IFSCC 2025 full paper (abstract reference number)

"Uncover Novel Mechanisms and Targets in Wound Healing through Artificial Intelligence Network-Based Prediction and In vitro Evaluation"

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1. Introduction

Wound healing is a complex process aimed at repairing damaged tissue and restoring skin integrity. Various medical and aesthetic interventions, such as laser treatments, dermabrasion, microneedling, and tattooing, can induce superficial or minor wounds that compromise the integrity of the epidermis, necessitating post-procedural wound care to facilitate the normal healing process[1]. Wound healing comprises four fundamental stages: hemostasis, inflammation, proliferation, and remodeling, which interact in a systematic manner. Topical compounds that support all stages of wound healing are particularly beneficial for treating minor and superficial wounds. Recent studies suggest that panthenol may exhibit activity across all stages of wound healing [2].

When applied topically to the skin, panthenol is well absorbed and rapidly converted into pantothenic acid, a crucial component of coenzyme A that is essential for the physiological functions of epithelial cells. panthenol supports skin regeneration by enhancing epidermal differentiation and promoting wound healing. It also exhibits properties that prevent biofilm formation and has anti-inflammatory effects. Additionally, panthenol serves as a moisturizer and enhances the skin barrier. In conditions of dry skin, it compensates for reduced hydration by increasing moisture content and positively influencing the molecular mobility of stratum corneum lipid lamellae and proteins[3]. These attributes have led to the development of various topical formulations containing panthenol, which are widely utilized in dermatology. Furthermore, topical panthenol is recommended for the treatment of minor and superficial wounds[4]. However, the mechanism by which panthenol promotes wound healing still needs further exploration.

The incorporation of AI models into bioinformatics has brought a revolutionary era in biological data analysis. Various AI models have been developed to search for similarity between compounds. The method for target prediction of SuperPred web server uses the similarity distribution among ligands for estimating the targets' individual thresholds and probabilities to avoid false positive prediction[5]. In this study, we employ SuperPred web server to uncover the potential targets and signaling pathways of panthenol in relation to wound healing. Furthermore, we utilized RNA sequencing to further validate the pharmacological mechanisms of panthenol.

2. Materials and Methods

2.1. Physicochemical properties of panthenol

Download the 2D structure of panthenol and obtain Isomeric SMILES from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

2.2. SuperPred web server Target prediction method

The method for the target prediction takes into consideration the 2D similarity between the query compound and the ligands associated to their respective targets. For each target set, the summation of all Tanimoto coefficients above a threshold of 0.45 is considered as raw score. To achieve comparability between raw scores of small and large target sets, the raw scores are normalized by dividing them by the number of ligands of the corresponding target. To further evaluate the specificity of a prediction, Z scores and E-values are computed. The Z-score is calculated by the formula:

$$Z_A = \frac{\left(\frac{(\text{raw score}_A)}{N_A} - \mu\right) \exp(0.335 \ln(N_A))}{\sigma}$$

Where A is a target set and NA represents the number of ligands of target set A. Similar to BLAST, μ and σ describe the random background noise of the database.

For diverse target sets, Z-scores tend to behave like high random scores. Therefore, a weighting factor λ_A is introduced which indicates the average similarity between the ligands within each target set:

$$\lambda_A = \exp\left(0.335 \ln\left(\frac{\text{raw score}_{AA}}{N_{AA}}\right)\right)$$

The weighting factor ranges between almost one for very uniform target sets to more than ten for very diverse target sets. The target prediction results are ranked according to the weighted Z-scores (Figure 1.).

