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Exploring the Mechanism of *Epimedium* on Osteonecrosis of the Femoral Head through Network Pharmacology

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Research Article

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ABSTRACT

Background: Traditional Chinese Medicine has consistently demonstrated promise in the prevention and management of Osteonecrosis of the Femoral Head (ONFH). *Epimedium*, historically revered in Chinese medicinal recipes, has been utilized for mitigating conditions such as osteonecrosis and symptoms of kidney yang deficiency.

Objectives: This study aimed to forecast the drug targets and associated pathways through which *Epimedium* exerts its therapeutic effects against ONFH. Additionally, we sought to delve deeper into its mechanism at the molecular level.

Methods: In this study, we identified the active constituents and targets of *Epimedium* using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform database. The Gene Expression Omnibus database (with accession number GSE123568) was consulted to pinpoint targets associated with steroid-induced osteonecrosis of femoral head. Differential gene expression was visually represented through volcano and heat maps, crafted using the R software. GO and KEGG analyzes of these target genes were also subsequently performed using R software.

Results: Five pivotal target genes were identified: *PTGS2, KCNH2, BCL2L1*, *ABCG2,* and *E2F2*. An exhaustive topological analysis was performed encompassing eight pathways and three genes.

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Conclusion: This study elucidates the fundamental constituents, specific targets, and molecular pathways that underlie the effectiveness of *Epimedium* in the treatment of osteonecrosis of the femoral head.

Keywords: *Epimedium*; Osteonecrosis of the femoral head; Network pharmacology; Mechanism

INTRODUCTION

Osteonecrosis of the Femoral Head (ONFH) is a debilitating skeletal condition in which patients experience deterioration of the joint cartilage and the femoral head, ultimately resulting in a loss of joint function. This descent is typically propelled by aberrations in the fibrinolytic system, compounded by disturbances in vascular perfusion [1-3]. Given the profound implications this relentless ailment has on one's quality of life, a significant contingent of the scientific community has been engrossed in unraveling the salient risk factors underpinning ONFH. With glucocorticoids finding burgeoning utility in addressing rheumatic, autoimmune, and hematopoietic-system maladies, the emergence of steroid-induced ONFH as the predominant subtype of clinical nontraumatic ONFH is unsurprising, constituting a staggering 24.1% of all ONFH diagnoses [4]. While a myriad of therapeutic strategies exists for countering osteonecrosis of the femoral head, each with its distinct efficacy profile, therapeutic decisions must be tailor-made to the individual. However, the overarching therapeutic objective remains unwavering: decelerating the disease's inexorable march. Presently, the primary medications employed for treating ONFH include bisphosphonates, statins, and anticoagulants, Although these drugs offer the promise of revitalizing bone cell function through various pharmacodynamics mechanism, they also bring with them potential side effects like gastrointestinal disturbances, nephrotoxicity, or mandibular joint necrosis, necessitating cautious use [5]. Numerous individuals have sought solace in the realm of Complementary and Alternative Medicine (CAM) as a means to address the treatment of ONFH, with Traditional Chinese Medicine (TCM) emerging as a notable choice in this regard [6-8].

The genus *Epimedium*, referred to as 'Yinyanghuo' in Chinese, is a member of the Berberidaceae Juss. family and was initially documented in the ‘Shen Nong Ben Cao Jing. Traditional Chinese herbal medicine, known for its impeccable safety profile and unerring efficacy, not only stands as a testament to enduring medical praxis but also, with the ascendancy of network pharmacology, has seen its bioactive constituents, molecular architectures, and action mechanisms meticulously deciphered by contemporary scientific inquiry [9]. *Epimedium* boasts an extensive phytochemical compendium; indeed, over 270 distinct entities spanning flavonoids, lignans, polysaccharides, alkaloids, and volatile essences have been elucidated from *Epimedium* species [10]. Historically, *Epimedium*, as a cornerstone of Chinese medicinal formulations, was sought for the amelioration of ailments such as osteoporosis and manifestations of kidney yang deficiency. However, as scholarly pursuits delved deeper into *Epimedium* and its pantheon of constituents, medical scientists discerned its latent potential in addressing cardiovascular disease, tumors, infuenza A through anti-inflammation, antioxidant, apoptosis and autophagy, modulating immunity [11-17].

