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Research article

Berberine improved the microbiota in lung tissue of colon cancer and reversed the bronchial epithelial cell changes caused by cancer cells

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ABSTRACT

Objective: The lung is a common organ for colon cancer metastasis, and the objective of this experiment was to explore the protective effect of berberine on lung tissue or alveolar epithelial cells induced by colon cancer.

Methods: Thirty-six BALB/c nude mice were used to establish a xenograft model of colon cancer with the HT29 cell line and were treated with berberine and probiotics. Human bronchial epithelial BEAS-2B cells were induced by conditioned medium (CM) from the colon cancer cell lines HT29 and RKO and were treated with berberine. Lung tissues were collected to detect the changes in the microbiota using 16S rDNA sequencing and the expression of inflammatory cytokines. The expression of E-cadherin and N-cadherin in BEAS-2B cells was detected by cellular immunofluorescence. The changes in cell proliferation were detected by the CCK-8 assay. Western blotting was used to detect E-cadherin, N-cadherin, collagen I, fibronectin, PDGF-β, and RAD51 expression in BEAS-2B cells.

Results: The richness and evenness of the microbiota in the lung tissues of mice with colon cancer were significantly lower than those of the control group. Berberine significantly increased the abundances of *Bacteroidetes*, *Bacteroidia*, *Bacteroidales*, *Lactobacillaceae*, *Lactobacillus* and *Acinetobacter* in the lung tissue of mice with colon cancer, with reduced abundances of *Actinobacteria*, *Bacillales*, *Staphylococcaceae* and *Staphylococcus*. Berberine or probiotics significantly increased the alpha diversity of the lung microbiota. Compared with probiotics, berberine significantly enhanced the abundance of microbiota involved in the metabolism of lysosomes, flavone and flavonol biosynthesis, glycosaminoglycan degradation, and glycosphingolipid biosynthesis-ganglio. Berberine increased IL-6 and IL-10 and decreased IL-17 and IFN-γ expression in lung tissue ($P > 0.05$), but berberine-probiotics significantly decreased IL-17 and IFN-γ and increased IL-10 expression ($P < 0.05$). Colon cancer cells could not induce BEAS-2B proliferation but

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decreased the expression of the epithelial marker E-cadherin and altered the expression of extracellular matrix-related proteins (collagen I, fibronectin, and PDGF-β), which were reversed by berberine. Berberine increased RAD51 expression in BEAS-2B cells, which had been decreased by HT29 and RKO CM treatment.

Conclusion: Berberine can selectively regulate the abundance of some microbiomes of lung tissue in colon cancer, improve the inflammatory response in lung tissue, and antagonize the cancerous stimulation of colon cancer cells to lung tissue cells by regulating the bronchial epithelial cell phenotype, extracellular matrix remodelling and the expression of the repair gene RAD51.

1. Introduction

Although the early screening and prevention of colorectal cancer (CRC) have improved significantly, the mortality rate of colorectal cancer is still a serious threat to human health around the world, mainly due to distant metastases. Clinical studies have shown that the incidence of pulmonary metastasis of colon cancer is second only to liver metastasis [1].

Although the liver and/or lungs are usually metastatic sites of colon cancer, a previous study has demonstrated that lung instead of liver metastasis is more likely to occur in primary rectal cancer than in colon cancer [2], which may be related to their anatomical location. However, the factors that influence the anatomic pattern of metastasis are unclear. Indeed, increasing evidence has identified the role of intestinal microorganisms or the gut microbiota in the development and metastasis of colon cancer through induction of inflammation and immune dysregulation [3–5], genomic instability [6–8], and the production of microbial metabolites [9,10].

The conventional wisdom is that human microbes have the greatest impact on the gut, skin and mucous membranes, and the lungs are supposed to be permanently sterile. Less research has been devoted to the lung microbiome. However, numerous studies have shown that the lungs have a unique microbial community. Moreover, many findings have reported the role of the lung and gut microbiota in colorectal cancer and lung cancer [11]. In addition, the lung-gut axis has been established through bidirectional communication by neural and humoral mechanisms between the lung and the intestine [12]. Indeed, in Traditional Chinese medicine, there has been the theory "lung and large intestine being interior-exteriorly related" since ancient times. Based on the "lung-gut axis" theory, a variety of lung diseases including pneumonia and asthma can be treated by regulating intestinal flora or gut function [13,14]. In recent years, some scholars have proposed a "lung-gut co-treatment" therapy for progression and metastasis of lung cancer [15]. However, the effect of the intestinal microbiota in colon cancer on the lung microbiome is not clear.

Berberine, an isoquinoline quaternary alkaloid (Fig. S1), is considered an epiphany against cancer [16], with multiple anticancer biological mechanisms, including the regulation of dysbacteriosis. Our previous study showed that berberine can not only regulate the intestinal microbiota in colon cancer rat models but can also mediate the level of sodium butyrate, a metabolite of the bacterial microbiota [17]. However, the effect of berberine on the lung microbiota and tissue microenvironmental architecture in colon cancer is unclear.

In this study, we aimed to determine the effects of berberine on lung tissue microorganisms in colon cancer and related mechanisms using a nude mouse xenograft model and colon cancer cell-derived conditioned medium (CM). We demonstrated that berberine could improve the dysbacteriosis of lung tissues in a colon cancer mouse model by mediating serum inflammatory cytokines. Colon cancer cell-derived CM did not promote alveolar epithelial cell proliferation but changed the cell phenotypes and matrix protein expression in cells, which was regulated by berberine.

