BMC Pharmacology and Toxicology

RESEARCH

Open Access

Integrated network toxicology, machine learning and molecular docking reveal the mechanism of benzopyrene-induced periodontitis

Wen Wenjie[1,2†], Li Rui[1,2†], Zhuo Pengpeng[1], Deng Chao[1,2*] and Zhang Donglin[1,2*]

Abstract

Background Environmental pollutants, particularly from air pollution and tobacco smoke, have emerged as significant risk factors. Benzopyrene (BaP), a Group 1 carcinogen, is ubiquitously present in these pollutants, yet its molecular mechanisms in periodontitis remain largely unexplored.

Methods We investigated these mechanisms through an integrated approach combining network toxicology, machine learning, and molecular docking analyses. Data from SwissTargetPrediction, CTD databases, and GEO datasets were analyzed to identify potential targets. Three machine learning algorithms (Support Vector Machine, Random Forest, and LASSO regression) were applied for core target identification, followed by Molecular docking analyses.

Results We identified 11 potential targets associated with BaP-induced periodontitis, primarily involved in cellular response to lipopolysaccharide, endoplasmic reticulum function, and cytokine activity, particularly in IL-17 and TNF signaling pathways. Machine learning analysis identified three core targets: CXCL12, CYP24A1, and HMGCR. Molecular docking demonstrated strong binding affinities between BaP and these targets (binding energies <-5.0 kcal/mol). A diagnostic nomogram based on these core targets achieved high prediction accuracy (AUC = 0.922).

Conclusions This first comprehensive analysis of BaP-induced periodontitis using an integrated computational approach elucidates potential molecular mechanisms and identifies specific therapeutic targets. The diagnostic nomogram developed offers a promising tool for clinical periodontitis risk assessment, providing new perspectives on understanding the impact of environmental pollutants on periodontal health.

Keywords Periodontitis, Benzopyrene, Network toxicology, Machine learning, Molecular Docking

†Wen Wenjie and Li Rui contributed equally to this work.

*Correspondence:
Deng Chao
20120015@wnmc.edu.cn
Zhang Donglin
19900005@wnmc.edu.cn

[1]Anhui Province Engineering Research Center for Dental Materials and Application, School of Stomatology, Wannan Medical College, Wuhu 241002, China
[2]Oral Disease Research Center, School of Stomatology, Wannan Medical College, Wuhu 241002, China

Introduction

Periodontitis, a complex inflammatory disease affecting the tooth-supporting tissues, represents one of the most significant oral health challenges globally. This chronic condition is characterized by progressive destruction of periodontal ligament, cementum, and alveolar bone, ultimately leading to tooth loss if left untreated [1]. According to the Global Burden of Disease Study 2019, severe periodontitis affects approximately 796 million people worldwide, with a standardized prevalence of 9.8% [2]. The disease not only compromises oral function and quality of life but also poses substantial economic burden on healthcare systems, with estimated direct treatment costs exceeding $30 billion annually in the United States alone [3]. The etiology of periodontitis is multifactorial, involving complex interactions between bacterial infection, host immune response, and environmental factors [4]. While the role of periodontal pathogens and host immune mechanisms has been well-established [5], accumulating evidence suggests that environmental pollutants play a crucial role in disease initiation and progression [6].

Benzopyrene (BaP), classified as a Group 1 carcinogen by the International Agency for Research on Cancer, is one of the most thoroughly studied PAHs [7]. Human exposure to BaP occurs through multiple routes, including inhalation of air pollution and tobacco smoke, ingestion of charred foods, and occupational exposure [8]. Recent epidemiological studies have revealed strong correlations between high levels of air pollution or tobacco smoke and increased prevalence of periodontal disease, particularly in populations [9].

The emergence of network toxicology has provided new opportunities for understanding the mechanisms of toxicant-induced diseases [10]. This approach enables the systematic analysis of complex interaction networks between environmental toxicants and biological systems, offering insights into both direct and indirect effects of xenobiotics [11]. Network toxicology has been successfully applied to investigate various environmental diseases, revealing previously unknown mechanisms and potential therapeutic targets [12]. Currently, there are many studies that combine network analysis with machine learning algorithms, which can predict new molecular targets and pathway interactions with high accuracy [13, 14].

Despite these technological advances, the complex molecular mechanisms underlying BaP-induced periodontitis remain largely unexplored through integrated computational approaches. The combination of network toxicology, machine learning, and molecular docking presents a promising strategy to decipher these mechanisms comprehensively. We constructed and analyzed molecular interaction networks to identify key protein targets and pathways involved in BaP-induced periodontal inflammation, developed and applied machine learning models to predict potential therapeutic targets and regulatory mechanisms, and validated the predicted molecular interactions through systematic molecular docking analyses. This study represents the first comprehensive analysis of BaP-induced periodontitis using an integrated computational approach, and our findings provide new insights into the pathogenesis of environment-related periodontitis while identifying potential therapeutic targets for future drug development.

Materials and methods

Data acquisition

The dataset (GSE10334) obtained from the periodontitis study was analyzed for differential expression using the limma package. Important genes that differentiate periodontitis samples ($n = 183$) from healthy controls ($n = 64$) were identified and confirmed, applying the thresholds of adj.P.Val < 0.05 (Benjamini-Hochberg correction) and|logFC| > 0.585 for differentially expressed genes (DEGs).

By searching for "benzopyrene" in the PubChem database, the standard structure and SMILE nodes of BaP were retrieved. Based on this search result, we used the SMILE node to retrieve potential targets of BaP from the SwissTargetPrediction database [15], setting the species filter to "Homo sapiens." We then filtered genes with an "Inference Score ≥ 20" from the CTD database [16] to identify target genes and chemicals for BaP. Finally, these targets were merged to construct the BaP target library.

Identifying signature genes related to environmental pollutants in periodontitis through machine learning

To identify potential candidate genes for diagnosing periodontitis, three machine learning prediction models were used: the Random Forest (RF) model, the Support Vector Machine (SVM) model, and the Least Absolute Shrinkage and Selection Operator (LASSO) regression model. The SVM modeling was performed using the "e1071" package. Feature elimination was used to identify the optimal key genes. Subsequently, we used the "randomForest" package in R to classify the important genes. We built a regression model using the "glmnet" package in R, setting the model to "binomial" and selecting the best λ (lambda) value using "lambda.min." A log-lambda curve of LASSO coefficients was plotted. After screening with the three algorithms, the intersection of the results from each algorithm was taken, and the intersecting genes were considered as potential core targets for BaP-induced periodontitis toxicity.

