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Soybean-derived proteins and peptides enhance probiotic adhesion to intestinal cells by modulating mucin secretion and adhesion-related pathways

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ABSTRACT: Dietary proteins influence the gut environment by modulating probiotic adhesion. This study investigated whether gastrointestinal-digested soybean protein isolate (dSPI) and peptides (dPEP[1]) enhance the adhesion of probiotics to intestinal LS174T cells. We found that 4 mg/mL dSPI and dPEP promoted the adhesion of *Lacticaseibacillus rhamnosus* GG (LGG) at 2 h and 4 h, and enhanced the adhesion of the weakly adherent strain *Lacticaseibacillus plantarum* K25 (K25) at 4 h and 6 h. This was associated with increased mucin secretion from LS174T cells. Transcriptomic analysis revealed that dSPI and dPEP regulated pathways related to cell adhesion molecules, inflammation, protein metabolism/transport, and glucose metabolism. Specifically, dSPI modulated all four pathways, whereas dPEP primarily targeted cell adhesion molecules by upregulating key genes to enhance adhesion. PCK1 and STAG3L3 were key genes regulating adhesion in dSPI and dPEP, respectively.

Keywords: Soybean protein isolates; Soybean peptides; Probiotic adhesion; LS174T cells; Mucin secretion; Cell adhesion mechanism

1. Introduction

Bowel diseases, particularly inflammatory bowel disease (IBD), are an increasing burden on global healthcare systems and society. Notably, there are more than 1.5 million cases of IBD in China by the end of 2025. The increased use of biologic therapies and the ageing population will bring new challenges and complexities to public health and the economy of society (Esteban Morales et al., 2017; Kaplan, 2015). In addition, IBD is a leading cause of colorectal cancer (CRC), which is the third most common cancer worldwide and second leading cause of cancer-related deaths worldwide (Dasari et al., 2023). The occurrence of these diseases was related to gut microbiota disorders, and many studies have shown that probiotics help maintain the balance of gut microbiota composition and function, playing a crucial role in maintaining host health and stability.

Therefore, people are increasingly concerned about methods to improve the gut microbiota, especially those involving probiotics, to prevent and treat intestinal diseases. Some clinical trials have shown that

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fermented milk containing *Lactobacillus casei* DN114001 can reduce the severity, duration and frequency of acute diarrhoea in children (Agarwal and Bhasin, 2002; Pedone et al., 2000). A European trial showed that giving oral *Lactobacillus* GG rehydration solution to children with acute diarrhoea resulted in a shorter duration of illness (Hidayat et al., 2025). So, probiotics are expected to become an effective method for treating this disease, and the prerequisite for probiotics to exert these effects was colonization in the intestine. Adhesion is a specific interaction between the surface components of bacteria and the complementary structures on the surface of host cells. If a certain nutrient could promote the adhesion between probiotics and the intestine, it would be beneficial to reduce the invasion of pathogens and maintain the balance of the intestinal microbiota.

Active peptides can regulate gut health through multiple pathways (Zhong et al., 2024). Our previous research has shown that soybean protein and soybean peptides can stimulate the proliferation of various probiotics such as *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis* (Zhang et al., 2022; Zhang et al., 2021; Li et al., 2020; Li et al., 2021a). *In vivo* experiments have also shown that soybean protein and soybean peptides increased the abundance of probiotics in the intestine of rats (Li et al., 2021b). Besides, we identified and analysed the digested soybean peptides after 36 h utilization by *L. rhamnosus* Lra05, and the results showed that *L. rhamnosus* Lra05 tends to utilize hydrophobic peptides with three to five amino acid residues and hydrophilic peptides with more than five residues. They also prefer peptides with proline at the penultimate C-terminal position or arginine at the ultimate C-terminal position (Zhang, et al., 2023). Therefore, based on the above research, we proposed that whether soybean protein isolates and soybean peptides could regulate the adhesion of probiotics to intestinal cells by processing them, and what are the ways to regulate adhesion. To comprehensively evaluate its regulatory effects, this study compared two probiotic strains with distinct inherent adhesion properties: *Lacticaseibacillus rhamnosus* GG (LGG), a model probiotic with strong adhesion capacity and well-defined mechanisms; and *Lacticaseibacillus plantarum* K25 (K25), a strain exhibiting weaker adhesion capacity. Transcriptomics technology was mainly used to study the overall gene transcription and regulatory patterns of cells, to reveal the molecular mechanisms of a specific biological process. Transcriptomics sequencing technology, especially RNA sequencing (RNA-seq), has the advantages of fast sequencing speed and high resolution, and has been widely used. This is one of the better means for us to explore the regulation of probiotics and intestinal cell patterns by proteins and peptides.

Therefore, this study aims to investigate whether soybean protein isolates and soybean peptides treatment of LS174T intestinal cells can promote the adhesion of probiotics with different adhesion abilities to it, and to explore the different modes of regulation of adhesion between probiotics and intestinal cells by soybean protein and soybean peptides at the transcriptional level. This is one of the better means to explore the patterns of protein and peptide regulation of adhesion between probiotics and intestinal cells.

2. Materials and methods

2.1. Materials and reagents

Soybean protein isolates and soybean peptides were provided by Shandong Xinyu Biotechnology Co., Ltd. (Shandong, China) and Rushan Hualong Food Co., Ltd. (Shandong, China), respectively. *Lacticaseibacillus rhamnosus* GG (LGG) and *Lacticaseibacillus plantarum* K25 (K25) were obtained from the Dairy Function Laboratory of Beijing Technology and Business University (Beijing, China). The LS174T cell line was acquired from the National Experimental Cell Resource Sharing Platform (Beijing, China). Beef extract, yeast extract, peptone, and other culture media components were sourced from Beijing Aoboxing Biotechnology Co., Ltd. (Beijing, China). Crystalline violet was purchased from Tianjin Fuchen Biotechnology Co., Ltd. (Tianjin, China). MEM medium, penicillin-streptomycin, fetal bovine serum (FBS), and 0.05% Trypsin-EDTA were procured from Gibco (Grand Island, NY, USA). All other chemicals and solvents used were of analytical grade.

2.2. In vitro gastrointestinal digestion

Soybean protein isolates and peptides require processing to modify their functional properties, and in vitro gastrointestinal digestion simulations better demonstrate their effects on gut probiotics (Hussain et al., 2024). *In vitro* simulated digestion was based on our previous methods. Soybean protein isolates and peptides were both digested by pepsin and pancreatin for 1 h and 2 h, respectively (Zhang et al., 2024). After enzyme inactivation, freeze for more than 48 h and then freeze-dry to obtain the powder of dSPI and dPEP.

2.3 Probiotics and cell culture

LGG and K25 were cultured in Man Ragosa Sharpe (MRS) broth for 24 h at 37°C in an incubator (HuaLida, Jiangsu, China). LS174T cells were grown in MEM supplemented with 5% fetal bovine serum and 1% penicillin-streptomycin, and passage 170 to 185 were used. Cells were grown at 37°C in a 5% CO_2 atmosphere (Zhang et al., 2025).

2.4 Adhesion assay of probiotics to LS174T cells

LS174T cells were cultured in the control group (CG), dSPI group, and dPEP group. The CG group used complete MEM medium. Cytotoxicity testing indicated that the optimal concentration for dSPI and dPEP was 4 mg/mL (Fig.S1). Cells were seeded out in 24 well plates at a density of 3×10^5 cells per well and grown for 24 h to full confluence. For adherence assays, K25 or LGG cultured to logarithmic or stable period was washed twice in PBS and resuspended in plain MEM without antibiotics at a viable count concentration of 1×10^7 CFU/mL. The cells in the 24 well plate was added 4 mg/mL of dSPI and dPEP after complete confluence, and the plate was washed twice in PBS after inoculated 2 h. Then, cell monolayers were inoculated with 1 mL K25 or LGG. After incubation for 2 h, 4 h, and 6 h, cells were washed three times in PBS to remove non-adherent bacteria. Adherent bacteria were quantified by lysing cell monolayers in 1% Triton X-100 (in PBS) for 10 min and diluted 10-fold with physiological saline. Then, the collected cells were plated in triplicate on the MRS agar medium for colonization and counting. Adherence was calculated according to the following equation: % adhesion = (number of adherent bacteria / numbers of inoculated bacteria) × 100 (Walsham et al., 2016).

2.5. Transcriptomic analysis

2.5.1. RNA extraction, library construction, and prokaryotic transcriptome sequencing of LS174T cells

Total RNA was extracted from the tissue using TRIzol® Reagent according the manufacturer's instructions (Invitrogen) and genomic DNA was removed using DNase I (TaKara). Then RNA quality was determined by 2100 Bioanalyser (Agilent) and quantified using the ND-2000 (NanoDrop Technologies). Only high-quality RNA sample ($OD_{260/280}$=1.8~2.2, $OD_{260/230}$≥2.0, RIN≥6.5, 28S:18S≥1.0, >1μg) was used to construct sequencing library.