In this research, the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/tcmsp.php) facilitated the assessment of *Epimedium*'s pharmacokinetics. We employed the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) to extract data on both *Epimedium*

and osteonecrosis of the femoral head chip. R language enabled the differential gene analysis. The Gene MANIA database played a pivotal role in constructing the Protein-Protein Interaction (PPI) network specific to the relationship between *Epimedium* and Steroid-Induced Osteonecrosis of the Femoral Head (SONFH). Employing an intricate process of integration and mapping, we successfully delineated the pivotal targets through which *Epimedium* exerts its therapeutic effects on SONFH. Moreover, we performed comprehensive Gene Ontology (GO) enrichment analysis and scrutinized the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways pertaining to these key genes. Our overarching objective was to elucidate the fundamental genes and molecular mechanisms that underlie the efficacy of *Epimedium* in the treatment of femoral head necrosis, thereby providing novel therapeutic targets and pathways for addressing this condition.

MATERIALS AND METHODS

Development of a database comprising active constituents and their targets within *Epimedium*

The chemical composition of the *Epimedium* herb was elucidated using TCMSP. From the TCMSP database platform, we can find important parameters on the absorption, distribution, metabolism, and excretion characteristics of traditional Chinese medicines, which detail parameters like Oral Bioavailability (OB), Drug-Likeness (DL), and half-life. In this study, compounds with OB value ≥ 30% and DL value ≥ 0.18 were classified as active compounds. In this study, we identified 23 active compounds. The potential targets for these compounds were ascertained from the Drug Bank database (http://www.drugbank.ca), which provides information on both approved and investigational drugs [18].

Analysis of GEO data was conducted to identify differentially expressed genes

The microarray dataset (GSE123568) and gene annotation file GPL15207, linked to femoral head necrosis, were sourced from the GEO database. This dataset encompasses 10 serum samples from healthy individuals and 30 from patients afflicted with steroid-induced femoral head necrosis. Genes exhibiting differential expression associated with SONFH were obtained from the GEO database (Series: GSE123568). This primary data was processed into a gene matrix utilizing the Perl tool [19]. Further analyses were executed using the package ('limma' and 'pheatmap') within the R software. Genes meeting the criteria of P-value<0.05 and |logFC|>1 were identified as osteonecrosis disease targets.

Predictive analysis of potential targets and building the 'active constituent-target' network

The potential targets of *Epimedium* for the treatment of SONFH were obtained by cross-setting the active constituents of *Epimedium* and the related targets of SONFH. The cytoscape software was employed to visualize the interaction network between active components and their potential targets.

Analysis of Protein-Protein Interaction (PPI) networks

The Gene MANIA database, which facilitates the extraction of gene-protein interactions, can discern interactions within gene sets based on literary textual date. This encompasses co-expression, co-localization, physical interaction, and both protein and genetic interactions [20]. Consequently, this database proves instrumental in analyzing genes and elucidating the associations of specific histone proteins. Initially, the study species was designated as '*Homo Sapiens*'. Subsequently, the aforementioned gene sets were entered into the search bar, enabling the construction of a PPI network. This assessed the functional genomics data, producing the primary target regulatory network for *Epimedium's* therapeutic role in femoral head necrosis.

