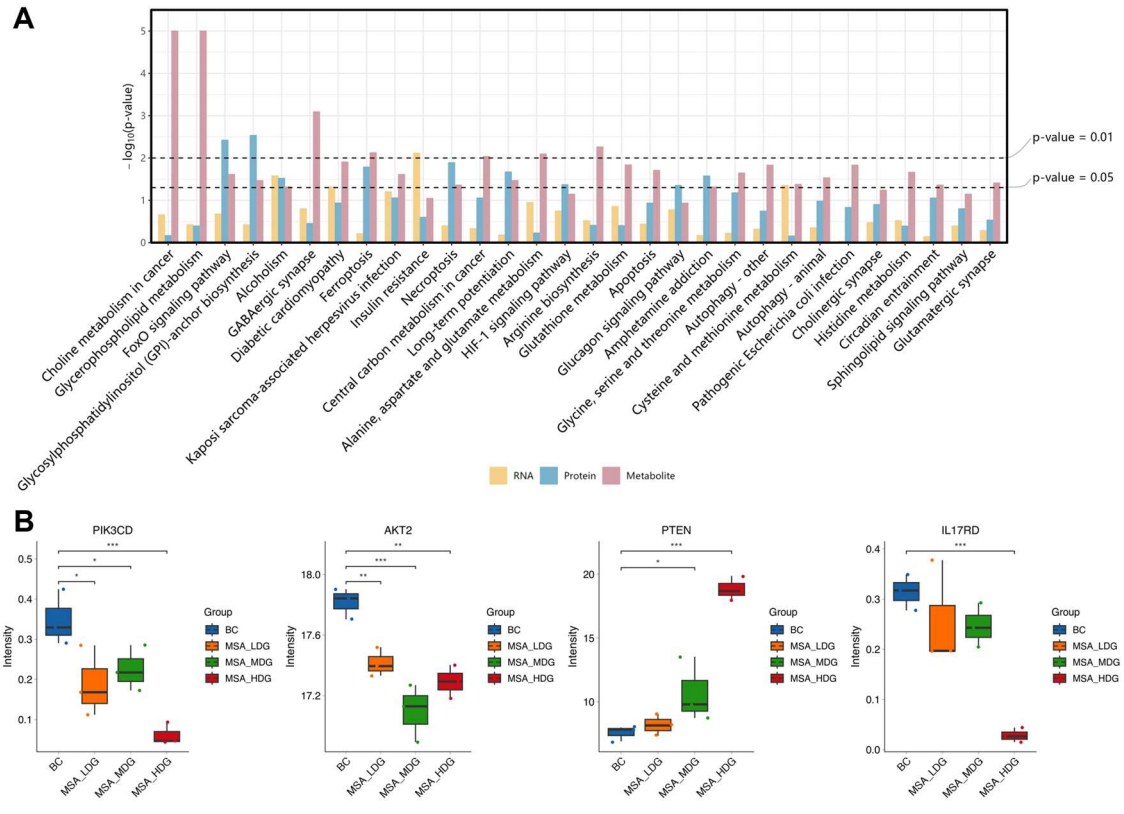

Figure 3. Mechanism of exfoliation and anti-inflammation efficacy of MSA in HaCaT cells. (A) The significance distribution of the common pathways in each omics. The x-axis represented KEGG pathways. The y-axis represented -log10(p value). (B) Boxplots of key genes and proteins.

3.4. The changes in the transcriptome, proteome, and metabolome of human sebocytes after MSA treatment

Through GO enrichment analysis of DEGs and DEPs in human sebocytes, we found that MSA was mainly involved in biological processes such as regulation of epidermal cell differentiation (Fig. 4A). Previous studies have shown sebaceous glands are formed by the continuous proliferation and differentiation of epidermal cells[11]. Therefore, MSA may be involved in the formation of sebaceous glands as well as the synthesis and secretion of sebum. From the boxplot, we could observe that testosterone treatment significantly increased the expression of hair follicle stem cell growth promoting factor SOX9, lipid metabolism regulatory factor PPARA, adipose differentiation related protein PLIN2 and fatty acid synthase FADS3. However, MSA could restore the expression of these genes and proteins (Fig. 4B). The changes in the expression of DEMs suggested that MSA could regulate lipid metabolism related pathways such as FoxO and AMPK (Fig. 4C&D).

