



Proteomic Profiling of Qi-blood Deficiency vs Damp-heat Syndrome in Colorectal Cancer: Therapeutic Targeting by Astragaloside IV-berberine*

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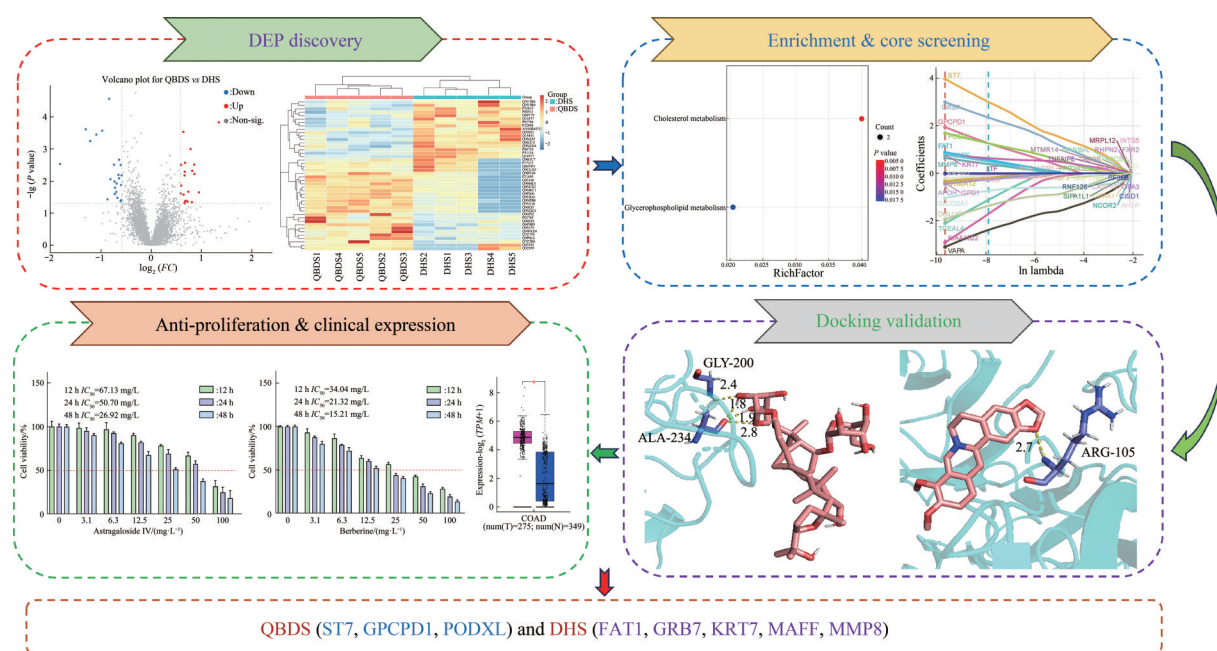
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Graphical abstract



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Abstract Objective Colorectal cancer (CRC) is a highly heterogeneous malignancy, and traditional Chinese medicine (TCM) syndrome differentiation is widely used in its clinical management. However, the biological basis underlying different TCM syndromes remains insufficiently understood. This study aimed to investigate the proteomic differences between qi-blood deficiency syndrome (QBDS) and damp-heat syndrome (DHS) in CRC patients and to establish a “syndrome-differential protein-herbal component” network for exploring potential molecular mechanisms and therapeutic targets associated with syndrome-specific treatment strategies. **Methods** Tumor tissue samples were collected from 10 patients with pathologically confirmed CRC, including 5 patients diagnosed with QBDS and 5 with DHS according to standardized TCM syndrome differentiation criteria. Tandem mass tag (TMT)-based quantitative proteomics was employed to identify differentially expressed proteins (DEPs) between the two syndrome groups. Functional enrichment analyses, including Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, were performed to characterize the biological functions and signaling pathways associated with the identified DEPs. Least absolute shrinkage and selection operator (LASSO) regression analysis was further applied to screen key syndrome-related proteins and construct a core protein prediction model. Subsequently, molecular docking was conducted to evaluate the binding affinities between representative herbal compounds, astragaloside IV and berberine, and the identified core proteins. Finally, cell counting kit-8 (CCK-8) assays were performed in HCT 116 and HT-29 colorectal cancer cell lines to validate the anti-proliferative activities of these compounds *in vitro*. **Results** A total of 44 DEPs were identified between QBDS and DHS tissues, including 21 upregulated and 23 downregulated proteins in the QBDS group relative to the DHS group. Functional enrichment analyses revealed that these proteins were primarily associated with antiviral immune responses, regulation of inflammatory signaling, NF- κ B signaling pathways, cholesterol metabolism, and other biological processes relevant to tumor progression and host immune regulation. LASSO regression analysis identified eight core proteins with potential syndrome-specific significance. Among them, ST7, GPCPD1, and PODXL were closely associated with QBDS, whereas FAT1, GRB7, KRT7, MAFF, and MMP8 were associated with DHS. Molecular docking demonstrated favorable interactions between astragaloside IV and QBDS-related proteins, with binding energies lower than -5 kcal/mol, indicating stable binding activity. Berberine exhibited even lower binding energies and stronger binding specificity toward DHS-related core proteins. *In vitro* experiments further confirmed that both compounds inhibited CRC cell proliferation, while berberine displayed significantly greater anti-proliferative efficacy. The lowest 48 h IC_{50} value of berberine reached 15.21 mg/L in CRC cells, indicating a stronger growth-inhibitory effect than astragaloside IV. **Conclusion** CRC patients with QBDS and DHS exhibit distinct proteomic characteristics, suggesting that different TCM syndromes possess unique molecular signatures. The identified core proteins may serve as potential biomarkers for syndrome differentiation, while astragaloside IV and berberine may exert therapeutic effects through targeting syndrome-specific molecular networks. These findings provide preliminary proteomic evidence supporting syndrome-guided precision integrative medicine in CRC and offer new insights into the biological basis of TCM syndrome differentiation and individualized therapeutic strategies.

Key words colorectal cancer, qi-blood deficiency syndrome, damp-heat syndrome, proteomics, astragaloside IV, berberine

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Colorectal cancer (CRC) is one of the most common and highly prevalent gastrointestinal malignancies, posing a serious threat to global public health. According to data released by the International Agency for Research on Cancer (IARC) in 2022, CRC ranks third in global incidence and second in mortality^[1]. In China, the disease burden of CRC is particularly severe, with its incidence ranking second and mortality fourth among all cancers in 2022^[2]. Additionally, data from 1990 to 2019 show that the age-standardized incidence and mortality rates of CRC in China increased by 144.01% and 36.15%, respectively, with growth rates significantly higher than the global average^[3]. This trend indicates that the prevention and treatment of CRC remain critical and require the exploration of more effective strategies.

Current treatment modalities for CRC primarily include surgical resection, radiation therapy, chemotherapy, immunotherapy, and targeted therapy^[4-7]. Surgical resection remains the core treatment for CRC, but factors such as surgical trauma and anesthesia may affect postoperative

gastrointestinal function recovery^[8-10]. Radiotherapy and chemotherapy have significant advantages in inhibiting tumor cell proliferation, but they are often accompanied by adverse effects such as immunosuppression, anemia, fatigue, and myelosuppression^[11-13]. Although immunotherapy and targeted therapy are highly specific, they are still in the stage of ongoing research and basic experimental validation due to high treatment costs and limited applicability^[14-17].

In recent years, traditional Chinese medicine (TCM) has demonstrated unique advantages in the prevention and treatment of CRC, particularly in inhibiting tumor cell growth, inducing apoptosis, overcoming drug resistance, and regulating immune function^[18-20]. *Astragali Radix*, known as the chief tonic herb, replenishes qi and blood^[20-22]; *Coptis chinensis*, a key herb for clearing heat and detoxifying^[23], shows good clinical efficacy in patients with qi-blood deficiency syndrome (QBDS) and Damp-Heat Syndrome (DHS). astragaloside IV, a major component of *A. Radix*, possesses anti-

inflammatory, antioxidant, and immunomodulatory activities^[24-26]. Berberine, the main active ingredient of *C. chinensis*, inhibits CRC cell proliferation and metastasis by regulating multiple signaling pathways^[27-29].

However, the core concept of TCM treatment for CRC lies in syndrome differentiation and personalized therapy, *i. e.*, tailoring treatment to different syndromes. According to the TCM Diagnosis and Treatment Guidelines for Tumors published by the Chinese Association of Chinese Medicine in 2008, CRC is classified into 6 syndromes, with this study focusing on QBDS and DHS^[30]. Despite abundant clinical experience in TCM treatment of CRC, the lack of objective and unified evaluation criteria has hindered a full understanding of its mechanisms. Therefore, exploring the biological basis of TCM treatment for CRC at the molecular level is crucial for promoting the modernization and internationalization of TCM.

Proteomics, a key research tool in the post-genomic era, aims to reveal the protein composition and dynamic changes of cells, tissues, or organisms at the global level^[31-32]. This approach aligns closely with TCM's philosophy of studying organisms through dynamic, dialectical, and holistic perspectives^[33-34]. The integration of proteomics with medical research not only provides new insights into disease mechanisms but also lays a foundation for precision medicine^[35-36]. Current quantitative proteomics techniques include label-free quantification, labeled quantification, and targeted quantification, which provide powerful technical support for in-depth molecular mechanism research in CRC^[37-38].

This study aims to systematically compare the protein expression differences in tumor tissues between QBDS and DHS using proteomics, screen key differentially expressed proteins (DEPs), and construct syndrome prediction models. Combined with molecular docking and *in vitro* cell experiments, we analyzed the potential targets and mechanisms of astragaloside IV and berberine in different CRC syndromes. For the first time, this study integrates the cross-scale association of TCM syndrome-differential protein-TCM component, providing objective molecular evidence for precision TCM treatment of CRC and promoting the deep integration of TCM syndrome differentiation with modern precision medicine.

1 Materials and methods

1.1 Basic data

This study included tumor tissue specimens from

10 CRC patients with two TCM syndromes who were treated at the Colorectal and Anal Surgery Department of the 940th Hospital of the Chinese People's Liberation Army Joint Logistics Support Force between September 2020 and October 2021, including 5 cases of QBDS and 5 cases of DHS. The study was approved by the hospital ethics committee (Ethics Approval No.: 2020KYLL075).