Analysis of GO and KEGG enrichment

To elucidate the biological functions and pathways connected to frequently expressed genes, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed with the R package "clusterProfiler" [17]. Visualization of the enrichment findings was achieved via bubble plots, with a significance threshold established at $P < 0.05$.

Chemical-gene-disease network Building

To explore the relationship between BaP and periodontitis, a network illustrating the interactions between BaP, genes, and periodontitis was constructed. Through this analysis, we identified and evaluated the chemicals associated with the key genes. First, an attribute table was built in Excel, containing information on the compounds, their corresponding targets, and the diseases related to these targets. Next, the attribute table was uploaded to Cytoscape, where network files were loaded, positions set, and image shapes modified. The compound-target-pathway network visually shows how compounds affect diseases through their targets.

Construction and validation of nomogram

The nomogram was constructed using the characteristic genes of the normal group and the periodontitis group, along with their corresponding expression levels. The nomogram can serve as an important tool for the clinical diagnosis of periodontitis. Additionally, a periodontitis diagnosis model based on diagnostic markers was constructed using the "rms" R software. The values of candidate genes were quantified as "points", and the total score was determined by summation. Subsequently, the cumulative scoring system was subsequently applied to calculate the periodontal disease occurrence probability. The diagnostic effectiveness of the nomogram for identifying periodontitis was assessed via the Receiver Operating Characteristic (ROC) curve and its corresponding Area Under the Curve (AUC). Furthermore, the clinical applicability of the nomogram was evaluated using the Decision Curve Analysis (DCA).

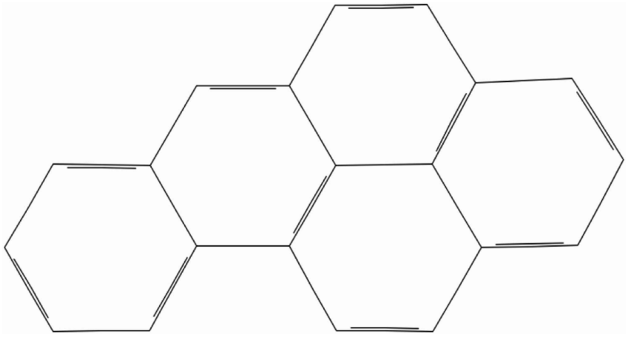

Fig. 1 Structure of BaP. The image sourced from the PubChem website

Development of protein-protein interaction network and target identification

Genes that intersected and indicated potential targets for BaP-related toxicity in periodontitis were entered into the STRING database. The analysis was confined to the species "Homo sapiens," with the "minimum required interaction score" parameter configured to "medium confidence > 0.4." Following this, the outcomes produced by STRING were transferred into Cytoscape software (version 3.10.0). Furthermore, the Network Analyzer plugin was employed to determine the Degree value for each individual target, after which the PPI network was visualized according to the Degree values' magnitude.

Molecular docking and molecular dynamics simulation

Molecular docking was carried out utilizing the CB-Dock2 online platform, a tool for molecular docking founded on AutoDock Vina (https://cadd.labshare.cn/cb-dock2) [18]. All settings were maintained at their default options. Molecular structures of the compounds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), whereas the protein structures for CXCL12 and HMGCR were acquired from the RCSB Protein Data Bank (https://www.rcsb.org/). The protein structure of CYP24A1 was sourced from the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/entry/Q07973).

All molecular dynamics (MD) simulations were performed using GROMACS 2023.2 software under physiological conditions (300 K, 1 bar). The AMBER14SB force field was employed for protein parameterization, with water molecules represented by the TIP3P explicit solvent model [19]. System charge neutralization was achieved through the addition of Na + and Cl – counterions using the genion utility. Energy minimization was carried out using both steepest descent. Equilibrium state simulations of NVT and NPT were subsequently performed. Formal simulations were run for 100 ns in 0.002 ps time steps, with a total of 50,000,000 steps performed. The stability of the simulated system was estimated by root mean square deviation (RMSD).

Result

Identification of targets for BaP-induced periodontitis toxicity

In this study, we retrieved BaP from the PubChem database and screened its standard structure (Fig. 1). Initially, we identified 100 BaP targets from the CTD and SwissTargetPrediction databases. Through differential analysis of the GSE10334 dataset, we determined 625 targets highly associated with periodontitis lesions, including 390 upregulated and 235 downregulated differentially expressed genes compared to the vehicle control group (Fig. 2A). A Venn diagram revealed 11 intersecting

targets from these two datasets as potential targets for BaP-induced periodontitis toxicity (Fig. 2B).

Network analysis of BaP-induced periodontitis

A target network diagram for BaP-induced periodontitis was constructed using Cytoscape, forming a " Chemical-gene-disease " network. As shown in Fig. 3, blue represents BaP, orange denotes key targets, and red signifies the disease. This diagram provides information on the relevant targets of BaP-induced periodontitis.

Functional enrichment analysis of co-associated genes

We further investigated the potential functions of these 11 intersecting targets through GO and KEGG analyses (Fig. 4A-B). GO enrichment analysis indicated that the intersecting targets primarily influence biological processes related to cellular response to lipopolysaccharide, endoplasmic reticulum lumen, cytokine activity, and cytokine receptor binding. Additionally, KEGG enrichment analysis showed that the intersecting targets mainly affect the IL-17 signaling pathway and TNF signaling pathway.

Identification of pivotal targets via multiple machine learning approaches

We employed three distinct machine learning methodologies (SVM, LASSO regression, and RF) to identify crucial target genes. The analysis through SVM yielded 5 promising target candidates (Fig. 5A). RF analysis generated a hierarchical ranking of genes based on their significance metrics (Fig. 5B). Concurrently, LASSO regression analysis revealed 5 potential key targets (Fig. 5C, D). To determine the most reliable targets, we analyzed the overlap between the SVM-identified candidates, the top 7 RF-ranked genes, and LASSO-selected targets using a Venn diagram (Fig. 5E). This integrative analysis identified three essential targets (CXCL12, CYP24A1, and HMGCR) in BaP-mediated periodontal damage.