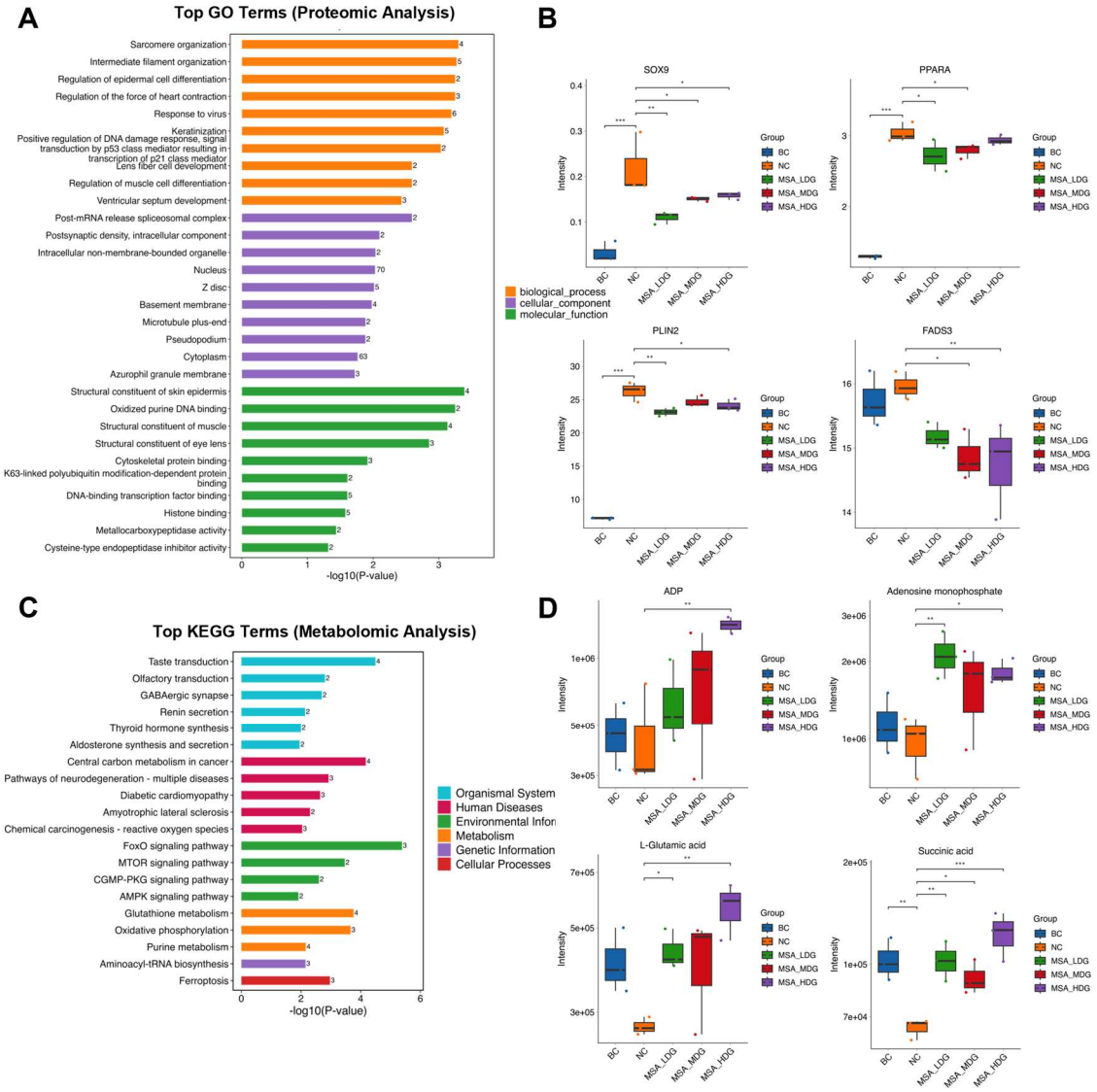

Figure 4. GO enrichment analysis and KEGG pathway analysis of DEGs, DEPs and DEMs in human sebocytes. (A) GO enrichment analysis of DEPs in MSA-HDG and NC. (B) Boxplots of typical DEGs and DEPs. (C) KEGG pathway analysis of DEMs in MSA-HDG and NC. The y-axis represented KEGG terms. (D) Boxplots of typical DEMs.