Bioinformatics data analysis

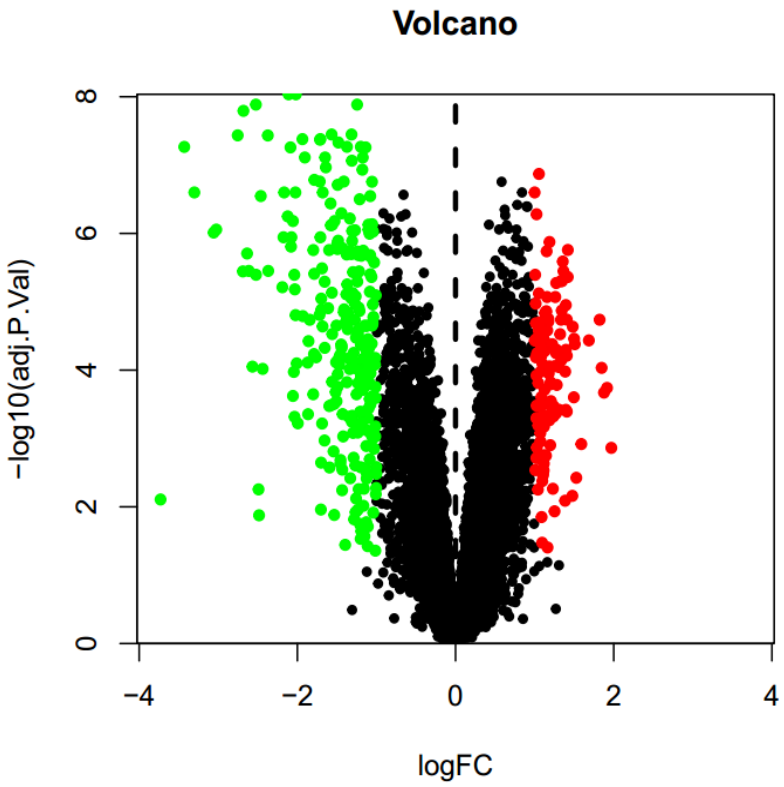
Gene Ontology (GO) enrichment of Biological Processes (BP) was performed using the Cluster Profiler package in the R software environment. This package facilitated both GO functional and KEGG pathway enrichment analyses of the pivotal target genes delineated in section 1.2. Through this method, the biological functions and pathway specifics of these key targets were extracted. An adjusted P-value of less than 0.05 indicated significant enrichment. Characterized by the lowest P-value, the results of the top 20 GO and KEGG are listed and visualized in the form of a bubble chart using R-studio. Subsequently, genes associated with notably regulated pathways underwent gene pathway network examination, underscoring the central target genes of Epimedii Herba for SONFH therapy.

RESULTS

Exploration of *Epimedium* active compounds and identification of targets associated with SONFH

From the TCMSP database, a comprehensive assortment of 130 active constituents intrinsic to *Epimedium* was meticulously extracted. Following a rigorous application of the predetermined selection benchmarks (OB ≥ 30%, DL ≥ 0.18), a select cadre of 23 paramount active compounds indigenous to *Epimedium* was discerned, as delineated. The characterization of the differentially expressed genes was illustrated using volcano plots, as shown in Figure 1. From the GSE123568 dataset, we identified 384 differentially expressed genes, which included 119 upregulated and 265 downregulated genes. Selection of the DEGs (Differentially Expressed Genes) from GSE123568 was based on their adjusted P-values, emphasizing the top 20 most notable upregulated and downregulated genes. These were further represented in heat maps, as illustrated as shown in Figure 1.

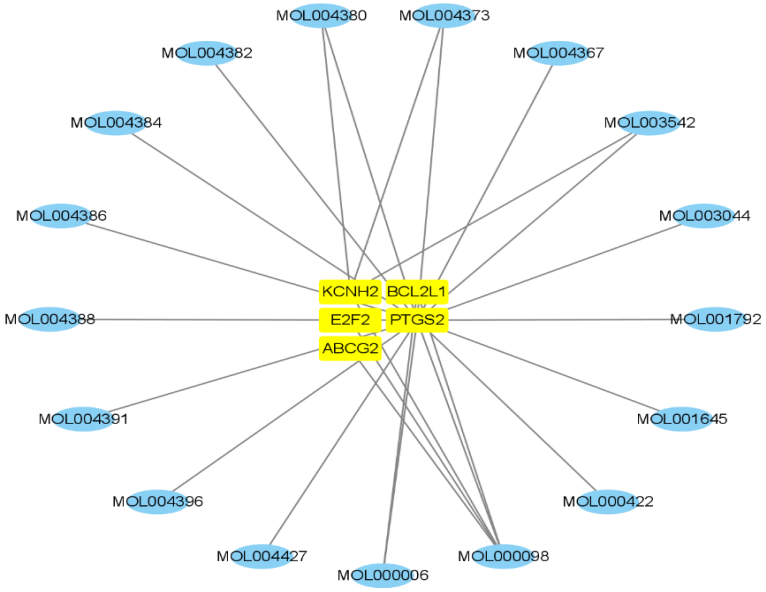
Figure 1. Volcano map of differently expressed genes. **Note:** Green dots represent the downregulated genes, and red dots represent the upregulated genes.