RNA-seq transcriptome library was prepared following TruSeqTM RNA sample preparation Kit from Illumina (San Diego, CA). Paired-end RNA-seq sequencing library was sequenced with the Illumina HiSeq xten/NovaSeq 6000 sequencer (2 × 150bp read length)

2.5.2 Differential expression analysis and Functional enrichment

Functional-enrichment analysis including GO and KEGG were performed to identify which DEGs were significantly enriched in GO terms and metabolic pathways at Bonferroni-corrected $P \leq 0.05$ compared with the whole-transcriptome background. GO functional enrichment and KEGG pathway analysis were carried out by Goatools.

2.5.3. Validation of LS174T cells adhesion key genes mRNA expression after regulation by dSPI and dPEP by RT-qPCR

The extracted RNA underwent reverse transcription under the following conditions: incubation at 50°C for 5 minutes, followed by heating to 85°C for 2 minutes. The resulting cDNA sample was subjected to RT-qPCR. The RT-qPCR reactions were performed according to the RT-qPCR reaction kit (TransStart Top Green qPCR SuperMix, +Dye I). The thermal cycling programme involves a denaturation step at 95°C for three minutes, followed by 40 cycles of 95°C for 10 seconds and 55°C for 30 seconds, and the primer sequences were presented in Table S1.

2.6 Statistical analysis

All data were normally distributed and are expressed as mean ± SD (n=3). Statistical significance ($P < 0.05$) was determined by one-way ANOVA in IBM SPSS Statistics 26 (IBM, Chicago, IL, USA), and Tukey's multiple comparison test and Duncan's test were used. Graphs were generated using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, USA).

3. Results and discussion

3.1 The adhesion of LGG and K25 to LS174T cells with different treatments

LGG from the logarithmic and stable phases were adhered to LS174T intestinal cells that had been treated in different ways. Fig.1A showed that the adhesion rates in the dSPI and dPEP groups were significantly higher than that in the MRS group when LGG from the logarithmic phase was adhered for 2 h. After 4 h, dSPI were still increasing the adhesion rate of LS174T intestinal cells to LGG from logarithmic

phase. However, after 6 h, there was no significant difference in the adhesion rate between the three groups. After 2 h of LGG adhesion in the stable phase (Fig.1B), soybean protein isolates and peptides also significantly promoted the adhesion of LS174T intestinal cells to them, and at 4 h of adhesion, the adhesion rates in the dSPI and dPEP groups were 1.50-fold and 1.41-fold higher than those in the MRS group, respectively. In summary, dSPI and dPEP can significantly promote the adhesion of LS174T intestinal cells to LGG at 2 h and 4 h, but previous studies have shown that dSPI and dPEP did not enhance the adhesion between LGG and LS174T intestinal cells when treated with LGG, indicating that dSPI and dPEP mainly affect the adhesion mode between LGG and LS174T intestinal cells by affecting the adhesion ability of LS174T intestinal cells. Combined with the previously measured adhesion results of LGG to different concentrations of mucin, it was speculated that dSPI and dPEP may affect the secretion of mucin in LS174T intestinal cells.

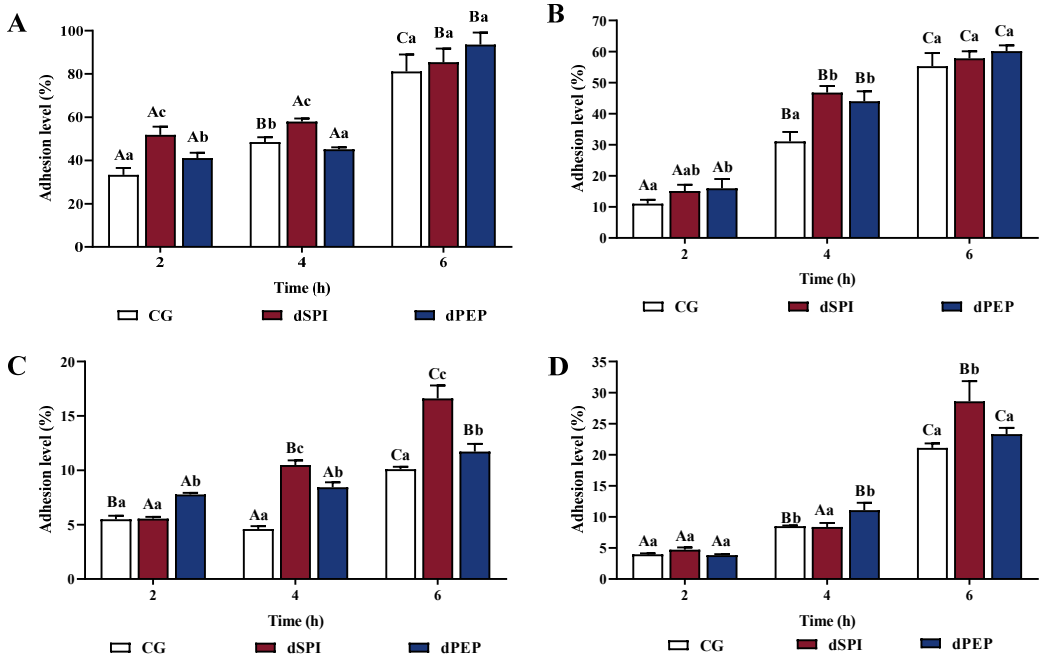


Fig. 1 Adhesion of probiotics to LS174T cells in different treatment groups (CG: control group, dSPI: gastrointestinal digested soybean protein isolate, dPEP: gastrointestinal digested soybean peptide). (A) Logarithmic stage of LGG and (B) Stationary stage of LGG, (C) Logarithmic stage of K25 and (D) Stationary stage of K25. Results are expressed as the mean ± standard error of triplicates. For each bioactivity tested, different small letters between different treatments and different capital letters between time points indicate significant differences between groups ($P < 0.05$)

To investigate the effects of dSPI and dPEP on the adhesion of probiotics with different adhesion abilities to LS174T intestinal cells, the probiotic K25 with weak adhesion ability was adhered to LS174T cells under different treatments (Fig.1C-D). Fig.1C illustrated the impact of dSPI and dPEP on the adhesion of LS174T intestinal cells to K25 in the logarithmic phase. After 2 h, dPEP significantly increased LS174T intestinal cell adhesion to K25, which is consistent with its increased adhesion to LGG from logarithmic phase. When adhering for 4 h, both the dSPI and dPEP groups had significantly higher adherence rates than the MRS group, and the adherence effect was maintained until 6 h. The results of K25 from the stable phase adhering to LS174T enterocytes with different treatments were presented in Fig.1D, and the adhesion rate of

the dSPI group was significantly higher than that of the other two groups after 2 h of adhesion. At 4 h of adhesion, the adhesion rate of the dPEP group was significantly higher than that of the dSPI and MRS groups. When the adhesion reached 6 h, dSPI significantly promoted the adhesion of LS174T cells to stable K25, and the adhesion rate was significantly higher than that of the dPEP and MRS groups. In summary, dSPI and dPEP elevated the adhesion of LS174T cells to LGG and K25. For LGG adhesion, dSPI and dPEP mainly regulated the role of LS174T cells at 2 h and 4 h of adhesion. For K25 adhesion, it mainly acted at 4 h and 6 h of adhesion.

3.2 Effects of dSPI and dPEP on mucin secretion in LS174T intestinal Cells

To investigate the way in which dSPI and dPEP promoted the adhesion of LS174T intestinal cells to LGG and K25, mucin secretion associated with adhesion was measured in enterocytes. Fig.2 showed the secretion of MUC2 and MUC by LS174T intestinal cells in different treatment groups. MUC2 secretion by LS174T intestinal cells was significantly higher in the dSPI and dPEP groups than in the CG group, and in combination with the adhesion rate analysis, dSPI and dPEP may promote mucin secretion, thereby facilitating the adhesion of LS174T intestinal cells to LGG and K25. LS174T intestinal cells also secreted significantly more MUC in the dSPI and dPEP groups than in the CG group, with the dPEP group residing in the highest position, which was consistent with the results of the pre-adhesion rate measurements, and with the findings reported in the literature on casein hydrolysate-stimulated intestinal mucin secretion in rats (Claustre et al., 2002).