2. Materials and methods

2.1. Animal experiments

As described in our previous studies [17], 36 BALB/c nude mice (half male and half female) obtained from Guangdong Yaokang Biotechnology Co., Ltd. were used to establish a xenograft model of colon cancer with the HT29 cell line (Genio, Guangzhou, China, JNO-21409). The mice were divided into 4 groups, namely, the model, model + berberine (BBR), model + probiotics, and model + combination of berberine with probiotics groups (each group had 9 animals). The probiotics (Leizhi, Guangzhou, China) were live combined Bifidobacterium (BNCC232112), Lactobacillus (BNCC336974) and Enterococcus (BNCC192631) powder. The mice in the berberine (Shenyang, China) and berberine with probiotics groups were treated with berberine (78 mg/kg) and probiotics ($7.8 \times 10^{\wedge}6$ CFU/kg) by gavage at the beginning of modelling for 4 weeks. Eight contemporaneous mice were used as normal controls. At the end of the experiment, all mice were anaesthetized by subcutaneous injection of 1% pentobarbital sodium (50 mg/kg) to obtain lung tissues and serum and then were killed by cervical dislocation. After treatment with pentobarbital sodium for 2–3 min, all mice showed anesthetic effects without peritonitis, pain or discomfort. The blood was collected after anesthesia by stabbing into the heart for 2–3 ml of blood. Animal feeding and management were performed according to the "laboratory animal environment and facilities" (GB 14925-2010) and relevant animal ethics guidelines. The microbiota in the lung tissues was detected using 16S rDNA sequencing (as described in our previous study [14]. ELISA was used to detect the level of inflammatory cytokines in the serum. The mouse IL-6 ELISA kit (ml024300), mouse IL-10 ELISA kit (ml024264), mouse IL-17 ELISA kit (JKl037866) and mouse IFN-γ (JK058350) were from Mlbio (Shanghai, China).

2.2. Establishment of xenograft model of colon cancer in nude mice

The mouse colon cancer model was established according to our previous studies [17]. In brief, the colon cancer cell line HT29 was conventionally cultured, digested, and re-suspended. 200 μl cell suspension was inoculated subcutaneously on the back of nude mice for 4 weeks. All animal performance was conducted in accordance with animal ethics.

2.3. In vitro cell experiments

The human bronchial epithelial cell line BEAS-2B (FH0319) and the human colon cancer cell lines RKO (FH0030) and HT29 (FH0024) were purchased from FocusHerb (Shanxi, China). Foetal bovine serum (FBS) was purchased from Gibco (10,099–141). HT29 and RKO cells (2–5 $\times$ 10^6/ml) were conventionally cultured with 10% FBS for 48 h, and the medium was collected and named conditioned medium (CM). Then, HT29-and RKO-derived CM (25%) and 0.07 μmol/ml, 0.15 μmol/ml, and 0.3 μmol/ml berberine (BBR) were added to the cultured BEAS-2B medium for treatment for 24 h, 48 h, and 72 h, respectively. Cell proliferation of each group was detected by the CCK-8 assay.

2.4. CCK-8 detection

Cell proliferation was detected by the CCK-8 assay according to the instructions. In brief, CCK-8 solution was added to each well and incubated at 37 °C for 2 h. OD values were detected at a wavelength of 450 nm.

2.5. ELISA of inflammatory cytokines in mouse serum

Ten microlitres of serum sample with 40 μl of sample diluent was mixed in each well of the enzyme plate for IL-6, IL-10, IL-17, and IFN-γ. Then, 100 μl of horseradish peroxidase (HRP)-labelled mouse antibody was added, incubated at 37 °C for 1 h, and washed 3 times. Next, 50 μl/well stop buffer was added for 5 min, and the OD values were detected at a wavelength of 450 nm.

2.6. Cell immunofluorescence assay

In the culture plate, the cells were washed in PBS 3 times for 3 min each time. The cells were fixed with 4% paraformaldehyde for 15 min. Triton X-100 (0.5%, prepared with PBS) was used to permeabilize the membrane at room temperature for 20 min. Normal goat serum was added to the cells at room temperature for 30 min. Sufficient amounts of diluted rabbit anti-E-cadherin and anti-N-cadherin antibodies (1:600) were incubated overnight at 4 °C in a wet box. Fluorescent AF488-labelled goat anti-rabbit antibody was incubated at 37 °C in a wet box for 1 h. DAPI was added to avoid light and incubated for 5 min. The acquired images were observed under a Zeiss fluorescence microscope.

2.7. Western blotting

The relative expression of related proteins in BEAS-2B cells was detected by Western blotting (WB) in accordance with strict procedures. The cells were treated with ristocetin-induced platelet agglutination (RIPA) (plus 1% phenylmethanesulfonyl fluoride (PMSF)) lysis solution to extract total protein, followed by centrifugation at 12,000 rpm at 4 °C for 15 min. After the protein concentration was measured using a Bradford kit or bicinchoninic acid (BCA) kit, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), transfer to membranes, and blocking were also carried out. The membranes were immunologically reacted with anti-E-cadherin, anti-N-cadherin, anti-collagen I, anti-fibronectin, anti-PDGF-β, anti-RAD51, and anti-β-actin antibodies (1:1000) and HRP-labelled secondary antibodies (1:10,000) for 1 h, followed by treatment with ECL exposure solution. The protein bands were observed, and the grey values were measured.

2.8. Statistical analysis

In alpha diversity analysis, the Kruskal test function in R language was used to compare samples from more than two groups, and the alpha diversity index of significant differences under different conditions was screened by the rank sum test. Qimme software was used to analyse the beta diversity using PCA, PCoA and NMDS maps. The differences in taxonomic phyla, classes, orders, families and genera between groups were compared by the Kruskal method of the R-STATS package. The results in Fig. 4 were analyzed using the statistical software package SPSS 23.0. One-way ANOVA was used to compare the levels of inflammatory cytokines and the cellular proliferation rate between groups, and least significant difference (LSD) was used to perform multiple comparisons. $P < 0.05$ was considered to be statistically significant.