Construction of an *Epimedium* active ingredients-target network for the treatment of SONFH

Figure 2 illustrates the interplay between the pivotal active constituents of *Epimedium* and the targets of SONFH. This suggests that *Epimedium* may exert a therapeutic effect by synergistically interacting with multiple SONFH targets through its diverse compounds. In this representation, the ovals signify the 17 chemical compounds, and the yellow rectangles denote the 5 shared genes as shown in Figure 2.

Figure 2. Network analysis of 'active component-target' in *Epimedium* for SONFH treatment. This network encompasses 17 compounds and 5 target genes within *Epimedium*, where potential target genes are denoted by yellow rectangles and active components are indicated by light blue ovals. The connections between the nodes signify their functional interrelations. A higher number of connections to a node underscores the greater significance of that particular target gene or compound within the network.



PPI network analysis

In order to further explore the pharmacological mechanism of *Epimedium* on SONFH, the PPI network map of 5 potential targets of *Epimedium* in the treatment of SONFH was constructed using GeneMANIA database. The interaction between differential genes of *Epimedium* and SONFH was analyzed. Finally, 25 targets of *Epimedium* Protein-Protein Interaction (PPI) network were found. The lines of different colors represent different features of interaction with each other. As be illustrated, the quintet of core target genes identified were *PTGS2*, *KCNH2*, *BCL2L1, ABCG2,* and *E2F2*.

Enrichment analyses of GO and KEGG pathways

Upon analyzing the five target genes within the domains of Cellular Component (CC), Biological Process (BP), and Molecular Function (MF), we successfully identified a total of 195 significantly enriched GO terms (P-value<0.05). This breakdown includes 170 terms for BP (The top 20 are shown in Figure 3), 7 for CC, and 18 for MF. Figure 3 illustrates these GO terms along with their associated adjusted P-value.

A subsequent exploration of the KEGG pathways highlighted 8 notably enriched pathways. These are visually represented. Among these, the clinically relevant pathways encompassed small cell lung cancer, NF-kappa B signaling, and human T-cell leukemia virus 1 infection.

In summary, we have established a gene pathway network by considering the significantly enriched pathways and the corresponding regulatory genes, as illustrated. This construction involved a comprehensive topological

examination of the 8 pathways and 3 genes. Within this network, squares represent target genes, whereas V-shapes indicate the respective pathways as shown in Figures 3 and 4.

Figure 3. The abscissa is the enrichment fraction. (A) Biological process; (B) cellular component. The larger the bubble, the more genes are enriched in the item. The redder the bubble color, the more significant the enrichment.