Construction nomogram

To estimate the prevalence of periodontitis patients, a diagnostic nomogram was drawn using the core target genes (CXCL12, CYP24A1, and HMGCR) (Fig. 6A). The area under the ROC curve of the histogram was 0.922, indicating a high prediction accuracy (Fig. 6B). The slope of the calibration curve was close to 1, indicating that the histogram had a significant predictive efficacy (Fig. 6C). In addition, the DCA results showed that the histogram had a good net benefit (Fig. 6D).

Molecular Docking and MD of bap and core target proteins

The potential interactions between BaP and the core target genes (CYP24A1, CXCL12, and HMGCR) were investigated through molecular docking analysis. The docking results were generated respectively using the CB - DOCK2 software (Fig. 7A-C). Among them, CYP24A1 and BaP showed relatively low binding energies (CYP24A1 - BaP: −10.9 kcal/mol; CXCL12 - BaP: −6.8 kcal/mol; HMGCR - BaP: −8.6 kcal/mol), indicating a strong affinity between the compound and the target. Building upon molecular docking predictions, we extended our investigation through 100ns MD simulations of BaP complexes with three critical targets: CYP24A1, HMGCR, and CXCL12. Comparative analysis of backbone RMSD trajectories revealed distinct stabilization patterns (Fig. 7D). The CYP24A1-BaP and HMGCR-BaP complexes demonstrated exceptional structural stability, maintaining RMSD fluctuations within 0.2–0.3 nm throughout the simulation. In contrast, the CXCL12-BaP system exhibited greater conformational flexibility, with RMSD values ranging from 0.2 to 0.6 nm. Notably, all systems achieved equilibrium within the first 60 ns of simulation, as evidenced

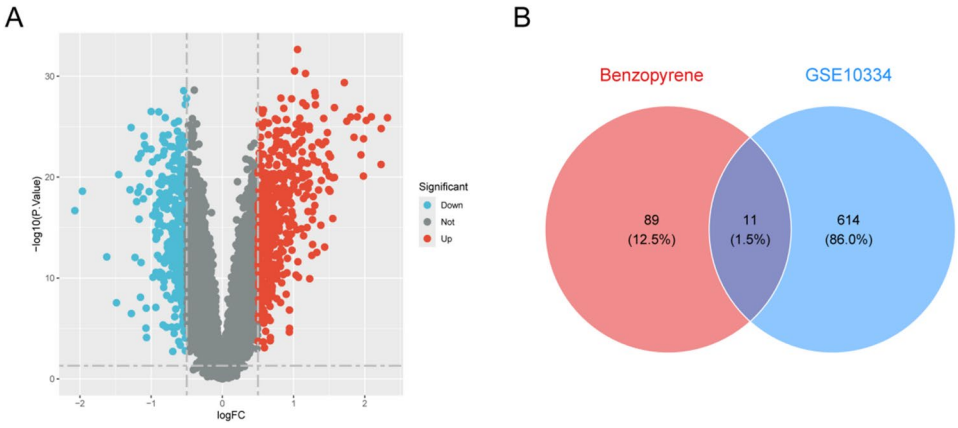

Fig. 2 Characterization of molecular signatures in periodontitis. (**A**) Differential gene expression profile visualized through volcano plot analysis. (**B**) The Venn diagram reveals overlapping genes between periodontitis-associated DEGs and benzopyrene-responsive targets

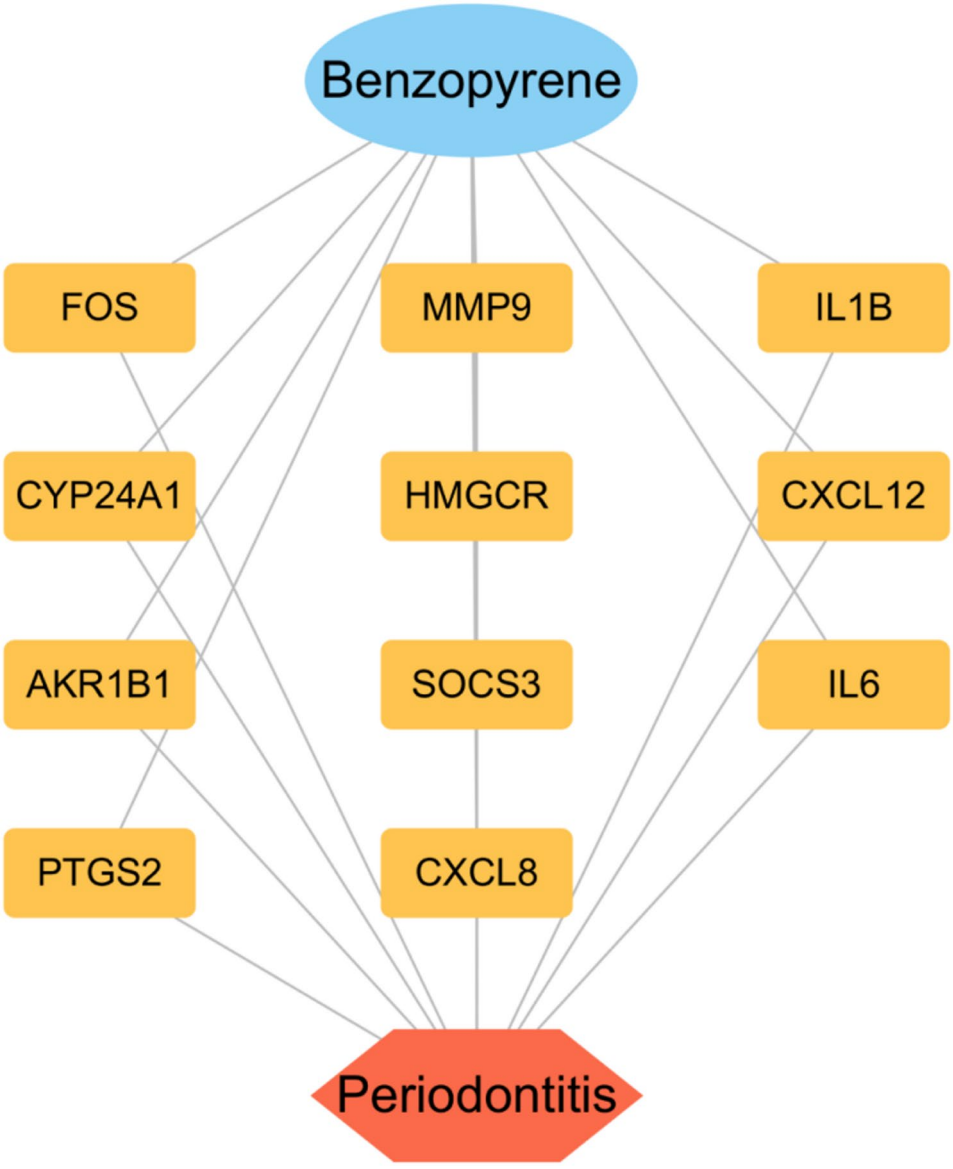

Fig. 3 Network analysis of Benzopyrene-induced Periodontitis. "Chemical-gene-disease" network diagram of Periodontitis induced by BaP