1.2 Inclusion and exclusion criteria

Inclusion criteria: (1) patients with primary CRC confirmed by clinical manifestations and biopsy pathology; (2) patients who had not received surgery, radiotherapy, chemotherapy, molecular targeted therapy, or immunotherapy; (3) patients who provided informed consent. Exclusion criteria: (1) patients with inconsistent syndrome differentiation results by three TCM physicians; (2) patients with concurrent tumors in other sites; (3) patients who used antibiotics, immunosuppressants, glucocorticoids, or drugs affecting liver/kidney function within the past week; (4) patients with severe underlying diseases incompatible with surgery; (5) patients with incomplete or erroneous clinical data that might affect subsequent analysis. TCM syndrome differentiation criteria: TCM syndrome classification for CRC referred to the TCM Diagnosis and Treatment Guidelines for Tumors (2008) issued by the Chinese Association of Chinese Medicine, which classifies CRC into 6 syndromes. This study focused on QBDS and DHS. Preoperative TCM syndrome differentiation for all patients was performed by two associate chief physicians in the Department of TCM of the 940th Hospital; in case of disagreement, a third associate chief physician or their superior was consulted until consensus was reached. Initially, 13 QBDS patients and 30 DHS patients were enrolled. Based on the principle that tandem mass tag (TMT) detection and proteomics require at least ≥ 3 biological replicates for clinical sample analysis, 5 samples were finally included for each syndrome^[39].

1.3 Collection of CRC patient tumor specimens

All specimens were derived from surgically resected tumor tissues during operations. After washing bloodstains with normal saline and wiping clean with dust-free paper, samples were rapidly frozen in liquid nitrogen for 5 to 15 min and then immediately transferred to an -80°C freezer for storage until shipment to a biotechnology company (Panomic, Suzhou, China) for subsequent testing and analysis.

1.4 Experimental methods for protein extraction from CRC tumor tissues and data processing

Samples were removed from -80°C and

subjected to sequential procedures including protein extraction, protein digestion, peptide labeling, labeled peptide fractionation, gradient elution using an Easy-nLC 1200 liquid chromatograph (Thermo Fisher Scientific, USA), and mass spectrometry data acquisition using an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific, USA). DEPs were screened *via* protein qualitative Venn diagram analysis, quantitative principal component analysis (qPCA) analysis, and hierarchical clustering analysis. The screening criteria were a protein fold change >1.5 and *P*-value <0.05. The identified DEPs were further analyzed *via* volcano plot, clustering heatmap, and functional enrichment analysis (including Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and keyword enrichment). GO enrichment and Circos plot analyses, along with the construction of a least absolute shrinkage and selection operator (LASSO) regression model associated with CRC pathogenesis, were also performed. Additionally, molecular docking was performed between DEPs of different syndrome groups and TCM compounds to determine binding energies, thereby elucidating the biological basis of integrative TCM-WM treatment for CRC and exploring specific targets for TCM treatment.

1.5 Molecular docking

Molecular docking studies were performed on 8 core targets screened by the LASSO regression model to validate the binding potential between TCM active components and target proteins. Astragaloside IV (the active component of *A. Radix* for QBDS treatment) and berberine (the active component of *C. chinensis* for DHS treatment) were selected as ligands. The specific procedures were as follows: two-dimensional molecular structures of the two compounds were obtained from the PubChem database, while the crystal structures of target proteins were downloaded from the PDB database (<https://www.rcsb.org/>). Target proteins were preprocessed using Pymol 3.0.3 to remove irrelevant ligands, water molecules, and repair structural defects. Polar hydrogen atoms were added to the receptor proteins using Autodock 4.2.6 to optimize the molecular force field. Docking simulations were executed *via* AutodockTools 1.5.7 in a Python environment with default parameters to complete ligand-receptor interaction calculations. Binding energy was used as the evaluation index, and a threshold of <-5.0 kcal/mol was set to determine effective binding, clarifying the interaction strength between TCM components and core targets^[40-41].

1.6 Bioinformatics analysis

Based on the 8 core targets screened by LASSO

regression, multi-dimensional bioinformatics databases were used for validation: Immunohistochemical data of target proteins in normal tissues and CRC tumor tissues were obtained from the Human Protein Atlas (<https://www.proteinatlas.org>) to compare and analyze expression differences. The GEPIA database (<http://gepia.cancer-pku.cn/index.html>) was utilized to analyze mRNA expression levels of core targets, their associations with overall survival (OS), disease-free survival (DFS), and expression patterns across different pathological stages, systematically exploring the clinical relevance and prognostic value of the targets.

1.7 In vitro cell experiments

1.7.1 Cell culture

HCT 116 and HT-29 cells (purchased from Shang'en Biotech) were cultured under the following conditions: maintained in a constant temperature incubator at 37°C, 5% CO₂, and 95% air, using RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% double-antibody solution (penicillin-streptomycin mixture, 10⁵ U/L). During culture, cell morphology and growth status were observed daily under a microscope. When cell density reached 80%–90% and entered the logarithmic growth phase, cells were harvested by digestion for subsequent experiments.

1.7.2 Cell proliferation assay

HCT 116 and HT-29 cells in the logarithmic growth phase were adjusted to a density of 1×10⁴ cells/ml and seeded into 96-well plates at 200 µl per well, with 3 parallel replicates per treatment. After pre-incubation in a 37°C, 5% CO₂ incubator for 24 h, drug treatments were applied: experimental groups received medium containing different concentrations (100.0, 50.0, 25.0, 12.5, 6.3, 3.1 mg/L) of astragaloside IV or berberine, while the blank control group received an equal volume of complete medium without drugs. After continuing culture for 24 h, 10 µl of CCK-8 reagent was added to each well, incubated for 4 h, and the absorbance (*A* value) was measured at 450 nm using a microplate reader to calculate relative cell viability.

1.8 Data statistics and analysis

Statistical analyses were performed with SPSS software, and graphs were generated using Origin 2024. The Shapiro-Wilk test was used for normality assessment of continuous variables, while the Levene test was applied to evaluate variance homogeneity. For two-group clinical comparisons, normally-distributed data were expressed as mean±standard deviation (mean±SD) and compared by independent-samples *t* test. Non-normally-distributed data were presented as

median (P_{25} , P_{75}) and analyzed using the Mann-Whitney U test with continuity correction. Categorical variables were reported as n (%) and compared *via* two-tailed Fisher's exact test. All statistical tests were two-tailed, and $P < 0.05$ was considered statistically significant.

2 Results

2.1 Basic data and differential protein analysis

2.1.1 Clinicopathological characteristics of CRC patients

Comparative analysis of clinicopathological characteristics was conducted between 5 CRC patients in the QBDS group and 5 patients in the DHS group. No significant inter-group differences were observed in sex, age, BMI, comorbidities (diabetes mellitus, hypertension), tumor location (left-sided/right-sided colon: 4 : 1 *vs.* 3 : 2), histological differentiation (poorly-differentiated *vs.* well-and-moderately-differentiated: 1 : 4 *vs.* 2 : 3), pathological stage (stage I–II *vs.* stage III–IV: 2 : 3 *vs.* 2 : 3), tumor markers (CEA, CA19-9), routine blood parameters, hepatic-renal function, and coagulation-related indices (fibrinogen, D-dimer). Categorical variables were assessed using two-tailed Fisher's exact test. Normally-distributed continuous variables were compared with independent-samples *t* test, whereas non-normally-distributed ALT and D-dimer were analyzed by Mann-Whitney U test with continuity correction. All variables yielded $P > 0.05$. These findings indicate satisfactory baseline comparability between groups, suggesting that intergroup differences in subsequent study outcomes are unlikely to be confounded by disparities in basic clinical characteristics (Table S1).

2.1.2 Sample data quality control

Comprehensive quality control tests were performed on data from both groups, covering key dimensions such as peptide length distribution, precursor ion mass deviation distribution, and Unique Peptide count distribution. All test indices strictly met preset standards, fully verifying the high reliability of the data. As shown in Figure 1a, peptide lengths were concentrated in the 6–25 amino acid residue range, highly consistent with the theoretical expectations of trypsin digestion, indicating standardized sample preparation processes that met experimental criteria. In terms of repeatability, the peptide length distribution curves of three biological replicate samples showed consistent patterns, further confirming the stability and consistency of sample preparation. Figure 1b demonstrates that precursor ion

mass deviations tightly clustered within 5×10^{-6} , indicating high-quality precision of the mass spectrometer, with deviations within an acceptable range to ensure accurate peptide and protein identification. In Figure 1c, the sufficient number of Unique Peptides in each sample not only corroborated the reliability of protein identification data but also reflected the high specificity of the protein identification process. Figure 1d shows that although there were slight fluctuations in the number of identified peptides and TMT-labeled peptides across samples, the overall values remained within reasonable ranges. Collectively, these quality control results confirm the reliable data quality in this study, with excellent performance in both sample preparation and mass spectrometry detection, laying a solid foundation for subsequent proteomics analysis.

2.1.3 Differential protein analysis

Through TMT proteomics analysis, a total of 5 055 proteins were identified in the QBDS and DHS groups, among which 44 were DEPs. This result indicates that although most proteins showed stable expression between the two TCM syndromes, a small number of proteins exhibited significant differences, which may serve as key factors distinguishing the biological characteristics of QBDS and DHS (Table S2). The original and normalized quantitative distributions show that data normalization effectively eliminated systematic technical bias between samples, enabling protein expression variation to be attributed to biological signals rather than technical deviation (Figure 1e, f). Sample correlation analysis revealed highly similar protein expression patterns within each group, confirming the experimental repeatability and biological rationality of syndrome grouping (Figure 1g).