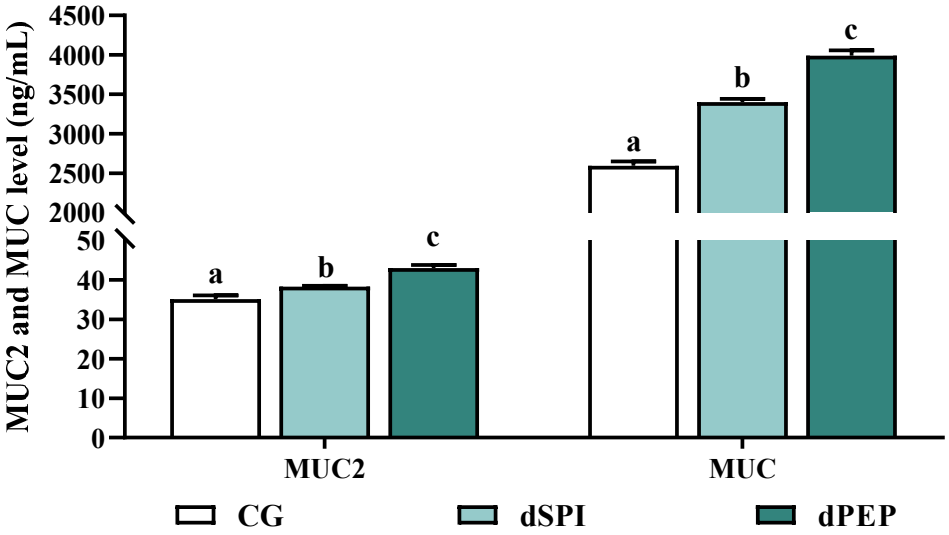


Fig. 2 The effects of soybean protein and peptides on the secretion of MUC2 and MUC by LS174T intestinal cells (CG: control group, dSPI: gastrointestinal digested soybean protein isolate, dPEP: gastrointestinal digested soybean peptide). different small letters between different treatments indicate significant differences between groups ($P < 0.05$)

3.3 Differentially expressed genes (DEGs) regulated by dSPI and dPEP

As shown in Fig.3. 1868 upregulated and 2576 downregulated DEGs were detected in the dSPI group vs the CG group, while only 688 upregulated and 771 downregulated DEGs were detected in the dPEP vs the CG group. This may explain the varying degrees of protein and peptide regulation of adhesion. As expected, 1117

upregulated and 1443 downregulated DEGs were detected in the dSPI group vs the dPEP group, these DEGs may be responsible for the different adhesion modes regulated by proteins and peptides.

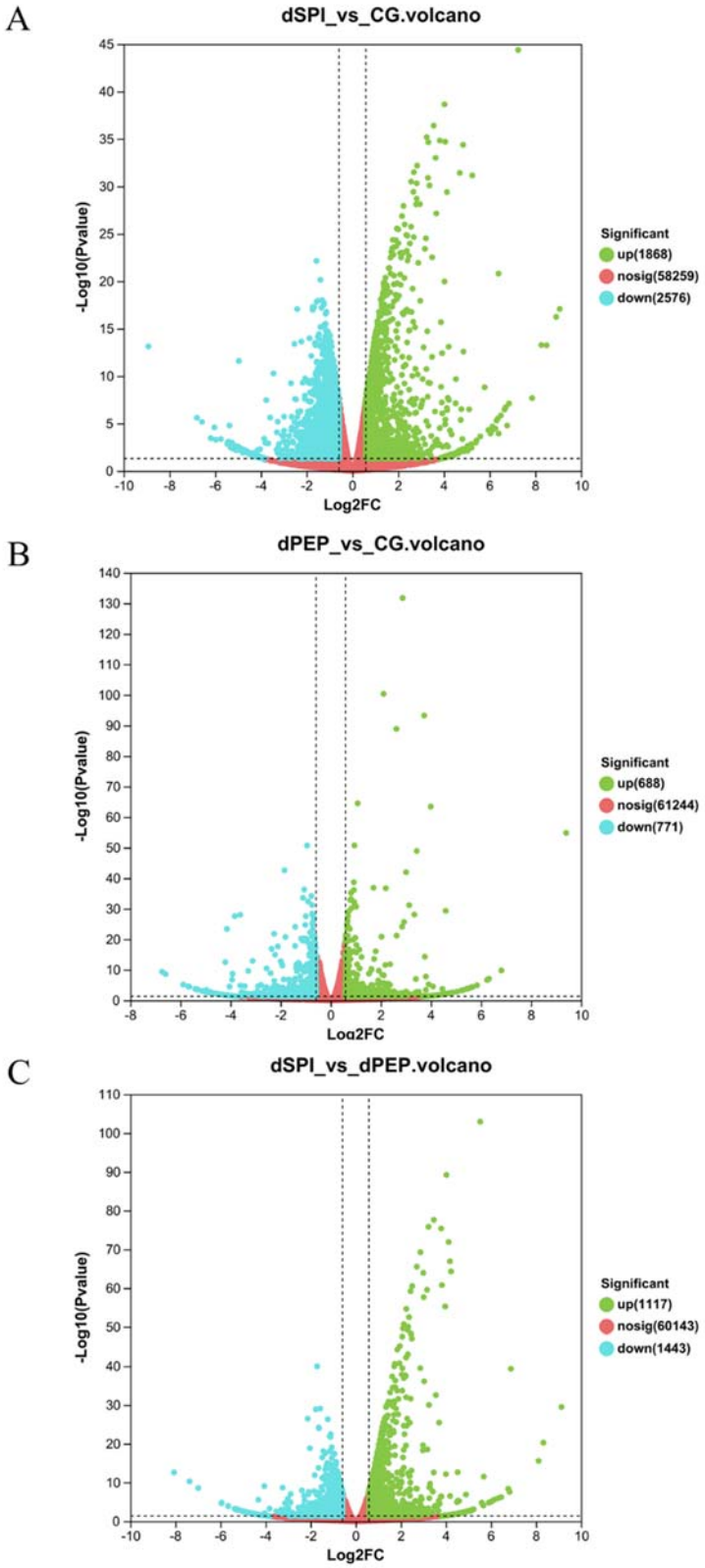


Fig. 3 Gene expression level in different comparisons of LS174T cells, (A) dSPI/CG, (B) dPEP/CG and (C) dSPI/dPEP. In the volcano plot, log2 (fold change) on the X-axis represents gene expression fold changes, while log10 (FDR) on the Y-axis represents statistically significant changes. The larger the value of log10 (FDR), the more significant the difference. The blue dots in the plot represent significantly downregulated genes, the pink dots represent genes with no significant differences, and the green dots represent significantly upregulated genes

3.4 GO enrichment analysis of DEGs

GO enrichment analysis was performed on the DEGs found in LS174T intestinal cells from various comparison groups. Fig.4A illustrated the partial enrichment pathways of the DEGs in the dSPI/CG comparison group. Differential gene enrichment in the comparison group included pathways such as regulation of epithelial cell differentiation, regulation of protein serine/threonine kinase activity, cell surface receptor signaling pathway et al. In addition, DEGs were enriched in pathways related to the regulation of proteins, such as regulation of cyclin-dependent protein kinase activity, regulation of protein metabolic process, protein targeting to membrane. It was also related to transmembrane and intracellular transport pathways, and the regulatory of cell growth pathway was also the main regulatory pathway of dSPI on LS174T intestinal cells. DEGs also accumulated in DNA-binding transcription factor activity, RNA polymerase II-specific, translational initiation and extracellular exosome pathways, and the regulation of the plasma membrane region pathway may be related to cell membrane synthesis. Further analysis of the GO enrichment pathways will be conducted.

Fig.4B showed the enriched pathways of DEGs in LS174T intestinal cells in the dPEP/CG comparison group. There were relatively few enriched pathways, with only 10 being significantly enriched. Among them, the regulation of cyclin-dependent protein kinase activity was also identified in the dSPI/CG comparison group. There were 25 DEGs enriched in the pathway of tissue formation, which was the result of cell differentiation. It was speculated that soybean peptides also affect the cell activity of LS174T intestinal cells by regulating their differentiation, thereby affecting their adhesion to probiotics.

GO enrichment pathway analysis of the DEGs in the dSPI/dPEP comparison group revealed that more than 100 pathways were significantly enriched (Fig.4C). Among them, there were pathways related to enzyme activity regulation, including regulation of cyclin-dependent protein serine/threonine kinase activity, regulation of cysteine-type endopeptidase activity, regulation of peptidase activity, positive regulation of kinase activity, regulation of endopeptidase activity, etc. DEGs were also enriched in pathways related to protein metabolism, including regulation of proteolysis and regulation of protein metabolic process. Pathways related to cell membrane synthesis and metabolism were also significantly enriched. These included the regulation of lipid metabolic processes and plasma membrane components. Additionally, the pathways most relevant to adhesion were significantly enriched. These include the regulation of intercellular and cell adhesion, as well as the response to molecule of bacterial origin. There was also a significant enrichment of many differentially expressed genes in pathways related to specific adhesion such as transmembrane receptor protein tyrosine kinase signaling pathways, cytokine-mediated signaling pathways, etc. Like the dSPI/CG comparison group, DEGs enrichment pathways were related to the regulation of epithelial cell proliferation and DNA-binding transcriptional activator activity. Further KEGG pathway analyses will be performed subsequently in conjunction with the above GO enrichment pathways to find key differential pathways.