by plateauing RMSD values that remained stable within ± 0.05 nm during the final 40 ns. This persistent stability suggests robust ligand-target interactions and validates the physiological relevance of our simulation conditions.

Discussion

Periodontitis is a chronic inflammatory disease, mainly manifested as gingival inflammation, periodontal tissue destruction, and alveolar bone resorption, which seriously affects global oral health [20]. There is a significant association between environmental pollutants and the development of periodontal diseases. BaP, as a representative substance of persistent organic pollutants, is widely present in cigarette smoke [21]. Epidemiological studies have shown that people exposed to smoking exhibit a higher incidence of periodontitis [22]. However, whether BaP in cigarette smoke has an inductive effect has not been fully elucidated. Our study, for the first time, systematically revealed the key molecular network related to BaP and periodontitis by integrating network toxicology and molecular docking methods. In this study, we systematically screened 11 potential targets related to BaP-induced periodontitis using the SwissTargetPrediction, CTD, and GEO databases. A "Chemical-gene-disease" network of BaP-gene-periodontitis was constructed. Subsequently, we employed three different machine learning methods to identify the target genes with the most significant BaP-related periodontitis toxicity among these 11 core targets. Finally, we identified three core genes (CXCL12, CYP24A1, and HMGCR) as the key targets in

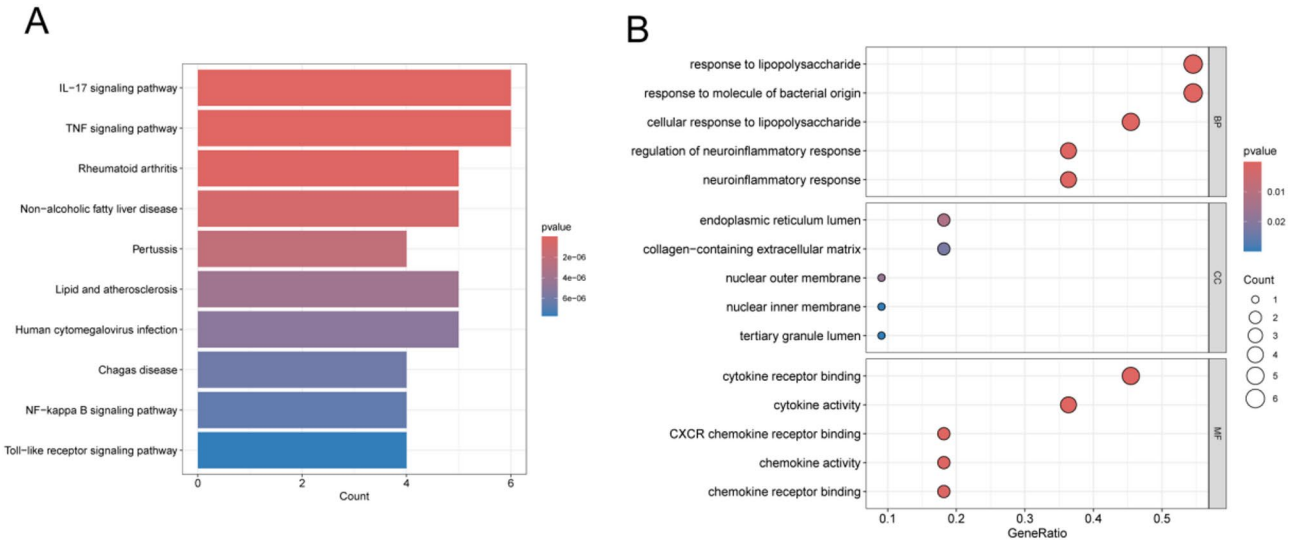

Fig. 4 Functional analysis of potential targets. (**A**) Top 10 pathways of the target genes in the KEGG enrichment analysis (**B**). the enriched entries for each GO category (BP: Biological Process, CC: Cellular Component, and MF: Molecular Function)

the context of BaP-induced periodontitis. These findings provide a new perspective for understanding the molecular mechanism of BaP-induced periodontitis, and also offer potential intervention targets for the development of prevention and treatment strategies.

In this study, network toxicology and bioinformatics tools were used to identify 11 potential co-associated genes related to periodontitis. GO enrichment analysis showed that they mainly affect biological processes related to the cell's response to lipopolysaccharide, endoplasmic reticulum lumen, cytokine activity, and cytokine-receptor binding. These results suggest that BaP may influence periodontitis through the endoplasmic reticulum and cytokine activity. KEGG enrichment analysis pointed to the IL-17 signaling pathway and the TNF signaling pathway. During the development of periodontitis, neutrophil extracellular traps (NETs) can trigger an up - regulation of the IL-17/Th17 response and bone destruction [23]. TNF is a pro - inflammatory cytokine that regulates various key pathways in inflammation, including the recruitment, activation, and survival of immune cells [24]. An increased expression of the TNF signaling pathway during the occurrence of periodontitis has also been widely reported. In addition, BaP enhances the expression of proteins related to the TNF-α pathway (TNF-α, NF-κB, Caspase3, and Caspase8) [25]. Therefore, we speculate that BaP may lead to the development of PD by affecting the IL – 17 signaling pathway and the TNF signaling pathway.