The volcano plot illustrates the overall distribution of a total of 44 DEPs, including 21 significantly upregulated proteins (red dots) and 23 significantly downregulated proteins (blue dots). The red upregulated proteins were predominantly distributed in the region with $\log_2(FC) > 0.5$ and $-\lg(P\text{-value}) \geq 1.3$ (corresponding to $P < 0.05$), whereas blue downregulated proteins were enriched in the region with $\log_2(FC) < -0.5$ and $-\lg(P\text{-value}) \geq 1.3$. Non-significantly altered proteins are shown in gray. Representative upregulated proteins included INTS5 and MTMR14, whereas typical downregulated proteins such as GPAA1 and FXR2 exhibited markedly decreased expression (Figure 1h). The clustering heatmap further indicated that the top 44 DEPs formed opposite expression patterns between the two groups: the protein cluster highly expressed

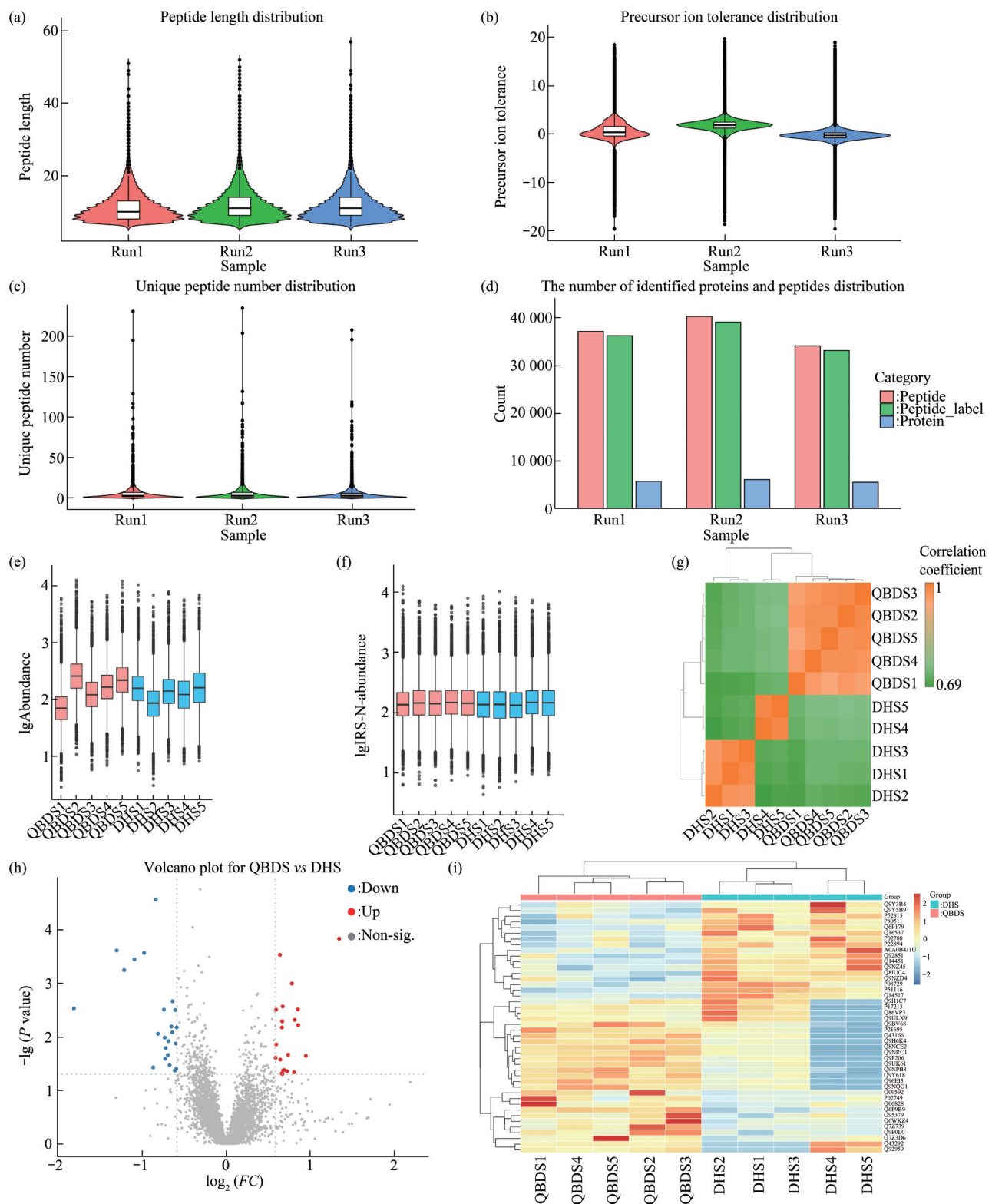


Fig. 1 Quality control and differential proteomic profiling between QBDS and DHS

(a) Peptide length distribution. (b) Peptide mass deviation. (c) Distribution of the number of differential peptides. (d) Distribution of identified peptides and TMT-labeled peptides. (e) Distribution of raw protein quantification values in QBDS and DHS. (f) Distribution of normalized protein quantification values in QBDS and DHS. (g) Spearman correlation analysis of protein quantification between samples. (h) Volcano plot of DEPs. (i) Clustering heatmap of the top 44 DEPs.

(red) in the QBDS group was lowly expressed (blue) in the DHS group, while the cluster lowly expressed (blue) in the QBDS group was highly expressed (red) in the DHS group. Samples clustered clearly by syndrome, demonstrating that these DEP expression profiles can distinguish samples belonging to the two different TCM syndromes at the molecular expression level (Figure 1i).

2.2 Functional enrichment and network analysis

2.2.1 GO enrichment analysis of differentially expressed proteins

This study analyzed the functional characteristics of differentially expressed proteins from three dimensions: biological processes (BP), cellular components (CC), and molecular functions (MF) through GO functional enrichment analysis. The enrichment results were sorted by ascending *P*-values, and the top 20 entries were visualized as bubble plots (Figure S1a–c). Biological processes: the top three enriched terms were negative regulation of viral genome replication, negative regulation by host of viral process, and positive regulation of I- κ B kinase/nuclear factor- κ B (NF- κ B) signaling. Differences in antiviral immune functions may affect the immune surveillance efficiency of CRC patients, suggesting that the immune status of QBDS or DHS syndromes influences tumorigenesis risk by regulating virus-related pathways. Excessive activation of the NF- κ B pathway is directly associated with CRC cell proliferation, metastasis, and chemoresistance, implying that differentially expressed proteins may promote or inhibit tumor progression by enhancing NF- κ B signaling (Figure S1a). Cellular components: differentially expressed proteins were significantly enriched in specific granule lumen, secretory granule lumen, and cytoplasmic vesicle lumen, indicating their primary localization in intracellular secretory structures. Abnormal secretory granule function may impair the ability of immune cells to kill tumors or promote tumor cell immune escape, which is closely related to differences in the immune microenvironment between QBDS and DHS syndromes (Figure S1b). Molecular functions: enrichment of lipopolysaccharide binding suggested that differentially expressed proteins can recognize lipopolysaccharide (LPS) and initiate innate immune responses, which is highly consistent with the damp-heat pathological characteristics of DHS (Figure S1c). Enrichment of prostaglandin transmembrane transporter activity indicated their role in regulating the transmembrane transport of inflammatory lipid mediators, which are key regulators of inflammatory responses and tumor angiogenesis—differences in

transporter activity may lead to variations in inflammation intensity or metabolic homeostasis between QBDS and DHS. Enrichment of D-glutamate cyclase activity suggested involvement in the cyclization modification of D-glutamate, potentially affecting the synthesis of neurotransmitters or peptide hormones, and influencing syndrome-related metabolic regulation through non-canonical pathways.

2.2.2 PPI network analysis of differentially expressed proteins

A protein-protein interaction (PPI) network of differentially expressed proteins was constructed using the STRING database, which included 44 differentially expressed protein nodes and interaction edges (Figure S1d). Upregulated proteins such as INTS5 and MTMR14 were closely interacted with NF- κ B pathway proteins, potentially promoting the formation of an immunosuppressive microenvironment in the QBDS syndrome by enhancing inflammatory signals. Downregulated proteins such as GPAA1 interacted with cell adhesion molecules, and their functional inhibition may be associated with reduced tumor cell adhesion in the QBDS syndrome. Downregulated proteins such as FXR2 interacted with spliceosome complex proteins, potentially affecting gene expression regulation in the DHS syndrome by interfering with RNA processing. Downregulated proteins such as MAF bZIP transcription factor F (MAFF) interacted with inflammatory cytokines, which may exacerbate chronic inflammation in the DHS syndrome.

2.2.3 UniProt keyword enrichment analysis of differentially expressed proteins

Functional classification of 44 DEPs using hierarchical controlled vocabulary revealed that antibiotic, phosphoprotein, and disulfide bond were the most significant keywords, highlighting the core roles of these proteins in antimicrobial defense, signal transduction, and protein structural stability (Figure S1e). Upregulation of antibiotic-related proteins in the QBDS group may enhance antiviral defense, while downregulation of related proteins in the DHS group may reduce antimicrobial capacity and exacerbate infection risk in damp-heat environments. Phosphoprotein enrichment indicated widespread involvement in phosphorylation signaling—a core mechanism of cellular regulation—abnormal signaling was closely related to CRC proliferation and metastasis. Upregulation of phosphoproteins in the QBDS group may enhance inflammatory signals, while regulatory imbalances in the DHS group may promote abnormal tumor cell proliferation. Differences in disulfide bond-related protein

expression may affect the mechanical properties and drug resistance of CRC cells, consistent with the invasive phenotype of the DHS syndrome.

2.2.4 KEGG pathway enrichment analysis of differentially expressed proteins

KEGG pathway enrichment analysis showed that differentially expressed proteins were significantly enriched in cholesterol metabolism and glycerophospholipid metabolism pathways, suggesting that lipid metabolic abnormalities are central to the biological mechanisms of syndrome differentiation (Figure S1f). GPD1, upregulated in the QBDS group, participates in glycerophospholipid synthesis and may influence tumor cell energy metabolism by regulating cholesterol metabolism intermediates. LTF, downregulated in the DHS group, inhibits tumor growth by suppressing cholesterol uptake—its reduced expression may lead to cholesterol metabolic imbalance and promote tumor progression. Upregulation of cholesterol metabolism-related proteins in the QBDS syndrome may enhance lipid synthesis capacity in tumor cells to adapt to increased energy demands in the “qi-blood deficiency” state, while downregulation of metabolic regulatory proteins in the DHS syndrome may exacerbate cholesterol accumulation and promote inflammation *via* NF- κ B pathway activation, consistent with the pathological characteristics of damp-heat accumulation.