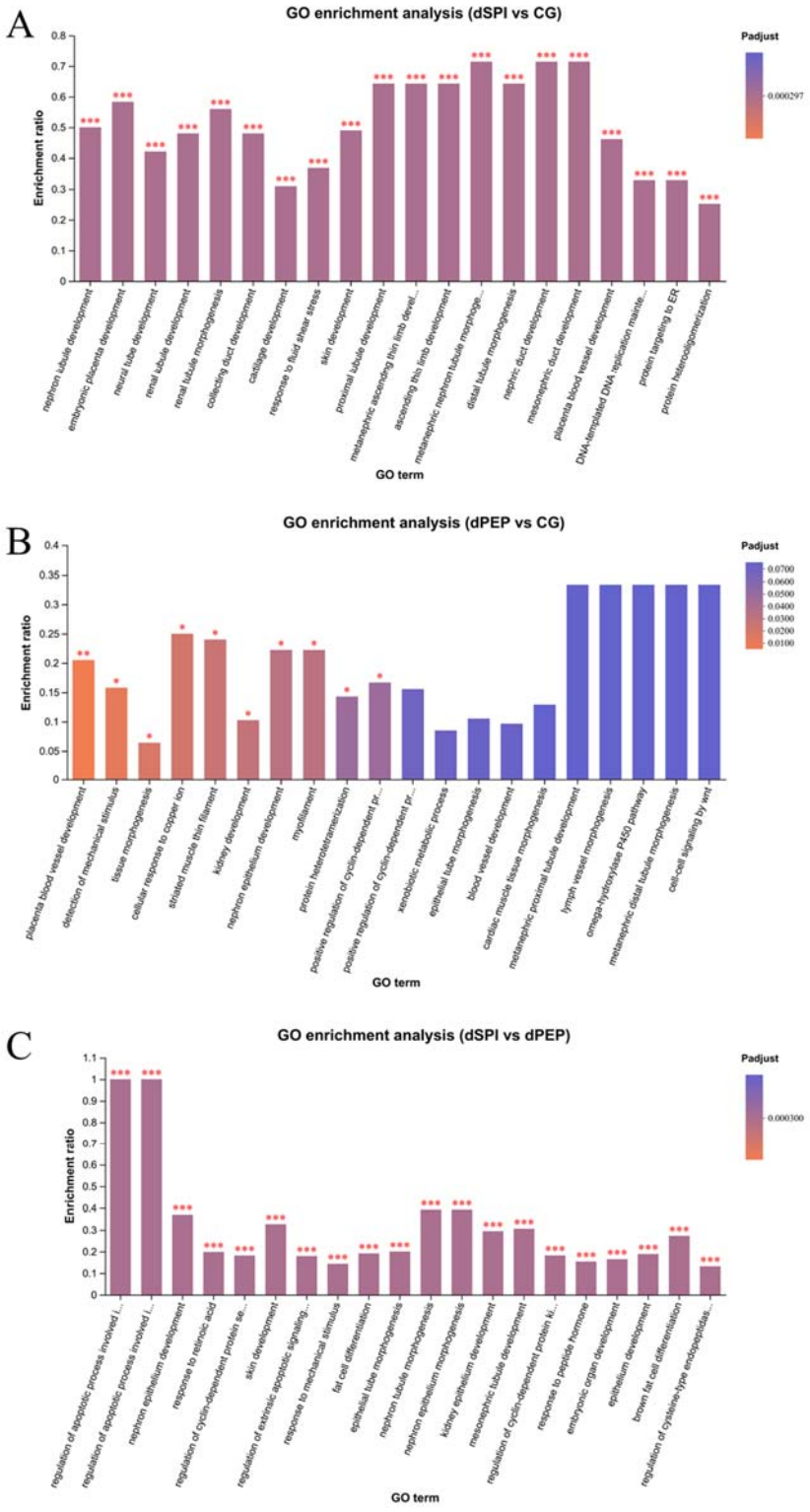


Fig. 4 GO enrichment analysis of differentially expressed genes of LS174T cells in different comparisons. (A) dSPI/CG, (B) dPEP/CG, (C) dSPI/dPEP. The horizontal axis represents the GO term, and the vertical axis represents the Rich factor. The larger the Rich factor, the greater the degree of enrichment. The color gradient of the column represents the significance of enrichment, with *P* adjust < 0.001 marked as***, *P* adjust < 0.01 marked as**, and *P* adjust < 0.05 marked as*

3.5 KEGG enrichment analysis of DEGs

Fig.5 showed the KEGG enrichment analysis of DEGs in LS174T intestinal cells in different comparison groups. Fig. 5A showed the 20 most significantly enriched pathways in LS174T cells in the dSPI/CG comparison group, including ribosome, oxidative phosphorylation, apoptosis, p53 signaling pathway.

Ribosomes were crucial for the survival and growth of cells as they served as the site of protein synthesis, and mucin synthesis facilitated cell adhesion (Buzovetsky and Klinge, 2025). Oxidative phosphorylation is the process by which ATP is synthesized through a series of biochemical reactions related to cellular metabolism. Studies have shown that tumour cells adapt to changes in their metabolic environment by converting glycolysis and oxidative phosphorylation (Elgendy et al., 2019). Meanwhile, KEGG also annotated many major pathways of concern, including pathways related to amino acid metabolism such as glycine, serine, and threonine metabolism, cysteine and methionine metabolism, arginine and proline metabolism, and glutathione metabolism. Several pathways related to glucometabolic have been identified, including the interconversion of pentose and glucuronic acid, the TCA cycle, and the biosynthesis of mannose-type O-glycans. Studies have shown that many of the enzymes involved in glycometabolism also function as 'moonlighting' proteins, which were associated with the adhesion of probiotic (Tsukagoshi et al., 2025). Pathways directly related to adhesion, such as the mucin-type O-glycan biosynthesis pathway and the cell adhesion molecule pathway, have also been annotated and were of particular interest. Specific pathways and proteins will be analyzed and validated in future.

Fig.5B showed the top 20 KEGG pathways enriched for DEGs in the dPEP/CG comparison group, including cytokine-cytokine receptor interaction related to specific adhesion, histidine metabolism pathways and ribosome pathways related to protein synthesis and metabolism, and pentose and glucuronic acid interconversion pathways related to sugar metabolism. In addition to the significantly enriched pathways mentioned above, KEGG also annotated many pathways of interest, including pathways related to amino acid metabolism such as tryptophan metabolism, β - alanine metabolism, glycine, serine, and threonine metabolism, arginine and proline metabolism, phenylalanine metabolism, etc. The pathways of protein export, protein digestion and absorption have also been annotated. At the same time, some pathways related to inflammation were also annotated, such as inflammatory bowel disease, TNF signaling pathway, etc.

Fig.5C showed the top 20 KEGG pathways enriched in the differentially expressed genes of the dSPI/dPEP comparison group. These included the cytokine-cytokine receptor interaction pathway, the p53 signaling pathway, the peroxisome proliferator-activated receptor (PPAR) signaling pathway, signaling pathways regulating pluripotency of stem cells, and the apoptosis pathway. In addition, KEGG also annotated many major pathways of concern, such as cell cycle pathways related to cell differentiation and metabolism, as well as inflammatory bowel disease pathways related to inflammation. Pathways related to protein and amino acid metabolism, such as protein digestion and absorption, arginine and proline metabolism, were also annotated, which was very similar to the differential gene enrichment in dSPI/CG and dPEP/CG comparison group. The tight junction and adherens junction pathways were also annotated, which were the most important pathways related to adhesion and the main reference pathways for subsequent analysis of key pathways.