An important finding of our study is that we screened out three core targets (CXCL12, CYP24A1, and HMGCR) through three machine learning methods. CXCL12 (CXC-chemokine ligand 12) is a potent chemotactic inducer and belongs to the CXC-chemokine family. During development, it is widely expressed in many tissues and is a powerful chemotactic inducer of hematopoietic cells. It has been shown to promote the migration of hematopoietic cells across the endothelial barrier [26]. During the development of periodontal diseases, the level of CXCL12 increases, which may recruit host defense cells to the site of inflammation, thus participating in the activation of the immune defense pathways of periodontal diseases [27, 28]. The observed downregulation of CXCL12 in smokers with high BaP exposure aligns with previous reports linking impaired CXCL12 signaling to dysregulated immune responses in chronic inflammatory conditions [29, 30]. Based on the above results, we hypothesized that BaP-induced downregulation of CXCL12 may decrease the chemotaxis of inflammatory cells (e.g., neutrophils, macrophages) to periodontal tissues and promote the development of inflammation. CYP24A1, cytochrome P450 family 24 subfamily A member 1, initiates the degradation of vitamin D through side-chain hydroxylation [31]. Vitamin D insufficiency represents a significant risk factor in periodontitis pathogenesis, potentially compromising alveolar bone density. During periodontal disease development, enhanced CYP24A1 expression may accelerate vitamin D degradation, potentially exacerbating disease progression [32]. Our results extend these observations, proposing that BaP may indirectly suppress periodontal tissue repair by enhancing CYP24A1 activity, thereby accelerating vitamin D catabolism and compromising bone homeostasis. HMGCR serves as the key regulatory enzyme in cholesterol biosynthesis via the mevalonate pathway [33]. Evidence suggests that NF-κB pathway activation through IKK enhances cholesterol synthesis by upregulating mevalonate pathway flux [34]. A large number of

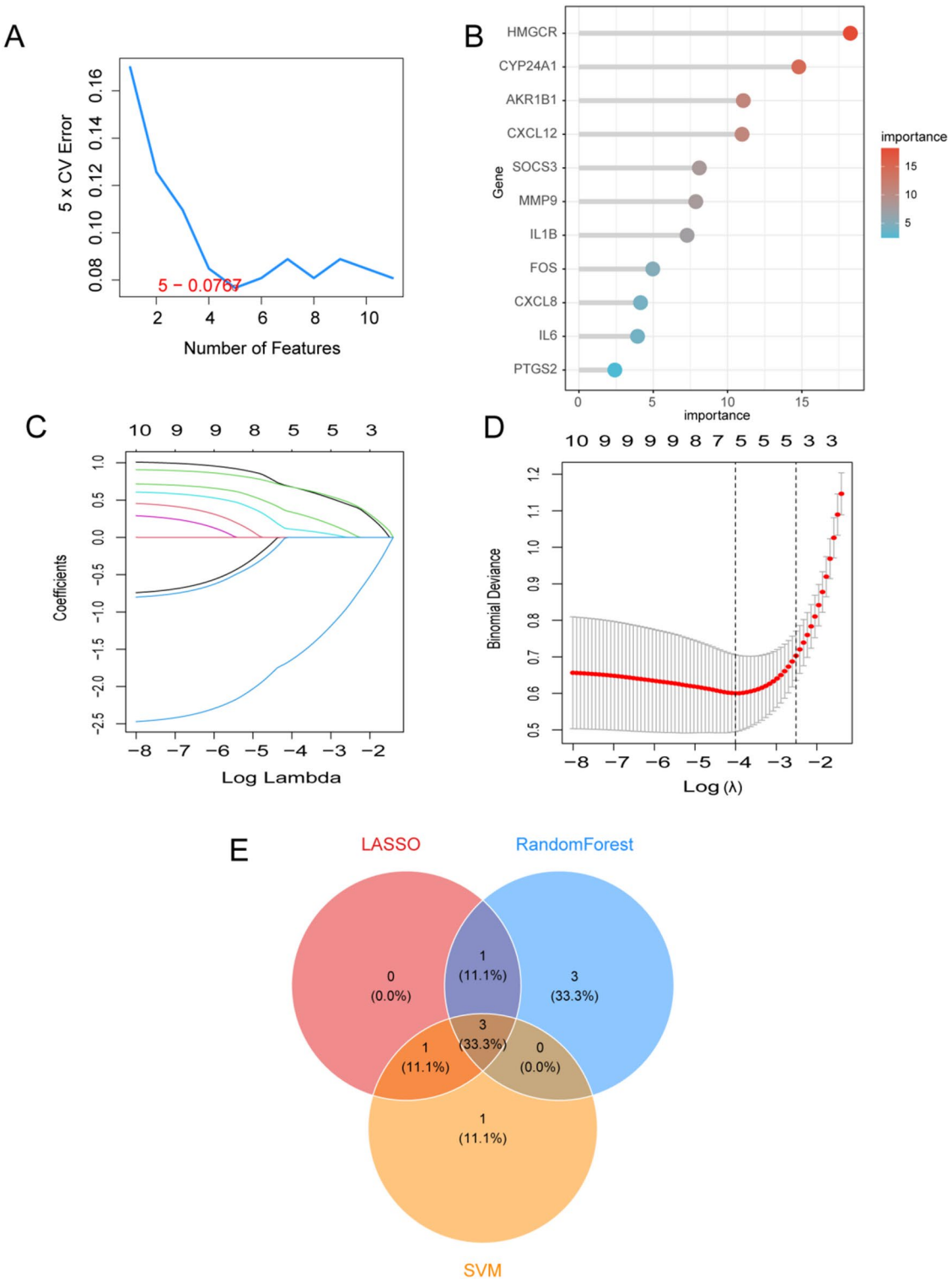

Fig. 5 Pivotal targets in benzopyrene-associated periodontitis. (**A**) SVM-RFE technique identified five high-confidence biomarkers. (**B**) RF classification algorithm-derived candidate targets. (**C**) and (**D**). LASSO regression coefficient trajectories demonstrating tuning parameter (λ) selection. The optimal model configuration (minimum cross-validation error) retained five predictive biomarkers. (**E**) the Venn diagram reveals consensus core targets established through integrative analysis of three distinct machine learning outputs