2.2.5 Circos analysis of differentially expressed proteins

A Circos plot was used to visually illustrate the correlation between DEPs and functional enrichment results: suppression of tumorigenicity 7 (ST7) and podocalyxin-like (PODXL), upregulated in the QBDS group, were closely linked to the functional term negative regulation of viral genome replication and connected *via* lines to GPD1 in the cholesterol metabolism pathway, suggesting a synergistic effect between antiviral immunity and lipid metabolism. LTF, downregulated in the DHS group, was simultaneously linked to lipopolysaccharide binding and cholesterol metabolism—its reduced expression may weaken antimicrobial capacity and cholesterol uptake regulation, exacerbating gut microbiota dysregulation and lipid accumulation in damp-heat environments. Phosphoprotein-related proteins were enriched in the phosphorylation signaling module and connected to phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway nodes, reflecting abnormal activation of cell proliferation signals in both syndromes. Disulfide bond-related proteins were associated with cytoskeletal stability functions, and

expression differences may affect tumor cell invasiveness—downregulation of keratin 7 (KRT7) in the DHS group may enhance cell migration potential by disrupting keratin networks. The highlighted cholesterol metabolism and glycerophospholipid metabolism pathways in the Circos plot were consistent with KEGG analysis results, further confirming that lipid metabolic abnormalities are the core mechanism of syndrome differentiation (Figure S2).

2.2.6 LASSO regression analysis of differentially expressed proteins

LASSO regression analysis was performed on 44 DEPs to screen core proteins most closely associated with CRC pathogenesis and construct syndrome-specific prediction models. Figure 2a shows the coefficient path diagram of the LASSO regression model. When the Binomial Deviance value was low, the minimum deviation point marked by the left dashed line and the larger λ value corresponding to the right dashed line both indicated that the optimal number of DEPs for the model was 19, which fully matched the 19 DEPs corresponding to the leftmost red dashed line in Figure 2b. Among the 19 DEPs screened by LASSO regression, 8 had positive coefficients and 11 had negative coefficients (may inhibit CRC or be associated with good prognosis) (Figure 2b). These results suggest that differentially expressed proteins exhibit complex regulatory patterns in CRC. Some positively correlated proteins may drive tumor development by activating pro-cancer pathways, while negatively correlated proteins might also be involved in tumor progression through loss of tumor-suppressive functions or other pro-tumorigenic mechanisms. Among the 8 potentially pro-cancer DEPs, the QBDS group included 3 proteins (ST7, glycerophosphocholine phosphodiesterase 1(GPCPD1), PODXL), and the DHS group included 5 proteins (FAT atypical cadherin 1 (FAT1), growth factor receptor bound protein 7 (GRB7), KRT7, MAFF, matrix metalloproteinase 8 (MMP8)).

2.3 Molecular docking analysis

To validate the correlation between the key proteins of QBDS (ST7, GPCPD1, PODXL) and DHS (FAT1, GRB7, KRT7, MAFF, MMP8) syndromes and active components of TCMs, this study selected astragaloside IV (the active component of *A. Radix* for treating QBDS) and berberine (the active component of *C. chinensis* for treating DHS) to perform molecular docking with the above core proteins, respectively.

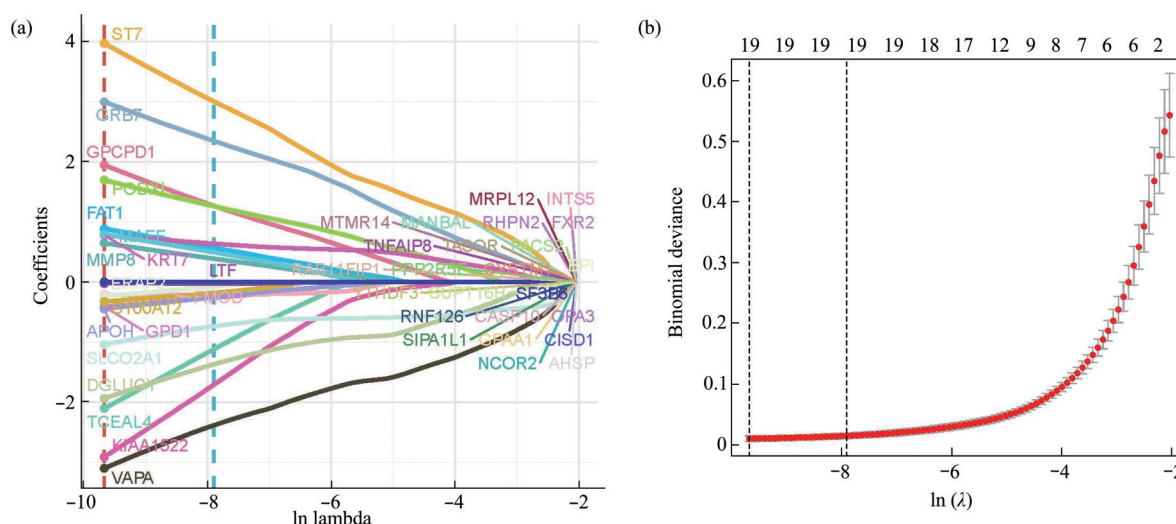


Fig. 2 LASSO regression analysis of differentially expressed proteins

(a) Coefficient path diagram of the LASSO regression model. Each colored curve depicts the trajectory of the regression coefficient of one protein as $\log(\lambda)$ changes, and the vertical dashed line marks the optimal λ value. (b) Plot of 10-fold cross-validation for the LASSO regression model. The numbers at the top represent the number of proteins with non-zero regression coefficients under the corresponding penalty parameter λ . The two vertical dashed lines indicate λ_{1se} (one standard error λ , the largest λ within one standard error of the minimum binomial deviance) and λ_{min} (minimum λ , the λ corresponding to the minimum binomial deviance), respectively. y-axis: binomial deviance; x-axis: $\log(\lambda)$.

2.3.1 Docking analysis of astragaloside IV with QBDS group proteins

According to the data in Table S3, the binding energies of astragaloside IV with the 3 proteins in the QBDS group were all less than -5 kcal/mol, indicating strong binding ability between the ligand and receptor (Figure 3). Specifically, astragaloside IV formed 4 stable hydrogen bonds with the amino acid residues GLN-51 and ARG-251 of PODXL protein, with a binding energy of -6.04 kcal/mol (Figure 3a); it formed 4 hydrogen bonds with ST7 protein through amino acid residues GLY-200 and ALA-234, with a binding energy of -5.62 kcal/mol (Figure 3b); and it interacted with GPCPD1 protein by forming 1 hydrogen bond with the amino acid residue GLY-175, with a binding energy of -5.24 kcal/mol (Figure 3c).

2.3.2 Docking analysis of berberine with DHS group proteins

As shown in Figure S3, the binding energies of berberine with the five proteins in the DHS group were all significantly lower than -5 kcal/mol, demonstrating stronger binding specificity (Table S3). As follows: it formed 3 hydrogen bonds with MAFF protein at amino acid residues ASP-158, GLY-56, and ASP-57, with a binding energy as low as -9.63 kcal/mol (Figure S3a); it formed 3 hydrogen bonds with MMP8 protein at amino acid residues ASP-158, ASP-57, and GLY-56, with a binding energy of -9.35 kcal/mol (Figure S3b); it formed 1 hydrogen bond with FAT1 protein through the amino acid residue ARG-105, with a binding energy of

-8.78 kcal/mol (Figure S3c); it formed 3 hydrogen bonds with GRB7 protein at amino acid residues HIS-525, CYS-527, and GLN-429, with a binding energy of -8.19 kcal/mol (Figure S3d); and it formed 1 stable hydrogen bond with KRT7 protein through the amino acid residue ARG-128, with a binding energy of -7.50 kcal/mol (Figure S3e). The hydrogen bonds formed during the docking process are key molecular forces driving the binding of proteins to active sites. The above results not only validate the direct interaction between the core proteins screened by proteomics and TCM components but also provide key evidence for interpreting the molecular mechanisms of TCMs acting on CRC with different TCM syndromes.

2.4 Integrated bioinformatics analysis

2.4.1 Analysis of key protein expression differences

Immunohistochemical analysis of 8 core proteins (QBDS group: ST7, GPCPD1, PODXL; DHS group: FAT1, GRB7, KRT7, MAFF, MMP8) screened by Lasso regression was performed based on the Human Protein Atlas (HPA) database. As shown in Figure 4a, the staining intensity of ST7 and PODXL (upregulated proteins in the QBDS group) in tumor tissues was significantly higher than that in normal tissues, suggesting that their high expression may be involved in CRC progression by promoting an immunosuppressive microenvironment, which is consistent with the pathological characteristics of the QBDS syndrome. The downregulated proteins in the DHS group, including FAT1, GRB7, KRT7, MAFF,