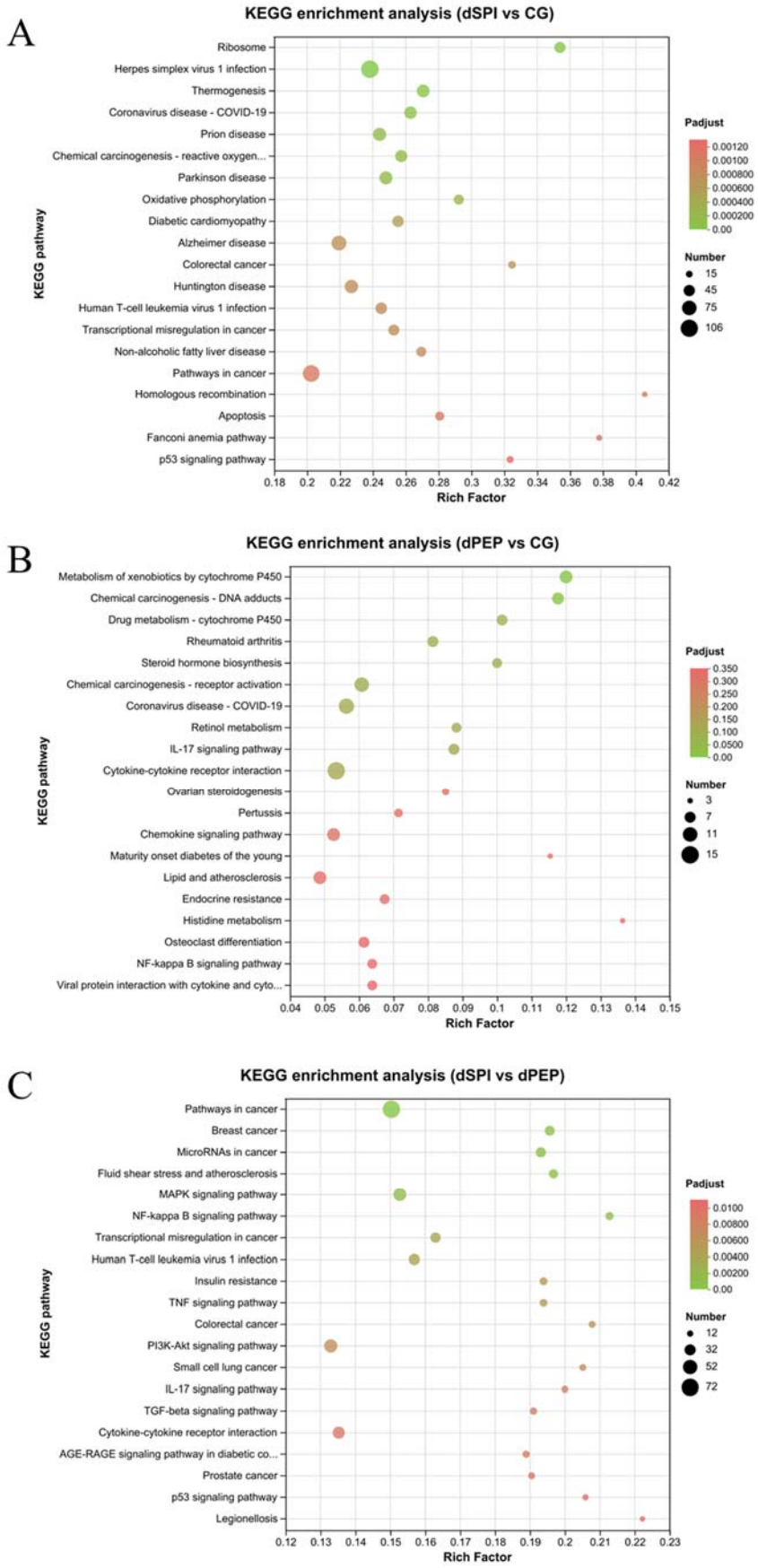


Fig. 5 KEGG enrichment analysis of differential genes of LS174T cells in different comparisons. (A) dSPI/CG, (B) dPEP/CG, (C) dSPI/dPEP. The vertical axis represents the name of the KEGG pathway, and the horizontal axis represents the Rich factor. The larger the Rich factor, the greater the degree of enrichment. The size of the dots represents the number of genes in this KEGG pathway, and the colors of the dots correspond to different P adjust ranges

3.6 Critical DEGs analysis under dPEP and dSPI treatment

3.6.1 dSPI, rather than dPEP regulated cell adhesion molecules in LS174T cells to promote their adhesion

To further explore the mechanism of dPEP and dSPI regulating LS174T cell adhesion, the pathways related to this process were first analyzed (Table 1). In the cell adhesion molecules pathway, the following genes were significantly upregulated in the dSPI/CG group: VSIR, ICOSL, PDCD1, CD276, ICAM2, CLDN23, NECTIN2 and SDC4 (Fig.6). The CLDN23 gene was also involved in the tight junction, which ensured that membrane proteins on the apical and basolateral surfaces of the cell performed different functions, and was significantly up-regulated 2.51-fold in the dSPI/dPEP comparison group and 2.31-fold in the dSPI/CG comparison group. Tight junctions can form barriers and provide mechanical support. The ACTG1 and ACTB genes encode actin and play a key role in tight junction formation. The ACTG1 gene was significantly upregulated by 2.02 and 2.23-fold in the dSPI/dPEP and dSPI/CG comparison groups, respectively, while the ACTB gene was significantly upregulated by 1.81- and 2.17-fold in the dSPI/dPEP and dSPI/CG groups, respectively. It has been demonstrated that ZO-1 protein surface condensates can specifically enrich G-actin monomers to the surface of biofilms, promoting G-actin multimerisation and bundling, and thus facilitating the extension and fusion of tight junction bands (Sun et al., 2024). Indicating that soybean protein isolates can affect the adhesion ability of LS174T cells by regulating their adhesion molecules and tight junctions. In addition, the genes ACTG1 and ACTB are also involved in the Adherens junction pathway. Adherens junctions mediated the process of intercellular recognition and adhesion, and played an important role in cellular localization, tissue formation and maintenance. ACTG1 and ACTB were mainly connected to adhesion proteins, and this connection enabled the cytoskeleton to connect the fibrous network of neighboring cells through adhesive junctions. This connection enabled the cytoskeleton to connect adjacent cells to the actin fibre network through adhesive links, and the contraction of actin regulated the degree of cellular tightness, indirectly regulating the adhesion capacity of cells (Choi et al., 2025). The calcium binding proteins (such as E-cadherin) that bind together with tightly connected transmembrane proteins (such as occludin and claudin) can be interconnected through some adaptor proteins (such as ZO protein), jointly mediating intercellular adhesion (Campbell et al., 2017). All adhesion-related genes were significantly expressed in the dSPI/dPEP and dSPI/CG comparison groups, suggesting that soybean proteins isolates regulate LS174T cell adhesion capacity through cell adhesion molecules, tight junctions and adhesive linkages. However, soybean peptides do not appear to be regulated through this pathway.

Table 1 Relative expression levels of adhesion related genes in different comparison groups

Gene_id	Gene name	Gene description	FC(dSPI/CG)	FC(dPEP/CG)	FC(dSPI/dPEP)
ENSG00000107738	VSIR	V-set immunoregulatory receptor [Source:HGNC Symbol;Acc:HGNC:30085]	2.07499762	-	1.776227788
ENSG00000160223	ICOSLG	inducible T cell costimulator ligand [Source:HGNC Symbol;Acc:HGNC:17087]	2.004846374	-	1.703817998
ENSG00000188389	PDCD1	programmed cell death 1 [Source:HGNC Symbol;Acc:HGNC:8760]	16.69070955	-	18.14248
ENSG00000103855	CD276	CD276 molecule [Source:HGNC Symbol;Acc:HGNC:19137]	1.510779862	-	-
ENSG00000243414	TICAM2	TIR domain containing adaptor molecule 2 [Source:HGNC Symbol;Acc:HGNC:21354]	2.26672	2.26672	-
ENSG00000253958	CLDN23	claudin 23 [Source:HGNC Symbol;Acc:HGNC:17591]	2.309750995	-	2.505680036
ENSG00000130202	NECTIN2	nectin cell adhesion molecule 2 [Source:HGNC Symbol;Acc:HGNC:9707]	1.651727081	-	-
ENSG00000124145	SDC4	syndecan 4 [Source:HGNC Symbol;Acc:HGNC:10661]	2.085939066	-	1.98747807
ENSG00000184009	ACTG1	actin gamma 1 [Source:HGNC Symbol;Acc:HGNC:144]	2.235315109	-	2.015653707
ENSG00000075624	ACTB	actin beta [Source:HGNC Symbol;Acc:HGNC:132]	2.174810192	-	1.816078042
ENSG00000177606	JUN	Jun proto-oncogene, AP-1 transcription factor subunit [Source:HGNC Symbol;Acc:HGNC:6204]	2.530806233	-	2.199002225
ENSG00000147168	IL2RG	interleukin 2 receptor subunit gamma [Source:HGNC Symbol;Acc:HGNC:6010]	2.854278037	-	2.24207105
ENSG00000092969	TGFB2	transforming growth factor beta 2 [Source:HGNC Symbol;Acc:HGNC:11768]	-	-	1.659107
ENSG00000175793	SFN	stratifin [Source:HGNC Symbol;Acc:HGNC:10773]	2.422265142	-	1.881186407
ENSG00000139410	SDSL	serine dehydratase like [Source:HGNC Symbol;Acc:HGNC:30404]	1.614961586	-	-
ENSG00000130005	GAMT	guanidinoacetate N-methyltransferase [Source:HGNC Symbol;Acc:HGNC:4136]	1.686071256	1.502338522	-
ENSG00000023330	ALAS1	5'-aminolevulinate synthase 1 [Source:HGNC Symbol;Acc:HGNC:396]	2.634794588	-	2.330181
ENSG00000164867	NOS3	nitric oxide synthase 3 [Source:HGNC Symbol;Acc:HGNC:7876]	1.877097604	-	1.767257121
ENSG00000172508	CARNS1	carnosine synthase 1 [Source:HGNC Symbol;Acc:HGNC:29268]		1.775934	0.514222032
ENSG00000115758	ODC1	ornithine decarboxylase 1 [Source:HGNC Symbol;Acc:HGNC:8109]	1.855821881	-	1.687459386
ENSG00000116649	SRM	spermidine synthase [Source:HGNC Symbol;Acc:HGNC:11296]	1.592015274	-	-
ENSG00000108602	ALDH3A1	aldehyde dehydrogenase 3 family member A1 [Source:HGNC Symbol;Acc:HGNC:405]	1.932365628	1.584307056	-
ENSG00000164825	DEFB1	defensin beta 1 [Source:HGNC Symbol;Acc:HGNC:2766]	3.065738871	-	3.049219443
ENSG00000132432	SEC61G	SEC61 translocon subunit gamma [Source:HGNC Symbol;Acc:HGNC:18277]	1.811152182	1.515784242	-