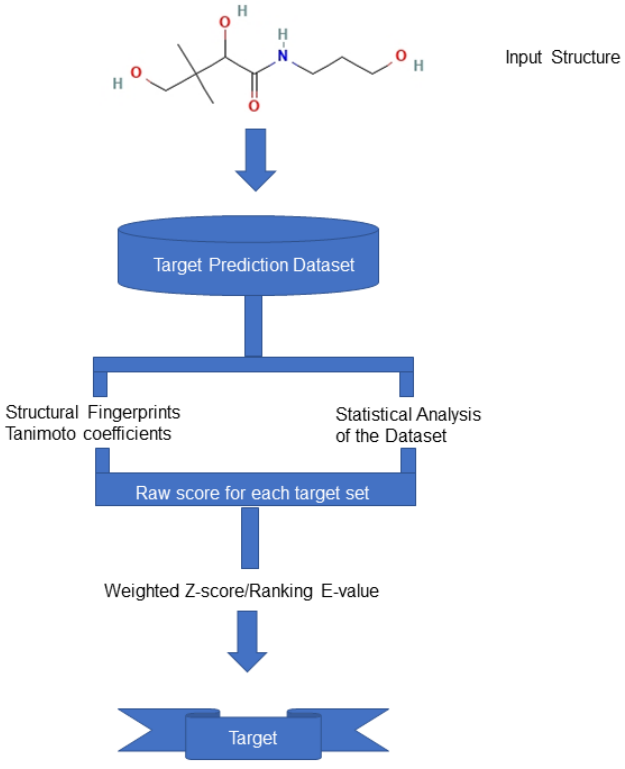

Figure 1. This diagram illustrates target prediction pipeline. The target prediction is carried out in three main steps. In the first step, the input compound is compared based on structural similarity (2D).The second step analyzed the statistical significance of the similarity score in comparison with precalculated statistics of the dataset. The last step computes the raw score for each target and finally the target is predicted with consideration of the weighted *Z*-score and *E*-value threshold.

2.3. Acquisition of potential targets for panthenol and wound healing

Potential targets for panthenol were obtained from SuperPred web server (https://prediction.charite.de/) by searching for the SMILES of panthenol. The GeneCards database (https://www. genecards.org/) was used to obtain wound healing -related targets. All acquired panthenol and wound healing targets will be standardized for gene names through the UniProt database (https://www. uniprot.org/).

2.4. Construction of interaction networks of intersecting genes

To find the relevant targets of panthenol acting on wound healing, the intersection targets of both were obtained using Venn diagrams. The intersecting targets were imported into the STRING database (https://cn.string-db.org/) to construct a protein–protein interaction (PPI) network. Set the minimum required interaction score = 0.4, species = Homo sapiens. The results of the analysis were imported into Cytoscape (Version 3.9.1) software for visualization.

2.5. Biological pathway enrichment analysis

To explore the biological mechanisms underlying the action of panthenol on wound healing, enrichment analysis was performed using genes that intersect panthenol with wound healing. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses were performed through the 'clusterProfiler' package of R software.

GO enrichment analysis was divided into biological process (BP), cellular composition (CC) and molecular function (MF), displaying the top 10 terms of each category, respectively. The results of all biological pathway enrichment analyses were visualized using the ‘ggplot2’ package of R software.

2.6. Establishing a ‘drug-target-pathway’ network

To further elucidate the specific mechanism of panthenol action on wound healing, Cytoscape was used to visualize the KEGG pathway enrichment results and construct a ‘drug-target-pathway’ network. The number of targets enriched in the pathway are shown.

2.7. Cell culture

The HaCaT cell line was provided by the National Collection ofAuthenticated Cell Cultures. We grew cells in DMEM supplemented with 10% FBS, 1% P/S at 37° C, 5% CO_2 and 75% of humidity.

2.8. Scratch assay

We seeded the HaCaT cells in a 24-well plate at a concentration of $1x10^5$ cells /well and we left them until they reached 80% of confluence. We scratched cell cultures with a 200 µL sterile pipette tip. We then washed away detached cells with PBS (1X). We made horizontal reference lines on the bottom of the plate with an ultrafine tip marker to have a grid for alignment to obtain the same field for each image acquisition run. Capture an image using a 4x microscope immediately after the scratch is made. After 24 hours, clean the area once with phosphate-

buffered saline (PBS) and then take another image under the 4x microscope. Calculate the cell migration rate according to the following formula.