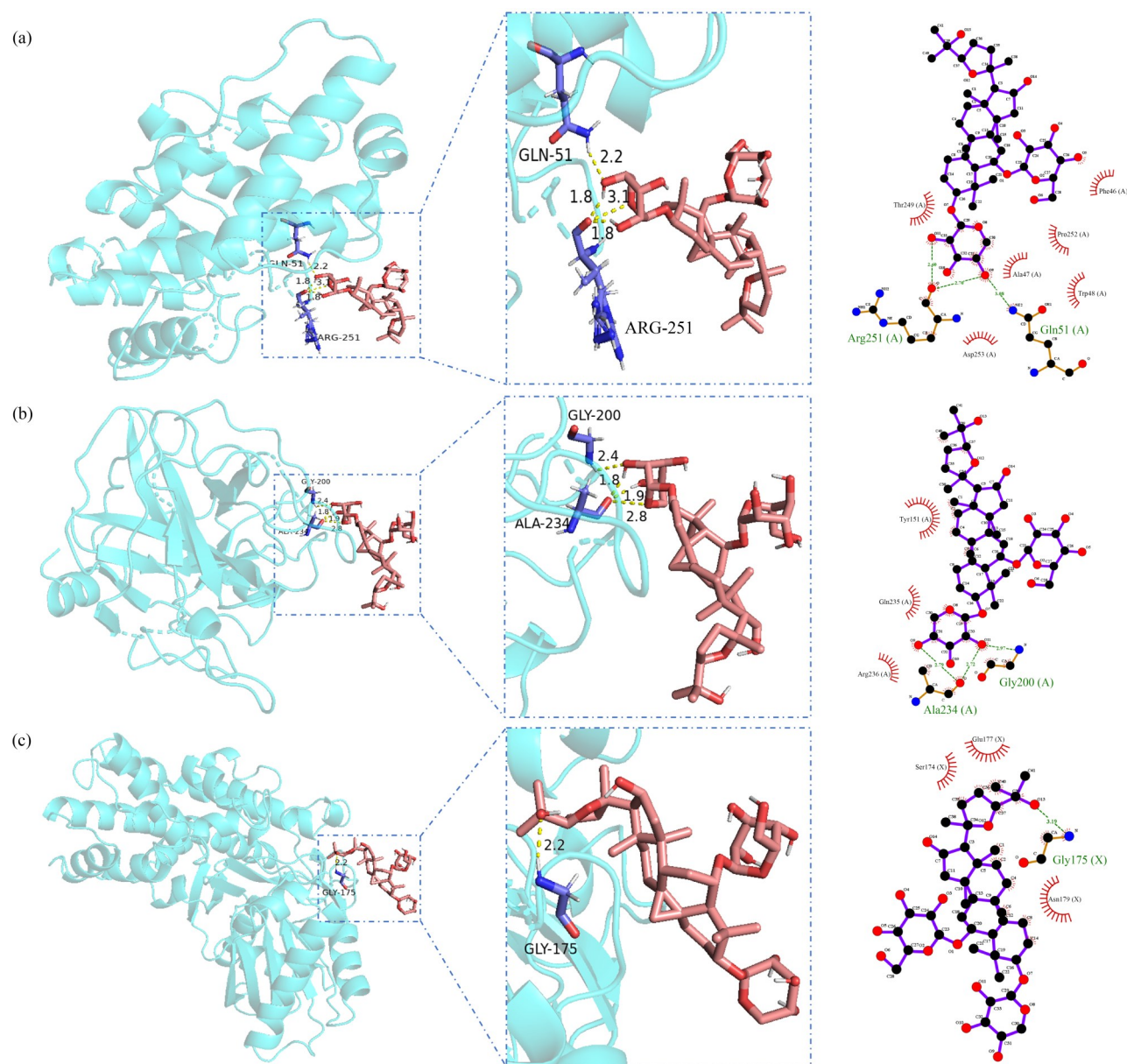


Fig. 3 Visual analysis of molecular docking results between astragaloside IV and key proteins in the QBDS group

(a) PODXL protein. (b) ST7 protein. (c) GPCPD1 protein.

and MMP8, showed significantly increased expression in tumor tissues. Their abnormal activation may drive the formation of a damp-heat microenvironment in the DHS syndrome by regulating extracellular matrix remodeling and signaling pathways (Figure S4). It is worth noting that no valid immunohistochemical data for GPCPD1 were found in the HPA database for CRC tissues, which may be due to the limited reliability of the available antibody or suboptimal staining conditions in the current HPA dataset, rather than a lack of GPCPD1 expression in CRC. Indeed, GPCPD1 expression has been reported in multiple other cancer types including breast, lung, and ovarian cancers, and

its elevated expression in colon cancer has been associated with poor prognosis. Further validation of expression differences at the mRNA level and clinical relevance using transcriptome databases such as GEPIA is required.

2.4.2 GEPIA database analysis of key proteins

Based on the GEPIA database, a correlation analysis was performed on the mRNA expression levels, survival prognosis, and pathological stages of the 8 core proteins screened by LASSO regression. The results showed that the mRNA expression of *ST7*, *PODXL*, and *GPCPD1* (upregulated proteins in the QBDS group) in CRC (COAD) tumor tissues was significantly higher than that in normal tissues (Figure

4b), suggesting that their high expression may be related to the tumor immune escape or metabolic adaptation mechanisms of the QBDS syndrome. The downregulated proteins in the DHS group, including FAT1, GRB7, KRT7, MAFF, and MMP8, all showed upregulated expression in tumor tissues, which was consistent with the pro-inflammatory and pro-invasive characteristics of the “damp-heat accumulation” syndrome in proteomics (Figure 4b, S5a). Among them, the expression differences of GRB7 and KRT7 proteins between tumor tissues and normal tissues were statistically significant.

Survival analysis showed that the high-expression and low-expression groups of ST7, PODXL, and MMP8 proteins had similar rates of survival decline in the early follow-up period, but the survival rate of the high-expression group was significantly lower than that of the low-expression group in the later period. There was no statistically significant difference in survival rates between the high-expression and low-expression groups of FAT1, GRB7, GPCPD1, KRT7, and MAFF proteins. Overall, there were no significant differences in survival curves between the high-expression and low-expression groups of all key proteins ($P>0.05$, Figure 4c, S5b), which may be attributed to tumor heterogeneity, unadjusted confounding factors, or the possibility that these proteins do not possess independent prognostic value.

Pathological stage analysis revealed differences in the expression distribution of proteins across stages (Figure 4d, S5c). Among them, ST7 and GRB7 showed distinct expression patterns across stages I–IV, with ST7 being relatively stable and GRB7 showing a gradual increasing trend from stage I to IV, although the differences were not statistically significant. The upregulated proteins in the QBDS group (ST7, PODXL, and GPCPD1) showed relatively stable expression levels across all stages, suggesting that their functions may be more focused on early metabolic regulation. FAT1 protein expression showed a slow downward trend with increasing stages, indicating a negative correlation with tumor progression and suggesting its key role in early tumor invasion. KRT7 protein expression levels increased with pathological stages, which was highly consistent with the highly invasive phenotype of the DHS.

2.5 Inhibitory effects of astragaloside IV and berberine on CRC cells

The proliferation inhibitory activities of astragaloside IV and berberine on HCT 116 and

HT-29 CRC cells were detected by CCK-8 assay (Figure 5). For HCT 116 cells, the cell viability of astragaloside IV showed a significant downward trend with the increase of drug concentration and the prolongation of action time. The IC_{50} values at 12, 24, and 48 h were 67.13, 50.70, and 26.92 mg/L, respectively (Figure 5a). The inhibitory potency showed a time-dependent increase, with the IC_{50} at 48 h being approximately 60% lower than that at 12 h, indicating nearly 2.5-fold enhanced efficacy. In HT-29 cells, the IC_{50} values of astragaloside IV at 12, 24, and 48 h were 61.59, 33.42, and 23.51 mg/L, respectively, indicating that the higher the drug concentration and the longer the action time, the stronger the inhibitory effect on cell proliferation (Figure 5c).

Berberine showed significantly stronger inhibitory activity against HCT 116 cells than astragaloside IV. Its IC_{50} values at 12, 24, and 48 h were 34.04, 21.32, and 15.21 mg/L, respectively (Figure 5b). The IC_{50} at 48 h was approximately 50% lower than that at 12 h, demonstrating a stronger dose-time synergistic effect. In HT-29 cells, the IC_{50} values of berberine at 12, 24, and 48 h were 43.68, 29.82, and 16.95 mg/L, respectively (Figure 5d). The inhibition efficiency gradually increased with the prolongation of time, and the half-maximal inhibitory concentration at 48 h was the lowest, indicating that the sustained effect of berberine was more significant. It is worth noting that the IC_{50} values of berberine against both cell lines were lower than those of astragaloside IV at the same time point, suggesting that berberine has a stronger inhibitory effect on the proliferation of CRC cells.

3 Discussion

In this study, TMT proteomics identified 44 DEPs in tumor tissues of QBDS and DHS, including 21 upregulated and 23 downregulated proteins in the QBDS group. GO enrichment analysis showed that DEPs were significantly enriched in pathways such as antiviral immunity, and inflammatory response, indicating that the biological differences between the two syndromes are closely related to immune-metabolic disorders. For example, ST7 and PODXL upregulated in the QBDS group may adapt to the energy demand in the qi-blood deficiency state by enhancing antiviral immunity and lipid synthesis, while LTF and KRT7 downregulated in the DHS group may exacerbate the inflammatory microenvironment and tumor invasiveness of damp-heat accumulation by weakening antibacterial ability and destroying cytoskeletal stability^[42].

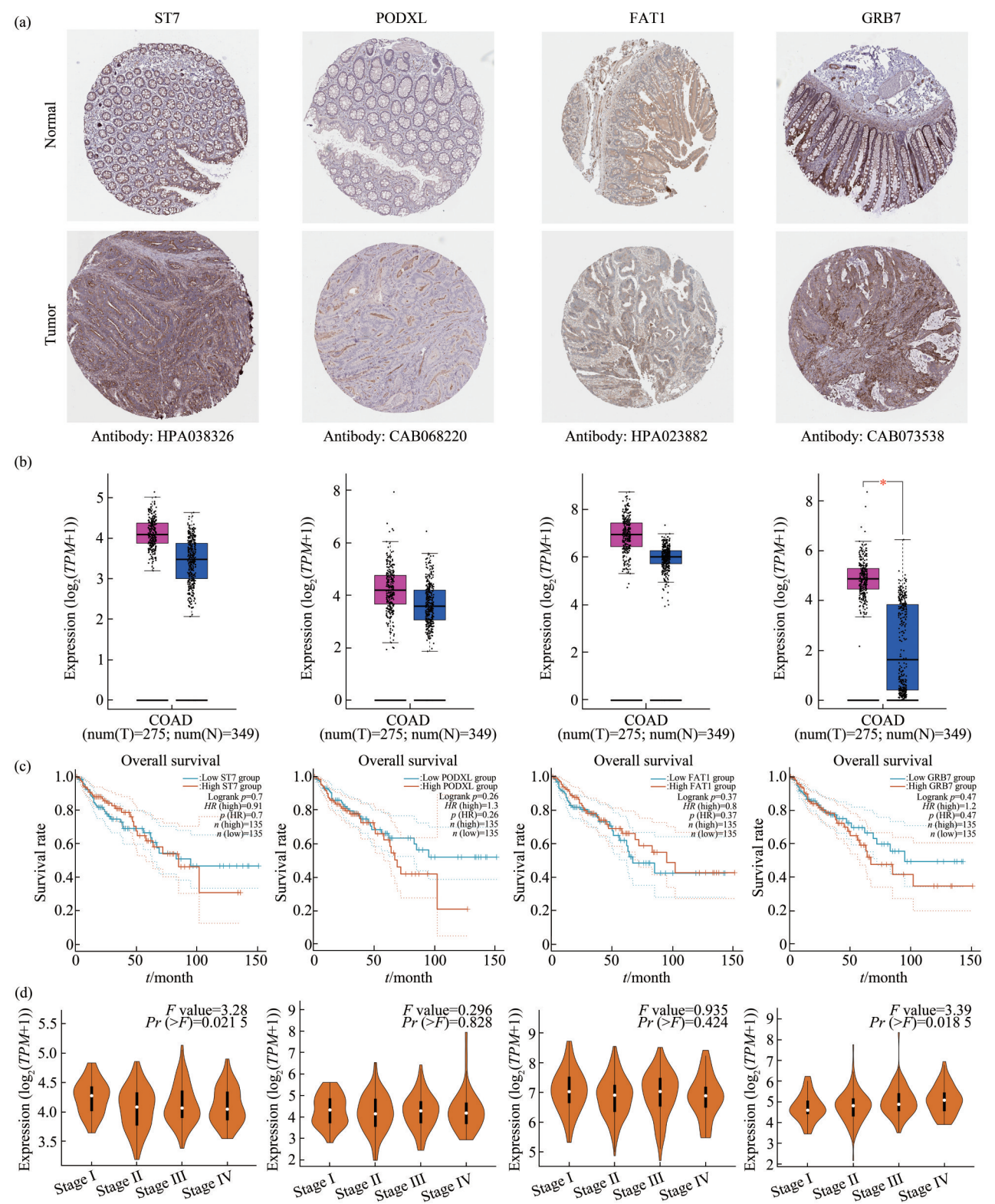


Fig. 4 Integrated bioinformatics validation of core protein expression, survival, and pathological stage associations