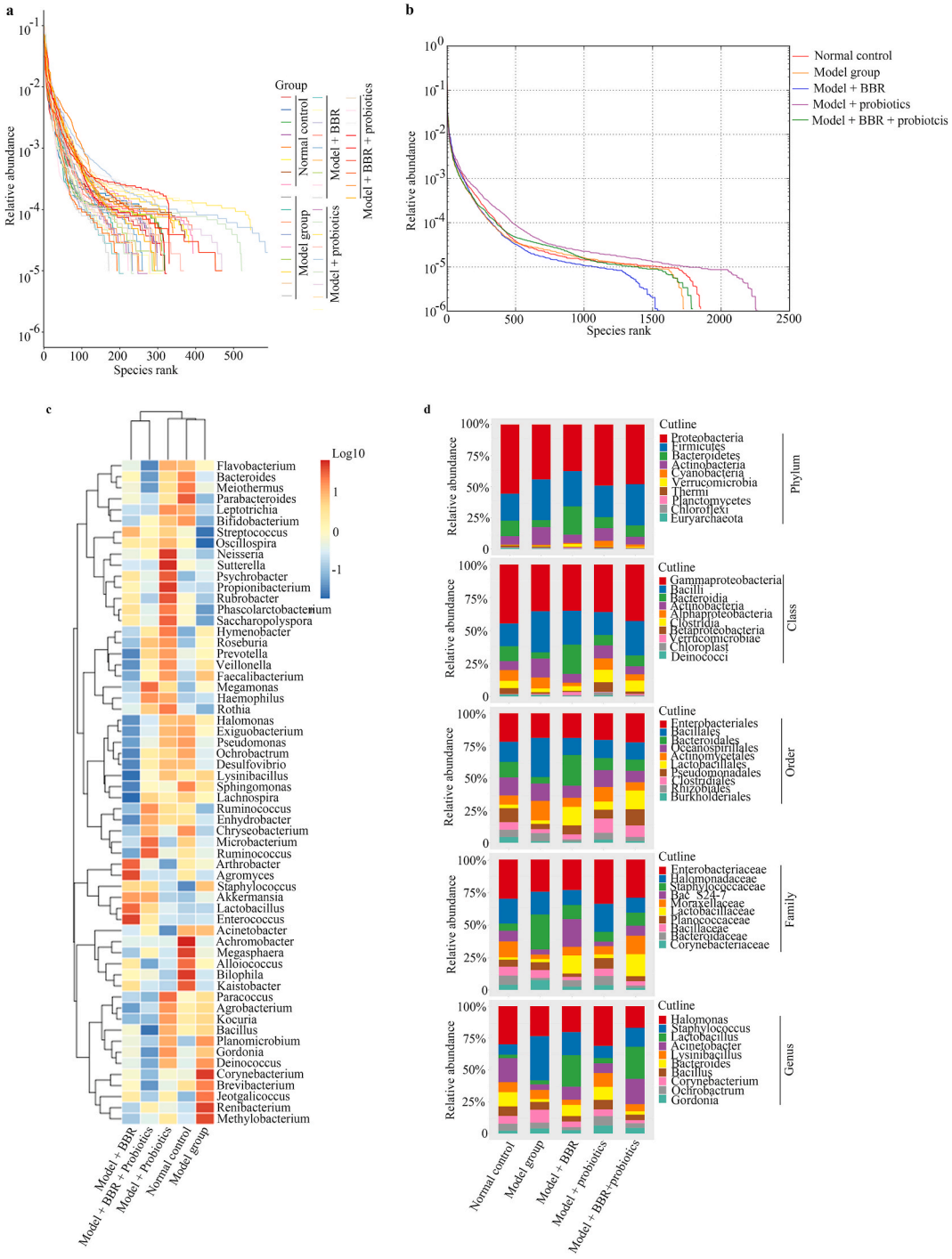

Fig. 1. Comparison of the microbiota richness and evenness in the lung tissue of colon cancer mice and the structure of the microbiota among the groups (n = 44). Abundance curves between samples (a) and groups (b). Cluster heatmap of the intergroup comparison at the species level (c). A bar chart of the top 10 species common to all samples (d).

3. Results

3.1. Berberine did not significantly improve the decreased richness and evenness of the microbiota in the lung tissue of the colon cancer mouse model, but it selectively regulated some microbiota

In our previous study [17], we reported the antitumor effect of berberine and probiotics on a mouse model of colon cancer. Specifically, the treatment of berberine and combination of berberine with probiotics reduced the growth of neoplasms in mice to a different extent ($P > 0.05$), especially at 14 days. The inhibitory effect of probiotics on tumor growth was more significant, especially at 11–21 days ($P < 0.05$). Therefore, in the present study, we further observed the effects of intervention of berberine and probiotics on intestinal microflora and studied the related mechanisms. As shown in Fig. 1a and b, the richness and evenness of the microflora in the lung tissue of the mouse model of colon cancer was significantly lower than that of the control group, which was further reduced by berberine treatment, while the intervention of probiotics significantly improved the richness and evenness, followed by the combined effect of berberine and probiotics.

As shown in Fig. 1c, at the genus level, the abundances of *Planomicrobium*, *Deinococcus*, *Corynebacterium*, *Brevibacterium*, *Jeotgalicoccus*, *Renibacterium* and *Methylobacterium* in the lung tissue of colon cancer mice increased significantly, while those of *Parabacteroides*, *Bifidobacterium*, *Streptococcus*, *Oscillospira*, *Phascolarctobacterium*, and *Saccharopolyspora* were significantly decreased compared with those of the control group. Cluster analysis showed that these phenomena were reversed in the berberine and berberine combined with probiotics (berberine-probiotics) group mice, and similar effects were found in the berberine and berberine-probiotics groups. In the probiotic mice, the abundances of *Parabacteroides*, *Bifidobacterium*, *Streptococcus*, *Oscillospira*, *Phascolarctobacterium* and *Saccharopolyspora* were significantly increased in lung tissue.