2.9. RNA sequencing

Total RNA was extracted from the Hacat cells. After quality control of RNA amount, purity, and integrity, cDNA library with 300 ± 50 bp size was generated from ~1 μg of total RNA. Then library was sequenced on an Illumina Novaseq 6000 using 2 × 150 bp paired-end sequencing chemistry. Differentially expressed genes were defined as fold change >2 or fold change <0.5 and $p < 0.05$. All services were provided by Shagnhai Biotechnology corporation (ShangHai, China).

2.10. Statistics

In GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis, the P-value was calculated from hypergeometric test to assess the statistical significance of functional term or pathway enrichment. Adjusted P-values were corrected from raw P-values using False Discovery Rate (FDR).

3. Results

3.1. Information about panthenol

The 2D picture structure of panthenol is shown in Figure 2a. The compound CID for panthenol in the PubChem database is 4678. The Isomeric SMILES of panthenol are CC(C)(CO)C(C(=O)NCCCO)O. The InChIKey of panthenol is SNPLKNRPJHDVJA-UHFFFAOYSA-N.

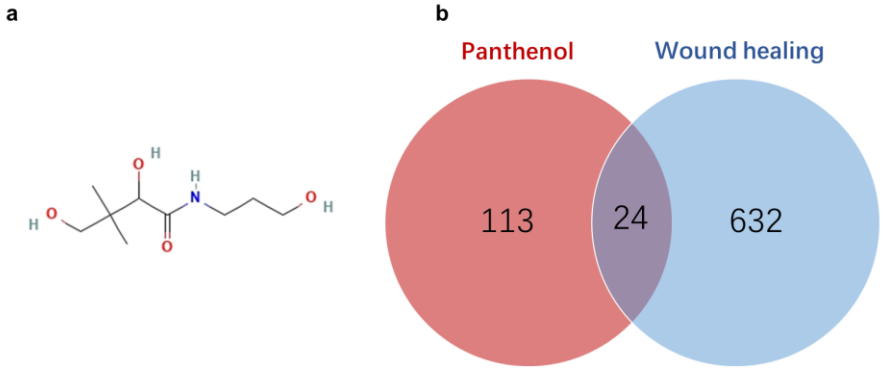

Figure 2. 2D structure of panthenol and identifying intersecting genes. (a) 2D structure of panthenol. (b) Identification of the intersecting genes of panthenol and wound healing. The red circles represent the action targets of panthenol, the blue boxes represent wound healing -related targets, and the intersection of the two is the intersecting target.

3.2. Target information for panthenol and wound healing

A total of 137 potential targets for panthenol were obtained from the SuperPred web server. All relevant targets for the wound healing were obtained from GeneCards database. All targets were compared by the UniProt database to obtain standardized Gene Symbols. After removing all duplicate targets, a total of 656 wound healing cor-related targets were obtained.

3.3. Establishing PPI network and identifying hub genes

After obtaining the relevant targets of panthenol and wound healing, the Venn diagram was used to screen the overlapping targets of both (Figure 2b). The intersecting 24 genes of

panthenol and wound healing were imported into STRING database to generate PPI network. The results were imported into Cytoscape for visualization (Figure 3), and the scores of MNC, DMNC, MCC, Degree, and Closeness were calculated using the CytoHubba plugin to filter the top 10 targets (Table 1). The intersections of the five scores were the hub genes of the PPI network: PIK3CA, HIF1A, PIK3CG, PIK3R1, NFKB1, PTPN11, SOS1 and KIT. These proteins are important targets for panthenol in the treatment of wound healing.