(a) Immunohistochemical comparison of ST7, PODXL, FAT1, and GRB7 protein expression between normal and tumor tissues from the HPA database (for citation of this figure, refer to <https://www.proteinatlas.org/>). (b) Box plots of mRNA expression levels of ST7, PODXL, FAT1, and GRB7 ($P<0.05$; red=tumor group, blue=normal group). (c) Survival curves of core targets in CRC patients with high (orange-red) and low (sky-blue) expression ($P<0.05$). (d) Violin plots of mRNA expression levels of core targets across different pathological stages of CRC.

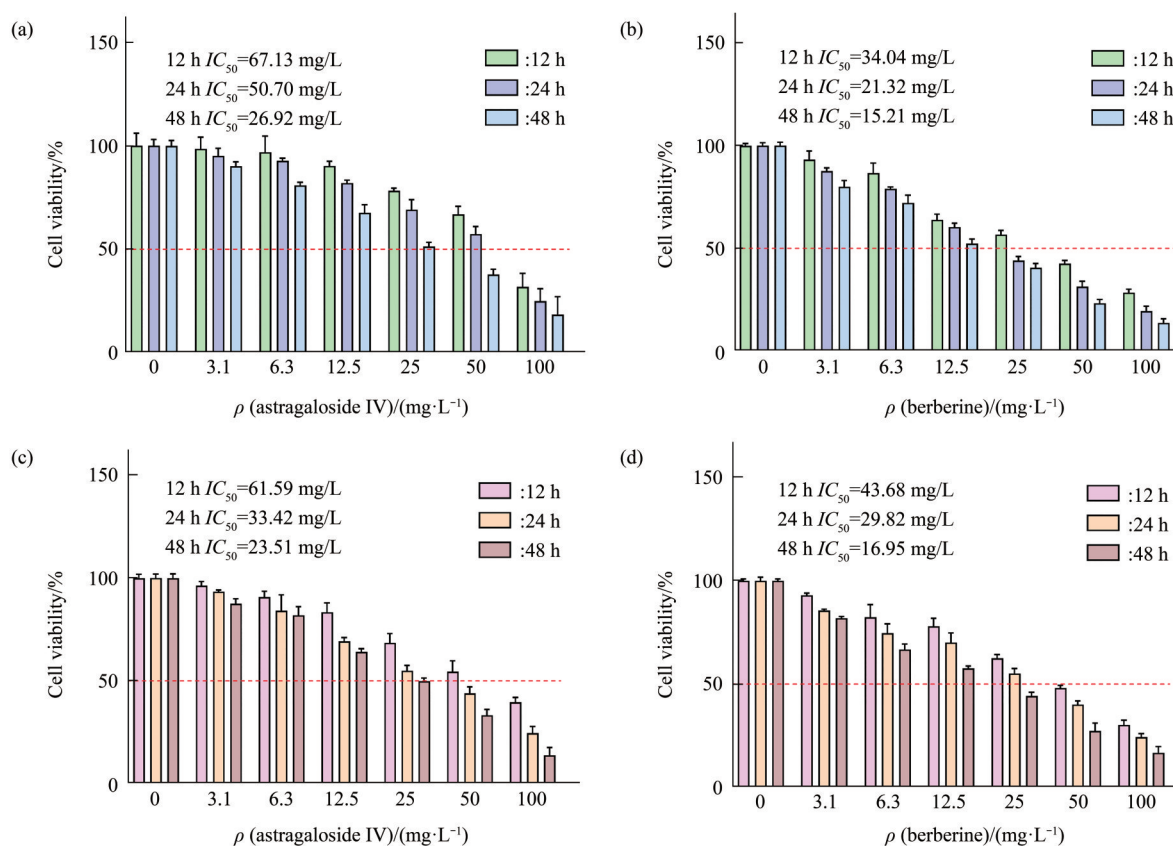


Fig. 5 Inhibitory effects of astragaloside IV and berberine on the proliferation of CRC cells (HCT 116 and HT 29)

(a) Inhibitory effect of astragaloside IV on HCT 116 cells. (b) Inhibitory effect of berberine on HCT 116 cells. (c) Inhibitory effect of astragaloside IV on HT-29 cells. (d) Inhibitory effect of berberine on HT-29 cells.

The LASSO regression model further screened out 8 core pro-cancer proteins, including ST7, GPCPD1, and PODXL in the QBDS group, and FAT1, GRB7, KRT7, MAFF, and MMP8 in the DHS group. These proteins interact with key pathways such as NF- κ B and PI3K/AKT in the PPI network, suggesting that they drive tumor progression by regulating inflammatory signals, cell adhesion, and matrix degradation. For example, as a transcription factor, MAFF interacts with IL-6 to exacerbate inflammation^[43], and MMP8 promotes metastasis by degrading the extracellular matrix, which is consistent with the highly invasive phenotype of the DHS syndrome^[44].

Molecular docking results showed that the binding energies of astragaloside IV with core proteins in the QBDS group were all < -5 kcal/mol, among which 4 hydrogen bonds were formed with PODXL, suggesting that it regulates immune-related targets through stable binding. Berberine had lower binding energies with DHS group proteins and formed hydrogen bonds with multiple amino acid residues, showing stronger binding specificity, which may exert its effects by inhibiting MAFF-mediated

inflammatory pathways and MMP8's matrix degradation function^[45-46].

Notably, berberine showed significantly stronger inhibitory effects on the proliferation of HCT 116 and HT-29 cells than astragaloside IV (48 h IC_{50} as low as 15.21 mg/L), and exhibited time-dose dependence, which was consistent with the higher binding energy in molecular docking, suggesting that berberine has better therapeutic potential in damp-heat syndromes. It should be noted, however, that HCT116 and HT-29 cells are conventional CRC lines and do not recapitulate the *in vivo* immune or inflammatory microenvironment characteristic of QBDS or DHS. Therefore, the observed anti-proliferative effects cannot be directly interpreted as syndrome-selective mechanisms; rather, the evidence for syndrome specificity primarily derives from the differential protein expression in clinical tissues and the binding specificity differences in molecular docking. Future studies using co-culture systems or animal models that simulate syndrome-specific microenvironments are needed to further validate these mechanistic links^[47].

Validation through GEPIA databases showed that ST7 mRNA was significantly upregulated in tumor tissues compared with normal tissues. The HPA immunohistochemical data for ST7 were used as a supplementary reference; however, given the potential limitations in antibody reliability and staining conditions within the database, these IHC results should be interpreted with caution. Our primary evidence for ST7 upregulation derives from the TMT proteomic data and GEPIA transcriptomic data. Similarly, PODXL was highly expressed in tumor tissues and had no significant association with pathological stages, suggesting that their functions may focus on early metabolic regulation of tumorigenesis^[48-49]; FAT1 expression showed a downward trend with increasing stages, possibly related to early tumor invasion^[50], while KRT7 expression was positively correlated with stages, consistent with its pro-metastatic role in tumor progression^[51]. Survival analysis showed no significant differences in survival rates between high-expression and low-expression groups of core proteins, which may be affected by sample size limitations or multifactorial regulation and requires verification in large sample cohorts. Given that the survival analysis yielded no significant differences between high- and low-expression groups for any of the eight core proteins ($P>0.05$), these findings do not support a prognostic role for these targets. Their primary value lies in their differential expression between TCM syndromes, and they should be considered as hypothesis-generating candidates for syndrome-related molecular differences rather than as established prognostic biomarkers. Future studies with larger cohorts and rigorous confounder control are needed to determine whether any of these proteins have independent prognostic significance. In addition, we acknowledge the value of protein-level survival analysis tools such as OSppc (based on CPTAC proteomic data) for future validation, which may complement our current mRNA-based GEPIA analysis and further strengthen the evidence for the prognostic relevance of these core proteins.

This study first constructed a TCM syndrome-differentially expressed protein-Chinese medicine component association network and found that astragaloside IV and berberine exerted their effects by targeting core proteins of QBDS/DHS groups, respectively. For example, astragaloside IV may improve the immunosuppressive microenvironment in the qi-blood deficiency state by regulating ST7-mediated immune surveillance and GPCPD1-involved lipid metabolism^[52-53]; berberine may reverse the pro-

inflammatory microenvironment of the DHS by inhibiting MAFF/NF- κ B pathways and MMP8's invasion-related functions. This finding provides molecular evidence for the syndrome-specific medication of Chinese medicines^[44, 54]. For example, berberine's strong binding capacity to MAFF is consistent with its anti-inflammatory effect, and astragaloside IV's regulation of PODXL is consistent with its immunomodulatory effect.