Furthermore, through the selection of the top 10 common species in all samples for bar chart analysis (Fig. 1d), at the phylum level, different from the probiotics and berberine-probiotics treatment group, although berberine did not increase the abundance of *Proteobacteria*, it could significantly increase the abundance of *Bacteroidetes* in the lung tissue of colon cancer mice, with a reduced

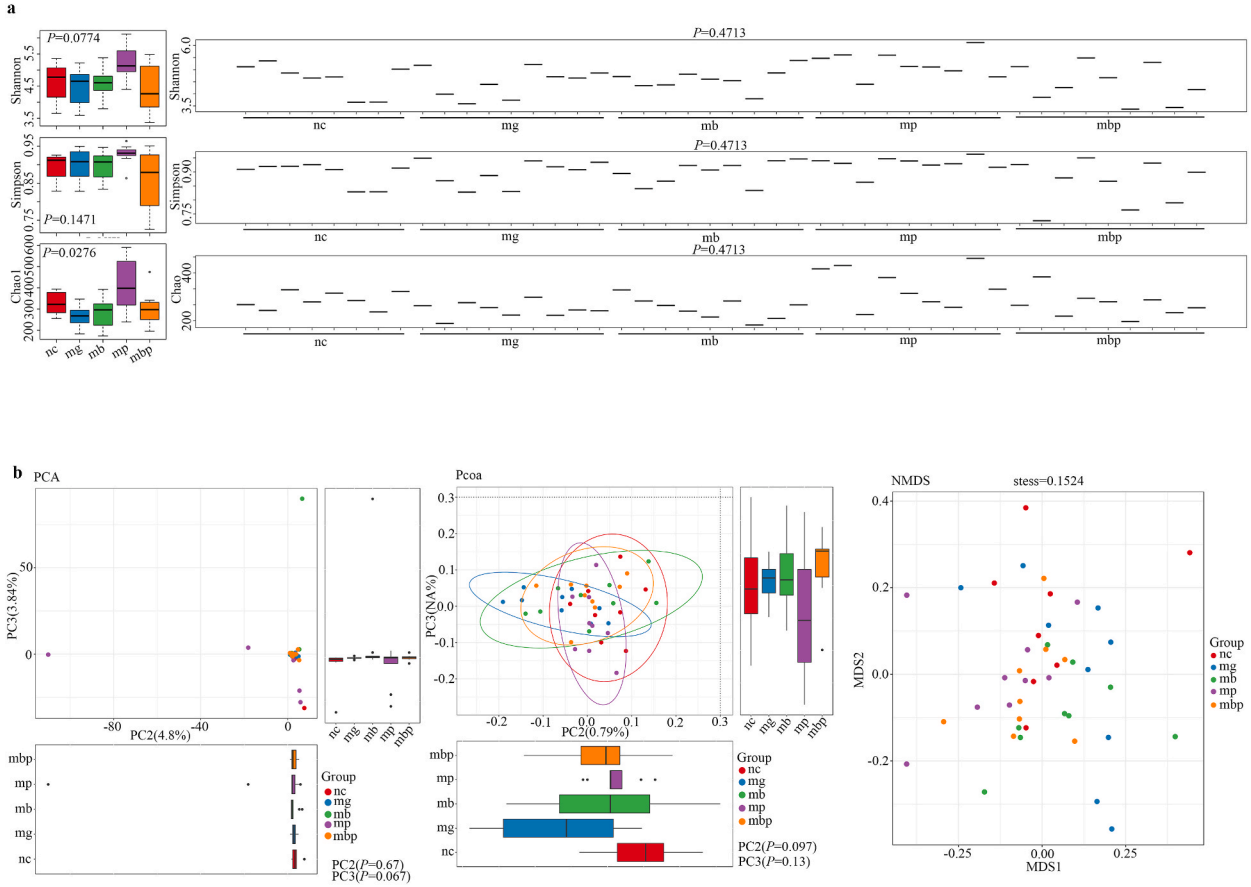

Fig. 2. Comparison of the biodiversity in the lung microbiota of colon cancer (n = 44). Alpha diversity index variance box plot (a). Beta diversity analysis (b). Differences between different samples (groups) were found based on multivariate statistical methods, including principal component analysis (PCA), principal coordinate analysis (PCoA) and NMDS analysis. For NMDS analysis, the stress function values should not be greater than 0.2.