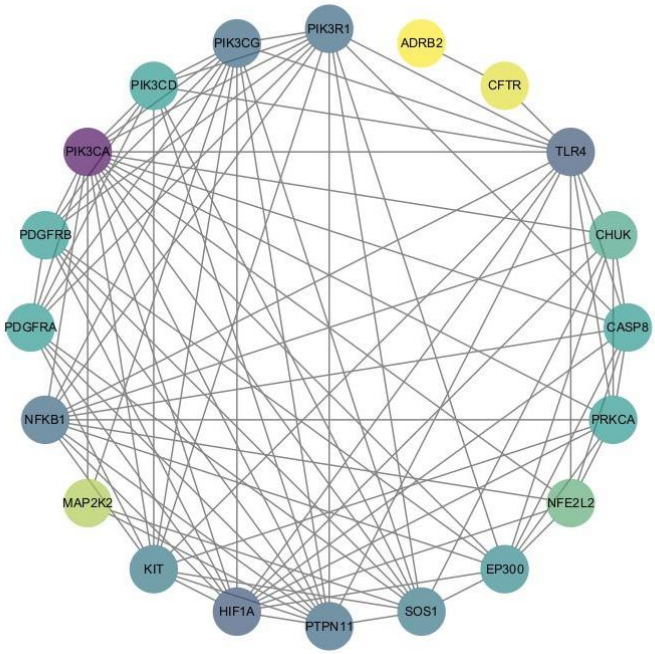

Figure 3. PPI network of intersecting genes. PPI network is constructed using intersecting genes, and each circle represents an intersecting gene. PPI: protein–protein interaction.

Table 1. The top 10 genes for the four algorithm scores.

MCC	DMNC	MNC	Degree	Closeness
PIK3CA	PIK3CD	PIK3CA	PIK3CA	PIK3CA
PIK3CG	PDGFRA	HIF1A	HIF1A	TLR4
PTPN11	NFE2L2	PIK3CG	TLR4	HIF1A
PIK3R1	CASP8	PIK3R1	PIK3CG	PIK3CG
PIK3CD	PRKCA	NFKB1	PIK3R1	PIK3R1
PDGFRA	KIT	PTPN11	NFKB1	NFKB1
SOS1	PDGFRB	TLR4	PTPN11	PTPN11
KIT	PIK3CG	SOS1	SOS1	KIT
PDGFRB	PTPN11	KIT	KIT	SOS1
NFKB1	NFKB1	EP300	EP300	EP300

3.4. KEGG and GO enrichment analysis

Biological pathway enrichment analysis of 24 intersecting targets was performed through the R 'clusterProfiler' package to explore the potential mechanisms of panthenol for wound healing. KEGG pathway enrichment analysis identified a total of 141 pathways, and Figure 4 demonstrates 10 of them with higher association with wound healing. Among them, PI3K-Akt

signaling pathway, MAPK signaling pathway, VEGF signaling pathway, Focal adhesion, Regulation of actin cytoskeleton, these pathways may be closely related to panthenol in the treatment of wound healing.