ENSG00000257594	GALNT4	polypeptide N-acetylgalactosaminyltransferase 4 [Source:HGNC Symbol;Acc:HGNC:4126]	1.82968289	-	2.049481769
ENSG00000008513	ST3GAL1	ST3 beta-galactoside alpha-2,3-sialyltransferase 1 [Source:HGNC Symbol;Acc:HGNC:10862]	2.570639408	-	2.30582354
ENSG00000136840	ST6GALNAC4	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 4 [Source:HGNC Symbol;Acc:HGNC:17846]	1.701096013	-	-
ENSG00000179913	B3GNT3	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 3 [Source:HGNC Symbol;Acc:HGNC:13528]	1.694207197	-	-
ENSG00000140297	GCNT3	glucosaminyl (N-acetyl) transferase 3, mucin type [Source:HGNC Symbol;Acc:HGNC:4205]	-	-	1.809931
ENSG00000086062	B4GALT1	beta-1,4-galactosyltransferase 1 [Source:HGNC Symbol;Acc:HGNC:924]	1.549329313	-	-
ENSG00000158850	B4GALT3	beta-1,4-galactosyltransferase 3 [Source:HGNC Symbol;Acc:HGNC:926]	1.569307984	-	-
ENSG00000198074	AKR1B10	aldo-keto reductase family 1 member B10 [Source:HGNC Symbol;Acc:HGNC:382]	1.967670023	-	1.545829383
ENSG00000169738	DCXR	dicarbonyl and L-xylulose reductase [Source:HGNC Symbol;Acc:HGNC:18985]	1.513076046	-	-
ENSG00000124253	PCK1	phosphoenolpyruvate carboxykinase 1 [Source:HGNC Symbol;Acc:HGNC:8724]	16.26532377	-	16.21344682

Note: FC (Fold Change) is calculated as treatment group expression/control group expression, such as FC(dSPI/CG) was dSPI group expression/control group expression, and all multiples expressed in the table 1 were statistically significant.

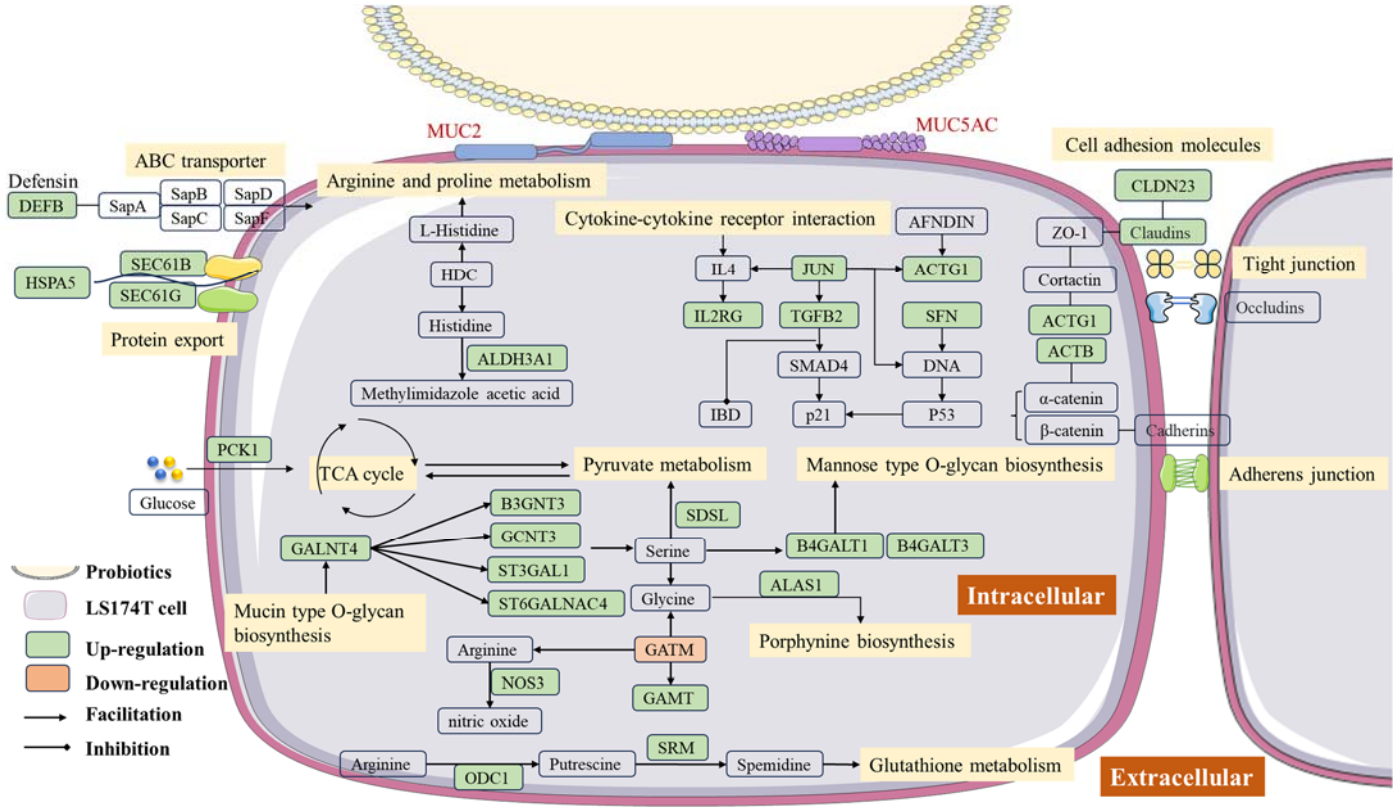


Fig. 6 Schematic diagram of DEGs involving key metabolic pathways related to LS174T adhesion

3.6.2 Compared with dPEP, dSPI has a stronger ability to promote DEGs involved in Inflammatory Cytokine Pathway

The c-Jun protein encoded by the JUN gene was not only involved in the tight junction process, but also in the inflammatory bowel disease pathway (Fig.6). The JUN gene was significantly upregulated by 2.20 and 2.53 times in the dSPI/dPEP and dSPI/CG comparison groups, respectively. Studies have shown that the JUN gene promotes the production of inflammatory cytokines such as IL-6 and TNF-α by regulating the expression of a variety of inflammation-related genes. These inflammatory cytokines not only exacerbate the inflammatory response in the intestine, but may also further disrupt the tight junctions of intestinal epithelial cells, leading to impairment of intestinal barrier function (Nitish Kumar et al., 2024). Soybean protein isolates may cause transient inflammatory response in intestinal cells, disrupting tight junctions, which may explain the effect of LGG adhesion to LS174T intestinal cells from logarithmic phase. The adhesion rate of dSPI group was higher than that of MRS group at 2 h and 6 h of adhesion, while it was significantly lower than that of MRS group at 4 h of adhesion (Fig.1A). However, when LGG from the stable phase adhered to LS174T intestinal cells, the adhesion rate was higher in the dSPI group than in the MRS group, so the up-regulation of the JUN gene did not predominate. The IL2RG gene in the inflammatory bowel disease pathway was upregulated 2.85 and 2.24 times in the dSPI/dPEP group and dSPI/CG group, respectively, and this gene is also involved in cytokine-cytokine receptor interaction pathway. The IL-2Rγ chain encoded by the IL2RG gene was a common component of multiple cytokine receptors, capable of secreting cytokines and expressing

adhesion molecules (Shouse et al., 2024). The TGFB2 gene in the inflammatory bowel disease pathway is associated with the cell cycle, which was significantly up-regulated 1.66-fold in the dSPI/dPEP group and not significantly expressed in the dPEP/CG group. The protein encoded by the TGFB2 gene is transforming growth factor β-2 (TGFβ-2), which acted by binding to cell surface receptors to activate the TGF-β signalling pathway, which in turn regulated the expression of cell adhesion molecules (Li et al., 2025). The SFN gene in the cell cycle pathway is also involved in the p53 signalling pathway, which was significantly up-regulated 1.88- and 2.42-fold in the dSPI/dPEP and dSPI/MRS groups, respectively. SNF are genes that encode the SWI/SNF complex, which can regulate the expression of a variety of genes, including some genes related to cell adhesion. It may affect the expression levels of adhesion molecules such as integrins and E-calmodulin, thereby indirectly regulating the adhesion properties of intestinal cells (Liu et al., 2019). Thus, soybean protein isolates may indirectly influence the adhesion properties of intestinal cells through their role in the regulation of inflammatory gene expression and in the modulation of cellular functions.