3.5. Multi-omics analysis reveals the molecular mechanism of the role of MSA in human sebocytes

Through the combined analysis of three omics, we speculated that MSA might increase the content of ADP/AMP and activate AMPK signaling pathway, which in turn further activated FOXO signaling pathway and upregulated the expression of a series of key proteins related to lipid metabolism (Fig. 5A). By regulating the synthesis, metabolism and transportation of lipid, MSA ultimately exerted the effect of controlling sebum production. In addition, we found that the expression of IL-17RD could also be adjusted back by MSA, indicating that MSA might also have an inhibitory effect on the inflammatory response of sebocytes (Fig. 5B).

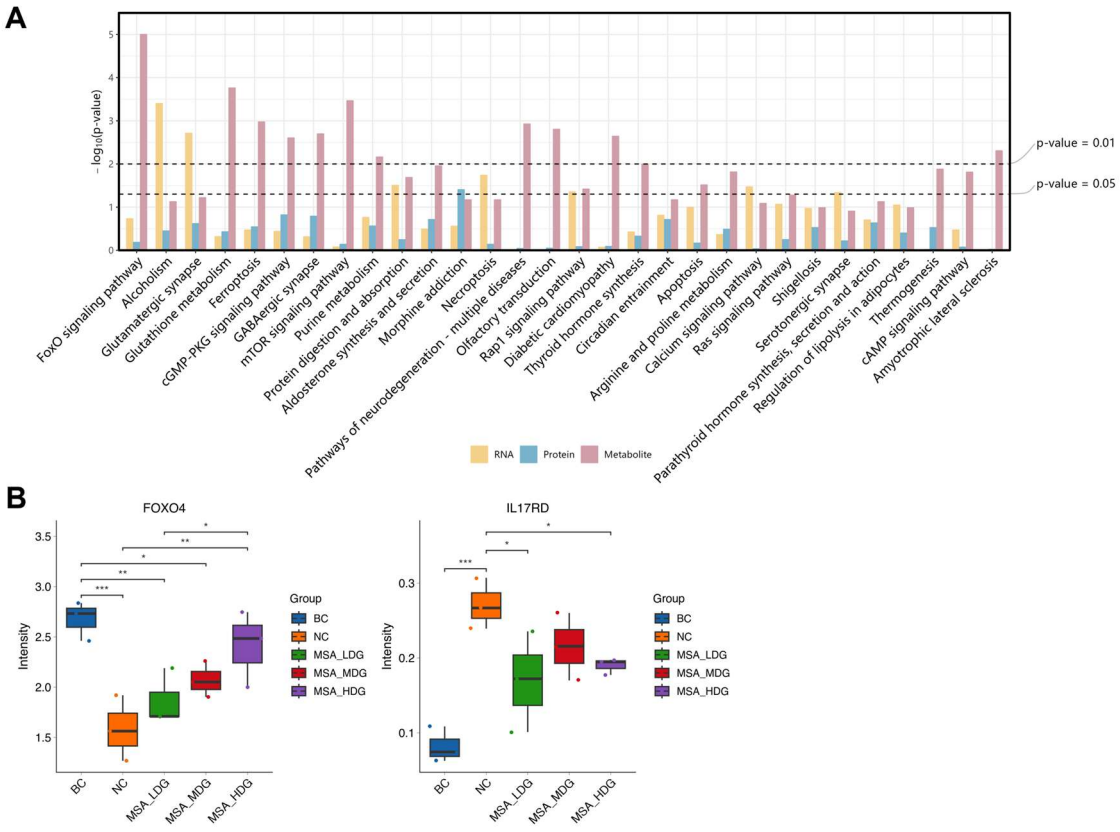

Figure 5. Mechanism of sebum production control efficacy of MSA in human sebocytes. (A) The significance distribution of the common pathways in each omics. The x-axis represented KEGG pathways. The y-axis represented -log10(p value). (B) Boxplots of key genes and proteins.