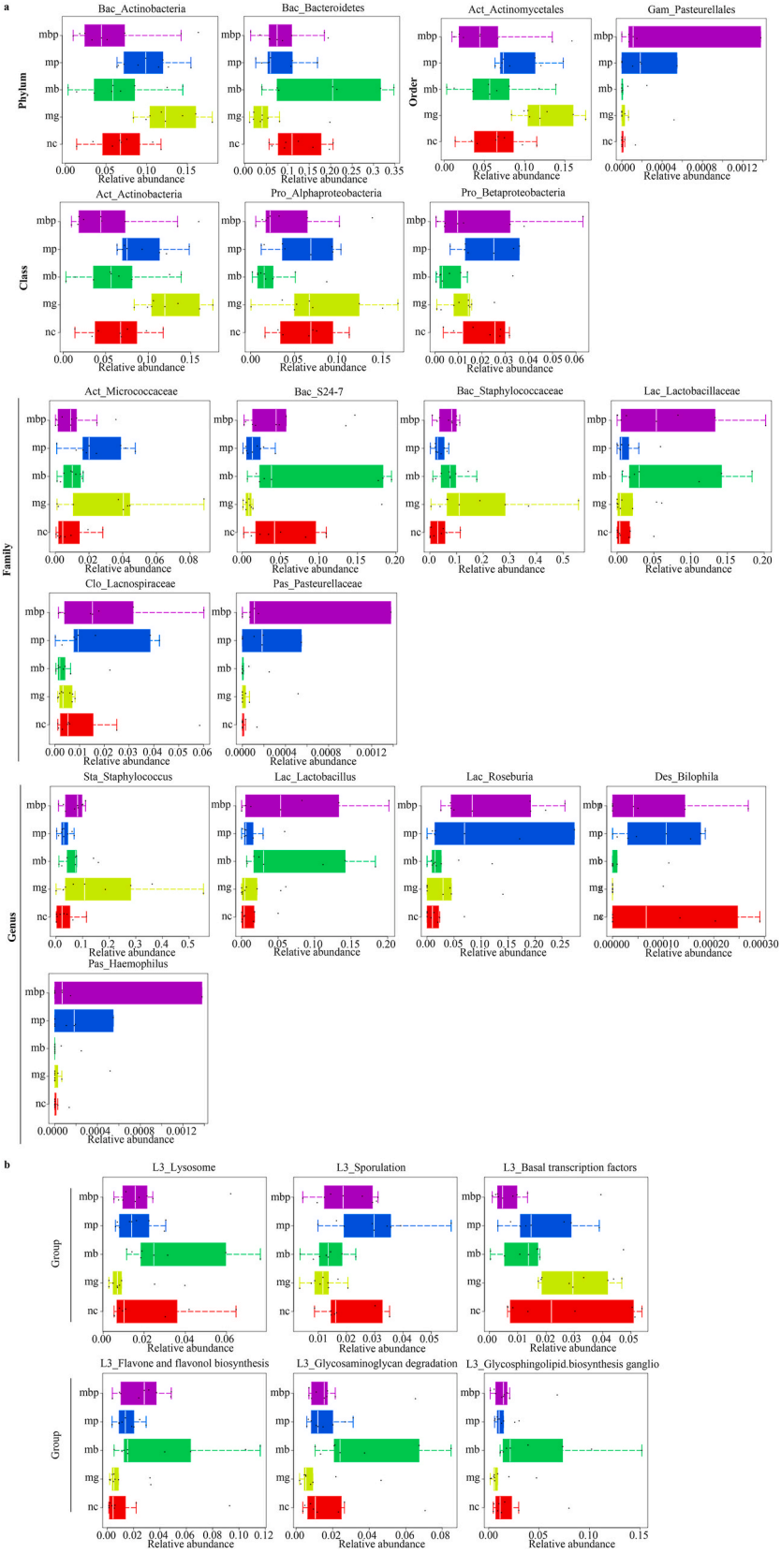

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Fig. 3. Comparison of species markers and metabolic functions of different levels of lung microbiota among the groups (n = 44). Species markers at different levels were selected for comparison of the microflora between the groups (a). Box diagram of the differences between groups predicting microbiota metabolic function based on KEGG level 3 (b).

abundance of *Actinobacteria*. At the class level, berberine-probiotics increased the abundance of Gammaproteobacteria and Bacilli, but probiotics showed a significant decrease in the abundance of Bacilli, followed by berberine. Simultaneously, the abundance of *Bacteroidia* increased significantly after berberine treatment and was even higher than that of the control group. At the order level, the abundance of *Bacillales* in the lung tissue of colonic carcinoma mice was obviously increased when treated with berberine or probiotics, but the abundance of *Bacteroidales* was obviously increased with berberine treatment. At the family level, the significantly increased *Staphylococcaceae* abundance in the lung tissues of colon cancer mice was significantly decreased with the treatment of berberine or probiotics, but berberine and berberine-probiotics could significantly increase the abundance of *Lactobacillaceae*. At the genus level, both berberine and probiotics significantly reduced the abundance of *Staphylococcus*, but berberine and berberine-probiotics significantly increased the abundances of *Lactobacillus* and *Acinetobacter*.

3.2. Berberine significantly increased the alpha diversity of the lung microbiota of colon cancer mice

The results of the box chart of the differences in three alpha diversity indices, namely, the Shannon, Simpson and Chao1 indices, showed that the difference in the alpha diversity index among the groups was statistically significant (Fig. 2a), and the lung microbiota alpha diversity of colon cancer mice treated with berberine and probiotics was significantly higher than that in the model group. However, for beta diversity analysis (Fig. 2b), principal component analysis (PCA) and principal coordinate analysis (PCoA) found no statistically significant differences among different samples (groups). For NMDS analysis of beta diversity, the value of STESS should not be greater than 0.2. In this study, the STESS value was 0.1524, indicating that berberine or probiotics did not significantly improve beta diversity differences between groups.

3.3. Berberine was selective in regulating the microbiota in colon cancer lung tissue and had a synergistic increasing effect with probiotics on the regulation of some microbiota

Species markers with statistically significant differences were selected by comparing the lung microbiota among groups (Fig. 3a). The results showed that at the phylum and class levels, the relative abundances of *Bac_Actinobacteria* and *Act_Actinomycetales* in the lung tissue of colon cancer model mice were significantly increased. However, berberine or berberine-probiotics significantly reduced these abundances, as did probiotics administration. The berberine group could significantly increase the relative abundance of *Bac_Bacteroidetes* compared with that in the probiotic group or berberine-probiotics group. At the order level, berberine or berberine-probiotics also significantly reduced the relative abundances of *Act_Actinobacteria* and *Pro_Alphaproteobacteria* in the lung tissue. Probiotics or berberine-probiotics increased the abundance of *Pro_Betaproteobacteria*, while berberine did not. At the family level, berberine or berberine-probiotics significantly reduced the abundances of *Act_Micrococcaceae* and *Bac_Staphylococcaceae*, but compared with probiotics and berberine-probiotics, the berberine group significantly increased the abundance of Bac_S24-7. Moreover, the abundance of *Lac_Lactobacillaceae* increased significantly under the influence of berberine or berberine-probiotics, while the effect of probiotics was not obvious. At the genus level, the abundances of *Sta_Staphylococcus* and *Lac_Roseburia* were significantly increased in the model group, while berberine, probiotics or berberine-probiotics significantly decreased the abundance of *Sta_Staphylococcus*. *Lac_Roseburia* increased significantly under probiotics or berberine-probiotics treatment. The abundances of *Des_Bilophila* and *Pas_Haemophilus* were significantly increased in the probiotic or berberine-probiotic group but not in the berberine group.