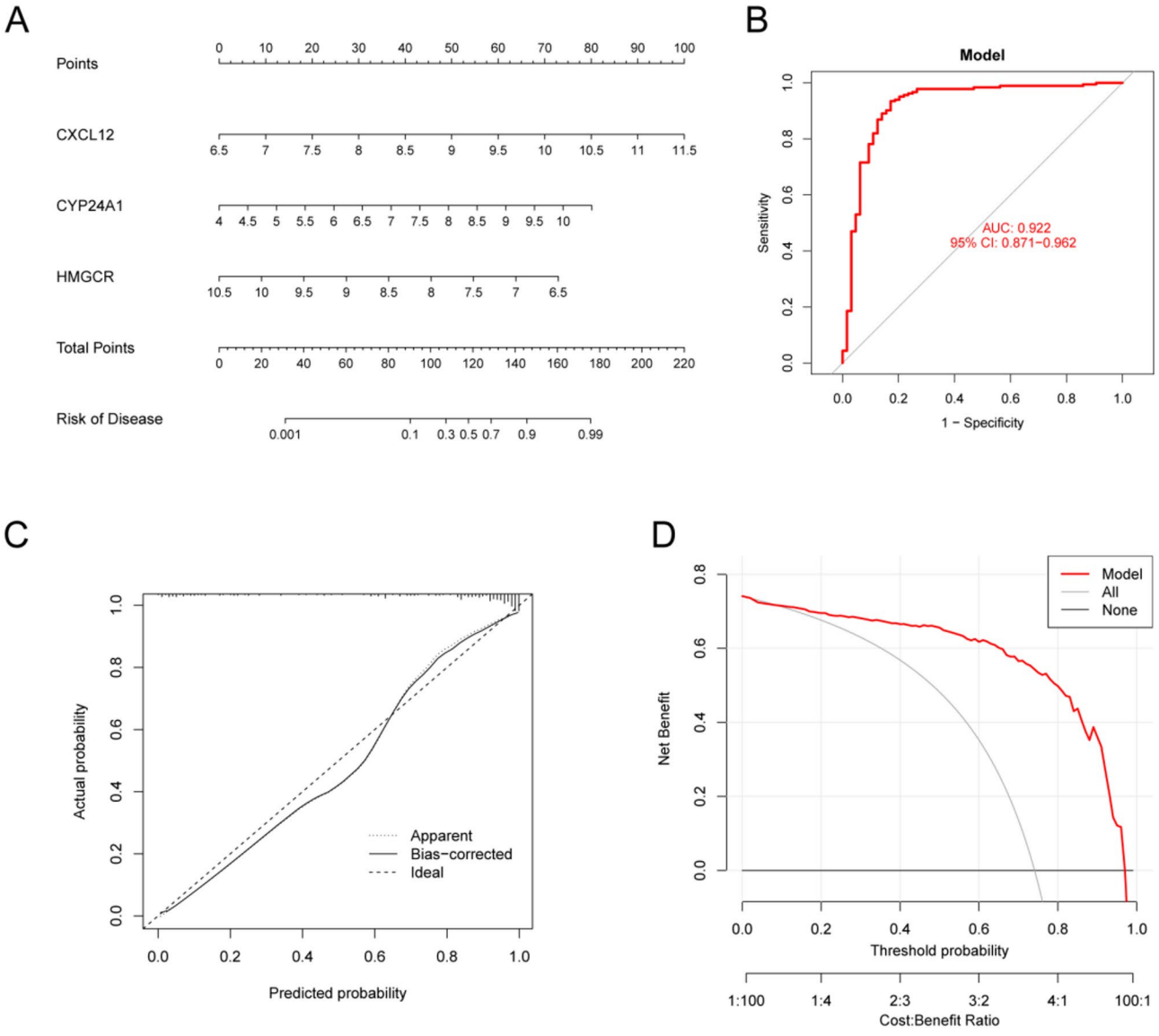

Fig. 6 Clinical predictive model development and performance validation. (**A**) Multivariate prognostic nomogram incorporating molecular signatures. (**B**) ROC analysis of GEO dataset demonstrating diagnostic accuracy. (**C**) Calibration curves demonstrating concordance between predicted (x-axis) and observed (y-axis) disease probabilities, with 45°reference line indicating ideal prediction. (**D**) DCA curve assessment nomogram model accuracy

studies have shown the activation of the NF-κB pathway in periodontitis. Therefore, we speculate that during periodontal diseases, NF-κB activation exerts an influence by increasing the mevalonate pathway. Finally, our molecular docking results showed that all three core target proteins had a strong binding to BaP, with binding energies all below − 5.0 kcal/mol [35, 36]. This indicates that BaP can spontaneously bind to each core target protein and plays an important role in the molecular mechanisms of BaP-induced periodontitis toxicology and damage.

Although this study has elucidated the molecular mechanism of BaP-induced periodontitis through multidimensional analysis, several limitations need to be considered. Firstly, our computational models are mainly based on known molecular pathways and protein structures, which may overlook some as yet undiscovered mechanisms of action. Secondly, due to technical limitations, this study was unable to validate all the predicted molecular targets in vivo. Especially when considering the complex physiological environment, the interactions between molecules may differ from those predicted in vitro. In addition, this study only focused on the direct effects of BaP and failed to fully investigate the possible impacts of its metabolites. These metabolites may have different toxic mechanisms in the organism. Finally, the prediction accuracy of machine learning models depends heavily on the quality and completeness of the training data, and there may be prediction biases, which