4. Discussion

Although acne is a disease that commonly occurs in the hair follicle sebaceous gland units, it may also involve the interfollicular regions. In addition to excessive sebum secretion, the epidermal barrier at the lesional sites of acne is severely damaged[12]. Therefore, we explored the mechanism of MSA against acne in two independent cell lines (keratinocytes and sebocytes). In all experiments, we treated the cells with three concentrations of MSA and found that the effects of MSA exhibited a good concentration dependent pattern. In HaCaT cells, we discovered that MSA promoted cell desquamation to improve hyperkeratosis and boosted barrier regeneration and wound healing by inhibiting PI3K/AKT signaling pathway. In sebocytes, MSA regulated the synthesis, metabolism and transportation of lipids by activating AMPK/FoxO signaling pathway, thereby inhibiting excessive sebum secretion.

PI3K/AKT/FoxO1/mTORC1 pathway can maintain skin homeostasis and regulate various physiological processes such as cell growth, proliferation, differentiation and apoptosis. This pathway is associated with various immune-mediated inflammatory and hyperproliferative skin diseases[13]. In acne, androgen and insulin-like growth factor 1 (IGF-1) can activate the signaling transduction of mammalian target of rapamycin (mTOR) through PI3K/Akt pathway. After androgen bind to the androgen receptor (AR) located in the cell nucleus, the phosphorylation level of mTOR increases. By forming the catalytic core of mTORC1, it activates sterol regulatory element binding protein-1 (SREBP-1) and stimulates lipid synthesis. Notably, mTOR is a key downstream factor of PI3K/Akt/FoxO1/mTORC1 pathway. FoxO1 is another core element in the pathogenesis of acne. Androgen and IGF-1 cause FoxO1 to translocate from the nucleus to the cytoplasm by stimulating PI3K/Akt pathway. This process finally affects lipid synthesis and sebaceous gland secretion through antagonizing the expression of SREBP-1 and inhibiting the activation of AR[14]. Additionally, FoxO1 can induce the activation of AMPK pathway, which is a key negative regulator of mTORC1[15]. It has been reported that the activation of PI3K/AKT/mTOR pathway is also closely related to the excessive proliferation and abnormal differentiation of keratinocytes. The over-activation of mTOR leads to abnormal terminal differentiation of keratinocytes, ultimately resulting in epidermal dysfunction[16].

Th17 pathway is noteworthy in the inflammatory processes observed in acne-related diseases. IL-17 is a key cytokine that recruits and attracts neutrophil infiltration and can target keratinocytes, endothelial cells, monocytes, and fibroblasts to induce pro-inflammatory mediators[17]. IL-17 receptors (IL-17Rs) are cell surface receptors that specifically bind to members of the IL-17 cytokine family and transmit signals from IL-17 cytokines into the cell. If the expression of IL-17R is inhibited, its downstream signaling pathway cannot be activated, and the expression of various inflammatory mediators, cytokines, and chemokines will be suppressed, ultimately affecting the inflammatory immune response[18].

Our study has revealed MSA may exert its anti-acne effects through the regulation of three signaling pathways (PI3K/AKT, IL-17, and AMPK/FoxO pathways). However, the accuracy of these three pathways and whether there are interactions among them still require further exploration and verification through molecular biology experiments.

5. Conclusion

Through transcriptomics, proteomics and metabolomics studies, we have found that MSA can regulate multiple biological processes to inhibit the excessive differentiation of keratinocytes, promote keratin exfoliation and barrier regeneration and suppress inflammation as well as the synthesis and secretion of sebum.The related network of the three omics revealed that matrine salicylate might alleviate acne-like lesions by regulating PI3K/AKT, IL-17 and AMPK/FoxO3 signaling pathways. Our research firstly elucidates the anti-acne mechanism of matrine salicylate, which providing theoretical basis for expanding its application in the field of skin care products in the future.

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