3.4. Berberine significantly improved the dysfunctional bacterial metabolism in colon cancer lung tissue

Based on the box diagram of the KEGG level 3 differences predicted by microbial metabolic function, we found that (Fig. 3b) the abundance of microbiota involved in lysosomes, sporulation, flavone and flavonol biosynthesis, glycosaminoglycan degradation, and glycosphingolipid biosynthesis-ganglio decreased significantly in the lung tissue of colon cancer mice, which was significantly enhanced by berberine. Although berberine also increased the abundance of bacteria involved in sporulation, the effects of probiotics or berberine-probiotics were more significant. In addition, treatment with berberine or probiotics did not seem to affect the abundance of the microflora involved in basal transcription factors, although the abundance of the model group was reduced.

3.5. Berberine improved the expression of inflammatory cytokines in colon cancer lung tissue, but it was more effective when combined with probiotics

Dysregulated expression of various inflammatory cytokines appeared in the lung tissues of colon cancer mice (Fig. 4a). Compared with levels in the control group, although the differences were not statistically significant, the expression levels of IL-6 and IL-10 were decreased (*P value* was 0.393 and 0.003) and the expression of IFN-γ and IL-17 was increased (*P value* was 0.006 and 0.009) in the model group. Compared with the model group, berberine treatment increased the expression of IL-6 and IL-10 (*P value* was 0.008 and 0.003) and decreased the expression of IL-17 and IFN-γ (*P value* was 0.009 and 0.006), while probiotic treatment significantly increased the expression of IL-10 (*P value* was 0.003 vs model group) and decreased the expression of IL-17 (*P value* was 0.009 vs model

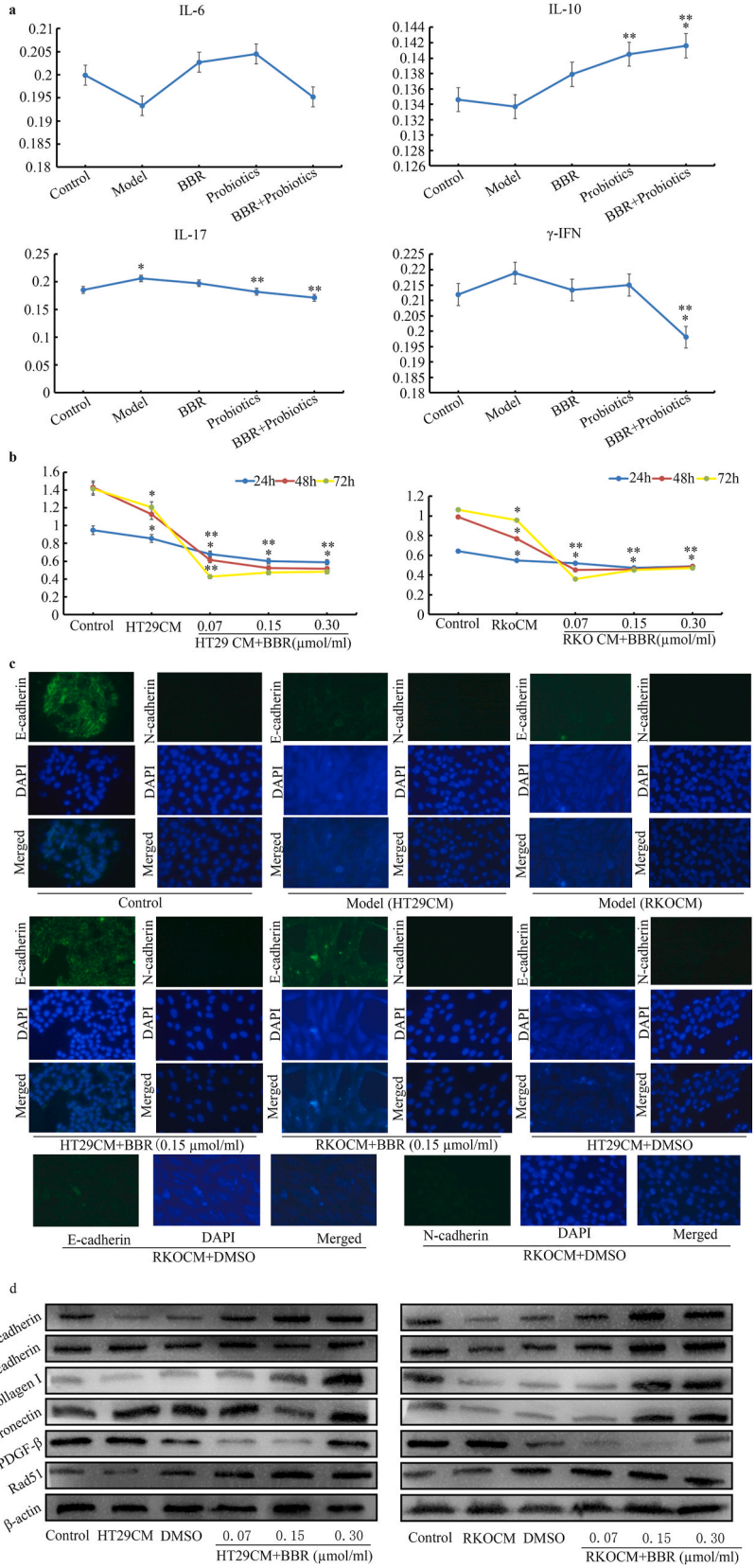

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Fig. 4. Changes in inflammatory cytokines in the lung tissue of colon cancer mice (n = 44) and *in vitro* experiments of bronchial epithelial cell induction by colon cancer cell lines. (a) Comparison of inflammatory cytokines (IL-6, IL-10, IL-17, and IFN-γ) in the lung tissue of colon cancer mice under different conditions. (b) Comparison of the proliferation ability of bronchial epithelial cell BEAS-2B cells induced by conditioned medium (CM) derived from the colon cancer cell lines HT29 and RKO. (c) Fluorescence detection of the phenotypic markers E-cadherin and N-cadherin in BEAS-2B cells induced by HT29- and RKO-derived CM. (d) Western blotting detected the expression of related proteins (E-cadherin, N-cadherin, collagen I, fibronectin, PDGF-β, and Rad51) in BEAS-2B cells induced by HT29 and RKO CM and berberine (As viewed in supplement file). *$P < 0.05$ vs. control. **$P < 0.05$ vs. model.

group). Besides, berberine-probiotics significantly decreased IL-17 and IFN-γ and increased IL-10 expression (*P value* was 0.009, 0.006, and 0.003 vs model group).