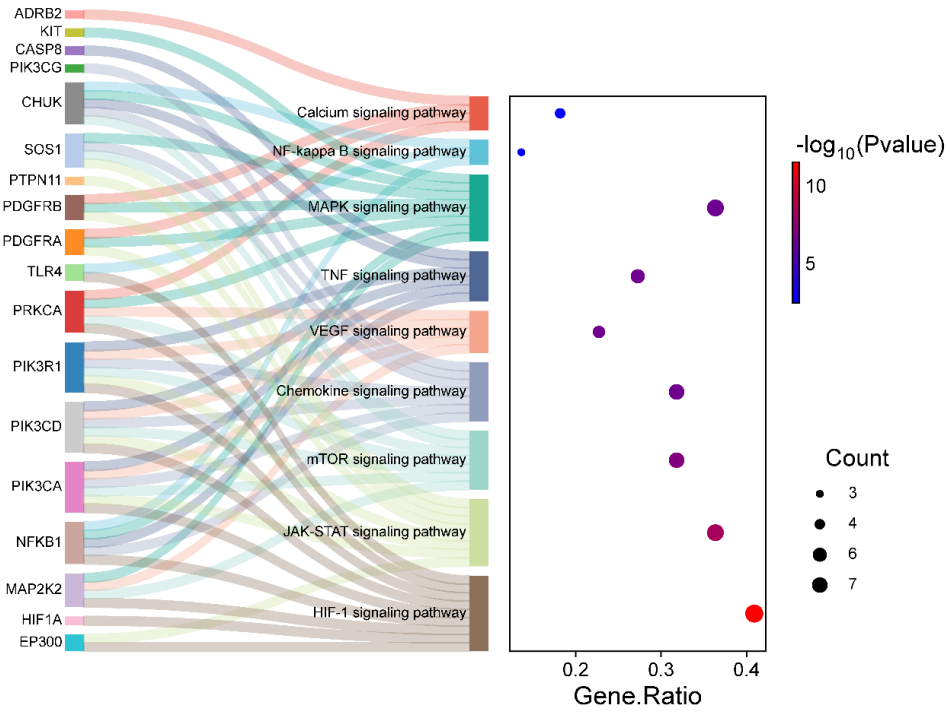

Figure 4. KEGG pathway enrichment analysis. The horizontal axis indicates the enrichment analysis of the pathway. The vertical axis is the pathway name.

3.5. Building drug-target-pathway networks

To further explore the potential pharmacological mechanism of panthenol on wound healing, the 'VEGF signaling pathway' was found to be the most closely related to panthenol in the treatment of wound healing by combining the results of hub gene and enrichment analysis. Figure 5 shows the genes in part of the pathway.

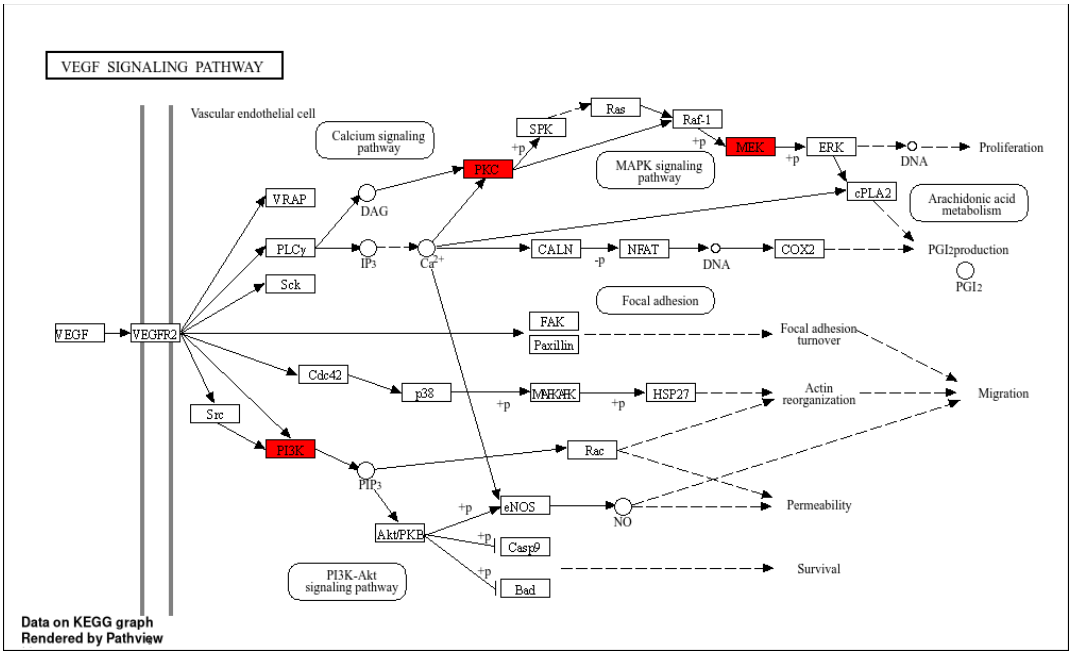

Figure 5. VEGF signaling pathway diagram. Each rectangle in the diagram indicates a gene. The dark blue genes indicate intersecting genes. Realized indicates direct activation. Dashed lines indicate indirect activation.