3.6.3 The regulation of LS174T cell adhesion by dPEP and dSPI may be associated with amino acid metabolism and protein transport

The protein metabolism and transport system were beneficial for cell construction and repair, enhancing immune function and energy supply (Fig.6). Analysis indicated that dSPI and dPEP enhanced multiple amino acid metabolic pathways in LS174T cells, with key genes participating in metabolic pathways and protein transport systems, and the expression of these genes may be related to the enhanced adhesion ability of LS174T intestinal cells. The genes SDSL, GAMT and ALAS1 were significantly up-regulated in the metabolic pathway of glycine, serine and threonine metabolism. The expression of SDSL was up-regulated 1.61 folds in the dSPI/CG comparison group and was not significantly expressed in the dSPI/dPEP and dPEP/CG groups. The proteins encoded by the SDSL gene also have protein-binding activity that contributes to the formation of protein complexes, and the gene was also involved in cysteine and methionine metabolism and promoted the synthesis of isoleucine through the enzymes it encoded, thus supporting cell growth and repair (Baker et al., 2021). The GAMT gene, which was up-regulated 1.50 and 1.69 folds in the dPEP/CG and dSPI/CG groups, respectively, encoded a methyltransferase enzyme that plays a key role in the biosynthesis of creatine in arginine and proline metabolism (Sun et al., 2025). In addition, genes involved in arginine and proline metabolism, such as NOS3, CARNS1, ODC1 and SRM, were also significantly expressed, with ODC1 significantly up-regulated by 1.69 and 1.85 folds in the dSPI/dPEP and dSPI/CG groups, and the SRM gene significantly up-regulated by 1.60-fold in the dSPI/CG group. Both ODC1 and SRM were also involved in the glutathione metabolism pathway, and ODC1 encodes ornithine decarboxylase, which was the rate-limiting enzyme for polyamine biosynthesis, and polyamines play an important role in cell proliferation, differentiation and migration (Kiely and Gilbert-Ross, 2021). The CARNS1 gene is also involved in Histidine metabolism and beta-Alanine metabolism, which was upregulated only in the dPEP vs CG group. The CARNS1 gene encoded an enzyme involved in the synthesis of myostatin and hyperinositide, dipeptides that play an important role. By reducing oxidative stress and inflammation, CARNS1 may indirectly support

enterocyte health and function, thereby maintaining normal intestinal cells adhesion (Zhang et al., 2021). The ALDH3A1 gene was involved in three metabolic pathways: histidine metabolism, β-alanine metabolism and phenylalanine metabolism, and the expression of this gene was up-regulated 1.58- and 1.93-fold in the dPEP/CG and dSPI/CG groups, respectively. This gene encoded aldehyde dehydrogenase and belonged to the aldehyde dehydrogenase superfamily, and the ALDH3A1 gene may play an indirect role in maintaining normal adhesion and function of intestinal cells by reducing oxidative stress and maintaining a healthy state of cells (Li et al., 2024). In summary, soybean protein isolates mainly regulated the metabolism of glycine, serine and threonine, cysteine and methionine, while soybean peptides mainly affect the adhesion of LS174T intestinal cells by regulating key genes involved in histidine metabolism.

ABC transporter and protein export were significantly annotated in the dSPI/MRS and dPEP/CG groups, respectively. ABC transporter act as molecular machines mainly through coupling ATP binding, hydrolysis and phosphate release and transmembrane transport of various substrates, and act as molecular machines (Geisler and Hegedus, 2020). The DEFB1 gene in this pathway encoded an antimicrobial peptide called β-defensin 1 (hBD-1). hBD-1 was continuously expressed on the surface of the intestinal mucosa and helps to maintain the integrity of the mucosal barrier against pathogens and harmful substances (Bonamy et al., 2018), suggesting that soybean protein isolates regulate the adhesion of LS174T intestinal cells. Protein export transported newly synthesised proteins from the cytoplasm to extracellular or specific regions of the cell (Philipp et al., 2022), and the gene SEC61G, a key gene in this pathway, was up-regulated 1.51- and 1.81-fold in the dPEP/CG and dSPI/CG groups, respectively. The SEC61G gene played an indirect role in maintaining the normal adhesion function of intestinal cells by ensuring normal expression of cell surface adhesion molecules and reducing inflammatory responses (Zhang, Chen, et al., 2023). In summary, soybean protein isolates and soybean peptides did not regulate LS174T cell adhesion in the same way; soybean protein isolates regulated genes in the ABC transporter molecule pathway and thus intestinal cell adhesion, whereas soybean peptides regulated the protein export pathway to influence LS174T cell adhesion.

3.6.4 Soybean protein isolates specifically regulated glucose metabolism to affect the adhesion properties of LS174T cells.

Research has shown that intestinal cell adhesion and glucose metabolism interact through multiple mechanisms (Fig.6). Glycosylation of mucins affected both the mucosal barrier function of the intestine and bacterial adhesion to intestinal cells. Metabolites and hormones secreted by the gut microbiota can regulate the energy metabolism of intestinal epithelial cells. Therefore, the analysis focused on the significantly enriched sugar metabolism pathways. Mucin type O-glycan biosynthesis pathway was significantly enriched in the dSPI/CG group, and the expression of the genes GALNT4, ST3GAL1, ST6GALNAC4, B3GNT3, and GCNT3 was significantly up-regulated in this pathway. The GALNT4 gene was significantly up-regulated by 1.82- and 2.05-fold in the dSPI/MRS and dSPI/dPEP groups, but not in the dPEP/MRS group. This gene was primarily responsible for mucin-type O-glycosylation, whereby mucins form complex glycan structures that acted as bacterial adhesion sites, influencing bacterial adhesion and the invasion of intestinal cells (Ye et al.,

2022). Soya proteins may promote mucin-type O-glycosylation by up-regulating the GALNT4 gene, thereby enhancing the adhesion capacity of LS174T cells. Shen et al. (2026) found that wheat peptides also exerted health benefits through multiple targets. The gene ST3GAL1 was significantly up-regulated 2.57 and 2.30-fold in the dSPI vs CG and dSPI vs dPEP groups, and the enzyme encoded by ST3GAL1 is responsible for the addition of α-2,3-sialic acid to glycan chains, a modification that can affect the structure and function of cell surface glycan chains. In the gut, this modification can regulate the interaction of gut microbes with intestinal epithelial cells (Shang et al., 2024). The ST6GALNAC4 gene was significantly up-regulated 1.70-fold in the dSPI/CG group, which can affect cell adhesion, migration and invasion by altering the structure of cell surface glycan chains (Man et al., 2023). Soybean protein isolates may affect the surface glycan chain structure of LS174T cells by up-regulating ST3GAL1 and ST6GALNAC4 genes, thereby enhancing their adhesion to probiotics. The enzymes encoded by B3GNT3 and GCNT3 are responsible for the addition of GlcNAc to glycan chains to extend Core-1 and Core-2 glycan chains, respectively, a process that can affect the structure of cell surface glycan chains and regulate cell adhesion and migration (Wang et al., 2024). Gene expression results showed that B3GNT3 was significantly up-regulated 1.69-fold in the dSPI vs CG group, and GCNT3 was significantly up-regulated 2.04- and 1.80-fold in the dSPI vs CG and dSPI vs dPEP groups, respectively, suggesting that soya proteins can alter the surface glycan structure of LS174T cells by extending the glycan chain, thereby enhancing their adhesion ability. Mannose type O-glycan biosynthesis was also significantly enriched in the dSPI vs CG group, B4GALT1 and B4GALT3, a member of the β-1,4-galactosyltransferase family, and B4GALT1 was in the dSPI vs CG and dSPI vs dPEP groups respectively were significantly up-regulated by 1.72- and 1.69-fold, B4GALT3 were up-regulated 1.57-fold in the dSPI vs CG group, and they catalyse the formation of the Galβ1→4GlcNAc glycosidic bond, a key step in N-glycosyl chain synthesis. N-glycosylation modifications, common on proteins, were essential for protein folding, stability and function. The activity of B4GALT1 and B4GALT3 affected the maturation and complexity of N-glycosylation chains, which in turn affected the structure and function of cell surface glycoproteins (Ma et al., 2024). Soybean protein isolates regulated mannose-type O-glycan biosynthesis in a similar way to the mucin-type O-glycan biosynthesis pathway by affecting the glycan chain and thus the adhesion capacity of intestinal cells.