3.6. Berberine reversed the phenotypic changes in bronchial epithelial cells indirectly induced by colon cancer cells and restrained cancerous stress by remodelling extracellular matrix proteins and cell damage repair

To further investigate the effect of colon cancer on lung tissue cells, we performed an *in vitro* experiment using BEAS-2B human bronchial epithelial cells, and the results showed that BEAS-2B cell proliferation was not enhanced but decreased after stimulation with conditioned medium (CM) derived from HT29 and RKO colon cancer cells (Fig. 4b). This effect was more obvious in the berberine group. The expression of two phenotype markers, namely, E-cadherin and N-cadherin, was further detected by cellular immunofluorescence, and the results showed (Fig. 4c) that although N-cadherin expression was not obvious in HT29 and RKO CM-treated cells, the expression of E-cadherin was significantly reduced compared with that in the control group. Berberine significantly increased the expression of E-cadherin instead of N-cadherin in BEAS-2B cells treated with HT29 and RKO CM.

The Western blot results showed (Fig. 4d) that the expression of E-cadherin in BEAS-2B cells treated with HT29 and RKO CM was significantly reduced, while the expression of E-cadherin was significantly increased by berberine, especially at high concentrations. In addition, HT29 and RKO CM decreased the expression of collagen I in BEAS-2B cells, while berberine at medium and high concentrations increased its expression. HT29 CM had no significant effect on fibronectin expression in BEAS-2B cells, but RKO CM significantly reduced fibronectin expression in BEAS-2B cells, and berberine reversed this effect. Although HT29 and RKO CM had no significant effect on the expression of PDGF-β in BEAS-2B cells, berberine significantly reduced its expression. In addition, HT29 and RKO CM decreased RAD51 expression in BEAS-2B cells, which was significantly increased by berberine.

4. Discussion

It is well known that a large number of highly diverse microbes inhabit humans. Less research has been devoted to the lung microbiome because of the conventional view that human microbes have the greatest impact on the gut, skin and mucous membranes and that the lung is supposed to be permanently sterile [18]. An increasing number of studies have suggested that the intestinal microbiota also has varying degrees of influence on the lung microflora. Our previous studies have confirmed that the gut microbiota in colon cancer mouse models is dysfunctional, showing structural and functional disorders [17]. It was further found in the present study that there were also many changes in the lung microbiota of the colon cancer mouse model including the richness, evenness and biodiversity. Therefore, after the administration of probiotics, the richness and evenness of the microbiota in lung tissue were significantly increased, and the microbial biodiversity was also improved.

The regulatory effect of berberine on intestinal microbes has become the focus of attention in recent years. The results of this study showed that although berberine did not significantly improve the richness and evenness of the microbiota in the lung tissues of a mouse model of colon cancer, berberine could significantly reverse the increased abundances of *Planomicrobium*, *Deinococcus*, *Corynebacterium*, *Brevibacteriu*m, *Jeotgalicoccus*, *Renibacterium* and *Methylobacterium* and reduced the abundances of *Parabacteroides*, *Bifidobacterium*, *Streptococcus*, *Oscillospira*, *Phascolarctobacterium*, *Saccharopolyspora*, etc. These were similar to the effect of berberine-probiotics, but the effect of probiotics was not significant, indicating that the regulation of these bacteria was mainly through berberine or that the regulation of berberine on the microbiota may be selective. In addition, berberine-probiotic treatment significantly increased the abundances of *Gammaproteobacteria*, *Lactobacillaceae*, *Lactobacillus*, and *Acinetobacter*, indicating that the regulation of some bacteria required the combined action of berberine and probiotics. A similar phenomenon was also been found by selecting species markers at each level. These results further indicated that berberine selectively regulates the microbiota in lung tissue.

The occurrence of colon cancer also affects metabolic function of lung microbiota. Some studies have clarified that the inhibition of carcinogenesis by berberine may be related to lipid metabolism [19]. We found that the abundance of microbiota participating in lysosomes, sporulation, flavone and flavonol biosynthesis, glycosaminoglycan degradation, and glycosphingolipid biosynthesis was significantly decreased in the lung tissue of colon cancer mice, which was significantly enhanced by berberine. Although berberine also increased the flora abundance involved in sporulation, the probiotics or berberine-probiotics treatment group was more significant. However, studies have shown that the metabolic functions of the microbiota that are changed in diseases that involve inflammation mainly include carbohydrates, phenols, benzoic acids, alcohols, and vitamins and can be regulated by berberine [20–23]. In the process of colitis-associated colorectal cancer, berberine can change butyric acid, acetic acid and propionic acid levels and the LPS content in stool [24], suggesting that the changes in metabolic function of the microbiome may vary with the disease conditions of the organism.

Studies have shown that dysregulation of the intestinal microbiota can alter intestinal metabolism and damage the intestinal mucosal barrier, thus further activating carcinogenic signalling pathways of colon mucosal epithelial cells, such as MAPK/ERK,

Hedgehog, IL-17 and Wnt/β-catenin [25–29]. The results indicated that the local microenvironment homeostasis of intestinal tissue was changed, which was beneficial to the further development of cancer. Similarly, dysfunction of the lung microbiome caused by colon cancer also triggered related homeostasis changes in lung tissue. An imbalance in the microbiota can cause an inflammatory response. As chronic inflammation is now recognized as an important carcinogen, the role of bacteria in the occurrence and development of lung cancer has attracted great attention from researchers worldwide [30].