This study has a small sample size and only includes two syndromes, which may affect the generalizability of the results. Nevertheless, to mitigate the risk of overfitting inherent in high-dimensional proteomic data with a limited cohort, we employed 10-fold cross-validation in the LASSO regression and selected core proteins using both $\lambda_{\min}$ and λ_{1se} criteria. In addition, independent validation in public database and cross-validation by molecular docking and *in vitro* CCK-8 assays further support the robustness of our findings. Thus, the eight core proteins identified by LASSO should be regarded as hypothesis-generating candidates that require independent validation in larger cohorts, rather than as definitive conclusions. In addition, the lack of immunohistochemical data for GPCPD1 in the HPA database requires supplementary verification by experiments such as Western blot. Regarding GPCPD1, although it was selected based on our TMT data (upregulated in QBDS, fold change >1.5 , $P=0.0031$) and LASSO regression, the lack of supporting IHC data from HPA and the non-significant mRNA difference in GEPIA suggest that its role as a core QBDS protein remains tentative and requires further validation. Furthermore, we acknowledge that direct validation of target protein regulation by astragaloside IV and berberine in cell lines was not performed in the current study. Due to limited research funding, we were unable to conduct these experiments at this stage. Nevertheless, our multi-level evidence—including molecular docking, CCK-8 assays, and public database analyses—provides substantial support for the proposed compound-target associations. We plan to perform systematic validation of protein-level regulation in future studies. The dynamic expression differences of core proteins in different syndromes and their associations with clinical prognosis still need to be verified by expanding the sample size and including multi-center data.

From a technical perspective, proteomics only reflects the protein expression status at a certain time point and lacks real-time monitoring of dynamic signaling pathways. In the future, phosphoproteomics

or single-cell sequencing technology can be combined to analyze the dynamic activation patterns of key pathways such as NF- κ B and PI3K/AKT in different syndromes and deeply interpret the time-sequential regulation mechanisms of astragaloside IV and berberine. In addition, although molecular docking confirms the binding potential between drugs and targets, experiments such as surface plasmon resonance (SPR) or fluorescence polarization are needed to verify the affinity, and *in vivo* efficacy verification in animal models is required to clarify the target delivery efficiency and toxicity differences of drugs in different syndromes. Furthermore, we acknowledge the value of tools such as OSTme for analysing tumour microenvironment components, which could be applied in future studies to further explore the immune-related contributions of these DEPs.

It should be noted that the primary objective of this study was to compare the proteomic profiles between two TCM syndromes within CRC; therefore, only tumor tissues were analyzed without paired adjacent non-cancerous tissues. This design was a deliberate choice to maximize the statistical power for inter-syndrome comparison under constrained sample resources, rather than an oversight. Although we validated the aberrant expression of the core proteins in tumor versus normal tissues using public databases and confirmed their functional relevance *via in vitro* assays, the lack of direct matched normal controls remains a limitation. Future studies incorporating paired adjacent tissues are warranted to further distinguish syndrome-specific alterations from general cancer-related changes and to elucidate the mechanistic roles of these core proteins in CRC pathogenesis.

In the TCM syndrome differentiation system, the associations between constitutional factors, gut microbiota, and syndromes have not been included in the study. In follow-up research, metabolomics and microbiomics data can be integrated to construct a multi-dimensional network of syndrome-molecule-microbiota to reveal the gut microecological characteristics of damp-heat/qi-blood deficiency syndromes and their interactions with Chinese medicine targets. For example, the “damp-heat” characteristics of the DHS syndrome may be related to LPS accumulation induced by gut microbiota dysregulation, and berberine’s antibacterial properties may indirectly inhibit tumors by regulating the microbiota.

In terms of clinical translation, it is necessary to

develop Chinese medicine compound formulas or nano-delivery systems targeting core targets to improve the drug targeting for specific syndromes. For example, based on the immunomodulatory properties of astragaloside IV, tumor microenvironment-responsive nanocarriers can be designed to enhance its enrichment in immunosuppressive areas; for berberine’s multi-target properties, structural optimization can be used to improve the binding specificity with proteins such as MAFF and reduce systemic toxicity.

4 Conclusion

Through proteomics, molecular docking, and *in vitro* cell experiments, this study systematically revealed the differential protein characteristics of CRC in QBDS and DHS and the action mechanisms of astragaloside IV and berberine. The study identified 44 DEPs, and 8 core targets were screened by LASSO regression. Among them, astragaloside IV regulates immunity and lipid metabolism by targeting proteins such as PODXL and ST7, while berberine inhibits inflammation and invasion-related proteins such as MAFF and MMP8 through strong binding energy, and *in vitro* experiments confirmed that berberine has a stronger inhibitory effect on the proliferation of CRC cells. Bioinformatics verification showed that core proteins were associated with tumor tissue expression and pathological stages. Although survival analysis did not show significant differences due to sample size limitations, their molecular mechanisms provide valuable proteomic clues for the cross-scale association of TCM syndrome-differentially expressed protein-Chinese medicine component, although further validation with normal tissue controls is needed to confirm the cancer-specificity of these targets. This study first established the proteomic basis for precision treatment by integrating traditional Chinese and Western medicine, laying a foundation for the targeted intervention of Chinese medicines in different syndromes of CRC. In the future, mechanism verification and clinical translation need to be further deepened through sample expansion, multi-omics integration, and *in vivo* experiments.

Data Availability The data associated with this paper (DOI: 10.57760/sciencedb.00zqj/CSTR:31253.11. sciencedb.00zqj) are available from the Science Data Bank (ScienceDB) database at <https://www.scidb.cn/detail?dataSetId=09f56c812dad4374a5ce79f67842c07d>.

Supplementary Available online (<http://www.pibb.ac.cn>, <http://www.cnki.net>):

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References

- [1] Bray F, Laversanne M, Sung H, *et al.* Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, 2024, **74**(3): 229-263
- [2] Han B, Zheng R, Zeng H, *et al.* Cancer incidence and mortality in China, 2022. *J Natl Cancer Cent*, 2024, **4**(1): 47-53
- [3] Tan Z, Sun W, Li Y, *et al.* Current progress of EMT: a new direction of targeted therapy for colorectal cancer with invasion and metastasis. *Biomolecules*, 2022, **12**(12): 1723
- [4] Saha S, Ghosh S, Ghosh S, *et al.* Unraveling the complexities of colorectal cancer and its promising therapies-an updated review. *Int Immunopharmacol*, 2024, **143**(Pt 1): 113325
- [5] Azarian M, Ramezani Farani M, C Cho W, *et al.* Advancements in colorectal cancer treatment: the role of metal-based and inorganic nanoparticles in modern therapeutic approaches. *Pathol Res Pract*, 2024, **264**: 155706
- [6] Han Q, Li J, Li Z, *et al.* Novel therapeutic strategies and recent advances in gut microbiota synergy with nanotechnology for colorectal cancer treatment. *Mater Today Bio*, 2025, **31**: 101601
- [7] El Omari N, El Fessikh M, Aboulaghras S, *et al.* The role of inflammation in colorectal cancer and the preventive potential of natural compounds. *J Funct Foods*, 2025, **129**: 106857
- [8] Gao X H, Li J, Liu L J, *et al.* Trends, clinicopathological features, surgical treatment patterns and prognoses of early-onset versus late-onset colorectal cancer: a retrospective cohort study on 34067 patients managed from 2000 to 2021 in a Chinese tertiary center. *Int J Surg*, 2022, **104**: 106780
- [9] Plasencia L D, Aparicio A B, Monzón E T, *et al.* Laparoscopic vs open surgery as surgical treatment of colorectal cancer with SEMS: feasibility of the procedure and results on survival and recurrence. *Eur J Surg Oncol*, 2024, **50**(2): 107638
- [10] Hahn S J, Sylla P. Technological advances in the surgical treatment of colorectal cancer. *Surg Oncol Clin N Am*, 2022, **31**(2): 183-218
- [11] Yazdimamaghani M, Kolupaev O V, Lim C, *et al.* Tumor microenvironment immunomodulation by nanoformulated TLR 7/8 agonist and PI₃k delta inhibitor enhances therapeutic benefits of radiotherapy. *Biomaterials*, 2025, **312**: 122750
- [12] Agboyibor C, Dong J, Effah C Y, *et al.* Epigenetic compounds targeting pharmacological target lysine specific demethylase 1 and its impact on immunotherapy, chemotherapy and radiotherapy for treatment of tumor recurrence and resistance. *Biomed Pharmacother*, 2023, **157**: 113934
- [13] Wang R, Liu Y, Liu M, *et al.* Combating tumor PARP inhibitor resistance: combination treatments, nanotechnology, and other potential strategies. *Int J Pharm*, 2025, **669**: 125028
- [14] Luo Y, Liang G, Zhang Q, *et al.* The role of cGAS-STING signaling pathway in colorectal cancer immunotherapy: mechanism and progress. *Int Immunopharmacol*, 2024, **143**(Pt 3): 113447
- [15] Goto N, Agudo J, Yilmaz Ö H. Early immune evasion in colorectal cancer: interplay between stem cells and the tumor microenvironment. *Trends Cancer*, 2025, **11**(9): 850-861
- [16] Rastin F, Javid H, Oryani M A, *et al.* Immunotherapy for colorectal cancer: rational strategies and novel therapeutic progress. *Int Immunopharmacol*, 2024, **126**: 111055
- [17] Chou M Y, Yang M H. Immunomodulation on tumor immune microenvironment in acquired targeted therapy resistance and implication for immunotherapy resistance. *Transl Oncol*, 2025, **54**: 102353
- [18] Jin Y, Liu H, Wang Y, *et al.* Pathogenesis and treatment of colitis-associated colorectal cancer: insights from Traditional Chinese Medicine. *J Ethnopharmacol*, 2025, **338**(Pt 2): 119096
- [19] Wang Y, Zeng Y, Chen X, *et al.* Gut microbiota modulation through traditional Chinese medicine (TCM) - improving outcomes in gastrointestinal (GI) cancer prevention and management. *Pharmacol Res Mod Chin Med*, 2024, **13**: 100528
- [20] Sue P H, Ng F L, Foo C N, *et al.* A systematic review on the traditional Chinese medicine body constitution in different types of cancer. *Eur J Integr Med*, 2025, **76**: 102481
- [21] Luan Z Q, Zhao P L, Cao W F. Research status of astragali radix on nerve cells and nerve system diseases. *Chin Herb Med*, 2012, **4**(4): 271-275
- [22] Li M, Han B, Zhao H, *et al.* Biological active ingredients of *Astragali Radix* and its mechanisms in treating cardiovascular and cerebrovascular diseases. *Phytomedicine*, 2022, **98**: 153918
- [23] Yang Y, Vong C T, Zeng S, *et al.* Tracking evidences of *Coptis chinensis* for the treatment of inflammatory bowel disease from pharmacological, pharmacokinetic to clinical studies. *J Ethnopharmacol*, 2021, **268**: 113573
- [24] Liu D, Qin H, Liu G, *et al.* Astragaloside IV inhibits atherosclerosis caused by ferritin autophagy in a lipid deposition environment by activating Nrf2. *J Funct Foods*, 2025, **126**: 106695
- [25] Li Y, Zhang Y N, Zhang P L, *et al.* Inhibition of citrate transport reduces HIF-1 α /GABA-T-mediated succinate accumulation in macrophages: the role of astragaloside IV in ischemic stroke. *Phytomedicine*, 2025, **139**: 156449
- [26] Gong P, Long H, Yang Q, *et al.* Astragaloside IV alleviates diabetic nephropathy by modulating the gut-kidney axis and AMPK/PI3K/AKT pathway. *Food Biosci*, 2024, **62**: 105448
- [27] Chen G, Zhang C, Zou J, *et al.* Coptidis rhizoma and berberine as anti-cancer drugs: a 10-year updates and future perspectives. *Pharmacol Res*, 2025, **216**: 107742
- [28] Wang M, Ma Y, Yu G, *et al.* Integration of microbiome, metabolomics and transcriptome for in-depth understanding of berberine attenuates AOM/DSS-induced colitis-associated