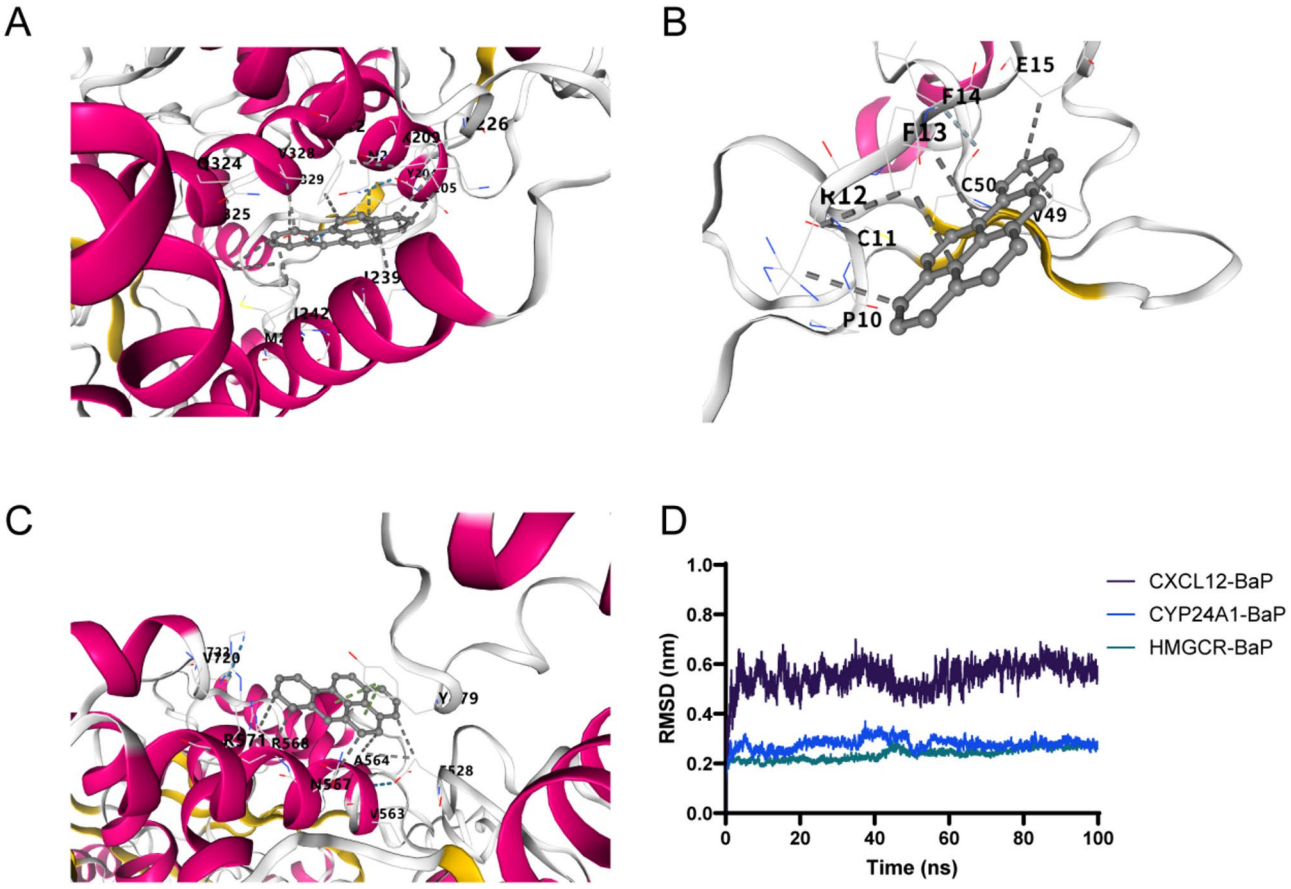

Fig. 7 Molecular docking and MD results of three key targets. The docking between BaP and CYP24A1 (**A**), BaP and CXCL12 (**B**), BaP and HMGCR (**C**). (**D**) Molecular dynamics simulation of RMSD of protein and ligand at 100 ns

require further experimental verification. Future research should focus on validating these findings through in vivo experiments.

Conclusion

In conclusion, this study has successfully integrated network toxicology, machine learning, and molecular docking approaches to comprehensively investigate the molecular mechanisms of BaP-induced periodontitis. Through systematic database screening and advanced computational analyses, we identified 11 candidate targets associated with BaP-induced periodontitis. The novel application of three machine learning algorithms refined these targets to three core proteins (CXCL12, CYP24A1, and HMGCR), whose interactions with BaP were validated through molecular docking analysis showing binding energies below −5.0 kcal/mol. Additionally, we developed a diagnostic nomogram based on these core targets that achieved high prediction accuracy (AUC = 0.922) for periodontitis diagnosis, demonstrating potential clinical utility. While our computational predictions require experimental validation, and the effects of BaP metabolites warrant further investigation, this study provides significant insights into the molecular mechanisms of BaP-induced periodontitis. Our findings not only advance the understanding of environmental pollutant-induced oral diseases but also demonstrate the effectiveness of integrated computational approaches in toxicological research. Future studies should focus on experimental validation of these findings and the development of targeted therapeutic strategies based on the identified molecular mechanisms, potentially leading to more effective treatments for environmental pollutant-induced periodontitis.

Abbreviations

BaP	Benzopyrene
DEGs	Differentially expressed genes
RF	Random Forest
SVM	The Support Vector Machine
LASSO	Least Absolute Shrinkage and Selection Operator
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
ROC	Receiver Operating Characteristic
AUC	Area Under the Curve
DCA	Decision Curve Analysis

Acknowledgements

Not applicable.

Author contributions
Data curation, Formal analysis and Funding acquisition: WWJ and ZPP; Project administration and Supervision: DC and LR; Writing—original draft: ZDL, WWJ; Writing—review and editing: ZDL. All authors have read and agreed to the published version of the manuscript.

Funding
This work was supported by the Doctoral Research Initiation Fund of Wannan medical college (Grant No: WYRCQD2023028), Anhui Province Engineering Research Center for dental materials and application (Grant No: 2024AMCD06), Program for Excellent Sci-tech innovation Teams of Universities in Anhui Province (Grant No: 2023AH010073), Anhui Key University Natural Science Research Project (No. 2024AH051905), and the Applied Basic Research Project of Wuhu Science and Technology Bureau (2022jc30).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 7 March 2025 / Accepted: 2 June 2025
Published online: 06 June 2025