3.6. Panthenol promotes wound healing in Hacat cells

Human epidermal keratinocytes (HEKα) is major cell type that is involved in the wound healing process and is responsible for restoring the integrity of the skin barrier. Therefore, the viability, proliferation and migration of human immortalized epidermal (Hacat) cells in response to panthenol were evaluated. Initially, we assessed the cytotoxicity of panthenol at various concentrations to determine the suitable amount for both in vitro and in vivo studies. As shown in Figure 6a, for panthenol concentrations of up to 5%, cell viability was higher than 80% after 24 h of incubation. A scratch assay was conducted to investigate the potential of panthenol to promote the migration of Hacat cells. Following a 24h incubation with EGF and panthenol, the percentage of wound area showed significant reduction as compared to the non-treated (NT) group (Figure 6b), respectively. Meanwhile, the cell migration rates of EGF and panthenol were significantly enhanced (Figure 6c). The regeneration of the epidermis and dermis relies on the proliferation and migration of keratinocytes. By promoting the survival, proliferation, and migration of the cells, panthenol could potentially accelerate wound closure rate and facilitate the formation of a stratified epithelium and granulation tissue. Consequently, the regenerated tissue quality could be further improved.

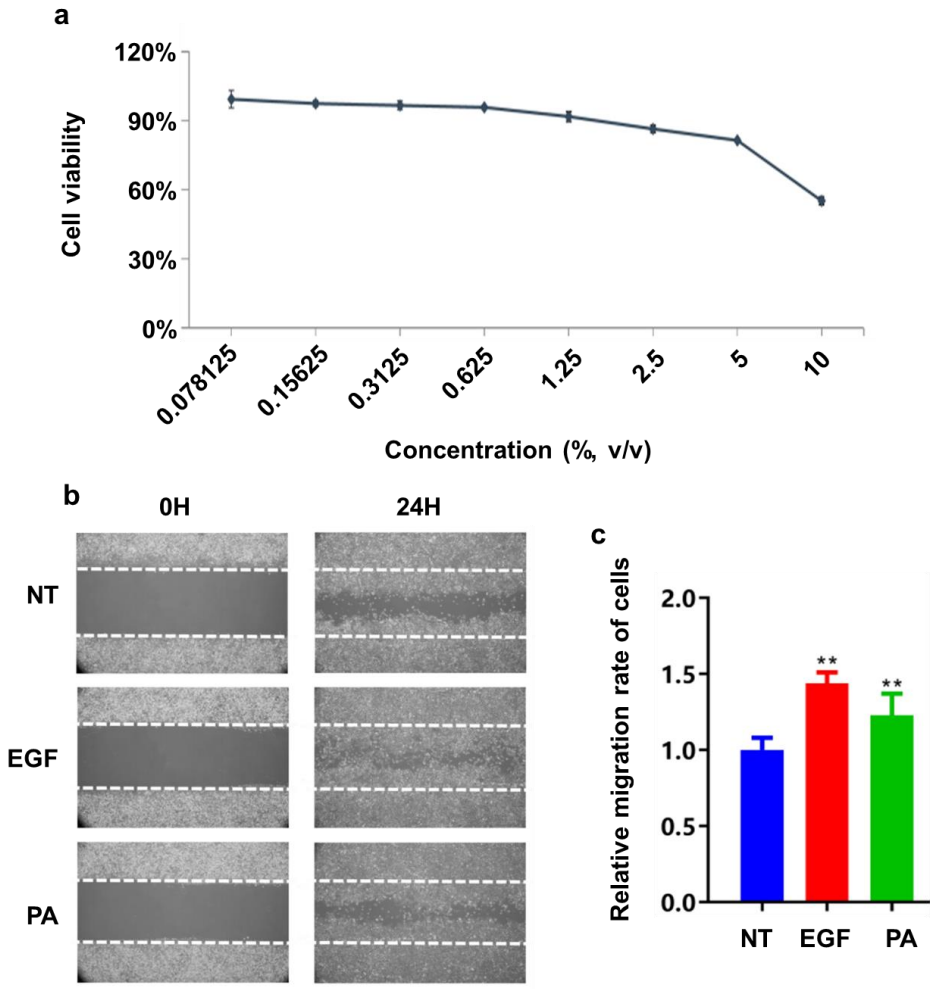

Figure 6. (a) Cell viability of Hacat cell after treated with different different concentrations of panthenol. (b) Time lapse (0 and 24 hours) images of wound healing closure in HaCaT cells**.** (c) Relative migration rate of cells. All the measurements and parameters were taken for 24 hours. n = 4 replicas per time, p-value<0.01**