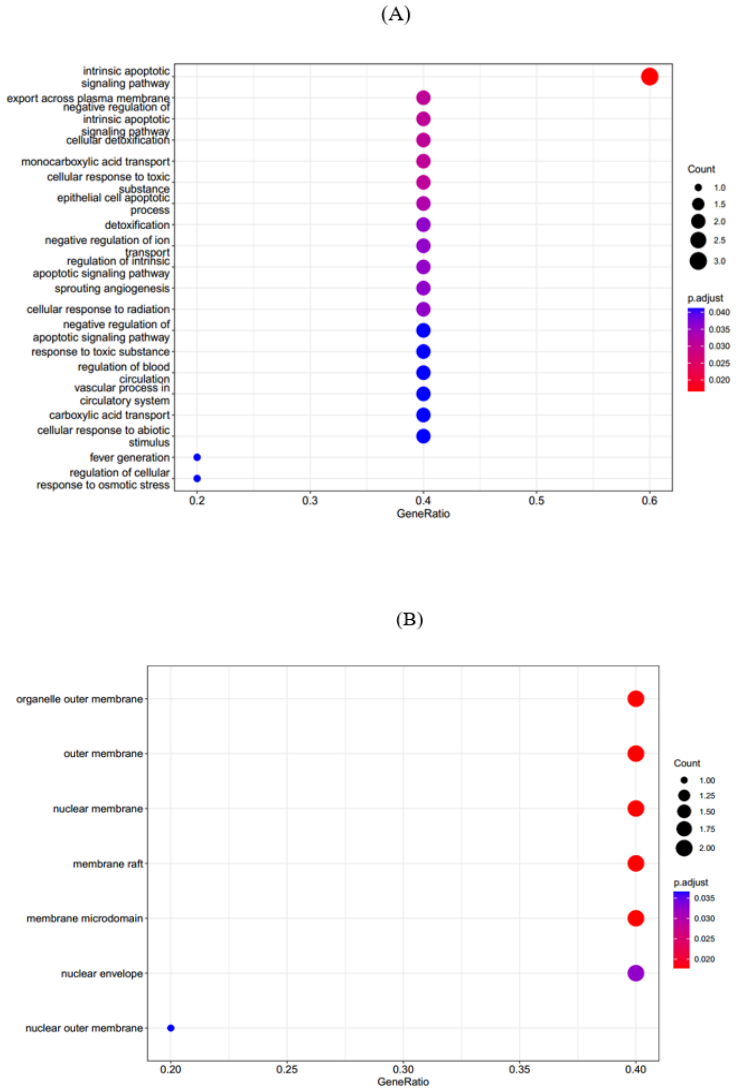
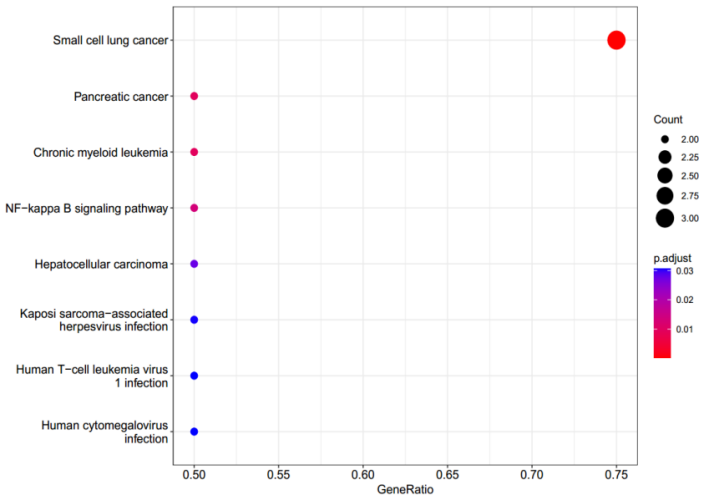


Figure 4. KEGG pathway enrichment of candidate targets of the *Epimedium* against SONFH.



DISCUSSION

Through the 'disease-gene-target-drug' interaction network, network pharmacology integrates disciplines such as systems biology and bioinformatics, offering a novel approach to study the mechanisms of drug active components. By systemically analyzing a drug's intervention and impact on disease networks, it reveals potential drug mechanisms at the molecular level, such as active ingredients and protein targets. This approach enables efficient exploration of traditional medicines in terms of their multi-component, multi-target, and multi-pathway mechanisms. Traditional Chinese medicine has indeed demonstrated considerable promise in the prophylaxis and therapeutic intervention of ONFH. Concurrently, the underlying mechanisms of traditional Chinese medicine in addressing osteonecrosis of the femoral head are increasingly gaining traction among the scientific community [21].