In addition to the O-glycan biosynthesis pathway, pentose and glucuronate interconversions and the citrate cycle were enriched in dSPI/CG group. AKR1B10 and DCXR were key up-regulated genes in the pentose and glucuronide interconversion pathway, and the DCXR gene was significantly up-regulated 1.51-fold in the dSPI/CG group, which was expressed on the cell surface and was involved in intercellular adhesion and signalling (Mills et al., 2025). The PCK1 gene was significantly expressed in the citric acid cycle and was significantly up-regulated 16.27 and 16.21-fold in the dSPI vs MRS and dSPI vs dPEP groups, respectively, and the enzyme encoded by this gene is mainly involved in the gluconeogenesis process, which is involved in the citric acid cycle by converting oxaloacetate (OAA) to phosphoenolpyruvate. Studies have shown that reduced expression of PCK1 in Crohn's disease correlated with disruption of the intestinal

epithelial barrier. This suggested that PCK1 played an important role in maintaining intestinal cell adhesion and epithelial integrity (Yang et al., 2024). Therefore, soy protein improves LS174T cell adhesion by affecting the structure of the sugar chain and indirectly influences this cell's adhesion capacity by regulating sugar metabolism. Research has shown that decreased expression of PCK1 in Crohn's disease is associated with disruption of the intestinal epithelial barrier, indicating that PCK1 played an important role in maintaining intestinal cell adhesion and epithelial integrity (Yang et al., 2024). Therefore, soybean protein isolates can increase the adhesion of LS174T cells by affecting the structure of glycans, as well as indirectly affecting the adhesion capacity of these cells by regulating the metabolism of glycans.

3.7 Real-Time Fluorescence Quantitative PCR (qRT-PCR) Verification

To ensure comprehensiveness and representativeness in qRT-PCR validation, genes significantly up- or down-regulated in key pathways, and genes involved in different regulatory pathways were analysed by qRT-PCR validation, and Fig.7A showed the relative expression of the differentially expressed genes in the dSPI/CG comparison group as determined by qRT-PCR and RNA-seq methodologies. SFN gene was involved in p53 signalling pathway and cell cycle pathway, this gene in RNA-seq method its expression was up-regulated 2.42-fold in dSPI/CG group and 1.37-fold by qRT-PCR verification. The relative expression of the SDSL gene, which was involved in glycine, serine and threonine metabolic pathways, was 1.61 and 1.32-fold in qRT-PCR and RNA-seq methods, respectively, and relative expression of other DEGs in qRT-PCR proved to be consistent with RNA-seq methods. The validation results of differentially expressed genes in the dPEP/CG control group are shown in Fig.7B. The relative expression levels of CXCL1 gene involved in cytokine cytokine receptor interaction pathway were 1.62 and 1.97 times in qRT-PCR and RNA seq methods, respectively. The relative expression levels of JUN gene involved in inflammatory bowel disease and tight junction pathway were 2.94 and 1.91 times in qRT-PCR and RNA-seq methods, respectively. Fig.7C showed the relative expression levels of differentially expressed genes in the dSPI/dPEP control group using qRT-PCR and RNA seq methods. The relative expression levels of CXCL2 gene involved in the cytokine cytokine receptor interaction pathway were 3.30 and 3.00-fold, respectively, using qRT-PCR and RNA-seq methods. The expression trends of other differentially expressed genes in qRT-PCR validation were also consistent with those determined by RNA-seq method. Fig.7D showed the correlation analysis between differential gene expression in LS174T cells using qRT-PCR and RNA-seq methods. The results showed that the differential gene expression levels obtained by qRT-PCR were significantly correlated with RNA seq data ($R=0.7452$, $P < 0.01$).

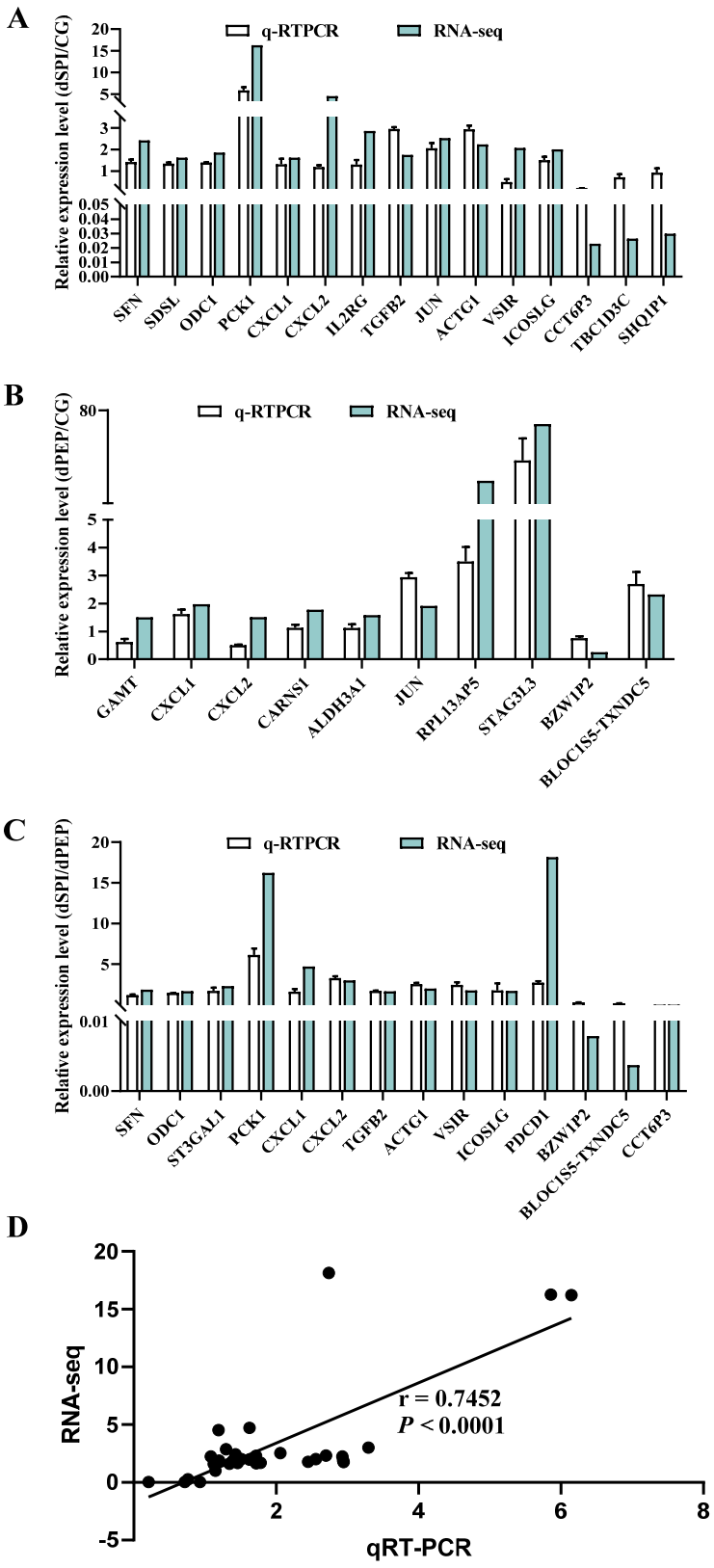


Fig. 7 RNA seq expression and qRT-PCR validation and correlation analysis of DEGs in LS174T cells in different comparison groups. (A) dSPI/CG, (B) dPEP/CG, (C) dSPI/dPEP, (D) Correlation analysis between RNA-seq and qRT-PCR.

4. Conclusion and Outlook

In summary, dSPI and dPEP differentially enhanced the adhesion of probiotics to LS174T intestinal cells in a time-dependent manner. This promotive effect was associated with elevated mucin secretion and the

regulation of key pathways, including cell adhesion molecules, inflammatory factors, protein metabolism and transport, and glycolysis. Notably, dSPI modulated all four pathways, whereas dPEP acted primarily through the cell adhesion molecules pathway. The key genes regulating adhesion in dSPI and dPEP were PCK1 and STAG3L3, respectively. Collectively, this study reveals that dSPI and dPEP enhance probiotic adhesion through distinct molecular pathways, such as modulating mucin secretion and cell adhesion molecules. These mechanisms provide a novel molecular basis for developing functional foods or ingredients aimed at improving gut microbiota colonization and barrier function. Building on these *in vitro* findings, a critical next step involves *in vivo* validation studies using animal models to assess the effects of these components on probiotic intestinal colonization, microbial community composition, and host health, thereby evaluating their translational potential.

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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