3.7. Panthenol stimulated the growth of cultured Hacat cells and reduced the inflammatory markers

To obtain a comprehensive overview of the transcriptional signature in panthenol treated and non-treated HaCat cells, we conducted RNA sequencing on the entire cell population. Differentially expressed genes in the skin between the panthenol group and the NT group included those associated with cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organismal systems (Figure 7a). Additionally, gene sets known to be affected in wound healing were highlighted, including cytokines (IL6, IL12, IL23A, TNF) and tissue repair-related genes (VEGFA, EGF, TGFBI) (Figure 7b). The RNA sequencing results were consistent with our AI analysis, revealing significant reductions in IL6, IL12, IL23A, and TNF, alongside increases in VEGFA, EGF, and TGFBI in the panthenol group compared to the NT group. This reflects a reduction in inflammation in the panthenol group compared to the NT group, while promoting cell repair and proliferation.

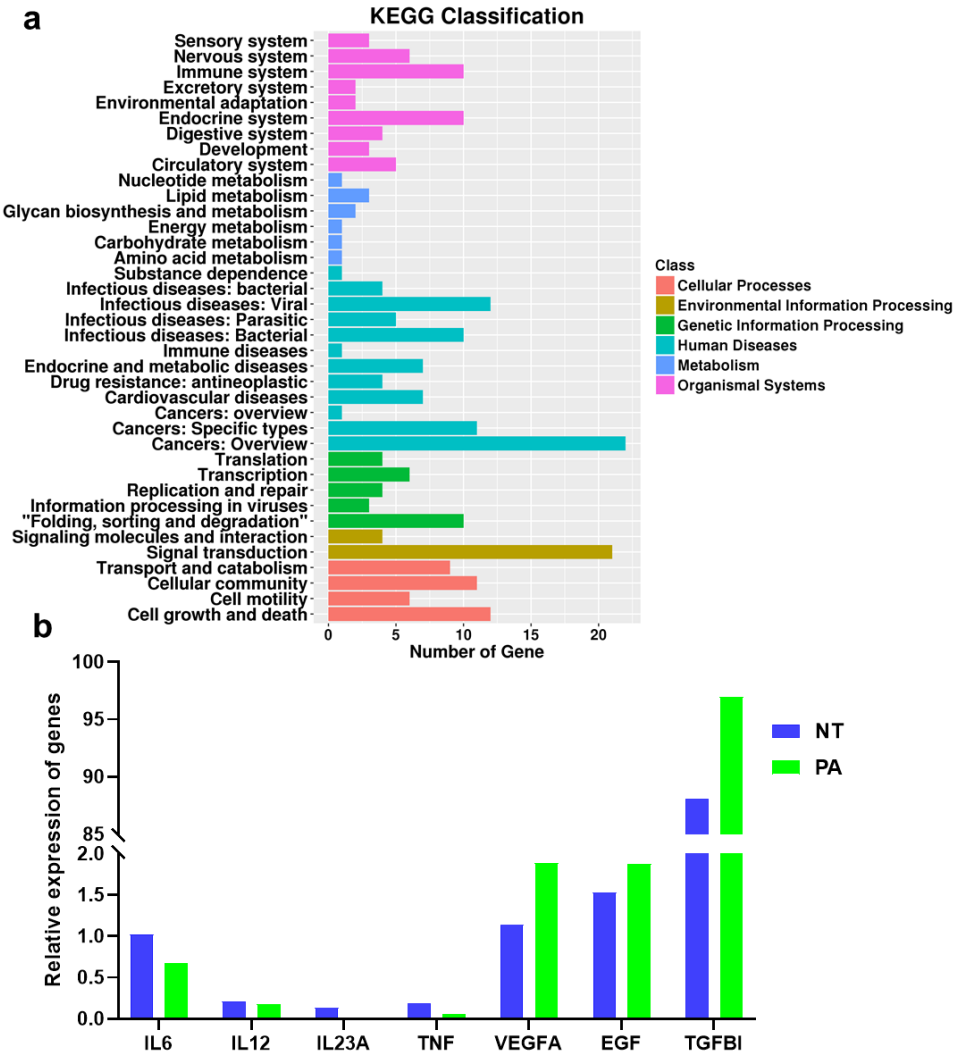

Figure 7. (a)RNA sequencing analysis of the whole cells showing differentially pathway associated with cellular processes, environmentalinformation processing, geneticinformation processing, human diseases, metabolism and organismal systems between NT group and panthenol group. (b)RNA sequencing analysis of the Hacat cells showing differentially expressed genes associated with inflammatory factors and growth factors between NT group and panthenol group

4. Discussion

Artificial intelligence (AI) has recently been proposed as a promising technique in learning and discovering pharmacological big data in active substance that has boosted the success rates of exploring the efficacy of active substances.In this study, we used AI tools to predict the target proteins of panthenol and conducted protein-protein interaction analysis between them and wound healing related proteins. The above-mentioned AI tools based target identification, binding site identification, docking prediction, develop ability predictions, affinity predictions, etc., are the whole or part of the work of mechanism exploration from existing active substances data. In mechanism exploration, accurate calculation of binding energy ligand–target, capturing structural and dynamical features of targets continue to rely on AI simulations. Researchers have recently used AI methods to explore the mechanism of action of the active substance and have made considerable progress.