The findings from the investigation intimate that *Epimedium* could exert its effects on the pathogenesis of SONFH *via* a multi-pronged strategy, encompassing numerous components, targets, and pathways. The five core genes of *Epimedium* in the treatment of SONFH were *KCNH2, BCL2L1, E2F2, PTGS2* and *ABCG2*. The protein produced by BCL2L1 is associated with the BCL-2 protein family. Members of the BCL-2 family act as regulators that promote or inhibit apoptosis. These regulators play pivotal roles in a multitude of cellular processes. The proteins encoded by *BCL2L1* are situated on the outer mitochondrial membrane and have been found to modulate the opening of the outer mitochondrial membrane channel (VDAC). Both these factors are powerful triggers for cell apoptosis. A study conducted by Komori et al reveals that overexpression of *BCL2L1* in osteoblasts leads to a significant augmentation in both trabecular and cortical bone volumes, maintaining their structural integrity. This effect is primarily attributed to the prevention of osteoblast apoptosis, especially in osteoporosis scenarios [22]. The enzyme prostaglandin-endoperoxide synthase (PTGS), commonly referred to as cyclooxygenase, is pivotal in prostaglandin biosynthesis. This enzyme is versatile, functioning both as a dioxygenase and a peroxidase. PTGS exists in two isozymic forms: The constitutive PTGS1 and the inducible PTGS2. Their distinction lies in the patterns of their expression and their distribution across tissues. Governed by specific induction paradigms, it is posited to be integral for the prostanoid synthesis implicated in inflammation and cellular proliferation. A work by Qingyu Zhang underscored PTGS2 as a pivotal biomarker for glucocorticoid-induced osteonecrosis of the femoral head, heralding it as an auspicious target for tissue regeneration and SONFH therapy. PTGS2's osteogenic implications on BMSCs were scrupulously evaluated in *in vitro* studies [23]. The protein encoded by the E2F2 is a member of the E2F family of transcription factors. This collective is instrumental in the meticulous regulation of the cell cycle, the functionalities of tumor-suppressive entities, and is also a prime target for the transformative proteins stemming from small DNA tumor viruses. A study on MiR-125a-5p-rich exosomes, sourced from mesenchymal stem cells, revealed their potential in thwarting chondrocyte degeneration by specifically targeting E2F2 in traumatic osteoarthritis contexts [24].

In a pathway enrichment analysis, *Epimedium* emerged as a salient player in the therapeutic approach to SONFH, exerting its influence predominantly through pathways related to apoptosis, immune responses, inflammation, cellular stress, and oncogenesis. A comprehensive enrichment assessment of the 5 targets gene was conducted using the GeneMANIA database. Biological processes are mainly manifested in intrinsic apoptotic signaling pathway, export across plasma membrane and epithelial cell apoptotic. Cellular component is mainly manifested in organelle outer membrane, outer membrane, nuclear membrane, membrane raft, and membrane micro domain. The molecular function is mainly manifested in death domain binding, ABC−type xenobiotic transporter activity, efflux transmembrane transporter activity, and inward rectifier potassium channel activity. KEGG signaling pathway

was mainly enriched in small cell lung cancer, pancreatic cancer and chronic myeloid leukemia and NF-kappa B signaling pathway.

Moreover, certain limitations in this study warrant mention. Initially, owing to the constraints of our screening parameters, only the predominant targets of *Epimedium* were subject to analysis, circumscribing the scope of our findings to an extent. Additionally, while network pharmacology and bioinformatics tools allow for an expansive delineation of targets and pathways, empirical corroboration through both *in vitro* and *in vivo* investigations remains imperative. This will elucidate the precise molecular modus operandi of *Epimedium* in addressing osteonecrosis of the femoral head, thereby catalyzing the modernization of traditional Chinese medicinal practices.

CONCLUSION

In summary, the key genes of *Epimedium* and SONFH were mined based on GEO database, and the interactions between *Epimedium* and SONFH key genes were explored from the global and molecular levels with the help of bioinformatics, which provided a feasible theory and method for studying the treatment of SONFH with *Epimedium*. GO and KEGG enrichment analysis predicted that the pathogenesis of *Epimedium* in the treatment of osteonecrosis of femoral head was not only involved in apoptosis, osteoclast activity, efflux transmembrane transporter activity, but also closely related to multi-targets and multi-signal pathways, which provided an important theoretical basis for the subsequent use of *Epimedium* drugs and clinical treatment of osteonecrosis of femoral head.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors hereby affirm that they have no conflicts of interest to declare.

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CONTRIBUTIONS OF THE AUTHORS

Yuxin Qi contributed to the investigation, methodology, original draft writing, resource management, and software development. Wei Hu was involved in the investigation and methodology. Jianzhong Wang and Pei Wang were responsible for funding acquisition, project administration, and manuscript review and editing. Chenyang Meng and Jingjing Wang were responsible for image creation and software development. Zhe Ge and Enze Jiang were involved in data curation and provided valuable guidance.

DATA AND MATERIAL ACCESSIBILITY

All data generated and scrutinized in the course of this study are comprehensively documented within this article and its accompanying supplementary materials.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This research is consistent with the principles of the Helsinki Declaration. All participants gave written informed consent for the use of personal data for research aims.

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