References

1. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, et al. Periodontitis: consensus report of workgroup 2 of the 2017 world workshop on the classification of periodontal and Peri-Implant diseases and conditions. J Periodontol. 2018;89(Suppl 1):S173–82.
2. Global burden. Of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the global burden of disease study 2019. Lancet. 2020;396(10258):1204–22.
3. Tonetti MS, Jepsen S, Jin L, Otomo-Corgel J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. J Clin Periodontol. 2017;44(5):456–62.
4. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015;15(1):30–44.
5. Meyle J, Chapple I. Molecular aspects of the pathogenesis of periodontitis. Periodontol 2000. 2015;69(1):7–17.
6. Pandics T, Major D, Fazekas-Pongor V, Szarvas Z, Peterfi A, et al. Exposome and unhealthy aging: environmental drivers from air pollution to occupational exposures. Geroscience. 2023;45(6):3381–408.
7. Bukowska B, Sicińska P. Influence of Benzo(a)pyrene on different epigenetic processes. Int J Mol Sci. 2021;22(24).
8. Kim KH, Jahan SA, Kabir E, Brown RJ. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ Int. 2013;60:71–80.
9. Akinkugbe AA, Slade GD, Divaris K, Poole C. Systematic review and Meta-analysis of the association between exposure to environmental tobacco smoke and periodontitis endpoints among nonsmokers. Nicotine Tob Res. 2016;18(11):2047–56.
10. Hartung T, FitzGerald RE, Jennings P, Mirams GR, Peitsch MC, et al. Systems toxicology: real world applications and opportunities. Chem Res Toxicol. 2017;30(4):870–82.
11. Spurgeon DJ, Jones OA, Dorne JL, Svendsen C, Swain S, et al. Systems toxicology approaches for Understanding the joint effects of environmental chemical mixtures. Sci Total Environ. 2010;408(18):3725–34.
12. Chen L, Cao Y, Zhang H, Lv D, Zhao Y, et al. Network pharmacology-based strategy for predicting active ingredients and potential targets of Yangxinshi tablet for treating heart failure. J Ethnopharmacol. 2018;219:359–68.
13. Jung YS, Kim Y, Cho YR. Comparative analysis of network-based approaches and machine learning algorithms for predicting drug-target interactions. Methods. 2022;198:19–31.
14. Toubiana D, Puzis R, Wen L, Sikron N, Kurmanbayeva A, et al. Combined network analysis and machine learning allows the prediction of metabolic pathways from tomato metabolomics data. Commun Biol. 2019;2:214.
15. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019;47(W1):W357–64.
16. Davis AP, Wiegers TC, Sciaky D, Barkalow F, Strong M, et al. Comparative toxicogenomics database's 20th anniversary: update 2025. Nucleic Acids Res. 2025;53(D1):D1328–34.
17. Yu G, Wang LG, Han Y, He QY. ClusterProfiler: an R package for comparing biological themes among gene clusters. Omics. 2012;16(5):284–7.
18. Liu Y, Yang X, Gan J, Chen S, Xiao ZX, et al. CB-Dock2: improved protein-ligand blind Docking by integrating cavity detection, Docking and homologous template fitting. Nucleic Acids Res. 2022;50(W1):W159–64.
19. Chen JH, Li KX, Fan CF, Yang H, Zhang ZR et al. Integration of machine learning and experimental validation reveals new lipid-lowering drug candidates. Acta Pharmacol Sin. 2025.
20. Walther KA, Gröger S, Vogler JAH, Wöstmann B, Meyle J. Inflammation indices in association with periodontitis and cancer. Periodontol 2000. 2024;96(1):281–315.
21. Zhao C, Jin H, Lei Y, Li Q, Zhang Y, et al. The dual effects of Benzo(a)pyrene/Benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide on DNA methylation. Sci Total Environ. 2024;950:175042.
22. Chaffee BW, Couch ET, Vora MV, Holliday RS. Oral and periodontal implications of tobacco and nicotine products. Periodontol 2000. 2021;87(1):241–53.
23. Kim TS, Silva LM, Theofilou VI, Greenwell-Wild T, Li L et al. Neutrophil extracellular traps and extracellular histones potentiate IL-17 inflammation in periodontitis. J Exp Med. 2023;220(9).
24. Neurath N, Kesting M. Cytokines in gingivitis and periodontitis: from pathogenesis to therapeutic targets. Front Immunol. 2024;15:1435054.
25. Zhao G, Wang Z, Huang Y, Ye L, Yang K, et al. Effects of benzoapyrene on migration and invasion of lung cancer cells functioning by TNF-α. J Cell Biochem. 2018;119(8):6492–500.
26. Imai K, Kobayashi M, Wang J, Ohiro Y, Hamada J, et al. Selective transendothelial migration of hematopoietic progenitor cells: a role in homing of progenitor cells. Blood. 1999;93(1):149–56.
27. Gao X, Guo Z, Wang P, Liu Z, Wang Z. Transcriptomic analysis reveals the potential crosstalk genes and immune relationship between IgA nephropathy and periodontitis. Front Immunol. 2023;14:1062590.
28. Gao J, Wu Z. M2 macrophage-derived exosomes enable osteogenic differentiation and inhibit inflammation in human periodontal ligament stem cells through promotion of CXCL12 expression. BMC Oral Health. 2024;24(1):1070.
29. Sahin Ersoy G, Zhou Y, İnan H, Taner CE, Cosar E, et al. Cigarette smoking affects uterine receptivity markers. Reprod Sci. 2017;24(7):989–95.
30. Khan F, Judge EP, Das JP, White D, Ingram C, et al. Effects of active chronic Cigarette-Smoke exposure on Circulating fibrocytes. Lung. 2024;202(4):431–40.
31. Tuckey RC, Cheng CYS, Li L, Jiang Y. Analysis of the ability of vitamin D3-metabolizing cytochromes P450 to act on vitamin D3 sulfate and 25-hydroxyvitamin D3 3-sulfate. J Steroid Biochem Mol Biol. 2023;227:106229.
32. Chen H, Peng L, Wang Z, He Y, Zhang X. Integrated machine learning and bioinformatic analyses constructed a network between mitochondrial dysfunction and immune microenvironment of periodontitis. Inflammation. 2023;46(5):1932–51.
33. McGovern AJ, González J, Ramírez D, Barreto GE. Identification of HMGCR, PPGARG and prohibitin as potential druggable targets of dihydrotestosterone for treatment against traumatic brain injury using system Pharmacology. Int Immunopharmacol. 2022;108:108721.
34. Heida A, Gruben N, Catrysse L, Koehorst M, Koster M, et al. The hepatocyte IKK:NF-κB axis promotes liver steatosis by stimulating de Novo lipogenesis and cholesterol synthesis. Mol Metab. 2021;54:101349.
35. Suttithumsatid W, Shah MA, Bibi S, Panichayupakaranant P. α-Glucosidase inhibitory activity of cannabidiol, tetrahydrocannabinol and standardized cannabinoid extracts from Cannabis sativa. Curr Res Food Sci. 2022;5:1091–7.

36. Wang N, Li Q. Simultaneous extraction and analysis of seven major saikosaponins from bupleuri Radix and the exploration of antioxidant activity and its mechanism. Molecules. 2023;28(15).

Publisher's note