Panthenol is a form of pantothenic acid with better biological activity and stability. Recently, the regulations of gene expression, cell activity, and pathophysiology by panthenol have been studied intensively. Panthenol have been identified to be involved in the regulation of wound healing[6]. There are many reports on the use of topical panthenol in wound healing after skin trauma, burns, and skin transplantation[7].In this study, relevant targets for panthenol and wound healing were obtained, and the overlap between the two represents a potential target for the action of panthenol in treating wound healing. The results of the enrichment analysis showed that multiple pathways may play a role in panthenol treatment of wound healing, among which VEGF signalling pathway was significantly enriched. A PPI network was constructed using overlapping targets to identify the hub genes (PIK3CA, HIF1A, PIK3CG, PIK3R1, NFKB1, PTPN11, SOS1 and KIT) for panthenol treatment of wound healing. These hub genes were all annotated to the VEGF signalling pathway in wound healing.

There is a strict balance between proangiogenic and antiangiogenic factors in healthy skin metabolism. VEGFA is arguably one of the most significant pro-angiogenic factors, playing a crucial role in angiogenesis. Typically, the angiogenic activity of Hacat cells is determined by the ratio of pro-angiogenic to anti-angiogenic factors. Consequently, the expression and secretion of VEGFA in HaCaT cells may primarily dictate their angiogenic activity[8]. To gain deeper insights into the molecular mechanisms underlying panthenol mediated promotion of angiogenic activity in HaCaT cells, we focused on investigating the regulation of VEGFA gene expression by panthenol. Our results indicated that the treated of panthenol in HaCaT cells led to an enhancement of VEGFA expression and simultaneously promotes the migration of HaCat cells. In contrast, it has been demonstrated that macrophage-specific ablation leads to delayed epithelialization, resulting in reduced expression of VEGF and TGF-β1, which is detrimental to wound angiogenesis and cell proliferation[9].

This study still has some shortcomings. The main component of the study was inflammation, and no validation was performed for the other components of the results of the AI analysis. A more diverse approach could be used to reflect the therapeutic effect of panthenol on wound healing, such as increasing in vitro research and deepening mechanism exploration. In the

future, we can combine different drug delivery materials to further improve the therapeutic efficiency of panthenol.

5. Conclusion

panthenol has a potential multi-target and multi-pathway molecular mechanism of action in the treatment of HW. PIK3CA, HIF1A, PIK3CG, PIK3R1, NFKB1, PTPN11, SOS1 and KIT may be the direct targets of panthenol. Panthenol may play a role in promoting wound healing by reducing the inflammatory response and promoting the VEGF signalling pathway.

6.Reference

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