- colorectal cancer. Biomed Pharmacother, 2024, **179**: 117292
- [29] Ling Q, Fang J, Zhai C, *et al.* Berberine induces SOCS1 pathway to reprogram the M1 polarization of macrophages *via* miR-155-5p in colitis-associated colorectal cancer. Eur J Pharmacol, 2023, **949**: 175724
- [30] China Association of Chinese Medicine. Guidelines for Traditional Chinese Medicine Diagnosis and Treatment of Tumors: ZYYXH/T136-156-2008. Beijing: China Press of Chinese Medicine, 2008: 25-29
- [31] Armengaud J, Cardon T, Cristobal S, *et al.* Novel model organisms and proteomics for a better biological understanding. J Proteom, 2025, **316**: 105441
- [32] Laurent A, Allard A, Fillet M. Cell surface proteomics: analytical challenges and clinical applications in cancer. Trends Anal Chem, 2025, **184**: 118143
- [33] Wang C, Zhou J, Jia P, *et al.* Joint proteomic and metabolomic analysis reveals renal metabolic remodeling of chronic heart failure mice. J Pharm Biomed Anal, 2025, **255**: 116641
- [34] Oh J W, Ahn S J, Jung J H, *et al.* Proteomic analysis of aqueous humor identified clinically relevant molecular targets for neovascular complications in diabetic retinopathy. Mol Cell Proteom, 2025, **24**(4): 100953
- [35] Yan S H, Hu L M, Hao X H, *et al.* Chemoproteomics reveals berberine directly binds to PKM2 to inhibit the progression of colorectal cancer. iScience, 2022, **25**(8): 104773
- [36] Gheybi E, Hosseinzadeh P, Tayebi-Khorrami V, *et al.* Proteomics in decoding cancer: a review. Clin Chim Acta, 2025, **574**: 120302
- [37] Koudelka T, Bassot C, Piazza I. Benchmarking of quantitative proteomics workflows for limited proteolysis mass spectrometry. Mol Cell Proteom, 2025, **24**(4): 100945
- [38] Peters-Clarke T M, Liang Y, Mertz K L, *et al.* Boosting the sensitivity of quantitative single-cell proteomics with infrared-tandem mass tags. J Proteome Res, 2025, **24**(4): 1539-1548
- [39] Kohler D, Staniak M, Tsai T H, *et al.* MSstats version 4.0: statistical analyses of quantitative mass spectrometry-based proteomic experiments with chromatography-based quantification at scale. J Proteome Res, 2023, **22**(5): 1466-1482
- [40] Guo J, Ho C T, Bai N. New utilization of *Fraxinus mandshurica* leaves: as a safe and promising natural antioxidant. J Agric Food Chem, 2024, **72**(37): 20470-20482
- [41] Guo J, Ho C T, Bai N. Utilizing HPLC-DAD, multivariate analysis, molecular docking, and *in vitro* validation methods to investigate phenolic compounds with potential antidiabetic properties in *Fraxinus mandshurica*. Microchem J, 2024, **207**: 111771
- [42] Waldner M J, Neurath M F. TGF β and the tumor microenvironment in colorectal cancer. Cells, 2023, **12**(8): 1139
- [43] Moon E J, Mello S S, Li C G, *et al.* The HIF target MAFF promotes tumor invasion and metastasis through IL11 and STAT3 signaling. Nat Commun, 2021, **12**(1): 4308
- [44] Juurikka K, Butler G S, Salo T, *et al.* The role of MMP8 in cancer: a systematic review. Int J Mol Sci, 2019, **20**(18): 4506
- [45] Xie Z, Lin H, Huang Y, *et al.* BAP1-mediated MAFF deubiquitylation regulates tumor growth and is associated with adverse outcomes in colorectal cancer. Eur J Cancer, 2024, **210**: 114278
- [46] Jiang Y, Li X, Yang Y, *et al.* LncRNA HOXC-AS1 sponges miR-99a-3p and upregulates MMP8, ultimately promoting gastric cancer. Cancers, 2022, **14**(14): 3534
- [47] Wan X B, Wang A Q, Cao J, *et al.* Relationships among *KRAS* mutation status, expression of RAS pathway signaling molecules, and clinicopathological features and prognosis of patients with colorectal cancer. World J Gastroenterol, 2019, **25**(7): 808-823
- [48] Zhang B, Cheng X, Zhan S, *et al.* MIB1 upregulates IQGAP1 and promotes pancreatic cancer progression by inducing ST7 degradation. Mol Oncol, 2021, **15**(11): 3062-3075
- [49] Lee H, Kong J S, Lee S S, *et al.* Radiation-induced overexpression of TGF β and PODXL contributes to colorectal cancer cell radioresistance through enhanced motility. Cells, 2021, **10**(8): 2087
- [50] Liang T, He J, Zhu W, *et al.* *FAT1* mutations in colorectal cancer patients are associated with the therapeutic benefit of immunotherapy. J Clin Oncol, 2022, **40**(16 suppl): e15523
- [51] Xie Y, Wang Y, Chen L, *et al.* Atractylenolide I regulates colorectal cancer cells' biological processes and glycolysis *via* KRT7. Pak J Pharm Sci, 2024, **37**(6): 1213-1222
- [52] Liang Y, Chen B, Liang D, *et al.* Pharmacological effects of astragaloside IV: a review. Molecules, 2023, **28**(16): 6118
- [53] Xia D, Li W, Tang C, *et al.* Astragaloside IV, as a potential anticancer agent. Front Pharmacol, 2023, **14**: 1065505
- [54] Zhu J R, Lu H D, Guo C, *et al.* Berberine attenuates ischemia-reperfusion injury through inhibiting HMGB1 release and NF- κ B nuclear translocation. Acta Pharmacol Sin, 2018, **39**(11): 1706-1715

结直肠癌气血两虚证与湿热证的蛋白质组学分析: 黄芪甲苷IV及小檗碱的靶向治疗作用*

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摘要 目的 探讨结直肠癌 (colorectal cancer, CRC) 气血两虚证 (qi-blood deficiency syndrome, QBDS) 与湿热证 (damp-heat syndrome, DHS) 的蛋白质组学差异, 构建“证候-差异蛋白-中药成分”网络。方法 采用串联质量标签 (tandem mass tag, TMT) 蛋白质组学技术分析 10 例 CRC 患者肿瘤组织 (QBDS 组 5 例、DHS 组 5 例), 筛选差异表达蛋白 (differentially expressed proteins, DEPs); 应用最小绝对收缩和选择算子 (least absolute shrinkage and selection operator, LASSO) 回归构建核心蛋白预测模型, 并结合分子对接及细胞计数试剂盒-8 (cell counting kit-8, CCK-8) 实验, 在人结肠肿瘤细胞系 116 (human colon tumor 116, HCT 116) 和人肿瘤细胞系 29 (human tumor 29, HT-29) 细胞中验证黄芪甲苷 IV 和小檗碱的结合能力及抗增殖作用。结果 共鉴定出 44 个 DEPs, 其中 QBDS 组 21 个上调、23 个下调, 主要富集于抗病毒免疫、核因子 κ B (nuclear factor κ B, NF- κ B) 信号通路及胆固醇代谢。通过 LASSO 回归分析筛选获得 8 个核心蛋白, 其中 QBDS 组包括肿瘤发生抑制因子 7 (suppression of tumorigenicity 7, ST7)、甘油磷酸胆碱磷酸二酯酶 1 (glycerophosphocholine phosphodiesterase 1, GPCPD1)、足萼蛋白样蛋白 (podocalyxin-Like, PODXL); DHS 组包括非典型钙黏蛋白 1 (FAT atypical cadherin 1, FAT1)、生长因子受体结合蛋白 7 (growth factor receptor bound protein 7, GRB7)、角蛋白 7 (keratin 7, KRT7)、MAF bZIP 转录因子 F (MAF bZIP transcription factor F, MAFF)、基质金属蛋白酶 8 (matrix metalloproteinase 8, MMP8)。分子对接显示, 黄芪甲苷 IV 与 QBDS 核心蛋白结合能均低于 -5 kcal/mol, 小檗碱与 DHS 核心蛋白结合能更低且特异性更强。小檗碱显著抑制 HCT 116 和 HT-29 细胞增殖, 48 h IC_{50} 最低为 15.21 mg/L, 优于黄芪甲苷 IV。结论 CRC 气血两虚证与湿热证具有不同的蛋白质组学特征, 黄芪甲苷 IV 和小檗碱可能分别靶向证候特异性核心蛋白发挥作用, 为基于证候的 CRC 精准整合医学研究提供初步蛋白质组学依据。

关键词 结直肠癌, 气血两虚证, 湿热证, 蛋白质组学, 黄芪甲苷 IV, 小檗碱

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