During inflammation, the lung microbiota becomes unstable, and its species composition often changes due to immune system activity. Therefore, in our study, we found that a variety of inflammatory cytokines in lung tissue were also changed, which could be reversed by intervention with berberine or probiotics. Both IL-6 and IL-10 are associated with inflammation and lung cancer, however, our results showed a slight reduction in IL-6 and IL-10 (compared with the control group), and the difference was not statistically significant, which may be related to the small sample size observed. We found that berberine-probiotics can significantly increase IL-10 and decrease IL-17 and IFN-γ levels ($P < 0.05$), suggesting that berberine alone has no significant effect on regulating inflammatory cytokines in lung tissue but can be significantly enhanced after combined treatment with probiotics. Therefore, berberine can be used as a dietary supplement (prebiotic) to improve immunity and inflammation [31]. These results indicate that the occurrence of colon cancer can affect the microenvironmental inflammatory state in lung tissue. Berberine can either change the inflammatory state in lung tissue by improving the intestinal microbiota or directly regulate the cells in the microenvironment of lung tissue.

To further explore the effect of colon cancer on lung epithelial cells and the related regulatory mechanism of berberine, we performed an *in vitro* experiment using BEAS-2B bronchial epithelial cells. We found that although CM from colon cancer cells could not promote the proliferation of BEAS-2B cells, the cell phenotype was changed, with reduced expression of the epithelial marker E-cadherin. The expression of E-cadherin was significantly upregulated after berberine intervention. Previous studies have shown that berberine can inhibit the inflammation-cancer transformation pathway, including TLR4/p-NF-κB p65/IL-6/p-STAT3, and can upregulate the epithelial markers Occludin and ZO-1 in cells [21,32]. Studies have shown that NF-κB p65 pathway activated in lung tissues can regulate the epithelial-to-mesenchymal transition (EMT) mechanism in cancer cells involving in metastasis and drug resistance [33]. These results indicate that berberine has a significant promoting effect on the maintenance of epithelial cell status, preventing malignant transformation of lung epithelial cells.

Although HT29 CM had no significant effect on fibronectin expression in BEAS-2B cells, RKO CM significantly reduced its expression in BEAS-2B cells, and berberine reversed the expression, suggesting that different cancer cell types may have different effects on the expression of extracellular matrix proteins. In addition, HT29 and RKO CM had no significant effect on the expression of PDGF-β in BEAS-2B cells, while berberine significantly reduced its expression. This finding suggests that berberine may change the local microenvironment of the lung-bronchial epithelial cells by influencing extracellular matrix remodelling when the influence of cancer cells on the lung-bronchial epithelial cells is not obvious.

RAD51 is a gene involved in cell damage repair and can act as a regulatory factor of replication stress to affect nucleases, helicases, DNA translocases, and signalling proteins [34]. Recent studies have found that the expression of Rad51 is significantly reduced in gastric cancer cells with positive expression of certain bacteria in the gastrointestinal tract, such as *Helicobacter pylori* [35]. We also found that colon cancer cell CM decreased the expression of the Rad51 protein in BEAS-2B cells, indicating that colon cancer induced DNA damage in BEAS-2B cells and that berberine significantly increased the expression of the Rad51 protein. This finding suggests that berberine can directly inhibit the local adverse reactions of colon cancer cells on lung/bronchial epithelial cells by enhancing the cell damage repair pathway in BEAS-2B cells. However, in other solid cancers, such as prostate cancer, breast cancer and oesophageal cancer, the high expression of RAD51 promotes the occurrence of cancer [36–39]. Defects in the DNA repair pathway are markers of cancer. During the cancer development process, cancer cells need to obtain residual DNA repair ability to repair the damage caused by replication stress and antitumor drugs. Therefore, to sustain the growth of cancer cells, the DNA repair ability will be continuously enhanced, thus increasing the expression of RAD51. In our study, colon cancer cells caused a decrease in Rad51 in BEAS-2B cells, and berberine significantly increased its expression, indicating that BEAS-2B cells stimulated by cancer cells are still in the early stage of cancerous transformation and that berberine protects lung cells against this stress by enhancing RAD51 expression.

5. Conclusions

Berberine can selectively regulate the abundance of some microflora changed in lung tissues during the growth and development of colon cancer cells *in vivo*, with improvement of inflammatory response. Besides, berberine can antagonize the cancerous stimulation of lung tissue from colon cancer cells by regulating the bronchial epithelial cell phenotype, extracellular matrix remodelling and the expression of the repair gene RAD51.

Ethics approval and consent to participate

We confirm that all methods were reported in accordance with ARRIVE guidelines (https://arriveguidelines.org) for the reporting of animal experiments. Because the Second Affiliated Hospital of Shenzhen University (People's Hospital of Shenzhen Baoan District) did not have the qualification of animal ethics review, this study was entrusted to the Hong Kong University of Science and Technology Medical Center for approval, and all experimental protocols were approved by the Ethics Committee on Laboratory Animals (Hong Kong University of Science and Technology Medical Center, Peking University, Shenzhen, No: 2022-860). Animal feeding and management were carried out in accordance with relevant guidelines and regulations (GB 14925-2010 and GB50447-2008).

Data availability statement

The data used to support the findings of this study are available from the corresponding authors upon request.

CRediT authorship contribution statement

Wei Yang: Writing – original draft. **Ting Yang:** Writing – original draft. **Bo Huang:** Visualization, Software. **Zhanjun Chen:** Software, Methodology. **Haosheng Liu:** Visualization. **Chao Huang:** Writing – review & editing, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24405.

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